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A Kinase Anchoring Protein 150 (AKAP150)

► Calcium Sparklets and Waves

AAP, Aminopeptidase from *Vibrio Proteolyticus (Aeromonas Proteolytica)*

► Zinc Aminopeptidases, Aminopeptidase from Vibrio Proteolyticus (Aeromonas proteolytica) as Prototypical Enzyme

Acclimatization

► Cadmium Exposure, Cellular and Molecular Adaptations

Accumulation

► Thallium, Distribution in Animals

ACD, N-Acetylcitrulline Deacetylase

► Zinc Aminopeptidases, Aminopeptidase from Vibrio Proteolyticus (Aeromonas proteolytica) as Prototypical Enzyme

Acireductone Dioxygenase

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Synonyms

1,2-Dihydroxy-3-keto-5-thiomethylpent-1-ene dioxygenase; ARD; E2; E2'; EC 1.13.11.54 & 1.13.11.53

Definition

Acireductone dioxygenase (ARD) catalyzes the penultimate reaction in the methionine salvage pathway (MSP), the oxidative addition of O_2 to 2-hydroxy-3-keto-thiomethylpent-1-ene. The ARD-catalyzed reaction represents a branch point in the MSP. The on-pathway reaction (Scheme 1) results in the formation of formate and the keto-acid precursor of methionine, 4-thiomethyl-2-ketobutyrate. An off-pathway reaction (Scheme 2) results in the production of formate, carbon monoxide and 3-thiomethylpropionate. It has been shown that the identity of the metal bound in the ARD active site controls which of these two reactions occur. In the case of the enzyme isolated from *Klebsiella oxytoca*, KoARD, the first ARD to be characterized, the on-pathway enzyme (KoARD') binds Fe^{+2} and that catalyzing the off-pathway reaction (KoARD) binds Ni^{+2}. Co^{+2} and Mn^{+2} can replace

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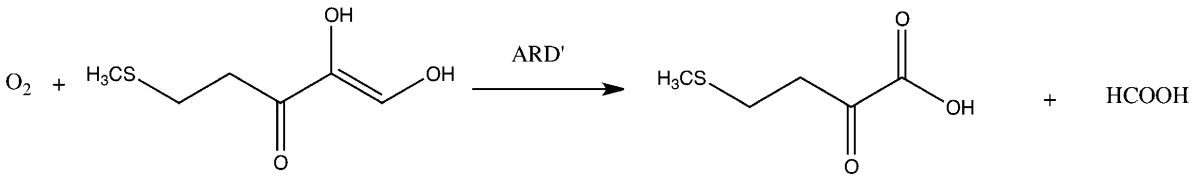
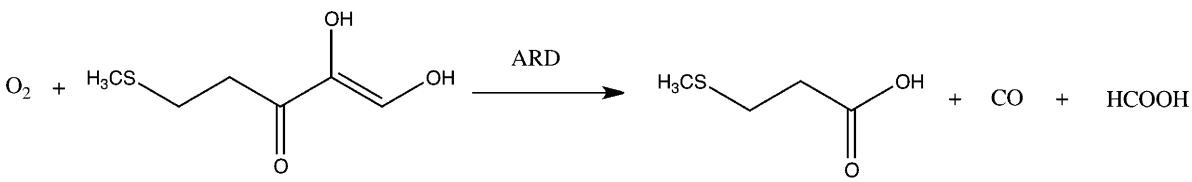


Scheme I

Acireductone Dioxygenase, Scheme 1 On-pathway reaction catalyzed by Fe-bound ARD' in the methionine salvage pathway



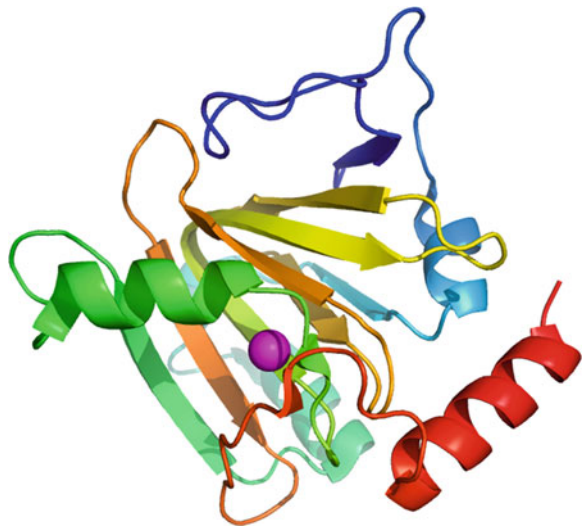
Scheme II

Acireductone Dioxygenase, Scheme 2 Off-pathway oxidation of acireductone catalyzed by Ni-bound ARD with carbon monoxide (CO) as product

Ni^{+2} in generating off-pathway activity, while Mg^{+2} confers partial on-pathway activity (Dai et al. 2001). The enzyme has since been identified in both prokaryotic and eukaryotic organisms, although catalytic activity and metal-binding preferences have only been thoroughly characterized for the *K. oxytoca* enzyme.

Structure

ARD is a member of the structural class of proteins known as cupins, or "jellyrolls." The core of the cupin structure is a β-helix formed from two antiparallel β-strands. If one were to twist a hairpin into a helix, one would obtain the cupin-folding topology. Several α-helices are arranged in pseudosymmetric fashion around the central β-helix in ARD (Fig. 1). The active site of ARD is located at the wide end of the β-helix, with access controlled by nearby helix and loop structures. The active site incorporates a three-histidine one-carboxylate pseudo-octahedral metal ligation scheme, with two His residues in adjacent equatorial positions around the metal, and the axial positions occupied by the third His and a glutamyl carboxylate group. The two equatorial positions closest to the opening of the active site are presumed to be the site of substrate binding to the metal. The ligation is reminiscent of and clearly related to the 2-His 1-carboxylate facial Fe(II) ligation found in many nonheme dioxygenases of the cupin superfamily, including those dependent on α-ketoglutarate.

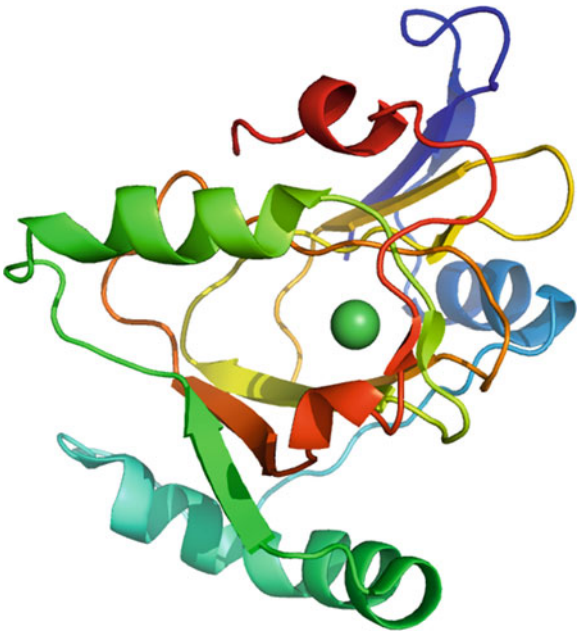


Acireductone Dioxygenase, Fig. 1 Crystallographic structure of MmARD, 1VR3, with active site in foreground. Presumed nickel ion is shown as *magenta sphere*

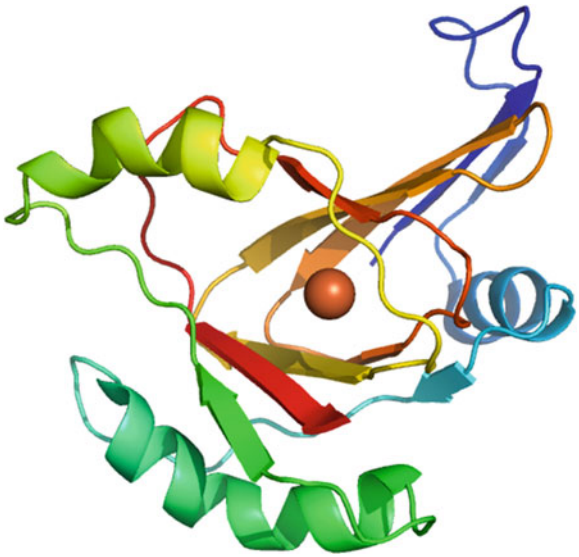
Three structures of ARD enzymes are currently available in the RCSB Protein Data Base. One is a crystallographic structure (Fig. 1), that of mouse ARD (MmARD, PDB ID 1VR3) (Xu et al. 2006), and two are NMR-derived, that of the iron-containing

KoARD' (2HJI) (Ju et al. 2006) and nickel-containing KoARD (1ZRR) from *Klebsiella oxytoca* (Pochapsky et al. 2002; Pochapsky et al. 2006). In the case of the MmARD structure, the ligand(s) occupying the presumed substrate binding site on the metal (which is presumed but not known to be Ni(II)) are unidentified, while the KoARD structures required XAS and homology modeling to characterize metal binding due to the paramagnetism of the metal in either case.

Comparison of the KoARD and KoARD' structures provide some insight into the origin of both the differential chemoselectivity of the two enzymes as well as their chromatographic separability (Ju et al. 2006). The ligation of Fe(II) and Ni(II) in both forms of the enzyme is similar, as determined by mutagenesis and XAS investigations (Chai et al. 2008). However, nickel-ligand bond lengths in KoARD are slightly shorter than the corresponding iron-ligand bonds in KoARD'. As the positions of three of the protein-based ligands (two histidine residues and one glutamate) are fixed within the β-helix, the differential bond lengths between the two forms are amplified in the position of the fourth ligand, a histidine that resides on a flexible loop. In KoARD, this loop is involved in several hydrogen bond–salt bridge interactions that stabilize a partially occluded active site entrance and permit formation of a 3,10 helix near the C-terminus of the polypeptide that sits atop the β-helix (Fig. 2). In KoARD', the active site is more open, the top helix is absent (Fig. 3), and the C-terminal peptide is disordered. This has been termed the result of a structural entropy switch, in that some parts of the iron-binding KoARD' are in fact more ordered than in the nickel-containing KoARD (Ju et al. 2006).



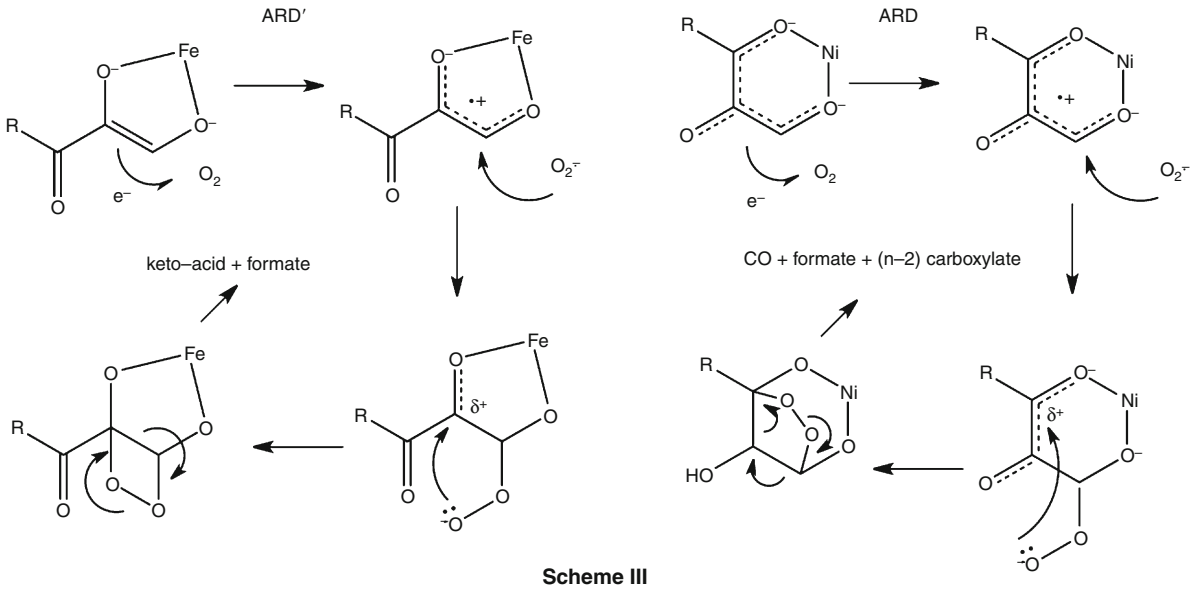
Acireductone Dioxygenase, Fig. 2 KoARD structure 1ZRR. *Green sphere* indicates position of Ni^{+2} ion. Note 3,10 helix on *top* (*red*)



Acireductone Dioxygenase, Fig. 3 KoARD' structure 1HJI. *Orange sphere* indicates the position of Fe^{+2}

Mechanism of Catalysis

The substrate of ARD, 1,2-dihydroxy-3-keto-5-thiomethylpent-1-ene, has not been synthesized de novo. However, the desthio analog, 1,2-dihydroxy-3-keto-5-hex-1-ene (acireductone) can be generated in situ by treatment of synthetically prepared 1-phosphonooxy-2,2-dihydroxy-3-oxohexane with E1 enolase phosphatase. This analog is turned over by both ARD and ARD' and is appropriate for activity assays and mechanistic studies. The acireductone substrate is quite reactive, and oxidative decomposition occurs in air with exposure to trace amounts of metal ions and/or peroxides, leading to the on-pathway products of keto acid and formate. Because of its reactivity, it was initially assumed that the on-pathway oxidation of acireductone was uncatalyzed in vivo, and it was



Acireductone Dioxygenase, Scheme 3 Proposed mechanisms for regioselectivity in the oxidation of acireductone by Fe-ARD' (left) and Ni-ARD (right)

only upon discovery of ARD that it was shown that the reaction is in fact catalyzed. However, it is clear that acireductone reacts readily with oxygen, and no evidence has yet been found for direct interaction between O_2 and the metal center in either form of ARD, as would be expected for oxygen activation (Dai et al. 2001). Nor is there evidence for even transient changes in metal oxidation state. As such, it is proposed that the role of the metal is to direct the regiochemistry of substrate oxidation by selective Lewis acid activation of either C-2 or C-3 on the substrate (Scheme 3). In the proposed mechanism, the first step for either on- or off-pathway reaction would be chelation of the metal by substrate at the two equatorial positions that do not bear proteinaceous ligands. Ligation would be to the oxygens at C-1 and C-3 in the case of the off-pathway reaction and C-1 and C-2 for on-pathway chemistry. In either case, substrate binding would be followed by initial electron transfer to molecular oxygen, generating a nucleophilic superoxide radical anion. The Lewis acidity of the metal ion would direct the attack of superoxide to form either the five-membered (in the case of ARD) or four-membered (ARD') cyclic peroxide (Ju et al. 2006).

The cyclic peroxides thus formed would undergo electrocyclic rearrangements to yield either the on-pathway (ARD') or off-pathway products (ARD). Note that in Scheme 3, there is a requirement for isomerization of 1-ene double bond in order to obtain the geometry required for ARD-type (C-1,C-3 oxygens) ligation. The mechanism of this isomerization is unclear. However, model studies of Ni-acireductone complexes support both the geometry of the ligation and demonstrate the production of carbon monoxide from such complexes (Rudzka et al. 2010).

Finally, the role of the structural rearrangement between ARD and ARD' in determining product distribution is unclear. The model complexes that have been synthesized to test the ARD reaction model are sterically quite hindered, suggesting that sterics may be important in controlling the type of chelation observed (Rudzka et al. 2010). Given that the ARD' active site is more accessible than that of ARD, it is possible that substrate is able to bind to ARD' a more extended conformation, yielding the C-1,C-2-attached oxygen ligation preferentially. However, this hypothesis is not supported experimentally as yet (Ju et al. 2006).

Alternative Functions of ARD

The fact that the on-pathway chemistry catalyzed by ARD' occurs spontaneously in vitro suggests that the

enzyme may have more complex roles to play than simply directing the last step of the MSP. Given that carbon monoxide has been implicated in a variety of biological signaling pathways, including neurotransmission, apoptosis suppression/induction, and bacterial quorum sensing, it is conceivable that the off-pathway functions of ARD are more significant than the on-pathway function in vivo. Some of these functions may not even require enzymatic activity: The human ARD ortholog (HsARD) was first identified as a permissive trigger for hepatitis infection in nonpermissive cell lines, not as an enzyme. ARD has since been implicated in carcinogenesis in testicular cancer (Oram et al. 2007) and regulation of activity of matrix metalloproteinase I (Hirano et al. 2005). While the role of the metal center in such activity is as yet unclear, we know that Ni(II) binding in KoARD represents a kinetic trap, since the protein must be denatured in order to remove nickel ion, while the iron form loses metal rather easily, and without denaturation of the protein. This would provide a potentially useful switching mechanism for controlling any regulatory binding functions that ARD might provide in nonenzymatic roles.

Cross-References

- ▶ Iron Proteins, Mononuclear (non-heme) Iron Oxygenases
- ▶ Nickel-Binding Sites in Proteins

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Actinide and Lanthanide Systems, High Pressure Behavior

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Synonyms

4f systems – lanthanide elements; *5f* systems – actinide elements; *f*-electron systems – lanthanide and actinide elements; Lanthanides – rare-earths

Definition

In the periodic table, the 15 elements starting from lanthanum (La) to lutetium (Lu) are called lanthanides and the other 15 elements starting from actinium (Ac) to lawrencium (Lr) are called actinides. Pressures above one atmosphere are called high pressure.

The effects of high pressure on these elements leading to changes in their physical behavior, especially structural behavior, are discussed in this entry.

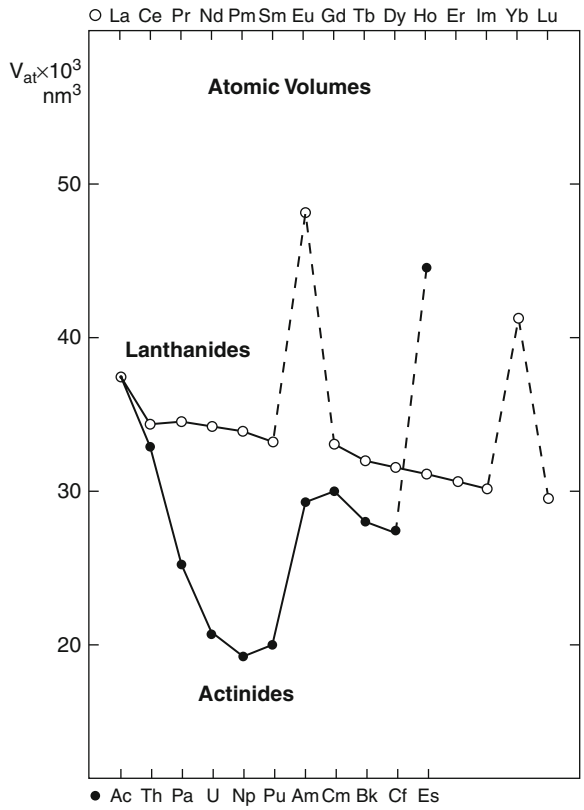
Introduction

The actinide and lanthanide elements occupy a special position in the periodic table. They exhibit very interesting physical and chemical properties and are of great technological importance.

The Lanthanides

The lanthanides comprise the 15 elements (La–Lu) in the periodic table. This series begins with the filling of the 4f electron shell having the electronic configuration: $[Xe]4f^{0}5d^{0\text{-}1}6s^{2}$. The 4f orbitals in the lanthanide series are inner and do not participate in chemical bonding. They are core-like (localized) in nature and the chemistry of the lanthanide ions are in general very similar (Temmerman et al. 2009; Johansson and Brooks 1993). The s and d valence electrons thus dominate in bonding and leave their respective atomic cores in a trivalent state, except for Eu and Yb, which exhibit divalency. This is because of the tendency of the 4f shell to be half-filled or filled, the lone d electron finds energetically more favorable to occupy the 4f shell. Thus the $[Xe]4f^{7/14}5d^{0}6s^{2}$ configuration in these two elements lead to divalency. The trivalent lanthanide elements are called regular lanthanides.

The atomic volumes of the lanthanides are shown in Fig. 1. In lanthanides, a gradual decrease of atomic volume across the series is found except for Eu and Yb, which are divalent. This gradual decrease is due to the increase in the nuclear charge and partial screening of the electronic charge by the inner 4f electrons seen by the nucleus due to the other outer electrons, leading to the well-known "lanthanide contraction." Their bulk properties also are expected to vary in a gradual manner across the series. For example, as we proceed from La to Lu with increasing atomic number (or decreasing atomic volume), the crystal structure sequence fcc → dhcp → Sm-type (Hexagonal) → hcp is observed at ambient conditions (Johansson and Rosengren 1975; Benedict and Holzapfel 1993; Holzapfel 1995).



Actinide and Lanthanide Systems, High Pressure Behavior, Fig. 1 The equilibrium atomic volumes of the lanthanide and actinide elements (Benedict and Holzapfel 1993). The deviations for Eu, Yb, and Es are due to their divalency

The Actinides

The actinide series comprises the 15 elements (Ac–Lr) in the periodic table and begins with the filling of the 5f electron shell having the electronic configuration: $[Rn]5f^{0}6d^{0\text{-}1}7s^{2}$. In actinides, since there is an inner 4f orbital, the electrons in the 5f orbital are pushed out due to core orthogonalization (5f orbitals orthogonal to 4f). The 5f orbitals are thus more extended compared to the 4f and are very close in energy to the 6d. However, as the atomic number increases, the spatial extension of the 5f orbitals shrink and become more localized. In fact, the 5f orbitals in early actinides (up to Pu) are itinerant (delocalized) and participate in bonding like the d orbitals in transition metals, and those in the late actinides (Am onward) are localized like the 4f orbitals in lanthanides (Johansson and Brooks 1993; Johansson and Li 2007; Moore and van der Laan 2009). Thus, the 5f orbitals in actinides exhibit dual nature, and the itinerant to localization transition takes place as a function of atomic number at Am. This can be clearly seen in their atomic volumes in Fig. 1. In early actinides, atomic volumes decrease

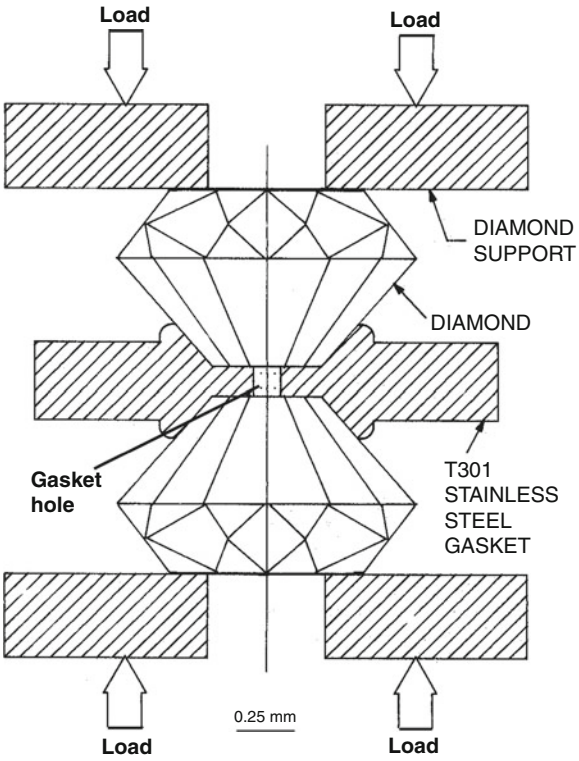
in a parabolic manner like the 3d/4d transition metal series because the itinerant 5f electrons are less effective in screening the nuclear charge. The late actinides (Am onward) exhibit a gradual decrease in their atomic volumes similar to the lanthanide series.

In actinide systems, most of the physical properties are governed by the nature of their 5f-electron states. For instance, the systems with itinerant 5f-electron states exhibit complex anisotropic physical properties and higher bulk modulus and stabilize in low symmetry crystal structures (tetragonal, orthorhombic, monoclinic, etc.). On the other hand, localized 5f-electron-based systems exhibit local magnetic moments and low bulk modulus and adopt high symmetry crystal structures (dhcp, fcc, etc.) (Johansson and Brooks 1993; Moore and van der Laan 2009).

High Pressure and Its Effect on Materials

Nowadays, high-pressure investigations up to pressures of few megabars (1 megabar = 100 GPa) can be carried out with the help of diamond anvil cells (DACs) using a variety of experimental techniques such as x-ray diffraction, neutron diffraction, Raman spectroscopy, optical absorption, and reflectivity, Mossbauer spectroscopy, NMR, and electrical resistivity (Jayaraman 1983, 1986). The basic principle of a DAC is shown in Fig. 2. It consists of a pair of gem quality diamond anvils with a metal gasket sandwiched between the two diamond flat culets. The metal gasket compressed to a thickness of about 50 μm has a central hole of diameter 100–200 μm, which contains a tiny sample immersed in a pressure-transmitting fluid. An external force is applied on the anvils to compress the fluid and thereby transmitting hydrostatic pressure to the sample. The diamond culets are small enough so that a few kN force is sufficient to generate pressures in the range of 100 GPa. Diamond being transparent to almost the entire electromagnetic spectrum, either an x-ray or laser beam can be focused on the sample through the diamond window to carry out a variety of in situ high-pressure investigations on the pressurized sample. Also, diamond, being the hardest material, enables achieving the highest static pressure (>500 GPa or 5 megabar) in a laboratory.

Application of external pressure on materials brings the constituent atoms closer and closer. This leads to



Actinide and Lanthanide Systems, High Pressure Behavior, Fig. 2 Basic principle of a diamond anvil cell for generating very high pressures

phenomenal changes in the materials which can be broadly classified into two categories: the lattice compression and the electronic structure change (Sahu and Chandra Shekar 2007). However, these changes are not totally independent, and often, one is associated with the other. The decrease in interatomic distances or increase in the density leading to changes in the phonon spectra, increase in the free energy (G) and the associated phase transitions stabilizing compact structures constitute the "lattice effects." As the interatomic distances decrease, the overlap of outer electronic orbitals in a solid increases. This leads to the following three principal effects in its electronic structure: (1) broadening of the electronic energy bands (or increase in the energy band widths), (2) shifting of the energy bands with respect to the Fermi energy E_F, and (3) shifting of the Fermi level itself to higher values. All these electronic effects lead to dramatic changes in their physical and chemical properties. For instance, closing of energy gaps lead to metal–insulator transitions, shift in energy bands lead to

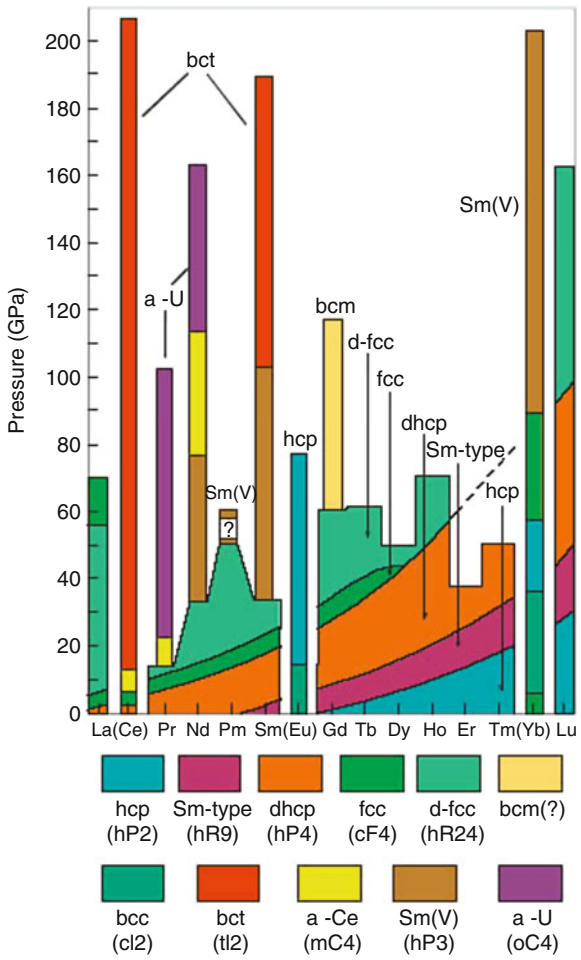
interband electron and valence transitions, change in the topology of the Fermi surface lead to Lifshitz type transitions and so on.

Effect of Pressure on Lanthanides and Actinides

Lanthanides

We have seen that the 4f orbitals in lanthanides are localized, do not participate in bonding, and contribute to the formation of local magnetic moments. The 5f orbitals in actinides on the other hand exhibit dual nature: itinerant in the first half of the series (up to Pu) and contribute to the bonding, localized in the next half (Am onward) resembling the lanthanides. The nature of these 4f/5f orbitals (i.e., whether itinerant or localized) depends on their f-orbital overlaps, which in turn depend on the interatomic distance in the solid. These interatomic distances or the orbital overlaps can be tuned in a controlled manner by applying external pressure. Thus in systems with localized f orbitals to start with, can be transformed into delocalized states by the application of pressure. Once the f orbitals become delocalized and participate in bonding, the systems exhibit very interesting changes in their crystal structures and physical and chemical properties. Generally, they transform to low-symmetry crystal structures associated with large volume collapse across the transition (Johansson and Rosengren 1975; Benedict and Holzapfel 1993; Holzapfel 1995). The stabilization of low-symmetry crystal structures is the direct manifestation of the highly complicated shaped f orbitals, and the volume collapse is due to their participation in bonding. Their physical properties also become highly anisotropic and the chemical nature changes due to increase in their valency. Also, their local magnetic moments get destroyed due to delocalization of the f orbitals.

In regular lanthanides, we have seen the structure sequence: hcp → Sm-type → dhcp → fcc as a function of decreasing atomic number at ambient conditions. The same structural sequence has been observed in lanthanide elements as function of increasing pressure (Johansson and Rosengren 1975; Benedict and Holzapfel 1993; Holzapfel 1995). This sequence is seen most completely for the heavier lanthanides, while the lighter elements start already at ambient pressure with the high-pressure structures of the heavier elements. However, this pressure-induced structural sequence should not be attributed to the effect of 4f electron states, because similar pressure-induced sequence has been seen in La which does not have any 4f occupancy. Later, energy band calculations show that the d-band contribution to the total energy is what actually drives the lattice through the observed crystal structure sequence (Duthie and Pettifer 1977). Lu, which crystallizes into an hcp structure at STP, has a d-band occupation number of about 1.5. La on the other hand, which crystallizes into a dhcp structure at STP, has a d-band occupation number of the order of 2.7. Pressure has the effect of increasing the energy of electrons in the s-band relative to the d-band, which initiates the s → d transfer and the number of d electrons per atom in the conduction band tends toward 3. A similar sequence has been observed in yet another non-4f element yttrium. Subsequent high-pressure investigations have revealed yet another high-pressure phase, known as the distorted fcc phase in La, Y, Pr, Nd, Pm, Sm, Gd, and Yb. This phase transition also is not because of the 4f effect, as both La and Y do not have any 4f occupancy. Hence, the correct high-pressure sequence in lanthanides is: hcp → Sm-Type → dhcp → fcc → dist. fcc. At still higher pressures, a number of anomalous low-symmetry phases (monoclinic, tetragonal, α-U type orthorhombic) have been found outside this sequence and their origins have been largely attributed to be the result of 4f-electron delocalization and/or hybridization (Benedict and Holzapfel 1993; Johansson and Brooks 1993). The 4f delocalization transitions are generally accompanied with large volume collapse. Ce with fcc structure at STP and having only one 4f electron on the verge of delocalization, in fact, undergoes an isostructural transition with a volume collapse of about 16% at 0.7 GPa due to delocalization of its 4f electron. However, it does not stabilize in any low-symmetry structure characteristic of delocalized 4f due to the dominant 5d states, which favors fcc structure. It transforms to low-symmetry bcm and then to bct phase at 5.3 and 12.5 GPa, respectively, due to the increased delocalization of the 4f states. The structural transitions observed in lanthanides under pressure are summarized in Fig. 3 (Schiwek et al. 2002). Some recent results on Pr, Eu, Gd, Tb, Dy, and Tm reporting more structural transitions at multimegabar pressures are not shown in Fig. 3 and it is beyond the scope of this entry to include these results and cite the relevant references.



Actinide and Lanthanide Systems, High Pressure Behavior, Fig. 3 Generalized high-pressure phase diagram of the lanthanides (Schiwek et al. 2002)

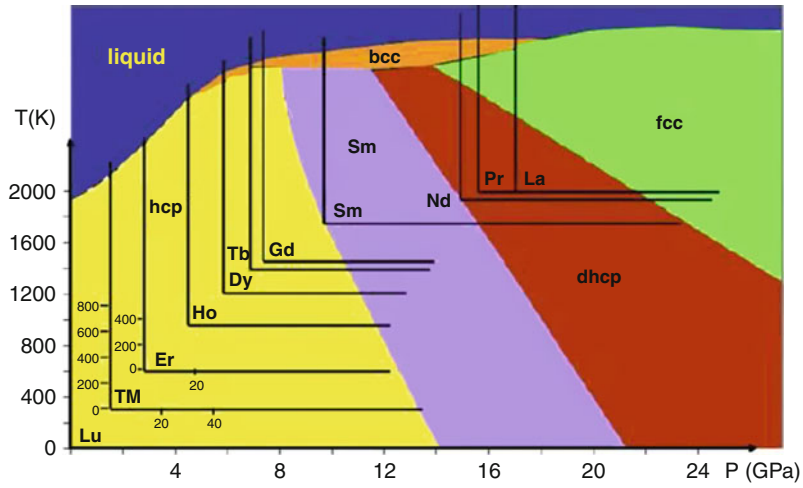
Johansson and Rosengren (1975) had constructed an interesting generalized P–T phase diagram for the lanthanides as shown in Fig. 4. The very fact that such a diagram could be constructed, based on the individual P–T phase diagrams of the lanthanides, gives very strong evidence for the close similarity between the lanthanide elements. In fact, this generalized phase diagram clearly demonstrates the inertness of the 4f electrons as regards their participation in the bonding. One can also clearly notice the absence of Eu and Yb in this diagram, the reason being their divalent metallic behavior in contrast to the common trivalent lanthanides. Also, the absence of Ce is due to the reason that high pressure destroys the inertness of its 4f electron, and therefore, it is no longer an adequate trivalent metal. Although, Pm has been found to exhibit the structural sequence: dhcp → fcc → trigonal under pressure and falls in line with other trivalent lanthanides (Benedict and Holzapfel 1993; Holzapfel 1995), it is not shown in this diagram.

The divalent lanthanide elements Eu and Yb, which stabilize in the bcc and fcc structure, respectively, at STP, are expected to exhibit normal behavior and enter the rare-earth crystal structure sequence at higher pressures. In fact, Eu transforms to the hcp structure at 12.5 GPa and Yb shows the structural sequence: fcc → bcc → hcp → fcc → hp3 at 4, 26, 53, and 98 GPa, respectively. The hp3 phase has been found to be stable up to 202 GPa. The divalent to trivalent transition in Yb takes place gradually and behaves like the regular trivalent lanthanides Sm and Nd (Chesnut and Vohra 1999).

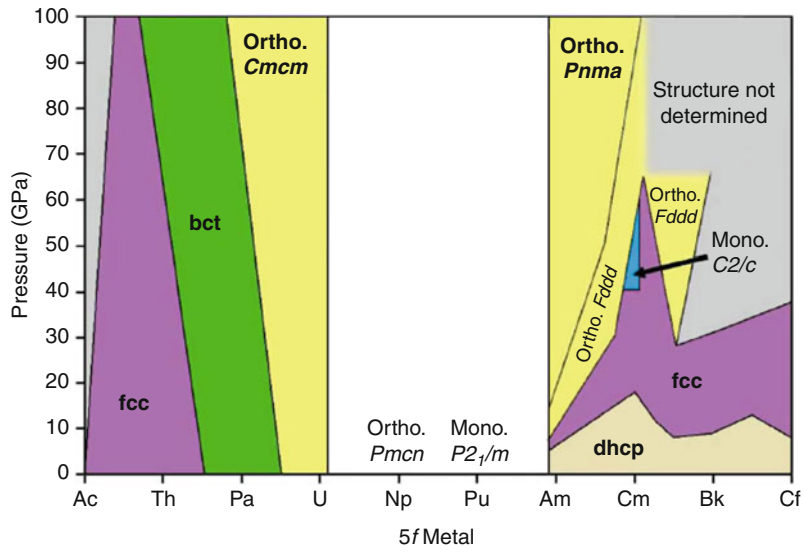
Actinides

The complex structures at atmospheric pressure of the lighter actinides are a consequence of itinerant 5f electrons. These metals are expected to be less compressible (i.e., exhibit larger bulk moduli) and exhibit few phase transitions under pressure. In contrast, the transplutonium metals (Am onward) have localized 5f electrons not contributing to bonding at atmospheric pressure, and are therefore expected to be "soft" (lower bulk moduli). Under pressure, the localized 5f electrons are expected to become itinerant due to more orbital overlap and acquire structures displayed by the light actinides (Benedict and Holzapfel 1993; Holzapfel 1995; Lindebaum et al. 2003; Moore and van der Laan 2009).

The structural transitions observed in actinides under pressure are summarized in Fig. 5. The light actinides with low-symmetry structures almost remain stable and show high bulk modulus (120–150 GPa). Pu, however, has a low bulk modulus of 40 GPa, as its 5f states are on the verge of localization and exhibit complex behavior. It transforms from its monoclinic to hexagonal phase at about 40 GPa. Pa also transforms from bct to α-U structure at 77 GPa. Th, which adopts the fcc structure at STP, has no 5f occupancy. However, its 5f level is located very close (about 1 eV above) to the Fermi level (E_F). Under high pressure, the 5f level gets lowered below E_F, giving rise to finite occupancy of the 5f states, and contributes to bonding. It transforms from its high-symmetry fcc to a low-symmetry bct structure at ~60 GPa and behaves like other light actinide metals.



Actinide and Lanthanide Systems, High Pressure Behavior, Fig. 4 A generalized P–T phase diagram of the trivalent lanthanides (Johansson and Li 2007). The empty spaces are for the missing elements Ce, Eu, Yb, and Pm (reasons given in section "Lanthanides")



Actinide and Lanthanide Systems, High Pressure Behavior, Fig. 5 A "pseudobinary" phase diagram of the 5*f* actinide metals as a function of pressure (Moore and van der Laan 2009). The pressure behaviors of Np and Pu are not shown, but the ground-state crystal structure of each metal is indicated. Under high pressure, Pu transforms to a hexagonal structure at 37 GPa, and Np remains stable in its orthorhombic phase up to 52 GPa. The area indicated by "structure not determined" represents α-U type structures

The late actinides, as expected, behave like the lanthanides under pressure. They transform from their dhcp to fcc/dfcc and eventually to low-symmetry (trigonal, α-U type orthorhombic) structures due to delocalization of their 5f states. The 5f delocalization with volume collapse takes place in one or two steps. For example, in Am, the fcc → fco (face centered oprthorhombic) transition at 10 GPa with a volume collapse of 2% takes place due to partial delocalization of 5f states, then it transforms to the α-U type structure at 16 GPa with a volume collapse of 7%. Similarly, in Cm and Cf, the 5f delocalization takes place in two steps, where as in Bk, it takes place in one step. Their bulk moduli, which are of the ~40 GPa and are similar to that of the lanthanides, dramatically increase to 120–150 GPa on delocalization of their 5f states and behave like the early actinides.

Their local magnetic moments also get destroyed due to delocalization of the 5f electrons. Some of the actinide elements like Th, Pa, and U also exhibit superconductivity at very low temperatures (Moore and van der Laan 2009). High pressure has very interesting effect on the superconducting transition temperature (T_C) of these metals. Whereas the T_C decreases with pressure in Th, it increases in case of Pa and U, and this difference in behavior has been attributed to the presence of 5f states.

Conclusions

The lanthanides having localized 4f states exhibit the structural sequence hcp → Sm-type → dhcp → fcc → dist. fcc as a function of increasing pressure or decreasing atomic number. At higher pressures, they exhibit volume collapse transitions due to delocalization of their 4f states and stabilize in low-symmetry structures. Their bulk moduli increase to very high values across the 4f delocalization transitions. In case of actinides, the light actinides with low-symmetry structures show high bulk modulus (120–150 GPa) and few structural transitions. The late actinides (Am onward) with localized 5f states and dhcp structure have very low bulk moduli (∼40 GPa) like the lanthanides. They transform from their dhcp structure to fcc/dfcc and eventually to low symmetry α-U type structures due to delocalization of their 5f states and exhibit very high bulk moduli, similar to the early actinides.

Cross-References

▶ Actinides, Physical and Chemical Properties
▶ Lanthanides, Physical and Chemical Characteristics

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Actinides, Interactions with Proteins

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Synonyms

Complexation of actinides by proteins; Coordination of actinides to proteins; Uptake of actinides by proteins

Definition

The coordination of actinides to proteins largely through functional groups including carboxyl, hydroxyl, phosphate, etc.

Introduction

Actinides are chemical elements with atomic numbers ranging from 89 (actinium) to 103 (lawrencium) (Connelly et al. 2005). Five actinides have been found in nature: thorium, protoactinium, uranium, neptunium, and plutonium. Uranium is a widely distributed and occurs in almost all soils. Thorium is present at low levels in rocks and soils.

Small quantities of persisting natural plutonium have also been identified in the environment. Transients amounts of protactinium and atoms of neptunium are produced from the radioactive decay of uranium and from transmutation reactions in uranium ores, respectively (Asprey 2012). The other actinides (curium, americium, etc.) are synthetic elements (Darling 2011). Naturally occurring uranium and thorium and synthetically produced plutonium are the most abundant actinides on earth.

The distribution of actinides in the environment is continuously incremented due to human activities including nuclear weapon production and nuclear energy production, the storage of radioactive wastes, etc. The risk assessment of the distribution of these radioactive elements in the environment on human health is needed and implies the understanding of the mechanisms of interaction of these toxic elements with living organisms including humans, animals, microbes, and their cellular components (e.g., proteins, ADN, and ARN).

Proteins are one of the most important biomolecules involved in the complexation of heavy metals, including actinides. Ionizable functional groups such as carboxyl, phosphoric, amine, and hydroxyl represent potential binding sites for the sequestration of metal ions. Of these groups, carboxyl and phosphate are the main binding sites of actinides (Merroun et al. 2005).

A good characterization of the actinide/protein system implies the determination of the structural parameters of the formed complexes (nature of neighboring atoms to actinide, bond distances, etc.). Among the analytical techniques used to determine these parameters, synchrotron-based techniques such as X-ray absorption spectroscopy (XAS) are of great importance. This technique has been used to determine the oxidation state (X-ray absorption near edge structure, XANES) and to identify the number of atoms and their distances in the local structural environment (extended X-ray absorption fine structure, EXAFS) of actinides within a variety of proteins (Merroun et al. 2005; Merroun and Selenska-Pobell 2008). In addition, XAS is among the few analytical methods that can provide information on the chemical environment of actinides in biological samples at dilute metal concentrations. XAS is a nondestructive method, and no sample reduction or digestion is required which would alter the chemistry of the element of interest.

The molecular scale characterization of actinide/protein interactions is of great interest to understand the potential transport of radionuclide inside living organism (Jeanson et al. 2009). In addition, the structural information obtained will help to design and synthesize potential specific chelating agents for actinides and their use in the elimination of incorporated radionuclides (Jeanson et al. 2009). It also provides insights on the role played by these biomolecules, particularly of microbial origin, on the mobility of these inorganic contaminants in the environment.

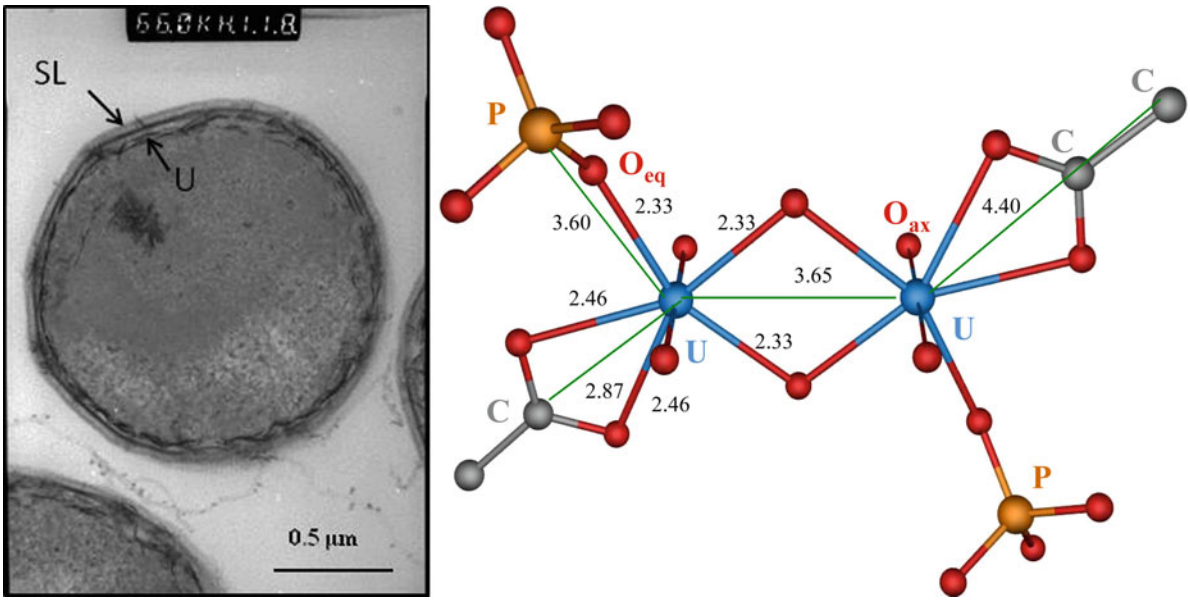
Interactions of Actinides with Microbial Proteins

Microbial cells may synthesize a variety of metal-binding peptides and proteins, e.g., metallothioneins and phytochelatins, which regulate metal ion homeostasis and affect toxic responses (Avery 2001). In addition, most archaea and a large number of bacteria possess proteinaceous layers external to their cell walls (Sleytr et al. 1996) which play a major role in the coordination of metal ions.

From the point of view of environmental impact, the interactions of actinides with microbial proteins are poorly studied, and most of these studies were focused on U. The best example of the proteins/uranium coordination studies is represented by bacterial surface layers proteins (S-layer).

Microbial S-Layers and Actinides

The crystalline bacterial S-layer, as probably the most abundant bacterial cellular proteins, represents the outermost cell envelope component of diverse types of bacteria and archaea (Sára and Sleytr 2000). S-layers are generally composed of identical protein or glycoprotein subunits, and they completely cover the cell surface during all stages of bacterial growth and division. Most S-layers are 5–15-nm thick and possess pores of identical size and morphology in the range of 2–6 nm (Beveridge 1994). As porous lattices completely covering the cell surface, the S-layers can provide prokaryotic cells with selective advantages by functioning as protective coats, as structures involved in cell adhesion and surface recognition, and as molecule or ion traps (Sleytr 1997). Surface layer sheets are, in some cases, glycosylated (Sleytr et al. 1996),



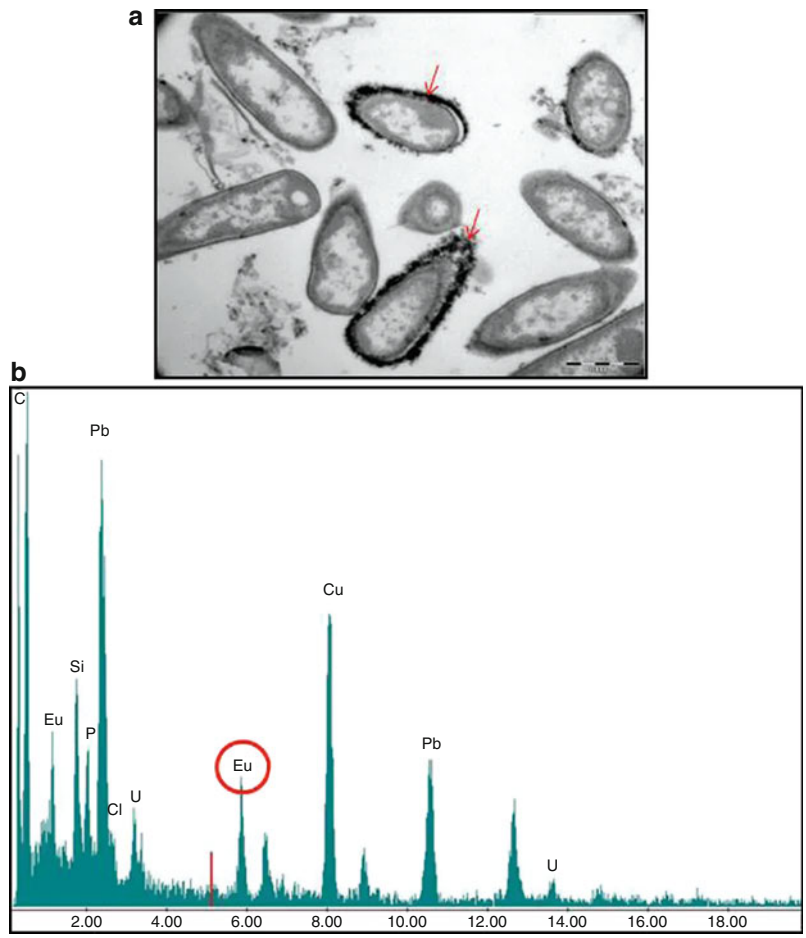
Actinides, Interactions with Proteins, Fig. 1 Transmission electron micrograph (*left*) of thin section of *L. sphaericus* JG-7B cells treated with uranium (U), where the metal is accumulated mainly within the inner side of S-layer protein (SL) of this bacterium. Model (*right*) used for the fit of the EXAFS spectrum of U complexes formed the S-layer of the strain JG-7B (Reprinted with permission from Merroun et al. (2005) American Society of Microbiology)

possess a large number of carboxylated amino acids (Sleytr et al. 1996), and can be also phosphorylated (Merroun et al. 2005). Johnson and Bardford (1993) reported that one-third of proteins in cells are phosphorylated. The phosphorylation of proteins is needed for the many cellular functions including regulation of gene transcription, signal transduction throughout the cell, regulation of enzyme activities, etc. (Johnson and Bardford 1993). These functions would be damaged by the coordination of proteins with heavy metals. Therefore, the interactions of heavy metals with proteins are of great importance (Fahmy et al. 2006).

The coordination of uranium to S-layer proteins of two uranium mining waste bacterial isolates, *Lysinibacillus sphaericus* (formerly *Bacillus sphaericus*) JG-A12 and JG-7B, has been extensively studied using molecular scale techniques (Merroun et al. 2005; Merroun and Selenska-Pobell 2008). X-ray absorption (XAS) studies showed that, in both bacterial S-layer proteins, U(VI) is coordinated to carboxyl groups in a bidentate fashion with an average distance between the U atom and the C atom of 2.88 ± 0.02 Å and to phosphate groups in a monodentate fashion with an average distance between the U atom and the P atom of 3.62 ± 0.02 Å (Merroun et al. 2005). The local coordination of U within the S-layer protein of these two bacteria is illustrated in the Fig. 1.

Analyses of the amino acid composition of the S-layer proteins of the strain JG-A12 demonstrated a high content of glutamic acid and aspartic acid and of other amino acids such as serine and threonine. The C-terminal part especially consists of stretches of glutamic acid and aspartic acid (both residues with carboxyl groups) and of serine and threonine (both residues with hydroxyl groups), the latter being potential phosphorylation sites. The carboxyl and phosphate groups of these amino acids are probably implicated in the complexation of uranium. Acidic amino acids (Asp and Glu) or the free carboxylate terminus has been demonstrated to be the binding sites for uranium in most of the structure of uranyl protein complexes found in the Protein Data Bank (PDB) (Van Horn and Huang 2006).

Compared to the S-layer protein of the closely related strain *L. sphaericus* NCTC 9602, the S-layer protein of the uranium mining waste pile isolate *L. sphaericus* JG-A12 contains about six times more phosphorus (Merroun et al. 2005). This difference may reflect an adaptation of *L. sphaericus* JG-A12 to its highly with uranium-contaminated environment.



Actinides, Interactions with Proteins,
Fig. 2 (**a**) Transmission electron micrograph of thin section of the cells of *Lysinibacillus sphaericus* JG-A12 treated with europium (III). The europium is coordinated mainly to S-layer proteins of this bacterium. (**b**) Spectrum of energy dispersive X-ray analysis of the Eu/S-layer protein accumulates

The high uranium affinity of phosphate groups on the surface of the cell may allow *L. sphaericus* JG-A12 to bind selectively large amounts of uranium before getting damaged by this toxic radionuclide. Figure 1 shows the cellular localization of the deposited U on the internal side of the S-layer protein, where the negatively charged groups are localized, and also into the cross-linked peptidoglycan layers of the cell wall.

The S-layer of *L. sphaericus* JG-A12 is involved in interactions not only with uranium but also with other radionuclides. Figure 2 shows, for instance, an example of accumulation of the inactive analog of the trivalent actinide curium(III), namely, the trivalent lanthanide europium(III), onto the surface of the strain. In this case, as well as in the case of U, the S-layer proteins may act as a molecular sieve, ion trap, or protective shell (Sleytr 1997). Because of the ability of S-layer to replace the "older" S-layer sheets on the cell surface, one can speculate about the mechanism of its protective function against uranium and other toxic metals. The saturation with metals (e.g., uranium) may lead to denaturation of the S-layer lattice, which is then replaced by freshly synthesized protein monomers (Merroun et al. 2005; Merroun and Selenska-Pobell 2008).

Coordination of Human/Animal Proteins to Actinides

In humans, the accidental release of actinides results in an internal contamination with these elements including uranium, thorium, neptunium, plutonium, and americium. Independently of the route of contamination, the radionuclide is absorbed into and then transported by blood (Jeanson et al. 2009). Several authors investigated the actinide/blood protein interactions in order to understand the behavior of actinides in biological tissues. Most of these studies were focused on uranium, and few investigations were performed to

elucidate the mechanisms of coordination of proteins to other actinides like Th, Np, Pu, Am, etc.

The coordination of tetravalent actinides (actinides (IV)) (thorium, neptunium, plutonium) by transferrin (the iron transport glucoprotein) was studied using a combination of spectroscopic techniques in including UV/Vis spectroscopy, X-ray absorption spectroscopy, etc. (Jeanson et al. 2010). The main objective of the later work is to compare the behavior of these elements to that of iron(III), the endogenous transferrin cation, from a structural point of view. There are some similarities between the behavior of Fe(III) and Np(IV)/Pu(IV). At least one tyrosine residue probably participate in the actinide coordination sphere, forming a mixed hydroxy-transferrin complex in which actinides are bound with transferrin both through actinide–tyrosine and through actinide–OH bonds (Jeanson et al. 2010). Grossmann et al. (1992) suggested that transferrin binds actinides(IV) in the iron complexation site which include two tyrosine, one histidine, and one aspartate. Th(IV) was not coordinated to ferritin under the experimental conditions of this work. In the case of calmodulin, Seeger et al. (1997) showed that plutonium (III) is coordinated to calcium site of the this protein.

In the case of uranium in the serum, the uranyl cation, a linear dioxo cation with an overall +2 charge (UO_2^{2+}), occurs as uranyl bis- and tris-carbonate complexes and UO_2/protein/carbonate complexes with human serum albumin (HSA, the most abundant serum protein implicated in the transport of essential divalent cations including Ni(II), Cu(II), Zn(II), etc.) and transferrin (TF). Of the two fractions, the uranyl–carbonate complexes are more diffusible into tissue (liver, kidneys, bones, etc.) while the portion bound to protein is the portion cleared from the serum via transport to and elimination from the kidneys (Van Horn and Huang 2006). The U(VI)–TF complexation constant values were slightly higher than those observed for U(VI)–HSA complex (Michon et al. 2010) which explain the high U binding capacities of the HSA protein(10 mol of U(VI) in comparison to that of the TF protein (5 mol of U(VI)).

Phosphovitin, from egg yolk, is another protein chosen as ideal model compound to study the complexation of organic phosphate residues of proteins with actinides. This protein is slightly glycosylated and highly phosphorylated being one of the most phosphorylated proteins in nature where 8–10% of the molecular weight composes of phosphorus (Byrne et al. 1984). The complexation of phosphovitin with U(VI) studied at different phosphate/uranium ratios using attenuated total reflection Fourier transform infrared (ATR FT-IR), indicated the implication of two phosphate groups in the coordination of this radionuclide (Li et al. 2010).

In addition to proteins, small peptides were used as models for studying the nature of proteins functional groups implicated in the coordination of actinides. Thus, a linear pentapeptide, acetyl-diaspartyl-prolyl-diaspartyl-amide, was studied as potential chelating ligand for thorium(IV), neptunium(IV), and plutonium(IV) cations forming polynuclear species with oxy- or hydroxo-bridged cations (Jeanson et al. 2009).

Conclusion

This entry described the molecular scale characterization of interactions between actinides and proteins of different origin (human, animal, and microbial). Carboxyl and phosphates groups are the main functional groups involved in the binding of actinides. Protein/actinides interaction research is an inherently multidisciplinary and requires a combination of wet chemistry, spectroscopy, microbiology, biochemistry, and radiochemistry. This emerging area of sciences poses many opportunities and challenges for microbiologists, biochemists, and toxicologists. Proteins could be used as biosorbent for bioremediation of actinide contaminated waters. A more immediate use of research results is in predicting the environmental and public health consequences of nuclear waste isolation.

Cross-References

▶ Toxicity

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Actinides, Physical and Chemical Properties

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Actinides are a group of metals following actinium in the periodic table. The first member is thorium (discovered in 1829) followed by protactinium (discovered in 1917), then uranium (discovered in 1789). The trans-uranium elements do not occur in nature and were prepared artificially. They became an important group of metals after the discovery of the phenomenon of fission in 1939.

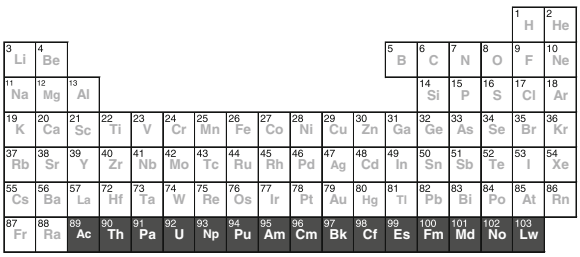
Element	Symbol	Atomic number	Atomic weight	Occurrence or preparation
Actinium	Ac	89	227	Relative abundance 3×10^{-14}
Thorium	Th	90	232.038	Relative abundance 1.1×10^{-3}
Protactinium	Pa	91	231	Relative abundance 8×10^{-11}
Uranium	U	92	238.03	Relative abundance 4×10^{-4}
Neptunium	Np	93	237	Bombarding ^{238}U by neutrons
Plutonium	Pu	94	242	Bombarding ^{238}U by deuterons
Americium	Am	95	243	Bombarding ^{239}Pu by neutrons
Curium	Cm	96	247	Bombarding ^{239}Pu by α-particles
Berkelium	Bk	97	247	Bombarding ^{241}Am by α-particles
Californium	Cf	98	249	Bombarding ^{242}Cm by α-particles
Einsteinium	Es	99	254	Product of nuclear explosion
Fermium	Fm	100	253	Product of nuclear explosion
Mendelevium	Md	101	256	Bombarding ^{253}Es by α-particles

(*continued*)

Element	Symbol	Atomic number	Atomic weight	Occurrence or preparation
Nobelium	No	102	254	Bombarding ^{243}Am by ^{15}Nor ^{238}U with α-particles
Lawrencium	Lw	103	257	Bombarding ^{252}Cf by ^{10}B or ^{11}B and of ^{243}Am with ^{18}O

Only thorium, uranium, and plutonium are of technical importance for nuclear energy.

Position in the Periodic Table



Physical and Nuclear Properties

	Thorium 232	Uranium 238	Plutonium 239
Meting point, °C	1,842	1,150	639.5
Density	11.7	19.1	19.8
Crystal structure	Face-centered cubic	Body-centered cubic	Body-centered cubic
Isotope percentage,%	100	99.28	
Half life, years	2.2×10^{10}	4.5×10^{9}	2.4×10^{4}
Type of decay	α-emission	α-emission	α-emission

Uranium 235 undergoes fission when bombarded by thermal neutrons; it breaks apart into two smaller elements and at the same time emitting several neutrons and a large amount of energy:

$$^{235}_{92}\mathrm{U} + {}^{1}_{0}\mathrm{n} \rightarrow {}^{89}_{36}\mathrm{Kr} + {}^{144}_{56}\mathrm{Ba} + 3{}^{1}_{0}\mathrm{n} + 200\ \mathrm{MeV}$$
$$\downarrow \qquad \downarrow$$
$$^{89}_{39}\mathrm{Y} + 3\mathrm{e}^{-} \quad {}^{144}_{60}\mathrm{Nd} + 4\mathrm{e}^{-}$$

Uranium 238 absorbs neutrons forming uranium 239 which is a beta emitter with a short half-life; its daughter neptunium 239 also emits an electron to form plutonium 239.

$$^{238}_{92}\mathrm{U} + {}^{1}_{0}\mathrm{n} \rightarrow {}^{239}_{92}\mathrm{U} \rightarrow {}^{239}_{93}\mathrm{Np} + \mathrm{e}^{-}$$
$$\downarrow$$
$$^{239}_{94}\mathrm{Pu} + \mathrm{e}^{-}$$

Plutonium 239 undergoes fission with the emission of several neutrons and can maintain a chain reaction. Thus, a nuclear reactor using uranium as a fuel, not only produces energy, but also produces another nuclear fuel. Under certain conditions it is possible to generate fissionable material at a rate equal to or greater than the rate of consumption of the uranium. Such a reactor is known as a *breeder reactor*.

Thorium absorbs neutrons and is transformed to uranium 233:

$$^{232}_{90}\mathrm{Th} + {}^{1}_{0}\mathrm{n} \rightarrow {}^{233}_{90}\mathrm{Th} \rightarrow {}^{233}_{91}\mathrm{Pa} + \mathrm{e}^{-}$$
$$\downarrow$$
$$^{233}_{92}\mathrm{U} + \mathrm{e}^{-}$$

Uranium 233 also undergoes fission and can be used to operate a breeder reactor in the presence of a thorium blanket according to the scheme:

$$^{233}_{92}\mathrm{U} + {}^{1}_{0}\mathrm{n} \rightarrow \text{Fission products} + \text{Energy} + \text{One neutron that is captured by } {}^{232}_{90}\mathrm{Th} \rightarrow {}^{233}_{90}\mathrm{Th} \rightarrow {}^{233}_{91}\mathrm{Pa} + \mathrm{e}^{-} \rightarrow {}^{233}_{92}\mathrm{U} + \mathrm{e}^{-} + \text{One neutron to continue chain reaction}$$

The electronic configuration of actinides is similar to the lanthanides in that the two outermost shells are the same (with minor exceptions due to quantum chemistry considerations).

Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lw
2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
18	18	18	18	18	18	18	18	18	18	18	18	18	18	18

(*continued*)

Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lw
32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
19	20	21	22	23	24	25	26	27	29	30	31	32	32	32
9	9	9	9	9	9	9	9	9	9	8	8	8	8	9
2	2	2	2	2	2	2	2	2	2	2	2	2	2	2

Main Compounds

- Thorium oxide, ThO_2, from which the metal is produced by reduction with calcium.
- Uranium tetrafluoride, UF_4, from which the metal is produced by reduction with magnesium
- Uranium hexafluoride, UF_6, a gas prepared from UF_4 by reaction with fluorine. Used for U 235 isotope separation
- Plutonium oxide, PuO_2, used for the production of the metal by reduction with calcium

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Activate: Stimulate

▶ Monovalent Cations in Tryptophan Synthase Catalysis and Substrate Channeling Regulation

Activation of Thyroxine (T4) by Deiodination to Triiodothyronine (T3)

▶ Selenoproteins and the Biosynthesis and Activity of Thyroid Hormones

Active Site Zinc

▶ Zinc Alcohol Dehydrogenases

Acute Phase

▶ Magnesium and Inflammation

Acute Promyelocytic Leukemia

▶ Arsenic in Therapy
▶ Promyelocytic Leukemia–Retinoic Acid Receptor α (PML–RARα) and Arsenic

ADAM

▶ Zinc Adamalysins

ADAMTS

▶ Zinc Adamalysins

AdcR

▶ Zinc Sensors in Bacteria

ADH1/ADC1

▶ Zinc Storage and Distribution in S. cerevisiae

AdoMet:Arsenic(III) Methyltransferase

▶ Arsenic Methyltransferases

Adsorption Studies

▶ Colloidal Silver Nanoparticles and Bovine Serum Albumin

Adverse Health Effects Related to Mn

▶ Manganese Toxicity

Aequorin

▶ Sarcoplasmic Calcium-Binding Protein Family: SCP, Calerythrin, Aequorin, and Calexcitin

Ag, MT

▶ Metallothioneins and Silver

AI-2 – Autoinducer 2

▶ Boron, Biologically Active Compounds

Al and Polyphosphoinositides

▶ Aluminum and Phosphatidylinositol-Specific-Phospholipase C

Alkali Cation Binding Sites and Structure–Functions Relations

▶ Potassium in Biological Systems

Alkali Cation Binding Sites and Structure-Functions Relationships

▶ Potassium-Binding Site Types in Proteins

Alkali Cations

▶ Potassium, Physical and Chemical Properties
▶ Sodium, Physical and Chemical Properties

Alkali Metals

▶ Potassium, Physical and Chemical Properties
▶ Sodium, Physical and Chemical Properties

Alkaline Earth Metal

▶ Strontium Binding to Proteins

Alkaline Metals

▶ Cesium, Therapeutic Effects and Toxicity

Alkaline-Earth Metal

▶ Barium, Physical and Chemical Properties
▶ Strontium, Physical and Chemical Properties

Allergy

▶ Chromium and Allergic Reponses

Allocation on Earth's Surface

▶ Lithium in Biosphere, Distribution

Allosteric: Other Site

▶ Monovalent Cations in Tryptophan Synthase Catalysis and Substrate Channeling Regulation

Alpha Rays

▶ Polonium and Cancer

Alpha-Radioactivity

▶ Polonium and Cancer

ALS

▶ Copper-Zinc Superoxide Dismutase and Lou Gehrig's Disease

ALS, Amyotrophic Lateral Sclerosis

▶ Zinc Aminopeptidases, Aminopeptidase from Vibrio Proteolyticus (Aeromonas proteolytica) as Prototypical Enzyme

Aluminum

▶ Aluminum in Plants

Aluminum and Bioactive Molecules, Interaction

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Synonyms

Aluminum complexes with low molecular mass substances; Aluminum speciation

Definition

Interaction of aluminum and low molecular mass substances of biological interest is a phenomenon that may explain the effects of aluminum in the environment and organisms.

Aluminum [Al(III)] is a potential neurotoxic agent implicated in the pathogenesis of many neurological disorders (Yokel et al. 2001). The possible role of Al(III) in the development of pathologies, such as Alzheimer's disease, has often been investigated. The interaction of this element with biologically related sites, represented by proteins, enzymes, and coenzymes as well as enzyme-active sites and low molecular mass organic substrates, is prevalent in biological fluids.

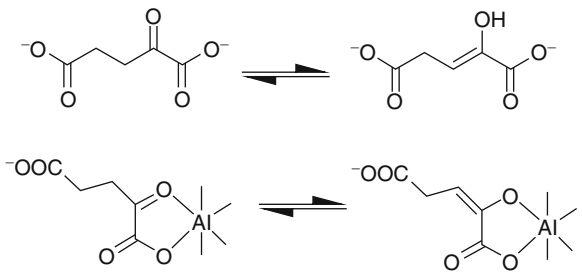
Al-α-KG: Constitutional Aspect

Alpha-Ketoglutate (α-KG) is common in many microorganisms, and can also be isolated from higher plants.

The α-KG was found to bind Al(III) in a bidentate manner at the carboxylate and carbonyl moieties. The mononuclear 1:1 ($AlLH_{-1}$, AlL^{+}, $AlHL^{2+}$) and 2:1 (AlL_2^{-}, $AlL_2H_{-2}^{3-}$) species, and dinuclear 2:1 (Al_2L^{4+}) species were found in acidic aqueous solution. Meanwhile, Al(III) can promote α-KG tautomerize to its enolic structure in solutions. Figure 1 shows α-KG and its Al(III) complexes in equilibrium between the *keto* and *enol* forms. There exists an equilibrium between the two forms: *keto*-form ↔ *enol*-form. The equilibrium is, however, shifted to the left or right, depending on the solvent type, solution acidity, and the concentration of Al–α-KG. The affinity of Al(III) for the enol-form is stronger than for the keto-form, especially at high Al(III) concentrations and high-pH solutions. Therefore, Al(III) can promote the keto–enol tautomerization of α- KG in aqueous solutions (Yang et al. 2003a, 2007).

Al-L-Glu: Configurational Aspect

Amino acids, like glutamic acid (Glu) and aspartic acid (Asp), are important acidic neurotransmitters in the central nervous system (CNS) (Delonce et al. 2002). Glu rather acts as a bidentate ligand, displaying similarity with simple carboxylate group.



Aluminum and Bioactive Molecules, Interaction, Fig. 1 Tautomerization of α-KG and its aluminum complexes (Yang et al. 2003a)

There is an electrostatic repulsive effect of the $-NH_3^+$ group and the Al^{3+} ion. The two carboxylate groups and one deprotonated amino group of L-glutamate are proposed binding sites for Al, and are thought to form the mononuclear 1:1 ($AlLH^{2+}$, AlL^+, and $AlLH_{-1}$) species and dinuclear 2:1 (Al_2L^{4+}) species in acidic aqueous solutions (Fig. 2). The tridentate binding mode of the likely five- + seven-membered joint chelate also existed in acidic aqueous solutions. The 1:1 species can be ascribed to only one reasonable tridentate binding mode ($-COO^-$, $-NH_2$, $-COO^-$) with two OH^- groups around Al(III) under the physiological condition. The formation of the tridentate Al–Glu complexes explains the above possible mechanism that Al(III) promotes the racemization from L-amino acid to D-amino acid, which is also very important in the food chemistry (Yang et al. 2003d).

Al-NAD, NADH, NADP: Conformational Aspect

NAD^+, and its reduced form, NADH, in the glutamate dehydrogenase reaction system, act as a coenzyme ubiquitously involved in biological redox processes and play an important role in the conversion of chemical energy to metabolic energy. Both of the coenzymes play crucial roles in oxidations, which are accomplished by the removal of hydrogen atoms in cells. The diphosphate bridge oxygen atoms of NAD^+ are the potential binding sites (Fig. 3). It was obvious that the conformation of NAD^+ not only depends on the Al(III) binding but also depends on the solvent effect.

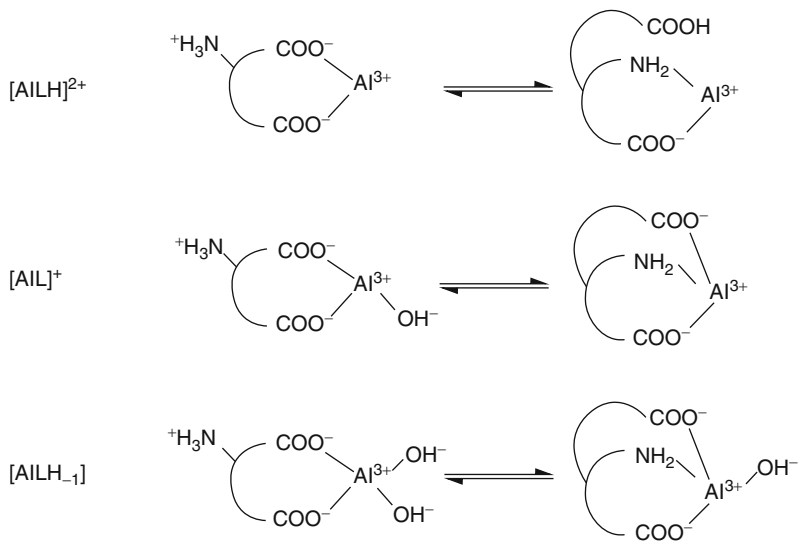
Al(III) can coordinate with NAD^+ through the following binding sites: N_7' of adenine- and pyrophosphate-free oxygen (O_A^1, O_N^1, O_A^2) to form various mononuclear 1:1 ($AlLH_2^{3+}$, $AlLH^{2+}$) and 2:1 (AlL_2^-) species, and dinuclear 2:2 ($Al_2L_2^{2+}$) species. The conformations of NAD^+ and Al-NAD^+ depend on the solvents and different species in the complexes. The occurrence of an Al-linked complexation causes structural changes at the primary recognition sites and secondary conformational alterations for coenzymes (Yang et al. 2003c).

Al(III) interacts with NADH by occupying the binding sites of pyrophosphate oxygen atoms and locks the adenine moiety of coenzyme in an *anti*-folded conformation. Meanwhile, the weak attractive interactions ("association") may occur between Al(III) and the hydroxyl groups of ribose rings through the intramolecular hydrogen bonding. The occurrence of Al(III)-linked conformational changes in these flexible molecules brings structural changes at the primary (by occupying the binding sites of phosphate oxygens O_{N1} and O_{A1}, hydroxyls of ribose rings) and secondary (restrict folded form of NADH in plant cell physiological pH and Al(III) concentration) recognition sites for substrates and enzyme. Furthermore, at biologically relevant pH and concentrations of Al(III) and NADH, Al(III) could increase the amount of folded forms of NADH, which will result in reducing the coenzyme NADH activity in hollow-dehydrogenases reaction systems. The presence of possible competing ligands such as citrate, oxalate, and tartate could detoxify the Al(III) toxic effect. Organic acids protecting the coenzyme NADH from Al(III) lesions are of a biological relevant value in this respect (Yang et al. 2003b).

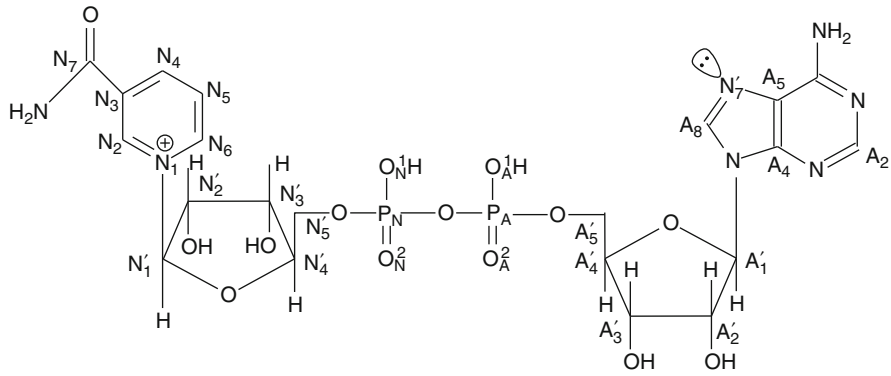
β-Nicotinamide adenine dinucleotide phosphate ($NADP^+$) and its reduced form NADPH is an important coenzyme in both plants and animals. Like other phosphate molecules of biological importance, such as adenosine monophosphate (AMP), diphosphate (ADP), and triphosphate (ATP), $NADP^+$ shows strong binding capability with Al(III) via the complexation of the nonbridging phosphate group (Murakami and Yoshino 2004). At μM concentration level and in neutral pH aqueous solutions, the 1:1 species, Al($NADP^+$), predominate.

Al, Al_{13}-GSH, GSSG: Species Aspect

Reduced glutathione (γ-L-glutamyl-L-cysteinylglycine, GSH) is a fundamental low molecular mass antioxidant that serves several biological functions. It is an essential



Aluminum and Bioactive Molecules, Interaction, Fig. 2 Bidentate and tridentate binding modes of the 1:1 Al-Glu species in acidic aqueous solutions (Al:Glu = 0.01M:0.01M) (Yang et al. 2003d)



Aluminum and Bioactive Molecules, Interaction, Fig. 3 The structure and atomic numbering of NAD^+

constituent of all living cells and usually the most abundant intracellular nonprotein thiol source. Upon enzymatic and non-enzymatic oxidation, GSH forms glutathione disulfide (GSSG).

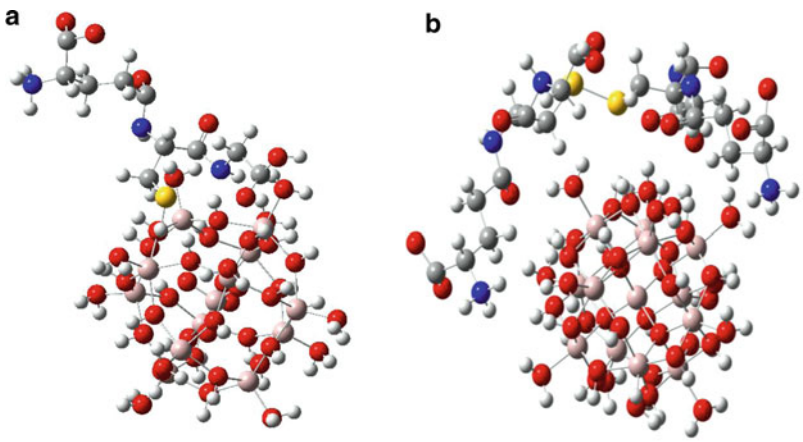
GSH is expected to display a greater affinity for Al^{3+} ions and thus interfere with aluminum's biological role more significantly than simple natural amino acids. Al^{3+} coordinates with the important biomolecule GSH through carboxylate groups to form various mononuclear 1:1 ($AlHL$, AlH_2L and $AlH_{-1}L$), 1:2 (AlL_2) complexes, and dinuclear ($Al_2H_5L_2$) species, where H^4L^+ denotes totally protonated GSH (Table 1). Besides the monodentate complexes through carboxylate groups, the amino groups and the peptide bond imino and carbonyl groups may also be involved in binding with Al^{3+} in the bidentate and tridentate complexes (Wang et al. 2009).

Oxidized glutathione (GSSG) exists in animal cells and in the form of phytochelatins in plant cells. It is, therefore, widely available in all living things. The coordination chemistry of Al(III) with glutathione is of vital importance, since it serves as a model system for the binding of this metal ion by larger peptide and protein molecules. Al(III) can coordinate with the important biomolecule GSSG through the following binding sites: glycyl and glutamyl carboxyl groups to form various mononuclear 1:1 ($AlLH_4$, $AlLH_3$, $AlLH_2$, $AlLH$, AlL, $AlLH_{-1}$, $AlLH_{-2}$) and several binuclear 2:1 (Al_2LH_4, Al_2LH_2, Al_2L) species (where H_6L^{2+} denotes the totally protonated oxidized glutathione) in acidic aqueous solutions. The carboxylate groups are effective binding sites for Al(III). The possible binding sites are the negative charged C-terminal Gly-COO^- and Glu-COO^- groups. The coordination

Aluminum and Bioactive Molecules, Interaction, Table 1 Proton and aluminum(III) complexes formation constants (logβ) of the hydroxo and low molecular mass substances at $T = 25 \pm 0.1$°C and $I = 0.10$ M KCl

Ligand species	logβ [a]	Hydroxo species	logβ	Complexation species	logβ [a]
α-KG		AlH_{-1}	−5.33	$AlLH^{2+}$	6.55
H_2L	2.41	AlH_{-2}	−10.91	AlL^{+}	3.83
HL^{-}	7.04	AlH_{-3}	−16.64	$AlLH_{-1}$	−0.87
		AlH_{-4}	−23.46	AlL_2^{-}	5.75
		Al_2H_{-2}	−7.15	$AlL_2H_{-2}^{3-}$	−4.58
		Al_3H_{-4}	−13.13	Al_2L^{4+}	6.32
		$Al_{13}H_{-32}$	−107.41		
L-Glu				$AlLH^{2+}$	10.68
H_2L	2.17			AlL^{+}	7.42
HL^{-}	6.15			$AlLH_{-1}$	2.46
L^{2-}	15.11			Al_2L^{4+}	9.56
NAD^{+}				$AlLH_2^{3+}$	15.60
H_2L	3.46			$AlLH^{2+}$	12.31
HL^{-}	10.95			$Al_2L_2^{2+}$	19.82
GSH		AlH_{-1}	−5.33	AlH_2L	21.85 ± 0.05
H_4L	24.12 ± 0.04	AlH_{-2}	−10.91	AlHL	17.70 ± 0.03
H_3L	21.10 ± 0.03	AlH_{-3}	−16.64	$AlH_{-1}L$	7.76 ± 0.08
H_2L	18.00 ± 0.02	AlH_{-4}	−23.46	AlL_2	24.72 ± 0.05
HL	9.53 ± 0.02	Al_2H_{-2}	−7.15	$Al_2H_5L_2$	49.6 ± 0.2
		Al_3H_{-4}	−13.13		
		$Al_{13}H_{-32}$	−107.41		
GSSG		AlH_{-1}	−5.33	$AlLH_4$	26.82 ± 0.03
H_6L	28.95 ± 0.02	AlH_{-2}	−10.91	$AlLH_3$	23.56 ± 0.03
H_5L	27.35 ± 0.01	AlH_{-3}	−16.64	$AlLH_2$	20.32 ± 0.03
H_4L	25.32 ± 0.02	AlH_{-4}	−23.46	AlLH	15.83 ± 0.03
H_3L	21.99 ± 0.02	Al_2H_{-2}	−7.15	AlL	11.92 ± 0.04
H_2L	18.23 ± 0.02	Al_3H_{-4}	−13.13	$AlLH_{-1}$	4.96 ± 0.01
HL	9.54 ± 0.02	$Al_{13}H_{-32}$	−107.41	$AlLH_{-2}$	−5.06 ± 0.01
				Al_2L	14.07 ± 0.04
				Al_2LH_2	21.93 ± 0.03
				Al_2LH_4	28.86 ± 0.05

[a]Averages (± standard deviations)



Aluminum and Bioactive Molecules, Interaction, Fig. 4 Binding mode of substances on the Al_{13} clusters. (**a**) GSH and (**b**) GSSG. Color codes: C, *gray*; H, *light gray*; O, *red*; N, *blue*; S, *yellow*; Al, *pink*

of Al(III) induces small conformational changes of peptide GSSG (Yang et al. 2008).

Nanometer-sized tridecameric aluminum polycation (nano-Al_{13}) is known as a new type clarifying agent, showing more effective coagulation effects and rapid aggregation velocity in a relatively wider pH range, and widely applied in the water treatment. Al_{13} clusters could exert strong effect to the conformation of GSH and GSSG, due to the significant interactions between the ligands and cluster surface (Fig. 4).

Cross-References

- ▶ Aluminum in Biological Systems
- ▶ Aluminum in Plants
- ▶ Aluminum Speciation in Human Serum
- ▶ Aluminum, Biological Effects
- ▶ Lead and Alzheimer's Disease
- ▶ Mercury and Alzheimer's Disease
- ▶ Zinc in Alzheimer's and Parkinson's Diseases

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Aluminum and Health

- ▶ Aluminum, Biological Effects

Aluminum and Phosphatidylinositol-Specific-Phospholipase C

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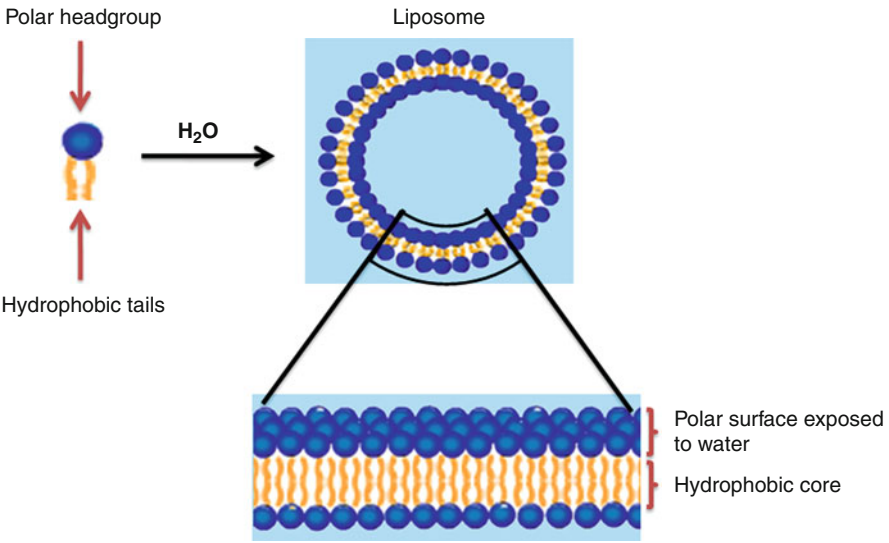
Synonyms

Al and polyphosphoinositides

Definitions

Lipid bilayer: Spontaneous arrangement of polar lipids, such as phospholipids, when in contact with water-containing solutions. Given that phospholipids have in their structure a hydrophilic headgroup and two hydrophobic tails, they adopt a disposition of a flat two-layered sheet with the headgroups facing the aqueous milieu and the hydrophobic tails grouped toward the inside of the sheet, isolated from water (Scheme 1).

Liposomes: Artificial membranes composed of lipids arranged as a bilayer (Scheme 1). Liposomes are custom made; thus by selecting their lipid and/or protein composition, liposomes become a useful tool



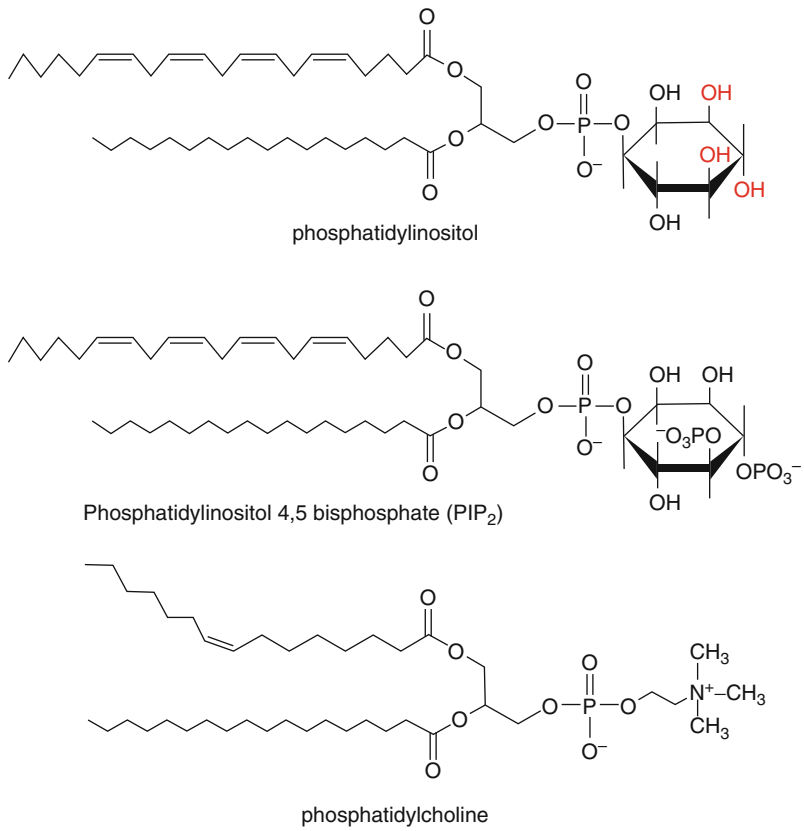
Aluminum and Phosphatidylinositol-Specific-Phospholipase C, Scheme 1 Amphiphilic lipids (such as phospholipids) spontaneously arrange into bilayers when dissolved in water-containing solutions

to assess lipid behavior under controlled conditions (such as changes in aqueous media composition, temperature, pressure, interaction with proteins or nucleic acids, etc.). Given that liposomes contain an internal water space, it is also possible to entrap water-soluble molecules. This property makes them useful as carriers (e.g., drug delivery).

Biological membrane: Naturally occurring membrane composed of lipids and proteins, which determines the boundaries of a cell. Biological membranes serve as selective barriers and prevent the leakage of molecules located within the cell. The outermost membrane of a cell, called the plasma membrane, participates in cell recognition and response, in cell-cell adhesion and interaction with other cellular and extracellular structures. It is also involved in cell signaling and contains identity markers. In addition to the plasma membrane, eukaryotic cells also have internal membranes that delimit the different intracellular compartments or organelles such as the mitochondria, nuclei, lysosomes, peroxysomes, and chloroplasts. The membrane of each organelle has characteristic lipid and protein compositions that create the adequate environment for them to exert their biological functions. Supporting a relevant role of biological membranes in cell function, alterations in membranes lipid or protein composition lead to a wide range of pathologies.

Membrane biophysical properties: Group of measurable properties that define the dynamics of a lipid bilayer. They include: (1) Membrane fluidity that is related to the viscosity of the bilayer. This property is determined by both the length of the acyl chains and the number of unsaturations of membrane fatty acids, and by the temperature. (2) Lateral phase separation (or lateral diffusion), a property closely related to membrane fluidity that reflects the velocity of lipids displacement along the membrane. (3) Membrane hydration reflects membrane water content. It is closely associated with membrane fluidity given that a more fluid membrane will allow water molecules to penetrate into deeper regions at the hydrophobic core of the bilayer. On the contrary, rigid membranes will extrude water from the hydrophobic core. (4) Membrane permeability, which determines how easily a given molecule will cross the bilayer. This property relies not only on the membrane composition but also on the chemical nature of the molecule permeating the bilayer. (5) Surface potential, determined by the different charged polar headgroup of phospholipids. (6) Topology, a property determined by the characteristic that not all phospholipids have the same length. Therefore, mixtures of relatively short phospholipids and long ones will determine membrane regions of different widths.

Cell signal transduction: Cascade of events that transmit the information from the environment to the inside of the cell. These events are frequently triggered by the interaction of a stimulus (e.g., proteins,



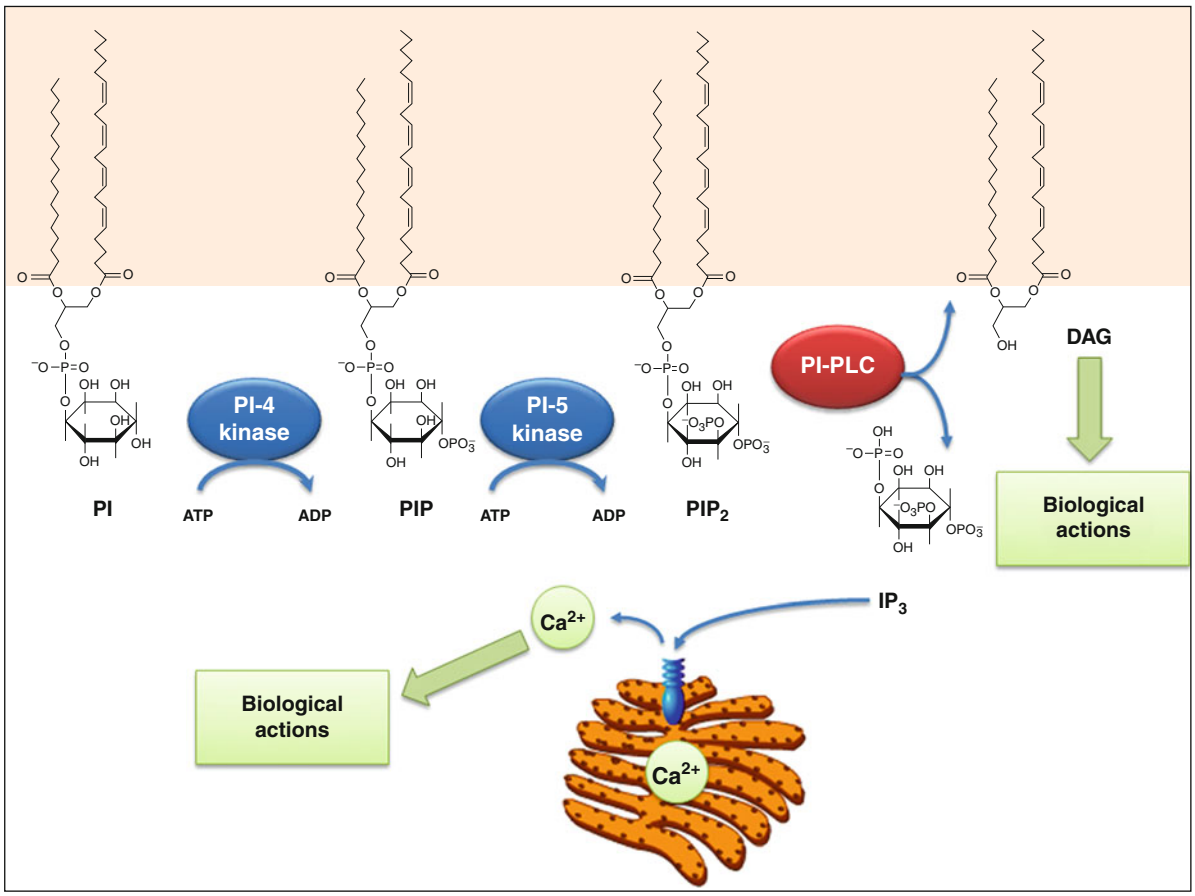
Aluminum and Phosphatidylinositol-Specific-Phospholipase C, Fig. 1 Chemical structure of phosphoinositides and phosphatidylcholine. The hydroxyl groups of phosphatidylinositol (*PI*) that can be phosphorylated by specific kinases to generate the different PPIs are indicated in *red*

chemical and physical stimuli) with cell surface or intracellular receptors. This interaction will determine the recruitment and activation of different intracellular proteins, which in turn, will lead to a biological action (e.g., changes in cell metabolism, changes in gene transcription).

Polyphosphoinositides (PPIs): Family of phospholipids that contain an inositol moiety in their headgroup. The simplest one is phosphatidylinositol (Fig. 1). Although this molecule has five hydroxyl groups (the sixth being involved in the O-P bond that connects phosphoinositol with diacylglycerol), only those at positions 3, 4, and 5 of the inositol ring can be phosphorylated by the action of specific kinases (Fig. 1). Each phosphoinositide has been associated with a variety of biological actions, being phosphatidylinositol (4, 5) bisphosphate (PIP_2, Fig. 1) the most studied. Research on the biological actions of this family of lipids is a growing field of interest in the areas of cell biology and metabolic regulation.

Introduction

Communication among cells, regardless if they are adjacent or distant, requires an intricate network of molecules that carry the chemical or electrical information to the target cell, where they trigger a biological action. The chemical nature of these molecules (stimuli) is diverse, from physical (e.g., light) or chemical (e.g., oxygen) molecules to peptides and proteins. At the target cell, they interact with specific receptors and drive the generation of one or more intracellular second messengers, an effect that depends on both the characteristics of the receptor and the cell type involved. In general, receptors can be classified in two groups, ionotropic and metabotropic. The first group is associated with the modulation of the opening/closing of ionic channels, leading to the controlled influx/eflux of inorganic ions. On the other hand, metabotropic receptors are associated with enzymes

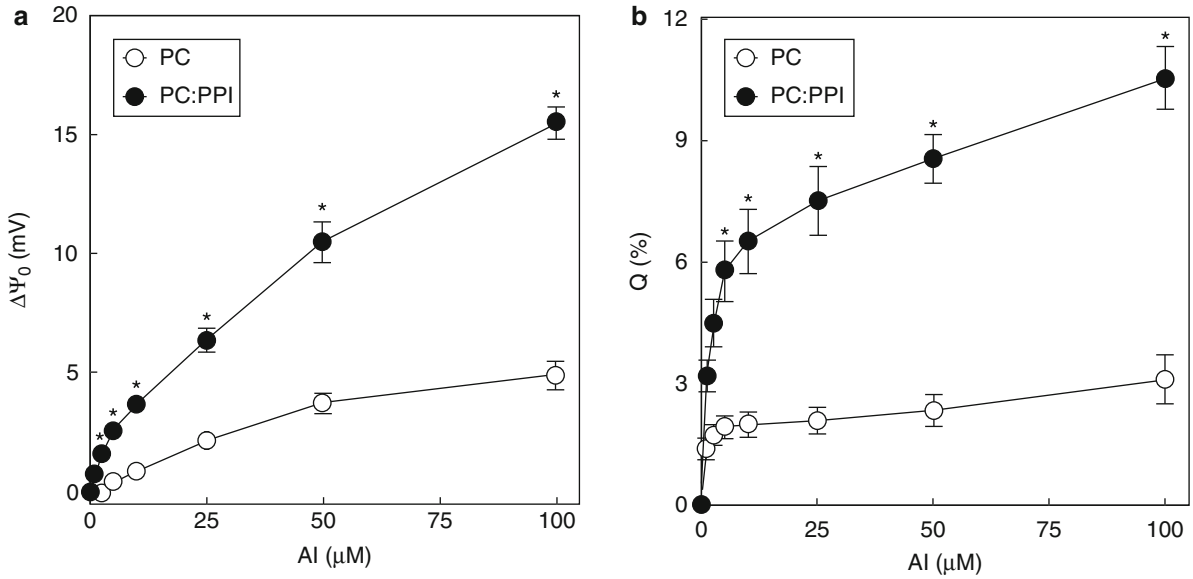


Aluminum and Phosphatidylinositol-Specific-Phospholipase C, Scheme 2 Summary of PPIs metabolism

that catalyze the generation of intracellular second messengers, each one having defined mechanisms of action.

A major signaling pathway in biological systems involves the binding of certain hormones, growth factors, and neurotransmitters to their corresponding membrane receptor which activates the turnover of polyphosphoinositides (PPIs). PPI comprises a group of phosphorylated species derived from phosphatidylinositol (PI). PI is a substrate for different kinases that act on the hydroxyl groups at the positions 3, 4, or 5 of the inositol ring (Fig. 1). Upon the proper stimuli, phosphatidylinositol (4, 5) bisphosphate (PIP_2, Fig. 1) is hydrolyzed by a specific phospholipase C (PI-PLC) rendering inositol trisphosphate (IP_3) and diacylglycerol, two molecules with relevant second-messenger function. Due to its lipidic nature, diacylglycerol remains within the bilayer and activates membrane-associated enzymes, such as protein kinase C (PKC) while the water-soluble IP_3 is released to the cytosol. IP_3 activates a family of IP_3 receptors (IP_3Rs) which are present in the plasma membrane, endoplasmic reticulum, Golgi apparatus, and nuclear membranes (Corry and Hool 2007). At the endoplasmic reticulum, binding of IP_3 to the IP_3R leads to the opening of the channel and to the consequent Ca^{2+} mobilization from this intracellular reservoir to the cytosol where it activates a number of signaling cascades (Scheme 2).

The capacity of aluminum (Al^{3+}) to inhibit the PPI-dependent signaling pathway has been reported several years ago (Shafer and Mundy 1995;



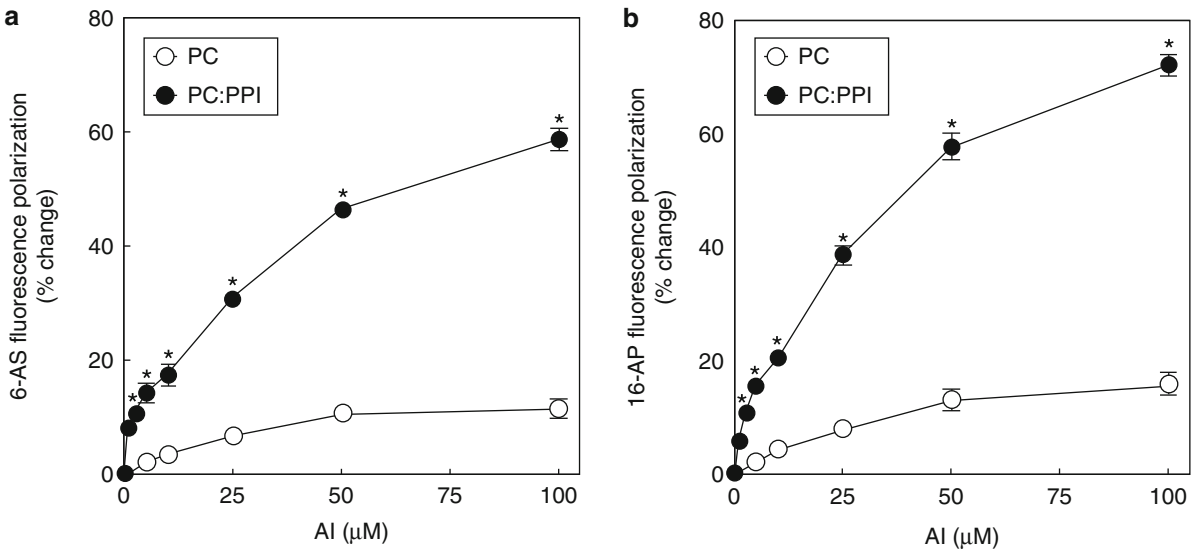
Aluminum and Phosphatidylinositol-Specific-Phospholipase C, Fig. 2 Al^{3+} modifies liposome surface potential and generates PPI-enriched domains. PC (○) or PC:PPI (60:40 molar ratio) (●) liposomes were incubated in the presence of variable amounts of Al^{3+}. (**a**) Liposome surface potential was evaluated using the fluorescent probe ANS. (**b**) Formation of lipid domains was assessed using the fluorescent probe C6-NBD-PC. Results are shown as the mean ± SEM of at least three independent experiments. * Significantly different from the value measured in PC liposomes at the same Al^{3+} concentration ($P < 0.001$, two-way ANOVA) (Adapted from Arch Biochem Biophys, 408, Verstraeten SV and Oteiza PI, "Al^{3+}-mediated changes in membrane physical properties participate in the inhibition of polyphosphoinositide hydrolysis", 263–271, 2002, with permission from Elsevier)

Nostrandt et al. 1996). This inhibition could be ascribed to the interaction of the metal with at least one of the four major components of this pathway: the membrane receptor, the G protein coupled to the receptor that activates the enzyme PI-PLC, PI-PLC itself, and its substrate PIP_2. So far, neither a direct interaction of Al^{3+} with the receptor nor the inhibition of the G protein have been proven to cause Al^{3+}-mediated inhibition of PIP_2 hydrolysis (Shafer and Mundy 1995). Therefore, the mechanism underlying this inhibition should reside in the interaction of the metal with PI-PLC and/or PIP_2. In the following sections, we will review current evidence showing that Al^{3+} can inhibit PI-PCL mainly due to the capacity of Al^{3+} to interact with PIP_2-containing membranes in a manner that impedes the availability of the substrate to enzyme, rather than to a metal-protein interaction which would directly alter PI-PLC activity.

Aluminum Interaction with PPI Causes Major Alterations in Membrane Physical Properties

In vitro, the binding of Al^{3+} to acidic phospholipids alters the dynamics of lipids at the bilayer (Verstraeten et al. 1997a). Given that phosphate is the main biological ligand for Al^{3+} (Martin 1986), metal interactions with phosphate groups present in the inositol moiety of PPI may enhance this ability of Al^{3+} and modify the biophysical properties of PPI-containing membranes. To investigate this hypothesis, a series of in vitro experiments using liposomes composed of a mixture of phosphatidylcholine (PC) (Fig. 1) and PPI in a 60:40 molar ratio were performed.

The binding of Al^{3+} to lipid bilayers occurs through the ionic interaction between the positively charged metal and the negative charges in the polar headgroup of phospholipids. Therefore, an overall increase in the superficial charge of liposomes is evidence of such metal-lipid interactions. In the absence of Al^{3+}, PC:PPI



Aluminum and Phosphatidylinositol-Specific-Phospholipase C, Fig. 3 Al^{3+} decreases membrane fluidity in PPI-containing liposomes. PC (○) or PC:PPI (60:40 molar ratio) (●) liposomes were incubated in the presence of variable amounts of Al^{3+} and the fluidity at the surface level (**a**) and at the hydrophobic core of the bilayer (**b**) was evaluated from the changes in the fluorescence polarization of the probes 6-AS and 16-AP, respectively. Results are shown as the mean ± SEM of at least three independent experiments. * Significantly different from the value measured in PC liposomes at the same Al^{3+} concentration ($P < 0.001$, two-way ANOVA) (Adapted from Arch Biochem Biophys, 408, Verstraeten SV and Oteiza PI, "Al^{3+}-mediated changes in membrane physical properties participate in the inhibition of polyphosphoinositide hydrolysis", 263–271, 2002, with permission from Elsevier)

liposomes are approximately 15 mV more negative than PC liposomes due to the presence of PPI phosphate groups (Verstraeten et al. 2003). In the presence of Al^{3+}, PC liposomes experience a slight increase in the surface membrane potential ($\Delta\Psi_0$) (Fig. 2a), indicating a weak ionic interaction between the metal and the membrane. This result is expected, based on the chemical structure of PC (see Fig. 1). PC is a zwitterionic phospholipid, with a negative charge conferred by the phosphate group esterifying the diacylglycerol molecule, and a positive charge from the quaternary ammonium of the choline residue. On the other hand, the presence of one or more phosphate groups in PPI positively affects the interaction of Al^{3+} with the membrane, as evidenced from the marked increase in the surface potential of PPI-containing liposomes (Fig. 2a).

Multivalent cations such as Al^{3+} have the tendency to interact with more than one binding site, with 1:2 or 1:3 stoichiometries (Martin 1986). In the case of PPI-containing membranes, Al^{3+} may bind to either phosphate groups located within a single phospholipid or to those located in neighbor molecules. In the case of PIP, the existence of only one phosphate group in the inositol ring favors the interaction of the metal with more than one phospholipid. On the other hand, PIP_2 contains two phosphate groups which, in principle, will favor the binding of one molecule of Al^{3+} with the two phosphates groups of a single phospholipid molecule. However, the different spatial disposition of those residues, facing the opposite sides of the inositol ring, would lead to the interaction of Al^{3+} with more than one PIP_2 molecule. If the latter occurs, PPI will segregate in the lateral phase of the bilayer, generating PPI-enriched domains. To assess this possibility, the generation of membrane domains was evaluated with the fluorescent probe C6-NBD-PC. When C6-NBD-PC molecules are in close proximity, fluorescence quenching occurs. In PC liposomes, the separation of

lipids into domains (fluorescence quenching, Q%) was minimal upon their interaction with Al^{3+} (Fig. 2b). However, when liposomes contained PPI, a marked and Al^{3+} concentration-dependent lateral phase separation was observed (Fig. 2b) (Verstraeten et al. 2003).

Membrane fluidity is another important biophysical property that has a profound impact on the functionality of membrane-associated proteins. Al^{3+} has been proven to promote a decrease in membrane fluidity both in diverse in vitro and in vivo models (Verstraeten et al. 1997a, b, 1998, 2002; Verstraeten and Oteiza 2000, 2002). It is important to point out that, even when the interaction of Al^{3+} with phospholipids occurs at the superficial level of the bilayer, membrane rigidification propagates from the surface to the deepest region of the bilayer (Fig. 3). However, the magnitude of the effect will depend on the nature of the phospholipid involved. In this way, in PC liposomes the effect of Al^{3+} promoting the rigidification of the membrane was almost negligible when evaluated either at the superficial level (probe 6-AS, Fig. 3a) or at the deepest hydrophobic region (probe 16-AP, Fig. 3b). The presence of PPI in the membrane resulted in an enhanced rigidifying effect of Al^{3+}. The magnitude of the effect was significantly higher at the hydrophobic portion than at the surface of the bilayer (Verstraeten et al. 2003). In accordance to the higher rigidity found in PPI-containing liposomes treated with Al^{3+}, membranes became partially dehydrated (Verstraeten and Oteiza 2002).

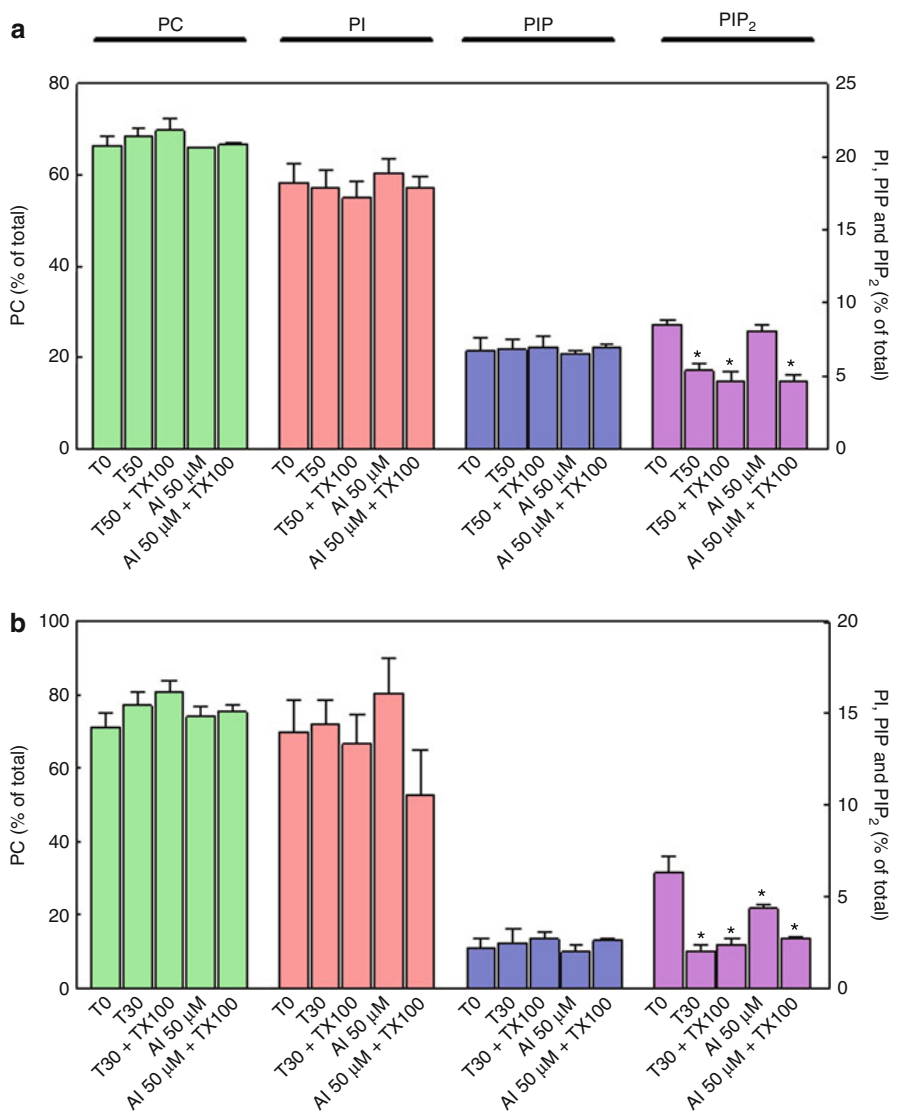
Aluminum-Mediated Changes in Membrane Physical Properties Determine the Ability of PI-PLC to Hydrolyze PIP_2

The findings discussed above raised the question if the binding of Al^{3+} to phosphate groups in PPI was the sole factor responsible for the previously described PI-PLC inhibition, or if Al^{3+}-mediated changes in membrane physical properties could contribute to such inhibition. To answer that question, PC:PPI liposomes were pre-incubated with Al^{3+} and subsequently submitted to the action of two PI-PLCs from different origin. One of the enzymes assessed was isolated from bovine brain and corresponds to the catalytic active subunit of the delta isoform (Verstraeten et al. 2003). The other was obtained commercially and was purified from *Bacillus cereus* (EC 3.1.4.10).

After the incubation of the samples in the presence of brain PI-PLC, the only specie hydrolyzed by the enzyme was PIP_2 (Fig. 4a) a result that is in accordance with the substrate specificity of this enzyme (Rebecchi and Pentyala 2000). A similar specificity was observed when the bacterial PI-PLC was assessed (Fig. 4b). It is important to stress that the extent of PIP_2 hydrolysis by both enzymes was similar in intact liposomes as well as in the micelles that were obtained by disrupting liposomes with the detergent Triton X-100. This finding indicates that both enzymes had a complete accessibility to their substrates even when they were incorporated in a bilayer.

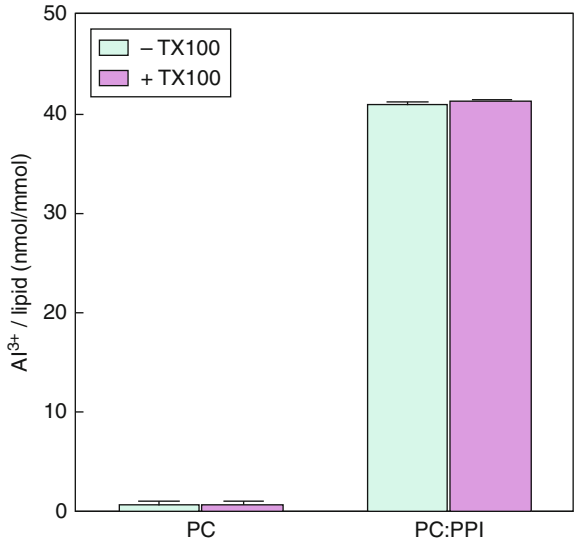
When liposomes were pre-incubated with 50 μM Al^{3+}, PI-PLC activity was abolished (brain PI-PLC) or significantly impaired (bacterial PI-PLC). Surprisingly, when the bilayer structure of liposomes was disrupted by the addition of Triton X-100, the activity of the enzymes was fully recovered (Fig. 4). However, it is possible to speculate that this treatment may have displaced Al^{3+} from its binding sites, increasing the accessibility of PI-PLC to its substrates. To investigate this hypothesis, the amount of Al^{3+} bound to lipids was measured. As expected from the weakness of Al^{3+} interaction with the zwitterionic phospholipid PC, the amount of Al^{3+} bound to PC liposomes was very low and close to the detection limit of the method. In PC:PPI liposomes the amount of Al^{3+} bound was 40-fold higher than in PC liposomes (Fig. 5). The disruption of liposomes with Triton X-100 did not affect Al^{3+}-phospholipid binding. These findings stress the strength of the ionic bonds established between Al^{3+} and the phosphate moieties in the inositol headgroups of PIP and PIP_2.

The catalytic domain of eukaryotic isoforms of PI-PLC is organized in a α/β TIM barrel that lodges the conserved amino acids His_{311}, His_{356}, Glu_{341}, Asp_{343}, and Glu_{390} involved in the binding and hydrolysis of the O-P bond that connects phosphoinositol with diacylglycerol (Rebecchi and Pentyala 2000). Surrounding the active site there is a ridge of hydrophobic residues that allows the enzyme to insert into the lipid bilayer, locating the active site close to the substrates. This is a common mechanism (called the interfacial activation mechanism) for several hydrophilic enzymes that act on hydrophobic substrates such as lipases and phospholipases (Aloulou et al. 2006). For the specific case of PI-PLC, it has been demonstrated that the insertion of the hydrophobic ridge into the bilayer strictly depends on the lateral pressure of the bilayer (reviewed in Rebecchi and



Aluminum and Phosphatidylinositol-Specific-Phospholipase C, Fig. 4 Al^{3+} inhibition of PI-PLC-mediated PIP_2 hydrolysis depends on membrane integrity. (**a**) PC:PPI (60:40) or (**b**) PC:PPI (90:10) liposomes were incubated for 10 min in the presence of 50 µM Al^{3+}. After incubation, portions of the samples were treated with or without 0.5% (v/v) Triton X-100 in order to disrupt membranes, and further incubated for (**a**) 60 min (T60) at 37°C in the presence of brain PI-PLC, or (**b**) 30 min (T30) at 37°C in the presence of PI-PLC from *Bacillus cereus*. Lipids were resolved by high-performance thin-layer chromatography, and PC, PI, PIP and PIP_2 were quantified. Results are shown as the mean ± SEM of at least three independent experiments. * Significantly different from the value measured in control liposomes (T0) ($P < 0.01$, one-way ANOVA) (Adapted from Arch Biochem Biophys, 408, Verstraeten SV and Oteiza PI, "Al^{3+}-mediated changes in membrane physical properties participate in the inhibition of polyphosphoinositide hydrolysis", 263–271, 2002, and Chem Phys Lipids, 122, Verstraeten SV and Oteiza PI, "Al^{3+}-mediated changes on membrane fluidity affects the activity of PI-PLC but not of PLC", 159–163, 2003, with permission from Elsevier)

Pentyala 2000). Therefore, in rigid membranes, where the lipids are tightly packed, PI-PLC is not able to penetrate the bilayer and unable to reach its substrates. This might be the mechanism operative in the inhibition of PI-PLC activity by Al^{3+}, which would act creating a lipid environment unfavorable for the docking of the enzyme rather than causing a direct inhibition.



Aluminum and Phosphatidylinositol-Specific-Phospholipase C, Fig. 5 The presence of PPI in the membrane enhances Al^{3+} binding. PC or PC:PPI (60:40 molar ratio) liposomes were incubated in the presence of 50 μM Al^{3+}. After incubation, portions of the samples were treated without (□) or with (■) 0.5% (v/v) Triton X-100 in order to disrupt membranes. The binding of Al^{3+} to the membrane was measured with the reagent lumogallion. Results are shown as the mean ± SEM of four independent experiments (Adapted from Arch Biochem Biophys, 408, Verstraeten SV and Oteiza PI, "Al^{3+}-mediated changes in membrane physical properties participate in the inhibition of polyphosphoinositide hydrolysis", 263–271, 2002, with permission from Elsevier)

Conclusions

Multivalent cations are documented to modify the activity of a myriad of enzymes. When evaluating the underlying mechanisms, it is important to consider that metal-protein interactions may not be the only cause for an altered functioning of membrane enzymes. Metals can interact with enzyme lipid substrates and alter their molecular organization and dynamics, making them less available to the enzyme.

Cross-References

- ▶ Aluminum and Bioactive Molecules, Interaction
- ▶ Aluminum, Biological Effects
- ▶ Calcium in Biological Systems
- ▶ Lipases

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Aluminum Complexes with Low Molecular Mass Substances

- ▶ Aluminum and Bioactive Molecules, Interaction

Aluminum in Biological Systems

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Definition

Aluminum is the third most abundant element in the Earth's crust and the most abundant metal (Exley 2003). Aluminum is neither required by biological systems nor is it known to participate in any essential biological processes. While today all living organisms contain some aluminum, there is no scientific evidence that any organism uses aluminum for any biological purpose. There is similarly no evidence from the proteome or genome that any organism has utilized aluminum at any time in the evolutionary record. Aluminum's abundance and paradoxical lack of biological function remains a biochemical enigma.

It is argued that aluminum's absence from biochemical processes can be best explained in terms of its "historical" absence from biochemical evolution (Exley 2009a). In spite of its abundance in the Earth's crust, aluminum was not biologically available for the greater part of biochemical evolution. It was not available to participate in the natural selection of the elements of life. It is shown that silicon, as silicic acid, is a geochemical control of the biological availability of aluminum and has probably played a significant role in keeping aluminum out of biota (Exley 1998).

If aluminum had been biologically available during early biochemical evolution then this encyclopedia entry would have been listing and describing myriad proteins and biochemical systems which are either built around or require aluminum. This can be anticipated as it is now known that when aluminum is biologically available it is a serious ecotoxicant and can act so at to displace or nullify biologically essential metals and, in particular, magnesium, calcium, and iron. It is known that aluminum is the principal antagonist in fish death in acid waters, in forest decline in acidified catchments, in poor crop productivity on acid soils, and it is also known that aluminum is both acutely and chronically toxic in humans (Exley 2009b). The scientific evidence indicates that biologically available aluminum is biologically reactive and not always to the detriment of the biochemical processes concerned. For example, while some phosphoryl-transferring enzymes, such as hexokinase, are potently inhibited by aluminum, others are not and appear at least to use Al-ATP as effectively as Mg-ATP as a biochemical substrate (Furumo and Viola 1989; Korchazhkina et al. 1999). In addition, while aluminum is not known to play any role in any enzyme it has been shown that when aluminum is engineered into the active site of a purple acid phosphatase the activity of this enzyme was not hindered by the presence of aluminum. This was described by the authors as the first example of an active aluminum enzyme (Merkx and Averill 1999). The lack of biochemical essentiality of aluminum is best explained as being due to it being excluded from biochemical evolution (Exley 2009a). If biologically available aluminum had been present, then aluminum would either be an essential element in modern biochemistry (the phenotype of which would not necessarily be similar to what is known today) or there would still be clues in the evolutionary record pertaining to the selection of aluminum out of biochemical processes. It is argued that today aluminum is a silent visitor to contemporary biota including human beings and is only now participating in the evolutionary process. These "visits" are not inconsequential and are already being manifested as both acute and chronic toxicity (Exley 2009b). The latter may prove to be an unrecognized burden upon biological systems including human health unless steps are taken to protect the environment and the body from its burgeoning presence in modern life and its increasing participation in modern biochemistry (Exley 1998).

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Aluminum in Plants

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Synonyms

Aluminum

Definition

Aluminum is a chemical element with atomic number 13 and atomic weight 26.98. In acid environments Al solubility increases and Al ions can be taken up by plants.

Presence of Aluminum in Plant's Life

Aluminum is an amphoteric element without any established biological function. Aluminum ranges third in abundance of the chemical elements in the lithosphere. In nature Al does not occur as free metal, but mainly in the form of only lightly soluble oxides and silicates. The availability of Al and, in consequence, the possibility of Al to interact with plants is mostly restricted to acid environments. In aqueous media with pH below 5, Al $(H_2O)_6^{3+}$ is the predominant monomeric Al species. This Al form, usually written simplified as Al^{3+}, is thought to be the main toxic Al species (Kochian et al. 2004). In the chemically complex soil solutions different inorganic (e.g., fluoride, sulfate, silicon) and organic ligands (e.g., organic acids, phenolics, hydroxamates, humic and fulvic acids) compete with OH to complex Al. Binding of Al to these ligands reduces the toxicity. Especially organic Al complexes are considered practically nontoxic to plants. Polymeric Al, especially in the tridecameric form ($[AlO_4Al_{12}(OH)_{24}(H_2O)_{12}]^{7+}$; simplified known as Al_{13}, has been claimed to be extremely toxic to plants and microorganisms. The environmental relevance of this polycation, however, is still under debate. In conclusion, acid environments are the main scenarios for potential Al toxicity. Among those acidic freshwaters, forests affected by acid deposition due to air pollution, and acid tropical soils have focused most research (Poschenrieder et al. 2008).

Phytotoxicity of Aluminum

Aluminum toxicity is among the most important abiotic stress factors limiting crop production on acid soils, especially in tropical regions. Plant roots explore the soils for acquiring water and essential mineral nutrients. In acid mineral soils roots are the first plant organ to contact with toxic Al^{3+} and inhibition of root elongation is a fast consequence in Al-sensitive plant species. The most Al-sensitive root part is the transition zone, the region where cells leaving the meristem (cell division zone) are about to enter the elongation zone (cell expansion zone). However, Al not only reduces root length but changes the entire root architecture. Aluminum-stressed roots show enhanced lateral root initiation. The elongation of these newly initiated laterals is quickly inhibited. The result is a stunted root system of bushy appearance. Affected plants are more sensitive to seasonal draught and low phosphorous availability.

As for other ions, the mechanisms of Al^{3+} toxicity are related to its binding to the organic molecules required for the proper functioning of the plant cells. The trivalent Al^{3+} is among the hardest Lewis acid and has high affinity for oxygen donor ligands. Aluminum strongly binds to phosphatidyl, carboxyl, and phenyl groups. This implies multiple toxicity targets both in cell walls and plasmalemma as well as inside the cells.

Cell Walls: Cell walls are main sites for Al binding in roots. Cross-linking by Al of the carboxyl groups of

cell wall pectins has been made responsible for Al-induced cell wall stiffening leading to inhibition of root cell elongation. Cross-linking of other polar wall constituents such as the hydroxyproline-rich glycoprotein (HRGP) extensin can also contribute to inhibition of root cell elongation. It remains to be established if such cross-linking of HRGPs is caused by direct binding of Al to the protein or is mediated by Al-induced production of reactive oxygen species (ROS) (Poschenrieder et al. 2008).

► *Plasma Membrane*: Carboxyl and phosphatidyl groups are potential binding sites for Al in the plasma membrane. Al binding to the plasmalemma can change key properties of this membrane affecting fluidity, lateral lipid phase separation, membrane potential, and ion channel activity (Ma 2007). Aluminum-induced alteration of Ca^{2+} homeostasis (Rengel and Zhang 2003), inhibition of proton adenosine triphosphatase (H^+-ATPase), and lipid peroxidation are characteristic features of the Al toxicity syndrome (Ma 2007). Aluminum by interacting with plasmamembrane components can alter different signaling pathways. ► Calcium signaling and inositol 1,4,5 triphosphate–mediated signal transduction are targeted by Al (Rengel and Zhang 2003; Ma 2007). Moreover, aluminum fluoride (AlF_4^-) is a well-known activator of G-proteins. Trimeric G proteins, which couple extracellular signals to internal effectors, are essential in plant signal transduction.

Symplastic Al Targets: In contrast to the classical view that the plasma membrane is almost impermeable to trivalent cations, it is now well established that Al penetrates into root tip cells within minutes upon exposure. Inside the cells Al^{3+} is expected to be immediately bound to the multiple ligands with high affinity for this metal ion. Binding to organic acids or phenolic substances renders nontoxic Al complexes. However, toxicity targets inside the cells are, among others, ATP, GTP, nucleic acids, and glutamate. Aluminum even at nanomolar concentrations can efficiently compete with Mg for binding to ATP (Ma 2007). Staining of root tips with the Al-specific fluorescent dye lumogallion revealed the presence of Al in the nucleoli of root tip cell nuclei after a few hours of Al exposure.

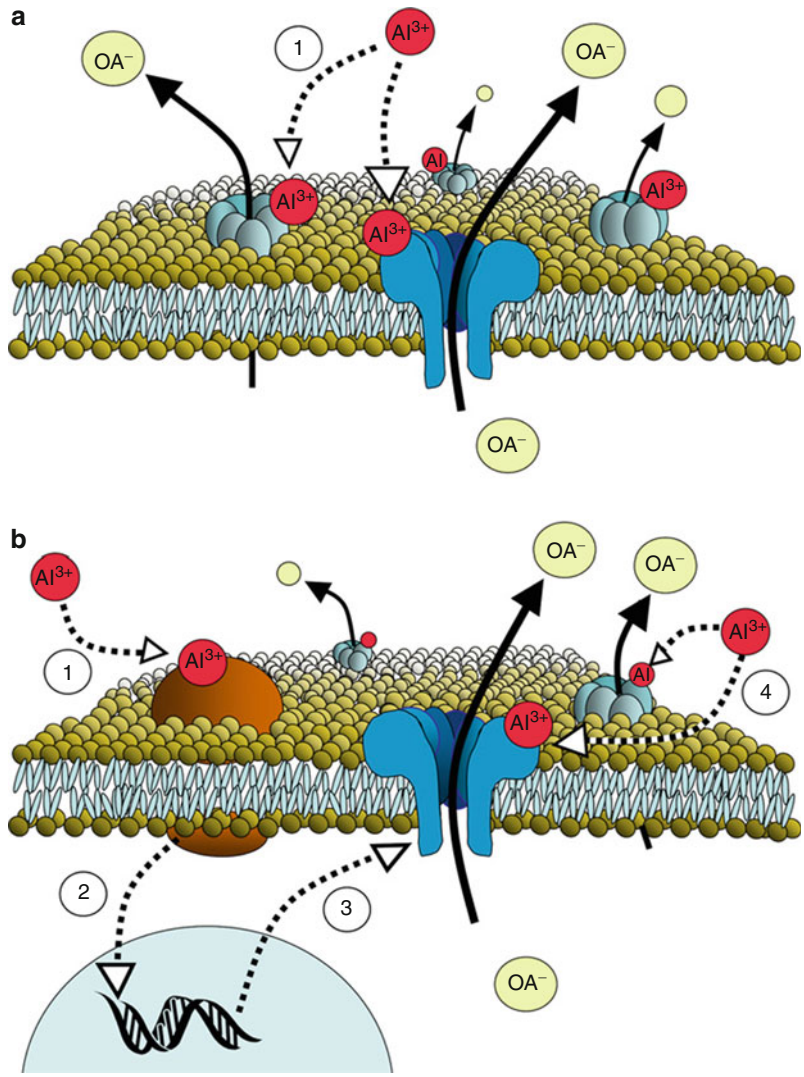
Root Growth Inhibition: In the view of both the multiple target molecules of Al and the fast Al effects on different cell signaling pathways, the primary mechanism or mechanisms of Al-induced inhibition of root growth is still under debate. Two basic mechanisms should be distinguished: the initial fast inhibition of root elongation that is reversible if Al is removed after short exposure times and the irreversible inhibition. The reversible inhibition is most probably caused by Al cross-linking of cell wall pectins causing stiffening of the walls and reduced elongation of root tip cells.

Contrastingly, the irreversible inhibition implies arrest of both root cell division and root cell elongation. Aluminum alters the patterning of root tip cells. Cell division in the apical meristem is inhibited, while stimulation of cell division in more mature, subapical root zones promotes the emergence of new lateral roots. This change in root architecture seems related to Al-induced inhibition of polar auxin transport in the transition zone of the root. Auxin is a growth-stimulating phytohormone that promotes lateral root formation. The inhibition of auxin transport may cause a local increase of auxin concentrations close to the transition zone inducing the formation of new lateral roots. The polar, cell-to-cell transport of auxin is mediated by intracellular vesicle trafficking. Vesicle trafficking, in turn is closely controlled by the actin cytoskeleton (Poschenrieder et al. 2009).

The cytoskeleton has for long been recognized as a target of Al toxicity both in plant root tip cells and astrocytes of the human brain. In vitro, Al induced rigor in plant actin filament network and aggregation of bovine brain cytoskeleton proteins has been observed. It remains to be established whether Al-induced cytoskeleton alterations in vivo are directly caused by Al binding, or are indirect consequences of other primary toxicity mechanisms of Al (e.g., alterations of the energy metabolism, prooxidant activity, and interference of protein phosphorylation).

Aluminum Resistance in Plants

There are considerable differences in Al resistance among crop species and varieties. Exclusion of Al from the root tips, the most Al sensitive plant part, is a fundamental strategy to avoid Al toxicity. In many species this is brought about by exudation of organic acids from the root tips into the rhizosphere (Kochian et al. 2004). The organic acids like malate, citrate, or oxalate bind Al in a nontoxic form that is not taken up by the roots. There are two different patterns of organic



Aluminum in Plants, Fig. 1 Hypothetical models for the Al^{3+}-activated efflux of organic anions by members of the ALMT and MATE families of proteins. (**a**) Pattern I: The protein is expressed constitutively in root apices with Al^{3+}-resistant genotypes showing greater expression than Al^{3+}-sensitive genotypes. Al^{3+} (*red cycles*) activates organic anion (OA^-) efflux by interaction directly with the preexisting protein in the plasma membrane. (**b**) Pattern II: Al^{3+} first induces the expression of proteins through a signal transduction pathway possibly involving a specific receptor (*arrows 1*, *2* and *3*) or nonspecific stress responses. Al^{3+} then activates organic anion efflux by interacting with the newly synthesized proteins (*arrow 4*) (Reproduced with permission from Delhaize et al. 2007)

acid exudation. Pattern I where the exudation of organic acids is triggered immediately upon Al exposure and pattern II where a lag time of several hours between start of exposure and organic acid release is observed (Fig. 1) (Delhaize et al. 2007). Wheat is the best studied pattern I plant; examples of pattern II behavior are rye, triticale, *Cassia tora,* and bean. Most genes responsible for Al resistance that have been isolated so far are genes encoding for organic acid efflux proteins. In cereals, proteins responsible for Al-activated efflux of organic acids are encoded by genes from two different families: *ALMT* encoding an Al-activated malate permease (*Al*uminum-induced *m*alate *t*ransporter) and *MATE* (*M*ultidrug *a*nd *T*oxic compound *E*xtrusion).

TaALMT1 is a major gene for Al resistance in wheat. Its constitutive expression in root tips is consistent with the pattern I type of Al-induced efflux of malate in Al-resistant wheat varieties (Fig. 1a). ALMT proteins, however, form a large, diverse family of plant proteins with transport functions not only restricted to Al resistance.

MATE proteins have been identified in plants, humans, animals, and microorganisms. In plants,

different members of this large family of efflux proteins have been related to Al resistance, Fe efficiency, vacuolar anthocyanin transport, and transport of secondary metabolites including alkaloids. Alt_{Sb}, the Al resistance gene of sorghum (*Sorghum bicolor*), encodes a citrate efflux protein of the MATE family. Aluminum exposure activates the expression of this Al resistance gene in root tips of sorghum. This is consistent with the pattern II type of Al-induced citrate efflux from root tip cells in this species (Fig. 1b) (Delhaize et al. 2007).

Not in all plant species Al-induced exudation of organic acids from root tips fully accounts for varietal differences in Al resistance and other mechanisms can play a role. Mutational analyses in the model plant *Arabidopsis thaliana* have identified two Al^{3+}-sensitive mutants, *als1* and *als3*. ALS1 and ALS3 are ABC-type transporters. ALS1 is located in the tonoplast of the vacuole of root tips and in the vascular system. ALS3 is a plasma membrane transporter found in root cortex cells, in the phloem, and in hydatodes. Hydatodes are special cells located in the leaf borders where plants can extrude water droplets under conditions of high root pressure, a process called gutation (Ryan et al. 2011). It has been suggested that these ALS proteins may transport Al in chelated form contributing to Al tolerance by detoxification of Al inside the plants.

The high constitutive Al tolerance in rice (*Oryza sativa*) is related to two genes, *OsSTAR1* and *OsSTAR2*, encoding ABC transporters. Both proteins STAR1 and STAR2 associate to form a protein complex bound to vesicle membranes. Not organic acids, but UDP-glucose is the transported molecule. Vesicle-mediated transport of UDP-glucose to the apoplast is a fundamental process required for the formation of the cell wall. It is still not clear whether the STAR proteins are involved either in Al exclusion by conferring special cell wall properties or in tolerance of high internal Al concentrations by promoting Al compartmentation inside the plant (Ryan et al. 2011). Still unknown are the mechanisms of the extraordinarily high Al resistance in *Brachiaria decumbens*, a forage grass species of African origin. Neither organic acid exudation nor root-induced changes in the rhizosphere pH seem responsible for hyperresistance to Al in *Brachiaria*.

Aluminum in Plants, Table 1 Aluminum concentrations in shoots of selected accumulator species from different botanical families. Potential Al ligands, present in high concentrations in the different families, are given

Family/species	Leaf Al concentrations (mg/kg)
Polygonaceae	Oxalate, malate, citrate, quercetin anthraquinones
Fagopyrum esculentum	480–15,000
Theaceae	Catechin epigallocatechin gallate
Camellia sinensis	1,000–30,000
Eurya acuminata	19,600
Hydrangeaceae	Delphidin (flowers) citrate, hydrangenol (leaves)
Hydrangea sp.	400–3,000
Melastomataceae	Citrate, oxalate, tannate
Melastoma malabathricum	590–10,000
Miconia lutescens	6,800
Miconia ferruginata	4,300
Acisanthera uniflora	20,200
Pternande caerulescens	16,600
Memecylaceae	Flavonoids
Memecylon laurinum	12,700–30,500
Rubiaceae	Quercetin, kaempferol
Urophyllum macrophyllum	23,100

Modified from Barceló and Poschenrieder (2002) and Jansen et al. (2002)

Hyperaccumulation of Aluminum

While Al exclusion is the main strategy for Al resistance in most crop plants, there is a considerable number of plant species, native to acid soils with high Al^{3+} availability, which accumulate extremely high concentrations of Al in their shoots (Table 1). Most of these plant species from different botanical families are not used as commercial crops. Notable exceptions are the tea plant (*Camellia sinensis*), buckwheat (*Fagopyrum esculentum*), and the ornamental *Hydrangea* species. Common characteristics of Al hyperaccumulator plants are high degree of mycorrhizal colonization and high internal concentrations of either or both organic acids

and phenolic substances. The mycorrhizal colonization seems crucial for phosphorus acquisition by these plants growing in soils with low P availability. To what extent mycorrhiza may contribute to the high accumulation of Al in nontoxic form is still unclear. Citrate seems to be the main ligand for Al in the xylem of *Melastoma*, tea, and buckwheat. After ligand exchange, Al can be stored in the leaves in the form of Al-oxalate. High concentrations of phenolic compounds can contribute to Al tolerance in these hyperaccumulating species both by binding Al in nontoxic form and by their antioxidant properties.

Localization studies of Al in leaves of hydroponically grown tea plants using low-energy X-ray fluorescence microscopy (LEXRF) have shown that Al mainly accumulates in the cell walls of leaf epidermal cells. Moreover, Al was identified in xylem and phloem tissue. Taken together, these results suggest that in tea plants Al preferentially moves apoplastically with the transpiration stream to the epidermal cells. Here water is evaporated to the air and the Al is stored in the cell walls. However, the Al signal detected in the phloem region indicates that some Al can move symplastically through the leaves.

Despite the high Al concentrations in tea leaves, no health risk for consumption of tea by humans has been shown. The high amount of phenolic substances present in tea-based beverages binds Al in nontoxic form and more than 99% of the Al passes the digestive tract unabsorbed. The small absorbed fraction is excreted by the kidney.

Beneficial Effects of Aluminum

Although most investigations on Al in plants address toxicity and resistance mechanisms, Al-induced stimulation of plant performance has also been reported. Aluminum is not an essential nutrient for plants and no specific function for Al has been established. In acid mineral soils, however, plants may benefit from the presence of Al^{3+} in the soil solution because the trivalent Al can ameliorate the toxicity of high proton concentrations (Kinraide 1993; Kidd and Proctor 2001).

Aluminum-induced stimulation of growth in Al hyperaccumulating plants seems related to enhanced phosphorous availability and uptake. Moreover, metal hyperaccumulation can be beneficial by inhibition of plant pathogens or as a feeding deterrent.

Cross-References

- ▶ Aluminum Speciation in Human Serum
- ▶ Aluminum and Bioactive Molecules, Interaction
- ▶ Aluminum in Biological Systems
- ▶ Aluminum, Genes Involved in Novel Adaptive Resistance in Rhodotorula glutinis

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Aluminum Speciation

- ▶ Aluminum and Bioactive Molecules, Interaction

Aluminum Speciation in Human Serum

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Synonyms

High molecular mass aluminum species in serum; Hyphenated techniques; Low molecular mass aluminum species in serum; Speciation analysis

Definition

The role of trace elements and their impact on the environment and living organisms depend not only on their total concentration but also on chemical forms in which they are actually present. Individual chemical species of trace elements in different samples are quantitatively determined by speciation analysis.

A fundamental tool for speciation analysis of aluminum (Al) is the combination of separation technique coupled to element-specific detector. For the time being the most powerful hyphenation is coupling of liquid chromatography to inductively coupled plasma mass spectrometry (ICP-MS). For identification of Al-binding ligands, mass spectrometry (MS)-based techniques exhibit the greatest potential.

Introduction

Al is the most abundant metal in the lithosphere, comprising 8% of the Earth's crust. The sparingly soluble nature of most Al compounds and its low bioavailability considerably decreases the probability of an Al body load in humans from environmental sources. The main route of entry of Al into the human body is through the consumption of food, beverages, and drinking water with higher Al concentrations. Due to effective urinary clearance, the whole body Al burden in adult person is low, about 35 mg. Also the physiological serum Al concentrations are low, around 2 ng Al mL^{-1} or less. Al is not considered to be an essential element in humans, but its toxic effects are known. In past decades Al represented potential overload in dialysis patients. Consequently, due to elevated Al burden, many clinical disorders were observed such as renal osteodystrophy, microcytic anemia, and dialysis encephalopathy. Concern over maintaining the high quality of water and fluids used for dialysis considerably prevented intoxication of dialysis patients with Al. Nevertheless, the absorption of Al via consumption of Al-based drugs and its accumulation in target organs is still a possible source of Al overload in renal patients. Al is also accumulated in brain of Alzheimer's patients. Risk for Al intoxication represent parenterally administered nutrients, contaminated with Al, particularly in infants with insufficient kidney function. Since Al salts are common adjuvants in vaccines, neurotoxic effects due to frequent vaccination may also develop. The outcomes of several epidemiological studies indicated on possible connection between elevated Al concentrations in drinking water and increased risk for different neurotoxic disorders (Milačič 2005; Yokel 2004).

To better understand the toxicity of Al in humans, it is essential to identify and quantify the chemical species in which Al is transported and stored in the body. For these reasons, among a variety of body fluids and tissues, Al speciation was the most frequently investigated in human serum (Milačič 2005; Harrington et al. 2010). Due to the complex chemistry of Al in serum, its low total concentration and the high risk of contamination by extraneous Al, speciation of Al in serum is a difficult task for analytical chemists. In the last two decades, analytical techniques for the determination of the amount and composition of high molecular mass Al (HMM-Al) as well as low molecular mass Al (LMM-Al) species in human serum has been progressively developed. For separation of Al species microultrafiltration, size exclusion chromatography (SEC) and anion-exchange chromatography were applied (Sanz-Medel et al. 2002; Milačič et al. 2009; Murko et al. 2011). Separated Al species were determined either by electrothermal atomic absorption spectrometry (ETAAS) or inductively coupled plasma mass spectrometry (ICP-MS). Identification of Al-binding ligand in separated serum fractions was performed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and mass spectrometry (MS)-based techniques (Kralj et al. 2004; Bantan et al. 1999; Murko et al. 2009).

Al-Binding Ligands in Human Serum and the Role of Al Speciation

Blood serum is a very complex matrix with pH 7.4, containing HMM and LMM compounds and high concentration of salts. LMM metal complexes are the most active in terms of bioavailability. One of the most important LMM serum constituents is citrate. It is considered to be one of the major LMM binding ligands in mammalian serum and as an important LMM ligand for metals transport in human body. In human serum citrate occurs in concentrations about 0.1 mmol L^{-1}. Al-citrate complex is formed through coordination binding of Al with hydroxyl group and the two terminal carboxylates of citrate, thus leaving a free carboxylate that is dissociated at physiological pH. The complex is very stable. In addition to citrate, the important LMM compound in serum is phosphate, present in concentration about 2 mmol L^{-1}. Al^{3+} readily forms sparingly soluble species with phosphate anion, which may precipitate from body fluids. Due to the sparingly soluble nature of phosphate species, soluble Al-phosphate complexes in serum occur in much lesser extent than Al-citrate, while other LMM constituents of blood serum like lactate, oxalate, and amino acids have significantly lower affinities for Al^{3+} than citrate and phosphate and were not considered to contribute in complex formation. The presence of Al $(OH)_4^-$ was predicted in human serum, but in negligible concentrations (Milačič 2005). The majority of Al^{3+} in human serum is bound to HMM proteins. The potential HMM Al-binding ligands of serum proteins are albumin (molecular mass 66,000 Da, average serum concentration about 0.6 mmol L^{-1}) and transferrin (Tf) (molecular mass 795,000 Da, average serum concentration about 37 μmol L^{-1}). Albumin is too weak as a metal ion binder at physiological pH values to be able to effectively compete for Al^{3+} with other much stronger carriers such as Tf and citrate. Al readily binds to the two high-affinity iron-binding sites, the N-terminal and C-terminal domains. Since each Tf molecule has two metal-binding sites, the total binding capacity of Tf is about 74 μmol L^{-1}. Under normal conditions Tf is only about 30% saturated with Fe, leaving about 50 μmol L^{-1} of unoccupied metal-binding sites. This excess is more than enough to complex Al in human serum at normal concentration levels (Al concentrations about 0.1 μmol L^{-1}) and even in serum of dialysis patients (Al concentrations up to 5 μmol L^{-1}). The Tf is characterized by the unique requirement that Al is only bound as a ternary complex between the metal, the protein, and a carbonate anion, which is referred to as the synergistic anion (Milačič 2005).

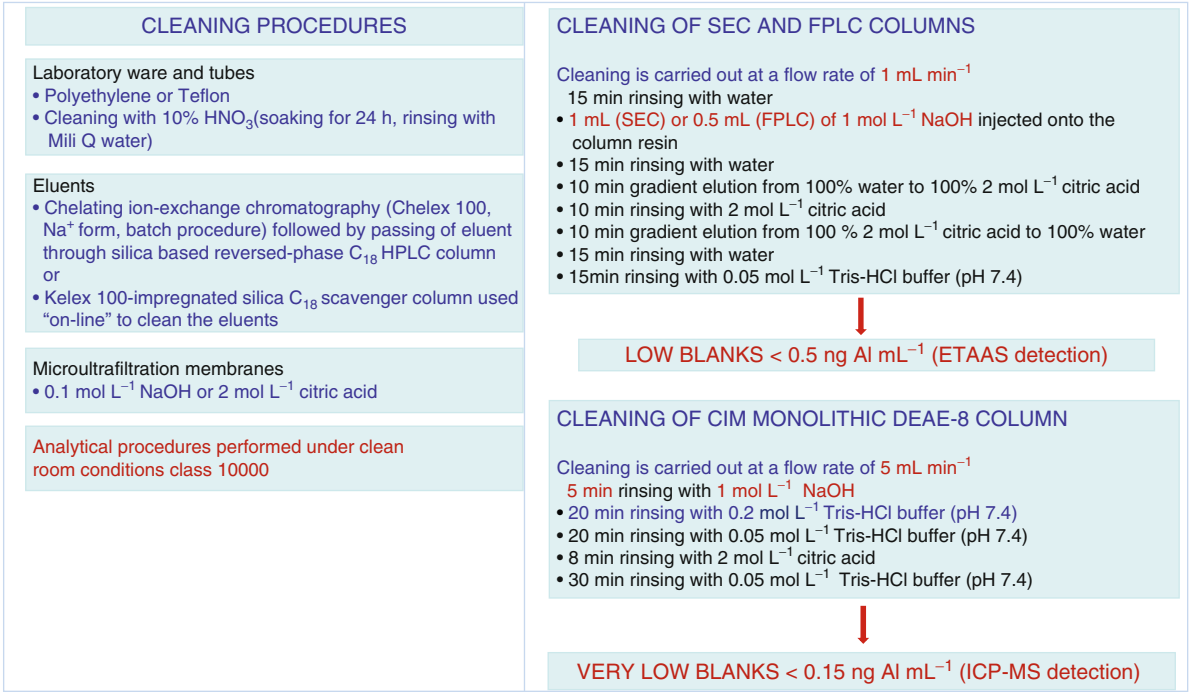
The chemical speciation of Al in human serum is of crucial importance in assessing which individual compounds contribute to Al neurotoxicity. Tf and citrate may facilitate Al transport across the blood–brain barrier, while Tf seems to mediate Al transport into neurons and glial cells (Yokel 2004). The speciation analysis is also very important in the evaluation of the efficiencies of chelation therapies in patients overloaded with Al (Murko et al. 2011). For these reasons efforts of many investigators were oriented to the development of reliable analytical procedures for the determination of Al species in human serum.

Al Speciation in Human Serum

Physiological concentrations of Al in human serum are very low, around 2 ng mL^{-1}. A decade ago elevated concentrations of Al up to 150 ng mL^{-1} were found in dialysis patients. Presently, these concentrations are appreciably lower due to intensive care to prevent Al overload in renal patients. Complexity of the serum constituents, low total concentration of Al, the high risk of contamination, and the tendency of Al to be adsorbed by different chromatographic supports make speciation of Al in human serum a difficult task for analytical chemists. In the following sections, problems related to Al speciation in human serum are discussed and the progress that was made through the development of the analytical procedures is presented.

Contamination: Sources and Elimination

To obtain reliable analytical data in Al speciation, it is of extreme importance to prevent all possible sources of contamination. The only appropriate material for laboratory ware is high-density polyethylene or Teflon that should be cleaned before use with 10% HNO_3. Traces of Al should be removed also from eluents, reagents, columns, and filtering devices. For effective cleaning of membranes of the filtering devices, NaOH was found to be the most convenient. To remove Al from eluents used in chromatographic separations,

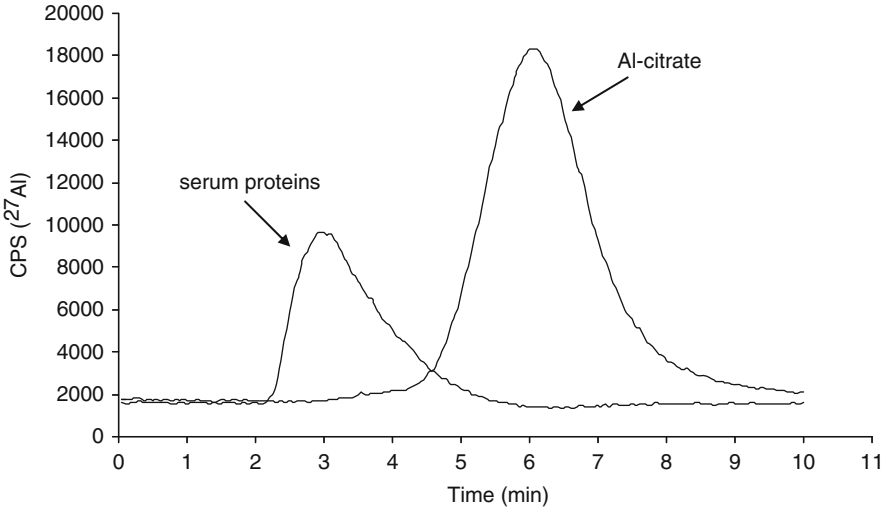


Aluminum Speciation in Human Serum, Fig. 1 The most frequently applied cleaning procedures in Al speciation

different chelating resins in combination with silica-based columns, which have strong affinity to adsorb Al, were applied (Sanz-Medel et al. 2002). Chromatographic supports used in the speciation procedure were cleaned by application of citric acid, acetic acid or sodium citrate, and NaOH (Milačič et al. 2009). In order to obtain reliable analytical data in speciation of Al in serum samples, the overall cleaning procedure and careful handling of samples to prevent contamination is of crucial importance. The most frequently applied cleaning procedures in Al speciation are schematically presented in Fig. 1.

Fractionation Procedures

Because of low Al concentration the speciation of Al in serum of healthy subjects was in general performed in spiked samples or in serum of renal patients with elevated Al concentrations. To separate Al bound to proteins (HMM-Al) from Al bound to LMM compounds, fractionation procedures based on microultrafiltration or size exclusion chromatography (SEC) were commonly applied. The microultrafiltration procedures were generally more liable to contamination problems than SEC procedures (Milačič et al. 2009). Microultrafiltration and SEC procedures in general gave the same results on the amount of HMM-Al and LMM-Al species. Studies of many researchers confirmed that about 90% of Al is bound to proteins, while the remaining Al corresponded to the LMM serum fraction (Sanz-Medel et al. 2002; Kralj et al. 2004; Milačič et al. 2009). Recently, a fractionation procedure was developed for rapid separation of proteins from the LMM-Al species. The fractionation was performed on HiTrap desalting SEC column coupled on-line to ICP-MS in 10 min (Murko et al. 2011). By this simple SEC procedure the partitioning of Al between serum proteins and the LMM-Al compounds may be quantified in both the HMM serum fraction (first 5 min of the separation procedure) and the LMM serum fraction (the following 5 min of the separation procedure). Typical elution profile of Al for separation of serum proteins and LMM species by HiTrap desalting SEC-ICP-MS procedure is presented in Fig. 2. In comparison to ultrafiltration and SEC procedures that take 0.5–1 h (Sanz-Medel et al. 2002, Milačič et al. 2009), the separation time of the HiTrap desalting SEC procedure is much shorter. In addition, ultrafiltration procedures enable quantification of Al

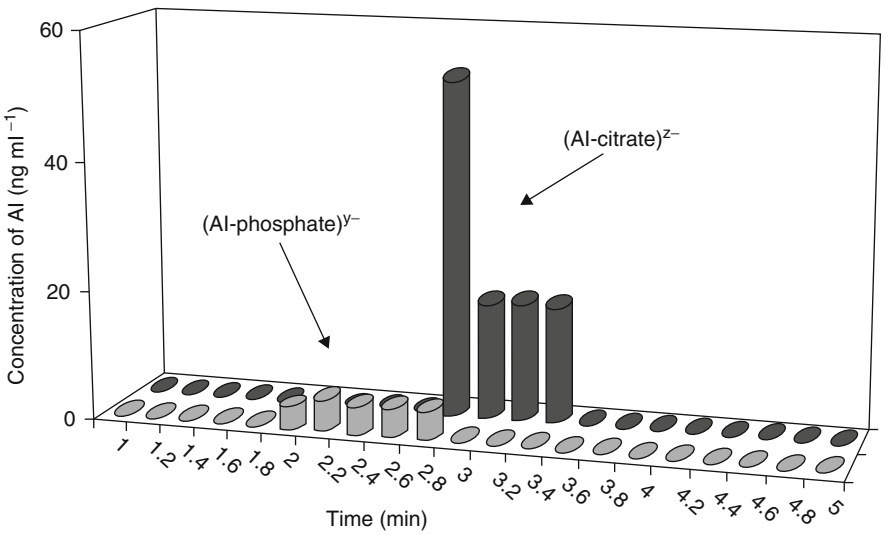


Aluminum Speciation in Human Serum, Fig. 2 Rapid separation of serum proteins and LMM species by HiTrap desalting SEC-ICP-MS procedure (Reproduced with permissions from The Royal Society of Chemistry (Murko et al. 2011))

only in the LMM serum fraction (Sanz-Medel et al. 2002; Milačič et al. 2009), while by the use of the conventional SEC procedures the quantification of Al is in general possible only in the protein fractions (Sanz-Medel et al. 2002; Kralj et al. 2004; Milačič et al. 2009). It is of great importance that the HiTrap desalting SEC fractionation procedure does not influence the chemical speciation of the serum constituents and therefore enables further speciation procedures to be applied in the separated HMM and LMM serum fractions. The fast fractionation procedure is also reliable and sensitive enough to perform fractionation of serum samples containing Al at physiological concentrations levels. It represents also a promising tool for studies of the efficiencies of the chelation therapies in patients overloaded with Al. Such therapies have been already performed in dialysis patients (Sanz-Medel et al. 2002) and are presently again actual in modalities for treatment of other clinical disorders. The latest discoveries on relationship between brain metal dishomeostasis and progression of Alzheimer's disease rendered chelation therapy as a promising option for curing this neurological disease (Hedge et al. 2009).

Chromatographic Procedures in Speciation of LMM-Al Species in Human Serum

Data obtained from computer modeling on the distribution of LMM-Al species in human serum predicted a variety of LMM-Al compounds, of which citrate was considered as one of the most important Al-binding ligand. Therefore, there was a need to experimentally determine the composition and content of LMM-Al species in human serum. This was rather difficult task for analytical chemists, since Al tends to adsorb on a variety of chromatographic supports. A big problem represented also contamination with Al arising from fillings of chromatographic columns (Sanz-Medel et al. 2002). The most powerful analytical procedure was developed by the use of strong anion-exchange Mono Q fast protein liquid chromatography (FPLC) column. It enabled quantitative separation of Al-citrate and soluble Al-phosphate species, applying gradient elution from water to 4 mol L^{-1} NH_4NO_3 in 10 min (Bantan et al. 1999). The use of appropriate cleaning procedures (NaOH and citric acid) lowered the column blanks below 1 ng mL^{-1} Al. In the analysis of serum samples, the microultrabiltrable fraction of spiked human serum and serum of dialysis patients was injected onto the column resin. 0.2 mL fractions were collected throughout the chromatographic run and Al determined by ETAAS. In fractions that contained Al, the binding ligand was presumed on the basis of the retention time and identified also by electrospray ionization tandem mass spectrometry (ESI-MS-MS). Based on the mass spectra and the corresponding daughter ion spectra, the presence of citrate (peak m/z 191 and the corresponding daughter ion spectra with m/z 111, 87 and 85), phosphate (peak m/z 97 and the corresponding daughter ion spectra with m/z

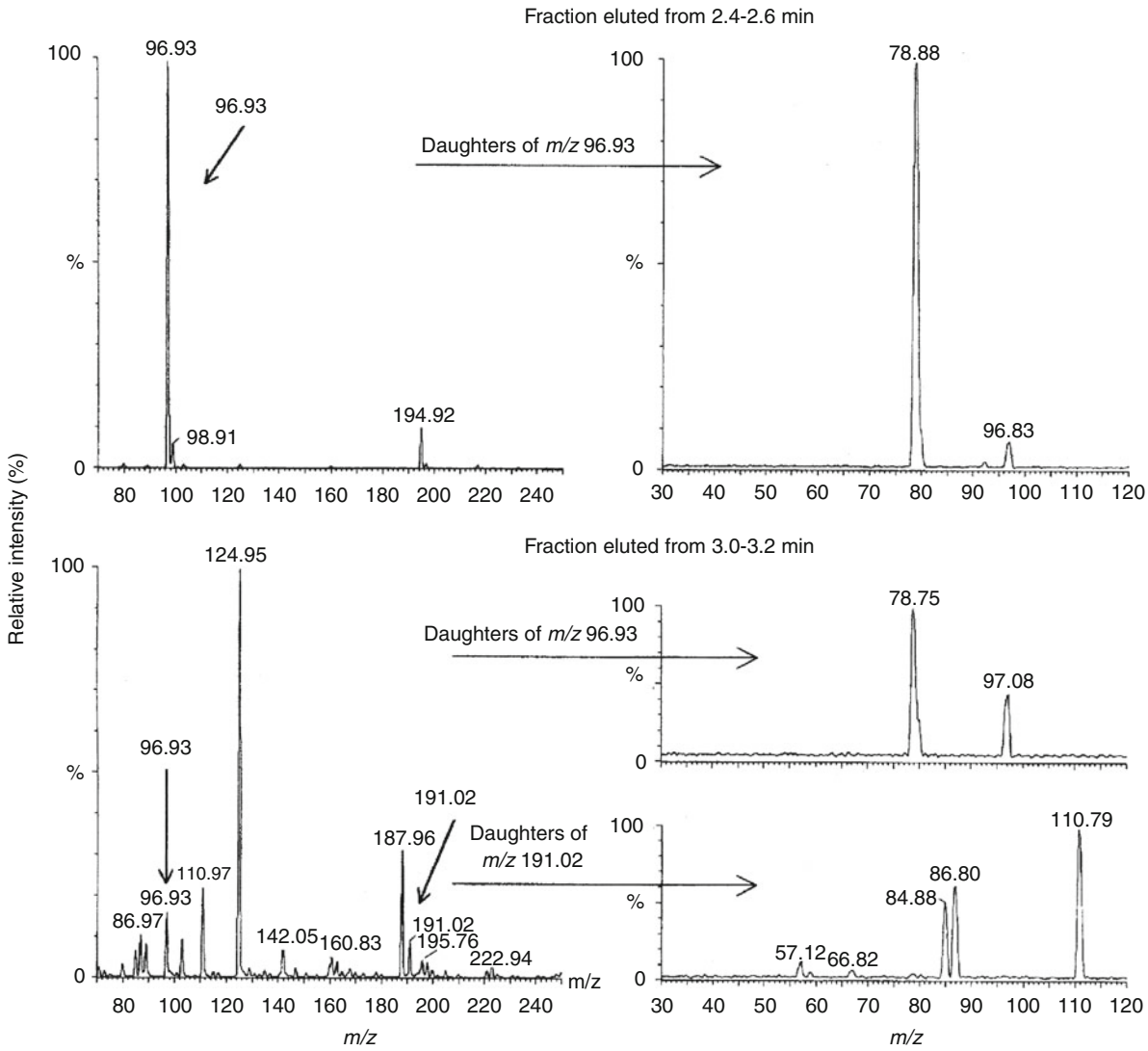


Aluminum Speciation in Human Serum, Fig. 3 Typical chromatograms of Al-citrate and Al-phosphate (100 ng mL^{-1} Al) at pH 7.4. Separation was performed on a Mono Q HR 5/5 anion-exchange FPLC column and separated species were detected by ETAAS (Reproduced with permissions from Elsevier (Bantan Polak et al. 2001))

97 and 79), and both phosphate- and citrate-binding ligands was confirmed in separated serum fractions. Typical chromatograms for separation of Al-citrate and Al-phosphate at pH 7.4 are presented in Fig. 3. An example of ESI-MS-MS spectra in separated serum fractions containing Al is presented in Fig. 4. By combining data from speciation of Al by FPLC-ETAAS procedure and further identification of the Al-binding ligands, it was experimentally proven that Al-citrate, Al-phosphate, and ternary Al-citrate-phosphate species were present in serum samples of healthy persons (serum spiked with Al) and dialysis patients. These data were in agreement with the modeling calculations in human serum. In serum of healthy adults, the percentage of LMM-Al species ranged from 14% to 20%, while in dialysis patients the LMM-Al fraction was higher, up to 50%.

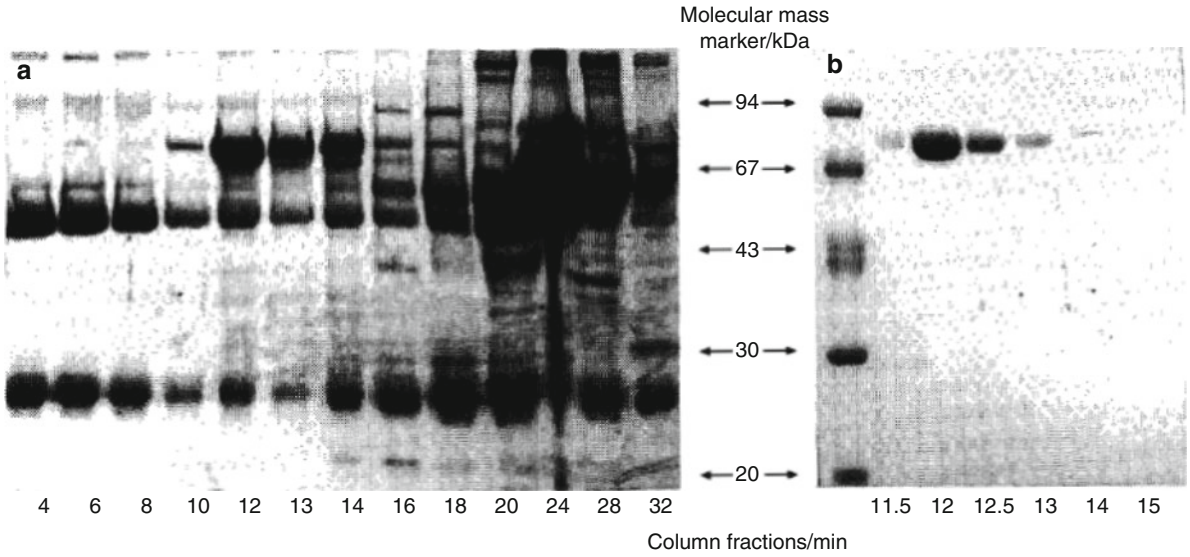
Chromatographic Procedures in Speciation of HMM-Al Species in Human Serum

SEC procedures were not selective enough to separate albumin and transferrin (Sanz-Medel et al. 2002; Milačič et al. 2009). Therefore, more powerful chromatographic procedures in combination with various specific detection techniques were needed to characterize and quantify the Al-binding proteins in human serum. To obtain higher resolution in separation of serum proteins, anion-exchange chromatographic columns were applied. One of the first promising chromatographic supports represented a polymeric anion-exchange Protein Pak DEAE-5-PW column (Sanz-Medel et al. 2002). Separated proteins were followed by UV detection, while Al in separated fractions was determined by ETAAS. The selected column fractions that contained Al were also characterized by SDS-PAGE (Fig. 5). Although immunoglobulin G (IgG) and Tf were not quantitatively separated, the results indicated that Al was eluted under the Tf peak. To obtain better separation of proteins, an anion-exchange FPLC Mono Q HR 5/5 column coupled to UV and element-specific ETAAS detectors was later used by several researchers. Results revealed that Al was bound exclusively to Tf. In order to lower the limits of detection, the same chromatographic column was further applied, while for detection of separated Al species quadruple ICP-MS and high-resolution ICP-MS instruments were used. Special attention was focused on efficient cleaning of eluents, while the cleaning of column supports remained the limiting factor for speciation of Al in unspiked human serum at physiological concentration levels (Milačič et al. 2009). The results of different researchers again confirmed that about 90% of Al in human serum was bound to Tf. Since there are no reference materials for speciation analysis of Al, the use of complimentary analytical procedures is desirable. As an alternative to

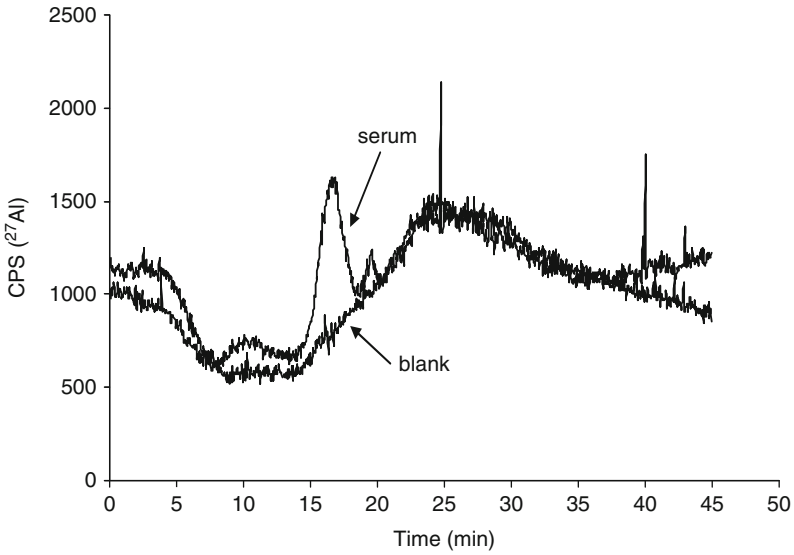


Aluminum Speciation in Human Serum, Fig. 4 ES-mass spectra and corresponding daughter ion mass spectra of m/z 97 for eluted fractions from 2.4 to 2.6 and m/z 97 and 191 for eluted fractions from 3.0 to 3.2 min, respectively, on an anion-exchange FPLC column for serum sample (Reproduced with permissions from The Royal Society of Chemistry (Bantan et al. 1999))

ion-exchange FPLC columns, ion-exchange separation supports based on convective-interaction media (CIM) were further applied. Al speciation was performed on CIM methacrylate-based monolithic anion-exchange diethyl amino ethane (DEAE) column. A great advantage of CIM DEAE column is its ability to sustain rigorous cleaning of the chromatographic support with NaOH (as presented in Fig. 1). Efficient cleaning of the column support and eluents used in the chromatographic procedure resulted in extremely low blanks (below 0.15 ng Al mL^{-1}). By coupling the CIM DEAE column to highly sensitive element

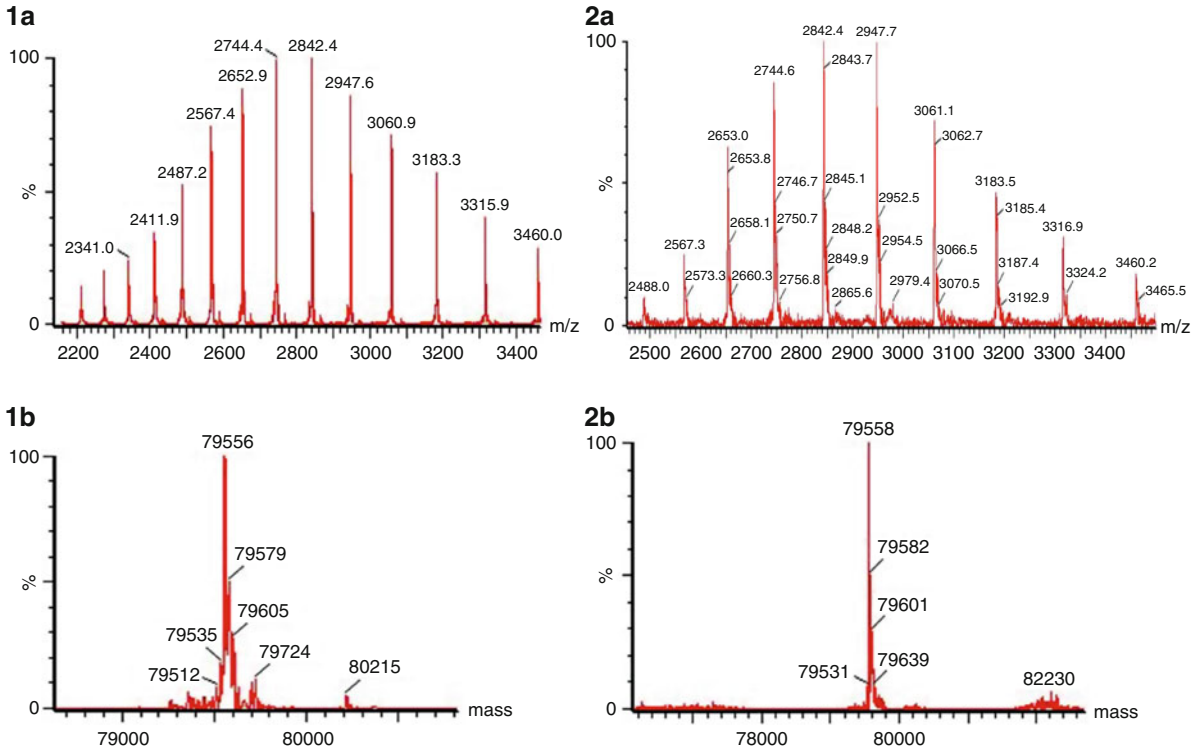


Aluminum Speciation in Human Serum, Fig. 5 SDS-PAGE of selected column fraction collected for elution of 500 µL of: (**a**) diluted spiked serum sample, and (**b**) Tf standard solution. Molecular mass markers are given in the center (Reproduced with permissions from The Royal Society of Chemistry (Wróbel et. al. 1995))



Aluminum Speciation in Human Serum, Fig. 6 The Al elution profiles for the separation of unspiked serum (1 + 4) and blank sample after overall cleaning procedure. The peak of Al in unspiked serum sample (1 + 4) corresponds to 1.04 ng mL^{-1} of Al (Reproduced with permissions from American Chemical Society (Murko et al. 2009))

specific detector such as ICP-MS, speciation of Al was possible at physiological concentration levels from 0.5 to 2 ng Al mL^{-1} (Fig. 6) (Murko et al. 2009). Data demonstrated that about 90% of Al in unspiked human serum was separated at the retention volume characteristic for Tf. To identify the Al-binding ligand, the column fraction that contained Al was further characterized by acquity ultra performance liquid chromatography–electrospray ionization mass spectrometry (UPLC-ESI-MS) (Fig. 7). The results again confirmed that Al-binding ligand was Tf. The data on the speciation of Al at physiological concentration levels represent an important basis for studies of Al distribution and its fate in human body.



Aluminum Speciation in Human Serum, Fig. 7 UPLC-ESI-MS analysis of transferrin standard (1**a** and 1**b**) and human serum transferrin after separation on CIM DEAE column (2**a** and 2**b**). (**a**) ESI-mass spectrum, (**b**) deconvoluted mass spectrum (Reproduced with permissions from American Chemical Society (Murko et al. 2009))

Conclusions

Al is involved in many clinical disorders. To understand Al transport through the human body and its accumulation in target organs, it is necessary to obtain information on the chemical species of Al in human serum. In last decades analytical procedures for speciation of Al have been progressively developed. Due to the very low physiological concentrations of Al in serum of healthy subjects and the environmental abundance of Al, there is a high risk of contamination during speciation analysis. To avoid contamination by extraneous Al, appropriate handling of samples and efficient cleaning procedures should be applied. Ultrafiltration and SEC procedures enable fractionation of Al in serum. With these procedures it is possible to distinguish only between the amount of LMM-Al and HMM-Al species. However, they can be successfully applied as an appropriate analytical tool, to follow the effectiveness of chelation therapies in patients overloaded with Al. For identification and quantification of Al chemical species present in human serum, more powerful analytical procedures are required. Anion-exchange FPLC columns in combination with element-specific detectors, mass spectrometry–based techniques, UV, and SDS-PAGE were found to be powerful analytical tools for speciation both LMM-Al as well as HMM-Al species in spiked human serum, or in serum of dialysis patients with elevated Al concentrations. The combination of microultrafiltration and speciation analysis of ultrafiltrable Al by anion-exchange FPLC and ESI-MS-MS techniques demonstrated that the LMM-Al fraction is composed of Al-citrate, Al-phosphate, and ternary Al-citrate-phosphate complexes. Anion-exchange FPLC columns were also applied in separation of serum proteins. Investigations were performed on human serum spiked with Al. As element-specific detectors ETAAS and ICP-MS techniques were used. In combination with UV detection and identification of Al-binding ligand by SDS-PAGE, it has been demonstrated that about 90% of Al in spiked human serum is

bound to Tf. Later, CIM DEAE monolithic column was applied in separation of serum proteins. By the use of rigorous cleaning of the column support and adequate cleaning of eluents used in the chromatographic procedure, the significant lowering of blanks and quantitative speciation of HMM-Al compounds at physiological concentration levels was possible. The identification of Al-binding ligand was performed by UPLC-ESI-MS. Data revealed that at physiological concentration levels the same amount of Al (about 90%) as in spiked human serum is bound exclusively to Tf. The possibility to perform speciation of Al at physiological concentration levels is of great importance in investigations of Al transportation and deposition in the target organs of the human body.

Cross-References

- ▶ Aluminum Complexes with Low Molecular Mass Substances
- ▶ Aluminum in Biological Systems
- ▶ Aluminum, Biological Effects

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Aluminum, Biological Effects

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Synonyms

Aluminum and health; Behavior of aluminum in biological systems

Definition

This entry refers to the ways of exposure of various biological systems to aluminum and to the mechanisms underlying the interactions of the element with these systems. Concerns on aluminum toxicity on ecosystems gain interest in view of the continuously increasing use of aluminum compounds in industry and acid rain.

Introduction

Despite the ubiquity of aluminum (Al) in the environment and its presence in living organisms, be it in small amounts (ppb-ppm range), no biological function has so far been attributed to the element. For this reason

aluminum is considered a nonessential metal. It has long been thought that aluminum was inert for living organisms and as such was not regarded as a toxic element until the 1960s. At that time, only a few reports dealt with the toxic effects of aluminum in humans and animals, which, however, did not receive much attention. In the 1970s, aluminum exposure was linked to particular disease states occurring in patients with end-stage renal failure particularly those treated by dialysis. Although aluminum toxicity is mainly concerned with dialysis patients and the element is adequately removed by the kidneys, occupationally exposed workers (e.g., welders) are also at risk for its deleterious effects. In the latter population some evidence has been provided for the element to cause pulmonary and neurological lesions. Aluminum exposure has also been linked to the development of Alzheimer's disease, an issue which is still controversial.

Occurrence

Aluminum is the most abundant metallic element in nature (8% of the earth crust) and the third most abundant element preceding iron (4.7%) but less abundant than oxygen and silicon. Aluminum exists primarily associated with silicates and oxides in minerals, which are rather insoluble, explaining the relatively low aluminum concentations in rivers, lakes, and sea water (<1 μg/L). Acidification of lakes as a result of acid rain enhanced aluminum solubility and hence toxicity for fish and even birds living in the immediate surroundings.

Human activities have also in other ways enhanced exposure of living organisms to aluminum since it is being used in industrial applications such as, for example, food additive, flocculant in water treatment, pharmaceuticals, etc. Especially, exposure to aluminum dust by welders and workers in aluminum-producing industries has resulted in neurological problems and lung diseases (Buchta et al. 2005; Donoghue et al. 2011).

Aqueous Chemistry

Aluminum, electronic structure: [Ne] $3\,s^2\,3p^1$, MM 27, exists only in the oxidation state III. It is amphoteric, combining with both acids and bases to form, respectively, aluminum salts and aluminates. It has a small ionic radius (54 pm) closer to iron (65 pm) than most other elements. From the combination of a small ionic radius with a high charge, it follows that in aqueous solutions the free Al^{3+} concentration is very small due to the formation of aluminum hydroxide complexes. In a solution with a pH below 5 aluminum exists as $Al(H_2O)_6^{3+}$. With rising pH, this complex deprotonates under the formation of the insoluble $Al(OH)_3$ at neutral pH, which redissolves at higher pH as the $Al(OH)_4^-$ complex.

Exposure to Aluminum

Because of its abundance in the environment, aluminum is frequently consumed as an incidental component of water or food, including infant formula. Aluminum is also intentionally added to food as a caking or emulsifying agent. As a result, bread made with aluminum-based baking powder can contain up to 15 mg aluminum per slice, and processed American cheese can contain as much as 50 mg aluminum per slice. It has been shown recently that exposure to aluminum in humans may also occur through vaccination as certain vaccines may contain specific aluminum salts (primarily aluminum hydroxide and aluminum phosphate) as an adjuvant. Aluminum adjuvants are important components of vaccines, since they stimulate the immune system to respond more effectively to protein or polysaccharide antigens that have been adsorbed to the surface of insoluble aluminum particles (Mitkus et al. 2011).

Till the early 1980s patients with chronic renal failure were exposed to high amounts of aluminum as at that time aluminum hydroxide was frequently used for phosphate control and was given in doses of grams/day during years. Moreover, when treated by hemodialysis the use of aluminum-contaminated dialysis fluids further increased the risk for aluminum overload and serious toxicological effects. Due to replacement of aluminum-based phosphate binders by nonaluminum-containing compounds together with the introduction of adequate water treatment installations caricatural aluminum overload nowadays is rarely seen although moderate exposure still frequently occurs in less developed countries and

with the "accidental" use of aluminum-leaking instrumental devices related to the dialysis treatment (D'Haese and De Broe 1999).

Aluminum Metabolism in Humans

Inhalation

A fraction of the aluminum present in dust remains indefinitely in the lungs after inhalation, thus without entering the systemic blood compartment. Especially in welders and workers in the aluminum industry, uptake of the element via this route has caused health problems related to lungs and brain.

Gastrointestinal Absorption of Aluminum

The gastrointestinal absorption of aluminum is importantly affected by the speciation of the element and values between 0.001% and 27% have been reported. This wide variation is mainly due to analytical difficulties, contamination, and differences in experimental protocols. With the application of the ^{26}Al radioisotope and its detection by Accelerator Mass Spectrometry (AMS) more reliable results on fractional gastrointestinal absorption varying between 0.04% and 1% could be obtained.

At least as important as the knowledge on the amounts of aluminum absorbed after oral intake is the exact mechanism that governs this process. No unified explanation has emerged till today. It is believed that intestinal absorption of aluminum includes both (1) paracellular pathways along enterocytes and through junctions by passive processes and (2) transcellular pathways through the enterocytes involving both passive and active processes.

Local factors altering aluminum absorption include citrate which has been reported to significantly enhance the element's absorption by opening the epithelial tight junctions although effects to a much lesser extent have been reported more recently by others using more sophisticated techniques. Other factors reported to alter aluminum absorption are fasting state, gastric pH, and silicon. With regard to the latter element, recent studies reported silicon to have therapeutic potential to prevent oral absorption and retention in mammals, particularly in the setting of Alzheimer's disease.

General factors which have been reported to enhance aluminum absorption are the uremic state, diabetes mellitus, hyperparathyroidism, and Down syndrome. During the last decades, the potentially increased bioavailability of aluminum resulting from an increased anthropogenic acidification of soils has become a matter of concern.

Tissue Distribution and Excretion of Aluminum

Following enteral absorption, aluminum is transported mainly in the plasma in association with the iron-binding protein transferrin. In adults with normal renal function, the total aluminum burden is estimated to be 30 mg with the highest levels found in the lungs and skeletal muscles. In patients with renal insufficiency, chronic accumulation will occur as the major elimination route, that is, the kidney is partially or even totally absent, and in the past, aluminum body burdens up to 1,660 mg have been reported in dialysis patients. In these patients also a different tissue distribution pattern is observed as compared to individuals with normal renal function with bone and liver as the major storage sites.

As only a very small fraction of ingested aluminum is absorbed, the feces represent the major excretion pathway. In the presence of a normal renal function, minute fractions that are absorbed are subsequently removed via the kidney. As for the absorption of aluminum, the exact mechanism of aluminum excretion by the kidney has not been unraveled up to now although it has been inferred from micropuncture experiments and other techniques that a significant fraction of aluminum filtered by the glomerulus is reabsorbed. Somewhat in contrast herewith is the statement made by some that the aluminum clearance is about 5–10% of the glomerular filtration rate, which in combination with the finding that in the blood compartment aluminum is about 90–95% protein bound would suggest that the unbound serum aluminum is filtered and excreted without tubular reabsorption.

Aluminum Toxicity

General

The chemistry and biochemistry of the aluminum ion (Al^{3+}) dominate the pathways that lead to toxic outcomes. The molecular targets of aluminum toxicity are multifaceted and appear to involve the disruption of essential metal homeostasis such as calcium,

magnesium, and iron. Aluminum has been demonstrated to replace calcium within the bone and interferes with calcium-based signaling events. Magnesium has been observed to be replaced by aluminum for binding to phosphate groups on the cell membrane, on ATP, and on DNA. Perhaps, the main targets of aluminum toxicity are iron-dependent biological processes. Interference of aluminum with iron homeostasis also leads to the production of reactive oxygen species (ROS), which in turn may also lead to toxicological effects.

Overall, experimental animal studies have failed to demonstrate carcinogenicity attributable solely to aluminum compounds and aluminum has thus not been classified with respect to carcinogenicity. *Aluminum production*, however, has been classified as carcinogenic by the International Agency for research on Cancer (IARC). Occupational limits exist in several countries for exposures to aluminum dust and aluminum oxide. For nonoccupational environments, limits have been set for intake in foods and drinking water.

Humans

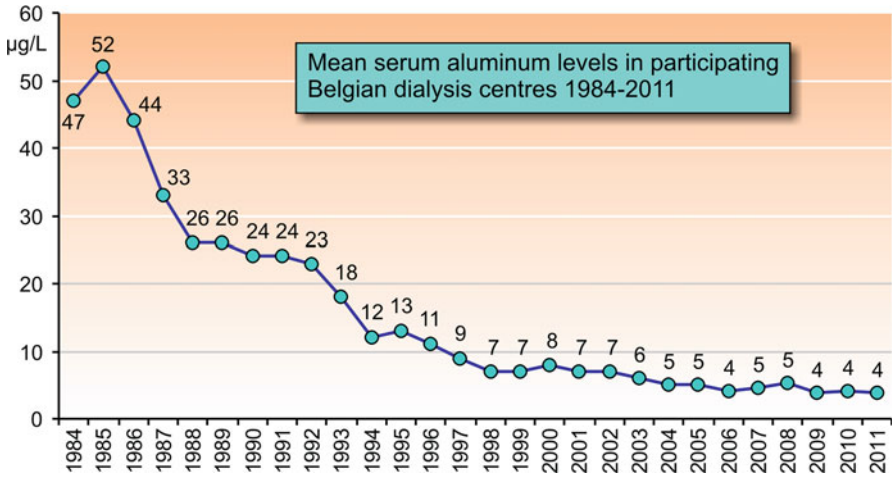
Subjects with Normal Renal Function

Neurological Effects In subjects with normal renal function, toxicity of aluminum has been mainly associated with neurological and lung diseases. Although in this population a definite role for aluminum in the development of neurological diseases remains elusive, the link between aluminum and Alzheimer's disease has been the subject of scientific debate for several decades. Following the findings from an epidemiological study of Martyn et al. (1989) showing a high incidence of Alzheimer's disease in areas with a high level of aluminum in the drinking water in England and Wales similar observations were made by others, while other studies revealed increased brain aluminum levels in patients with Alzheimer's disease. The discovery of aluminum in the brain of Alzheimer's patients and the association of aluminum with amyloid plaques provided some further evidence to the connection between the metal toxin and Alzheimer's disease, although it should be mentioned that such associations could not be confirmed by some other groups. Mounting evidence has suggested the significance of oligomerization of β-amyloid protein and neurotoxicity in the molecular mechanism of Alzheimer's disease pathogenesis. In their excellent overview, Kawahara and Kato-Negishi (Kawahara and Kato-Negishi 2011) revisit the link between aluminum and Alzheimer's disease and integrate aluminum and amyloid cascade hypotheses in the context of β-amyloid oligomerization.

Amyotrophic lateral sclerosis (ALS) along with Parkinsonism dementia has been seen in certain populations with great frequency, such as in the Chamorros of Guam, in the Kii Peninsula of Japan and in southern West New Guinea, three regions of the Western pacific region. Studies later on revealed an abnormal mineral distribution in the soil and drinking water of these areas consisting of a virtual lack of calcium and magnesium coupled with high levels of aluminum and manganese. Based on experimental studies in rats it was hypothesized that long-term intake of low calcium and high aluminum water and/or diet reduces magnesium in the body and may cause degenerative neurological disorders by altering the normal biological effects of magnesium.

Increased exposure to aluminum by the use of aluminum hydroxide as an adjuvant in the anthrax vaccine has also been linked to the Gulf War Syndrome, a multisystem disorder afflicting many veterans of Western armies in the 1990–1991 Gulf War. A number of those showed neurological deficits including various cognitive dysfunctions and motor neuron disease, the latter expression virtually indistinguishable from classical amyotrophic lateral sclerosis (ALS) except for the age of onset as the great majority of diseased individuals were less than 45 years of age.

Pulmonary Effects Following inhalation exposure, the effects of aluminum are mainly exerted on the respiratory system. Reports have been published dealing with the development of asthma, cough, or decreased pulmonary function (Nayak 2002). To which extent these effects are solely due to aluminum or rather are the consequence of multielement exposure or have a multifactorial etiology remains questionable. A small minority exposed to powdered metallic aluminum appear to develop an alveolitis that progresses to fibrosis, a lung disease known as aluminosis, which was already described in the 1930s and 1940s. Although it was assumed that under today's working conditions lung fibrosis induced by aluminum dust could not occur anymore, several severe cases of aluminum-induced lung fibrosis have been reported in



Aluminum, Biological Effects, Fig. 1 Evolution of the mean serum aluminum levels of dialysis patients from different centers in Belgium

more recent years. Experimental studies provided some evidence for a causal role of the element be it that in most of these studies exposure was much higher than that encountered in environmental or even occupational settings and, thus, have to be interpreted with caution.

Subjects with Impaired Renal Function, in Particular Dialysis Patients

As the urinary excretion route is the major pathway of elimination of aluminum, impairment of the kidney will be an important determination of the element's accumulation in the human body. In the past, aluminum-based phosphate binders were frequently used in these subjects, which led to the development of severe aluminum-related diseases such as osteomalacia and dialysis encephalopathy (De Broe and Coburn 1990), particularly when patients were treated by hemodialysis. Indeed, according to the concentration gradient between the ultrafiltrable amount of aluminum in the blood (i.e., serum-) compartment (<5% of the total concentration), a transfer of the element from the dialysis fluid toward the patient thus may already occur at dialysate aluminum levels as low as 2 µg/L (D'Haese and De Broe 1999).

It is now well recognized that the critical localization of aluminum at the mineralization front underlies the mineralization defect in aluminum-related osteomalacia. While this type of bone disease is accompanied by distinctly elevated levels of the element in bone (>15 µg/g wet weight vs <2 µg/g in normals), much lower levels were reported in the brain of dialysis patients with encephalopathy (2.3 ± 0.83 µg/g vs <0.60 ± 0.37 µg/g in dialysis patients without dementia) indicating the brain to be much more vulnerable to the toxic effects of aluminum as compared to other organs.

Due to the introduction of adequate water treatment systems and aluminum-free phosphate-binding agents together with the establishment of regular monitoring programs chronic, caricatural aluminum overload nowadays is rarely seen and in most developed countries serum values now vary around 3 µg/L (Fig. 1). Nevertheless, the risk for accumulation/intoxication should not be neglected (D'Haese and De Broe 1999). Indeed, as demonstrated in a recent survey, may moderate aluminum accumulation still occur in chronic renal failure patients, either or not treated by dialysis, from particular regions in the world. With such levels more subtle disorders at the level of the parathyroid gland function and bone turnover, resistance to erythropoietin therapy, and anemia may be observed. The anemia is typically microcytic and the presence of microcytosis with normal serum ferritin levels suggests that aluminum intoxication may be the causative factor. It is thought that aluminum might induce a defect in iron utilization or interfere with the bioavailability of stored iron for erythropoiesis.

Research on the speciation and protein-binding characteristics of aluminum has led to a better insight in the mechanisms underlying the element's tissue distribution and it has been hypothesized that iron depletion due to the widespread use of erythropoietin may result in an increased binding of aluminum to transferrin (Van Landeghem 1997). Consequently, the uptake of the aluminum-transferrin complex by

cells via endocytosis of transferrin receptors increases. This will cause a preferential accumulation of aluminum in transferrin receptor expressing tissues such as, for example, the parathyroid gland and the osteoblast which may result in a reduced PTH secretion and cellular proliferation. Insight in the speciation of aluminum in cerebrospinal fluid of severely intoxicated dialysis patients also put some light on the mechanisms underlying the dialysis-related encephalopathy which, as mentioned above, may already occur at much lower levels than those seen in bone or other organs. Indeed, it has been shown that whereas in serum of dialysis patients up to 90% of the total concentration appears bound to transferrin, the remaining fraction most probably occurring as (a) low molecular mass compounds such as citrate or silicate, in the brain evidence has been presented for the element not to circulate as a protein-bound complex. This could be explained by the fact that the molar concentration of citrate in cerebrospinal fluid is 900-fold higher than that of transferrin by which aluminum in the latter biological fluid will preferentially bind to citrate in contrast to the situation in serum, where the molar citrate/transferrin ratio is only 4 (Van Landeghem 1997). To a certain extent, this may also provide an answer to the question as to why the incidence of neurological diseases such as Alzheimer's disease and cognitive impairment is not increased in dialysis patients despite the relatively higher exposure of this population to aluminum, an issue which often has been used as a major argument for disregarding such an association. Indeed, literature data have shown silicon levels in both CSF and serum of dialysis patients to be increased up to 100-fold as compared to subjects with normal renal function. Case studies furthermore demonstrated that also in the CSF, dialysis patients have much higher silicon levels as compared to subjects with normal renal function; CSF and serum silicon levels are quite identical. This together with data attributing a protective effect of silicon against aluminum toxicity by either affecting the bioavailability of aluminum or reversing the aluminum-induced conformational changes of neurofibrillary tangles characteristic of Alzheimer's disease may put this controversial issue in another perspective.

Due to its high protein binding, aluminum can hardly be removed during dialysis even when serum aluminum levels are high (>100 μg/L). Therefore, in cases of aluminum intoxication/overload, chelation therapy using desferrioxamine is applied. With this treatment aluminum stored in bone and tissues is picked up by the chelator under the formation of the aluminum-desferrioxamine complex, that is, aluminoxamine (MW 583 Da), which by its relatively low molecular mass can be removed efficiently from the body during dialysis (or the urine in subjects with intact renal function). Care should be taken during desferrioxamine therapy, however, as in severely intoxicated patients (serum aluminum levels >300 μg/L) a neurologic syndrome similar to aluminum-related encephalopathy has been reported. The exact mechanism is unknown but is theorized to be due to redistribution of aluminum mobilized by desferrioxamine into the brain, hereby assuming that the formed aluminoxamine complex is able to pass the blood-brain barrier (D'Haese and De Broe 2007).

Aquatic Systems and Plants

Aluminum toxicity has also been reported in aquatic systems. So has it been demonstrated that aluminum is a strong inhibitor of the mullet cytochrome P450 reductase activity while exposure to environmentally relevant concentrations of aqueous aluminum at neutral pH was reported to have a negative effect on the immunocompetence of the crayfish *Pacifastacus leniusculus*, specifically in the ability of the hemocytes to remove bacteria from the circulation. It is likely that the impairment of immunocompetence is due to hypoxia rather than direct toxicity of aluminum. Prolonged exposure to aluminum abolished this effect, indicating that the crayfish is able to adapt to exogenous aluminum. More or less in line with observations in humans has a protective role against aluminum toxicity been attributed in these organisms also. While in most cases this has been described to be due to ex vivo silicon–aluminum interactions by the formation of inert hydroxyaluminosilicates, evidence has been presented more recently for the existence of a silicon-specific intracellular mechanism for aluminum detoxification in aquatic snails involving regulation of orthosilicic acid (White et al. 2008).

Concerns on aluminum toxicity on ecosystems gain in interest in view of the continuously increasing use of aluminum compounds in industry and acid rain which will result in an increased solubility of aluminum in aquatic systems and in soil. Aluminum toxicity is considered a major constraint for crop production in acidic soil worldwide. When the soil pH is lower than 5, aluminum enters the root tip and causes inhibition of

cell elongation and cell division leading to root stunting as well as a deep change in the entire root architecture.

Cross-References

- ▶ Aluminum and Bioactive Molecules, Interaction
- ▶ Aluminum in Biological Systems
- ▶ Aluminum in Plants
- ▶ Aluminum Speciation in Human Serum
- ▶ Aluminum, Physical and Chemical Properties
- ▶ Iron Homeostasis in Health and Disease
- ▶ Iron Proteins, Transferrins and Iron Transport
- ▶ Lead and Alzheimer's Disease
- ▶ Magnesium

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Aluminum, Genes Involved in Novel Adaptive Resistance in *Rhodotorula glutinis*

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Synonyms

Aluminum-resistant genes related to cation homeostasis

Definition

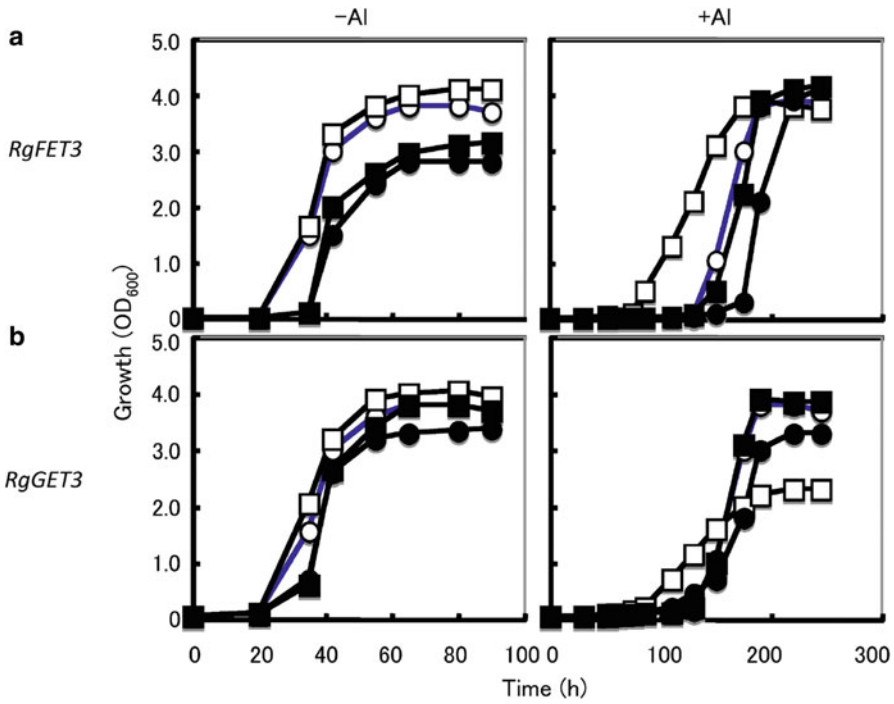
Three genes probably related to cation homeostasis are involved in adaptive acquirement of novel heritable aluminum (Al) resistance in *Rhodotorula glutinis* IFO1125.

So far, no nutrient role is known for aluminum (Al), which has toxic effects not only on plants and microorganisms, but also on human beings. The strong toxicity of Al is caused by inorganic monomeric ion (Al^{3+}, $Al(OH)^{2+}$, and $(Al(OH)_2^{+})$, especially Al^{3+}, which is maintained at low pH below 4.5, but at higher pH, it is gradually converted to harmless forms. In what follows, Al toxicity is associated with Al^{3+} below pH 4.0. The acidification of soil causes the dissolution of Al salts from soils and even a micromolar range of cationic Al ions shows severe inhibition of plant growth. Resistant plant species exist and, in some, the lowering of toxicity is caused by complexation of Al with organic acids originating from the metabolic pathways. Since the acidification of soil and concomitant dissolution of Al causes serious agricultural and environmental problems, extensive studies have been carried out on the mechanism of plant resistance to Al. On the other hand, screening of

highly Al-resistant microorganisms resulted in isolation of super-resistant microorganisms, belonging to fungi, *Aspergillus flavus*, *Penicillium* sp., *Penicillium janthinellum*, and *Trichoderma asperellum* (Kawai et al. 2000). Usually such filamentous fungi are known to resistant to acidic pH and Al. The other isolates belong to yeasts, *Rhodotorula glutinis* and *Cryptococcus humicola*. Since yeasts of high resistance to Al are not known, we focused on one of the isolates, *Rhodotorula glutinis* Y-2a. Strain Y-2A was resistant up to 200 mM Al, the concentration of which does not occur in natural environments. A normal counterpart *R. glutinis* in culture collections (IFO1125) was, however, sensitive to Al toxicity, and it showed retarded growth in the presence of 50–100 μM Al in liquid culture. Curiously, supplementation of Al resulted in longer lag phase, but not in decreased growth rate. Namely, when the growth started in the presence of Al after long lag phase, the growth ratio was comparable to that in the absence of Al. It seemed that the strain adapted to Al during the lag phase. When the strain was pre-grown in liquid culture in the absence of Al and spread onto Al-containing agar plates, colony-forming unit (CFU) varied depending on Al concentration, but the colonies once appeared on Al-containing plate showed variable CFU to Al concentrations added to agar plates. These results suggested that the resistance obtained by one round of Al treatment was somewhat unstable, and that this resistance cannot be explained by simple mutation. When the strain was cultivated in the presence of Al at more than 50 μM repeatedly by ten times in liquid culture, the acquired resistance was heritable and stable, which was the first report on the novel adaptive Al resistance (Tani et al. 2004). In addition, when the stable resistant cells were repeatedly exposed to stepwise increments in Al concentration, the resistant level increased up to more than 5 mM. The growth yield of resistant strains decreased as their resistance increased. It is known that Al ions inhibit Mg-uptake systems, but resistant cells obtained the improved Mg-uptake system under Al stress, since they could grow in the presence of Al and low Mg. When the wild type was repeatedly treated by Cu, heritable and stable Cu resistance (50 μM to 1.6 mM) similar to Al resistance was acquired, but the Cu-resistant cells did not show Al resistance and vice versa. These results suggested that the underlying resistance mechanisms of Al and Cu resistance are different, but the strain is capable of adapting to such increased metal stress.

The crucial role of mitochondrial regulation in adaptive Al resistance was found (Tani et al. 2008). Resistant cells contained Al when grown in the presence of Al, but the content of which was significantly low compared to Al added to the medium, suggesting that the cells have a barrier to Al. The concentration and ionic form of Al (Al^{3+}) in the media did not change during the cultivation, suggesting that the cells neither chelate Al^{3+} nor make a complex with it to detoxify. Transmission electron microscopic analyses revealed a greater number of mitochondria in resistant cells. The formation of small mitochondria with simplified cristae structures was observed in the wild type strain grown in the presence of Al and in resistant cells grown in the absence of Al. Addition of Al to cells resulted in high mitochondrial membrane potential and concomitant generation of reactive oxygen species (ROS). Exposure to Al also resulted in elevated levels of oxidized cellular proteins and lipids. Addition of the antioxidants such as α-tocopherol and ascorbic acid alleviated the Al toxicity. These results suggested that ROS generation is the main cause of Al toxicity. Differential display analysis indicated upregulation of mitochondrial genes in the resistant cells. Resistant cells were found to have 2.5- to 3-fold more mitochondrial DNA (mtDNA) than the wild type strain. Analysis of tricarboxylic acid cycle and respiratory-chain enzyme activities in wild type and resistant cells revealed significantly reduced cytochrome *c* oxidase activity and resultant high ROS production in the resistant cells. The adaptive increased Al resistance resulted from an increased number of mitochondria and increased mtDNA content, which is a compensatory response to reduced respiratory activity caused by a deficiency in complex IV function.

Three nuclear genes (*RgFET3, RgGET3* and *RgCMK*) were also found to be upregulated in the resistant cells. They code for proteins homologous to *Saccharomyces cerevisiae* FET3p (ferrooxidase), GET3p (guanine nucleotide exchange factor for Gpa1P), and CMK1p and CMK2p (calmodulin-dependent protein kinases), respectively. Their expression was promoted in the presence of Al. These three genes were cloned from the wild type *R. glutinis* and introduced into *S. cerevisiae* BY4741 and its derivatives (*fet3*Δ, *get3*Δ, *cmk*Δ and *cmk2*Δ) (Tani et al. 2010).



Aluminum, Genes Involved in Novel Adaptive Resistance in *Rhodotorula glutinis*, Fig. 1 Growth of *S. cerevisiae* and its transformants with *RgFET3* (*top panel*) and *RgGET3* (*lower panel*). The wild type *S. cerevisiae* and its derivatives *fet3*Δ and *get3*Δ were grown in the absence (*left panels*) and presence (*right panels*) of Al (50 μM). Open symbols, wild type *S. cerevisiae;* closed symbols, mutant derivatives; *circles*, vector control (*p*YES2); *squares*, transformants (Cited from Tani et al. 2010)

The introduction of *RgFET3* improved the growth of the wild type and *fet3*Δ of *S. cerevisiae* in the presence of Al (Fig. 1a). This result indicated that RgFet3p functioned as high-affinity Fe uptake system as Fet3p (Stearman et al. 1996). Fet3p receives copper in a late or post-Golgi compartment into which Ccc2p, a P-type copper-translocating ATPase, and Gef1p, CLC chloride-transport protein, supply copper ions. CCC2 expression is regulated by iron and Gef1p is involved in cellular cation homeostasis. The RgFet3p-GFP protein is localized at the cell periphery in *S. cerevisiae*. The supplementation of Fe (III) promoted *R. glutinis* growth under Al stress. From these results, the role of RgFet3p in *R. glutinis* would be iron uptake, and the high expression level in resistant cells under Al stress would reflect cellular demand for iron. This is in accordance with the idea that iron ion should be of great importance for its mitochondrial activity under Al stress and that iron competes with Al. Thus, RgFet3p from *R. glutinis* is considered to have relevance to Al resistance through the cellular Fe requirement (Fig. 1a).

RgGet3p is similar to bacterial ArsA ATPase, and *GET3* mutant of *S. cerevisiae* was found to be sensitive to various metal ions and temperature. Later, it was shown that Get3p is involved in Golgi-to-ER trafficking (Schuldiner et al. 2005). Get3p binds to Gef1p, which is required for efficient copper supply to the Golgi, and cytosolic copper supports this interaction. Furthermore, in *gef1*Δ, Fet3p does not mature normally because of copper shortage. In addition, genes involved in the retrograde transport (*COG6, COG8, RGP1, RIC1, TLG2* and *YPT6*) were identified as Al-tolerance genes (Kakimoto et al. 2005), with which Get3p interacts in *S. cerevisiae*. Thus, Get3p is important for copper loading to Golgi, and copper is necessary for Fet3p maturation. *RgGET3* complemented the slow growth rate of *get3Δ* under Al (Fig. 1b). The RgGet3p had a tendency to form punctate bodies in the cytosol under Al stress, which was very similar to the localization of Get3p in *S. cerevisiae*. These results suggested that RgGet3p functions as Get3p. Thus, RgGet3p was considered to localize at Golgi-ER trafficking, which is important for Al tolerance. RgGet3p may enable copper loading to RgFet3p, through as yet unidentified Gef1 homologue.

The roles of calmodulin-dependent protein kinases (Cmk1p and Cmk2p) in *S. cerevisiae* have not been fully understood yet. They are considered to be

involved in stress responses for weak organic acids such as sorbate or benzoate, and in the acquisition of thermotolerance. The wild type yeast shows growth retardation followed by adaptation to weak organic acids, but *cmk1* cells are resistant. The wild type yeast can tolerate exposure to high temperature, but *cmk1*Δ mutants show decreased levels of induced thermotolerance. Cmk2p becomes independent of Ca^{2+} and calmodulin via autophosphorylation, but Cmk1p does not. *CMK2* is induced in a calcineurin-dependent manner. Calcineurin controls cell wall biosynthesis and cation homeostasis. One of the phosphorylation target of calcineurin is Crz1p, whose binding site is 5′-GNGGC(G/T)CA-3′. Interestingly, in *C. neoformans*, calcineurin activity is dependent on cyclophilin A, and cyclophilin A is required for laccase (Fet3p homologue) and is also the target of cyclosporin A. Furthermore, calmodulin from chicken was shown to bind aluminum. *RgCMK* is induced in both the wild type and resistant cells of *R. glutinis* in the presence of Al. *S. cerevisiae cmk1*Δ and *cmk2*Δ mutants exhibited no clear deficiency in growth under Al stress, and introduction of *RgCMK* into them did not show any distinct effect. But the response to calcium ion and cyclosporin A was clearly different between the wild type and the resistant cells of *R. glutinis*. These results suggested altered Ca ion homeostasis in resistant cells and the involvement of calmodulin-dependent signaling pathway in Al resistance. The adaptation of *Rhodotorula* cells to Al necessitates repeated cultivation and its resistance was not stable in earlier treatment. Such behavior of tolerance is quite similar to the induced thermotolerance and adaptation to weak organic acids seen in *S. cerevisiae*, which is mediated by calmodulin-dependent kinases. The complete growth inhibition caused by cyclosporin A in the wild type and no effect in the resistant cells in the presence of Al will be the key to understand the Ca-mediated mechanism of adaptive Al resistance, and this difference may further be linked to RgFet3p again through cyclophilin A. The consensus sequence for Crz1p binding was found in the promoter region for *RgFET3* (5′-GAGGCGCA-3′, 169 bp upstream from transcription initiation site) and *RgCMK* (5′-CTGGCGCA-3′ [possible] and 5′-GAGGCGGA-3′, 548 and 393 bp upstream, respectively). This also implies the link between RgCmkp and RgFet3p mediated by calcineurin, although FET3 is not the target of Crz1p in *S. cerevisiae*.

Thus, at least two genes (*RgFET3* and *RgGET3*) were considered to have relevance to Al resistance in *R. glutinis*. The relevance of these genes to Al resistance has not been reported previously. The relevance of *RgCmk* has not been clarified yet, but interestingly, RgGet3p and RgCmkp may have links to RgFet3p, through as yet unidentified mechanisms. Although these three genes were not recognized as Al-tolerance gene in the work of mutant screening of *S. cerevisiae*, RgGet3p and RgCmkp are supposed to interact with these tolerance genes directly or indirectly, which suggests the common tolerance mechanisms between *S. cerevisiae* and *R. glutinis*. The difference is that the tolerance level of *R. glutinis* became higher and higher by repetitive cultivation under increased concentration of Al. In the process of this adaptation, cellular cation homeostasis and the regulation of mitochondrial activity and amount have been considerably changed.

In conclusion, the novel adaptive Al resistance found in *R. glutinis* IFO1125 may be attributable to altered cation homeostasis and the regulation of mitochondrial activity and amount in the resistant cells. Besides, Mg-uptake system and calcium signaling probably play significant roles in this adaptation. The inference may be reasonable because Al competes with Mg, Fe, and Ca in various cellular systems. In other words, the microorganisms able to alter their cation homeostasis and mitochondrial regulation in response to Al may become resistant to increased Al stress. The novel adaptive Al resistance found in *R. glutinis* may be widespread in various yeasts, as yet undiscovered.

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Aluminum, Physical and Chemical Properties

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Aluminum is the most abundant metallic element in the earth's crust. It occurs in a variety of minerals combined with oxygen, silicon, the alkali and alkaline-earth metals, and fluorine, and as hydroxides, sulfates, and phosphates. Many applications of aluminum are based upon its low density, high electrical and thermal conductivities, and resistance to corrosion. Pure aluminum is soft but it can be alloyed with other elements to increase strength and impart a number of useful properties.

Physical Properties

Atomic number	13
Atomic weight	26.98
Relative abundance,%	8.13
Melting point, °C	660.5
Boiling point, °C	2,494
Heat of fusion, J/g	397
Heat of vaporization, kJ/g	10.8
Heat capacity, J g^{-1} K^{-1}	0.90
Density, g/cm^3	2.699
Density of liquid, g/cm^3	
at 700°C	2.357
at 900°C	2.304
Crystal structure	Face-centered cubic
Atomic diameter, m	2.86×10^{-10}
Lattice constant (length of unit cube) at 25°C, m	4.0496×10^{-10}
Coefficient of expansion at 20°C, K^{-1}	23×10^{-6}
Thermal conductivity at 25°C, W cm^{-1} K^{-1}	2.37
Electrical resistivity of pure aluminum at 25°C, μΩ·cm	2.5
Surface tension at melting point, N/cm	8.68×10^{-3}
Viscosity at melting point, Pa·s	0.0012
Magnetic susceptibility at 25°C, mm^{-3} mol^{-1}	16×10^{-3}
Thermal neutron cross section, cm^2	$(2.32 \pm 0.03) \times 10^{-25}$
Barn	0.232 ± 0.003
Nuclear magnetic moment, A·m^2	1.84×10^{-26}

(*continued*)

Chemical Properties

Aluminum is a typical metal in the sense that when it loses its three outermost electrons, it will have the electronic structure of an inert gas. Although it is one of the most reactive metals, it is widely used as a material of construction because of the continuous adherent thin oxide film that rapidly forms on its surface. Aluminum is used to store nitric acid, concentrated sulfuric acid, organic acids, and many other reagents. However, it dissolves in alkaline solutions:

$$2Al + 2OH^- + 6H_2O \rightarrow 2\left[Al(OH)_4\right]^- + 3H_2$$

Aluminum is amphoteric; it reacts with mineral acids to form soluble salts with hydrogen evolution:

$$2Al + 6H^+ \rightarrow 2Al^{3+} + 3H_2$$

Aluminum powder reduces many oxides when heated to produce metals and alloys:

$$3MO + 2Al \rightarrow 3M + Al_2O_3$$

where M is a divalent metal.

All aluminum compounds are colorless. Alumina is a major aluminum compound produced mainly from bauxite. It is the starting material for producing the metal by fused salt electrolysis. It is also used as a catalyst, as an adsorbent, as abrasive, in ceramics, etc. Aluminum sulfate is an important flocculating agent for purifying water. Anhydrous aluminum

chloride is an important *Friedel–Crafts catalyst* in the chemical and petrochemical industries. Aluminum compounds are considered nontoxic.

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Aluminum-Resistant Genes Related to Cation Homeostasis

▶ Aluminum, Genes Involved in Novel Adaptive Resistance in *Rhodotorula glutinis*

Amine N-Ethylcarbamoylborane

▶ Amine-Boranes

Amine Oxidase (Copper-Containing)

▶ Copper Amine Oxidase

Amine-Boranes

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Synonyms

Amine *N*-ethylcarbamoylborane; Amine-carbamoylborane; Amine-carbomethoxyborane; Amine-carboxyborane; Amine-cyanoborane; Borane; Borano-amine; Boronated amine

Definition

Amine-boranes are complexes of a Lewis acid borane (e.g., BH_3, BH_2CN, BH_2COOH, BH_2COOCH_3, $BH_2CONHCH_2CH_3$) and an amine (e.g., R,R′,R″N) where the lone electron pair on the nitrogen atom forms a coordinate covalent bond by donating both of its electrons into the vacant orbital of borane.

Introduction

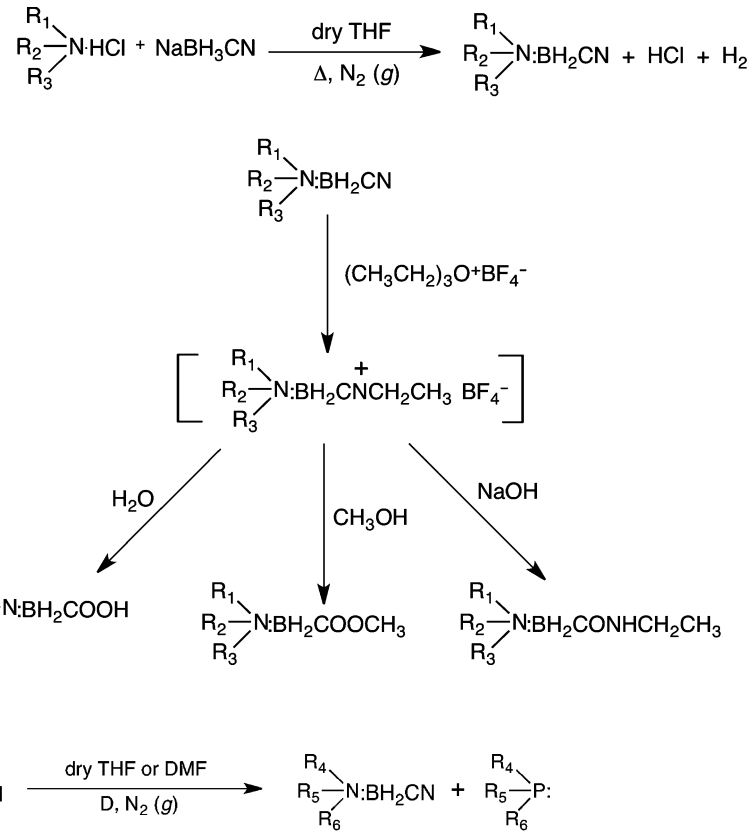
Chemistry of Boron

Boron is in column three of the periodic table and has three valence electrons. This leaves the boron atom with a vacant orbital capable of accepting a pair of electrons acting as a Lewis acid. Typically, molecules with atoms containing lone pairs of electrons will form complexes with boron such as nitrogen, oxygen, and phosphorus (Spielvogel 1988).

When coordinated to amino groups, the borane becomes tetrahedral making it isosteric to a tetrahedral carbon atom. Since the borane is more lipophilic, this type of isosteric replacement is attractive in the design of therapeutic agents since this property enhances their ability to cross cell membranes (Spielvogel 1988).

Synthesis

The most common borane functionalities present in the pharmacologically active compounds are borane (BH_3), cyanoborane (BH_2CN), carboxyborane (BH_2COOH), carbomethoxyborane (BH_2COOCH_3), and *N*-ethyl carbamoylborane ($BH_2CONHCH_2CH_3$). The cyanoborane can be conveniently prepared by two methods, see Figs. 1 and 2: (1) by refluxing the amine hydrochloride and sodium cyanoborohydride in an inert solvent such as tetrahydrofuran (THF) or (2) via a Lewis acid exchange reaction, either by using equimolar or excess amounts of a cyanoborane and the amine heated to at least 60–70°C in an inert solvent. In the Lewis acid exchange reaction, a weakly basic or bulky amine or phosphine, as its substituted borane adduct, is exchanged for a more basic or less bulky amine. The Lewis acid exchange reactions must be carried out under anhydrous conditions since water would complex with the borane. The Lewis acid exchange method is also a general route, which has also been used in the preparation of other borane



Amine-Boranes, Fig. 1 Synthesis of the cyano-, carboxy-, and *N*-ethylcarbamoylborane adducts of amines

Amine-Boranes, Fig. 2 Synthesis of the cyanoborane adducts of amines via Lewis acid exchange

adducts (e.g., BH_3 and BH_2COOH). Subsequently, the cyanoborane can be converted to the carboxyborane through the formation of the nitrilium salt by refluxing the cyanoborane with triethyloxonium tetrafluoroborate in dichloromethane. Then, addition of water hydrolyzes the nitrilium salt to the carboxyborane. The nitrilium salt can alternatively be treated with methanol to yield the carbomethoxyborane or with a hydroxide base to yield the *N*-ethylcarbamoylborane. Standard coupling conditions (e.g., a carbodiimide with triethylamine or CCl_4 with Ph_3P) have also been used to prepare the ester and amide derivatives from the carboxyborane (Burnham 2005).

The aforementioned isosteric replacement makes amine-boranes attractive functional groups in designing drugs, and such compounds have demonstrated anticancer, antiviral, hypolipidemic, and anti-inflammatory activities (Burnham 2005). One of the reasons amine-boranes were investigated as therapeutic agents was their potential use in boron neutron capture therapy (BNCT), which is a treatment for inoperable tumors. BNCT is based on the principle that ^{10}B, when exposed to thermal neutrons, will split and therefore emit locally ionizing radiation. The natural abundance of the stable ^{10}B isotope is 19.8%. When a ^{10}B atom is bombarded with low kinetic energy thermal neutrons of approximately 0.025 eV, it splits into ^{7}Li and ^{4}He (α-particles). In 94% of the neutron captures, a 0.48-MeV gamma ray is emitted, and the remaining 2.31 MeV is the average kinetic energy between the ^{7}Li (0.84 MeV) and the ^{4}He particles (1.46 MeV). The path length of the ^{7}Li and ^{4}He particles produced in situ in tissue are 5 and 9 μm, respectively, which is less than the diameter of a mammalian cell (~10 μm). Therefore, this process can selectively deliver high-energy ionizing radiation to a single cell while minimizing collateral damage to the surrounding cells that do not contain ^{10}B atoms. For BNCT to achieve therapeutic success, the boronated compounds must be selectively distributed into the neoplastic tissue. It has been determined that

5–30 μg ^{10}B per gram of tissue is needed for effective neutron capture and that the response is enhanced twofold if the ^{10}B is localized in the cell nucleus (Perks et al. 1988).

Amino Acid Derivatives

Anticancer Activity

An initial strategy toward employing amine-borane isosteres was the synthesis of analogues of the amino acids glycine (H_2NCH_2COOH) or betaine ($(CH_3)_3N^+CH_2COO^-$) where the amino methylene (H_2N-CH_2- or $(CH_3)_3N^+CH_2$-) would be replaced by an amine-borane (H_2N:BH_2- or $(CH_3)_3N$:BH_2-) (Hall et al. 1979). These derivatives of amino acids were designed to inhibit protein biosynthesis or other cell processes utilizing glycine or betaine, hence stopping cell replication. The most potent of the early series was trimethylamine-cyanoborane ($(CH_3)_3N$:BH_2CN). However, it was quite toxic to normal mice with an LD_{50} of 70 mg/kg, I.P. Of the series of aliphatic amine-borane, amine-cyanoborane, amine-carboxyborane, amine-carbomethoxyborane, and amine *N*-ethylcarbamoylborane, trimethylamine-carboxyborane proved to be the most potent and least toxic of the compounds synthesized. The substitution of the methylene for the borane resulted in an increase in the pK_a of the carboxylic acid group from 1.83 to 8.14–8.38 making it the weakest known simple carboxylic acid of zero net charge further illustrating the enhanced lipophilicity of the amine-borane derivatives (Spielvogel 1988). Trimethylamine-carboxyborane demonstrated in vitro cytotoxicity against suspended murine and human-cultured cells including L_{1210}, Tmolt^3, and HeLa-S^3 cell lines. An in vivo Ehrlich ascites antitumor screen in CF_1 mice demonstrated that Me_3N:BH_2CO_2H inhibited tumor growth of 82% at 20 mg/kg/day, I.P. Mixed Walker-246 carcinosarcoma ascitic tumor showed a treated-to-control (T/C) value of 174% at 2.5 mg/kg/day, I.P. Some activity was observed in the B-16 melanoma with a T/C value of 134%, and in the Lewis lung, with a T/C value of 144% at 20 mg/kg/day, I.P., in C_{57}BL/6 mice. No acute toxicity was observed in mice at 100 mg/kg/day, I.P., and the LD_{50} value in CF_1 mice was determined to be 1,800 mg/kg, I.P. In L_{1210} cell culture, the inhibition of DNA, RNA, and protein syntheses by Me_3N:BH_2CO_2H occurred at 1, 2, and 3 times the ED_{50} value after incubation for 60 min. Mode of action (MOA) studies in L_{1210} cells showed that the decrease in DNA synthesis occurred due to inhibition of the following enzyme activities: thymidine kinase, TDP kinase, PRPP amidotransferase, IMP dehydrogenase, dihydrofolate reductase, and ribonucleotide reductase. The combined effects of inhibiting regulatory pathways in de novo purine biosynthesis (PRPP amidotransferase, IMP dehydrogenase, and dihydrofolate reductase), together with inhibition of the activities of thymidine kinases and ribonucleotide reductase, will lead to the overall inhibition of DNA synthesis (Hall et al. 1990). The amine-carboxyborane did demonstrate an inhibition of the phosphorylation of topoisomerase II by protein kinase C (PKC) resulting in decreased activity of topoisomerase II, which results in DNA strand scission after 8 h. The inhibition of PKC phosphorylation was shown to be mediated in a similar manner to TNF-α since $Me_3NBH_2CO_2H$ was shown to competitively bind with [^{14}C]-TNF-α to high-affinity TNF-a receptors on L_{1210} and L_{929} cells (Miller et al. 1998). In vivo boron distribution studies with Me_3N:BH_2CO_2H, in which the agent was administered at an I.P. dose of 40 mg ^{n}B/kg to BALB mice with Harding-Passey melanoma, demonstrated tumor boron concentrations of 45.7 and 17.5 μg ^{n}B/g of tissue. These concentrations are adequate for BNCT, but the tumor:blood and tumor:brain ratios were only ~1:1, which is not sufficient selectivity for BNCT use (Spielvogel 1988).

Hypolipidemic Activity

A number of the amine-borane amino acid analogues were shown to have inhibitory activity against HMG-CoA reductase, the rate-limiting enzyme in de novo cholesterol biosynthesis. The inhibition of HMG-CoA reductase is a target for the statins (e.g., atorvastatin, simvastatin, etc.), which are clinically used to lower cholesterol concentrations in the blood. It was found that amine-boranes were found to lower serum lipid in rodents. Of the amino acid derivatives, the most effective agents were trimethylamine-carboxyborane, trimethylamine-carbomethoxyborane, trimethylamine-*N*-*n*-octyl-carbamoylborane, and *N*, *N*-dimethyl-*N*-octadecylamine-borane. The most potent agent was the methyl ester trimethylamine-carbomethoxyborane, which lowered serum cholesterol and triglyceride levels to 44% and 77% of control

values, respectively, in mice at a dose of 10 mg/kg/day, I.P. In vitro MOA studies on CF_1 mouse liver homogenates revealed that this compound inhibited the activities of *sn*-glycerol-3-phosphate acyl transferase, ATP-dependent citrate lyase, and acyl-CoA cholesterol acyl transferase. In studies on lipid levels of serum lipoproteins from rats, cholesterol and triglyceride levels were reduced in the chylomicron, VLDL, and LDL fractions. Importantly, the largest reduction occurred in the LDL fraction. In this fraction, cholesterol levels were decreased to 57% of control values; triglyceride levels were decreased to 44% of control values. Also, this carbomethoxyborane adduct increased serum HDL cholesterol levels by 95% (Hall et al. 1987).

Anti-inflammatory Activity

Potent anti-inflammatory activity was observed in rodents for the glycine and betaine analogues of amine-boranes including trimethylamine-borane, trimethylamine-cyanoborane, and ammonia-cyanoborane were potent anti-inflammatory agents in rodents. These agents demonstrated activity in the carrageenan-induced edema of mouse foot pads (Winter's test), writhing reflex, and rat chronic adjuvant arthritis screen. Key enzymes involved in inflammation whose activities were inhibited included acid phosphatase, cathepsin, and prostaglandin synthetase. The derivatives with the best activities were ammonia-cyanoborane and trimethylamine-cyanoborane; however, those compounds were quite toxic with LD_{50} values of 30 and 70 mg/kg, I.P., respectively, in mice (Hall et al. 1980).

Amine-Borane Derivatives of Peptides

The logical extension of amino acid analogues was the use of the amine-borane adducts to prepare peptides. Di- and tripeptides with $Me_3N{:}BH_2CO_2H$ or $H_3N{:}BH_2CO_2H$ at the *N*-terminus have been prepared using standard carbodiimide coupling procedures (Sood et al. 1990). These peptides showed weaker antineoplastic activity than the previously discussed α-amino acid analogues. The dipeptides' cytotoxic effects were due to the inhibition of DNA synthesis, primarily affecting de novo purine biosynthesis inhibiting the activities of PRPP amidotransferase and IMP dehydrogenase.

Boron-containing dipeptides also demonstrated hypolipidemic activity in mice (Sood et al. 1990). The active peptides were L-serine or L-leucine coupled to trimethylamine-carboxyborane as the *N*-terminus (Miller et al. 1999).

Dipeptides in the class mentioned above demonstrated anti-inflammatory activity but were less potent than the simple amino acid analogues. The more potent derivatives were those containing a tyrosine or tryptophan at the C-terminus (Miller et al. 1999).

Amine-Borane Derivatives of Proteins

Early attempts to attach *p*-boronophenylalanine to monoclonal antibodies (mAb) specific for tumor cells were successful but did not yield a high enough concentration of ^{10}B. Borane cage compounds ($B_{10}H_{10}$) have been attached to mAb's yet rendered the mAb ineffective. A strategy to use the Merrifield solid-phase peptide synthesis was successful in polymerizing *closo* and *nido* borane cages with subsequent coupling to a mAb. Again, while the chemical synthesis was successful, the immunogenicity on the mAb was lost. Another method employed was boronating a DL-polylysine polymer with trimethylamino-octahydrodecaborane via an isocyanato linkage. These conjugated mAb did retain 40% to 90% of their immunogenicity; however, the boronated mAb had increased liver uptake over the nonboronated mAb (Soloway et al. 1993).

Other Biomolecule Amine-Borane Derivatives

Since amino acids are used as building blocks for other biomolecules, amine-cyano-, carboxy-, carbomethoxy-, and carbamoylborane adducts of simple heterocyclic amines (e.g., morpholine, piperidine, piperazine, and imidazole) and nucleosides have been synthesized and evaluated for pharmacological activity. The most potent compounds in vitro and in vivo were the heterocycles piperidine-carboxyborane and *N*-methylmorpholine-carboxyborane, the nucleosides 2′-deoxycytidine-N^3-cyanoborane and 3′,5′-*O-bis* (triisopropylsilyl)-2′-deoxyguanosine-N^7-cyanoborane, and the deprotected 2′-deoxyguanosine-N^7-cyanoborane. The heterocyclic compounds as well

as the nucleosides targeted DNA synthesis and de novo purine biosynthesis at PRPP amidotransferase and IMP dehydrogenase. 2′-Deoxycytidine-N^3-cyanoborane showed increased uptake in vitro in Tmolt3 cells over Bg-9 fibroblast cells. However, in vitro BNCT studies with V-79 Chinese hamster cells showed no uptake of the unlabelled compound into the cells at concentrations up to 100 *mM* (Spielvogel et al. 1992).

Summary

Borane (BH_3) and its Lewis acid derivatives (BH_2CN, BH_2COOH, BH_2COOCH_3, and $BH_2CONHC_2H_5$) can form adducts with amines forming an amine-borane where the B–N bond is a coordinate covalent bond. Such compounds can be easily synthesized from $NaBH_3CN$ and Lewis acid exchange reactions. Amine-borane adducts resembling the amino acids glycine and betaine demonstrated anticancer activity as well as hypolipidemic activity. Thus far, their use in BNCT has not been established due to either low or nonselective tumor cell uptake. Some anti-inflammatory effects were observed but for the more toxic cyanoborane adducts.

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Amine-Carbamoylborane

▶ Amine-Boranes

Amine-Carbomethoxyborane

▶ Amine-Boranes

Amine-Carboxyborane

▶ Amine-Boranes

Amine-Cyanoborane

▶ Amine-Boranes

Amyotrophic Lateral Sclerosis

▶ Copper-Zinc Superoxide Dismutase and Lou Gehrig's Disease

Anaerobic Prokaryotes

▶ Zinc and Iron, Gamma and Beta Class, Carbonic Anhydrases of Domain Archaea

Analytical and High Resolution Electron Microscopy

▶ Palladium, Colloidal Nanoparticles in Electron Microscopy

ANCA

▶ Silicon Exposure and Vasculitis

Anemia

▶ Iron Homeostasis in Health and Disease

Angiotensin I-Converting Enzyme

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Synonyms

Carboxycathepsin; Dipeptide hydrolase and peptidase P; Dipeptidylcarboxypeptidase I; Kininase II

Definition

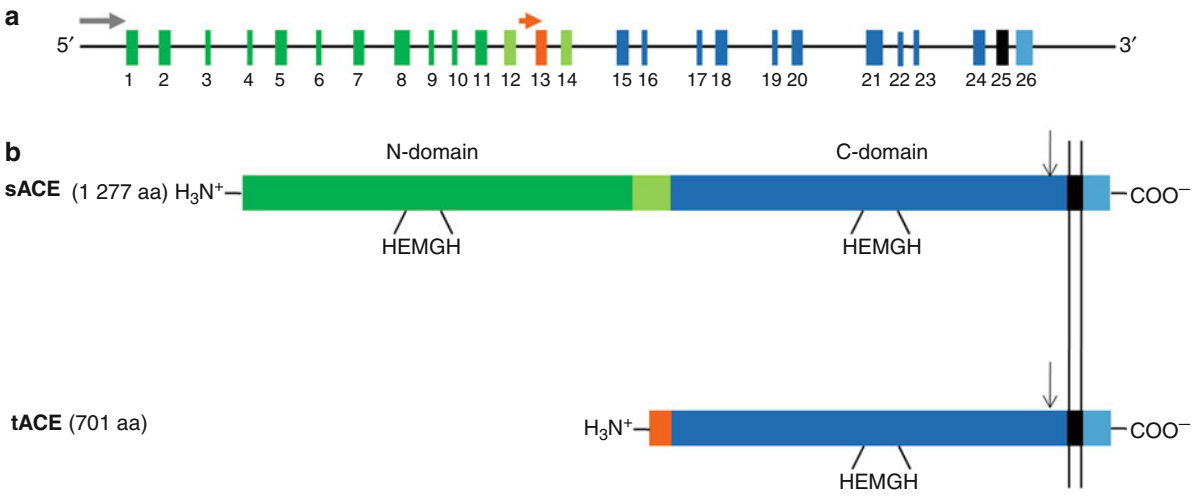
Angiotensin-converting enzyme (ACE) is a metallopeptidase that utilizes a zinc ion as a crucial cofactor in its catalytic mechanism. ACE has wide human tissue distribution and plays a key role in the regulation of blood pressure, cardiovascular function, electrolyte balance, hematology, and tissue fibrosis. Potent inhibitors of ACE have been developed and are clinically useful in the treatment of hypertension, post-myocardial infarction and diabetic nephropathy.

Background

ACE is a zinc metallopeptidase that is responsible for the hydrolysis of the penultimate peptide bond of several substrates of physiological consequence. This dipeptidyl peptidase is best known for its conversion of inactive decapeptide angiotensin I to active octapeptide hormone angiotensin II, leading to sodium retention, aldosterone release, and ultimately vasoconstriction. The central role that ACE plays in blood pressure regulation emphasizes both the enzyme's physiological importance and its therapeutic importance as a target for the treatment of cardiovascular disease.

ACE is widely expressed in many human tissues, especially on vascular endothelial cells. It is anchored to the cell surface by means of a single hydrophobic transmembrane region. While ACE is targeted for extracellular surface localization, a region proximal to the transmembrane region can be proteolytically cleaved and therefore result in a soluble form of the protein. This posttranslational processing is generally referred to as "ectodomain shedding." Two isoforms of ACE exist: a widely expressed somatic form (somatic ACE) and a truncated form, testis ACE, that is expressed in male germinal cells and plays an important role in fertility.

The *Ace* gene is located on the 17q23 locus and is 21 kilobases in length with 26 variably sized exons. Both ACE isoforms are the products of the same gene, with the truncated testis ACE arising from a testis-specific intronic promoter. Thus, testis ACE is approximately half the size of somatic ACE. Somatic ACE is expressed as a 1, 277 amino acid mature protein while testis ACE is 701 amino acids in length. Molecular cloning of the somatic isoform revealed an unusual feature for enzymes: The enzyme consisted of two homologous domains (designated N- and C-domains depending on their location on the polypeptide chain), each containing a fully functional active site. In contrast, testis ACE contained only one domain and was identical to the C-domain of somatic ACE with the exception of a 36-amino acid region at the N-terminus (Fig. 1). Comparison of the two somatic ACE domains reveals high sequence identity between the two domains (approximately 60% identity). Analysis of residues present in the active sites shows even higher position similarity (approximately 90% identity). Elucidation of the three-dimensional



Angiotensin I-Converting Enzyme, Fig. 1 Schematic representation of the Ace gene and the two isoform products. (**a**) The *Ace* gene consists of 26 exons all of which are transcribed for somatic ACE with the exception of exon 13. The promoter for somatic ACE expression is indicated as a *gray arrow*. Testis ACE arises from the presence of a germinal cell–specific promoter in intron 12 (*orange arrow*) and translation of exon 13 results in a unique 36 amino acid N-terminus of testis ACE whereas the remainder of the protein is identical to the C-domain of sACE. (**b**) Somatic ACE N- and C-domains with catalytic HEMGH motifs are shown in *green* and *blue*, respectively, the inter-domain linker region in *light green*, transmembrane region in *black*, and cytoplasmic C-terminus in *light blue*. The *black arrow* indicates the approximate cleavage site of ACE to be solubilized into the surrounding medium

structures of each domain has further revealed highly conserved structural topology between the two domains.

Another noteworthy feature of ACE biochemistry is the presence of glycosylation (carbohydrate or glycan chains) on the protein surface. The presence of these glycans is important for proper folding of the protein and thus key in the production of active enzyme (Acharya et al. 2003).

Biological Function

ACE is one of the central proteases in the renin-angiotensin-aldosterone system (RAAS), an important system for blood pressure regulation and electrolyte homeostasis. In the classical linear system, 55-kDa plasma protein angiotensinogen is N-terminally cleaved by the aspartyl protease renin to yield angiotensin I. ACE is then responsible for the conversion of this inactive decapeptide into the active octapeptide hormone by cleavage of the penultimate C-terminal peptide bond. The product of ACE hydrolysis, angiotensin II, mediates effects through the angiotensin receptor to induce a signaling cascade to ultimately increase sodium retention, aldosterone release, and vasoconstriction. While the classical system appears relatively simple, research performed in recent decades underlines the complexities of the RAAS (see (Fyhrquist and Saijonmaa 2008) for additional information). The physiological significance of ACE action is emphasized in studies involving transgenic mice. ACE null mice display low blood pressure, renal defects, anemia, reduced ability to concentrate urine, and decreased fertility. Overexpression of ACE in heart tissue specifically resulted in mice with atrial enlargement, cardiac arrhythmia, and a tendency to die of sudden cardiac death due to ventricular fibrillation (Bernstein et al. 2005).

In addition to angiotensin I hydrolysis, ACE is able to cleave a variety of physiologically relevant peptides. These include vasodilator peptides bradykinin, angiotensin1–7, and substance P as well as other non-vasoactive peptides such as amyloid β-peptide and anti-fibrotic peptide *N*-acetyl-Ser-Asp-Lys-Pro (AcSDKP) (Table 1). ACE can also operate outside of its classical dipeptidyl peptidase function in vitro. However, the in vivo actualities

Angiotensin I-Converting Enzyme, Table 1 An overview of selected ACE substrates. Cleavage sites by ACE are indicated by an arrow. The broken arrow indicates the sequential cleavage site after the removal of the first dipeptide

Substrate name	Biological action of substrate	Substrate peptide sequence and ACE cleavage sites	Biological action of product
Angiotensin I	Inactive	D – R – V – Y – I – H – P – F – H – L	Vasoconstriction Hypertrophy Fibrosis
Bradykinin	Vasodilation	R – P – P – G – F – S – P – F – R	Inactive
Angiotensin (1–7)	Vasodilation	D – R – V – Y – I – H – P	Inactive
Substance P	Vasodilation Pain response	R – P – K – P – Q – Q – F – F – G – L – M -NH_2	Inactive
Gonadotropin-releasing hormone	Sexual development	pyro-E – H – W – S – Y – G – L – R – P – G -NH_2	No in vivo data published
N-acetyl-SDKP	Anti-fibrosis	Ac-S – D – K – P	Inactive

of this property remain to be properly elucidated (Bernstein et al. 2011).

In addition to its role in hormone cleavage, ACE has also been shown to play a role in immunological function. Studies suggest that ACE has a physiological function in editing the carboxyl termini of proteasome produced major histocompatibility complex class 1 antigenic peptides (Shen et al. 2011). Further, modification of sACE domain activities results in marked changes of inflammatory cytokine levels, implying a function in cytokine regulation.

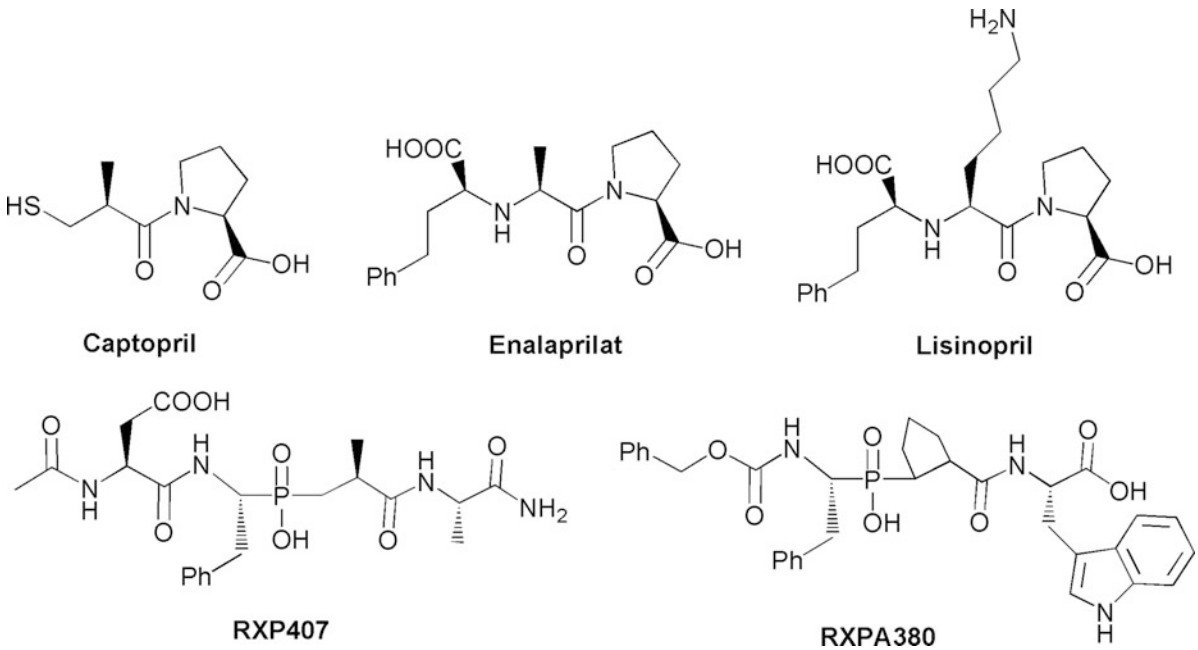
While the exact details of the mechanism of ACE substrate hydrolysis are not currently fully characterized, it is presumed to be a general base-type mechanism similar to thermolysin. The substrate is positioned and stabilized through interactions with residues in the enzyme active site (particularly with the C-terminal carboxylate of the substrate and Gln, Lys, and Tyr residues) and the peptide bond carbonyl oxygen coordinating the zinc ion. Proper positioning of the substrate displaces the zinc ion–associated water molecule, resulting in a nucleophilic attack of the carbonyl carbon of the peptide bond by water. After proton exchange, the resultant tetrahedral intermediate promptly collapses to form the product pair (Sturrock et al. 2004).

While ACE is classically a metalloenzyme, other works have shown it to play an interesting "non-catalytic" role. Binding of substrate and inhibitors tended to result in phosphorylation of the cytoplasmic tail of ACE which led to an induction of the JNK/c-Jun pathway. Other works have shown that angiotensin II binding specifically resulted in Ca^{2+} release while other inhibitors and substrates did not elicit this effect. Therefore, while ACE is popularly referred to as such due to its documented conversion of angiotensin I, ACE has the ability not only to process other physiologically relevant substrates but to function in a receptor-like role as well (Lambert et al. 2010).

Domain Substrate Specificities

Somatic ACE contains two homologous domains that have high sequence identity and conserved structural topology between their active sites. Despite this marked similarity, the two domains show different in vivo substrate cleavage abilities. As examples, the C-domain has been shown to be the prominent site for the conversion of angiotensin I. Meanwhile, the N-domain is the major domain for the cleavage of anti-fibrotic peptide AcSDKP. Both domains cleave bradykinin with similar efficiencies (Bernstein et al. 2011).

The two domains, while having discreet substrate cleavage abilities, do not operate in a completely independent manner. Biochemical analysis indicates that in an in vitro system, the domains tend to exhibit a negative cooperative effect on the other domain. That is, the domains in isolation have improved



Angiotensin I-Converting Enzyme, Fig. 2 Chemical structures of a selection of ACE inhibitors. Captopril, enalaprilat, and lisinopril are clinically approved ACE inhibitors for the treatment of hypertension, heart failure, and diabetic nephropathy. RXP407 and RXPA380 are phosphinic peptidomimetic inhibitors that are selective for the N- and C-domains, respectively

activities compared to the full-length somatic ACE. The total activity of somatic ACE is therefore approximately the mean of the two isolated domain activities. The exact physiological significance of this observation is not yet known.

ACE Inhibitors

With ACE playing a central role in cardiovascular functioning, it is perhaps not surprising that ACE inhibitors are successfully used in the treatment of hypertension, congestive heart failure, post-myocardial infarction, left ventricular dysfunction, and diabetic nephropathy. First-generation ACE inhibitors were based on bradykinin-potentiating peptides from the snake venom of *Bothrops jararaca* and, with modifications to the zinc-binding group (to contain a sulfhydryl as a binding group), led to approval of the first inhibitor captopril for clinical use in 1981. Since the finding that ACE inhibition significantly contributes to reduction of blood pressure in the human system, a total of 17 ACE inhibitors containing different zinc-binding groups and functionalities have been designed, synthesized, and approved for the clinic (Fig. 2) (Redelinghuys et al. 2005).

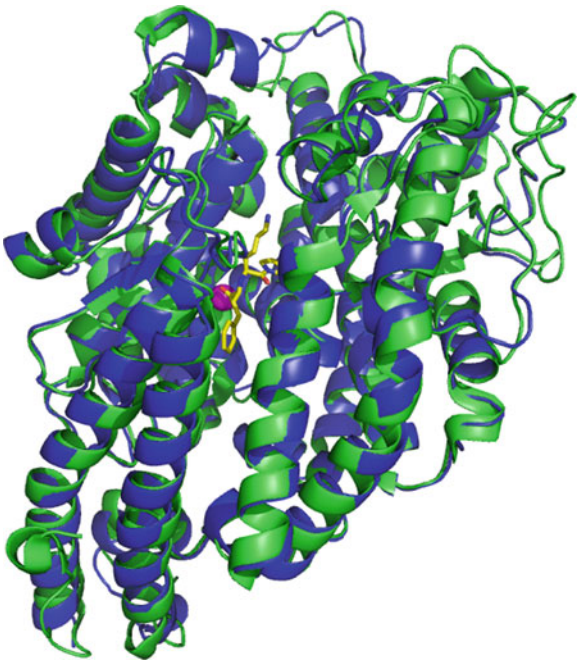
It is important to note that first-generation current clinical ACE inhibitors were designed with neither the knowledge of the two-domain ACE composition nor the crystal structures. Since these findings, research has been carried out, and continues to be, on attempting to develop inhibitors that are selective for one domain. Such efforts have resulted in the production of phosphinic peptidomimetic inhibitors RXP407 and RXPA380 that are selective for the N- and C-domains, respectively (Fig. 2). These developments are important since adverse drug events (such as persistent dry cough and angioedema) associated with clinical ACE inhibitor treatment are possibly due to elevated plasma bradykinin levels. Currently, all small molecule ACE inhibitors block both domains with approximately equal binding affinities. Thus, inhibitors that are selective for the C-domain could allow for treatment of blood pressure with lower elevations of bradykinin levels (and therefore reduced incidence of side effects), while N-domain-selective inhibitors

could result in the treatment of conditions related to tissue fibrosis due to AcSDKP buildup without affecting blood pressure regulation (Bernstein et al. 2011).

Three-Dimensional Structures of ACE

The overall structure of both the N- and C-domains of ACE represents an ellipsoid divided into two subdomains by a deep central cleft. The catalytic zinc-containing active site is located deep within this cleft, and investigations have shown that substrates could perhaps gain access through hinge movement by twisting the N- and C-termini of the protein. The structures are predominantly α-helical with only six β-sheets seen in the resolved structures. The determination of such structures has allowed for an understanding of ACE inhibitor binding at the atomic level and allowed researchers to identify obligatory binding sites within the active site (Fig. 3). Side chains of substrates or inhibitors have traditionally carried the nomenclature of $P_N \ldots P_2, P_1, P_1', P_2' \ldots P_N'$ based on the insertion and interaction with the corresponding $S_N \ldots S_2$, S_1, S_1', $S_2' \ldots S_N'$ subsites. The resolution of both domain structures with ACE inhibitor lisinopril revealed a very similar overall binding mode within the active sites. The inhibitors' carboxyl-alkyl group in both cases is coordinating the catalytic zinc ion. The P_1 phenylalanine extends into the corresponding S_1 subsite to interact with residues Thr496/Val518 of the N- and C-domains respectively, while the P_1' lysyl moiety interacts with unique C-domain residues Glu162 and Asp377 (replaced by Asp140 and Gln355 in the N-domain). In the S_2' subsite, the P_2' prolyl carboxylate has significant interactions with conserved residues Gln259/281, Lys489/511, and Tyr498/520 (N/C numbering) and these residues have been suggested as important for substrate and inhibitor positioning.

In addition to an appreciation of overall topology and inhibitor binding mode, these structures allowed for the identification of amino acids that were present in the active sites but differed in chemical nature between the N- and C-domains. This finding has been useful in assessment of the contribution of these unique amino acids to the selective binding of inhibitors and processing of specific substrates. In particular, residues Tyr369 and Arg381 in the N-domain (replaced by a Phe and Glu in the C-domain) appear to be important



Angiotensin I-Converting Enzyme, Fig. 3 Three-dimensional structures of the somatic ACE N- and C-domains. The N- (*green ribbon*) and C-domains (*blue ribbon*) display marked overall structural topology. Potent ACE inhibitor lisinopril and the catalytic zinc ion are shown in *yellow sticks* and *magenta sphere*, respectively. A deep central cleft can be noted down the center of the molecules resulting in two subdomains

in the selective binding of RXP407 while the S_2' subsite and Phe391 (replaced by more bulky hydrophilic residues and Tyr respectively in the N-domain) seem to be the major contributors toward the selective binding of RXPA380. The crystal structures have therefore provided important insights as to the structure-function relationship of this significant enzyme (Anthony et al. 2012, Watermeyer et al. 2010).

Concluding Remarks

ACE is an important metalloenzyme in its physiological contribution to cardiovascular function and is therefore a good drug target for the improvement of human health. Furthermore, the presence of two homologous catalytic domains that have different properties and play diverse functional roles in immunology, hematology, and fibrosis adds another dimension to the function of this ubiquitous enzyme. The role

of the zinc ion as crucial to the catalytic mechanism emphasizes the importance of metal ions in biological function and stresses the need for thorough understanding of the role of bioinorganics in health and disease.

Cross-References

- ▶ Angiotensin I-Converting Enzyme
- ▶ Thermolysin
- ▶ Zinc Carboxypeptidases

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Annexin

- ▶ Calcium-Binding Proteins, Overview

Annexins

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Synonyms

Calpactins; Chromobindins; Endonexins; Lipocortins

Definition

Calcium binding sites: Structural motifs, which enable the binding of a calcium molecule. Type I is defined as EF-hand motif, which consists of a 12-residue loop between two helices. Type II and type III binding sites describe structural propensities for calcium binding which do not correspond to the EF-hand motif and consist of shorter loops. The type II calcium binding site differs in its conformational contribution of individual or paired amino acids from the type III site, which is considered to be a "minor" calcium binding site.

Introduction

The annexins are a multigene family of Ca^{2+}-dependent membrane-binding proteins. They are structurally related and expressed in most phyla and species of eukaryotes. In vertebrates, 12 annexins are expressed (A1–11 and A13), displaying different splice versions (Gerke and Moss 2002). The annexins have been implicated in physiological processes, which are generally concerned with the regulation of plasma membrane organization and membrane-related signaling events, such as the control of vesicle trafficking during endo- and exocytosis (Raynal and Pollard 1994; Gerke and Moss 2002). In addition, they have been credited with extracellular functions, among them prominently featuring anticoagulative and anti-inflammatory properties (Dassah et al. 2008; Babbin et al. 2008).

In general, the annexins act as intracellular Ca^{2+} sensor/effector proteins; however, they have also been assigned Ca^{2+}-independent functions

(Raynal and Pollard 1994; Gerke and Moss 2002). In this entry, only the Ca^{2+}-dependent functions of this protein family will be considered: Intracellular interactions of the annexins which occur in a Ca^{2+}-independent way, as well as extracellular functions of annexins, which take place at saturating Ca^{2+} concentrations, are beyond the scope of this entry.

Annexins and Molecular Structure

The annexins share a core structure made up of four homologous domains, each of approximately 70 amino acids in length (except for annexin A6, which, as a result of gene duplication, possesses eight). Each of the four highly conserved domains consists of five α-helices, wound in a tight right-handed superhelix and connected by short loops (Raynal and Pollard 1994). In contrast, the NH_2-terminal domains of the annexins are highly variable and harbor phosphorylation sites, which are important for the interaction with other proteins (Gerke and Moss 2002). It is this variability which is thought to influence the specificity of individual annexins, whereas the conserved core domain unites the annexins as a family of structurally related proteins.

The annexin core shows both intramolecular and intermolecular homology (Gerke and Moss 2002) and harbors type II and type III Ca^{2+} binding sites. Originally identified by its resistance to proteolysis, the core forms a curved disk with a central hydrophobic pore. Ca^{2+} also coordinates carbonyl and carboxyl groups of the protein and phosphoryl groups at the glycerol backbone of membrane phospholipids. Annexin-membrane interactions occur via the formation of a ternary complex between the convex surface of the annexin core, Ca^{2+}, and the negatively charged phospholipids (rev by Gerke and Moss 2002). Ca^{2+} binding leads to the conformational changes in the core, particularly in repeat III (rev by Gerke and Moss 2002).

The Ca^{2+} Sensitivity of Annexins

Biochemical analysis in vitro revealed that the annexins have individual Ca^{2+} requirements for their interaction with negatively charged phospholipids (rev by Raynal and Pollard 1994; Monastyrskaya et al. 2009).

In vivo, at low $[Ca^{2+}]_i$ in resting cells, annexins are diffusely distributed throughout the cytoplasm. After stimulation, they translocate to the plasmalemma. Direct comparison of the Ca^{2+} sensitivity of several annexins by live cell imaging techniques established that annexin A2 shows the highest Ca^{2+} sensitivity of plasmalemmal translocation, followed by annexin A6, A4, and A1 (rev Monastyrskaya et al. 2009). Recent data have shown that the half maximal $[Ca^{2+}]_i$ required for annexin A2 translocation in vivo was 0.5 μM (Potez et al. 2011), whereas that of annexin A6 and annexin A1 was 5 and 10 μM, respectively (rev by Draeger et al. 2011).

Annexin A2 translocates to the plasma membrane in response to physiological stimuli, which lead to a transient Ca^{2+} release from intracellular stores (rev by Monastyrskaya, et al. 2009). Simultaneous Ca^{2+} and annexin imaging demonstrated that the duration and timing of this association precisely corresponded to the elevation in $[Ca^{2+}]_i$. Thus, it is highly likely that this molecule takes part in membrane-associated signaling processes. Annexin A2 is particularly abundant in endothelial and in smooth muscle cells, cell types which have a highly varied and intricate relationship with each other and with their surroundings (Babiychuk and Draeger 2000). Annexin A6 translocates to the plasmalemma only after a sustained influx of Ca^{2+}, such as is induced by the activation of store-operated channels during cellular stress responses (rev by Monastyrskaya et al. 2009). The expression of plasma membrane–anchored annexin A6 led to a downregulation of store-operated Ca^{2+} entry, implicating that this protein may play a role in the maintenance of intracellular Ca^{2+} homeostasis in the environmentally stressed cells (Monastyrskaya et al. 2009). Annexin A1 translocates to the plasmalemma only after a massive elevation in $[Ca^{2+}]_i$ such as occurs during plasmalemmal injury. Its role in the repair of plasmalemmal lesions caused either by mechanical damage or by pore-forming toxins has recently been established (rev by Draeger et al. 2011). Hence, the intracellular translocation of annexins might represent an important mechanism for the control of numerous physiological and pathological cellular functions.

For the investigator, annexins are useful tools which allow the monitoring of changes in $[Ca^{2+}]_i$. Especially in damaged cells, their differential Ca^{2+} sensitivity of membrane binding makes them more reliable Ca^{2+}

sensors than fluorescent Ca^{2+} dyes which diffuse out of the cytoplasm once the plasmalemma is perforated (rev by Draeger et al. 2011).

The annexins' Ca^{2+} sensitivity of membrane binding can be modulated via the NH_2-terminal interaction with other proteins (rev by Gerke and Moss 2002; Monastyrskaya et al. 2009). Especially well investigated in this respect are annexins A1 and A2. These annexins exist either as monomers or heterotetramers in which two molecules of annexin A1 are associated with two molecules of S100A11 (p10) or, in the case of annexin A2, with dimers of S100A10 (p11). NH_2-terminal cleavage of annexins A1 and A2 abrogates the formation of heterotetramers leading to opposite effects on Ca^{2+} sensitivity in annexin A1 and A2: It increases the Ca^{2+} sensitivity of annexin A1 and decreases that of annexin A2 (rev by Monastyrskaya et al. 2009). Binding to S100A10 has also been demonstrated to enhance annexin A2's properties to aggregate membranes at micromolar concentrations (rev by Gerke and Moss 2002; Monastyrskaya et al. 2009). Seven members of the S100 protein family (S100A1, S100A4, S100A6, S100A10, S100A11, S100A12, and S100B) are known to interact with at least one of the 12 annexin proteins (rev by Rintala-Dempsey et al. 2008). In addition, some S100 proteins, i.e., S100A6, have been shown to form complexes with several annexins (A2, A5, A6, and A11) (rev by Rintala-Dempsey et al. 2008). Thus, it is possible that the Ca^{2+} sensitivity not only of annexins A1 and A2 but also of other annexins is modulated accordingly.

An additional layer of complexity is added by the observation that annexins are able to adapt their individual Ca^{2+} sensitivity to match changes in the lipid composition within the plasmalemma. All annexins associate with negatively charged phospholipids, but several members of this protein family prefer sites of distinct lipid composition (rev by Monastyrskaya et al. 2009; Draeger et al. 2011). Apart from their association with distinct lipids, they can specifically bind to – or specifically avoid – distinct lipid microdomains or certain assemblies of lipids. One example is the association of annexin A1 with ceramide during apoptosis. As a consequence of cellular stress, an elevation in $[Ca^{2+}]_i$ leads to an increased hydrolysis of plasmalemmal sphingomyelin to ceramide, which self-associates thereby coalescing into large membrane platforms. At these conditions, annexin A1 preferentially associates with the newly formed ceramide platforms (rev by Draeger et al. 2011). Another example is annexin A2, which was identified as a phosphatidylinositol (4,5)-bisphosphate-binding protein being recruited to sites of actin assembly at the plasma membrane and to endocytic vesicles (rev by Gerke et al. 2005).

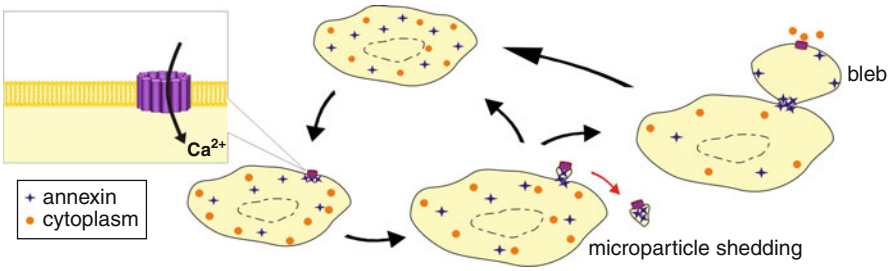
Thus, several annexins present within any one cell, endowed with a distinct Ca^{2+} threshold for membrane translocation and specific lipid targeting properties, can be considered as a broad-range Ca^{2+} and lipid sensing/effecting system (rev by Monastyrskaya et al. 2009; Draeger et al. 2011). The advantages of this mechanism are obvious: By expressing a unique set of annexins, each cell type can develop its characteristic annexin "profile" and adjust its Ca^{2+} homeostasis to its specific functional requirements. Nonetheless, it is conceptually difficult to understand why – apart from annexins A2 and A6 – all other annexins investigated so far (annexins A1, A4, A5, A7, and A11) have Ca^{2+} sensitivities, which are too low to be compatible with cell survival.

In order to resolve this, one needs to consider yet another of the annexins' functional properties: their ability to fuse and aggregate membranes.

Aggregation and Fusion of Membranes

Membrane fusion is of vital importance for the life of cells; it regulates the contacts with the extracellular environment via the exo- and endocytosis of substances, and it is instrumental in intracellular protein transfer. Annexin A7 (formerly "synexin") was initially identified in a search for proteins that promote the aggregation of chromaffin granules of the adrenal medulla (rev by Raynal and Pollard 1994).

Vesicle association and involvement in vesicular transport have since been shown for multiple annexins (rev by Raynal and Pollard 1994; Futter and White 2007; Monastyrskaya et al. 2009). Annexin A1 is targeted to endosomes and contributes to EGF-mediated membrane inward vesiculation. Annexin A2 contributes to intracellular vesicle movement and regulates endosomal functions. Annexin A3 has been credited with a role in phagosomal aggregation, annexin A5 has been colocalized with late endosomes, and annexin A6, which is associated with endocytic transport, is capable of binding to two adjacent phospholipid membranes. Annexins A1, A2, and A6 play



Annexins, Fig. 1 Annexin-mediated plasma membrane repair and damage control mechanisms. Annexins are diffusely distributed within the cytoplasm and translocate to the plasmalemma in response to Ca^{2+} influx via a toxin- or complement-induced pore. The injured region is isolated by annexin molecules, which fuse the adjoining membranes which are then released in the form of a microvesicle or microparticle. Alternatively, cellular subcompartments (blebs) can be sealed off from the cell body. This mechanism allows the regional compartmentalization of $[Ca^{2+}]$ and facilitates membrane repair

a role in the regulation of endocytic membrane traffic and in the biogenesis of multivesicular bodies.

In fact, it is notable that those annexins, which have been probed for membrane aggregation properties, all appear to support vesicle aggregation. However, the mechanism by which annexins link two membranes is not completely understood. Recent reports, which have closely monitored membrane fusion, have largely focused on annexins A1 and A2 heterotetramers. A structural model of membrane binding and membrane fusion has implicated the amphipathic helices of the annexin A1 NH_2-terminus (Gerke et al. 2005). They interact directly with one membrane while the core domain binds to the second membrane (Gerke et al. 2005). However, at least in vitro, isolated annexin cores have the ability to aggregate membranes without the N-terminal domain (Raynal and Pollard 1994). Cryo-electron microscopy of the annexin A2 heterotetramer suggested that in the presence of Ca^{2+} and at physiological pH, the complex forms membrane bridges in chromaffin granules and large unilamellar vesicles.

Annexins in Membrane Injury

The combination of Ca^{2+} sensing with fusogenic properties shared by the proteins of the annexin family are of considerable practical importance for a cell as soon as it suffers membrane injury. The integrity of the plasma membrane can be compromised by mechanical or chemical injury, by pore-forming toxins or even by the organism's internal defenses such as blood complement complexes or perforins (rev by McNeil and Steinhardt 2003).

Depending on the size of the lesion and on the nature of the injury, the cell has to adopt different strategies for resealing the lesion. Cells suffering mechanical damage, which causes large physical defects (>0.2 μm), cannot reseal their plasma membrane spontaneously but have to cover the defect with vesicles which originate from the cell's cytoplasmic compartment. During this so-called exocytotic repair mechanism, membrane patches are created by the fusion of lysosomes into large lipid segments, which are transported to the plasma membrane and inserted into the lesion (Idone et al. 2008). It is likely that – apart from lysosomes – other intracellular membrane reservoirs can also be recruited for this purpose. Well investigated in plasma membrane repair are the protein families of the SNAREs, synaptotagmins, and ferlins (rev by Draeger et al. 2011). A potential cooperation of the annexins with these proteins has frequently been invoked.

In contrast to the repair of a defect characterized by "free lipid edges," the protein-lined pores caused by toxins or complement need first to be quarantined and subsequently excised and discarded. Depending on the nature of the toxin or on the cell type, the pore-containing membrane segments are either taken up by endocytosis and neutralized intracellularly or shed extracellularly in the form of microvesicles or microparticles (rev by Draeger et al. 2011) (Fig. 1).

In damaged cells, the sudden influx of extracellular Ca^{2+} constitutes the initial trigger for the activation of cellular membrane repair responses (rev by Draeger et al. 2011). A limited increase in $[Ca^{2+}]_i$ is thought to induce transcriptional activation and numerous physiological activities (rev by McNeil and Steinhardt 2003). However, a surfeit of Ca^{2+} is detrimental to the cell and ultimately leads to cell death.

Plasmalemmal repair appears to be most effective at Ca^{2+} concentrations between 5 and 10 μM (rev by Draeger et al. 2011). If [Ca^{2+}] rises above 10 μM, the cells are unable to complete membrane repair, and persistent changes in the architecture of the plasmalemma are brought about by the externalization of phosphatidylserine, which leads to the loss of membrane lipid asymmetry; simultaneously, sphingomyelin is hydrolyzed to ceramide, which self-associates into large membrane platforms (rev by Monastyrskaya et al. 2009; Draeger et al. 2011). Coinciding with the shift of phosphatidylserine from the inner to the outer leaflet of the plasma membrane, newly assembled ceramide platforms from the outer leaflet move into the opposite direction – being internalized by so-called massive endocytosis as the steric properties of the plasmalemma are significantly altered (Lariccia et al. 2011).

These profound changes in plasma membrane architecture are initiated by an unrestrained influx of Ca^{2+} and ultimately cause cell death. At the "hot-spots" of Ca^{2+} entry, the local [Ca^{2+}] rises to above 10 μM, leading to a surge of annexins, which translocate from the cytoplasm to the injured plasma membrane. An essential role of annexin A1 in membrane repair was demonstrated by laser damage to selective regions of the plasmalemma of HeLa cells, which triggered a translocation of annexin A1 followed by membrane repair. Membrane repair could be specifically blocked by antibodies against annexin A1 or by a dominant-negative annexin A1 protein mutant, which was incapable of Ca^{2+} binding (McNeil et al. 2006). This was the first experimentally confirmed demonstration of a member of the annexin protein family being involved in membrane repair (McNeil et al. 2006). More recently, annexin A1 has been shown to be instrumental in the repair of membrane lesions after an attack by streptolysin O (SLO), a bacterial pore-forming toxin. The downregulation of annexin A1 by siRNA significantly decreased the ability of human embryonic kidney cells (HEK 293) to withstand a SLO attack (rev by Draeger et al. 2011). By fusing membrane sites on either side of the SLO pore, annexin A1 imprisoned the lesion, which was subsequently pinched off and discarded into the extracellular space as a microvesicle or microparticle (rev by Draeger et al. 2011) (Fig. 1). These remnants of the plasma membrane consist of uni- or multilamellar vesicles and are largely devoid of cytoplasm. They have been detected in all bodily fluids investigated to date and are considered to be a sequel of cell stress or cell injury, often in the wake of systemic disease. They contain annexins A1 (rev by Draeger et al. 2011) and A6 (Potez et al. 2011) in addition to distinct cell surface proteins. Microvesicles are known to be released in response to Ca^{2+} influx into blood cells and platelets and have been shown to contain annexin A7 (rev by Draeger et al. 2011).

In addition to the diverse membrane repair mechanisms described above, early warning systems are operative in cells that are about to suffer a toxin or a complement attack on their plasma membrane. Sublytic concentrations of toxins elicit the release of Ca^{2+} from intracellular stores and trigger the detachment of the subcortical cytoskeleton from the plasma membrane in the form of blebs (rev by Draeger et al. 2011). Blebbing is described as the appearance of multiple membrane protrusions in a cell, which increase the cell surface and create additional intracellular compartments. If the membrane of such a compartment is perforated and flooded with extracellular Ca^{2+}, annexin A1 will move into the bleb and effect a fusion of the membranes of the neck of the bleb. The cell is thus able to save its life by sacrificing the bleb, which can be locally detached from the membrane or – if repair is successful, meaning that physiological $[Ca^{2+}]_i$ is restored – annexin A1 will back-translocate to the cytoplasm and the connection between cell body and bleb will be reestablished (rev by Draeger et al. 2011) (Fig. 1).

Cells can exhibit widely differing phenotypes. Displaying multiple protrusions, cell types such as glia and neurons are prone to rapid changes in $[Ca^{2+}]_i$, in particular within their thin-necked appendages. They are thus at high risk of experiencing an uncontrollable surge of Ca^{2+} after a membrane perforation. Since $[Ca^{2+}]_i$ can change very rapidly, membrane protection must be realized under different conditions. The annexin protein family is ideally suited to provide such a shield: Several annexins work in concert in order to protect the plasma membrane. Depending on the site of injury and the extent of the rise in local $[Ca^{2+}]_i$, the translocation of different annexins is being triggered: Initially, highly Ca^{2+}-sensitive ones, and – if the attack persists or if initial damage limitation mechanisms fail – annexins with lower Ca^{2+} sensitivity will translocate to the injured site and undertake membrane repair.

Conclusion

An incidental appearance of annexins in biochemical assays has been recorded by many scientists and frequently led to a lasting interest in these proteins. The annexins are a family of Ca^{2+}-regulated phospholipid-binding proteins. Presumably attributable to their involvement in numerous membrane-associated processes, they have been credited with a bewildering range of functions. It appears that their structural similarity and ubiquitous expression have obscured rather than furthered the understanding of their functional role.

Annexins display distinct Ca^{2+} sensitivities of plasmalemmal translocation and differential lipid affinities. Their capability to react to a surge in $[Ca^{2+}]_i$ by translocating to the plasma membrane in addition to their fusogenic properties enable them to perform a variety of membrane surgical operations: excising and shedding a protein-lined perforation or patching a membrane hole by fusing the "sticky lipid edges."

In order to prevent a lethal outcome of plasmalemmal perforation, several members of the annexin family working in concert can provide rapid and efficient protection. Functioning as a membrane repair emergency team also explains why most annexins investigated so far operate in a range of $[Ca^{2+}]_i$ which can be considered nonphysiological and indeed lethal for normal cellular function. Annexins A2 and A6, which display a higher Ca^{2+} sensitivity, are presumably fulfilling additional functions in cell signaling, membrane transport, and regulation of intracellular Ca^{2+} homeostasis (Fig. 1).

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Anti-apoptotic

▶ Lithium, Neuroprotective Effect

Antiarrhythmics

▶ Sodium Channel Blockers and Activators

Anticancer Characteristics of Gold(III) Complexes

▶ Gold(III) Complexes, Cytotoxic effects

Anticancer Drug

▶ Gold(III), Cyclometalated Compound, Inhibition of Human DNA Topoisomerase IB

Anticancer Metallodrugs

▶ Zinc, Metallated DNA-Protein Crosslinks as Finger Conformation and Reactivity Probes

Antimicrobial Action of Silver

▶ Silver as Disinfectant

Antimicrobial Action of Titanium Dioxide

▶ Titanium Dioxide as Disinfectant

Antimony, Impaired Nucleotide Excision Repair

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Synonyms

Inhibition of DNA repair

Definition

Antimony interferes with the repair of a specific DNA lesion which is induced by UVC radiation. The nucleotide excision repair, a major DNA repair pathway, is responsible for the removal of this lesion and is accomplished by the concerted interaction of more than 30 proteins. Two of these proteins, XPE and XPA, both playing a crucial role in the recognition of the DNA lesions, appear to be affected by antimony via different mechanisms.

Antimony

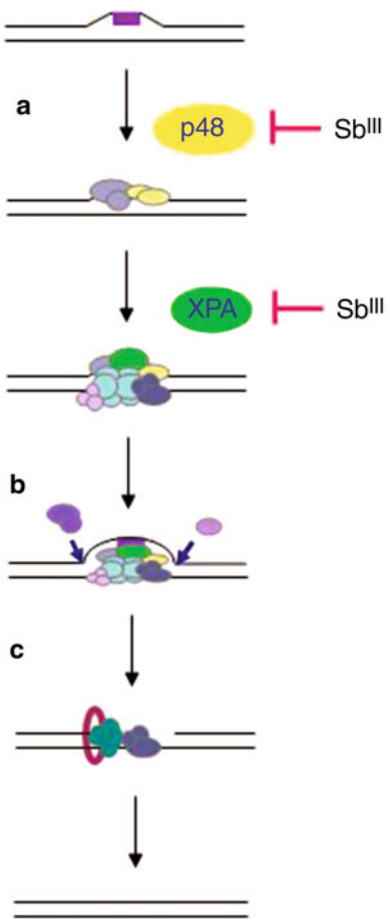
Essential biological functions for the metalloid antimony have not been identified so far. Nevertheless, the toxicity is in the center of interest, especially since antimony shares toxicological features with arsenic, a human carcinogen. Epidemiological data indicate that antimony might be carcinogenic in humans, too, sufficient evidence, however, exists only in experimental animals. Thus, in two inhalation studies, an increased number of lung tumors were observed in rats after long-term exposure toward antimony trioxide dust (for review see De Boeck et al. 2003).

The toxicity of arsenic and antimony highly depends on their oxidation state: The trivalent species are much more cytotoxic than the pentavalent ones and cause DNA damage while the pentavalent do not. Since both elements are not mutagenic, it is supposed that the trivalent species do not directly interact with DNA but mediate their genotoxic properties via an indirect mechanism such as inhibition of DNA repair. Since DNA lesions are continuously generated, the inhibition of their repair gives rise to an accumulation of DNA damage, too. Several toxic metals such as cadmium, cobalt, or arsenic are known to inhibit the repair of DNA lesions and antimony seems to act in this way as well. In the case of antimony, an inhibition of the repair of DNA double strand breaks was shown, even though at comparatively high concentrations (reviewed in Beyersmann and Hartwig 2008).

A striking feature of the trivalent species is their high affinity toward thiol groups of peptides and proteins. It has been therefore speculated that antimony might interact with proteins involved in the DNA repair process (Schaumlöffel and Gebel 1998; De Boeck et al. 2003).

The Nucleotide Excision Repair Pathway

In response to various types of DNA lesions, highly specialized repair systems have evolved to maintain genomic stability. One of them, the nucleotide excision repair (NER) pathway, is responsible for the removal of bulky, helix-distorting DNA lesions typically induced by environmental mutagens such as UVC radiation or benzo[*a*]pyren. The removal of these lesions comprises several steps including lesion



Antimony, Impaired Nucleotide Excision Repair, Fig. 1 Molecular targets for trivalent antimony within nucleotide excision repair, which comprises of (**a**) lesion recognition and formation of a pre-incision complex, (**b**) removal of the damaged DNA strand by dual incision up- and downstream of the DNA lesion and (**c**) gap-filling by polymerization and ligation

recognition, excision of the damaged oligonucleotide, polymerization, and ligation of the new fragment (Fig. 1).

Altogether more than 30 proteins are involved in this process, sequentially assembling and disassembling at the lesion site. Of special importance are the so-called XP proteins A-G. Their name is derived from the severe human genetic disorder xeroderma pigmentosum, which is caused by defects in these proteins and is characterized by extreme UV sensitivity and enhanced risk for skin cancer (Friedberg et al. 2006).

Inhibition of Nucleotide Excision Repair

Diverse methods exist to measure the removal of lesions by nucleotide excision repair. UVC radiation is frequently used as a model. It specifically generates two kinds of lesions: the 6-4 photoproducts and the cyclobutane pyrimidine dimers both formed by covalent binding between two adjacent pyrimidine bases. An elegant way to detect these lesions is immunofluorescence labeling: Cultured human cells previously radiated with UVC are fixed in formaldehyde solution and exposed to antibodies directed against the respective lesion. The fluorescence of the antibodies is finally visualized under the microscope and quantified with special software. By choosing different repair times (the time between irradiation of the cells with UVC and fixation), the decline in signal intensity, respectively lesion number, can be traced. With respect to the well-documented inhibitory effect of arsenic on nucleotide excision repair, a possible impact of antimony was of high interest. Therefore, the test system was used to quantify the removal of lesions in the presence of the trivalent antimony compound $SbCl_3$. Interestingly, the removal of the 6-4 photoproducts was not affected while the removal of the cyclobutane pyrimidine dimers was. In comparison to cells not treated with antimony, significantly, more pyrimidine dimers remained in the presence of $SbCl_3$ 24 h after lesion induction with UVC, consistent with a repair inhibition of up to 50 %. Most notably, the effect on the repair of cyclobutane pyrimidine dimers was already observed at noncytotoxic concentrations of antimony.

Lesion Recognition

The lesion-specific impact of antimony might be explained by an interference with lesion recognition, a very crucial step in nucleotide excision repair. Most NER lesions present in nontranscribed DNA regions are directly recognized by the repair protein XPC, which binds to the DNA damage and recruits further repair proteins. In case of UVC-induced DNA lesions, however, a further protein complex, named UV-DDB complex, appears to be involved in this process and acts as an initial sensor. Especially the cyclobutane pyrimidine dimers, which cause only a very subtle distortion of the DNA helix, are recognized and repaired more efficiently in the presence of this

complex. It has been shown that XPC does not bind to the pyrimidine dimers in the absence of XPE, which is part of the UV-DDB complex, whereas the recognition of the 6-4 photoproducts is not impaired. Due to its tight transcriptional and proteasomal regulation, XPE is assumed to be a limiting factor for the recognition of these lesions (Sugasawa 2010). Actually, quantification of the cellular XPE level revealed that treatment with trivalent antimony caused a decrease in the amount of XPE protein which was due to a reduction of the XPE gene expression. Also, antimony diminished the UVC-induced expression of XPE. In contrast to that, no impact was observed on the XPC protein level.

Experimental results indicate that besides XPE, also XPA might become limiting for the repair of cyclobutane pyrimidine dimers (Cleaver et al. 1995; Shell et al. 2009). Exposure toward trivalent antimony did not alter the expression and protein level of XPA; nevertheless, antimony impaired the association of XPA at the DNA lesion. The association of XPA was examined by generating local spots of DNA damage via UVC irradiation through a porous filter. Labeling of the XPA protein with fluorescent antibodies revealed a diminished assembly of the protein at the lesion site 10 min after irradiation in the presence of antimony trichloride. After 60 min, a time point at which half of the protein normally had already dissociated from the lesion, more XPA remained at the DNA lesion when co-treated with antimony, indicating a delayed association of XPA. Again, no effect was observed on XPC, which binds to the damaged DNA prior to XPA. Also an impairment of XPE as underlying mechanism can be ruled out since effects on XPA were observed after a short treatment with antimony for only 2 h while effects on the XPE protein level were restricted to later time points.

Zinc-Binding Motif of XPA

Like many other proteins involved in maintaining genomic integrity, e.g., transcription factors and repair proteins, XPA contains a zinc-binding motif in its structure. Zinc-binding motifs are formed by cysteine or histidine side chains coordinating one or more zinc ions and mediate protein-protein interactions or DNA binding. Metal ions known for their affinity toward thiols and imidazoles are able to interact with zinc-binding motifs and thus pose a risk for the correct conformation and function of the protein (Hartwig 2001). With respect to these data, the zinc-binding domain of XPA appears to be a promising target for trivalent antimony species, too.

A direct interaction of antimony with the repair protein was investigated by measuring zinc release from a 37 amino acids containing peptide saturated with zinc, which resembles the zinc-binding domain of human XPA. Liberated zinc was quantified via formation of a colored complex with 4-(2-pyridylazo)-resorcinol (PAR) and spectrometric detection. Zinc release was provoked in a dose-dependent manner already at a less than equimolar ratio of antimony trichloride and the peptide. In great excess, the antimony compound was even as effective as hydrogen peroxide which was used as positive control. Although no direct conclusions can be drawn from the isolated zinc-binding domain to what might happen to the whole protein in the cell, these results support the assumption that antimony interacts with XPA and impairs its function within nucleotide excision repair.

Conclusions

DNA repair inhibition is a common feature of toxic metals and offers at least one explanation for their mutagenicity and carcinogenicity. Regarding first results on nucleotide excision repair, this also seems to be true for antimony. Remarkable is, however, the lesion specificity of the repair inhibition observed in case of UVC-induced DNA damage. This might be explained by two different mechanisms affecting lesion recognition (Fig. 1). On the one hand, the association of the repair protein XPA is altered in the presence of trivalent antimony. With respect to its high affinity toward thiol and imidazole groups, antimony may directly interact with the zinc-binding domain of XPA and impair its function within nucleotide excision repair; investigations with the isolated zinc-binding domain of XPA strongly support this assumption. On the other hand, the expression of XPE, a limiting factor for the recognition of UVC-induced DNA lesions, is diminished after treatment with antimony. Interestingly, XPE is transcriptionally regulated by the tumor suppressor protein p53, which also contains a zinc-binding motif. It is known from

literature that the conformation of the protein as well as its transcriptional activity is sensitive toward zinc-chelating compounds, oxidation, and also metals as demonstrated for cadmium (Meplan et al. 1999). Therefore, it can be assumed that although different target proteins within lesion recognition might be responsible for the inhibitory effect of antimony on NER, both interactions may be due to the interaction with thiol and imidazole groups of zinc-binding motifs. This issue needs to be further investigated.

Cross-References

- ▶ Arsenic
- ▶ Zinc-Binding Proteins, Abundance
- ▶ Zinc-Binding Sites in Proteins
- ▶ Zinc Cellular Homeostasis

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Antimony, Physical and Chemical Properties

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Antimony is a metalloid; its outermost electrons are not free to move in the crystal structure because they are fixed in position in a covalent bond. It has metallic luster but is brittle with no useful mechanical properties. Sulfide ores with antimony contents between 5% and 25% are roasted to give volatile Sb_2O_3, which can be reduced directly to the metal. The oxide forms between 290°C and 340°C in an oxidizing atmosphere:

$$2Sb_2S_3 + 9O_2 \rightarrow 2Sb_2O_3 + 6SO_2.$$

If too much oxygen is available, the nonvolatile antimony (V) oxide is formed:

$$Sb_2S_3 + 5O_2 \rightarrow Sb_2O_5 + 3SO_2.$$

Therefore, oxygen supply must be kept low to inhibit its formation. Metallic antimony may form when Sb_2O_3 reacts with the sulfide:

$$2Sb_2O_3 + Sb_2S_3 \rightarrow 6Sb + 3SO_2.$$

Antimony metal is used mainly in alloys with lead or other metals. Nearly 50% of the total demand is accounted for by storage batteries, power transmission devices, communications equipment, type metal, solder, and ammunition. The compounds of antimony have a wide range of industrial uses, including uses in flame retardants, industrial chemicals, rubber, plastics, ceramics, and glass.

Physical Properties

Atomic number	51
Atomic weight	121.76
Relative abundance in the Earth's crust, %	1×10^{-4}
Isotopes	121, 123
Density	
Solid at 20°C, g/cm^3	6.688

(*continued*)

Liquid at 630.5°C, g/cm^3	6.55
Melting point, °C	630.5
Boiling point at 101.3 kPa, °C	1,325
Heat of fusion, kJ/mol	10.49
Tensile strength, N/mm^2	10.8
Modulus of elasticity, N/mm^2	566
Hardness, on the Mohs scale	3
Crystal form	Rhombohedral
Surface tension, mN/m	
Solid at 432°C	317.2
Liquid at 630°C	349
Liquid at 1,200°C	255
Molar heat capacity at 630.5°C, J mol^{-1} K^{-1}	
Solid	30.446
Liquid	31.401
Coefficient of linear expansion [0–100°C], °C^{-1}	10.8×10^{-6}
Thermal conductivity, W m^{-1} K^{-1}	
At 0°C	18.51
At 100°C	16.58

There are unstable forms of metallic antimony: yellow antimony, black amorphous antimony, and what is known as explosive antimony. Yellow antimony is formed when air or oxygen is passed through liquid stibine. Black amorphous antimony is obtained by rapidly cooling antimony vapors and is also formed from yellow antimony at −90°C. At room temperature, black antimony slowly reverts to metallic antimony, and at 400°C, reversion is spontaneous. Black amorphous antimony ignites spontaneously in air. Explosive antimony is obtained by electrolysis of antimony (III) chloride solution in hydrochloric acid at a high current density with an antimony anode and a platinum cathode. It consists of black amorphous antimony contaminated with antimony trichloride. In the vapor state, antimony exists as Sb_4; at higher temperature, it splits into Sb_2.

Chemical Properties

When antimony loses its outermost electrons, it forms trivalent compounds, as in $SbCl_3$, or pentavalent, as in $SbCl_5$. It can also add electrons in the outermost shell forming trivalent compounds as in SbH_3. Antimony tetroxide, Sb_2O_4, is according to x-ray diffraction a double oxide, Sb_2O_3 and Sb_2O_5, or antimony antimonate, $Sb^{III}Sb^{V}O_4$. Pure antimony does not change in air at room temperature, and it is not tarnished in humid air or pure water. If heated in air, the molten metal ignites. Above 750°C, steam oxidizes liquid antimony to antimony trioxide, and hydrogen is evolved. Fluorine, chlorine, bromine, and iodine react violently with antimony at room temperature to form trihalides. Antimony (III) sulfide is the product of the reaction with sulfur, hydrogen sulfide, or dry sulfur dioxide. It is soluble in alkali sulfide solution.

Antimony is resistant to concentrated hydrofluoric, dilute hydrochloric, and dilute nitric acids. It is readily soluble in a mixture of nitric and tartaric acids and in aqua regia. At room temperature, it is not attacked by dilute or concentrated sulfuric acid. It is attacked at 90–95°C by concentrated sulfuric acid, and sulfur dioxide is evolved. Pure antimony is resistant to solutions of ammonium and alkali-metal hydroxides and to molten sodium carbonate. If heated to redness, it reacts with molten sodium or potassium hydroxide to form hydrogen gas and antimonites. Poisoning with antimony and its compounds can result from exposure to airborne particles in the workplace.

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Antimony-Based Therapy of Leishmaniases, Molecular and Cellular Rationale

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Synonyms

Pentavalent antimonial therapy against leishmaniases, molecular bases

Definition

Pentavalent antimonials have been used for decades as first-line compounds against leishmaniasis. The search for the molecular basis of antimony-based treatment against the parasite has long time eluded all investigators' efforts. In the last two decades, many causes of both antimonials action and resistance toward these drugs on a molecular level have been identified. Both mechanisms of action and of resistance are multifactorial, the first depending on the effect on different targets, and the second on different strategies used by the parasite to escape the killing actions of antimonials.

Leishmaniasis

The Leishmaniases are a group of vector-borne diseases caused by the infection with protozoans belonging to the at least 21 species of parasite of the genus Leishmania. These include the *Leishmania* (*L.*) *donovani* complex with two main species (*L. donovani* and *L. infantum*), the *L. mexicana* complex with three main species (*L. mexicana, L. amazonensis*, and *L. venezuelensis*), *L. tropica*, *L. major*, *L. aethiopica*, and the subgenus *Viannia* (*V.*) with four main species (*Leishmania (V.) braziliensis, L. (V.) guyanensis, L. (V.) panamensis*, and *L. (V.) peruviana*). Animals and humans themselves are the reservoirs of the disease. Leishmaniasis is transmitted through the bite of phlebotomine sand flies infected with the protozoan. The parasite can then be internalized via macrophages in the liver, spleen, and bone marrow of the mammalian host. Leishmania parasites are dimorphic organisms, that is, with two morphological forms in their life cycle: promastigotes in the digestive organs of the insect vector, and amastigotes in the mononuclear phagocytic system of the mammalian host.

Leishmaniasis affects about 13 million people in at least 88 countries, and is a poverty-related disease. The clinical manifestations range from cutaneous leishmaniasis (CL), characterized by ulcers which can heal spontaneously, often leaving scars, to the destruction of cutaneous and subcutaneous tissues in mucocutaneous forms, to the involvement of liver and many organs in the most severe form, visceral leishmaniasis (VL) or kala-azar, caused by *L. donovani* and *L. infantum*. In VL, patients develop fever, splenomegaly, hypergammaglobulinemia, and pancytopenia; VL is almost always fatal if untreated, and causes about 500,000 cases and 80,000 deaths per year.

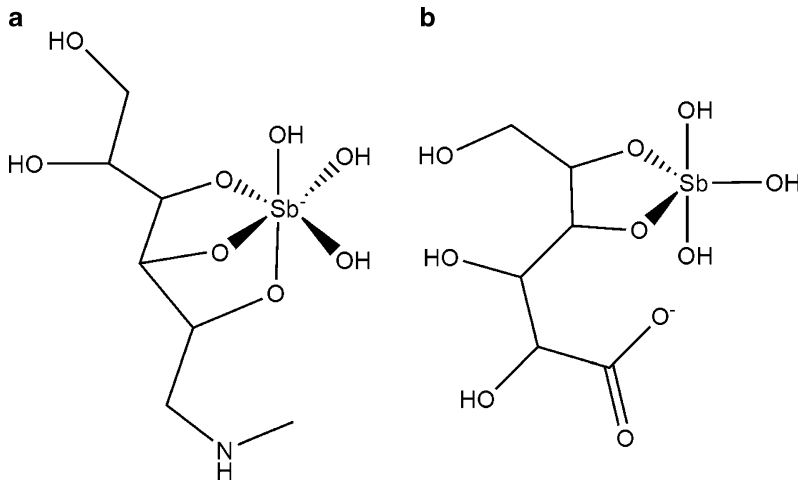
Anti-Leishmanial Drugs

No vaccine is available yet against leishmaniasis, and chemotherapy is the main option against the disease. The existing weapons against leishmaniasis are limited, because companies invest only a limited budget on the research against neglected diseases, which affect mostly people in the developing world. Pentavalent antimonials are in use since the first half of the twentieth century, and constitute the first-line drugs in many countries. However, the increase of clinical resistance, especially in the region of Bihar (India) prevents the use of antimonials in these regions. Pentavalent antimonial drugs are administered parenterally, at doses of 20–30 mg of Sb/(kg day) for at least 20 days, and present several side effects, which include nausea, vomiting, weakness and myalgia, abdominal colic, diarrhea, skin rashes, and hepatotoxicity. Pancreatitis and cardiotoxicity can be important problems of antimonial treatment of leishmaniasis.

Second-line drugs include pentamidine and lipid formulations of amphotericin B, a less toxic antifungine. Though amphotericin B preparations have high cure rate against leishmaniasis, their high cost impairs their extensive use in developing countries. Miltefosine, originally developed as anticancer drug, is the first oral drug against leishmaniasis; it is effective also against antimony-resistant leishmaniases, but high costs, severe side effect, long treatment schemes, long half-life, and potential teratogenicity limit their use. Paromomycin, an aminoglycoside antibiotic, is a promising drug that is currently in clinical trial; it is cheap and effective, but needs long-term parenteral treatments for VL, and it may generate resistance (Croft and Coombs 2003).

Antimony-Based Drugs Against Leishmaniasis

Potassium antimony tartrate (tartar emetic), a trivalent antimonial, was used against leishmaniasis for the first time by Vianna in 1913, but Sb(III) compounds have low efficacy and stability, while being highly toxic, with serious side effects.



Antimony-Based Therapy of Leishmaniases, Molecular and Cellular Rationale, Fig. 1 Structural formula in aqueous solution of 1:1 Sb-NMG and Sb-SG complexes in Glucantime (**a**) and Pentostam (**b**), according to Frézard et al. (2008)

Pentavalent antimonials have been used against leishmaniasis since the 1920s, when Brahmachari successfully used urea stibamine against VL. Subsequently, other Sb(V) compounds have been synthesized and used, such as stibosan, neostibosan, antimony gluconate (Solustibosan) in 1937; and sodium stibogluconate (SSG, or Pentostam) in 1945.

Nowadays the two main pentavalent antimonials in clinical use are the highly water-soluble complexes of Sb(V) with *N*-methyl-D-glucamine (NMG, in Glucantime) and sodium gluconate (SG, in Pentostam). The structure of these complexes has been unknown for half a century, because of their amorphous state. Both compounds are a mixture of oligomeric structures with the general formula (sugar-Sb)*n*-sugar, with the prevalence of 1:1 Sb-NMG and Sb-SG complexes in diluted samples. They are mostly zwitterionic in solution (Fig. 1).

Antimony Metabolism in Leishmania

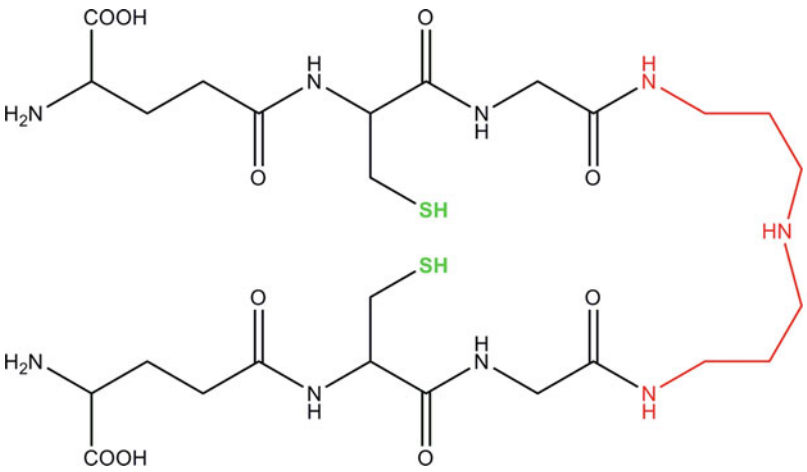
Both Sb(V) and Sb(III) accumulate, in antimony-sensitive and antimony-resistant strains of different *Leishmania* species. Entry of antimonials in the parasite appears to take place via different routes for Sb(V) and Sb(III). Sb(III) uptake is competitively inhibited in parasite cells by As(III), belonging to the same group, and depends on a parasitic transporter, aquaglyceroporine (AQP1), involved in the entry of several metabolites.

The route of entry of pentavalent antimonials is currently unknown. Since gluconate competitively inhibits the uptake of Sb(V), while As(V) and phosphate, which are known to enter via a phosphate transporter, do not compete with Sb(V) transport, pentavalent antimonials are believed to enter *Leishmania* cells by means of a transporter which recognizes the sugar moiety of the drug.

Sb(V)-containing compounds are more effective and about tenfold less toxic than trivalent antimony derivatives. According to the recent literature, pentavalent antimonials are considered to be mainly prodrugs. Sb(V) can be reduced by the host macrophages or by the parasite to the more toxic Sb(III) form, both enzymatically and non-enzymatically (Ashutosh et al. 2007; Haldar et al. 2011).

Leishmania parasites reduce Sb(V) to Sb(III) in a stage-specific fashion. Amastigotes reduce Sb(III) more efficiently than promastigotes, and are therefore more susceptible to pentavalent antimonials.

Nonenzymatic reduction is carried out by thiols. Reduced glutathione, the main thiol present in the host cytosol, can reduce Sb(V) to Sb(III) within the phagolysosome containing *Leishmania* amastigotes. In vitro, such conversion is quite slow, although pentavalent antimony reduction is favored by the acidic pH and temperature of the macrophage organelle. Cysteine and cysteinyl-glycine, which are the predominant thiols within lysosomes, also reduce Sb(V) to Sb(III) at 37°C, with a higher rate, compared with the glutathione-mediated reaction. However, reduction of Sb(V) to Sb(III) in the macrophage cannot be very high in vivo, because Sb(III) is very toxic, and concentrations of 25 μg/mL kill 50% of the macrophages. Probably the majority of the highly toxic Sb(III) is generated inside the parasite.



Antimony-Based Therapy of Leishmaniases, Molecular and Cellular Rationale, Fig. 2 Structure of reduced trypanothione (T$(SH)_2$), formed by conjugation of two glutathione molecules (in *black*, with the two -SH groups colored *green*) and a spermidine (*red*). In the oxidized form of trypanothione, the two -SH groups are oxidized to form a -S-S- disulfide bridge

The most important thiol involved in Sb(V) reduction in *Leishmania* is trypanothione (N1,N8-bis (glutathionyl)spermidine, T$(SH)_2$), which is the predominant thiol within the parasite (Fig. 2).

T$(SH)_2$ has been found to rapidly reduce Sb(V) to Sb(III), especially under acidic conditions and at slightly elevated temperature. Such reaction occurs preferentially in the intracellular amastigotes, which have a lower intracellular pH and live at a higher temperature than promastigotes, although promastigotes contain higher intracellular amounts of T$(SH)_2$ and glutathione than amastigotes.

Two enzymes have been demonstrated to catalyze the reduction of Sb(V) in *Leishmania*. The thiol-dependent reductase TDR1 is a parasite-specific tetrameric protein found highly abundant in the amastigotes, which uses glutathione as a source of reducing power to reduce Sb(V) to Sb(III). LmACR2, homologue to yeast arsenate reductase, identified in *L. major*, is also able to reduce Sb(V), by using reduced glutathione and glutaredoxin as cofactors. LmACR2 is inhibited by trivalent antimony and As(III), and its overexpression increases sensitivity to Pentostam.

Reduction of Sb(V) to Sb(III) is a critical event for the parasite, and a decrease of reductive activity in the parasite can be associated with antimony resistance.

Antimony Targets

Sb(III), as other thiophilic metals such as As(III) and Bi(III), strongly bind thiols. When sulfhydryls bind these metals, they form completely deprotonated and highly stable thiolate anions, whose nucleophilicity is attenuated upon formation of metal complexes with high thermodynamic stability, which dissociate with very slow rates.

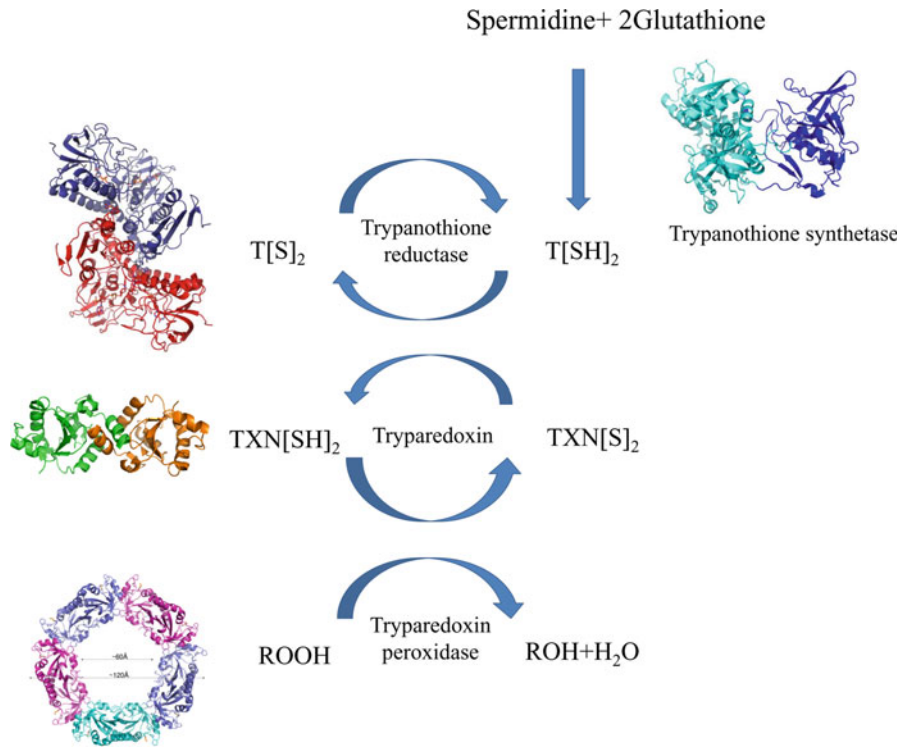
The mechanism of action of Sb(III) against leishmaniasis is mainly due to its interaction with low-molecular thiols, cysteine-containing peptides and, in particular, thiol-dependent enzymes.

Trypanothione Metabolism: T$(SH)_2$ and Trypanothione Reductase

The trypanosomatids (*Leishmania* spp., *Trypanosoma* spp., and other parasites) possess a unique redox metabolism, based on the trypanothione and on thiol-dependent enzymes, which is the main target of pentavalent antimonials (Fig. 3) (Colotti and Ilari 2011).

The trypanothione-dependent redox metabolism is considered as a highly potential drug target against leishmaniases, because of the absence of the T$(SH)_2$ system in mammals, the lack of a functional redundancy within the redox metabolism, and the sensitivity of Trypanosomatids against oxidative stress (i.e., most enzymes of the pathway are essential for the survival of all *Leishmania* species). In addition, *Leishmania* parasites lack mammalian redox enzymes such as glutathione reductase, selenocysteine-containing glutathione peroxidases and thioredoxin reductase, and catalase.

T$(SH)_2$ is synthesized by trypanothione synthetase, by conjugation of two glutathione molecules with a spermidine, and its concentration is very high (1–2 mM) in all parasite forms and growth phases. T$(SH)_2$ is the electron donor in several metabolic



Antimony-Based Therapy of Leishmaniases, Molecular and Cellular Rationale, Fig. 3 The trypanothione metabolism in *Leishmania*. Trypanothione is synthesized by Trypanothione synthetase, is kept reduced by Trypanothione reductase (TR), and provides reducing equivalents via Tryparedoxin to Tryparedoxin-dependent peroxidase for the reduction of the peroxides

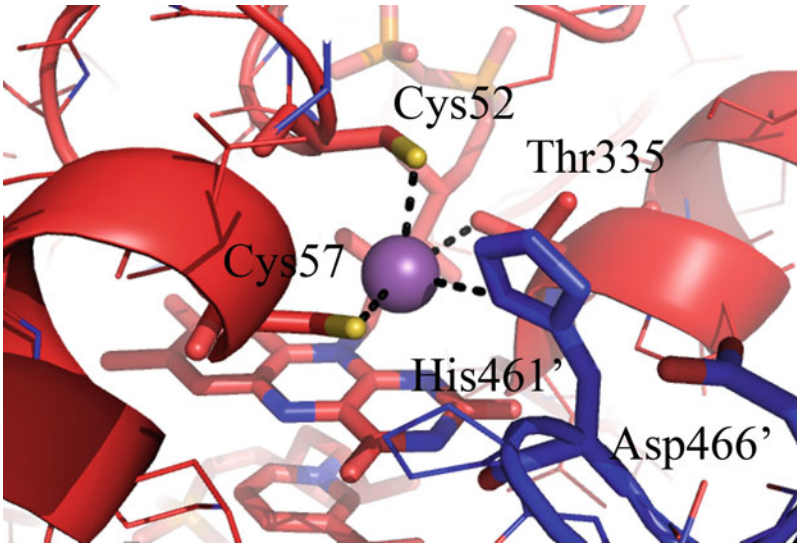
pathways, where it delivers reducing equivalents to oxidized glutathione, dehydroascorbate, and in particular to the dithiol tryparedoxins (TXN1, cytosolic; and TXN2, mitochondrial) which, in turn, reduce different enzymes and participates in hydroperoxides elimination, together with the tryparedoxin-dependent peroxidases (TDPXs).

Tryparedoxins and TDPXs are homologues of thioredoxin and thioredoxin peroxidase, respectively. Tryparedoxins, reduced by $T(SH)_2$, operate as sources of reducing electrons during removal of peroxides by the TDPXs, which catalyze the reduction of H_2O_2 and organic hydroperoxides to water or alcohols, thereby detoxifying the *Leishmania* parasites.

$T(SH)_2$ is kept reduced by trypanothione reductase (TR), thereby maintaining the oxidoreductive balance in *Leishmania* parasite, protecting the parasites from oxidative damage and toxic heavy metals, and allowing delivery of reducing equivalents for DNA synthesis. The $T(SH)_2$/TR system replaces many of the antioxidant and metabolic functions of the glutathione/glutathione reductase and thioredoxin/thioredoxin reductase systems present in mammals and is necessary for the survival of all *Leishmania* species.

TR, as well as glutathione reductase, is a member of the family of FAD-dependent NAD(P)H oxidoreductases, that is, dimeric flavoenzymes that catalyze the transfer of electrons between pyridine nucleotides and dithiol compounds. Each TR monomer is formed by three different domains, such as the FAD-containing domain (residues 1–160 and 289–360, *L. infantum* TR numbering), the NADPH-binding domain (residues 161–289), and the interface domain (residues 361–488). TR and human glutathione reductase share structural and mechanistic similarities, although they are mutually exclusive for their dithiol substrates. Substrate specificity is largely determined by only five amino acids at the substrate binding site: the active site pocket of TR is wider, more hydrophobic, and negatively charged than that of glutathione reductase, and can, therefore, select oxidized trypanothione, bulkier and more positively charged, with respect to oxidized glutathione.

The first step of the reaction catalyzed by TR is the binding of NADPH, which transiently reduces the flavin. Reduction of the Cys52-Cys57 disulfide in the active site by the reduced flavin follows, with the formation of a short-lived covalent intermediate with Cys57, and subsequently of a stable charge-transfer



Antimony-Based Therapy of Leishmaniases, Molecular and Cellular Rationale, Fig. 4 Antimony (Sb(III), in *purple*) binds to the active site of Trypanothione reductase. Sb(III) inhibits TR activity by binding to the two active cysteine residues (Cys52 and Cys57), to Thr335 and to His461′, belonging to the second monomer of the enzyme dimer. The residues belonging to the two TR monomers are drawn in *red* (first monomer) and in *blue* (second monomer) (Authors: Colotti Gianni, Ilari Andrea)

complex between the flavin and the Cys57 thiolate. Following the generation of the charge-transfer complex, $NADP^+$ dissociates and is replaced by another NADPH. Cys52, which is activated similarly to serine and cysteine proteases by the His461′-Glu466′ pair (belonging to the second subunit of the TR dimer) can then react with the oxidized trypanothione to produce a mixed disulfide, followed by attack of Cys57 on Cys52 and full Trypanothione dithiol reduction.

Fairlamb and collaborators demonstrated that the antimonials interfere with the trypanothione metabolism in two ways: Sb(III) can form stable complexes with $T(SH)_2$ and glutathione, in 1:1 and 1:3 antimony-thiol ratios, respectively, or ternary Sb (III)- $T[SH]_2$-glutathione complexes. These complexes can be sequestered inside a vacuole or are rapidly extruded by ATP-binding cassette (ABC) transporters, thereby decreasing the thiol buffering capacity of the parasite. Antimonials also strongly inhibit TR in vivo and in vitro, resulting in the lack of reduction and regeneration of the thiols and the consequent accumulation of the disulfide forms of trypanothione and glutathione. Together, these two mechanisms impair the thiol redox potential and all thiol-dependent processes in both amastigote and promastigote stages of the life cycle of *Leishmania* (Wyllie et al. 2004).

Colotti, Ilari et al. solved X-ray crystal structure of the reduced *Leishmania* TR in complex with Sb(III) and NADPH, thus disclosing the molecular basis of TR inhibition (Baiocco et al. 2009).

Sb(III) inhibits TR by binding strongly to the catalytic pocket of the enzyme, engaging the residues involved in the catalytic mechanism in the formation of a complex. Sb(III) binds to Cys52, to Cys57, to Thr335, and to His461′ of the twofold symmetry–related subunit, thus disallowing the hydride transfer from the protein to the trypanothione and therefore, $T(SH)_2$ reduction (Fig. 4).

Trivalent antimony may also bind to and inhibit other enzymes of the trypanothione metabolism, but only preliminary results are available on the interaction with tryparedoxin and TDPXs and their possible inhibition.

The $T(SH)_2$ metabolism, compromised by antimonials, is essential for many different cellular functions, such as the detoxification of peroxides via the tryparedoxin-dependent peroxidase system, the biosynthesis of deoxyribonucleotides via ribonucleotide reductase, the detoxification of methylglyoxal to D-lactate via $T(SH)_2$-dependent glyoxalase I and glyoxalase II, and the detoxification of xenobiotics via trypanothione *S*-transferase. All these functions are strongly impaired upon pentavalent antimonials administration. In addition, oxidized trypanothione inhibits ribonucleotide reductase that is essential for deoxyribonucleotides synthesis.

Further, survival of amastigotes within host macrophages requires them to withstand oxidant stress from

potentially lethal macrophage responses, such as increased production of superoxide, hydrogen peroxide, hydroxyl radicals, and nitric oxide.

Impaired antioxidant defenses caused by inhibition of trypanothione reductase in amastigotes and by thiol efflux are probably the main determinants of parasite's death upon antimony treatment.

Zinc Finger Proteins

Other targets for antimonials have been identified by Frézard, Demicheli, and coworkers. They have shown that Sb(III) binds to CCHC and CCCH zinc finger peptide models, determining the ejection of Zn(II) and stabilizing the structure of the motif. These structural elements are contained in many proteins, interacting with nucleic acids and proteins and involved in diverse functions, including DNA recognition, RNA packaging, protein folding and assembly, lipid binding, transcriptional activation, cell differentiation and growth, and regulation of apoptosis. Several zinc finger proteins containing CCCH or CCHC motifs are involved in different cellular functions in trypanosomatids. In *L. major*, the protein HEXBP, containing nine CCHC motifs, binds to the hexanucleotide repeat sequence found in the intervening region of the GP63 gene cluster, the most abundant surface glycoprotein of the parasite, and it is likely to be involved in DNA replication, structure, and repair. The interaction between Sb(III) and these motifs may, therefore, modulate or mediate the pharmacological action of antimonial drugs (Frézard et al. 2012).

Other Targets of Sb(III)

Treatment of *L. infantum* amastigotes with Sb(III) induces an increase in intracellular Ca^{2+} via nonselective cation channels in the host and in the parasite, and apoptosis (programmed cell death), with the presence of DNA fragmentation, a marker of late events of apoptosis.

Studies on the effect of arsenite, chemically similar to Sb(III), show important alterations in the parasites microtubules, and possibly in several signal-transduction pathways, upon As(III) treatment. In particular, expression of α- and β-tubulin in promastigotes is altered upon arsenite administration, while it remains unaltered in arsenite-resistant parasites. Microtubule polymerization can be regulated by α- and β-tubulins phosphorylation, which is highly increased in the arsenite-resistant mutant.

Sb(V) Targets

Sb(V) has also been shown to possess intrinsic antileishmanial activity, that is, to have drug-like effects without the need for reduction to Sb(III). Pentavalent sodium stibogluconate inhibits glycolysis and fatty acid beta oxidation, but the specific targets in these pathways have not been identified.

Sodium stibogluconate, but not Sb(III), specifically inhibits type I DNA topoisomerase from *L. donovani*, thus inhibiting DNA unwinding and cleavage. The formation of this metal-protein complex has been shown to correlate with the levels of in vivo sensitivity toward antimonial drugs.

Formation of Sb(V)-ribonucleoside complexes, both in the ratio of 1:1 and 1:2 has also been reported. NMR studies suggest that antimony binds to ribose hydroxyl groups, probably via ring chelation at C2′ and C3′. Formation of such complex is kinetically favored in acidic conditions, such as those present in the macrophage phagolysosome, while its dissociation is very slow at neutral pH. Sb(V)-ribonucleoside complexes might inhibit the *Leishmania* purine transporters, or might enter the parasite, where it may interfere with the purine salvage pathway.

Effects on the Host Macrophages: Activation of the Host Immune Responses

Antimonials also contribute to parasite elimination by activating both innate as well as adaptive host immune responses (Haldar et al. 2011).

Microbicidal molecules, like nitric oxide (NO) or hydrogen peroxide (H_2O_2) generated by the host macrophage upon antimony treatment, play a role in the leishmanicidal activity of antimonials.

Treatment with Pentostam induces reactive oxygen species (ROS) generation and NO production in murine macrophages infected by *L. donovani* amastigotes, by promoting two waves of killing. The first wave is due to the induction of ROS, while the second wave is mediated by NO generation.

ROS generation is mediated by phosphorylation of ERK via PI3K-PKC-Ras/Raf pathway, while NO production depends on activation of the PI3K/Akt pathway and p38MAPK. In particular, p38MAPK induces TNFα production, which in turn induces iNOS2 (nitric oxide synthase) expression and subsequent NO generation. Parasite killing is inhibited by treatment with antiTNFα antibodies.

Leishmania infection can increase PTPase activity, with the consequent dysregulation of PTK-dependent signaling in the macrophages. Pentostam inhibits SHP1 and SHP2 classes of PTPases, such that dephosphorylation of ERKs, a mechanism by which *Leishmania* parasites can escape activation of PI3K-PKC-Ras/Raf-ERK1/2 pathway for ROS generation and the PI3K-Akt-p38 MAPK pathway for NO generation, is not available.

A Pentostam-dependent increase of IL-12 production has been observed in macrophages: IL-12 induces Th cells to produce IFN-γ, which activates TNF-α synthesis and, consequently, NO production. Efficacy of Pentostam treatment of VL depends on the endogenous levels of IL-2, IL-4, and IL-12.

Pentavalent antimonials also contribute to activation of cell-mediated response. This effect, together with the generation of ROS and NO, potentiates the ability of macrophages to eliminate *Leishmania* parasites and also protect from relapse.

Pentostam induces T-cell response, while B-cell response is not activated. Cytotoxic T lymphocyte response is increased by pentavalent antimonials, which increase the expression and the presentation of MHC I class-mediated antigens. Th1 cells are essential for a complete antileishmanial effect of pentavalent antimonials. Patients with HIV-VL co-infections have poor response upon antimony treatment.

Resistance Toward Antimonials

Pentavalent antimonials have been used for decades for the treatment of leishmaniases, and remain the first choice drugs in Africa, Asia (except the Bihar district, in India), and South America.

In the last three decades, however, widespread resistance toward these compounds has been reported for *L. donovani*–dependent VL in the Bihar district, where in the late 1970s the original low-dose treatment based on the administration of 10 mg/kg/day for 6–10 days was found to be ineffective for 30% of the patients.

Although several revisions of antimony-based treatment recommendations have been emanated, with increases in daily regimens and in treatment length, and temporary increases in cure rates, resistance toward Sb(V)-based drugs has continued to spread, such that in some villages in the Bihar district 100% resistance toward antimonials has been observed.

Pentavalent antimonials, however, continue to be effective in the rest of the world, although small areas of resistance are spreading in small regions of Nepal and of India, surrounding Bihar. The reasons for resistance are not fully understood. Misuse and overuse of antimonial drugs and the presence of high amount of arsenic in the water have determined long-term exposure of the parasite (and of the hosts) to the metals. Another reason for the spread of resistance to antimonials is the high number of persons infected in Bihar, that makes the transmission of the parasite in that region mostly anthroponotic (humans are the reservoirs of the parasite), rather than zoonotic (in most part of the world, animals such as dogs and horses are the main reservoirs). In zoonotic transmission, drug-resistant parasites kill the animals and are eliminated preferentially, while in anthroponotic transmission, drug-sensitive parasites are eliminated while drug-resistant parasites continue to circulate, and resistance continue to develop in patients with relapses (Perry et al. 2011; Haldar et al. 2011).

Resistance can be generated in vitro by exposing the parasite cultures to increasing concentrations of drugs.

Mechanisms of Resistance Toward Pentavalent Antimonials

Many Gene Expression Profiling studies and some genomic studies have been carried out on antimony-resistant and antimony-sensitive strains of several *Leishmania* species, reporting differential expression for many different proteins, in accordance with the multiplicity of mechanisms leading to antimony resistance. At least four mechanisms of resistance to antimonial drugs have been identified (Carter et al. 2006; Ashutosh et al. 2007; Haldar et al. 2011).

1. Restricted entry of antimonials into the parasite cell
   AQP1, responsible for entry of Sb(III) into *Leishmania* cells, has been associated with resistance toward antimonials. Overexpression of AQP1 increases sensitivity toward both Sb(III) and pentavalent antimonials in *Leishmania* cells. Further, AQP1 has been found to be downregulated in both promastigote and amastigote stages in antimony-resistant clinical isolates and mutants.
2. Low reduction of Sb(V) to Sb(III).
   Both alterations in enzymatic and in nonenzymatic Sb(V) reduction have been associated to antimony

resistance. Transfection of LmACR2 in *L. infantum* promastigotes has been found to increase sensitivity to Pentostam in amastigotes. In addition, the levels of thiols in the infected host macrophage and in the parasite are related to antimony resistance. The expression of γ-glutamylcysteine synthetase (γ-GCS), the enzyme which catalyzes the rate-limiting step in glutathione synthesis, is downregulated in the macrophages of antimony-resistant isolates, determining the reduction of glutathione concentrations in the host macrophage and promoting a less reducing environment, with a consequent low ability to reduce Sb(V) to the more toxic Sb(III).

3. Increased expression of proteins of redox metabolism in *Leishmania*.
   Overexpression of TR has been observed in antimony-resistant strains, and high levels of tryparedoxin and TDPX, enzymes in hydroperoxide detoxification, have been reported in parasites resistant to antimonials. Such increased levels determine an increased metabolism of peroxides, and link clinical resistance with enhanced antioxidant protection.
4. Sequestration and/or efflux of the drug.
   The ABC transporter MRPA has been found to be amplified in Leishmania mutants resistant to Sb (III), Sb(V), and As(III). Its overexpression seems to confer resistance by sequestration of metal-thiol conjugates, rather than by increasing metal efflux. At least eight proteins belong to the MRP1 family and may be associated in antimony resistance dependent on thiol-based efflux, but no precise identification is available.

Increase of metal efflux has been observed in *Leishmania* strains expressing high amounts of glutathione and trypanothione, for example, overexpressing ornithine decarboxylase, responsible for spermidine synthesis, and γ-GCS, responsible for glutathione synthesis. Increases in the intracellular level of $T(SH)_2$ induce resistance toward Sb(III), since an increased level of the Sb-trypanothione complexes can be sequestered or can be extruded.

Other genes and proteins able to confer antimony resistance have also been identified. These enclose genes coding for proteins that belong to the superfamily of the leucine-rich repeat proteins, histone H2A, and kinetoplastid membrane protein-11 (KMP-11).

Cross-References

- ▶ Antimony, Impaired Nucleotide Excision Repair
- ▶ Antimony, Physical and Chemical Properties
- ▶ Arsenic and Aquaporins
- ▶ Arsenic
- ▶ Arsenic in Nature
- ▶ Arsenic, Mechanisms of Cellular Detoxification
- ▶ As
- ▶ Ribonucleotide Reductase

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Antineoplastic

- ▶ Tin Complexes, Antitumor Activity

Antioxidant Enzymes

▶ Manganese and Catalases

Antiproliferative Characteristics of Gold(III) Compounds

▶ Gold(III) Complexes, Cytotoxic Effects

Antiproliferative Compounds

▶ Tin Complexes, Antitumor Activity

Antithrombotic

▶ Nanosilver, Next-Generation Antithrombotic Agent

Antitumor Drug – Anticancer Drug

▶ Osmium Complexes with Azole Heterocycles as Potential Antitumor Drugs

Antitumor Effects of Metal-Based Drugs

▶ Gold(III) Complexes, Cytotoxic Effects

Antiviral Metallodrugs

▶ Zinc, Metallated DNA-Protein Crosslinks as Finger Conformation and Reactivity Probes

Apoferritin, Activation by Gold, Silver, and Platinum Nanoparticles

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Definition

The effect of silver, gold, and platinum nanoparticles on the ferroxidase activity of apoferritin showed a respective increase in specific activity of 110-fold, ninefold, and ninefold over the control at the molar ratio of 1,000:1 (500:1 for Au:apoferritin). Typical color change, from light yellow to orange (Ag), light yellow to a dark brown/black (Au), or light orange to dark brown (Pt) when apoferritin was mixed with $AgNO_3$, $AuCl_3$, or K_2PtCl_4 followed by sodium borohydride to afford respective M:apoferritin nanoparticle complexes in a ratio of between 250:1 and 4,000:1. These complexes were characterized by UV/Vis, inductively coupled plasma optical emission spectroscopy (ICP-OES), Fourier transform infra red (FTIR), transmission electron microscopy (TEM), and energy dispersive X-ray spectroscopy. TEM revealed that the size of nanoparticles increased as the molar ratio of metal to apoferritin increased with an average size of 3–6 nm generated with M:apoferritin molar ratios of 250:1 to 4,000:1. FTIR showed no structural changes of the apoferritin when the nanoparticles were attached to the protein.

Introduction

The multidisciplinary field of "nanotechnology" embraces applications of nanoscale(10^{-9} m) functional materials including particles in cross disciplinary studies such as health care, environmental biology, chemical and material sciences, and communications (Rao et al. 2004; Rai et al. 2009). One of the fundamental principles that sets this type of technology apart

from traditional standard protocols is the fact that as the particle itself becomes smaller and smaller its association with biomacromolecules changes which, inevitably, leads to these molecules behaving differently to the native ones. The biological syntheses (Cao 2004) of noble metal nanoparticles through the bioreduction of metal salts by both prokaryotic and eukaryotic organisms (Whiteley et al. 2011; Fendler 1998) is the most cost-effective, simple, and eco-friendly process even though a limitation still remains with control of the mechanism that determines the particle size and shape (Riddin et al. 2006, 2009, 2010; Rashamuse and Whiteley 2007; Whiteley et al. 2011). A successful synthesis of platinum nanoparticles from both a eukaryote (*Fusarium oxysporum*) and a prokaryote (*Desulphovibrio*) (Riddin et al. 2006; Rashamuse and Whiteley 2007; Rashamuse et al. 2008; Govender et al. 2009, 2010), showed no specific control on their shape and size and a mixed "bag" of triangles, squares, spheres, trapezoids, and pyramids of varying size was produced. The shape-and size-controlled synthesis of functional metal nanoparticles (Konetzka 1977) has attracted great attention since these particles have unique electronic, magnetic, catalytic, and optical properties. One promising approach of size-controlled nanomaterials synthesis is a biomimetic one using organic assemblies as templates for directed fabrication. The use of a protein cavity or cage as a limited growth field for nanoparticles is not novel but serves as an ideal template to confine particle growth in a homogeneous distribution as well as a stabilizer against particle aggregation. One such cage is apoferritin, a globular protein of approximately 440 kDa in 24 identical subunits that has ferroxidase activity and catalyzes the oxidation of Fe^{2+} to Fe^{3+} in the presence of molecular oxygen as an electron acceptor. The subunits form a spherical protein shell of 12 nm with an internal aqueous cavity of 8 nm. Apart from Fe^{3+} several other zero-valent apoferritin encapsulated metal nanoparticles have been prepared within this protein cage and these include palladium, platinum, copper, cobalt, nickel, cadmium, chromium, and zinc. Apoferritin from horse spleen (HSAF) was the first protein cage to be used as a template for synthesis of inorganic nanoparticles, and it was probably the most intensively studied and best understood example. One of the most important features of this protein cage is the perforation of the protein shell by small channels (0.5 nm diameter), found at the junctions of the subunits, which allow the introduction of several metal cations and metal complexes into the cage.

Although several studies of protein-nanoparticle interactions have appeared in the past few years none have actually addressed what actually occurs at the interface between the protein and the nanoparticle. Perhaps the most promising suggestion that has materialized is an understanding of the effective "unit" as that of being a dynamic layer or "corona." The binding between nanoparticles and protein changes the structure of the latter and as the particle gets smaller not only does its interaction with the protein change but the composition of the protein itself changes. Consequently, the "nanoparticle-enzyme corona" may have totally different biological properties in comparison to a native protein or enzyme.

In view of the foregoing discussion regarding the anticipated different properties exhibited by enzymes/proteins in the presence of nanoparticles and since no reports had appeared in the literature there was special interest in the activity of apoferritin in the presence of nanoparticles. It was expected that apoferritin associated with platinum nanoparticle may have a wide range of affinities and activities dependent on the size of the nanoparticle.

This preparation of nanoparticles within a biomacromolecular apoferritin cage has lead to some unprecedented enhancement of its ferroxidase activity.

M:HSAF Nanoparticle Complex

Synthesis

M:HSAF nanoparticle complexes of silver, gold, or platinum were formed after HSAF was incubated with varying concentrations of respective metal salt [$AgNO_3$, $AuCl_3$, or K_2PtCl_4] followed by treatment with $NaBH_4$ (Deng et al. 2009). The preparation of the complex is carried out at pH 8 in order to increase the electronegativity of the interior of HSAF thereby improving electrostatic attraction between positive metal cations and the core of HSAF. The change in nanoparticle size was determined by TEM analysis and a variation in molar ratios of M:HSAF was used to determine the saturation point of HSAF in terms of the amount of metal that the protein was capable of encapsulating.

Nanoparticle formation was monitored visibly by color change from light yellow to orange (Ag), light yellow to a dark brown/black (Au) or light orange to dark brown (Pt). Color change is the initial evidence of nanoparticle formation. The intensity of color increased as the amount of metal salt added to the aqueous solution of HSAF increased. Since more particles were stabilized as the precursor platinum to HSAF increased it suggested an increase in the encapsulation of metal atoms with increasing metal concentration that was available in solution. Studies on metals nanoparticles stabilized by HASF have suggested divalent precursor metal salts were preferably accommodated within the core of HSAF. This may be due to the fact that apoferritin in its normal biological function takes up iron in its Fe^{2+} state which is later converted to Fe^{3+} within its core.

The M:HSAF complex was purified by size exclusion chromatography and the elution profile indicated a single peak coincidental with a peak of pure apoferritin which indicated that HSAF remained intact after incubation with metal salt and that the metal was bound to HSAF. Analysis with native PAGE (5 %) showed a co-migration of HSAF only and M:HSAF nanoparticles complexes implying that the metal nanoparticles were attached to HSAF.

Characterization

Synthesized nanoparticles were characterized by UV-visible spectroscopy, ICP-OES, FTIR, TEM, and EDX analysis.

The synthesized nanoparticles showed similar absorption spectra for HSAF and for all M:HSAF ratios typical of surface plasmon resonance bands consistent with metal nanoparticles encapsulated by protein shells. There was an increase in absorption maxima with increasing platinum nanoparticles around 230–280 nm due to the attachment of the platinum nanoparticles to HSAF.

With Au:HSAF nanoparticle complexes there were two absorbance maxima, one at 280 nm, corresponding to pure HSAF, and the other at 520 nm that represented the characteristic surface plasmon resonance band of a spherical gold nanoparticle <20 nm in diameter. The intensity of these two absorbance peaks increased with increasing gold nanoparticle concentration.

With Ag:HSAF nanoparticle complexes there was, apart from the obvious protein absorbance peak present at 280 nm, a rather prominent surface plasmon resonance band at 414 nm the intensity of which increased with increasing silver nanoparticle concentration.

ICP-OES showed an increase in platinum nanoparticle concentration but a decrease in gold and silver nanoparticle concentration with increase in molar ratio of metal salt, respectively, to a fixed HSAF concentration. The M:HSAF ratio based on protein content of HSAF before and after synthesis, respectively, was also estimated. The greatest number of platinum atoms stabilized was 404; that of gold and silver were 357 and 125, respectively, based on the starting HSAF concentration and 920 based on the final concentration of HSAF after synthesis (gold and silver were 661 and 202, respectively).

The biologically synthesized M:HSAF nanoparticle complexes were analyzed by FTIR spectroscopy in order to investigate if there were any functional groups on HSAF that were responsible for the stabilization and/or coordination of the nanoparticles as well as to study the structural integrity of HSAF after the synthesis of the nanoparticles. Noticeable findings were seen between 1,200–1,800 cm^{-1} indicating that the integrity of HSAF remained intact. The presence of amide I (1,590–1,650 cm^{-1}) and amide II (1,500–1,560 cm^{-1}) bands which are characteristic of proteins and peptides, in both spectra of HSAF and M:HSAF nanoparticle complexes indicated protein stability. Furthermore the presence of an amide I peak (1,630–1,650 cm^{-1}), often ascribed to predominantly α-helical structured protein (like HSAF) in aqueous solutions, all pointed to the fact that the synthesis of metal nanoparticles in the presence of HSAF may not have compromised the overall structure of HSAF. With a more detailed analysis of the FTIR spectra of the M:HSAF nanoparticle complexes it was noticed that additional peaks appeared in the amide I region at 1,737–1,740 cm^{-1}, which were not seen in the spectrum of HSAF, suggesting the binding of the metal (before reduction to nanoparticles) to the carboxylate side chains of acidic amino acids. These residues predominantly line the interior of HSAF and give it its net negative charge at physiological pH, implying a possibility of metal nanoparticle synthesis within the cavity of HSAF.

M:HSAF nanoparticle complexes were characterized by TEM in order to determine particle size and distribution, position of the nanoparticle in the HSAF and to confirm the synthesis of metal nanoparticles in the presence of HSAF. The nanoparticles were

predominantly spherical that agreed, obviously, with the spherical shape of the HSAF interior. Analysis revealed a general increase in the size of the nanoparticles with an increase in M:HSAF ratio. It was noticed, however, that there was no significant increase in the size of the nanoparticles as the M:HSAF increased implying that the cavity of HSAF restricted metal nanoparticle nucleation within its core. The average size of the metal nanoparticles obtained with different molar concentration of metal salt was between 2 and 6 nm and since the size obtained was below that of the interior diameter of HSAF (8 nm), it gave further evidence that the metal nanoparticles formed within the core of HSAF.

Confirmation of the presence and stabilization of metal in M:HSAF nanoparticle complexes was made by EDX analysis.

Activation

There is a ninefold increase in ferroxidase activity with an increase in the molar ratios of Pt:HSAF nanoparticles seen at a molar ratio of 1,000:1 [Pt:HSAF]. At a Pt:-HSAF ratio of 500:1 there is a fivefold increase while at a ratio of 250:1 there is only a twofold increase. Platinum is a widely used catalyst that exhibits increased catalysis with increased surface area and so it is not surprising that this metal, at the nanoscale level, showed enhanced activity of ferroxidase.

Furthermore, the release of platinum nanoparticles after the uptake of iron further confirmed that platinum nanoparticles were initially present within the core of the HSAF. It may also suggest the ability of the interior of HSAF to accommodate more than one type of metal atom (Fe and Pt) within its cavity. The functional groups responsible for the coordination of both platinum and iron within the core of HSAF may not be involved in the storage and oxidation of iron in the HSAF core. A different mechanisms of Fe^{2+} uptake and oxidation by apoferritin proposed that the basic amino acids residues within the core of apoferritin were responsible for its ferroxidase activity. This site was different from that containing acidic amino residues reported to be implicated in the uptake, nucleation, and stabilization of nanoparticles.

There is also a ninefold increase in ferroxidase activity with an increase in the molar ratio of Au:HSAF nanoparticles seen at a molar ratio of 500:1 [Au:HSAF]. At Au:HSAF ratios of 250:1 and 1,000:1, respectively, there is a six- and eightfold increase, while at a ratio of 2,000:1 there is a 2.7-fold increment.

As far as silver nanoparticles were concerned there is a 110-fold increase in ferroxidase activity with an increase in the molar ratio of Ag:HSAF nanoparticles of 1,000:1. At Ag:HSAF ratio of 500:1 there is a 90-fold increase, while at a ratio of 2,000:1 there is a 67-fold increase; at a ratio of 250:1 there is only a 29-fold increase.

Future Directions

Furthermore novelty manifests itself with possible clinical applications in the treatment of diseases associated with poor iron absorption and possible decrease in oxidative stress associated with the toxic levels of iron in biological systems.

Gold and silver nanoparticles have shown extensive popularity in nanomedicine for their anticancer/antitumor and antimicrobial properties. From a structural and mechanistic point of view it is not clear why there is this considerable increase in activity of ferroxidase in the present study. Though it may be considered speculation to propose tentative suggestions it is probable that one, or more, of the following may occur:

1. Noble metallic nanoparticles, especially Au and Ag have catalytic properties.
2. The distances between the glutamic acid, aspartic acid, glutamine, and water molecule within the active site of ferroxidase are about 8–10 Å – well within the range for the Au/Ag nanoparticles to bind. This would, in turn, enhance the negativity of the amino acids in the core increasing the binding of Fe^{2+}.
3. The Au/Ag nanoparticles interact with the di-ferrous [Fe–Fe] complex in the enzyme reactive core increasing the rate of removal of electrons and oxidation to [Fe^{3+}–Fe^{3+}].
4. The nanoparticles facilitate the addition of a water molecule to enhance the formation of the hydrolysis product [FeOOH] and in so doing interact with the released protons to drive the reaction.
5. The nanoparticles interact with polar amino acids at the reactive site changing the conformation of the reactive core increasing the rate of addition of oxidant.

6. The nanoparticles chelate to the cysteine sulfur atoms and/or the zinc at the subunit interface disrupting any structural conformations (cysteine bridges) forming and potentially blocking the access of oxidant through channels to the active region.

Cross-References

► Gold and Nucleic Acids

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Apoptosis

► Arsenic and Primary Human Cells
► Tin Complexes, Antitumor Activity

Apoptosis, Programmed Cell Death

► Calcium and Viruses
► Chromium(VI), Oxidative Cell Damage

Apoptotic Cell Death

► Selenium and Glutathione Peroxidases

Aquaporin-Mediated Arsenic Transport

► Arsenic and Aquaporins

Aquaporin-Mediated Boron Transport

► Boron and Aquaporins

Aquaporin-Mediated Selenite Transport

► Selenium and Aquaporins

Aquaporin-Mediated Silicon Transport

► Silicon and Aquaporins

Aquaporins and Transport of Metalloids

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Synonyms

Major Intrinsic Proteins; Metalloid transport; Solute channels; Water channels

Definition

Aquaporins or major intrinsic proteins (MIPs) are channels facilitating the passive diffusion of small uncharged solutes across biological membranes. In addition to water, several aquaporin isoforms were shown to transport gases (NH_3 and CO_2) or other uncharged solutes including metalloid compounds (arsenite, boric acid, silicic acid, selenite, and antimonite).

Aquaporin Discovery and Phylogeny

Aquaporins are membrane proteins belonging to the ancient major intrinsic protein (MIP) family whose members are found in all kingdoms of life. Although the term aquaporin was initially restricted to MIPs transporting water molecules, it is now used to describe all MIPs.

The first demonstration of the water channel activity of a MIP protein was published in 1992 when Peter Agre and coworkers heterologously expressed in Xenopus oocytes a 28-kDa protein (CHIP28) from human erythrocyte membrane (Preston et al. 1992). This protein was renamed AQP1 and became the archetypal membrane water channel. Peter Agre shared the 2003 Nobel Prize in chemistry for the discovery of water channels and their molecular and physiological characterization of AQP1. While 13 aquaporin genes have since been identified in mammals, higher plants contain a larger number of isoforms with 33, 35, 36, 37, and 55 homologs in rice, *Arabidopsis*, maize, tomato, and poplar, respectively (reviewed in Gomes et al. 2009; Hachez and Chaumont 2010; Maurel et al. 2008). This large multiplicity of isoforms probably offers better adaptive advantages for plants to grow in different environmental conditions as a result of divergent substrate specificity, localization, and regulation.

Based on their amino acid sequences, aquaporins can be divided into three classes, as proposed for mammalian aquaporins (Ishibashi et al. 2009). Class I originally regroups water-specific channels but also includes aquaporins shown to be permeable to other solutes such as carbon dioxide (CO_2) and nitric oxide (NO). Class II aquaporins, also named aquaglyceroporins, can facilitate the transport of solutes such as glycerol and urea, as well as water and, for some of them, metalloids. Class III regroups the most divergent aquaporins (less than 20% identity with class I and class II aquaporins) and includes the more recently discovered mammalian AQP11 and AQP12 and the plant SIPs (small basic intrinsic proteins). However, as the number of available aquaporin sequences is increasing, their phylogenetic analyses become more complex. Nowadays, a total of 13 subfamilies can be distinguished whereof five are plant-specific (Danielson and Johanson 2010).

Aquaporin Topology and Pore Selectivity

Aquaporins are channels with a molecular mass around 30 kDa, typically containing six transmembrane domains (TM1–TM6) linked by five loops (A–E), with the N- and C-termini facing the cytosol (reviewed in Chaumont et al. 2005; Gomes et al. 2009). The two halves of the protein show an obvious structural symmetry. The cytosolic loop B and extracytosolic loop E both form short hydrophobic α-helices that dip halfway into the membrane from opposite sides forming two hemipores. These two loops contain the generally conserved asparagine-proline-alanine (NPA) motifs, which contribute to the substrate selectivity and proton exclusion mechanism. Aquaporin molecular structures have been determined with a high resolution (5–2.2 Å) by electron microscopy and X-ray crystallography using 2D and 3D crystals. Aquaporins assemble as tetramers in the membrane in which each monomer forms an independent channel (Murata et al. 2000). The common channel structure consists in three topological parts, an extracellular and a cytoplasmic vestibule connected by a narrow pore acting as a selectivity filter. In spite of this highly conserved structure, differences in channel specificity derive from size

exclusion mechanisms at two constriction regions (the NPA motif and the aromatic/arginine (ar/R) constriction) as well as from stereoscopic interaction between the substrate and the amino acid residues facing the pore.

The narrow NPA region is positively charged because of the dipole moments of the two short α-helices formed by the loops B and E, and it induces a reorientation of the water molecules within the constriction by promoting an interaction of these latter with the asparagine residues (Murata et al. 2000). The ar/R constriction, located ~7–8 Å from the NPA motifs toward the extracellular vestibule, also constitutes a steric barrier which measures ~2 Å in diameter in most of the water-selective aquaporins and is ~1 Å larger in aquaglyceroporins. This constriction is formed by interaction of four amino acid residues, two from the TM2 and TM5 and two from the loop E, that play a key role in controlling the passage of uncharged molecules. Substitutions at these positions and in their neighborhood modulate the polarity and the size of the pore and therefore the specificity of the channel.

Aquaporins and Metalloids

As mentioned above, aquaporins are not solely water channels but are involved in the transport of a wide range of small uncharged solutes including metalloids. Aquaporins channel activity and specificity are generally tested by heterologous expression in Xenopus oocytes or in yeast strains (reviewed in Hachez and Chaumont 2010). Transport assays in oocytes include cell swelling measurements to determine the water or solute channel activity and measurements of the accumulation of radioactively labeled substrates into the cell according to time. In yeast, growth tests in the presence of a toxic solute, complementation assays of strains deleted in specific transporters, or membrane permeability measurement using stopped-flow spectrometry allow the determination of the intrinsic permeability of a single aquaporin isoform. These systems have been used to demonstrate that several aquaporins facilitate the diffusion of metalloids.

Aquaporins facilitating the transport of metalloids in animals, plants, prokaryotes, and protozoan generally belong to class II (aquaglyceroporins). In plants, most of them are members of the nodulin-26-like intrinsic protein (NIP) family and of the recently discovered X intrinsic protein (XIP) subfamilies, although some plasma membrane intrinsic proteins (PIPs) belonging to the class I (aquaporin) were also found to be involved in this process (reviewed in Ishibashi et al. 2009; Bienert and Chaumont 2011; Bienert et al. 2011; Danielson and Johanson 2010). In general, NIP aquaporins are physiologically essential metalloid transporters in plants, responsible for the uptake, translocation, or extrusion of uncharged metalloid species including boron, silicon, arsenic, and antimony (Bienert et al. 2008) (see ▶ Arsenic and Aquaporins, ▶ Boron and Aquaporins, ▶ Selenium and Aquaporins, ▶ Silicon and Aquaporins). Selenium is occasionally included in the list of metalloid as selenite shares chemical features with other hydroxylated metalloid species. This is in accordance with the observation that these compounds share a common NIP-facilitated transmembrane pathway (see ▶ Selenium and Aquaporins).

Interestingly, the properties of the four amino acid residues of the ar/R filter essential for the pore specificity appear to be an important feature for metalloid transport. Accordingly, plant NIPs are further subdivided into three subgroups, the NIPIs, NIPIIs, and NIPIIIs (Danielson and Johanson 2010; Ma 2010). NIPI proteins have been reported to transport water, hydrogen peroxide, glycerol, lactic acid, or the metalloids antimonite and arsenite (Bienert and Chaumont 2011). NIPII proteins are permeable to solutes such as water, ammonia, urea, or the metalloids boric acid, antimonite, and arsenite (Bienert and Chaumont 2011). Finally, NIPIII subgroup isoforms have a distinct selectivity filter consisting of relatively small amino acid residues and forming a potentially larger constriction region compared with other NIP subgroups or microbial and mammalian aquaglyceroporins (Danielson and Johanson 2010; Ma 2010). The pore diameter and channel path of NIPIIIs were modeled to be among the widest of all aquaporins. This is in accordance with the fact that NIPIII proteins allow the passage of silicic acid which is larger than typical MIP substrates such as water, glycerol, or other transported metalloid species (Ma 2010).

Aquaporin Regulation

Water and/or solutes movement through aquaporins is mainly dependent on the concentration gradients of

these compounds across the membranes, as aquaporins only facilitate their diffusion and do not act as active transporters. Therefore, the amount of active channels in the membranes has to be tightly regulated according to the metabolic demand (reviewed in Chaumont et al. 2005; Gomes et al. 2009; Maurel et al. 2008). The first regulation step is through regulation of mRNA and protein expression level according to the cell type, developmental stage, and environmental cues. For instance, expression of different boron transporters belonging to the NIP subfamily is upregulated in plants grown under boron-limited conditions, and this seems to be cell-type specific (see ▶ Boron and Aquaporins). Once translated, aquaporins have to be sent to their target membrane, a mechanism that depends on their phosphorylation status and on physical interactions with different aquaporin isoforms as well as with regulatory proteins of the secretory pathway. These trafficking regulation mechanisms probably explain the polarization of metalloid NIP transporters in specific plasma membrane regions in specialized root cells (see ▶ Boron and Aquaporins, ▶ Silicon and Aquaporins). Finally, gating of aquaporins through reversible phosphorylation, pH, osmotic gradient, and hydrostatic gradients has been reported for different isoforms and represents a rapid way to control the membrane permeability to different solutes in response to developmental and environmental constraints. To our knowledge, the gating of metalloid-transporting aquaporins has not yet been studied in detail.

Concluding Remarks

The discovery that aquaporins are channels facilitating the diffusion of a wide range of small uncharged solutes including metalloids has definitely opened new perspectives concerning their roles in cell physiology. Further characterization of the molecular basis defining the pore selectivity of metalloid-transporting aquaporins through the generation of 2D or 3D crystals would definitely reveal essential amino acid residues. Several aquaporins from very different organisms have been shown to transport metalloids, but their involvement in cell and/or organism physiology has still to be determined for most of them. A better understanding of their function and regulation is therefore required before envisioning their use to improve metalloid homeostasis in living cells.

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Cross-References

▶ Arsenic and Aquaporins
▶ Boron and Aquaporins
▶ Selenium and Aquaporins
▶ Silicon and Aquaporins

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ARD

▶ Acireductone Dioxygenase

ArgE, ArgE-Encoded N-Acetyl-L-ornithine Deacetylase

▶ Zinc Aminopeptidases, Aminopeptidase from Vibrio Proteolyticus (Aeromonas proteolytica) as Prototypical Enzyme

Argyrophilia

▶ Silver Impregnation Methods in Diagnostics

Arsen

▶ Arsenic in Pathological Conditions
▶ Arsenic, Free Radical and Oxidative Stress

Arseniasis

▶ Arsenicosis

Arsenic

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Synonyms

Arsenic metabolic pathway; Arsenic toxicity; Human health effects of arsenic exposure

Definition

Arsenic (As) is a naturally occurring element widely distributed in the Earth's crust. Exposure to excess As, principally from contaminated drinking water, is considered one of the top environmental health threats worldwide. Most of this exposure is from natural geological sources of As that contaminate groundwater (Mukherjee et al. 2006).

As has the common oxidation states −3, +3, and +5. Trivalent As is more soluble and mobile than the pentavalent form. The log K_{ow} value for As is 1.3, indicating lipophilicity. For the oxidation and reduction of As can be responsible also microorganisms such as *Bacillus* and *Pseudomonas*. As fungi can produce toxic and highly volatile As. Methylation of As to organoarsenicals occurs in vertebrates (including humans) and invertebrates.

Exposure to As

In the environment, As is combined with oxygen, chlorine, and sulfur to form inorganic As compounds. For the general human population, the main exposure to organic and inorganic As is through ingestion. Both organic and inorganic As are present in varying amounts in water and food (meat, poultry, fish, shellfish, dairy products, and cereals) (Chakraborti 2011). Inorganic forms of As can exist as either arsenate or arsenite. Although arsenate is less toxic, it can be converted to arsenite in humans through metabolism. The major metabolic pathway for inorganic As in humans is methylation. After absorption, inorganic As is transported by blood to other organs. A single oral dose of inorganic As results in increased As concentrations in liver, kidneys, lungs, and intestinal mucosa. Some As, predominantly in the pentavalent form is excreted directly in feces. Once absorbed, pentvalent As is excreted also by the kidneys. Trivalent As is readily excreted in bile to the intestines, where it is available for reabsorption or feces elimination.

Effects of As

As affects breathing, hearth rhythm, and increases the risk of bladder cancer. Its exposure can also cause gastrointestinal disturbances, liver and renal diseases,

reproductive health effects, and dermal changes (Balabanič et al. 2011; Chakraborti 2011). As causes oxidative damage by the production of reactive oxygen species which produces different bioactive molecules (Flora et al. 2007; Zhang et al. 2011). Chronic exposure to As has been associated with increased numbers of spontaneous abortions in contaminated water users and women working at or living in close proximity to smelters, as well as women whose partners worked at the smelters (Balabanič et al. 2011). The rate of spontaneous abortion was even higher when both partners worked at the smelters. Chronic exposure to high level of As elevated serum lipid peroxide levels and lowered nonprotein sulfhydryl levels suggesting oxidative stress (Flora et al. 2007).

Tests for genotoxicity have indicated that As compounds inhibit DNA repair and induce chromosomal aberrations, sister-chromatid exchanges, and micronuclei formation in both human and rodent cells in culture and in cells of exposed humans (Chakraborti 2011; Kesari et al. 2012). The mechanism of genotoxicity is not known, but may be due to the ability of arsenate to inhibit DNA replicating or repair enzymes, or the ability of arsenate to act as a phosphate analog.

Cells accumulate As by using an active transport system normally used in phosphate transport. Once absorbed, As toxicity is generally attributed to the trivalent form. Toxic effects are exerted by reacting with sulfhydryl enzyme systems. The tissue rich in oxidative metabolism, such as the alimentary tract, liver, kidney, lung, and epidermis, are therefore most affected (Chakraborti 2011).

One of the mechanisms by which As exerts its toxic effect is through impairment of cellular respiration by the inhibition of various mitochondrial enzymes and the uncoupling of oxidative phosphorylation. Most toxicity of As results from its ability to interact with sulfhydryl groups of proteins and enzymes and to substitute phosphorous in a variety of biochemical reactions (Zhang et al. 2007).

Most of the inorganic As is metabolized to dimethylarsinic acid [DMA (V)] and monomethylarsonic acid [MMA (V)] before excretion in the urine. Methylation of As involves a two electron reduction of pentavalent [As (V) and MMA (V)] to trivalent [As (III) and MMA (III)] As species followed by the transfer of a methyl group from a methyl donor, such as *S*-adenosylmethionine (Thompson 1993; Tchounwou et al. 2003). This methylation mechanism has been widely accepted and the metabolites MMA (V) and DMA (V) have been consistently observed in human urine. A key intermediate for the methylation of MMA (V) to DMA (V) is the MMA (III) species (Suzuki et al. 2004; Reichard et al. 2007).

The generally held view of As carcinogenesis in the past was that arsenite was the most likely cause of carcinogensis and that methylation of As species was a detoxification pathway. The present view of As carcinogenesis is that there are many possible chemical forms of As that may be causal in carcinogenesis and that methylation of As may be a toxification pathway. MMA (III) has been found in urine of humans exposed to As without and with concomitant treatment with chelators (Aposhian et al. 2000). Some of the biological activities that MMA (III) is known to possess in various experimental systems include enzyme inhibition, cell toxicity, and genotoxicity (Kesari et al. 2012; Lin et al. 2012). MMA (III) is an excellent choice as a cause of As carcinogenesis. DMA (III) has been detected in human urine of As-exposed humans administered a chelator.

Various hypotheses have been proposed to explain the carcinogenicity of inorganic As. Nevertheless, molecular mechanisms by which this arsenical induces cancer are still poorly understood. Results of previous studies indicated that inorganic As does not act through classic genotoxic and mutagenic mechanisms, but rather may be a tumor promoter that modifies signal transduction pathways involved in cell growth and proliferation (Simeonova and Luster 2000). Inorganic As (III) has been shown to modulate expression and/or DNA-binding activities of several key transcription factors, including nuclear factor kappa B, tumor suppressor 53 (*p53*), and activating protein-1 (AP-1) (Hu et al. 2002; Chang et al. 2009). Mechanisms of AP-1 activation by trivalent inorganic As include stimulation of the mitogen-activated protein kinase (MAPK) cascade with a consequent increase in the expression and/or phosphorylation of the two major AP-1 constituents, *c-Jun* and *c-Fos* (Hu et al. 2002; Sanchez et al. 2009). Several studies have examined *p53* gene expression and mutation in tumors obtained from subjects with a history of As ingestion. The *p53* participates in many cellular functions, cell-cycle control, DNA repair, differentiation, and apoptosis (Yu et al. 2008; Kim et al. 2011). The tumor suppressor protein *p53* is one component of the DNA damage response

pathway in mammalian cells. Some of these normal cellular functions of *p53* can be modulated and sometimes inhibited by interactions with either cellular proteins or oncoviral proteins of certain DNA viruses (Yu et al. 2008; Qu et al. 2009).

Conclusions

Integration of the available scientific information on As indicates that geogenic and anthropogenic contamination of natural resources represents a major public health problem in many countries of the world. Exposure to As occurs via ingestion, inhalation, and dermal contact. Such exposure has been associated with a significant number of systemic health effects in various organs and tissue systems including skin, lung, liver, kidney, bladder, gastrointestinal tract, reproduction system, respiratory system, and hematopoietic system. Evidence from recent studies has linked As consumption in drinking water with two noncancer health conditions (hypertension and diabetes mellitus) that are a major cause of morbidity and mortality.

Experiments in animals and in vitro have demonstrated that As has many biochemical and cytotoxic effects at low doses of human exposure. Those effects include induction of oxidative damage, altered DNA methylation and gene expression, changes in intracellular levels of *p53* protein, inhibition of thioredoxin reductase, inhibition of pyruvate dehydrogenase, induction of protein-DNA cross-links, altered regulation of DNA-repair genes, glutathione reductase and other stress-response pathways, and induction of apoptosis. Additionally, the evidence of carcinogenicity in humans is very strong, especially for cancer of the skin, lung, liver, kidney, and bladder. Further epidemiological studies are highly recommended to investigate the dose-response relationship between As ingestion and noncancer endpoints. Because of the very large populations exposed, these endpoints are common causes of morbidity and mortality, even small increases in relative risk at low doses of As exposure could be of considerable public health significance. Such information is also important in developing a comprehensive risk assessment and management program for As.

Cross-References

- ▶ Arsenic in Nature
- ▶ Arsenic in Pathological Conditions
- ▶ Arsenic Methyltransferases
- ▶ Arsenic, Free Radical and Oxidative Stress
- ▶ Arsenic-Induced Stress Proteins

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Arsenic Accumulation in Yeast

► Arsenic and Yeast Aquaglyceroporin

Arsenic and Alcohol, Combined Toxicity

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Synonyms

Combined toxicity of arsenic and alcohol drinking; Synergistic toxicity of arsenic and ethanol

Definition

Combined toxicity of arsenic and alcohol refers to harmful effects resulting from exposures to arsenic and alcohol (or ethanol) at the same time. In some cases, the combined toxicity is synergistic. The most common form of the exposures is intake of water and alcohol.

Introduction

Historically, co-exposures to arsenic and alcohols caused severe diseases. In the early 1900s, there were over 6,000 cases and 70 deaths from heart diseases attributed to drinking arsenic-contaminated beer in England (Cullen 2008). Wine fermented from grapes treated with arsenical fungicides resulted in peripheral vascular disease and cardiomyopathies in a group of German vintners in the 1920s (Engel et al. 1994). A recent study reported the presence of As in sweet little Gabonese palm wine in Gabon (Mavioga et al. 2009). In the USA, arsenic-contaminated moonshine was implicated as the cause of a dozen cases of cardiovascular diseases in the state of Georgia (Gerhardt et al. 1980). In these incidents, had the patients taken the amount of arsenic in their drinks alone or the amount of alcohol alone, such severe damages would not have occurred. This indicates that the co-exposure made alcohol and arsenic toxic at their nontoxic concentrations.

To date, there are no epidemiological studies on the population who are co-exposed to arsenic and alcohol and on how severe a public health problem it is. Nonetheless, high prevalence of arsenic exposure and the alcohol epidemic make it highly possible that certain populations are co-exposed to arsenic and alcohol (Bao and Shi 2010).

Alcohol and Arsenic Pharmacokinetics

It is well established that alcohol consumption on a regular basis increases deposition of arsenic and copper in liver and kidney. Higher total urinary arsenic has been reported in alcohol consumers than nonalcoholics (Hsueh et al. 2003). It has also experimentally proven that ethanol increases the uptake and retention in the liver and kidneys (Flora et al. 1997).

Alcohol may facilitate the uptake of arsenic through cell membranes that are damaged and changed in their molecular composition by the direct effect of alcohol. Alcohol consumption may also alter the methylation of arsenic, affecting its distribution and retention in tissues (Tseng 2009).

Action of Alcohol and Arsenic on Proteins and Their Activities

Kinases. Experimentally, co-exposure, but not exposure to arsenic or alcohol alone, increased active Fyn tyrosine kinase, phosphorylation of important proteins such as PKCδ, membrane localization of phospholipase Cγ1, vascular endothelial cell growth factor, and insulin-like growth factor-1 (Klei and Barchowsky 2008).

Redox proteins. Arsenite activates NADPH oxidase (Lynn et al. 2000), which is a major source of intracellular superoxide anion radical ($O_2^{\bullet -}$). Methylated arsenic species can release redox-active iron from ferritin and have synergic effect with ascorbic acid to do so while alcohol elevate serum ferritin levels (Ioannou et al. 2004). Ethanol induces cytochrome P450 2E1 (CYP2E1), which has a high NADPH oxidase activity and produces $O_2^{\bullet -}$, H_2O_2, and hydroxyethyl radicals. Chronic ethanol consumption can result in a 10–20-fold increase in hepatic CYP2E1 in animals and humans. In addition, NO production is increased by the effect of ethanol as well as arsenic on inducible nitric oxide synthase (Kao et al. 2003).

Arsenic reduces plasma and cellular GSH levels. For instance, arsenic drinking (12 mg/kg) for 12 weeks depletes GSH levels in the liver and the brain of rats (Flora 1999). Similarly, possible contributions of impaired antioxidant defenses to ethanol-induced oxidative stress have been extensively investigated. Besides impairing catalase and superoxide dismutase (SOD) activities, alcohol depletes cellular GSH. Fernandez-Checa et al. have found that alcohol intake can lower the hepatic mitochondrial GSH content by 50–85% in rats (Fernandez-Checa et al. 1991). A combined exposure decreased hepatic and plasma GSH more markedly than ethanol or arsenic alone (Flora et al. 1997).

Mitochondrial proteins. Mitochondria are important targets of arsenic and ethanol. Arsenic is accumulated in mitochondria via phosphate transport proteins and the dicarboxylate carrier. Once accumulated in mitochondria, arsenic uncouples the oxidative phosphorylation because ATP synthase undergoes oxidative arsenylations (Crane and Lipmann 1953). Ethanol metabolism promotes a substantial reduction of both cytosolic and mitochondrial NAD, substantially increasing the NADH level. This increase poses an acute metabolic challenge for energy metabolism. Meanwhile, ethanol increases utilization of oxygen mainly through ethanol oxidation, resulting in localized and transient hypoxia. In recent studies, the mitochondria permeability transition has been identified as a key step for the induction of mitochondrial cytochrome c release and caspase activation by ethanol (Kurose et al. 1997; Higuchi et al. 2001). The harmful effects of arsenic on the enzymes of antioxidant defense systems such as thioredoxin reductase will potentiate ethanol's effect on membrane permeability, mtDNA damage, and mitochondrial dysfunction, which will cause more mitochondrial dysfunction and damage.

Proteins in DNA methylation, damage, and repair. DNA methylation is an important determinant in controlling gene expression whereby hypermethylation has a silencing effect on genes and hypomethylation may lead to increased gene expression. Ethanol can interact with one carbon metabolism and DNA methylation. It reduces the activity of methionine synthase which remethylates homocysteine to methionine with methyltetrahydrofolate as the methyl donor. It inhibits the activity of DNA methylase which transfers methyl groups to DNA in rats.

Arsenic is also able to induce DNA hyper- and hypomethylation (Lee et al. 1985). Arsenic interferes with DNA methyltransferases, resulting in inactivation of tumor suppressor genes through DNA hypermethylation. When co-exposed to alcohol and arsenic, cells are exposed to combined effects on modification of DNA methylation.

Acetaldehyde is the immediate product of the ethanol metabolism by alcohol dehydrogenase. Acetaldehyde causes point mutations in the hypoxanthine phosphoribosyltransferase 1 locus in human lymphocytes, and induces sister chromatid exchanges and gross chromosomal aberrations. It can bind to proteins such as *O*-6-methylguanine-DNA methyltransferase.

It is well known that arsenic interferes with DNA repair system. A link between the enhancing effects

and inhibition of DNA repair processes has been documented by Okui and Fujiwara (1986). The interaction of arsenite with the removal of DNA damage induced by *N*-methyl-*N*-nitrosourea (MNU) has been characterized by Li and Rossman (1989). They observed an accumulation of DNA strand breaks after MNU treatment in the presence of arsenite in permeabilized V79 cells, indicating the inhibition of a later step of base excision repair.

Summary

Alcohol consumption and arsenic are two high risk factors for human health. Co-exposure to alcohol and Arsenic has potential synergistic effects on many protein activities, which play important role in maintaining cellular functions such as phosphorylation, redox homeostasis, mitochondrial functions, and DNA repair. Besides necessary epidemiological studies to assess the prevalence of the co-exposure, future studies are needed to clarify the exact mechanisms and identify potential pathways for prevention and therapeutic treatments for co-exposure of arsenic and alcohol.

Cross-References

- ▶ Arsenic
- ▶ Arsenic in Nature
- ▶ Arsenic Methyltransferases
- ▶ Arsenic and Primary Human Cells
- ▶ Arsenic, Biologically Active Compounds
- ▶ Arsenic, Free Radical and Oxidative Stress

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Arsenic and Aquaporins

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Synonyms

Aquaporin-mediated arsenic transport; Arsenic channels; Major intrinsic proteins and arsenic transport

Definition

Aquaporins or major intrinsic proteins (MIPs) are transmembrane channel proteins, which facilitate the passive and bidirectional diffusion of water and a variety of small and noncharged compounds across biological membranes. Aquaporins are found in organisms of all kingdoms of life and are present in all main subcellular membrane systems. The substrate specificity/spectra of aquaporins are highly isoform-dependent. Several isoforms from bacteria, protozoa, yeasts, mammals, fishes, and plants were shown to facilitate the transmembrane diffusion of diverse arsenic species (i.e., arsenite, monomethylarsonous acid, monomethylarsonic acid, and dimethylarsinic acid). All organisms face the challenge to handle considerable variations in the concentration of metalloids which they are exposed to, in terms of either the demand to acquire sufficient amounts for their metabolism or, conversely, the necessity to extrude them to prevent toxicity. This is achieved through homeostatic processes that require, among others, aquaporin-mediated transport across membranes at the cellular level.

Chemical Prerequisites for Metalloid Substrates to Pass Through Aquaporins

Aquaporins are membrane proteins known as diffusion facilitators for water and small uncharged solutes (see ▶ Aquaporins and Transport of Metalloids). Among the latter, the inorganic undissociated arsenite ($As(OH)_3$) molecule fulfills all requirements to pass through certain aquaporins. The molecular volume, electrostatic charge distribution, polarity, and capacity to form hydrogen bonds strikingly resemble the glycerol molecule, the typical substrate of aquaglyceroporins (Porquet and Filella 2007). Furthermore, with pKa_1, pKa_2, and pKa_3 values of 9.2, 12.1, and 13.4, respectively, arsenite is mostly undissociated at normal physiological pH conditions (>94% undissociated molecules at $pH < 8$) and, therefore, represents a typical substrate for aquaporins (Zhao et al. 2010b; Bienert et al. 2008b). Antimonite ($Sb(OH)_3$) is highly similar to arsenite both in terms of (bio) chemical reactivity and in physicochemical parameters (Porquet and Filella 2007).

Aquaporin-Mediated Arsenic and Antimony Transport in Bacteria

Important contributions to metalloid and in particular arsenic transport pathways and detoxification systems in bacteria were made by Rosen and collaborators. In a screen aiming at identifying metalloid transporters in *Escherichia coli*, they isolated a mutant that was resistant to extracellularly applied antimonite. The gene carrying the mutation turned out to be the GlpF aquaglyceroporin. EcGlpF is the major player for arsenite and antimonite uptake into *E. coli* (Rosen and Tamas 2010). As aquaglyceroporins are widespread in prokaryotes, it is likely that bioavailable arsenite is adventitiously taken up by these channels. Consequently, active bacterial arsenic efflux pumps have to transport it out of the cells again. It is also obvious that aquaglyceroporins can be used to detoxify bacteria from arsenite, especially when grown in oxidizing conditions. In these conditions, arsenate is the predominant arsenic species and is taken up by phosphate transporters, which cannot distinguish between arsenate and phosphate molecules due to chemical similarity. Arsenate is reduced to arsenite by arsenate reductases, which then has to be pumped out actively in a process costing metabolic energy (Rosen and Tamas 2010). However, export could occur without the cost of metabolic energy via passive diffusion through aquaglyceroporins driven by the naturally occurring chemical gradient towards the outside of the cells. That bacterial aquaglyceroporins might be used for arsenic detoxification stayed a hypothesis until Rosen and coworkers discovered that an aquaglyceroporin (AqpS) is encoded by a gene found

in the arsenic resistance (*ars*) operon in specific bacteria such as *Sinorhizobium meliloti*. In this bacterial strain, it replaces the typically occurring *arsB* gene, which encodes an active arsenite extrusion transporter (Rosen and Tamas 2010). The combination of AqpS and the arsenate reductase ArsC forms an effective pathway for cell arsenate detoxification. This provides a clear evidence that aquaporin-mediated arsenic transport is not only adventitious and responsible for arsenic influx but plays a physiological role in arsenite efflux and detoxification. The exchange of *arsB*, encoding an ATP-driven arsenite extrusion pump found in many bacterial *ars* operons, by *aqp*S, encoding an aquaglyceroporin, is an interesting example of how bacteria develop genetic and metabolic strategies to be optimally adapted to all kinds of ecological niches and environments. Another exciting discovery of such an evolutional optimization event in bacteria with respect to arsenic detoxification was made by Beitz and coworkers. They identified that actinobacteria from soil (*Frankia alni*) and marine environments (*Salinispora tropica*) genetically combined the two-step process of arsenate reduction by ArsC and the extrusion by the ATP-driven arsenite extrusion pump (ArsB) to a one-step resistance process mediated by a single gene (Wu et al. 2010). This gene is translated into a protein which consists of the fusion of an aquaglyceroporin to a C-terminal arsenate reductase domain of a phosphotyrosine-phosphatase origin. These bacteria engineered a functional system, which is constituted by one protein enzymatically generating the substrate of its transport capacity. This allows increasing the concentration of the toxic substrate only locally at the place of transport, preventing toxic cellular accumulation (Wu et al. 2010). This genetic fusion of a metabolic arsenate-reducing enzyme to an arsenic-permeable aquaglyceroporin is another example for the physiological involvement of aquaporins in arsenic detoxification and transport.

Aquaporin-Mediated Arsenic Transport in Yeast

The best-characterized aquaglyceroporin from yeast is the glycerol facilitator Fps1 from *Saccharomyces cerevisiae*, which is the functional homolog of the GlpF aquaglyceroporin from *E. coli*. The major physiological role of Fps1p is the regulation of intracellular levels of glycerol in response to changing osmotic conditions and stresses (Rosen and Tamas 2010). In addition, based on both genetic and direct transport measurements, it was shown that Fps1p mediates the bidirectional transmembrane transport of arsenite and antimonite (Rosen and Tamas 2010; Wysocki and Tamás 2010; Maciaszczyk-Dziubinska et al. 2012). Interestingly, regulation of Fps1 at the transcriptional and posttranscriptional levels physiologically controls its channel function with respect to different substrates. When *S. cerevisiae* is externally exposed to either antimonite or arsenite, the transcription of the *Fps1* gene is rapidly downregulated to decrease the total amount of proteins and reduce the entry of metalloids. Furthermore, the activity of plasma membrane-localized Fps1 proteins is inhibited through phosphorylation of an amino acid residue located in the N-terminus, resulting in channel closure (Rosen and Tamas 2010; Wysocki and Tamás 2010). It is assumed that metalloids activate the mitogen-activated protein kinase Hog1p which subsequently phosphorylates and closes Fps1p. Altogether, these data indicate that Fps1p is involved in the physiological regulation of metalloid transport processes in *S. cerevisiae* (Rosen and Tamas 2010; Wysocki and Tamás 2010).

Aquaporin-Mediated Arsenic and Antimony Transport in Protozoan Species

The adventitious metalloid transport capacity of microbial aquaglyceroporins represents one of the molecular bases for an effective use of arsenic and antimony-containing drugs. Such drugs have been used against protozoan parasitic infections since the pre-antibiotic era. The arsenic-containing drug Melarsoprol and the antimony-containing drugs Pentostam and Glucantime are still used as the first-line treatments against leishmaniasis and sleeping sickness caused by trypanosomes and have not been replaced by other therapeutic agents since more than 40 years (Mukhopadhyay and Beitz 2010). All three aquaglyceroporins identified in *Trypanosoma brucei* are proposed to be involved in osmoregulation and glycerol transport and have been demonstrated to channel antimonite and arsenite (Mukhopadhyay and Beitz 2010). Whether the metalloid transport capacity is responsible for the uptake of metalloid-containing drugs and whether drug resistance is due to a reduced

drug uptake into the parasites are not known yet. However, this link has been revealed for aquaglyceroporins from *Leishmania* species. For example, antimony-resistant isolates of *Leishmania donovani* showed a downregulation of one of their aquaglyceroporins, resulting in a reduced uptake of antimony (Mukhopadhyay and Beitz 2010). *Leishmania major* possesses five aquaporins including LmAQP1, which is permeable to arsenite and antimonite. Disruption of *LmAQP1* gene conferred increased antimony resistance while *Leishmania* overexpressing this isoform became hypersensitive to antimony-containing drugs (Mukhopadhyay and Beitz 2010). Altogether, these data suggest that adventitious metalloid transport by parasitic aquaglyceroporins crucially contributes to their high sensitivity to metalloid-containing drugs. On the other hand, parasites may develop drug resistance by suppressing or minimizing channel abundance under stress conditions or by changing channel properties. This latter adaptation might be achieved by maintaining selectivity of the channel for physiologically essential substrates while making it impermeable to detrimental metalloid species.

Aquaporin-Mediated Arsenic Transport in Mammals

Human and other mammalian genomes encode 13 aquaporins (AQP0–AQP12). AQP3, AQP7, AQP9, and AQP10 are aquaglyceroporins (Verkman 2011). The ability of AQP7 and AQP9 from rat, mouse, and human to transport arsenite and antimonite species was first demonstrated in transport assays performed in heterologous expression systems (*S. cerevisiae* and *Xenopus laevis* oocytes) (Liu 2010). In contrast, human AQP3 and AQP10 are not able to significantly transport metalloids in these systems. AQP9 displays the highest metalloid transport capacity and became a convenient model to further study metalloid transport of mammalian aquaglyceroporins (Liu 2010). Biochemical transport assays and mutagenesis approaches revealed that metalloids and glycerol use the same translocation pathway through AQP9. Moreover, AQP9 transports the organic methylated arsenic species monomethylarsonous acid (MMAs(III)), monomethylarsonic acid (MMAs(V)), and dimethylarsinic acid (DMAs(V)) (Liu 2010). In humans, arsenite is methylated in the liver to MMAs(V), MMAs(III), DMAs(V), and dimethylarsinous acid DMAs(III); and these compounds are then excreted into the bile and urine and cleared from the body (Liu 2010). This led to the hypothesis that, in addition to its role in arsenite uptake from the blood into the liver, AQP9 is also involved in the export of methylated derivatives of arsenic out of the liver back to the blood (Liu 2010). The characterization of AQP9-null mice, which do not express AQP9 anymore, revealed that these mice are more sensitive to arsenite exposure, accumulate more arsenite, and excrete less arsenic in the feces and urine compared to the wild-type mice (Carbrey et al. 2009), supporting the hypothesis that aquaglyceroporins are involved in arsenic detoxification in mammals.

Arsenic- and antimony-containing drugs have been used as pharmaceuticals for more than 2,000 years and were developed and applied systematically since the nineteenth century. Despite being proven human carcinogens, arsenicals are still used nowadays in therapies against a wide range of tumors which are resistant to other treatments. Arsenic trioxide (As_2O_3) is used as first-line therapeutics against acute promyelocytic leukemia in which an abnormal accumulation of immature leucocytes takes place in the bone marrow (Mukhopadhyay and Beitz 2010). As in the case of microbial aquaglyceroporins, the adventitious transport capacity of mammalian aquaglyceroporins for metalloids might be one of the reasons why arsenite trioxide represents such a powerful antitumor agent both in vitro and in vivo (Mukhopadhyay and Beitz 2010; Bienert et al. 2008b). Human leukemia cells overexpressing AQP9 accumulate more metalloids and concomitantly become hypersensitive to arsenic and antimony agents (Mukhopadhyay and Beitz 2010). AQP9 expression in leukemia cells of different cell lines correlated directly to arsenic trioxide sensitivity (Mukhopadhyay and Beitz 2010). Downregulation of aquaglyceroporins in cancer cells might turn them insensitive to arsenic-containing drugs. As an example, a human lung adenocarcinoma cell line, which became significantly more drug-resistant compared to the parental cell line, accumulated less arsenic which was correlated to a concomitant downregulation of AQP3 (Mukhopadhyay and Beitz 2010). The development of pharmacological compounds able to increase the expression or activity of aquaglyceroporins could increase the efficiency of arsenic-containing drugs. Two such substances have already been described: vitamin D and all-trans-retinoic acid, which both

increase the expression of AQP9 in cancer cells and, concomitantly, their hypersensitivity to arsenic trioxide due to an increased arsenic uptake rate (reviewed in Bienert et al. 2008b; Mukhopadhyay and Beitz 2010).

Aquaporin-Mediated Arsenic and Antimony Transport in Plants

Physiological studies in plants suggested that channel-mediated passive diffusion is a major uptake mechanism for the noncharged arsenite molecule, and it was suggested that aquaporins are responsible for this transport capacity (Meharg and Jardine 2003). Among the five subfamilies of aquaporins found in higher plants (the plasma membrane intrinsic proteins (PIPs), the tonoplast intrinsic proteins (TIPs), the small-basic intrinsic proteins (SIPs), the nodulin26-like intrinsic proteins (NIPs), and the X-intrinsic proteins (XIPs) (▶ Aquaporins and Transport of Metalloids), NIPs represent the structural and functional analogs of microbial and mammalian aquaglyceroporins in terms of steric channel properties and substrate spectra (reviewed in Bienert et al. 2008b). NIPs mediate the transport of a wide range of small uncharged molecules including ammonia, hydrogen peroxide, urea, lactic acid, glycerol, boric acid, and silicic acid (Bienert and Chaumont 2011) and, therefore, were obvious candidates for testing their ability to channel arsenite and antimonite across membranes. When expressed in *S. cerevisiae* mutants which are highly resistant to elevated (externally applied) arsenite levels, NIPs from *Arabidopsis thaliana* (AtNIP5;1, AtNIP6;1, and AtNIP7;1), *Oryza sativa* (OsNIP2;1, OsNIP2;2, and OsNIP3;2), and *Lotus japonicus* (LjNIP5;1 and LjNIP6;1) significantly increased the cell sensitivity to arsenite and antimonite (Bienert et al. 2008a). It was further shown that this increased sensitivity is directly linked to an increased uptake of the toxic metalloids.

Other transport studies using Xenopus oocytes demonstrated a permeability to arsenite of NIPs from *A. thaliana* (AtNIP1;1, AtNIP1;2, AtNIP5;1, and AtNIP7;1) and *O. sativa* (OsNIP1;1, OsNIP2;1, OsNIP2;2, and OsNIP3;1) (Bienert and Jahn 2010; Zhao et al. 2010b). The impact of NIPs on arsenite uptake, accumulation, and tolerance was demonstrated to be of major biological relevance using *nip* knockout mutants in both Arabidopsis and rice. In Arabidopsis, a forward genetic screen testing the growth behavior of mutagenized lines on medium containing root growth-inhibiting concentrations of arsenite led to the isolation of three independent arsenite-tolerant lines, all of them having a mutation in the *AtNIP1;1* gene (Kamiya et al. 2009). Further experiments clearly confirmed the dominant role of AtNIP1;1 in arsenite uptake.

In rice, mutated lines for OsNIP2;1 (an aquaporin which functions as an essential silicon influx channel in roots (▶ Silicon and Aquaporins)) were isolated and exhibited a significantly decreased arsenite uptake capacity. This indicated that OsNIP2;1 represents a major uptake pathway for arsenite. At the same time, in arsenate stress condition, OsNIP2;1 promoted arsenic detoxification by channeling arsenite out of the roots (Ma et al. 2008; Zhao et al. 2010a). Information on arsenite entry routes into rice plants is highly valuable for strategies aiming at breeding or engineering low-arsenic-accumulating cultivars. Arsenic contamination of rice-based food products is a public health issue as rice actually accounts for the largest contribution to dietary intake of inorganic arsenic for the population (Zhao et al. 2010b; Bienert and Jahn 2010). High arsenic accumulation in rice is probably promoted by the following facts: (1) rice is cultivated in regions where bioavailable arsenic is present at high levels in soils. (2) Rice is grown in paddy soils, in which arsenite is the predominant arsenic species due to the reductive conditions. Arsenite is more mobile and more bioaccessible than arsenate, which is predominant in oxidative field conditions. (3) Last but not least, rice uses NIP-mediated uptake pathways to ensure assimilation of large amounts of silicon, a physiologically highly beneficial metalloid (▶ Silicon and Aquaporins). However, adventitious uptake of toxic arsenite molecules through these channels cannot be prevented. Moreover, other known metalloid transporters are present and active such as Lsi2 protein which in addition to silicon also transports arsenic species (▶ Silicon and Aquaporins).

OsNIP2;1 also mediates the uptake of undissociated pentavalent MMA and DMA (Zhao et al. 2010b). The *lsi1* rice mutant (standing for "*low silicon 1*") carrying a loss-of-function mutation in OsNIP2;1 took up less than 50% of these organic arsenic species compared to wild-type plants. In nature, these methylated arsenic species derive most probably from soil microorganisms.

In summary, permeability to arsenite (and maybe to some organic arsenic species) seems to be a common feature to all three different subclasses of plant NIPs (NIPI, NIPII, and NIPIII) even though they differ in their amino acid residue composition of their selectivity filter (Danielson and Johanson 2010). Whether some NIPs are involved in arsenic detoxification mechanisms in certain plant species has to be resolved. Differential regulations of NIPs at the transcriptional and post-translational level in response to arsenic and/or other physiologically relevant substrates would suggest such a role.

When heterologously expressed in Xenopus oocytes, three members of the PIP subfamily of rice aquaporins (OsPIP2;4, OsPIP2;6, and OsPIP2;7) significantly increase arsenic uptake compared to control oocytes (Mosa et al. 2012). These PIP isoforms are highly expressed both in the roots and the shoots in standard conditions. Overexpression of these PIPs in Arabidopsis resulted in an increased arsenite tolerance and higher biomass accumulation. Moreover, the lines overexpressing OsPIP2;6 exhibited both influx and efflux capacity of arsenite depending on the metalloid concentration gradient between the external medium and the root (Mosa et al. 2012). Whether other PIPs or plant aquaporin isoforms have the ability to transport arsenic species needs to be investigated as well as their role in plant arsenic homeostasis.

Concluding Remarks

Aquaporins constitute indispensable and high-capacity transport systems for reduced and uncharged forms of the toxic metalloids arsenic and antimony. These channels fulfill physiologically relevant roles in arsenic homeostasis, which is supported by several findings: (1) aquaporin-mediated arsenite transport is conserved in all kingdoms of life, (2) mutants lacking aquaporins are impaired in uptake or extrusion of various arsenic species, (3) aquaporins are regulated at the transcriptional and post-translational level in response to arsenic stress, and (4) aquaporins are part of arsenic resistance operons and form fusion proteins with arsenate reductases in some bacteria, observations providing genetic evidence for their role in arsenic resistance mechanisms.

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Cross-References

▶ Aquaporins and Transport of Metalloids
▶ Boron and Aquaporins
▶ Selenium and Aquaporins
▶ Silicon and Aquaporins

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Arsenic and Primary Human Cells

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Synonyms

Apoptosis; Arsenical compounds; Mode of action

Definition of the Subject

The subject of arsenic and primary human cells refers to the role of arsenical compounds in the physiology of cells of primary origin. The importance of this subject resides in the fact that most of the studies reported in the literature focus on the role of arsenic in cancer cells or in immortalized cancer cell lines, but very few deal with the arsenic-primary cell interaction. Primary cells are those that are directly isolated from blood (immune origin) or isolated from a tissue or organ (nonimmune origin) like epithelial or hepatocyte cells. Because arsenical compounds may be toxic or, paradoxically, could be used in the treatment of diverse diseases, it is important to better understand how these compounds alter primary cells of different origins.

Generalities

Metalloids are elements that are neither metals nor nonmetals and this group includes boron, silicon, germanium, tellurium, antimony, and arsenic. Arsenic (As) is among the most intensively studied elements in the field of metal toxicology. The term "arsenic" is, however, frequently erroneously used in the literature since it includes several distinct arsenical compounds, including the potent anticancer drug arsenic trioxide (ATO). Humans can be exposed to arsenic via air and food, but the major exposure route is through the contaminated drinking water where millions of humans are exposed worldwide to this environmental toxicant (Yoshida et al. 2004). It is well established that arsenic poisoning via groundwater is a worldwide problem; arsenic-contaminated groundwater has been found in aquifers in Bangladesh, China, India, Nepal, Argentina, Mexico, and Taiwan (Nicolli et al. 2012; Yoshida et al.2004). In addition to causing significant problems in the provision of safe drinking water, elevated concentrations of arsenic in water raised also concern regarding food safety. Long-term exposure to arsenic is associated with cancers of the lungs, liver, skin, urinary tract, kidneys, etc. Epidemiologic and experimental evidences support the conjecture that arsenic could play a role in hypertension and cardiovascular diseases (Chen et al. 1995). For example, a positive relationship between inorganic arsenic exposure from drinking water and hypertension was reported in epidemiologic studies conducted in arsenic-endemic areas in Taiwan and Bangladesh (Chen et al. 1995, 2007; Rahman et al. 1999).

Paradoxically, arsenic compounds have been used in traditional oriental medicine to treat a variety of diseases other than cancers, such as inflammatory diseases (Sears 1988). Experimental studies have indicated that arsenic exposure may be involved in the development of hypertension through the promotion of inflammation, oxidative stress, and endothelial dysfunction (Aposhian et al. 2003; Balakumar et al. 2008). However, it was only in the 1970s that arsenic agents were found to be highly effective for treating several types of leukemia (Sears 1988). To date, ATO is considered to be one of the most potent drugs for chemotherapy of cancers. In addition, arsenic was recently

reported to be a potential candidate for the treatment of a variety of diseases, including arthritis (Mei et al. 2011) and asthma (Chu et al. 2010).

Route of Transport of Arsenic Through the Cell Membrane

Like other metalloids, arsenic passes through cell membrane via channels involved in passive transport, such as aquaporins and aquaglyceroporins (Zangi and Filella 2012). While aquaporins selectively conduct water molecules, aquaglyceroporins will transport also other small uncharged molecules. They are bidirectional channels operating via passive diffusion. The direction of the transport depends on the concentration gradient across the membrane; if the intracellular concentration of the solute is too high and causes damage, the channel will facilitate elimination of the compound out of the cell if the extracellular concentration is lower. Therefore, there are no specific receptors identified for arsenical compounds. Nevertheless, the role of aquaporin 9 in accumulation of arsenic and its cytotoxicity in primary mouse hepatocytes was reported (Shinkai et al. 2009). Aquaporin 9 is a member of the aquaglyceroporin subfamily of aquaporins involved in the transfer of water and small solutes, including arsenite. Since arsenic toxicity is largely associated with intracellular accumulation of this metalloid inside the cells, pretreatment with sorbitol, a competitive inhibitor of aquaporin 9, and siRNA-mediated knockdown of aquaporin 9 were found to significantly decrease the arsenite uptake in the cell and its cytotoxicity, suggesting that aquaporin 9 is a channel for arsenite in primary mouse hepatocytes.

Toxicity and Mode of Action

The toxicity of arsenic compounds is partly explained by their ability to bind and inactivate several sulfhydryl-containing proteins and enzyme systems. Oxidative stress is certainly among the more documented mechanisms of arsenic toxicity. Arsenic acts by causing a marked imbalance between production of reactive oxygen species (ROS) and antioxidant defense resulting in alteration of the cellular redox status. This is explained by the fact that it is the balance between ROS production and antioxidant defenses that determines the degree of oxidative stress. However, the precise mechanisms by which arsenic induces oxidative stress are not yet fully understood but it is well accepted that ROS exert their effects by modulating cell apoptosis. Thus, the principal mode of action of arsenic is via its ability to induce cell apoptosis via ROS but also via caspases activation (Miller et al. 2002). The cytoskeleton, especially the microtubules, is an important cellular target for arsenic, probably due to the high sulfhydryl protein content of cytoskeletal components. However, in addition to microtubules, intermediate filaments and microfilaments are also important targets of arsenic. For example, ATO induces the cleavage of several microfilament-associated proteins, including myosin, paxillin, gelsolin, and actin as well as of the two intermediate filament proteins lamin B1 and vimentin. The cleavage of these cytoskeletal proteins occurs via caspase activation since treatment of cells with caspase inhibitors reversed the ability of arsenic to induce the cleavage of these proteins (Binet et al. 2006). Of note, ATO induces cell differentiation when used at a low concentration (ranging between 0.5 and 5 μM), while it induces cell apoptosis at concentrations ranging from 5 to 20 μM in a variety of cell types. Treatment of acute promyelocytic leukemia (APL) with ATO is associated with the disappearance of the PML-RARα fusion transcript, the characteristic APL gene product of the chromosomal translocation t(15;17). ATO can affect the PML portion of the aberrant protein since the PML is characterized by the presence of a cysteine-rich region.

Cell Signaling

Arsenic is known to activate the mitogen-activated protein kinase (MAPK) pathway in a variety of cancer cells. For example, ATO-induced U937 cell apoptosis was found to be dependent on activation of p38 and inactivation of extracellular signal-regulated kinases-1/2 (ERK-1/2). The p38 mitogen-activated protein kinase pathway was also involved in ATO-induced human acute promyelocytic leukemia (APL) NB-4 cells, K562 CML-blast crisis cell line as well as in the MCF-7 human breast carcinoma and the LNKAP prostate carcinoma cell lines (Binet et al. 2009). Activation of c-jun N-terminal kinase (JNK) is also an event occurring in ATO-induced apoptosis in APL. Recently, it was demonstrated that ATO could downregulate survivin, a member of the inhibitor of apoptosis family that is highly expressed in various cancer cells, via activation of both p38 and JNK in apoptotic human lung adenocarcinoma cell line

H1355. In neutrophils, ATO was found to recruit p38 mitogen-activated protein kinase and/or c-jun NH_2-terminal MAPK but not ERK-1/2 (Binet and Girard 2008). Therefore, ATO can act by modulating all the three major MAPK pathways, namely, p38, Erk-1/2, and JNK, but a certain selectivity exists depending on the cell types since the activation of these three MAPKs is not necessarily systematically observed.

Arsenic and Primary Cells

Although the vast majority of studies investigating the role of arsenic compounds in cell physiology have been conducted with cancer cell lines or in immature cancer cells isolated from patients, their roles on primary cells have retained less attention (Binet et al. 2009). Due to its high efficiency for the treatment of APL, ATO is certainly one of the most studied arsenical compounds. Cells of immune origin, including B or T lymphocytes, monocytes, macrophages, and, more recently, neutrophils, are important targets of arsenical compounds.

As for a variety of cancer cell lines, the main mechanism of action of arsenic in primary immune cells is also via induction of cell apoptosis. Inorganic arsenic was found to significantly repress major functions of human T-lymphocytes and macrophages (Lemarie et al. 2006, 2008). Arsenic blocks the differentiation of blood monocytes into functional macrophages by inhibiting survival signaling pathways and, at non-cytotoxic concentrations, arsenic reverses, at least partially, the phenotypic and genotypic features of mature macrophages (Bourdonnay et al. 2009). While both ATO and ascorbic acid mediate cytotoxicity in chronic lymphocytic leukemia B lymphocytes when used alone, the efficacy of ATO is enhanced by ascorbic acid (Biswas et al. 2010). In vitro experiments conducted with peripheral blood mononuclear cells from healthy individuals indicated that low concentrations of arsenic tended to increase the number of natural T regulatory lymphocytes. In contrast, higher concentrations (>5.0 μM) decreased the cell number (Hernandez-Castro et al. 2009). In neutrophils, ATO induced apoptosis by a mechanism involving caspase activation, cytoskeletal breakdown, ROS production, and de novo protein synthesis. ATO also induced an endoplasmic reticulum stress in these cells (Binet et al. 2006, 2009, 2010). Using pharmacological inhibitors, the proapoptotic activity of ATO was found to occur by a MAPK-independent mechanism. In contrast, the ability of ATO to enhance adhesion, migration, phagocytosis, release, and activity of gelatinase, but not azurophilic granules, is dependent upon activation of p38 and/or JNK.

Recently, the role of arsenic in a variety of cells of primary origin other than immune cells has been documented. Arsenite (sodium meta-arsenite, $NaAsO_2$) was found to decrease the expression of tumor necrosis factor-alpha and vascular endothelial growth factor, two important inflammatory mediators, in primary human hepatocytes (Noreault-Conti et al. 2012). In another study, this arsenical compound inhibited glutamate metabolism in human primary cultured astrocytes probably responsible or partially involved in arsenic-induced neurotoxicity (Zhao et al. 2012). Investigating the differential sensitivity of primary human cultured chorion and amnion cells prepared from fetal membranes to As^{3+}, aquaporin 9 and multidrug resistance-associated protein were found to be involved in the control of cellular As^{3+} accumulation (Yoshino et al. 2011). While ATO is known to induce apoptosis in various cancer cells including lung cancer cells, little is known about the toxicological effects of ATO on normal primary lung cells. In one study, the cellular effects of ATO on human pulmonary fibroblast cells in relation to cell growth inhibition and death were reported (Park and Kim 2012). ATO was found to inhibit the cell growth with an IC(50) of ~30–40 μM at 24 h and induced cell death. This was accompanied by the loss of mitochondrial membrane potential. Human pulmonary fibroblasts were considered to be highly resistant to ATO. The mode of action involved an increased expression of p53 and decrease of the antiapoptotic Bcl-2 protein. Interestingly, ATO activated caspase-8 but not caspase-3 in these cells and administration of caspase-8 siRNA attenuated pulmonary fibroblast cell death whereas caspase-3 siRNAs did not, indicating that ATO induced the growth inhibition and death in these cells via caspase-8 and not caspase-3. A possible involvement of arsenic in Alzheimer's disease has been recently proposed (Gong and O'Bryant 2010). In this respect, a study reported that sodium arsenite and its main metabolite, dimethylarsinic acid, affected the expression and processing of the amyloid precursor protein, using primary neuronal cells. It was demonstrated that sodium arsenite and dimethylarsinic acid up-regulated the expression and processing of

amyloid precursor protein in vitro. After proteolysis, this precursor protein generates beta amyloid, the primary component of amyloid plaques found in the brains of Alzheimer's disease patients (Zarazua et al. 2011). In an immunotoxicological study, ATO was administered in mice (inhalation exposure) and spleen was used for several assays. No spleen cell cytotoxicity was observed and there were no changes in spleen cell surface marker expression for B and T lymphocytes, macrophages, and natural killer cells (Burchiel et al. 2009). Also, the cell proliferation of both B and T lymphocytes in response to mitogens was not affected by arsenic treatment, and no changes were found in the natural killer-mediated lysis of Yac-1 target cells. However, the primary T-dependent antibody response was highly susceptible to arsenic suppression. Because arsenic is associated with cardiac toxicity, a study was conducted in order to evaluate the cytotoxic effect of ATO on cardiac myocytes (Raghu and Cherian 2009). To do so, primary culture of rat myocytes was treated with different concentrations of ATO for various periods of time and the cardiac toxicity was assessed by monitoring cell viability, mitochondrial and deoxyribonucleic acid integrity, ROS generation, calcium overload, and cell apoptosis. ATO was found to alter mitochondrial integrity, generation of ROS, calcium overload, and apoptosis in a concentration- and time-dependent manner. However, no DNA fragmentation was observed. Therefore, it was concluded that ATO induces apoptosis in cardiomyocytes by generation of ROS and the induction of calcium overload. Exposure of mouse fetal liver cells to sodium arsenite ($NaAsO_2$) was found to induce adaptive responses and aberrant gene expression (Liu et al. 2008). For example, expression of genes related to steroid metabolism, such as 17beta-hydroxysteroid dehydrogenase-7 and Cyp2a4, was increased approximately twofold. This study indicates that the aberrant gene expression observed in response to arsenic insults could alter genetic programming very early in life, potentially contributing to tumor formation later in life. Primary rat vascular smooth muscle cells were isolated from aortic explants from adult Sprague Dawley rats and were exposed to arsenic ($NaAsO_2$) in order to elucidate cell signaling events occurring in response to arsenic insults (Pysher et al. 2008). It was observed that arsenic can alter focal adhesion protein co-association leading to activation of downstream pathways. More specifically, arsenic caused a sustained increase in focal adhesion kinase-Src association and activation, and stimulation of downstream PAK, ERK and JNK pathways known to be involved in cellular survival, growth, proliferation, and migration in vascular smooth muscle cells.

All of the above studies clearly indicated that arsenical compounds can alter the physiology of primary cells of a variety of origins. This includes cells of immune origin like B and T lymphocytes, natural killer cells, monocytes, macrophages and neutrophils and in cells of nonimmune origin like hepatocytes, astrocytes, chorion and amnion cells prepared from fetal membranes, pulmonary fibroblasts, neuronal cells, myocytes, fetal liver cells, and vascular smooth muscle cells. Also, these studies demonstrate the importance to pursue investigations of primary cells in response to arsenic insults in order to better control potential arsenic toxicity in cells of primary origin and, in parallel, to better elucidate the complex mode of action of arsenical compounds, including the cell signaling pathways involved in a biological response.

Utilization of Arsenical Compounds in Combination with Other Agents

In addition to its direct effect on cells of primary origin, the utilization of arsenic in combination with other agents is becoming a very interesting avenue of research for the development of therapeutic strategies. The main objective is to decrease the concentration as much as possible of one arsenical compound and to mix it with another agent, limiting potential toxic effects. For example, ATO has been recently used in combination with silibinin, a natural polyphenolic flavonoid, in glioblastoma multiform cell line, U87MG and the results showed that ATO, in some cases, improved and/or complemented the anticancer effects, suggesting a new combination therapy for the highly invasive human glioma treatment (Dizaji et al. 2012). Combined administration of suberoylanilide hydroxamic acid and ATO was recently found to be an effective approach to the treatment of lung cancer (Chien et al. 2011). In combination with blocking monoclonal antibodies against CD154 and LFA-1, ATO was found to prolong heart allograft survival in allo-primed T cells–transferred mice (Lin et al. 2011). Genistein, an isoflavone known to inhibit tyrosine kinases, was reported to potentiate the effect of ATO in human hepatocellular carcinoma (Ma et al. 2011).

Interestingly, one study reported that Imatinib Mesylate induced mainly the intrinsic pathway of cell apoptosis, whereas ATO induced the endoplasmic reticulum stress-mediated pathway of cell apoptosis and that the combination of these two anticancer drugs was more effective for inducing the intrinsic, extrinsic, and ER stress-mediated pathways of cell apoptosis, resulting in a more effective and efficient induction of apoptosis in K562 cancer cells (Du et al. 2006). Therefore, in cancer therapy, it could be possible to further accelerate cell apoptosis using drugs that when combined together will activate different cell apoptotic pathways. In human peripheral blood lymphocytes, a combination of curcumin with arsenic was found to ameliorate the toxic effect of arsenic when used alone by reducing the frequency of structural aberrations, hypoploidy and primary DNA damage (Tiwari and Rao 2010).

This strategy is not restricted to ATO, since other arsenical compounds are known to act in synergy with different drugs. Arsenic disulfide (As_2S_2) was reported to synergize with a phosphoinositide 3-kinase inhibitor (PI-103) to eradicate acute myeloid leukemia stem cells by inducing their differentiation (Hong et al. 2011). Tetra-arsenic oxide (As_4O_6), in combination with paclitaxel, was found to increase apoptosis in vitro, in gastric, cervix and head and neck cancer cell lines (Chung et al. 2009). A combination of arsenic sulfide (As_4S_4) and Imatinib was found to possess more profound therapeutic effects than As_4S_4 or Imatinib used alone in a mouse model of chronic myeloid leukemia (Zhang et al. 2009).

Cross-References

- ▶ Arsenic and Aquaporins
- ▶ Arsenic in Nature
- ▶ Arsenic in Therapy
- ▶ Arsenic, Free Radical and Oxidative Stress

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Arsenic and Vertebrate Aquaglyceroporins

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Synonyms

Arsenic channels; Arsenic transporters

Definitions

Arsenic (As): A column VI element sharing similar properties with phosphate (P) and antimony (Sb). Dissolved arsenic usually exists in trivalent (As^{III}) and pentavalent forms (As^{V}) which are common contaminants in aquatic environments and human agriculture. Arsenic is widely and unevenly distributed

globally and poses a major health concern, with drinking water and food sources in many areas far surpassing the 10 ppb (parts per billion) arsenic limit set by the World Health Organization.

Aquaglyceroporin: A highly conserved, evolutionarily ancient member of the major facilitator superfamily (MFS) and subgroup of the aquaporin family of membrane channels that permeate water, glycerol, and other noncharged molecules (Liu 2010). Channels in this family present in all domains and play important roles in osmolarity regulation and nutrient uptake. Recently, aquaglyceroporins were identified as major means of access for metalloids into cells and play critical roles in metalloid uptake and detoxification.

Arsenic chemical forms and toxicology: Most immobile arsenic coexists with other elements in ores. Soluble arsenic exists naturally or is produced by microbial activity. Arsenic can have −3, +3, and +5 valence and exists in many forms of compounds. The inorganic soluble forms include trivalent arsenite (As^{III}, $As(OH)_3$, or As_3O_3), and pentavalent arsenate (As^V, AsO_4^{3-}). Metabolism of these inorganic species by prokaryotes and eukaryotes produces a profile of organic species including MMA^{III} (monomethylarsonous acid, $CH_4As(OH)_2$), MMA^V (monomethylarsonic acid, $CH_4AsO(OH)_2$), DMA^{III} (dimethylarsonous acid, $(CH_3)_2AsO(OH)$), TMA (trimethylarsine, $(CH_3)_3As$ (gas)) and TMAO (trimethylarsine oxide, $(CH_3)_3AsO$). Marine organisms can metabolize inorganic arsenic into the same species in addition to nontoxic arsenobetaine (ArsB) (Thomas 2007). Each arsenic species has different structures, cellular targets, carcinogenicity, and overall toxicity. In general, the cellular toxicity is ranked from most to least toxic as DMA^{III}, $MMA^{III} > As^{III} > As^V > DMA^V$, MMA^V (Liu 2010). TMAO and ArsB are not considered to cause notable toxicity (Mandal 2002).

Inorganic arsenic causes toxicity through causing dysfunction in many cellular targets. As^V can inactivate ATPases by substituting with the chemically similar phosphate (ref). As^{III} can strongly bind vicinal cysteines, which are present in the active site of many enzymes (Mandal 2002). Inorganic arsenic causes oxidative stress by creating reactive oxygen species (ROS). This can occur directly during its metabolism, and perhaps most significantly, by inhibiting mitochondrial enzymes and leading to increases in the ROS leakage which occurs with oxidative phosphorylation. As the mechanisms of arsenic toxicity are so numerous, a key factor in determining overall arsenic toxicity becomes the capacity to metabolize inorganic arsenic to other forms and the ability of the organism to extrude arsenic from the body.

Depending on the type of arsenic exposure and dosage, arsenic can either manifest acute toxicity and lethality or increase risk of cancer and other pathologies. Acute exposure leads to vomiting and cardiac abnormalities. At the cellular level, arsenic induces apoptosis. Chronic arsenic exposure frequently occurs in areas with contaminated drinking water and agriculture, particularly in Bangladesh and southern India. It is also elevated in developing countries with increased industrial mining activity. Chronic arsenic exposure is linked to hyperpigmentation of skin and extensive cancer risk, such as skin and bladder cancers.

Inorganic arsenite (believed to be As^{III}) was applied in traditional Chinese medicine and other cultures have also used arsenic to treat infections. Currently, this old drug has new applications with promising results in multiple cancer treatments. The trivalent form is currently an FDA-approved drug clinically applied to treat acute promyelocytic leukemia. Anthropogenic sources of arsenic contamination in the environment have been common but are decreasing out of concern for toxicity to animals. Pentavalent arsenate (as chromated copper arsenate) has been used in wood preservation. Additionally, a variety of arsenic compounds have been widely applied as herbicides and pesticides in past decades.

Arsenic detoxification in living organisms: Due to the widespread presence of arsenic, all living organisms – from prokaryotes to eukaryotes – developed systems for arsenic detoxification throughout evolution. The general steps involved in cellular arsenic metabolism are (a) membrane transporter–mediated uptake, (b) cellular metabolism, and (c) efflux. Among these steps, membrane transport is the first and likely rate-limiting step for downstream responses.

Previous studies of metalloid transport and aquaglyceroporins: Transporter-mediated uptake for As^V (AsO_4^{3-}) has been established since the early 1950s where it was found As^V can be assimilated by microbes and mammalian cells via phosphate transporters. AsO_4^{3-} and PO_4^{3-} share very close structural similarity and compete for PO_4^{3-} in enzymes and biological molecules (Ballatori 2002). The transport

pathway for the more toxic As^{III} has been found only relatively recently. In 1997, using a transposon mutation system in *E. coli*, mutation of aquaglyceroporin GlpF was identified by leading to an antimonite resistance phenotype. Antimonite shares very close physical and chemical properties with arsenite, and it soon followed that GlpF was identified as the first membrane transporter to facilitate As^{III} uptake. This has been proved by later experiments that showed GlpF deletion leads to decreased As^{III} accumulation in *E. coli*. Following this study, a yeast homolog, FPS1, was shown to facilitate As^{III} transport. The genetic deletion of *FPS1* leads to a dramatic increase in As^{III} tolerance as well as a decrease in As^{III} accumulation (Liu 2010). In 2002, the mammalian homologues, AQP7 and AQP9, were overexpressed in yeast and oocytes from *X. laevis*, and both were found to transport As^{III} and Sb^{III}, with AQP9 having the greatest As^{III} transport capacity. Studies using mammalian cell lines showed that AQP3 is also an efficient transporter for As^{III} (Liu 2010). Using AQP9 knockout mice, AQP9 was shown to be a critical transporter and to be required for overall As^{III} clearance. The AQP9 knockout mice exhibited higher arsenic retention in tissues and less tolerance for As^{III}. Thus, AQP9 was found to serve as the major As^{III} transporter in mammals and many homologs in other organisms were found to share this function (Carbrey et al. 2009). Studies showed that in addition to As^{III}, the internally generated arsenic metabolite, MMA^{III}, is a popular substrate for aquaglyceroporins. Mammalian AQP9 and many homologs exhibit robust transport for MMA^{III}, and AQP9 also transports other As^{III} metabolites. As aquaglyceroporins are bidirectional channels, they likely provide efflux of As^{III} metabolites rather than only having function in uptake.

Experimental methods used to study metalloid transport by aquaglyceroporins: The most popular and direct method to study aquaglyceroporin function is by transport assays to measure activity of functionally expressed aquaglyceroporins. Aquaglyceroporin expression can be modified for overexpression and reduced expression in in vitro systems. Cell lines can be transfected to overexpress target proteins or silence endogenous expression with siRNA. This system allows assessment of the relative contribution of aquaglyceroporins to cellular transport and their ability to function in the presence of a relatively normal cell environment. Another effective expression system is the oocytes of the African clawed frog, *Xenopus laevis*, which express high amounts of proteins following injection of the proteins RNA. Compared to cell lines, the *X. laevis* expression system often allows clearer interpretation of results as the oocytes have almost no background transporters or other activities which cause indirect effects. To perform transport assays in these systems, the cells are exposed to the substrate of interest in a buffered system to simulate various conditions, followed by quantification of internalized substrates. Precise quantification of metalloids may be achieved by using inductively coupled plasma mass spectrometry (ICP-MS) which is capable of measuring metalloids in parts-per-trillion range to parts-per-billion range. ICP-MS can also be coupled with HPLC in the front end to determine the chemical form of metalloids. To assay non-metalloid substrates (such as glycerol), radiolabeled isotopes are incorporated into the substrate as a tracer and the accumulation of substrate can be quantified by scintillation counting (Liu 2010).

As most metalloids are toxic, cell lines are also useful to observe phenotypes and detailed mechanisms of toxicity. In the case of arsenic, it is common that the overexpression of aquaglyceroporins will increase arsenic sensitivity and deletion or silencing of endogenous aquaglyceroporins will increase arsenic tolerance. Studies not directly related to function, such as mechanisms of aquaglyceroporin regulation, localization, activation, and specialized roles are also studied in these models.

Cellular roles of aquaglyceroporins: Aquaglyceroporins belong to the aquaporin family. Orthodox aquaporins are known as water channels which allow very rapid diffusion of H_2O in response to osmotic gradients, while aquaglyceroporins have larger channel entrances and pore diameters which allow transport of H_2O and a variety of small, uncharged molecules which are often nutrients.

Aquaporins are ubiquitously present to maintain cellular osmolarity and for various other roles that are tissue specific. Thirteen aquaporins have been identified in mammals, designated AQP0-12. Four out of 13 human aquaporins (AQP3, AQP7, AQP9, and AQP10) are aquaglyceroporins and share 37–45% sequence identity with each other (Liu 2010). These AQPs are highly conserved among vertebrates and respective AQPs of one species have closer identity with orthologous AQPs than they do with other family

members in their own species. Aquaglyceroporins exhibit wide expression patterns. AQP3 is detected in skin, kidney, testis, and erythrocytes. AQP7 expresses in testis, adipose tissue, heart, and kidney. In rat and human, AQP9 is abundantly expressed in liver. In contrast with rat, human AQP9 is also expressed in peripheral leukocytes and in tissues that accumulate leukocytes, such as lung, spleen, and bone marrow. Studies also found that human AQP9 is present in duodenum, jejunum, and ileum as well as in brain astrocytes. Human AQP10 is expressed in the duodenum and jejunum. Much of the in-depth expression patterns are based on rodent studies and these have mostly been consistent with human expression patterns, but with some variability.

All aquaglyceroporins appear capable of glycerol and H_2O transport but vary in the efficiency of water transport and range of other substrates. Cell swelling assays show that aquaglyceroporins transport H_2O much less rapidly than orthodox aquaporins; thus, a common opinion is that while H_2O is transported, this is not their normal physiological role. However, despite the lower efficiency in H_2O transport, AQP9 activation and water transport has been shown to be required in the volume-dependent motility of neutrophils (Karlsson et al. 2011). Other substrates are considerably broad and aquaglyceroporins vary in selectivity and efficiency of transport. AQP9 has the most identified substrates which include polyols, purines, pyrimidines, carbamides, monocarboxylic acids, and gaseous CO_2.

The known physiological roles of aquaglyceroporins are understood from their tissue expression and substrate specificity, and confirmation by genetic ablation and rescue experiments. AQP3 has been shown to transport H_2O_2, which enables AQP3-dependent local responses to H_2O_2 signaling (Miller et al. 2010). AQP7, located in adipocytes, mediates efflux of glycerol produced under fasting conditions, which can coordinate with uptake into hepatocytes mediated by AQP9 (which is upregulated by fasting) for gluconeogenesis. The AQP9 knockout mice showed a significant malfunction in glycerol metabolism (Rojek et al. 2007; Jelen et al. 2011). Given the wide substrate specificity of these channels and their complex expression patterns, their physiological roles are multifunctional and quite diverse. Though numerous roles have been found, complete roles remain under investigation. Many aquaglyceroporin roles are been difficult to pinpoint as there is some redundancy in their functions and many roles may only be noticed in specialized models. Many new roles of aquaglyceroporins are still under ongoing investigations.

Aquaglyceroporin structure and translocation mechanisms: The three-dimensional structures of several aquaporins including human aquaporin-1 (AQP1) and *E. coli* aquaglyceroporin GlpF have been solved with water in the channels and with or without glycerol within GlpF. All aquaporins are homotetramers and each monomer consists of six transmembrane alpha-helical segments. Aquaglyceroporin entrances are lined with exposed residues capable of hydrogen bonding to reduce the energy costs of desolvating molecules which have water hydration shells. These residues also play a stereoselective role by aligning substrates as they proceed further down the channel to several restriction and filtering regions. The narrowest region of the pore contains a highly conserved aromatic histidine residue closely oriented to the positively charged hydrogen of a conserved charged arginine. This tight region only allows one molecule to pass at a time while also creating an energy barrier for protons. Another conserved feature is two asparagine-proline-alanine (NPA) signature motifs which also act as filters, first by imposing size restrictions and second, creating an electrostatic field forming an energy barrier to protons and ions. Comparing the structures of aquaporins and aquaglyceroporins, it is clear that they have different pore diameters. The narrowest region of AQP1 is 2.8 Å, which is just large enough for water molecules, while a 4.0 Å diameter is observed in GlpF, accommodating transport of larger molecules (Liu 2010).

Aquaglyceroporins have sites for serine phosphorylation to regulate their activity. In some cases, aquaglyceroporins are normally trafficked to the plasma membrane and are always active, but in others, they may remain docked in scaffolds until intracellular signals cause phosphorylation and membrane trafficking. Phosphorylation sites may also affect localization and colocalization with other membrane proteins.

Metalloid transport by aquaglyceroporins: Inorganic metalloids are commonly found in tri- and tetra-hydroxylated forms, such as with arsenite ($As(OH)_3$), antimonite ($Sb(OH)_3$), and silicate ($Si(OH)_4$). These small, polar, and neutral molecules are expected to favorably interact with hydrogen bonding residues

at the pore entrance and pass through channel restriction regions. Because of this chemical similarity in terms of size, solvation, and neutral charge, such metalloids are classified to be transported as molecular mimics of normal substrates in vertebrates. There is an exception to this case known in plants, as aquaglyceroporin homologs known as NIPs (nodulin-like intrinsic proteins) fulfill a need in boron and silicon transport. In vertebrates, transport of boron and silicon by AQPs does not appear to be essential. In support of molecular mimicry and a shared transport mechanism between metalloid and nutrient substrates, arsenite transport by AQP9 can be reduced by mutating residues which show correspondingly negative effects on glycerol transport. However, molecular mimicry does not completely explain As^{III} transport, as AQP3, AQP7, AQP9, and AQP10 all transport glycerol, but AQP10 does not transport As^{III} and AQP3 and AQP7 transport As^{III} at a lesser rate than AQP9.

Impact of AQP9 arsenic transport on human health and cancer treatment: Aquaglyceroporins appear to be the major cellular transporters of inorganic metalloids as well as several organic forms. It is widely believed this transport of metalloid substrates is purely adventitious, as there appears to be no cellular benefit in allowing uptake of such toxins. Many cell culture experiments show increased AQP9 expression sensitizes cells to As^{III}. However, while clearly increasing toxicity on the cellular level, arsenic transport by AQP9 may have some benefit to arsenic exposure on a physiological scale. AQP9 expression in the liver may help arsenic processing, as the liver is more equipped to methylate As^{III} and perform other metabolic modifications which may detoxify As^{III} and improve its clearance. AQP9 also transports the products of As^{III} methylation, MMA^{V} and MMA^{III}, and may efflux them from hepatocytes after their synthesis. In support of a beneficial role in arsenic exposure, AQP9 knockout mice exhibited less As^{III} tolerance as well as greater arsenic accumulation than wild-type mice.

Arsenic cellular toxicity has made As^{III} a suitable chemotherapeutic agent in certain types of cancers. As^{III} (under the trade name Trisenox (As_2O_3)) is used to treat acute promyelocytic leukemia (APL) with great success, being capable of causing complete remission with tolerable levels of side effects. A critical property of As^{III} effectiveness comes from its ability to be concentrated in AQP9-expressing myelocytes. As AQP9 efficiently transports arsenite, it follows that activity and expression of AQP9 in this cancer type impacts the degree of selective toxicity of arsenite to these cells. Western analysis of clinical APL samples shows that AQP9 expression is a critical predictor of Trisenox therapeutic effectiveness in this cancer type. In the future, As^{III} may become a viable treatment for other tumor types and AQP9 expression should be considered prior to drug application (Agre and Kozono 2003).

Perspectives: The adventitious uptake of toxic metalloids via nutrient transporters represents a popular phenomenon in many organisms. The major role of aquaglyceroporins in metalloid uptake and subsequent cellular toxicity has been firmly established. It is less clear how much aquaglyceroporins contribute to metalloid detoxification or the exact reason of AQP9 knockout mice experiencing higher lethality in As^{III} exposure. The simplest hypothesis is that AQP9 allows arsenic extrusion by efflux of arsenic metabolites, but future studies are needed to verify the exact mechanisms responsible. A better understanding of the role of aquaglyceroporins in this process may allow design of intervention strategies to modulate aquaglyceroporin expression to alleviate metalloid toxicity.

Cross-References

- ▶ Arsenic
- ▶ Arsenic and Aquaporins
- ▶ Arsenic in Nature
- ▶ Arsenic in Pathological Conditions
- ▶ Arsenic in Therapy
- ▶ Arsenic, Free Radical and Oxidative Stress
- ▶ Arsenic, Mechanisms of Cellular Detoxification
- ▶ Boron and Aquaporins
- ▶ Silicon and Aquaporins

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Arsenic and Yeast Aquaglyceroporin

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Synonyms

Arsenic accumulation in yeast; Glycerol channel Fps1; Glycerol facilitator Fps1

Definition

Aquaglyceroporins are ubiquitous integral membrane proteins which belong to the MIP (major intrinsic protein) superfamily and mediate passive transport of water and/or small solutes, like glycerol and urea. In the budding yeast *Saccharomyces cerevisiae*, there are two aquaglyceroporins: Fps1 (fdp1 suppressor 1) involved in osmoregulation by controlling the intracellular level of glycerol and Yfl054 of unknown physiological role. In addition, Fps1 constitutes a major uptake route for trivalent inorganic arsenic and antimony metalloids into the yeast cells and plays a role in arsenic export out of the cells down the concentration gradient of metalloid.

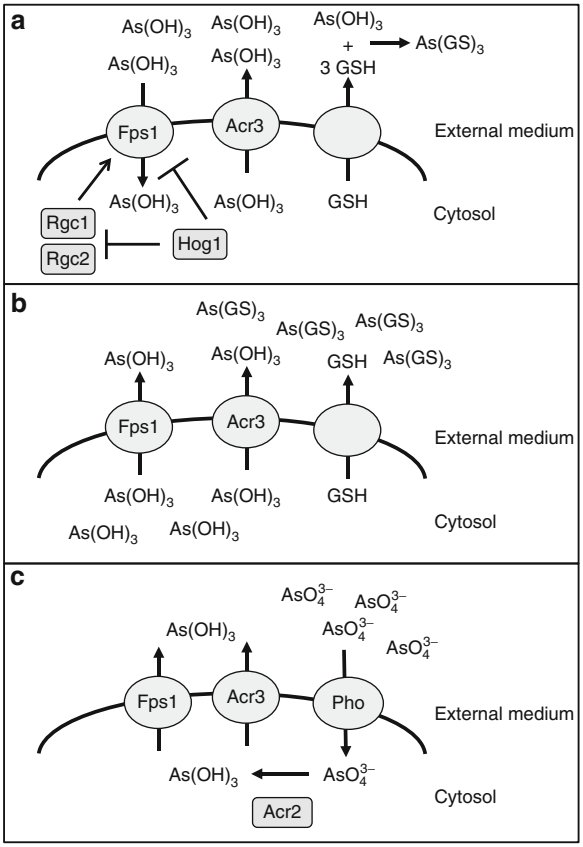
Arsenic and the Role of Yeast as a Model Organism to Study Metalloid Transport

Arsenic is a highly toxic metalloid which is ubiquitously present in the environment, sometimes in quite high concentrations. What is more, arsenic is easily accumulated by living organisms and imposes a major health and agriculture problem in many areas worldwide. On the other hand, arsenic is also used in the modern therapy as an anticancer drug. Thus, knowledge of cellular uptake pathways of arsenic is crucial for understanding how to minimize arsenic accumulation in normal cells as well as how to increase arsenic intake into cancer cells or organisms used for remediation of regions polluted by this metalloid.

Baker's yeasts are unicellular fungi which serve as an excellent eukaryotic model organism to study various biological processes, including mechanisms of arsenic transport. Studies in yeast allowed to identify and characterize major routes of arsenic uptake and efflux (Wysocki and Tamás 2010, 2011). Thanks to the fact that arsenic transport pathways are conservative, this helped to understand how other organisms, including humans, acquire tolerance to metalloids. Importantly, the yeast mutant cells devoid of arsenic uptake and efflux systems are used as a biological tool to identify plant and human genes involved in arsenic transport (Maciaszczyk-Dziubinska et al. 2012).

Routes for Arsenic Uptake into Yeast Cells

Arsenic is able to enter the cells in a pentavalent form of arsenate anion AsO_4^{3-} and in a trivalent state as arsenite, which exists as an uncharged form of $As(OH)_3$ at the neutral pH in solution. Arsenate structurally resembles phosphate anion and permeates the cells via transporters dedicated for phosphate uptake. $As(OH)_3$ is similar to glycerol that enables arsenite uptake through the glycerol transporters. In addition, $As(OH)_3$ was also demonstrated to enter yeast and mammalian cells using glucose transporters (Maciaszczyk-Dziubinska et al. 2012). In yeast, at least two phosphate permeases, Pho84 and Pho87, are involved in accumulation of arsenate. In the absence of glucose, arsenite uptake is catalyzed by any of 18 glucose transporters present in the yeast cell. However, in the presence of glucose, the main route for arsenite uptake in yeast is constituted by the glycerol



Arsenic and Yeast Aquaglyceroporin, Fig. 1 A dual role of the aquaglyceroporin Fps1 in accumulation and extrusion of arsenite in the yeast cells. (**a**) When there is a high concentration of arsenite [$As(OH)_3$] in the environment, the aquaglyceroporin Fps1 is a major route for $As(OH)_3$ accumulation into the yeast cell. To prevent $As(OH)_3$ uptake, the Fps1 channel closes as a result of Hog1-dependent phosphorylation of its N-terminal tail and inhibition of the positive regulators of Fps1, Rgc1, and Rgc2. (**b**) When the external concentration of free $As(OH)_3$ drops due to extrusion of glutathione (GSH) which forms $As(GS)_3$ complexes, Fps1 facilitates $As(OH)_3$ export from the cytosol to the environment. (**c**) In the case of pentavalent arsenic (AsO_4^{3-}) treatment, arsenate enters the cell through the phosphate transporters (Pho). In the cytosol, arsenate is rapidly reduced to arsenite by the arsenate reductase Acr2. Then, arsenite is transported out of the cell via the Fps1 channel down the concentration gradient. (**a**–**c**) In all cases, arsenite efflux is also mediated by a specific arsenite transporter Acr3

channel Fps1. More importantly, arsenite transport via Fps1 is bidirectional and regulated at both transcriptional and posttranscriptional levels either to reduce arsenite intake or increase arsenite export out of the cell (Fig. 1) (Wysocki and Tamás 2010, 2011).

Fps1 is a Glycerol Channel Involved in Osmoregulation

The yeast glycerol channel Fps1 is a member of the MIP superfamily which comprises integral membrane proteins including a large family of water-transporting aquaporins and glycerol facilitators called also aquaglyceroporins (Benga 2009). The MIP channels are usually less than 300 amino acid long and contain six transmembrane domains connected by five loops named A, B, C, D, and E. The loops B and E form two half-transmembrane domains and carry a highly conserved MIP channel motif asparagine-proline-alanine (NPA). In the membrane, MIP channels exist as homotetramers but each subunit constitutes an individual pore. Fps1 is an unusual example of MIP channel as the NPA motif is changed into asparagine-proline-serine (NPS) in the loop B and asparagine-leucine-alanine (NLA) in the loop E. In addition, the length of Fps1 is extended to 669 amino acids due to the presence of large N- and C-terminal tails, about 250 and 150 amino acid long, respectively (Hohmann 2002). Fps1 is located in the plasma membrane and catalyzes both uptake and efflux of glycerol. The physiological role of Fps1 is regulation of intracellular glycerol content in response to changes in the external osmolarity (Tamás et al. 1999). In microorganisms, accumulation of compatible solutes, like glycerol, increases the internal osmolarity. Thus, upon a hyperosmotic shock, yeast cells inhibit glycerol transport out of the cell as well as increase biosynthesis of glycerol. On the contrary, in response to a hypoosmotic stress, yeast cells rapidly release glycerol to the external medium to prevent bursting. Fps1-mediated transport of glycerol plays a crucial role during osmoadaptation (Hohmann 2002). In response to a hyperosmotic stress, Fps1 channel closes to maintain a high concentration of glycerol in the cytosol and opens to allow glycerol release when the external osmolarity drops. Thus, the yeast deletion mutant lacking *FPS1* gene (*fps1*Δ) loses viability upon transfer from high to low osmolarity conditions. It has been demonstrated that the Fps1 mutants with the truncated N-terminal cytosolic domain (fps1Δ-1) exhibit unregulated transport of glycerol, cannot close in response to hyperosmotic shock, and thus are unable to grow in the presence of high concentrations of salts (Tamás et al. 1999). Further studies showed that two short stretches of amino acids, 222–238 in the N-terminal tail and 535–546 in the C-terminal

extension, are required for the regulation of Fps1 transport activity and deletion of any of these sequences results in a constitutive open channel.

Identification of Fps1 as a Major Uptake System for Antimonite and Arsenite

First, it has been observed that mutation in the *Escherichia coli glpF* gene encoding for the glycerol channel, which is similar to Fps1, renders bacteria highly resistant to antimony, suggesting that the glycerol transporters might be involved in antimony uptake. Next, a phenotypic analysis of the yeast mutant lacking the aquaglyceroporin Fps1 revealed that both antimonite and arsenite permeate the yeast cells via the glycerol channel (Fig. 1a) (Wysocki et al. 2001). The *fps1*Δ mutant is highly resistant to antimonite and arsenite and accumulates less arsenite as demonstrated by direct transport studies using a radioactive arsenite. Moreover, applying a hyperosmotic stress to wild type cells in order to close the Fps1 channel also results in resistance to these metalloids. On the other hand, expression of a constitutive open mutant form of Fps1 (fps1Δ-1) increases sensitivity to antimonite and arsenite. It is important to mention that the yeast aquaglyceroporin Fps1 is the first reported eukaryotic transporter involved in antimony and arsenite accumulation into the cells (Wysocki et al. 2001).

Aquaglyceroporin Fps1 is a Bidirectional Metalloid Channel

The MIP channels mediate a passive diffusion of solutes across the membranes down the concentration gradient. Thus, the aquaglyceroporin Fps1 should be able to facilitate arsenite transport out of the cell when the cytosolic concentration of arsenite is higher than outside the cell. Interestingly, arsenate, which enters the yeast cells via the phosphate transporters, is rapidly reduced to the trivalent form by the action of arsenate reductase Acr2 present in the cytosol (Fig. 1c). Reduction of arsenate is an important step of arsenic detoxification because the trivalent arsenic is the only substrate for the plasma membrane transporter Acr3 which efficiently extrudes arsenite from the yeast cells against its concentration gradient (Fig. 1a) (Wysocki and Tamás 2010, 2011). However, during arsenate poisoning as a result of arsenate reduction, the concentration of arsenite in the cytosol becomes higher than in the surroundings and arsenite could diffuse via the aquaglyceroporin Fps1 out of the cell. Recent studies proved that Fps1 is indeed the bidirectional channel involved in both uptake and efflux of arsenite and antimonite (Maciaszczyk-Dziubinska et al. 2010). The yeast cells containing multiple copies of *FPS1* gene and thus overexpressing the Fps1 protein exhibit an elevated extrusion of arsenic compared to normal control cells. In contrast, the yeast mutant lacking the aquaglyceroporin Fps1 shows a significant decrease of arsenic transport out of the cell and is more sensitive to arsenate than wild type cells. This demonstrates that the aquaglyceroporin Fps1 contributes to metalloid tolerance of yeast cells by extrusion of arsenite formed after arsenate reduction (Fig. 1c). Surprisingly, it was observed that overexpression of Fps1 causes increased resistance to arsenite due to a lesser accumulation of arsenite within the cells. This strongly indicates that the aquaglyceroporin Fps1 facilitates arsenite efflux in the presence of high concentration of this metalloid in the medium. The question arises how the Fps1 channel can mediate arsenite transport against its concentration gradient. It was shown that in response to arsenite treatment, the yeast cells secrete a tripeptide called glutathione to bind arsenite outside the cells and limit arsenite influx as glutathionylated arsenite is not able to enter the cells (Wysocki and Tamás 2010, 2011). Over time the concentration of free arsenite in the vicinity of the cells becomes lower than in the cytosol which allows a passive diffusion of arsenite via the aquaglyceroporin Fps1 out of the cell (Fig. 1b).

Regulation of Arsenic Transport via the Aquaglyceroporin Fps1

Although toxic metals usually permeate the cells via the membrane proteins developed for accumulation of essential metals and nutrients using molecular mimicry, cells are not defenseless and employ several strategies to downregulate such entry pathways and prevent accumulation of unwanted substances. First, cells can reduce the number of transporter molecules in the plasma membrane by limiting the production of transporter proteins at the level of transcription or by inducing endocytic removal of transporters from the plasma membrane followed by their degradation in the vacuole. The alternative and much faster response involves inhibition of transport activities of these proteins which are already present in the plasma membrane. At least two such responses have been described for the

aquaglyceroporin Fps1 during arsenite stress. A short-term exposure to arsenite leads to decrease of *FPS1* mRNA production (Wysocki et al. 2001). The mechanism of negative regulation of *FPS1* gene transcription is not known. However, in the presence of arsenite, the level of Fps1 protein remains constant and Fps1 is not removed from the plasma membrane (Maciaszczyk-Dziubinska et al. 2010). This suggests that accumulation of arsenite into the yeast cells is blocked by inhibition of Fps1 transport activity. Indeed, in response to arsenite stress, Fps1 undergoes phosphorylation on threonine 231 located in the N-terminal tail which leads to closing of Fps1 channel (Thorsen et al. 2006). Arsenite-induced phosphorylation of Fps1 depends on a mitogen-activated protein kinase (MAPK) Hog1 (High osmolarity glycerol 1), which is involved in response to several stresses by regulating various aspects of metabolism to adapt the cells to unfavorable conditions. By a yet unknown mechanism, arsenite activates the HOG signaling pathway to transduce a stress signal by phosphorylating and activating the Hog1 kinase within 15–30 min from arsenite addition to the yeast cells. Then activated Hog1 remains localized in the cytoplasm to phosphorylate and close the aquaglyceroporin Fps1 in order to restrain arsenite accumulation. That is why the cells lacking the Hog1 kinase or expressing Fps1 with threonine 231 mutated into alanine (Fps1-T231A) are highly sensitive to arsenite and accumulate more arsenite than normal cells. Interestingly, deletion of two genes *RGC1* and *RGC2* (Regulator of the Glycerol Channel 1 and 2), which encode for two pleckstrin homology (PH) domain proteins of unknown function, suppresses the arsenite sensitivity of *HOG1* deletion mutant (Beese et al. 2009). In addition, cells lacking Rgc1 and Rgc2 are defective for glycerol efflux via the aquaglyceroporin Fps1, suggesting that they are involved in a positive regulation of Fps1 by promoting the open state of Fps1 channel. It was shown that in response to arsenite and hypo-osmotic stress, Rgc2 undergoes hyperphosphorylation, which is markedly reduced in the *HOG1* deletion mutant (Beese et al. 2009). Thus, Hog1 may downregulate the activity of Fps1 channel not only by a direct phosphorylation of N-terminal tail of Fps1 but also indirectly by affecting Rgc2, a positive regulator of Fps1 (Fig. 1a). However, arsenite-induced phosphorylation of Hog1 is only maintained up to 180 min after arsenite addition to the cells, suggesting that negative regulation of Fps1 is released after this time despite the presence of metalloid in the medium. This coincides with the upregulation of *FPS1* gene expression and accumulation of Fps1 mRNA (Maciaszczyk-Dziubinska et al. 2010). Such switch from the negative to the positive regulation of Fps1 channel activity is in a good agreement with a dual role of the aquaglyceroporin Fps1 in both uptake and efflux of metalloids in the yeast cells.

Yeast as a Tool for Identification of Arsenic Transporters

Yeast are often used as a host for heterologous expression and functional analysis of proteins from other organisms. Detailed understanding of arsenic transport pathways, including the aquaglyceroporin Fps1, the plasma membrane arsenite transporter Acr3, and the ABC (ATP-Binding Cassette) pump Ycf1 mediating a vacuolar accumulation of arsenite complexed with glutathione, made the yeast *Saccharomyces cerevisiae* a perfect model for studies of foreign proteins involved in arsenic transport and tolerance. The yeast triple mutant lacking *ACR3*, *FPS1*, and *YCF1* genes, and thus exhibiting no arsenic transport activity and high resistance to arsenite due to the lack of arsenite uptake, was successfully used for identification of plant and mammalian aquaglyceroporins involved in metalloid accumulation (Wysocki and Tamás 2010, 2011). For example, based on the reversion of arsenite resistance and the increased accumulation of radioactive arsenite in the *FPS1* deletion strain upon expression of the rat AQP9 on a plasmid, it could be concluded that aquaglyceroporins constitute a major uptake pathway for arsenic also in mammals. A similar approach was used to identify plant aquaporins involved in arsenic accumulation. Importantly, it was noticed that expression of plant aquaporins not only sensitizes yeast cells to arsenite but also improves the growth of yeast in the presence of arsenate. This observation allowed to propose that aquaglyceroporins are bidirectional channels which are capable of both uptake and efflux of arsenic depending on its concentration gradient.

Cross-References

▶ Arsenic
▶ Arsenic and Aquaporins

▶ Arsenic and Vertebrate Aquaglyceroporins
▶ Arsenic in Nature
▶ Arsenic in Therapy
▶ Arsenic, Biologically Active Compounds
▶ Arsenic, Mechanisms of Cellular Detoxification

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Arsenic Black

▶ Arsenic in Pathological Conditions
▶ Arsenic, Free Radical and Oxidative Stress

Arsenic Channels

▶ Arsenic and Aquaporins
▶ Arsenic and Vertebrate Aquaglyceroporins

Arsenic in Nature

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Synonyms

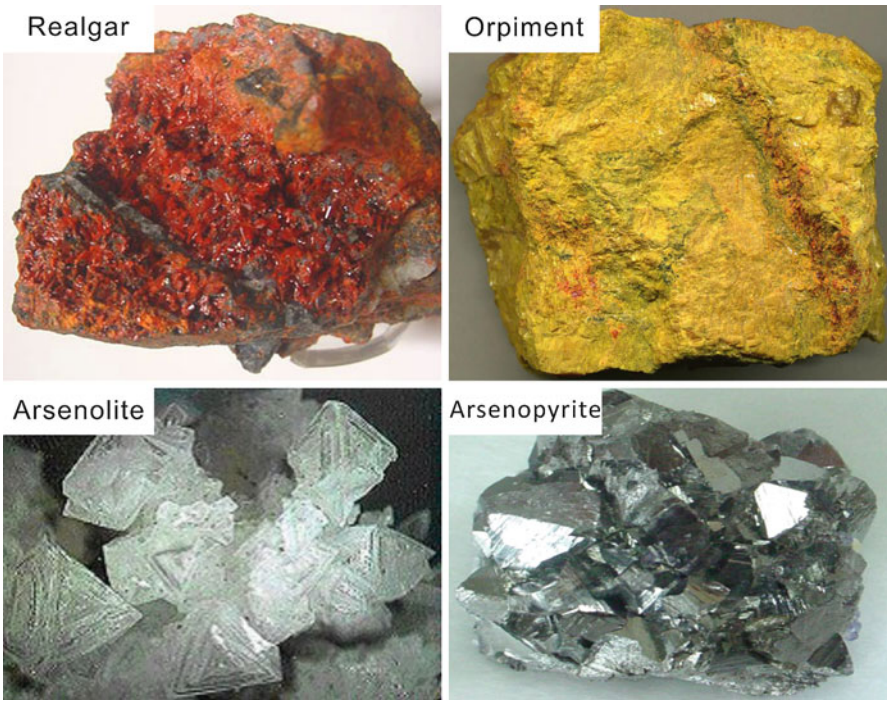
Arsenicals; Organic and inorganic arsenic

Definition

Arsenic is a chemical element with the symbol As on the periodic table along with nitrogen, phosphorus, antimony, and bismuth. The atomic number of arsenic is 33 and the relative atomic mass is 74.92. Naturally occurring arsenic is composed of one stable isotope, ^{75}As. Many radioisotopes of arsenic have also been synthesized. Arsenic was first documented by Albertus Magnus in 1,250. The term "arsenic" probably originates from the Persian word az-zarnikh or other modifications of its root word, "zar," which refers to yellow or gold orpiment. Arsenic is ubiquitous in nature and ranks twentieth among the elements in abundance in the Earth's crust, fourteenth in seawater, and twelfth in the human body (Jomova et al. 2011).

Arsenic Compounds

Arsenic is a metalloid element, meaning that it displays some properties of both a metal and a nonmetal. It is widely distributed in the biosphere with inorganic or organic forms. The most common valence states of arsenic are −3 (arsine), 0 (elemental arsenic), +3 (arsenite), and +5 (arsenate). Under reducing conditions, arsenite (As^{III}) is the dominant form; arsenate (As^{V}) is generally the stable form in oxygenated environments. Although very rare in nature, elemental arsenic may form in hydrothermal deposits at low temperatures (50–200°C) under very anoxic and low-sulfur conditions. Solid samples of elemental arsenic tend to be brittle, nonductile, and insoluble in water.



Arsenic in Nature, Fig. 1 Arsenic minerals

Three most common solid forms of elemental arsenic are metallic gray, yellow, and black arsenic (Gorby 1988). Gray arsenic is the most common and important for use in industry. It has a metallic sheen and conducts electricity. Yellow arsenic is metastable, is a poor electrical conductor, and does not have a metallic sheen. Black arsenic is glassy, brittle, and a poor electrical conductor. Arsenic can exist in many different chemical forms in combination with other elements. Most pure arsenic compounds have no smell or special taste and are white or colorless powders that do not evaporate. Arsenic salts exhibit a wide range of solubilities depending on pH and the ionic environment. Inorganic arsenic is found usually to combine with sulfur, oxygen, halogen elements, and metals (e.g., copper, nickel, cobalt, iron, lead). It occurs naturally in the minerals and ores (Fig. 1), such as realgar (As_4S_4, "red arsenic"), orpiment (As_2S_3, "yellow arsenic"), arsenolite (As_2O_3, "white arsenic"), arsenopyrite (FeAsS), cobaltite (CoAsS), and niccolite (NiAs). Organic arsenic is an arsenic compound containing one or more arsenic-carbon bonds that can be found in nature, in water, natural gas, and shale oil, with the most important general types being those which contain methyl groups. Examples of organic arsenic are methylarsine (CH_3AsH_2), dimethylarsine [$(CH_3)_2AsH$], trimethylarsine [(CH3)3As], monomethylarsonic acid (MMA^V) [$CH_3AsO(OH)_2$], monomethylarsenous acid (MMA^{III}) [$CH_3As(OH)_2$], dimethylarsinic acid (DMA^V) [$(CH_3)_2AsO(OH)$], dimethylarsenous acid (DMA^{III}) [$(CH_3)_2AsOH$], trimethylarsinic oxide (TMAO) [$(CH_3)_3AsO$], tetramethylarsonium ion (TMA^+)[$(CH_3)_4As^+$], and others (United States Environmental Protection Agency 2000; Ng 2005).

Occurrence and Source

The Earth's crust is an abundant natural source of arsenic. It is present in more than 320 different minerals, the most common of which is arsenopyrite (Foster 2003). Arsenic may enter the air, water, and soil from wind-blown dust and may get into water from runoff and leaching. Volcanic eruptions are the most important source of arsenic in atmosphere. Arsenic may enter the environment during the mining and smelting of arsenic-containing ores. Small amounts of arsenic also may be released into the atmosphere from coal-fired power plants and incinerators because

coal and waste products often contain some arsenic. Many common arsenic compounds can dissolve in water. Thus, arsenic can get into lakes, rivers, or underground water by dissolving in rain or snow or through the discharge of industrial wastes. Some of the arsenic will stick to particles in the water or sediment on the bottom of lakes or rivers, and some will be carried along by the water. Ultimately, most arsenic ends up in the soil or sediment. Arsenic is also ubiquitous in the plant kingdom and all living organisms. The main dietary forms for human are seafood, rice, mushrooms, and poultry. Although some fish and shellfish take in more arsenic, which may build up in tissues, most of this arsenic is in an organic form called arsenobetaine (commonly called "fish arsenic") that is much less harmful.

Environmental levels of arsenic vary. Mean total arsenic concentrations in air from remote and rural areas range from 0.02 to 4 ng/m^3. Mean total arsenic concentrations in urban areas range from 3 to about 200 ng/m^3; much higher concentrations (>1,000 ng/m^3) have been measured in the vicinity of industrial sources, although in some areas, this is decreasing because of pollution abatement measures. In water, levels of arsenic are lowest in seawater, typically 1–2 μg/L. It is higher in rivers and lakes where the concentrations are generally below 10 μg/L. Arsenic levels in groundwater can range up to 3 mg/L in areas with volcanic rock and sulfide mineral deposits. Background concentrations in soil range from 1 to 40 mg/kg, with mean values often around 5 mg/kg, which increase if there are natural and/or man-made sources of arsenic contamination present (Scientific Facts on Arsenic 2004).

Applications

Industrial processes can contribute to the presence of arsenic in air, water, and soil. Environmental contamination of arsenic also occurs because it is used in agricultural pesticides and in chemicals. In the past, inorganic arsenic compounds were predominantly used as wood preservatives, pesticides, herbicides, and paints, and now can no longer be used in agriculture for its environmental issues. However, organic arsenic compounds, namely, cacodylic acid, disodium methylarsenate (DSMA), and monosodium methylarsenate (MSMA), are still used as pesticides. Some organic arsenic compounds are used as additives in animal feed. Small quantities of elemental arsenic are added to other metals to form metal mixtures or alloys with improved properties. In addition, arsenic is used in alloys (primarily in lead-acid batteries for automobiles) and in semiconductors and light-emitting diodes.

Toxicity

Arsenic is an essential trace element for some animals; however, arsenic and many of its compounds are especially potent poisons. The toxicity of an arsenic-containing compound depends on its valence state, its form (inorganic or organic), its solubility, and the physical aspects governing its absorption and elimination. Generally, inorganic arsenic species are more toxic than organic forms to living organisms, and trivalent arsenite is more toxic than pentavalent arsenic (arsenate). The reported lethal dose of arsenic ranges from 120 to 200 mg in adults and is 2 mg/kg in children (Ellenhorn et al. 1997). Since arsenic is found naturally in the environment, people may be exposed to some arsenic by eating food, drinking water, or breathing air. For drinking water, the World Health Organization (WHO) has set the International Drinking Water Standard for arsenic concentration at 10 μg/L in 1993. For air, the Occupational Safety and Health Administration has established limits of 0.01 mg/m^3 for inorganic and organic arsenic compounds.

Arsenic can cause skin lesions, hepatic injury, hemorrhagic gastroenteritis, cardiac arrhythmia, cancers and psychiatric disease (Hughes 2002; Jomova et al. 2011). Intensive studies have been carried out to elucidate the underlying mechanisms (Chen et al. 2011). The results show that trivalent arsenic (As^{III}) can firmly bind the sulfhydryl groups of biomolecules such as glutathione and lipoic acid and the cysteinyl residues of many proteins and enzymes and therefore inhibits the activities of enzymes such as glutathione reductase, glutathione peroxidases, thioredoxin reductase, and thioredoxin peroxidase. Arsenic also affects flavin enzymes such as NAD(P)H oxidase and NO synthase isozymes. Consequently, arsenic exposure leads to production of reactive oxygen species (ROS). Arsenic interferes with many signal transduction cascades and activates (or inactivates) transcription

factors by alteration of global histone H3 methylation. Another mechanism of arsenic toxicity involves substitution of pentavalent arsenic (As^V) for phosphorus in many biochemical reactions, leading to rapid hydrolysis of high-energy bonds in compounds such as ATP.

Cross-References

- ▶ Arsenic
- ▶ Arsenic in Pathological Conditions
- ▶ Arsenicosis
- ▶ As

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Arsenic in Pathological Conditions

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Synonyms

Arsen; Arsenic black; Arsenicals; Colloidal arsenic; Gray or grey arsenic; Metallic arsenic; Sodium arsenate; Sodium arsenite

Definition

Arsenic (As), the 33rd element of the periodic table, is classified as a metalloid and is also ubiquitous and highly abundant in nature. Being odorless and colorless, its presence is not immediately obvious and thus, serious human health hazard exists. The use of arsenic as a poison has been known and reported for many years. Arsenic has long been used worldwide as poison, in medicines and pesticides, which still prevails in many countries. The very name of arsenic is thus synonymous with poison. Arsenic exists in the environment as pentavalent (As^{5+}, arsenate) and trivalent (As^{3+}, arsenite) forms, and arsenite is considered as more toxic than arsenate. Human exposure to arsenic may occur from inhalation, skin absorption, and primarily by water ingestion and food like rice, mushrooms, seafood, and poultry, which are reported to have the highest concentrations of arsenic. Environmental contamination of arsenic, particularly in drinking water sources mainly because of anthropogenic activities, is a major cause of concern for human arsenic exposure (Jomova et al. 2011). Small amounts of arsenic absorbed over a period of time may result in chronic poisoning which may produce nausea, headache, coloration and scaling of the skin, hyperkeratosis, anorexia, and white lines across the fingernails. These common symptoms are followed by significant pathological abruptions with or without involvement of internal organs. Ingestion of large amounts (acute poisoning) of arsenic results in severe gastrointestinal pain, diarrhea, vomiting, and swelling of the extremities. Acute poisoning causes renal failure and shock which may ultimately be fatal. Arsenic-induced generation of free radicals and oxidative stress can cause DNA damage, and inhibition of various proteins mainly including transcription factors, regulatory proteins, and induction and/or inhibition of apoptosis. These oxidative stress biomarkers correlated with determination of arsenic concentration in urine, hair, or fingernails of the exposed population that are common diagnostic tools for epidemiological studies. Chronic arsenic exposure has been reported to .induce several pathological conditions such as abnormal skin pigmentation, vasculopathy resulting in dry gangrene of extremities, keratosis, ischemic heart disease, respiratory disease, diabetes, gastrointestinal disturbances, splenomegaly, and neurological defects.

Arsenic (III) binds to sulfhydryl groups leading to the inhibition of a number of enzymes in cellular energy pathway (including pyruvate dehydrogenase), and DNA synthesis and repair, etc. Arsenic is metabolized by reduction and methylation, which was earlier considered as a detoxification mechanism. These reactions are catalyzed by glutathione-S-transferase omega-1 and arsenic (III) methyltransferase. Highly reactive and carcinogenic trivalent arsenic intermediates (monomethylarsonous and dimethylarsonous), are present in urine of arsenic-exposed human subjects more than the pentavalent forms confirming toxicity-mediated through biotransformation. Arsenic is also known to induce cancer of skin, lung, kidney and bladder. Cancer initiation involves the promotion of oxidative stress, in which the antioxidant capacity of the living organism is overwhelmed by arsenic-induced reactive oxygen species (ROS). Latter results in molecular damage to proteins, lipids, and most significantly DNA. Although the toxic effects of arsenic on humans and environment have been well documented, the mechanism by which arsenic induces adverse health effects is not well characterized. The antidotes for arsenic poisoning are dimercaprol, succimer, and recently proposed monoester of succimer.

Arsenic-Induced Clinicopathological Effects

Dermal Effects

Clinical cases of arsenic exposure are most commonly known and identified with dermal toxicity. Appearance of skin pigmentation following exposure is an early indication of initial stage of arsenic poisoning. Chronic exposure of arsenic results in the development of skin lesions, including hyperkeratosis and hyperpigmentation. These symptoms are often used as a diagnostic feature for arsenicosis. Skin cancer induced by arsenic may take a long time to appear, sometimes taking several decades to develop symptoms. Arsenic penetrates through epithelium and causes allergic contact dermatitis in subjects exposed to contaminated water.

Mees' lines: Formation of single, solid white transverse bands of about 1 or 2 mm in width completely crossing the nail of all fingers at the same relative distance from the base.

Melano-Keratosis

Melanosis: It is dark pigmentation of skin surface which initially is visible in palms and spreads to the whole body gradually.

Keratosis: In this condition, rough, dry, and spotted nodules are observed in palms and/or soles.

Both melanosis and keratosis are the chief symptoms of arsenical dermatitis (ASD) and combination of these two features – melanosis and keratosis – in the same patient points to the diagnosis of arsenical dermatitis.

Spotted melanosis (*Spotted or raindrop pigmentation*) is commonly defined as "raindrops in the dust," and is usually seen on chest, back, or limbs.

Spotted and Diffuse Keratosis of palms indicates moderate to severe toxicity. This condition is characterized by rough, dry, spotted nodules which appear after 5–10 years of arsenic exposure. Later (after 10 years), the skin appears dry and gets thickened. This stage is called diffuse keratosis. Gradual thickening of soles may lead to cracks and fissures, a condition termed as "hyperkeratosis."

Leucomelanosis: It is the advanced stage of the disease in which development of pigmentation and depigmented spots (white and black in color) occurs on legs or trunk. Leucomelanosis is common in advanced stage of arsenicosis or condition where arsenic consumption is stopped but had spotted melanosis earlier.

Dorsal keratosis: In severe case of long-term arsenic exposure, skin becomes rough and dry often with the development of palpable nodules (spotted keratosis) on the dorsal skin of hands, feet, legs, or even other parts of the skin, resulting in whole body keratosis.

Gastrointestinal Disturbances

Gastrointestinal symptoms are observed more during acute exposure to inorganic arsenic, which occurs within 30 min of exposure, than chronic exposure. Clinically, acute arsenic poisoning occurs in two distinct forms: acute paralytic syndrome and acute gastrointestinal syndrome. While acute gastrointestinal syndrome leads to dry mouth and throat, heartburn, nausea, abdominal pain and cramps, and moderate diarrhea; chronic arsenic ingestion results in symptomatic gastrointestinal irritation or may produce gastritis, esophagitis, or colitis. Symptoms of acute arsine gas exposure are nonspecific and include headache, weakness, nausea, vomiting, accompanied with abdominal pain. Within a few hours of exposure, dark red urine is seen and within 1–2 days, jaundice is evident. The trio abdominal pain, hematuria, and jaundice are characteristic of arsine gas poisoning.

The gastrointestinal tract appears to be the critical target of toxicity following oral exposure to monomethylarsenous acid (MMA). A dose level of 72.4 mg MMA kg^{-1} per day led to a thickened wall; edema; and hemorrhagic, necrotic, ulcerated, or perforated mucosa in the large intestine and a significant increase in the incidence of squamous metaplasia of the epithelial columnar absorptive cells in the colon and rectum.

Hematological Effects

Acute and chronic arsenic exposure leads to alterations in hematopoietic system. Anemia (normochromic, normocytic, aplastic, and magaloblastic) and leucopoenia (granulocytopenia, thrombocytopenia, myeloid, myelodysplasia) are common effects of arsenic poisoning which may be due to a direct hemolytic or cytotoxic effect on the blood cells. High levels of arsenic have been linked with bone marrow depression in humans and it also leads to red blood cell hemolysis which can cause death within hours. Blood cells undergo hemolysis in the presence of arsenic. Arsenic lowers the GSH levels, which leads to oxidation of sulfhydryl groups in the hemoglobin. The formed hemocyanin then combines with arsenic, which reduces oxygen uptake by cells.

Hepatotoxic Effects

Long-term exposure to arsenic leads to liver damage and the exposed subject may report bleeding esophageal varices, ascites, jaundice, or simply an enlarged liver. Clinical examination reveals swollen, tender liver and elevated levels of hepatic enzymes. These effects are often observed after chronic arsenic exposures to as little as 0.02–0.1 mg As/kg/day.

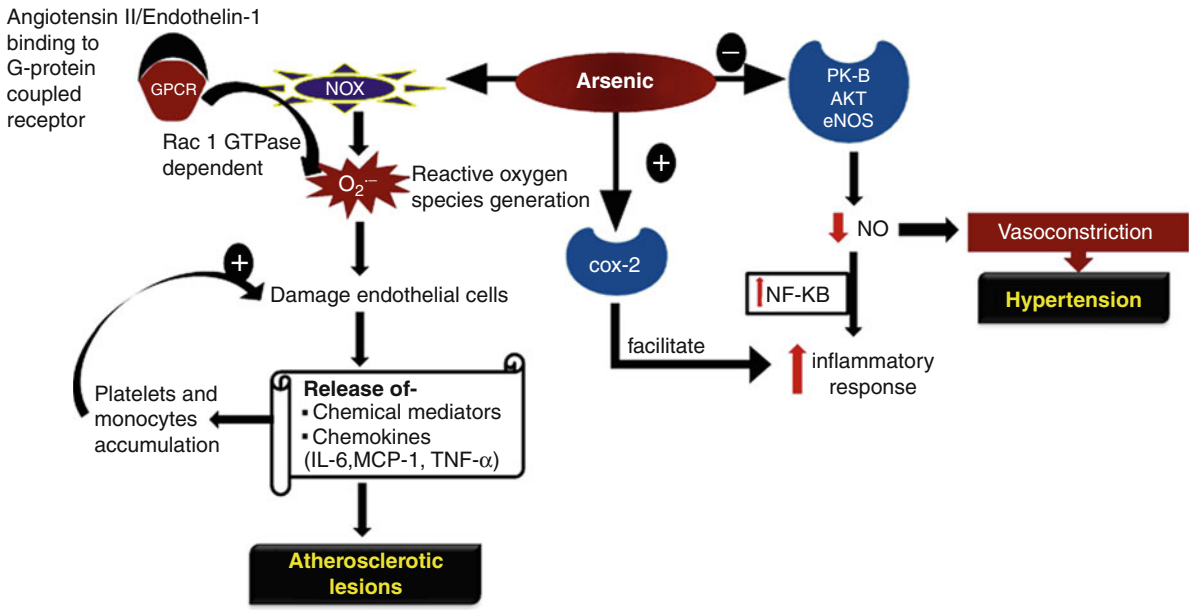
A correlation between chronic arsenic exposure and abnormal liver function, namely, hepatomegaly, hepatoportal sclerosis, liver fibrosis, and cirrhosis, is well known.

- *Abnormal liver function* is manifested by gastrointestinal disturbances like abdominal pain, indigestion, loss of appetite, and by clinical elevation of serum enzymes following chronic exposure to arsenic.
- *Hepatomegaly* is defined as the abnormal enlargement of liver. More than 75% of the arsenic-exposed subjects in West Bengal, India, report hepatomegaly which is also positively correlated with hepatic arsenic content and arsenic concentration in drinking water.
- *Hepatoportal sclerosis* (*Noncirrhotic portal hypertension*), also known as noncirrhotic portal fibrosis, idiopathic portal hypertension, and Banti syndrome, is a rare arsenic-related condition characterized by portal hypertension but without liver cirrhosis. Initial clinical symptoms of hepatoportal sclerosis are manifested by splenomegaly, anemia, and episodes of gastrointestinal hemorrhage.
- *Liver fibrosis and cirrhosis* is generally present in cirrhotic patients who consume "homemade brew" made with water highly contaminated with arsenic. Liver cirrhosis is one of the major causes of arsenic-related mortality in Guizhou, China, and is potentially associated with hepatocellular carcinoma (HCC).

The susceptibility of liver to arsenic is a consequence of its primary role in arsenic metabolism. In liver, conversion of As^{3+} to As^{5+} results in ROS generation, which leads to mitochondria-mediated liver cell death. Arsenic causes mitochondrial damage and impairs mitochondrial functions. Additionally, toxic methylated arsenic species such as MMA^{III} show greatest binding with hepatocytes. Arsenic involves several toxic mechanisms such as ROS-mediated oxidative stress, inflammatory response, or metabolic hindrances to induce its hepatotoxic effect.

Cardiovascular Effect

Arsenic is also associated with various cardiovascular diseases such as Raynaud's disease, myocardial infarction, myocardial depolarization, cardiac arrhythmias, hypertension, carotid atherosclerosis, ischemic heart disease, and vascular disease (States et al. 2009). Black Foot Disease (BFD) causing ischemic heart disease (ISHD) is one of the major complications following arsenic exposure. This peripheral neuropathy and vascular disease is characterized by severe systemic atherosclerosis and dry gangrene in the lower extremities featuring blackening of feet and hands at end stages (Mazumder 2008). Although cardiovascular effects of arsenic are well defined, the mechanism requires exploration. Arsenic via excessive ROS generation alters the regulation of gene expression, inflammatory responses, and endothelial nitric oxide homeostasis which is important in maintaining vascular tone, leading to cardiovascular endpoints. Arsenic may also induce cardiovascular effects in infants



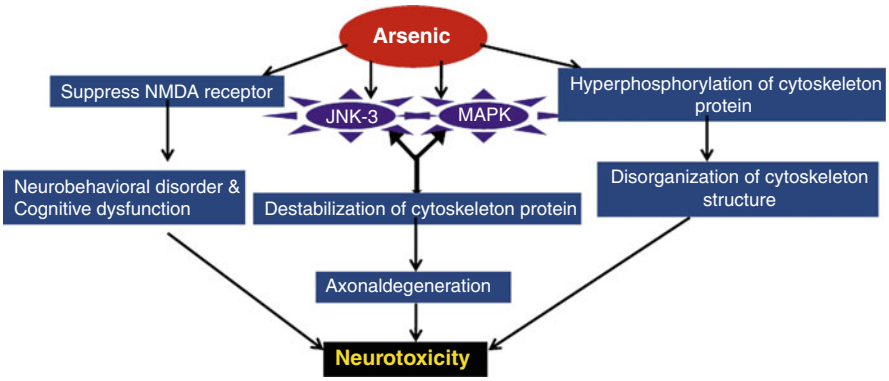
Arsenic in Pathological Conditions, Fig. 1 Arsenic-induced cardiovascular pathologies

following in utero exposure. Although with limited evidence, human data and animals studies suggest that arsenic triggers the onset of atherosclerosis in infants. Arsenic-induced alteration in plasma triglycerides and impaired vasorelaxation response are major observations reported.

Mechanism Involved for Cardiovascular Lesions

Arsenic induces cardio-toxicity that is a result of both cardiac and more importantly vascular effects is mediated through multiple mechanisms. Chronic arsenic exposure causes hypertension due to elevated peripheral resistance resulting from stiffness and lower compliance of the vessel wall. These altered vascular functions are caused by perturbed regulation of vasomotor function and/or structural remodeling of blood vessels. Arsenic-induced ROS or altered redox signaling in both the vascular endothelial and the smooth muscle cell forms the foundation of underlying toxic mechanisms. NADPH oxidase (NOX) enzyme complex involved in hypertension and other vascular disease by superoxide generation is stimulated by arsenic. In the process, various endogenous hypertensive peptides, such as angiotensin II or endothelin-1, interact with membrane linked G-protein-coupled receptors (GPCR) to initiate Rac1-GTPase–dependent superoxide generation by NOX enzymes. Arsenic-induced ROS generation damages the endothelial cells, thereby affecting the endothelial nitric oxide homoeostasis, which play an important role in maintaining vascular tone. Nitric oxide (NO) is a vascular endothelial factor involved in vasodilatation, anti-inflammation, inhibition of platelet adhesion and aggregation, smooth muscle cell proliferation and migration. Exposure to sodium arsenite inactivates protein kinase B/Akt and eNOS, resulting in endothelial cytotoxicity and reduced generation of NO subsequently decreasing the endothelium-mediated vasorelaxation (Balakumar and Kaur 2009). Arsenic also increases the phosphorylation of myosin light chain and calcium flux in the blood vessel. Further, arsenic disrupts the blood pressure regulatory mechanisms by virtue of its effect on hepatic, renal, and neurological system (Fig. 1).

Atherosclerosis is another cardio-pathology closely associated with arsenic exposure. Arsenic-mediated atherosclerosis is initiated by ROS-induced oxidative insult to endothelial vascular cells, instigating activation of inflammatory chemical mediators, adhesion molecules, and chemokines. These chemokines and proinflammatory cytokines including monocyte



Arsenic in Pathological Conditions, Fig. 2 Role of arsenic in neuropathology

chemoattractant protein-1 (MCP-1), interleukin-6 (IL-6), and tumor necrosis factor alpha, presence of which was detected at high concentrations in atherosclerotic lesions. Arsenic is known to induce the overexpression of MCP-1 and IL-6 in vascular and smooth muscle cells, thereby leading to generation of atherosclerotic lesions. Release of these mediators attract platelets and monocytes which facilitates their activation, forming a continuous process. Foam cell formation by scavenging of oxidized low-density lipoprotein (LDL) by macrophages initiates vascular plaque formation. Physiologically these oxidized LDLs modulate intracellular signal transduction but also generate ROS and peroxides. Arsenic increases accumulation of protein adducts of malondialdehyde (MDA) and 4-hydroxy-*trans*-2-nonenal (HNE) in vascular lesions. Arsenic-induced low NO bioavailability supports the inflammatory processes by increased transcription factors like NF-κB and plaque formation by vasoconstriction. Arsenic is also known to upregulate cyclooxygenase-2 (COX-2) expression in endothelial cells facilitating inflammatory processes. Finally, arsenic promotes the coagulation processes by inhibiting tissue type plasminogen activator, thus reducing fibrinolysis. Progressive development of atherosclerosis which is either initiated or facilitated by arsenic ultimately continues till congestion of cardiac blood vessel by plaque formation (Flora 2011).

Neurological Effects

Ingestion of inorganic arsenic results in neural injury and damage to both peripheral and central nervous system. During acute exposure, encephalopathy with symptoms such as persistent headache, mental confusion, hallucination, seizures, coma, diminished recent memory, distractibility, abnormal irritability, lethargy restlessness, loss of libido, and increased urinary urgency is observed. This is often associated with anxiety, panic attacks, somatizations, and secondary depression. Repeated arsenic exposure in individuals causes polyneuropathy of sensorimotor. Arsenic exposure results in the polyneuropathy, particularly in the lower limbs which is characterized by tingling, numbness, burning soles, and weakness.

Arsenic-induced neurotoxicity causes changes in cytoskeletal protein composition and hyperphosphorylation which may lead to disorganization of the cytoskeletal structure, a potential cause of neurotoxicity (Fig. 2). Peripheral neuropathy and the cytoskeletal defects are typical neurological features (Mathew et al. 2010). Arsenic-induced neurotoxicity involves induction of apoptosis in the cerebral neuron by activating p38 mitogen-activated protein kinase and JNK3 pathway. Destabilization and disruption of nerve cytoskeletal proteins leading to axonal degeneration is another mechanism involved in arsenic-induced neurotoxicity. These nerve cytoskeletal proteins serves as flexible scaffold for cells that is responsible for the communication between cell parts and other functions. The major components of myelinated neurons are neurofilaments (NF-H, NF-M, NF-L). Among them NF-L is crucial and is required by both NF-H and NF-M to form a heteropolymer in the cytoskeleton of the neuronal cell. Within the peripheral nerves, arsenic by virtue of its affinity for -SH moiety, binds and subsequently degrade neurofilaments (NF-L). This ultimately leads to arsenic recirculation, and rendering it available for further targeting NF-L. Arsenic and its metabolite (monomethylarsonic acid and monomethylarsonous) suppress the NMDA receptors in hippocampus, leading to neurobehavioral disorders and cognitive dysfunction.

Renal Effects

Arsenic causes acute renal failure and chronic renal dysfunction. Pathologically, arsenic-induced manifestations are due to hypotensive shock, hemoglobinuric or myoglobinuric tubular injury. Arsenic during its excretion via kidneys gets converted into less soluble but more toxic trivalent arsenic. The capillaries, tubules, and glomeruli of kidneys are involved in this process and, thus, are also the sites of arsenic induced damage. Arsenic induces lesions such as loss of capillary integrity and increase in glomerular capillary permeability. Mitochondrial damage and arsenic-induced hemolysis leads to tubular necrosis with a high risk of renal failure. The damaged tubular cells lead to proteinuria, oligouria, hematuria, acute tubular necrosis (ATN), renal insufficiency, or frank renal failure. Dimethylarsenic acid (DMA) also causes renal damage which is characterized by increased volume and pH of urine, urinary calcium level, increase in water consumption, and decreased levels of electrolytes. Increased kidney weights and minimal tubular epithelial cell degeneration, tubular casts, and focal mineralization have also been associated with arsenic exposure. The urinary system seems to be a more recessive target for DMA than for MMA (Jomova et al. 2011).

Respiratory Effects

Acute arsenic exposure leads to the development of pulmonary edema, adult respiratory distress syndrome (ARDS), along with respiratory failure from muscle weakness, and phrenic nerve damage leading to apnea. Laryngitis, bronchitis, pharyngitis, rhinitis, tracheobronchitis, shortness of breath, nasal congestion, conjunctiva congestion, redness of the eyes, chronic cough, chronic asthmatic bronchitis and asthma, and perforation of the nasal septum have been associated with exposure to arsenic.

Arsenic accumulates in lungs along with other target sites following ingestion and biotransformation by liver. Although mechanisms for arsenic-induced lung toxicity and cancer are not well defined, one of the hypothesis is that high oxygen partial pressure in lungs facilitates arsenic-induced oxidative stress. In the presence of high oxygen, a DMA metabolite, dimethylarsine which is a gas and thus excreted by lungs reacts with oxygen to generate free radical. Arsenic exposure through inhalation forms an important route to understand arsenic-induced respiratory defects. Respiratory tract absorbs more arsenic than gastrointestinal tract. The fate of inhaled arsenic particles is different from ingested arsenic. Arsenic particles have low solubility and thus show slow body clearance retaining for longer time compared to ingested arsenic. Inhaled arsenic causes inflammation of the lung tissue due to mechanical interaction along with other mechanisms such as oxidative stress, insult to biomolecules especially DNA, suppression of p53 leading to manifestations like cancer (Celik et al. 2008).

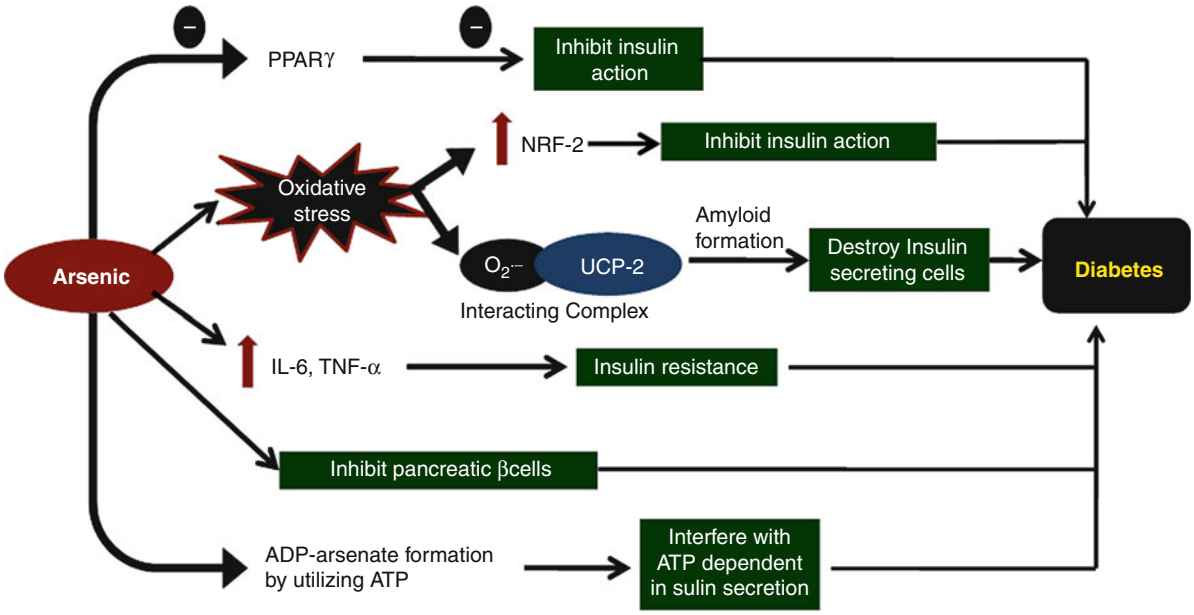
Reproductive and Developmental Effects

Arsenic-induced reproductive toxicity has not been widely studied, thus limited information is available. Arsenic-induced male reproductive toxicity includes low sperm count, postmeiotic spermatogenesis, abnormal hormonal secretion, and altered enzyme activity. Female reproductive system is also adversely affected by arsenic exposure. Major toxic effects include ovarian steroidogenesis, prolonged diestrus, degradation in ovarian, follicular, and uterine cells; and increased meiotic aberrations in oocyte. Sporadic human, while contradictory animal data suggest arsenic and its methylated metabolites can cross the placenta. Arsenic exposure increases the incidence of preeclampsia in pregnant women; decreases birth weight of newborn infants; and increases the risk of malfunctions and stillbirths, spontaneous abortions, preterm births, and infant mortality. On the other hand, laboratory studies suggest an increase in malformations and stillbirths in animals. All these data are indicative but not conclusive. Thus, a properly designed epidemiologic study in a sufficiently large population is required in order to assess the potential adverse effects of arsenic in human reproduction (Jomova et al. 2011; Flora et al. 2011).

Arsenic causing oxidative insult to the placental cells which interferes with the communication between the mother and the developing embryo and adversely affects the nutritional translocation through placenta. Arsenic generates ROS and reduces important placental antioxidants such as thioredoxin reductase. Arsenic induces anemia in pregnant female due to it hematological adverse effects (Flora et al. 2011).

Diabetes Mellitus

Diabetes mellitus observed in patients exposed to arsenic is similar to Type II diabetes mellitus characterized



Arsenic in Pathological Conditions, Fig. 3 Role of arsenic in inducing diabetes mellitus

by both insulin resistance and a relative deficiency in insulin secretion (Flora 2011). Diabetogenic effects of arsenic are known; however, the exact mechanism remains unclear. Arsenic substitutes phosphate in the formation of adenosine triphosphate (ATP) and other phosphate intermediates involved in glucose metabolism. This slows down the normal metabolism of glucose, thus interrupting the production of energy and interfering with the ATP-dependent insulin secretion (Fig. 3).

Chronic arsenic exposure leads to increased oxidative stress and upregulation of tumor necrosis factor-a (TNF-α) and interleukin-6 (IL-6). These cytokines have been known for their effect on the induction of insulin resistance. Arsenic also shows inhibitory effect on the expression of peroxisome proliferator-activated receptor-g (PPAR-g), a nuclear hormone receptor important for activating insulin action. A major pathogenic link between insulin resistance and cell dysfunction is oxidative stress which exerts its action through activation of nuclear factor-kB (NF-kB). Superoxide production induced by arsenic can impair insulin secretion by interaction with uncoupling protein-2 (UCP-2). This may lead to amyloidal formation in the pancreas, which could progressively destroy the insulin-secreting cells (Tseng 2004). Arsenic inhibits glucose-stimulated insulin secretion (GSIS) by pancreatic β cells, besides increasing Nrf_2-mediated oxidative response, and decreasing glucose-stimulated peroxide accumulation. These observations suggest that low levels of arsenic triggers a cellular adaptive response, impairing ROS signaling involved in GSIS, and thus disturbs β-cell function.

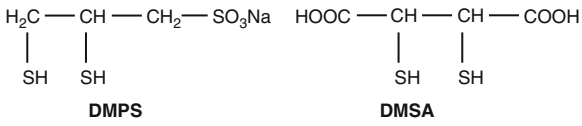
Carcinogenic Effects

Inorganic arsenic exposure induces cancer in human lungs, urinary bladder, skin, kidney, and liver, with the majority of deaths from lung and bladder cancer. Trivalent form of arsenic is more genotoxic than pentavalent form. Arsenic induces cancer by various mechanisms: (1) arsenic causes impairment of cellular respiration by the inhibition of various mitochondrial enzymes, and the uncoupling of oxidative phosphorylation, (2) arsenic potentially interacts with sulfhydryl groups of proteins and enzymes, and it substitutes phosphorous in a variety of biochemical reactions, and (3) arsenic can potentially induce the release of iron from Ferritin, an iron storage

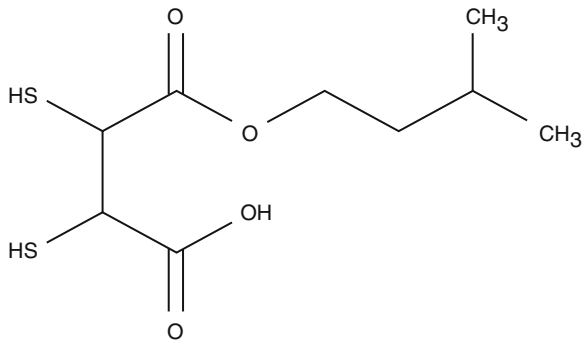
protein, which regulates intracellular concentration of iron. Once released free iron may catalyze the decomposition of hydrogen peroxide via the Fenton reaction, thereby generating the reactive hydroxyl radical which causes DNA damage. 8-Hydroxy-2′-deoxyguanosine (8-OhdG) is one of the major ROS-induced DNA damage products and used as biomarker of oxidative stress to DNA. Hepatic 8-OhdG levels increase on exposure to organic and inorganic arsenic, suggesting that arsenic elevates rate of free radical attack on DNA. Some clastogenic effects of arsenic are also mediated via free radicals (e.g., peroxynitrite, superoxide, hydrogen peroxide, and possibly free iron). Arsenic methylation leads to formation of more toxic, reactive, and carcinogenic trivalent methylated arsenicals (MMAIII and DMAIII). No carcinogenic effect of MMA (V) has been observed so far in in vivo studies. DMA(V) and MMA(V) are stored and reduced to form DMA (III) and MMA(III) in the lumen of the bladder; this organ is much more susceptible to cancer than liver and kidney (Cohen et al. 2006; Jomova et al. 2011). DMA (III) does not directly interact with DNA to exert genotoxicity but follows indirect pathway, i.e., formation of ROS. ROS formation activates a cascade of transcription factors (e.g., AP-1, c-fos, and NF-kB), and oversecretion of pro-inflammatory and growth-promoting cytokines, resulting in increased cell proliferation and ultimately carcinogenesis. In summary, it can be suggested that the exact mechanism for arsenic-induced carcinogenicity is still unclear; however, genetic and epigenetic changes, the role of oxidative stress, enhanced cell proliferation, and modulation of gene expression are some current recommendations.

Therapeutic Strategies

British Anti-Lewisite (BAL) or 2,3-dimercaptopropanol (Dimercaprol) is one of the oldest chelating agents for treating acute arsenic poisoning following ingestion, inhalation, or absorption. BAL however, is rather considered most toxic chelator available that restricts its application to few acute poisoning cases. Later, few derivatives of BAL were introduced which were safe and effective. Meso 2,3-dimercaptosuccinic acid (DMSA) and sodium 2,3-dimercaptopropane 1-sulfonate (DMPS) are water-soluble dithiols with safer drug profiles (Fig. 4). Both these drugs show predominantly extracellular distribution with DMPS



Arsenic in Pathological Conditions, Fig. 4 Chemical structures of DMSA and DMPS



Arsenic in Pathological Conditions, Fig. 5 Chemical structure of MiADMSA

showing some intracellular distribution also (Flora 2011). A major drawback associated with DMSA is its extracellular distribution, since it is unable to cross the cell membrane. Thus, esters of DMSA were synthesized to enhance chelation from intracellular compartments. Monoisoamyl DMSA (MiADMSA), a C5 branched chain alkyl monoester of DMSA, is one such investigational chelator which exhibits lipophilicity as compared to the parent DMSA. MiADMSA thus has been identified as a promising drug against arsenic-induced pathological lesions in experimental animals. Safety of MiADMSA has been well established in preclinical in vitro and in vivo models with copper depletion as the only prominent reversible side effect (Fig. 5). Combination therapy, administering a chelating agent either with an antioxidant or another structurally different chelator formulates effective therapeutic strategies against chronic arsenic toxicity. The advantages include providing an immediate and additional antioxidant effects and more pronounced arsenic excretion from different body compartments, respectively. Such combination therapy with a lipophilic and lipophobic (MiADMSA and DMSA) chelating agents would limit drawbacks like metal redistribution and form a safer therapy regime. Experimental studies revealed that such strategies result

not only in better reduction of body arsenic burden but also more effective recoveries in biomarkers, and neurological defects (Flora and Pachauri 2010). Thus, advanced therapeutic strategies may exhibit superior results as compared to traditional chelation monotherapy.

Cross-References

- Arsenic
- Arsenic and Aquaporins
- Arsenic in Nature
- Arsenic in Therapy
- Arsenic in Tissues, Organs, and Cells
- Arsenic Methyltransferases
- Arsenic, Free Radical and Oxidative Stress
- Arsenic, Mechanisms of Cellular Detoxification
- Arsenic-Induced Diabetes Mellitus
- Arsenicosis
- As

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Arsenic in Therapy

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Synonyms

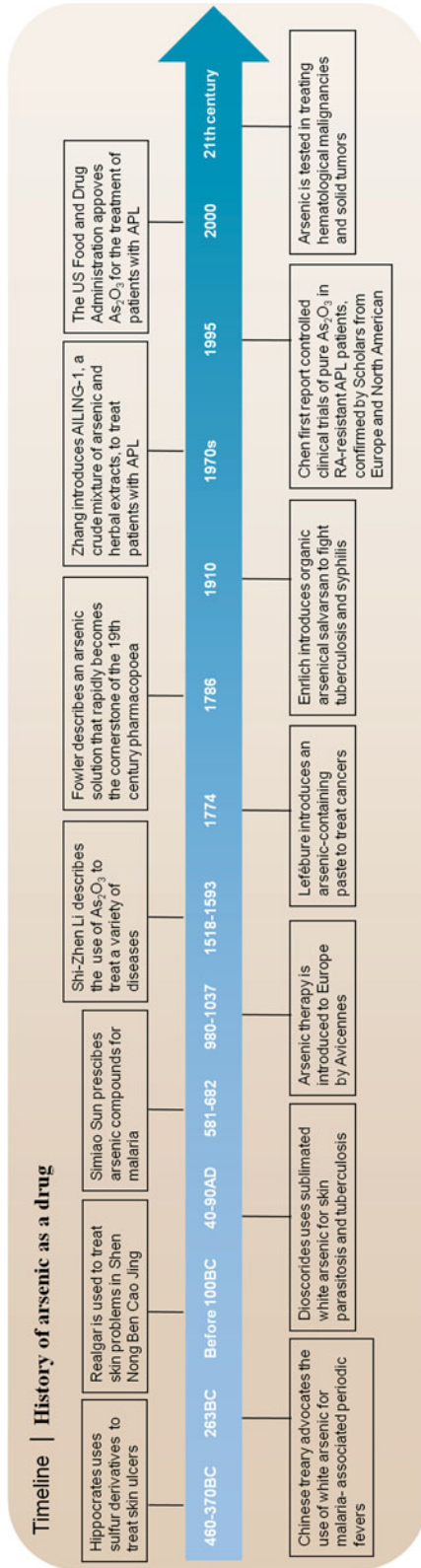
Acute promyelocytic leukemia; Arsenic sulfide; Arsenic trioxide; Darinaparsin; Melarsoprol

Definition

Arsenic is a natural substance and a traditional poison. The toxicity of arsenic is a double-edged sword. In fact, it has also been used as a drug with appropriate application for over 2,400 years in both traditional Chinese medicine and the Western world to treat many diseases from syphilis to cancer. However, the medical use of arsenic faced an embarrassment partly due to its low effect compared to modern therapy, but mostly because of concerns about the toxicity and potential carcinogenicity. In the modern era, interest in arsenic as a chemotherapy is rekindled after reports from China describing a high proportion of hematologic responses in patients with acute promyelocytic leukemia (APL) treated with arsenic trioxide. Numerous studies in the last two decades have confirmed arsenic trioxide as a successful treatment for APL. These results provided new insights into the pathogenesis of this malignancy and raised hopes that arsenicals might be useful in treating other cancers.

An Old Remedy

Although elemental arsenic was isolated about 700 years ago, arsenic compounds have been used as medicines by Greek and Chinese healers since more than 2,400 years ago (Fig. 1). Despite its carcinogenicity and the toxic effects associated with long-term exposure, scientists and physicians have used the



Arsenic in Therapy, Fig. 1 History of arsenic as a drug

poison successfully in practice to treat numerous ailments and diseases (Chen et al. 2011; Zhu et al. 2002). The first mention of arsenicals was made by Hippocrates (460–370 BC), who used realgar and orpiment pastes to treat ulcers. In China, arsenic pills for the treatment of malaria-associated periodic fever were recorded in the Chinese Nei Jing Treaty (263 BC). Realgar-containing pastes were used in the treatment of certain skin problems like carbuncle and were historically recorded in Shen Nong Ben Cao Jing (before 100 BC). Dioscorides (40–90 AD) later noted that arsenicals could cause hair loss but clear scabies, lice, and many skin growths that might have been cancers. Si-Miao Sun (581–682 AD) purified a medicine composed of realgar, orpiment, and arsenic trioxide in treating malaria. Later, the pharmacopoeia of Shi-Zhen Li (1518–1593 AD) in the Ming Dynasty described the use of As_2O_3 to treat a variety of diseases. Arsenic therapy was introduced to Europe by Avicenna (980–1037 AD) and Paracelsus (1493–1541 AD).

During the eighteenth, nineteenth, and twentieth centuries, a number of arsenic compounds have been used as medicines, including arsphenamine, neosalvarsan, and arsenic trioxide (Antman 2001; Zhu et al. 2002). In the 1700s, Lefébure introduced an arsenic-containing paste proposed to be an "established remedy to radically cure all cancers." Later, English inventor Thomas Fowler developed a solution of arsenic trioxide in potassium bicarbonate (KH_2AsO_3, 1% w/v), aptly named "Fowler's solution," that was used to treat asthma, chorea, eczema, pemphigus, and psoriasis. In the nineteenth century, arsenides and arsenic salts were used in the form of external pastes to treat ulcers and cancer. They were also prescribed as antiperiodics, antipyretics, antiseptics, antispasmodics, caustics, cholagogues, depilatories, hemantinics, sedatives, and tonics. The drugs were in liquid or solid form and could be inhaled as vapor, injected, administered intravenously, or given as enemas to treat systemic illnesses. In 1878, Fowler's solution was used to lower white blood cell counts in patients with chronic myelogenous leukemia (CML) and became a mainstay in the treatment of leukemia until it was succeeded by radiation in the twentieth century. Nevertheless, Fowler's solution resumed treating CML in 1931 after a report showing the remarkable response in nine patients to the treatment. However, this period was short because of the reports of arsenic's chronic toxicity and the efficacy of busulfan in 1950s. In 1910, a Nobel laureate, physician, and founder of chemotherapy, Paul Ehrlich introduced salvarsan, an organic arsenic-based product which was screened from 500 organic arsenic compounds. This compound was shown to be effective in treating tuberculosis and syphilis. Other organic arsenicals, such as melarsoprol, are still used to treat trypanosomiasis. Since this time, the use of the arsenicals in medicine declined because newer drugs had a greater therapeutic index and the toxicity of arsenic was concerned.

A New Trick

In the early 1970s, a group from Harbin Medical University in China studied arsenic oxide in a number of cancer types (Chen et al. 2011; Wang and Chen 2008). They found that intravenous infusions of Ailing-1 containing 1% As_2O_3 and trace amounts of mercury chloride could be used to treat patients with APL and achieved complete clinical remission (CR) in two-thirds of patients. From 1994, clinical trials with pure As_2O_3 were conducted by our group in Shanghai Second Medical University, in collaboration with the group from Harbin (Chen et al. 2011; Wang and Chen 2008). The efficacy of pure As_2O_3 has been demonstrated in patients with not only newly diagnosed APL but also APL cases relapsed after retinoic acid (RA) plus chemotherapy. A molecular remission is obtainable in a relatively high proportion of the patients, from 70% to 90% in different studies. Further studies conducted in Japan, Europe, and North America reported similar results. In 2000, the US Food and Drug Administration (FDA) approved As_2O_3 for the treatment of patients with APL that are resistant to ATRA. The efficacy of As_4S_4 in APL was also investigated (Chen et al. 2011; Wu et al. 2011). In traditional Chinese medicine, combination therapy containing multiple drugs with distinct but related mechanisms, called formulas, have been used for more than 2,000 years to amplify therapeutic efficacies of each agent and to minimize adverse effects. On the basis of traditional Chinese medicine (TCM) theories, a patented Realgar-Indigo naturalis formula (RIF) was designed in the 1980s, in which a mined ore realgar was the principal element, whereas Indigo naturalis, Salvia miltiorrhiza, and radix

pseudostellariae were adjuvant components to assist the effects of realgar. Multicenter clinical trials showed that a CR rate of more than 90% and a 5-year overall survival (OS) rate of about 85% were achieved in patients with APL receiving the RIF.

The remarkable therapeutic efficacy of As_2O_3 in APL rekindled the enthusiasm of the experts in hematology and oncology. The therapeutic efficacy of arsenicals in other hematologic cancers and solid tumors is under intensive investigation (Cui et al. 2008; Emadi and Gore 2010; Hu et al. 2005). Arsenic has been tested in treating hematological malignancies such as CML, multiple myeloma (MM), myelodysplastic syndrome, non-APL acute leukemia, myelofibrosis/myeloproliferative disorder, and lymphoid malignancies (including non-Hodgkin lymphoma) and has displayed beneficial effects in some cases, but the therapeutic efficacy was limited as compared to the APL miracle. Solutions containing arsenicals have been used as therapeutic agents since ancient times for CML, a malignant myeloproliferative disease of pluripotential hematopoietic stem cells that is characterized by BCR–ABL fusion protein with constitutively activated tyrosine kinase activity, and the results suggested that arsenic exhibits some inhibitory effects on proliferation of BCR–ABL–expressing cells. Clinical trials are underway to test the efficacy of arsenicals (As_2O_3 and As_4S_4) in combination with tyrosine kinase inhibitor imatinib for CML, because these regimens have showed synergetic effects in vitro and in vivo. Arsenic holds therapeutic promise in the treatment of MM, with data showing growth inhibitory and apoptotic effects of arsenic on myeloma cells and mice model. Clinical trials have demonstrated arsenic trioxide's activity in advanced refractory or high-risk MM. Several trials have demonstrated promising results in human T cell lymphotropic virus type I (HTLV-I)-associated adult T cell leukemia-lymphoma (ATL). A high synergistic effect between IFN-α and As_2O_3 in ATL-derived cell lines has been demonstrated. Similar results have been obtained with fresh leukemia cells derived from an ATL patient. Accordingly, an association between IFN-α and As_2O_3 has shown therapeutic effects in patients with ATL that have become refractory to other treatments.

There are more than 100 recently completed or ongoing clinical trials listed on www.clinicaltrials.gov evaluating As_2O_3 alone or in combination with other agents for treatment of cancers, excluding APL. As_2O_3 is under investigation as treatment for a variety of solid tumors including bladder cancer, glioma, breast cancer, hepatocellular carcinoma, pancreatic adenocarcinoma, cervical cancer, colorectal cancer, esophageal cancer, germ cell tumors, liver cancer, lung cancer, and melanoma. Limited clinical activity as a single agent has been reported in a small number of patients with hepatocellular carcinoma, melanoma, and renal cell carcinoma; As_2O_3 in combination with chemotherapy has shown promising activity in osteosarcoma and Ewing sarcoma.

Besides As_2O_3 and As_4S_4, some newly developed organic arsenic compounds have demonstrated anticancer efficacy in vitro, such as melarsoprol, dimethylarsinic acid, GSAO, and darinaparsin (Elliott et al. 2012; Mann et al. 2009). GSAO, 4-(*N*-(*S*-glutathionylacetyl)amino) phenylarsonous acid, has potential antiangiogenic capability with application in cancer where tumor metastasis relies on neovascularization. The phase I clinical study of GSAO is ongoing in patients with solid tumors refractory to standard therapy. Darinaparsin (ZIO-101) is an organic arsenical composed of dimethylated arsenic linked to glutathione and has significant activity in a broad spectrum of hematologic and solid tumors in preclinical models. The phase I/II clinical studies of darinaparsin are ongoing in patients with refractory solid tumors and demonstrate antitumor activity.

Cross-References

- Arsenic in Nature
- Arsenic, Biologically Active Compounds
- Promyelocytic Leukemia–Retinoic Acid Receptor α (PML–RARα) and Arsenic

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Arsenic in Tissues, Organs, and Cells

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Synonyms

Arsenic metabolism; Arsenic methyltransferases; Arsenic pharmacokinetics; Biomonitoring

Definition

As contained in water, soil, food, or atmosphere, the primary routes of arsenic entry into the body are ingestion and inhalation. Very little internal exposure to arsenic occurs via the material passing through the skin into the body. After absorption, arsenic is widely distributed by the blood stream throughout the body. Most tissues rapidly clear arsenic, except for skin, hair, and nails. The absorbed As undergoes biomethylation primarily by the liver. Inorganic As in the body is metabolized by the reduction of arsenate (As^{III}) to arsenite (As^{V}), followed by sequential methylation to monomethylarsonic acid (MMA) and dimethylarsenic acid (DMA). These methylation reactions have traditionally been regarded as a detoxification mechanism since the methylated metabolites exert less acute toxicity and reactivity with tissue constituents than inorganic As. Approximately 70% of arsenic is excreted, mainly in urine. Smaller amounts may be eliminated in the feces, sweat, hair, or nails. The metabolic process and distribution of arsenic in human body are closely associated with the paradoxical effects of arsenic: poison and medicine.

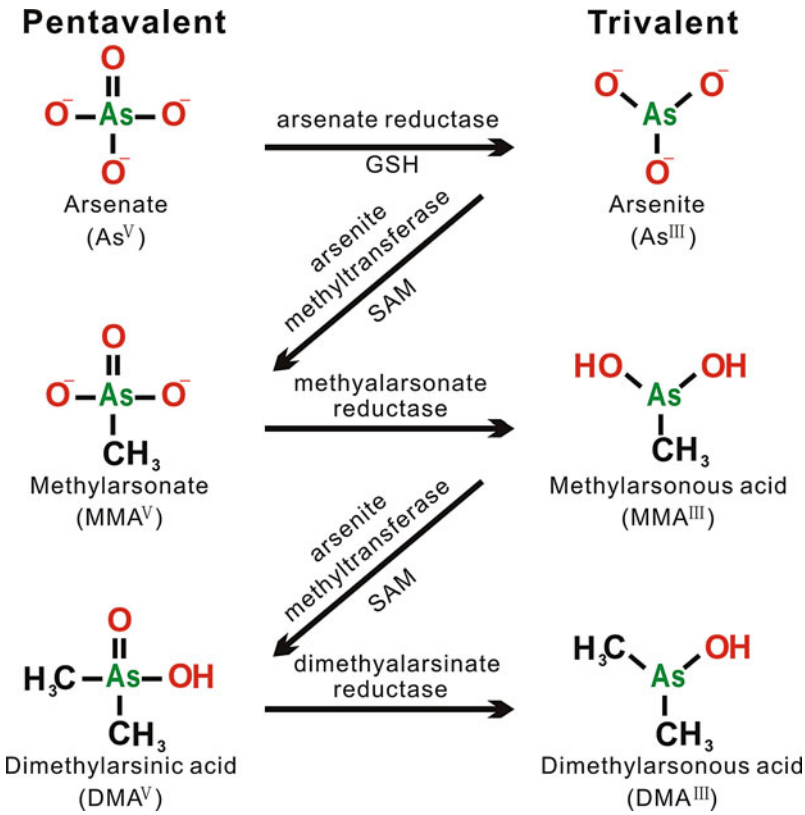
Sources and Route of Exposure

Humans are exposed to arsenic in the environment through ingestion of food and water, inhalation of polluted air, and, to a much lesser extent, dermal absorption. Food is usually the largest source except in areas where drinking water is naturally contaminated with arsenic. The daily intake of arsenic from food and beverages is generally between 20 and 300 μg/day (http://www.greenfacts.org/en/arsenic/ 2004). Arsenic in food is mainly in the form of organic arsenic. About one-quarter of the arsenic present in the diet is inorganic arsenic, mainly from foods such as meat, poultry, dairy products, and cereals. In drinking water, arsenic is present in the more toxic, inorganic form. The predominant form in water from deep wells is arsenite. Surface water contains mostly the arsenate, and the airborne pollutant is predominantly the unhydrated trivalent compound As_2O_3 (Rossman and Klein 2011).

Absorption

Correspondingly, the primary routes of arsenic entry into the body are ingestion and inhalation. Dermal absorption also occurs, but to a lesser extent. In the gastrointestinal (GI) tract, soluble arsenic compounds from food and beverages are rapidly and extensively absorbed into the blood stream. The amount of arsenic absorbed into the body from inhaled airborne particles is highly dependent on two factors, the size of particles and their solubility. The amount of arsenic absorbed by inhalation has not been determined precisely, but it is thought to be within 60–90% (ATSDR 2009).

The majority of arsenic enters the body in the trivalent inorganic form via a simple diffusion



Arsenic in Tissues, Organs, and Cells, Fig. 1 The methylation process of arsenic metabolism in humans

mechanism. Only a small amount of pentavalent inorganic arsenic can cross cell membranes via an energy-dependent transport system, after which it is immediately reduced to trivalent arsenic. Arsenic is absorbed into the blood stream at the cellular level and is taken up by red blood cells, white blood cells, and other cells that reduce arsenate to arsenite (ATSDR 2009). Since cells would have no reason to evolve uptake systems for toxic elements, both trivalent arsenite and pentavalent arsenate are taken up adventitiously by existing transport systems. Arsenate is taken up by phosphate transporters, while arsenite is taken up by aquaglyceroporins (AQP7 and AQP9 in mammals) and hexose permeases (GLUT1 and GLUT4 in mammals) (Rosen and Liu 2009).

Distribution and Metabolism

After absorption through the lungs or GI tract, arsenic is widely distributed by the blood throughout the body. Most tissues rapidly clear arsenic, except for keratin-rich tissues such as skin, hair, nails, and, to a lesser extent, in bones and teeth. The absorbed As is metabolized primarily by the liver and excreted by the kidneys into the urine. Smaller amounts may be eliminated in the feces, hair, nails, sweat, and skin desquamation. The metabolism of arsenic is a particularly complex pathway. In human, arsenic metabolism is characterized by two main types of reactions (Jomova et al. 2011; Nicolis et al. 2009; Rossman and Klein 2011): (1) reduction reactions of pentavalent to trivalent arsenic and (2) oxidative methylation reactions in which trivalent forms of arsenic are sequentially methylated to form methylated products using S-adenosyl methionine (SAM) as the methyl donor and glutathione (GSH) as an essential cofactor. The postulated scheme is as follows: $As^{V} \rightarrow As^{III} \rightarrow MMA^{V} \rightarrow MMA^{III} \rightarrow DMA^{V} \rightarrow DMA^{III}$ (Fig. 1). The enzyme catalyzing the biomethylation has been identified as arsenite methyltransferase (also named Cyt19, encoded by the gene *AS3MT*). Several factors have been suggested to influence the metabolism of arsenic, such as age, gender, genetic polymorphisms in genes coding for enzymes involved in arsenic metabolism,

exposure level, nutrition, smoking, alcohol use, and diseases (Cui et al. 2008). The metabolism and disposition of inorganic arsenic may be influenced by its valence state. In vitro studies have shown that the cellular uptake of As^{III} is greater and faster than that of As^{V} (Cui et al. 2008). Ingested organoarsenicals such as MMA, DMA, and arsenobetaine are much less extensively metabolized and more rapidly eliminated in urine than inorganic arsenic in both laboratory animals and humans. Studies show that, regardless of the As concentration, methylated metabolites can be detected in the liver cancer cell line HepG2, whereas no such metabolites are found in the leukemia cell lines NB4 and U937 (Cui et al. 2008). This observation suggests that leukemia cells have special metabolic system of arsenic which may exert antileukemia effects. Methylation has long been considered the main route of arsenic detoxification, but more recently, there has been a growing body of literature supporting other detoxification mechanisms. For example, a number of animal species lack arsenic methylation and excrete inorganic arsenic. The implication is that there may be other important arsenic detoxification mechanisms in mammals.

Excretion

Absorbed As is rapidly cleared from the blood, most of which is cleared with a half-life of about 1 h. In samples collected from patients for three weeks after the last intravenous administration of 0.15 mg As_2O_3/kg body wt, the arsenic content in blood cells is measured 6–10 times higher than plasma levels. Pentavalent arsenic is found in blood only transiently at the end of therapy and rapidly disappears. Trivalent arsenic concentration of blood rapidly decreases after the end of administration falling below detection limits 10 h after end of infusion. Methylated metabolites appear in blood 6 h after arsenic infusion and decreased more slowly over a few days. An elimination half-life of 70 h is reported for As_4S_4 oral administration. DMA is the primary As species in blood plasma and red blood cells with lower concentrations of inorganic As and MMA. Arsenic concentrations in blood from people with no unusual exposures range from 0.3 to 2 μg/L, much lower than those in urine, hair, or nails (Nicolis et al. 2009; Orloff et al. 2009).

The main route of arsenic excretion is in the urine through the kidneys. During the first 2–4 h after intravenous administration of As_2O_3, trivalent inorganic arsenite is the main compound found in urine, while pentavalent metabolites monomethylarsonic and dimethylarsinic acids become the major urine arsenic species after the first 24 h, dimethylarsinic acid being generally the most important one in percentage. Only small amounts of pentavalent inorganic arsenate are detected in urine. Approximately 50% of excreted arsenic in human urine is dimethylated and 25% is monomethylated, with the remainder being inorganic. However, there may be individual variations in percentage. After a single intravenous injection of radiolabeled trivalent inorganic arsenic in human volunteers, most of the arsenic are cleared through urinary excretion within 2 days, although a small amount of arsenic is found in the urine up to 2 weeks later. Fish arsenic is largely not biotransformed in vivo, but it is rapidly excreted unchanged in the urine. The biologic half-life of ingested fish arsenic in humans is estimated to be less than 20 h, with total urinary clearance in approximately 48 h. The concentration of metabolites of inorganic arsenic in urine reflects the absorbed dose of inorganic arsenic on an individual level. Generally, it ranges from 5 to 20 μg/L, but may even exceed 1,000 μg/L (Nicolis et al. 2009; Orloff et al. 2009).

Arsenic in the blood is in equilibrium with As in the hair root, so that As is incorporated into the hair shaft as the hair grows out. Arsenic (As^{III}) has a high affinity for sulfhydryl groups that are prevalent in keratin and other proteins in hair. The binding of As to keratin may be regarded as an excretory pathway since once As is incorporated into the hair matrix, it is no longer biologically available. Arsenic in hair is predominantly inorganic and less than 10% is organic. Arsenic in water or dust that comes into contact with the hair can also bind to the sulfhydryl groups in keratin and other proteins in hair. In individuals with no known exposure to As, the concentration of As in hair from multiple studies is generally in the range of 0.02–0.2 μg/g (Orloff et al. 2009; Rossman and Klein 2011).

The nail bed has a rich blood supply, and As is incorporated into the nail as it grows out. As with hair, inorganic As^{III} binds to sulfhydryl groups in keratin proteins in the nail matrix. External contamination of nails with As is a potential problem for biomonitoring, as it is with hair. Inorganic As is the primary As species in fingernails, although lower concentrations of DMA and MMA are also detected.

Normal As concentrations in nails are reported to range from 0.02 to 0.5 μg/g (Orloff et al. 2009; Rossman and Klein 2011).

Cross-References

- ▶ Arsenic and Aquaporins
- ▶ Arsenic and Primary Human Cells
- ▶ Arsenic and Vertebrate Aquaglyceroporins
- ▶ Arsenic in Nature
- ▶ Arsenic in Pathological Conditions
- ▶ Arsenic Methyltransferases
- ▶ Arsenic, Biologically Active Compounds
- ▶ Arsenic, Mechanisms of Cellular Detoxification

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Arsenic Metabolic Pathway

- ▶ Arsenic

Arsenic Metabolism

- ▶ Arsenic in Tissues, Organs, and Cells

Arsenic Methyltransferase

- ▶ Arsenic Methyltransferases

Arsenic Methyltransferases

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Synonyms

AdoMet:arsenic(III) methyltransferase; Arsenic methyltransferase; Arsenite methyltransferase; Arsenite S-adenosylmethionine methyltransferase; Cyt19; Monomethylarsonous acid methyltransferase; S-adenosyl-L-methionine:arsenic(III) methyltransferase

Definition

Arsenic methyltransferase (EC # 2.1.1.137) is an enzyme that catalyzes reactions that convert inorganic arsenic into mono-, di-, and tri-methylated products. Examples of arsenic methyltransferases have been identified in archaea, prokaryotes, and eukaryotes. Conversion of inorganic arsenic into methylated metabolites affects the environmental transport and fate of arsenic and its metabolism and disposition at cellular and systemic levels.

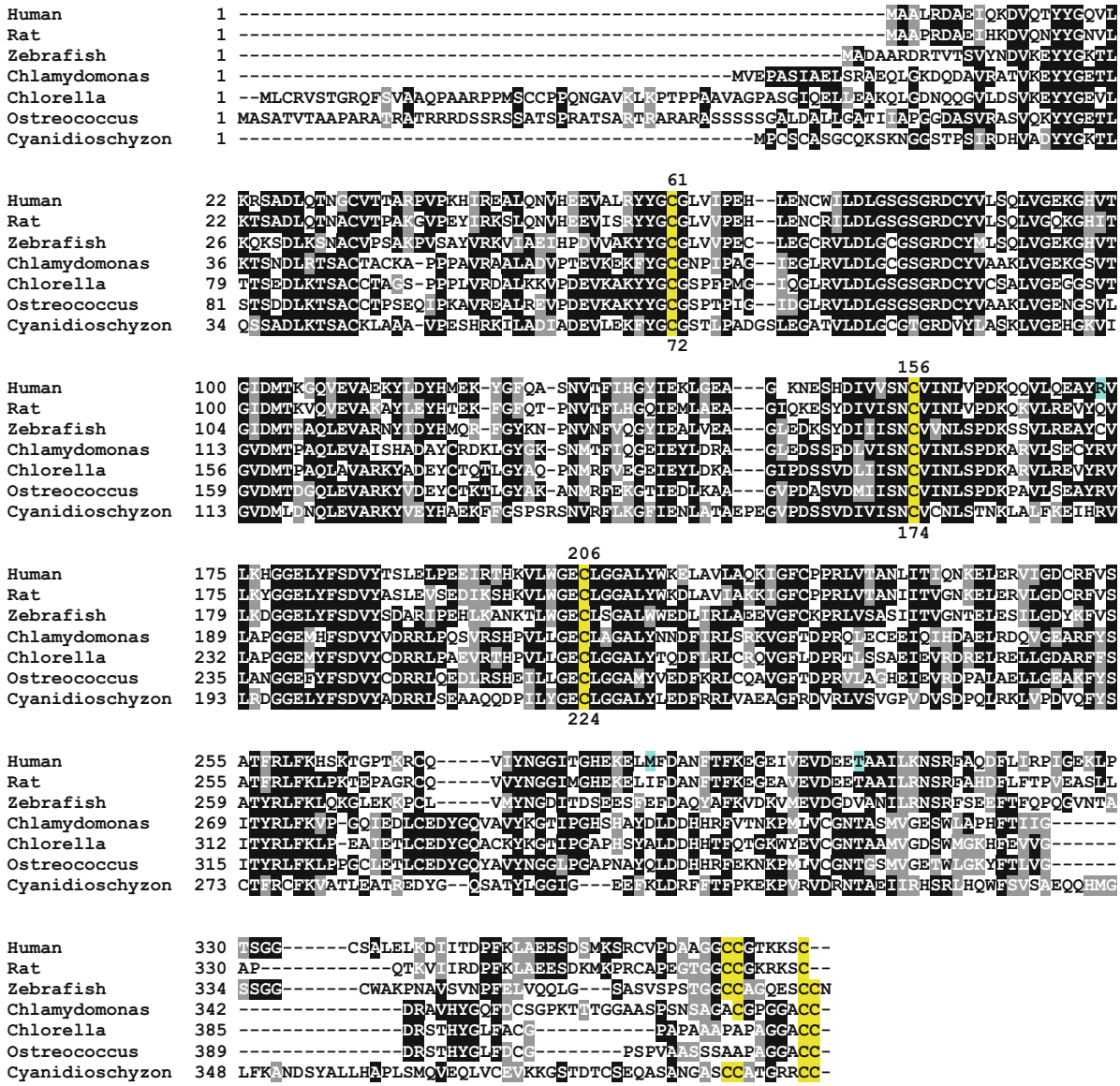
The metalloid arsenic enters the environment by natural processes (volcanic activity, weathering of rocks) and by human activity (mining, smelting, herbicides, and pesticides). Although arsenic has been exploited for homicidal and suicidal purposes since antiquity, its significance as a public health issue arises from its potency as a human carcinogen. In addition, considerable epidemiological evidence shows that chronic exposure to inorganic arsenic

contributes to increased risk of other diseases (Hughes et al. 2011). Interest in the biomethylation of arsenic as a factor in its environmental fate and its actions as a toxicant and a carcinogen originated in the nineteenth century with observations that microorganisms converted inorganic arsenicals used as wallpaper pigments into Gosio gas, a volatile species that is released into the atmosphere and that Gosio gas was trimethylarsine. Subsequent detection of methylated arsenicals in natural waters and in human urine suggested that biomethylation of arsenic was a widespread phenomenon (Cullen 2008).

These studies piqued interest in understanding the molecular basis of biomethylation of arsenic. Studies in mammals demonstrated methylation of arsenic to be enzymatically catalyzed and culminated in isolation and purification of an arsenic methyltransferase from rat liver cytosol and cloning and expression of cognate genes from rat, mouse, and human (Thomas et al. 2007). This gene was initially identified as *cyt19* but is now designated as arsenic (+3 oxidation state) methyltransferase (*As3mt*). The human *AS3MT* gene (accession number NP_065733.2) encodes a 375 amino-acid protein (41,745Da, EC # 2.1.1.137) that contains sequence motifs commonly found in non-DNA methyltransferases. Putative *As3mt* genes have been identified in genomes of deuterostomes ranging in complexity from purple sea urchin (*Strongylocentrotus purpuratus*) to *Homo sapiens*. The central role of As3mt in methylation of arsenic has been demonstrated by *As3mt* gene silencing by RNA interference and by heterologous expression of the *As3mt* gene in a human cell line that does not methylate arsenic (Thomas et al. 2007). Studies in *As3mt* knockout mice show capacity for arsenic methylation to be greatly diminished, albeit not eliminated, in these animals. Residual capacity for arsenic methylation in *As3mt* knockout mice may reflect methylation of arsenic by gut microbiota or arsenic methyltransferase activity of other methyltransferases. Arsenic methylation catalyzed by As3mt is central to the processes that control the distribution, retention, and clearance of arsenicals in tissues after exposure to inorganic arsenic. For example, reduced methylation of arsenic in *As3mt* knockout mice is associated with increased accumulation of inorganic arsenic in urinary bladder and exacerbation of injury to this organ. Conversely, there is evidence that methylated arsenicals containing trivalent arsenic formed during enzymatically catalyzed methylation of arsenic are more potent than inorganic trivalent arsenic (arsenite) as enzyme inhibitors, cytotoxins, and genotoxins (Thomas et al. 2007). Hence, methylation of arsenic in higher organisms has aspects that can be considered simultaneously as pathways for both activation and detoxification of this metalloid.

Parallel studies have demonstrated arsenic biomethylation in bacteria, archaea, fungi, and eukaryotic algae. In these organisms, *ars*enite S-adenosyl*m*ethyltransferase (ArsM) orthologues of As3mt catalyze arsenic methylation. Typically, prokaryotic and archaeal *arsM* genes are downstream of an *arsR* gene that encodes the archetypal arsenic-responsive transcriptional repressor that controls expression of *ars* operons suggesting that ArsM genes evolved to confer arsenic resistance. The first identified *arsM* gene was cloned from the soil bacterium *Rhodopseudomonas palustris* (Qin et al. 2006) and encodes for a 283-residue ArsM protein (29,656 Da) (accession number NP_948900.1). Its expression in an arsenic hypersensitive strain of *Escherichia coli* conferred resistance to arsenite. *E. coli* cells expressing recombinant *arsM* biotransformed medium arsenic into the methylated species, dimethylarsinic acid, trimethylarsine oxide, and trimethylarsine. These results clearly demonstrate that *arsM* gene expression is necessary and sufficient for arsenic detoxification. Purified recombinant ArsM catalyzed transfer of methyl groups from S-adenosylmethionine to arsenite, forming di- and trimethylated species, with the final product being trimethylarsine gas.

Commonalities and differences have been identified in the structure and catalytic function of arsenic methyltransferases from highly divergent species. Figure 1 shows that amino acid sequences are highly conserved in arsenic methyltransferases from species ranging from human (hAS3MT) to the thermophile *Cyanidioschyzon* strain 5,508, an environmental isolate of the eukaryotic red alga *Cyanidioschyzon merolae* from Yellowstone National Park (CmArsM). In all known arsenic methyltransferase orthologues, three cysteine residues are strictly conserved. These conserved cysteine residues (61, 156, and 206 in hAS3MT and 72, 174, and 224 in CmArsM7 from *C. merolae*) are required for catalytic function (Song et al. 2009; Marapakala et al. 2012). Loss of catalytic



Arsenic Methyltransferases, Fig. 1 Conserved cysteine residues are highlighted in *yellow*

activity in As3mt in which these critical cysteine residues have been replaced has been attributed to disruption of critical disulfide bonds between these cysteine residues (Fomenko et al. 2007). In contrast, formation of disulfide bonds is not observed in CmArsM during catalysis (K. Marapakala and B.P. Rosen, unpublished results). A final judgment on the role of critical cysteine residues will depend on structural characterization of the enzymes and elucidation of the catalytic process. In addition to these three strictly conserved cysteine residues, all arsenic methyltransferase orthologues have multiple C-terminal cysteine residues and pairs that are not absolutely conserved. These cysteine residues could form a site for initial binding of arsenite before its transfer to the catalytic cysteine residues or function to regulate enzyme activity.

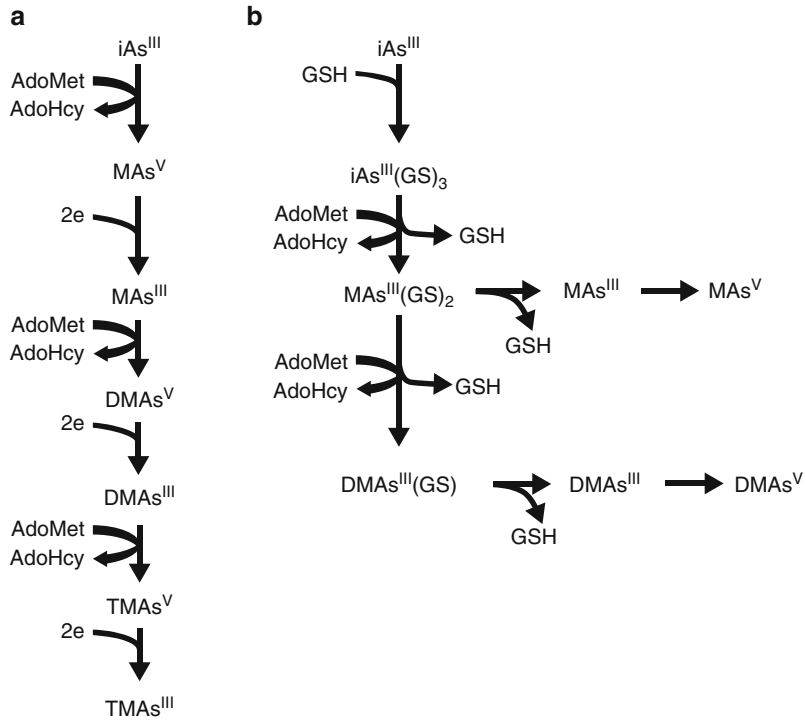
There are notable differences between As3mt and ArsM in reductants that support catalytic function. Arsenic methylation catalyzed by recombinant rat

As3mt (rAs3mt) shows a strong dependence on the presence of dithiol reductants, with activity maximized by addition of a coupled system containing the endogenous reductant thioredoxin (Tx), thioredoxin reductase (TxR), and NADPH. The monothiol glutathione (GSH) does not support methylation catalyzed by rAs3mt but does increase the overall rate of methylation when added to reaction mixtures containing the Tx/TxR/NADPH coupled systems and reduces the capacity of rAs3mt to catalyze formation of trimethylated arsenicals (Thomas et al. 2007). In contrast, studies with hAS3MT have shown GSH supports conversion of inorganic arsenic into mono- and dimethylated products (Hayakawa et al. 2005). Methylated products were detected in the presence of at least 2 mM GSH, and there was a positive relation between the percentage of arsenite present in an arsenite-$(GSH)_3$ complex and the extent of hAS3MT-catalyzed methylation. Although direct comparisons of the results with rAs3mt and hAS3MT are problematic due to differences in assay conditions and analytical procedures, it is notable that yields of methylated metabolites were much higher in rAs3mt-catalyzed reactions with the Tx/TxR/NADPH coupled systems than in hAS3MT-catalyzed reactions with GSH. In contrast, GSH alone is sufficient to support methylation of arsenic catalyzed by CmArsM7 (Marapakala et al. 2012). Arsenite-$(GSH)_3$ or methylarsonous acid-$(GSH)_2$ are bound to the enzyme faster than are arsenite or methylarsonous acid, suggesting that complexation of substrate by GSH accounts for its role in CmArsM7-catalyzed reactions. Because GSH is the only thiol present in cells at millimolar concentrations, it is possible that spontaneously formed $As(GS)_3$ is a direct donor of metalloid to CmArsM. In CmArsM-catalyzed reactions, the first round of methylation converts arsenite to methylarsonous acid and a second round of methylation converts methylarsonous acid to dimethylarsinous acid faster than methylarsonous acid dissociates from the enzyme. The latter product with a relatively lower affinity for the enzyme dissociates faster than it undergoes the third round of methylation. This model posits binding of a glutathionylated trivalent arsenical to the enzyme during repeated cycles of methylation and predicts dimethylarsinous acid to be the principal metabolic product.

Differences in requirements for specific reductants in reactions catalyzed by arsenic methyltransferases suggest two distinct models for the pathway of arsenic methylation. The Challenger scheme for arsenic methylation was originally developed as a chemically plausible pathway for the conversion of inorganic arsenic to methylated products based on studies in microorganisms (Challenger 1951). As shown in Fig. 2a, this pathway involves alternating rounds of oxidative methylation in which a methyl group is added to a trivalent arsenical. The resulting pentavalent arsenical is reduced to trivalency for additional cycles of oxidative methylation. An alternative pathway proposed by Hirano and coworkers involves reductive methylation of arsenic (Hayakawa et al. 2005). Here, trivalent arsenicals present as GSH conjugates or bound to protein thiols undergo repeated rounds of methyl group addition, and the appearance of methylated products containing pentavalent arsenic in urine is due to nonenzymatic oxidation of the glutathionylated intermediates or products (Fig. 2b). This pathway is supported by recent studies with CmArsM7 (Marapakala et al. 2012). Further studies of molecular interactions between reductants and arsenic methyltransferases or of the role of arsenothiol complexes as preferred substrates are essential to delineating the relevant pathway for arsenic methylation.

Variation in capacity for enzymatically catalyzed arsenic methylation can have practical consequences. A common genetic variant in human *AS3MT (*NCBI rs11191439) that occurs at a frequency of about 10% of most populations results in the replacement of a methionyl residue in position 287 with a threonyl residue (AS3MT/M287T). In population-based studies of the effect of AS3MT genotype on urinary arsenical profiles, AS3MT/M287T genotype has been associated with a higher concentration of methylated arsenic than was found with the wild-type AS3MT genotype (Engstrom et al. 2011) and with increased disease susceptibility (Hsieh et al. 2011) Thus, a specific AS3MT genotype may be linked to an arsenic methylation phenotype and to a disease susceptibility phenotype through increased production of methylated arsenic, a reactive and toxic metabolite of inorganic arsenic.

The bacterial *arsM* gene has the potential use for improving food safety. Rice, the primary source of nutrition for more than half of the world's population, is a natural arsenic accumulator. Because consumption of arsenic-containing rice could increase cancer risk



Arsenic Methyltransferases, Fig. 2 Alternative pathways for biomethylation of arsenic. (**a**) Oxidative methylation of arsenic converts trivalent arsenicals to methylated species containing pentavalent arsenic. Donation of a methyl group donor from S-adenosylmethionine (AdoMet) yields S-adenosylhomocysteine (AdoHcy). Pentavalent arsenic in methylated arsenicals is reduced to trivalency before each round of methylation (**b**). Reductive methylation converts a trivalent oxyarsenical into a glutathione (GSH) complex. This complex is the substrate for repeated rounds of methylation. Dissociation of arsenical-GSH complexes yields trivalent arsenicals that can be oxidized to pentavalency. iAs^{III} arsenite, $iAs^{III}(GS)_3$ arsenite-triglutathione, MAs^{V} methylarsonic acid, MAs^{III} methylarsonous acid, $MAs^{III}(GS)_2$ methylarsonous acid-diglutathione, $DMAs^{V}$ dimethylarsinic acid, $DMAs^{III}$ dimethylarsinous acid, $DMAs^{III}(GS)$ dimethylarsinous acid-glutathione, $TMAs^{V}$ trimethylarsine oxide, $TMAs^{III}$ trimethylarsine

(Williams et al. 2007), it would be useful to reduce arsenic accumulation in this food crop. One approach to reducing arsenic levels has been to clone the *R. palustris arsM* gene into rice to create transgenic plants that methylate arsenic (Meng et al. 2011). Although the process requires optimization, it confirms the validity of the approach and demonstrates that rice plants can be engineered to biotransform arsenic. This process might be optimized by using a plant gene rather than a bacterial one to transform rice. Although higher plants do not have *arsM* genes and do not methylate arsenic, *arsM* genes from lower plants, especially from eukaryotic algae, are candidates for use in rice modification (Yin et al. 2011). With the goal of increasing biomethylation and volatilization of arsenic, future experiments will be conducted with rice transformed with the *CmarsM* gene from the red alga *C. merolae*.

Disclaimer

This manuscript has been reviewed in accordance with the policy of the National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. BPR acknowledges support from United States Public Health Service NIH Grant R37 GM55425.

Cross-References

- ▶ Arsenic
- ▶ Arsenic and Aquaporins
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- ▶ Arsenic Methyltransferases
- ▶ Arsenic, Biologically Active Compounds
- ▶ Arsenic, Mechanisms of Cellular Detoxification

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A

Arsenic Pharmacokinetics

- ▶ Arsenic in Tissues, Organs, and Cells

Arsenic Sulfide

- ▶ Arsenic in Therapy
- ▶ Promyelocytic Leukemia–Retinoic Acid Receptor α (PML–RARα) and Arsenic

Arsenic Toxicity

- ▶ Arsenic

Arsenic Transporters

- ▶ Arsenic and Vertebrate Aquaglyceroporins

Arsenic Trioxide

- ▶ Arsenic in Therapy
- ▶ Promyelocytic Leukemia–Retinoic Acid Receptor α (PML–RARα) and Arsenic

Arsenic, Biologically Active Compounds

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Synonyms

DMA^{III} – dimethylarsenite; DMA^{V} – dimethylarsenate; MMA^{III} – monomethylarsenite; MMA^{V} – monomethylarsenate

Definition

Arsenic is widespread in the environment and many of its derivatives are highly toxic and carcinogenic. Toxicity of arsenic-containing compounds is primarily derived from their ability to form protein adducts via a reaction with cysteine residues and to deplete the cellular glutathione levels. Arsenic in living organisms is reduced and methylated to form less toxic organoarsenic compounds. Arsenic is also incorporated into arsenolipids, arsenosugars, and other types of organic compounds.

Inorganic and Organic Arsenic Compounds in the Environment

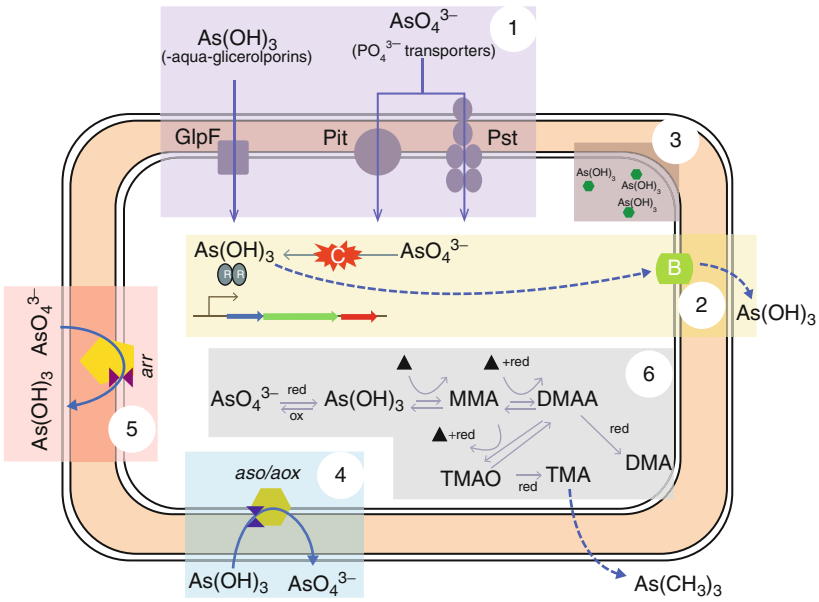
Arsenic is the 20th most abundant element on Earth and is often present together with sulfide minerals (Rezanka and Sigler 2008). In inorganic compounds, arsenic is present in a variety of oxidation states with As^{3+} and As^{5+} being the most common. Arsenic is released into the environment during burning of coal and other fossil fuels and during smelting of arsenic-containing ores. Arsenic derivatives have been routinely used as pesticides, wood preservers, and fungicides further contributing to release of this element although they are being phased out due to their relatively high toxicity. In the beginning of the twentieth century, organoarsenic compounds have been used as antibiotics although their use in this capacity has been discontinued due to availability of more effective and less toxic alternatives.

Arsenic is considered an essential element for humans and other mammals (Fig. 1). It is present in serum at the concentrations around 10 ng/ml. Lower levels of arsenic in blood (5–7 ng/ml) were observed in patients with CNS and cardiovascular diseases (Mayer et al. 1993). Low arsenic diet (0.1 mg/kg or less) leads to slower growth and higher risk of birth defects in several types of experimental animals. Arsenic is believed to be important in promotion of glutathione biosynthesis and metabolism of several amino acids including arginine and methionine (Uthus et al. 1983). However, the exact mechanism of its action is unknown.

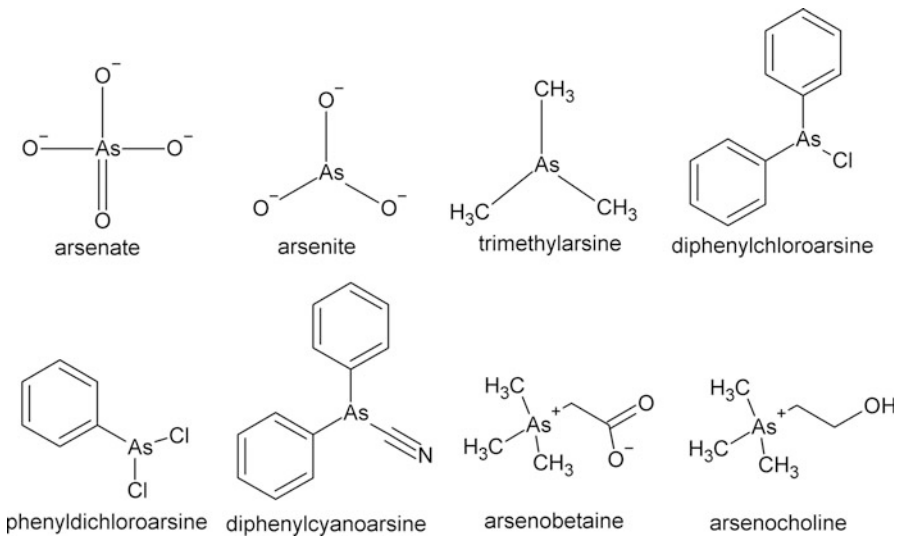
At higher concentrations, many arsenic derivatives are both highly acutely toxic and carcinogenic. Arsenates and elemental arsenic are not particularly toxic but toxicity of arsenites and many organoarsenic compounds (especially As^{3+} organoarsenics) is substantial. High abundance of arsenic in soil leads to its persistent presence in water at the level of several ppb (part per billion). WHO has determined that levels of arsenic in water above 10 ppb are unsafe for human health (Fawell and Nieuwenhuijsen 2003). However, in some water sources, arsenic levels are significantly higher, exposing over 100 million people in the world to the risk of increased incidence of cancer and other diseases. Several organoarsenic compounds (diphenylchloroarsine, diphenylcyanoarsine, phenyldichloroarsine, and lewisite) have been used as chemical weapons, and dimethylarsinic acid has been used as a defoliant (Fig. 2). Arsenic derivatives (especially arsenites) have also been used as poisons since at least the Middle Ages.

The most important mechanism of toxicity of arsenites and trivalent organoarsenic compounds involves their reactions with thiols. Arsenic derivatives rapidly react with cysteine residues of proteins such as enzymes and metal carrier proteins to form the As adducts (Fig. 3). These adducts are highly stable, and their formation inhibits normal function of enzymes and other proteins. Especially stable adducts are formed when As complexes with two neighboring sulfhydryl groups in a single protein. While excess glutathione can restore the protein function, prolonged exposure to arsenites depletes glutathione levels and leads to increased ROS production. Confirming its effect on ROS production, As^{3+} (1–25 μM) increased mRNA levels of antioxidant enzymes such as heme oxygenase-1, thioredoxin peroxidase 2, NADPH dehydrogenase, and glutathione *S*-transferase P subunit (Hirano et al. 2003). These enzymes are overexpressed as a defense mechanism against oxidative stress.

Toxicity of arsenate occurs via a different mechanism and stems from its ability to replace inorganic phosphate and form unstable arsenyl esters that hydrolyze spontaneously (Fekry et al. 2011; Nielsen and Uthus 1984). During glycolysis, this spontaneous hydrolysis of arsenate esters prevents formation of highly energetic phosphate esters that are later converted to ATP. Thus, energy produced during glycolysis is lost if arsenic substitutes for phosphorus.



Arsenic, Biologically Active Compounds,
Fig. 1 Diagram of the different processes involved in microbial arsenic metabolism. (*1*) Arsenic enters the cells. (*2*, *5*) Reduction of arsenate to arsenite and extrusion of arsenite. (*3*) Reaction of arsenite with thiols. (*4*) Oxidation of arsenite to arsenate. (*6*) Methylation of arsenate and arsenite (Reproduced with permission from Paez-Espino et al. (2009))

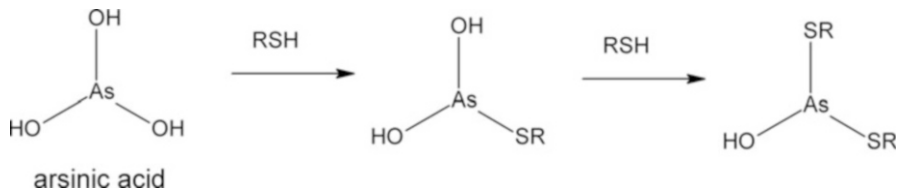


Arsenic, Biologically Active Compounds,
Fig. 2 Common arsenic-containing compounds in vivo: arsenate, arsenite, trimethylarsine, diphenylchloroarsine, phenyldichloroarsine, diphenylcyanoarsine, dimethylarsinic acid, arsenobetaine, arsenocholine

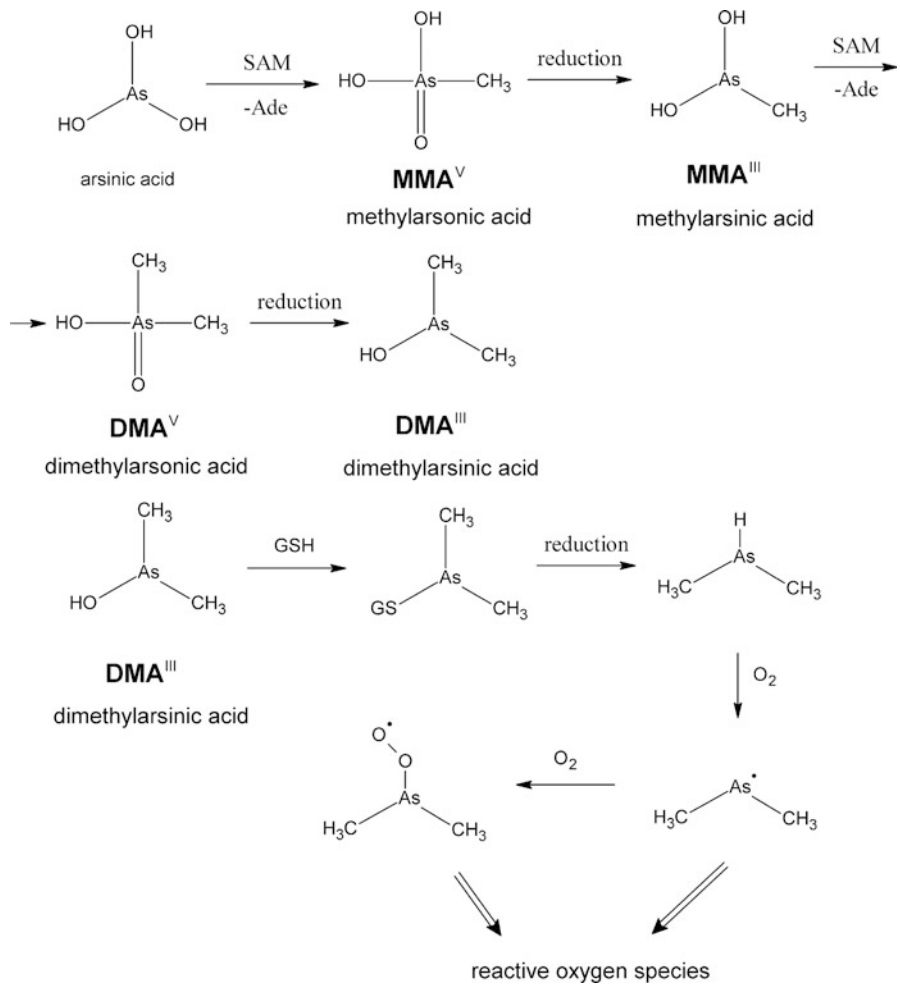
Metabolism of Arsenate and Arsenite

Metabolism of inorganic arsenate and arsenite in mammals involves four steps. A two-electron reduction of arsenate to arsenite is followed by methylation of arsenite to form monomethylarsonic acid (MMA^V). Another two-electron reduction of MMA^V to form monomethylarsenic acid (MMA^{III}) occurs, which is methylated to form dimethylarsonic acid (DMA^V) (Fig. 4).

The first step is the reduction of arsenate to arsenite. It is catalyzed by arsenate reductases. There are three types of this enzyme with two found exclusively in bacteria. Prokaryotic arsenate reductases (ArsC) utilize reduced glutathione and a thiol transfer protein glutaredoxin as reducing



Arsenic, Biologically Active Compounds,
Fig. 3 Reaction of arsenite with thiol



Arsenic, Biologically Active Compounds,
Fig. 4 Reductive methylation of arsenite and decomposition of DMA^V to dimethylarsine

agents. ArsC has a single catalytic Cys residue that forms a covalent intermediate with arsenate. Glutathione reacts with this intermediate, forming a mixed disulfide and displacing arsenite. Glutaredoxin reduces the mixed disulfide, regenerating the active site cysteine and reduced glutathione. Eukaryotic arsenate reductase has been identified in yeast. It is not closely related to bacterial arsenate reductases but instead is similar to tyrosine phosphatases. It has the same cofactor requirements as ArsC and appears to operate via the same mechanism. Glutathione and other thiols can also reduce arsenate to arsenite nonenzymatically but this reaction is quite slow in physiological conditions.

In addition, some phosphorylases are capable of reducing arsenate to arsenite. Purine nucleoside phosphorylase, glyceraldehyde-3-phosphate

dehydrogenase (GAPDH), glycogen phosphorylase, and ornithine carbamoyl transferase (Nemeti and Gregus 2009) have been identified as such enzymes although it is likely that other phosphorylases can catalyze this reaction as well. Phosphorylases add phosphate to their substrates, resulting in the cleavage of substrate into two products, one of which is phosphorylated. Arsenate can replace phosphate in these reactions, leading to an arsenylated metabolite. For example, purine nucleoside phosphorylase cleaves inosine with arsenate to yield hypoxanthine and ribose-1-arsenate. The resulting arsenate ester is rapidly reduced to arsenite by thiols in nonenzymatic fashion (Gregus et al. 2009).

Reverse reaction (oxidation of arsenite to arsenate) is catalyzed by the arsenite oxidase. It is a bacterial enzyme that consists of two subunits (aoxA and aoxB). This enzyme contains both molybdenum bound to pterin cofactor and iron-sulfur clusters (Santini and vanden Hoven 2004) and is similar to other molybdenum-containing redox enzymes. Azurin is used as an electron donor. Since arsenate is significantly less toxic compared to arsenite, this enzyme is common in the bacteria living in arsenic-rich environments (Santini and vanden Hoven 2004). For some of those bacteria, arsenite oxidation serves as a source of energy.

Conversion of arsenite to organoarsenic derivatives starts from its methylation to yield monomethylarsonic acid (MMA^V). This reaction is catalyzed by arsenite methyltransferase with *S*-adenosyl methionine (SAM) serving as a methyl donor (Fig. 4). Arsenate can also be methylated by this enzyme but the reaction is significantly slower. This enzyme is common in bacteria and animals but it is not present in plants. In some mammalian species, the enzyme is present in inactive truncated form. Methylation likely evolved as detoxification pathway as As(V) methylated species are relatively nontoxic and are rapidly cleared (Hall and Gamble 2012; Thomas et al. 2004).

MMA^V can be reduced to form monomethylarsenic acid (MMA^{III}). This reaction is catalyzed by MMA^V reductase, a member of glutathione *S*-transferase superfamily (Zakharyan et al. 2001). MMA^{III} can be methylated further to dimethylarsonic acid (DMA^V) by arsenite methyltransferase. DMA^V is reduced to DMA^{III} by MMA^V reductase although a specific DMA reductase may be present as well. DMA^{III} may be methylated further to trimethylarsine oxide although this reaction is inhibited by the presence of thiols and is thus uncommon in vivo. DMA^V is a major arsenic metabolite in humans (Mandal et al. 2001). While it does not react with thiols as rapidly as As^{3+} derivatives, both DMA^V and DMA^{III} can decompose to produce dimethylarsine (Fig. 4). Dimethylarsine rapidly reacts with oxygen to produce the dimethylarsenic peroxyl radical and other ROS (Yamanaka et al. 2009). These ROS are capable of damage to DNA and proteins and may be carcinogenic.

In general, organoarsenic compounds are significantly less toxic compared to arsenite. For example, LD_{50} for arsenite is 0.014 g/kg while that for MMA^V is 0.7 g/kg and for DMA^{III} is 3.3 g/kg (Paez-Espino et al. 2009). Formation of organoarsenic compounds is clearly a part of detoxification process for arsenic.

Bacterial Arsenite Extrusion System

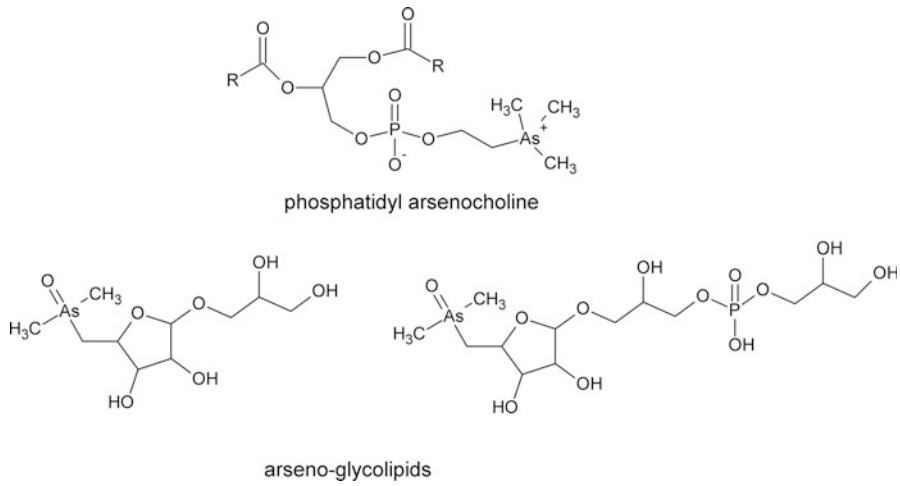
Many bacteria are resistant to arsenic toxicity. In addition to the methylation/reduction process described above, arsenite extrusion system contributes to this resistance. This system is composed of several proteins encoded by the *ars* operon: transcriptional repressor ArsR, arsenite efflux pump ArsB, and arsenate reductase ArsC (Paez-Espino et al. 2009). Additional components of the arsenite extrusion system (ArsA, ArsD, ArsH) are present in some bacteria as well. ArsA is an ATPase that serves as a source of energy for the arsenic pump ArsB and ArsD is an arsenic chaperon for this pump (Lin et al. 2007). ArsH is oxidoreductase that contributes to As reduction in some bacteria.

In its resting state, ArsR is bound to the promoter of *ars* operon inhibiting its transcription. Upon binding to As(III) derivatives, ArsR is activated and dissociates from DNA, allowing the transcription of arsenic extrusion proteins to occur. ArsR repressor is activated by covalent interaction of As(III) with three cysteines. ArsA ATPase is activated in the same manner. The role of ArsD is to transfer As(III) species to ArsA using the same three cysteine arrangement (Paez-Espino et al. 2009). The role of ArsH in the system is unclear.

Arsenic in Plants, Fungi, and Marine Organisms

Many species of mushrooms and other fungi have elevated concentrations of arsenic. In many of these

Arsenic, Biologically Active Compounds,
Fig. 5 Arsenolipids: phosphatidyl arsenocholine, dimethylarsenylribose derivatives



species, arsenic is primarily present in the form of arsenobetaine (up to 60–90% of total arsenic). In marine animals, arsenic is primarily present as arsenobetaine as well (Fig. 2). In addition, other arsenic-containing compounds have been found in marine organisms including arsenocholine, MMA, DMA, and a variety of arsenolipids (Fig. 5). Arsenolipids can be divided into two structural classes: hydrocarbon-based lipids and glycerolipids. Hydrocarbon-based arsenolipids usually contain dimethylarsinoxide moiety attached to a hydrocarbon chain. The mechanism for the biosynthesis of these compounds is unclear, and it has been suggested that dimethylarsinoyl-propionic acid might be a substrate for a fatty acid synthase. Arsenic-containing glycolipids include phosphatidylarsenocholine (presumably derived from arsenobetaine) and various derivatives of ribose with dimethylarsinoxide moiety in a 5′ position (Rezanka and Sigler 2008). These lipids, widespread in marine organisms, are believed to be synthesized by algae. Complex organoarsenic compounds do not react with thiols and are thus essentially nontoxic. Their formation is presumably a detoxification pathway for these organisms that live in the environment with relatively high arsenic concentrations.

Can As Replace Phosphorus in DNA?

A recent publication claimed that a strain of *Halomonadacea* bacteria (GFAJ-1) isolated from the arsenic-rich waters of Mono Lake, California, is able to substitute arsenic for phosphorus in its macromolecules including DNA and RNA and small molecule metabolites (Wolfe-Simon et al. 2011). The authors showed that these bacteria can grow at very low phosphate concentrations (0.02% of dry weight). In addition, EXAFS data indicated that arsenic is present in these bacteria as arsenate with geometry resembling that of phosphate in nucleic acids. These findings were hotly disputed with other researchers pointing out the half-life of the model arsenate triester is 0.06 s under physiological conditions. In addition, it was pointed out that arsenate esters are easily reduced to arsenite (Fekry et al. 2011). Other researchers have shown that very low phosphate concentrations observed in GFAJ-1 may be sufficient for synthesis of nucleic acids, given very slow propagation of these bacteria. In addition, mass spectroscopy showed that DNA isolated from GFAJ-1 bacteria contained no covalently bound arsenic. Growth of these bacteria at low phosphate levels was independent of arsenic levels and stability of DNA was inconsistent with the presence of highly labile arsenate ester bonds (Reaves et al. 2012).

Overall, it can be concluded that there is no evidence that arsenic is incorporated into the bacterial DNA.

Conclusions

Arsenic is a ubiquitous element, and wide variety of arsenic compounds is present in the environment. The most important feature of many arsenic compounds is their toxicity due to formation of stable thiol adducts.

Metabolism of arsenic derivatives is dominated by detoxification pathways attempting to convert toxic arsenite to less toxic organoarsenic derivatives. Despite their toxicity, arsenic compounds are important components of the food chains of many organisms and may play important biological roles.

Cross-References

- ▶ Arsenic
- ▶ Arsenic in Nature
- ▶ Arsenic Methyltransferases
- ▶ Arsenic, Mechanisms of Cellular Detoxification
- ▶ As

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Arsenic, Free Radical and Oxidative Stress

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Synonyms

Arsen; Arsenic black; Arsenicals; Free radicals; Hydroxyl radicals; Metallic arsenic; Oxidative stress; Reactive oxygen species; Sodium arsenate; Sodium arsenite; Superoxides anions

Definition

Arsenic, a naturally occurring metalloid, is known to generate free radicals that play a fundamental role in inducing oxidative stress–mediated toxicity.

Oxidative stress refers to serious imbalance between level of free radicals generated and cellular antioxidant defense that ultimately alters cellular redox status. Thus, oxidative stress may be induced either by accelerating the pro-oxidant species (direct) or by depleting the antioxidant defense (indirect). Free radicals are chemical entities with unpaired electrons or an open shell configuration. Most of these are highly reactive chemically, and play important role in normal physiology and in pathologic manifestations. Arsenic-induced oxidative stress is a multifactorial phenomenon produced by both direct and indirect mechanisms. Directly arsenic causes free radical generation (ROS) during its biotransformation processes or by facilitating through cellular pathway disruption. Arsenic also inhibits the antioxidant mechanisms of the body, thereby indirectly causing elevated ROS and oxidative stress. Arsenic-induced ROS results in the damage to biomolecules that are important cell components and modulates various cell signaling pathways which ultimately results in apoptosis or proliferative defects like cancer.

Arsenic: The Toxicant

Arsenic is a toxic metalloid acquiring most common oxidation states of +3, +5, and −3 existing in both organic and inorganic forms. Inorganic arsenic (iAs) is most abundant in nature existing as trivalent (As^{III}) or pentavalent forms (As^{V}). Arsenic in Earth's crust forms complex with pyrite which under favorable conditions, subjected to oxygen, pH, redox conditions, etc., gets oxidized to mobilize arsenic and release to groundwater. Elevated concentrations of arsenic above the WHO guideline level of 10 mg/l are present in most countries, although the prevalence and concentrations vary considerably. Southeast Asia is among the most severely affected regions, with highest ground water arsenic concentration reported from Bangladesh. Inorganic arsenic is a well-documented potent human carcinogen, causing cancer in skin, lungs, urinary bladder, kidney, and, possibly liver. Chronic exposure to arsenic through drinking water is associated with detectably increased risk of several non-cancer diseases (e.g., hyperkeratosis, pigmentation, cardiovascular diseases, hypertension, respiratory, neurological, liver and kidney disorders, and diabetes mellitus). Arsenic exposure induces diverse adverse effects at cellular and subcellular/molecular levels. This entry focuses on: (1) arsenic-induced free radical generation and oxidative stress, (2) role of methylation in arsenic-induced oxidative stress and toxicity, (3) cellular pathways, proteins, and biomolecules affected, and (5) the role of antioxidant in therapy against arsenic toxicity.

Direct Oxidative Stress

Arsenic-Induced Free Radicals Generation

Arsenic-induced free radical generation forms the foundation for producing oxidative stress. The most important players in the process of oxidative signaling include superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and peroxyl radical ($LOO^{\bullet}$). Thus oxygen based radicals form the most important class and superoxide anions are considered the "primary" reactive oxygen species (ROS). These radicals may further interact through enzyme- or metal-catalyzed reactions to form "secondary" ROS such as H_2O_2 and $^{\bullet}OH$ by $O_2^{\bullet -}$ dismutation and Fenton reaction. Mitochondria being directly involved in arsenic-induced ROS generation forms a crucial site for induction as well as important target for ROS-induced oxidative insult. Respiratory or the Electron Transport Chain (ETC) present at mitochondria physiologically functions to generate mitochondrial membrane potential (MMP). The MMP is a proton gradient generated by pumping out protons by utilizing energy produced during transfer of electron through ETC. At ETC, electron is transferred from NADH and succinate through enzymatic series of donors and acceptors to ultimately reach oxygen that is reduced to water. MMP is then utilized to generate ATP by oxidative phosphorylation. Free radical superoxide is generated when some electrons leak to directly reach oxygen, bypassing the complete ETC transactions. These radicals then attack the phospholipids of mitochondrial membrane damaging it to further disrupt the respiratory chain, thus forming a vicious circle. Arsenic promotes deflection of electrons from ETC, thus inducing ROS generation and a decline of MMP that ultimately leads to apoptosis (see later sections). Arsenite increases the free radical generation especially at the

ubiquinone site (Complex I) of the respiratory chain. Increase in cellular superoxide anions following arsenic exposure also involves cytosolic, peroxidase enzymes like cytochrome P-450, that sequentially transfer two electrons from NADPH to molecular oxygen. Flavin enzymes such as NAD(P)H oxidase and NO synthase isoforms also mediate arsenic-induced ROS generation. NAD(P)H oxidase physiologically activates during respiratory outburst for generation of superoxide anions for phagocytosis in neutrophils. Arsenic increases the NAD(P)H oxidase activity by either upregulating the enzyme at different levels like gene expression and translocation of its subunit $p22_{phox}$ and Rac1 respectively, or by enzyme activation. NO synthase isoforms physiologically catalyze generation of NO from substrate L-arginine involving essential cofactors. Under stress conditions like limited availability of substrate or cofactor, NO synthase results in reduced NO production along with superoxide anion generation. Arsenic depletes heme tetrahydrobiopterin (BH_4), one of its five cofactors possibly due to destruction by superoxide anions. In view of increased superoxide generation, depleted levels of NO during arsenic exposure may also be attributed to peroxynitrite (ONOO−) formation following reaction between NO and $O_2^{\bullet -}$. Peroxynitrite is not a free radical but rather a highly reactive anion, a powerful oxidant and nitrating agent. In addition to mitochondria, other important sources of arsenic-induced ROS generation include (1) superoxide anion production in the process of arsenic biotransformation

$$H_3AsO_3 + H_2O + O_2 \rightarrow H_3AsO_4 + H_2O_2 \left(\Delta rG\theta = -\ 40.82\ \text{kcalmol}^{-1}\right)$$

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + \bullet OH + OH^- \ \text{(Fenton reaction)};$$

(2) during oxidation of arsenic from its As^{III} to As^{V} form, the intermediary free radical arsenic species are generated such as dimethylarsinic peroxyl $[(CH_3)_2AsOO^{\bullet}]$ and dimethylarsinic radicals $[(CH_3)_2As^{\bullet}]$; (3) methylated arsenic species especially dimethylarsinous acid (DMA^{III}) release redox-active iron from ferritin. Arsenic induces heme oxygenase isoform 1 (HO-1) leading to production of additional free iron, CO, and biliverdin. Free iron plays a central role in ROS generation via Fenton type and/or Haber–Weiss reaction that combines a Fenton reaction, such as facilitating conversion of $O_2^{\bullet -}$ and H_2O_2 into highly reactive $^{\bullet}OH$.

$$O_2^{\bullet -} + H_2O_2 \rightarrow O_2 + {}^{\bullet}OH + OH^-$$

Arsenic increases oxygen consumption by cell, thereby contributing to an increased metabolism and ultimately ROS generation. These effects are due to uncoupled oxidative phosphorylation. ROS is critically associated with various tightly regulated physiological pathways and processes essential for integrity of cell and synchronization of biological system. ROS acts as a secondary messenger and, by modulating intracellular redox status, induces signaling pathways, downstream gene expression, and cell proliferation or death. Thus, arsenic by induction of ROS disrupts various physiological processes (Flora 2011; Jomova et al. 2011).

Arsenic Methylation and Toxicity

Virtually every organism possesses an arsenic detoxification/biotransformation mechanism involving transport systems and enzymatic pathways possibly due to its ubiquitous occurrence in nature. Since arsenic species have no physiological role, they utilize the existing cellular transports by mimicking electrochemical characteristics of essential ions rather than any dedicated system. In pH range of 4–10, pentavalent arsenate species are negatively charged while trivalent arsenic compounds remain neutral, and thus they readily cross the cell membrane. The pentavalent arsenate is reduced to trivalent arsenite in blood stream before entering cell for further metabolism. The reduction of arsenate to arsenite was initially believed to occur nonenzymatically utilizing GSH which is now known as enzymatic reaction through arsenate reductase. Arsenate reductase however requires inosine and thiol compound for the reaction. Reduction of arsenate can also be catalyzed by human liver MMA^{V} reductase/ hGSTO-1. Arsenite was initially believed to enter cell by simple diffusion, being a neutral species at physiological pH. However, it is now known to be transported inside cell via aquaporin isozyme 7 or 9 (AQP7/9), a member of

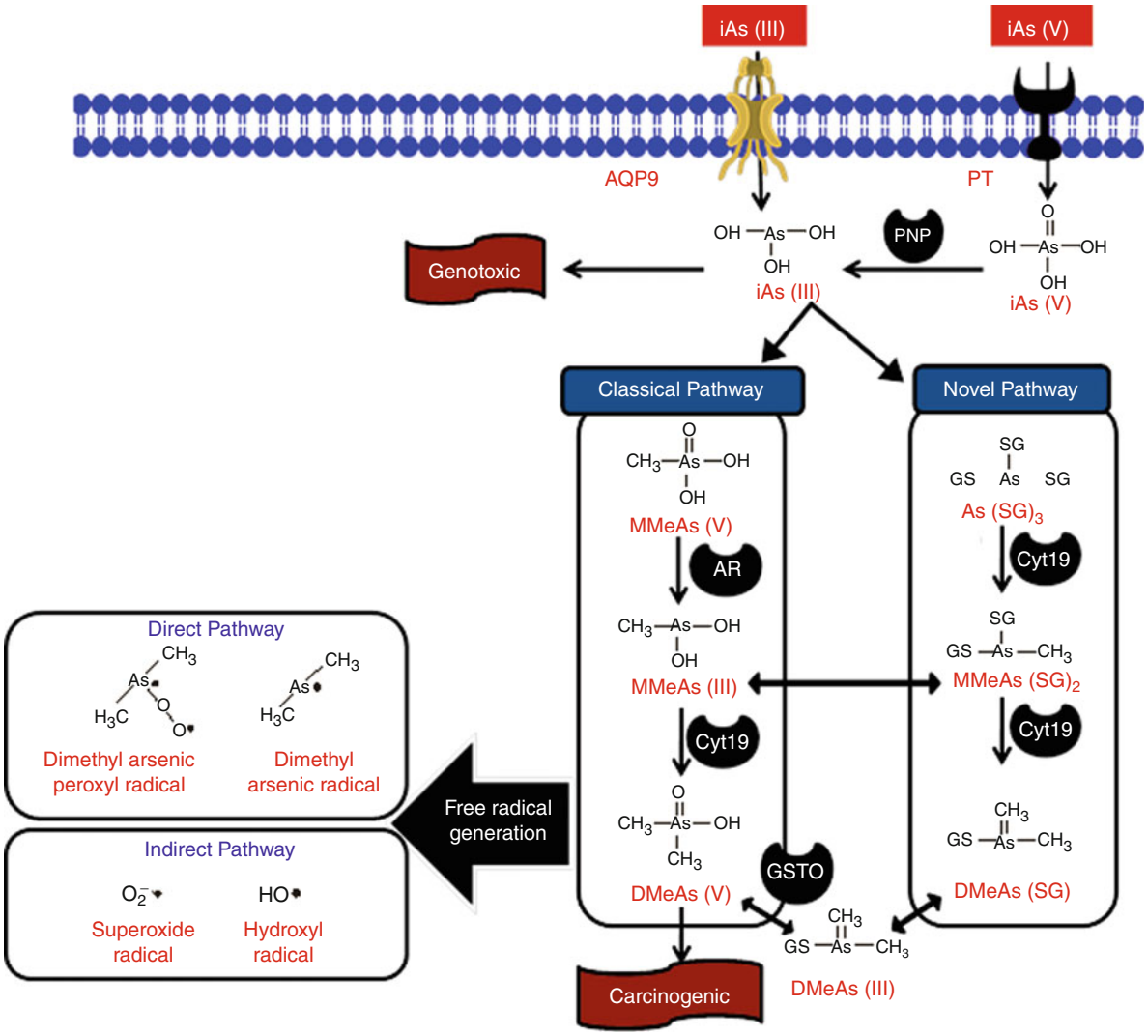
aquaglyceroporins. Arsenate (iAs^V) enters cell by phosphate carrier system and competes with phosphate inside the cell, for example, binding with polyphosphates like ADP.

Arsenic metabolism follows two possible pathways. The classically known pathway follows reduction and oxidative methylation and the second novel pathway involves GSH conjugation reactions and methylation. In the classical pathway, pentavalent arsenate or its methylated species such as monomethylarsenate (MMA^V) or dimethylarsenate (DMA^V) is reduced to its corresponding arsenite through arsenate reductases. These arsenate reductases are omega isoform of GSH S-transferase (GSTO-1) and purine nucleoside phosphorylase (PNP). Subsequently, the trivalent arsenite (iAs^{III}) or its methylated species, monomethylarsenite (MMA^{III}), then undergoes oxidative methylation through arsenite methyltransferase and Cyt19 to form pentavalent monomethylarsenate (MMA^V) or dimethylarsenite (DMA^V), respectively. The MMA^V reductase is the rate-limiting enzyme for the inorganic arsenic methylation/classical pathway. MMA^V reductase has an absolute requirement for GSH not replaceable by any other thiol. In the novel pathway, inorganic trivalent arsenite (iAs^{III}) forms GSH conjugates to produce arsenite triglutathione [$As(SG)_3$] and subsequent methylation by Cyt 19 to yield a monomethylarsonic diglutathione [$MMS(SG)_2$] followed by dimethylarsinic glutathione [DMS(SG)]. The GSH-conjugated species however can undergo spontaneous degradation to form intermediates of the classical pathways. For example, the $MMA(SG)_2$ may either (1) be degraded to MMA^{III}, followed by oxidation to MMA^V, or (2) methylate to DMA(SG) through Cyt 19, followed by DMA^{III} to DMA^V. DMA^V is the major metabolite excreted in urine. In the process enzymatic methylation of arsenic requires S-adenosylmethionine as the methyl donor and reducing agents such as GSH and cysteine. Unlike most xenobiotics arsenic biotransformation/methylation does not result in its detoxification. The trivalent arsenic species especially the MMA^{III} are most toxic among its methylated forms and represent obligatory intermediates to ultimate end products like MMA^V and DMA^V. Therefore, Cyt19 polymorphism forms an important factor capable of influencing arsenic toxicity in humans.

The iAs^{III} possesses unshared 4s electron pair that renders it capable to interact with biological molecules, which is lacking in the iAs^V. The methylated arsenite possesses one, two, or three methyl substituents that significantly alter the chemical behavior of the moiety, in addition to the unshared electrons. Quantum mechanical calculations predict that addition of methyl group to MMA^V increases the dipole moment and the ionization energy and decreases the molecular volume available for delocalization of electron density. Since dipole moment is directly correlated to log P, it is speculated that log P will increase following methylation of MMA^V. Although iAs^{III} species are not free radicals themselves, they may give rise to free radical species. Dimethylarsine, a trivalent arsenic species, can react with molecular oxygen to produce dimethylarsenic [$(CH_3)_2As^{\bullet}$] radical and superoxide anion. Addition of another oxygen molecule to the dimethylarsenic radical forms dimethylarsenic peroxyl [$(CH_3)_2AsOO^{\bullet}$] radical (Fig. 1). Superoxide and hydrogen peroxide, generated through superoxide dismutase from superoxide anion, produces hydroxyl radicals (Cohen and Arnold 2006; Kumagai and Sumi 2007; Aposhian et al. 2004).

Indirect Oxidative Stress

Other than accelerating the pro-oxidant mechanism, arsenic induces oxidative stress also by inhibiting or depleting the antioxidant defense. The major components of antioxidant defense system are nonenzymatic and enzymatic free radical scavengers, inhibitors to free radical generating enzyme and metal chelators. Reduced glutathione (GSH) is an important nonenzymatic antioxidant that plays an important role in maintaining cellular redox status. Arsenic alters cellular GSH concentration to induce oxidative stress. Arsenic-induced oxidative stress is mediated through decrease in GSH concentration by three important mechanisms: (1) GSH is utilized during arsenic metabolism, (2) arsenic by virtue of thiol-binding property shows high affinity for GSH, and (3) arsenic-induced free radicals oxidize GSH which gets converted to glutathione disulfide (GSSG). However, arsenic-induced GSH modulation depends on arsenic concentration and cellular response to arsenic attack. During acute arsenic exposures, increase in GSH concentration occurs possibly as cellular response to combat arsenic-induced elevated



Arsenic, Free Radical and Oxidative Stress, Fig. 1 Role of biotransformation in arsenic toxicity

ROS levels. However, GSH depletion occurs following chronic arsenic exposures (Shi et al. 2004). Other nonenzymatic cellular antioxidants affected by arsenic include vitamin C and E, flavonoids, carotenoids, essential elements (zinc, selenium), and amino acids such as taurine, methionine, cysteine, and thiol compounds. Thiols constitute the most important class of compounds involved in arsenic-induced toxic mechanism. These include N-acetylcysteine, thioredoxin, α-lipoic acid other than GSH. Enzymatic antioxidants such as superoxide dismutase (SOD), catalase, and glutathione peroxidase also show immediate increase followed by decrease in their activity following arsenic exposure subjected to dose and duration of exposure. Arsenic alters the antioxidant status of the cell by its action either at the molecular level, i.e., inhibition of antioxidant activity by structural alterations, such as binding to thiol groups and displacement of essential metal cofactors, and/or at the genetic level by affecting the antioxidant expression. For example, arsenic induces downregulation of SOD2 expression in patients with arsenic-induced skin lesions. These subjects were also indicated of deficient wound healing. Arsenic possibly causes suppression of chemokine response pathway by downregulation of TNF and CCL20 expression.

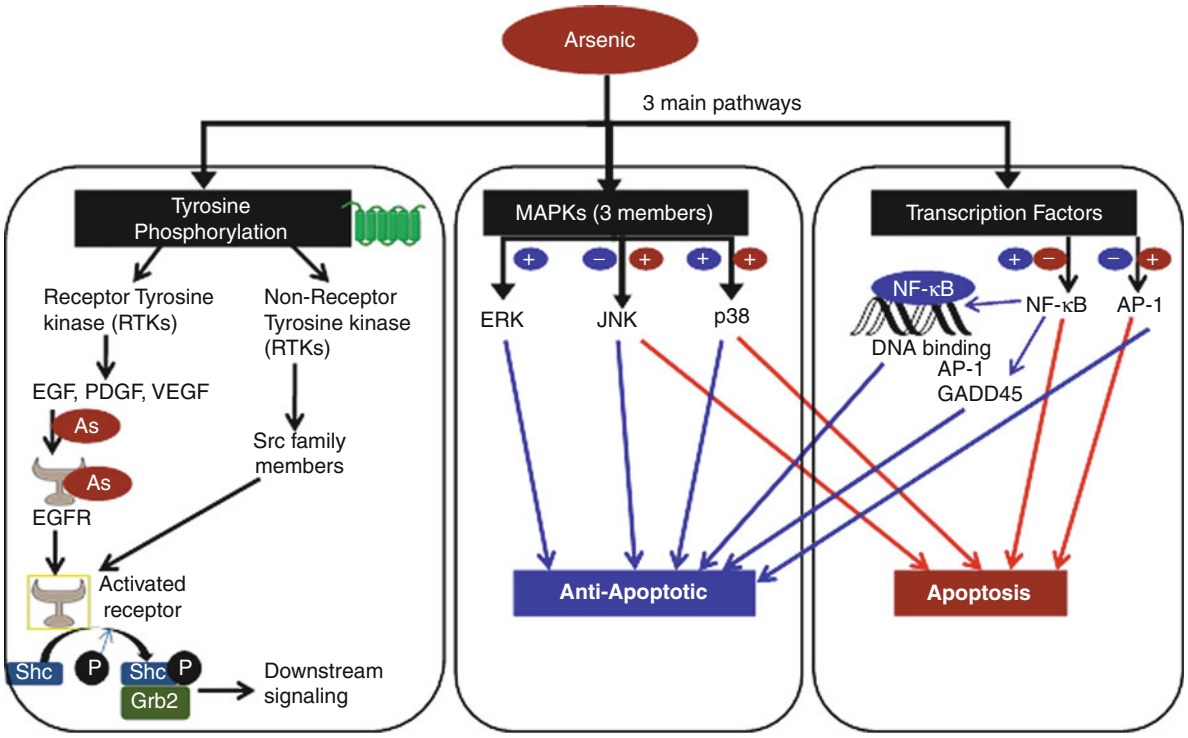
Arsenic also affects other important cellular enzymes such as thioredoxin reductase which is depleted in a concentration-dependent manner especially by methylarsine(III), ultimately resulting in induction of apoptosis. Thioredoxin reductase physiologically functions to catalyze reduction of many disulfide-containing substrates and is NADPH dependent. Heme oxygenase (HO-1) is a cytoprotective enzyme, an oxidative stress protein, expression of which is regulated by Nrf-2. Arsenic induces upregulation of HO-1 expression which is suggested as cellular adaptive mechanism to combat its toxicity (Vizcaya-Ruiza et al. 2009).

Arsenic Disrupts Signal Transduction Pathways

Cell signal transduction pathways are tightly regulated orchestra that operate crucial physiological functions and dictate paraphrase like cell differentiation, apoptosis, and proliferations. Intrinsic interaction of arsenic with the components of cell signaling pathways either directly or through ROS determines its toxicity and fate of the cell. Major pathways affected by arsenic include tyrosine phosphorylation system, mitogen-activated protein kinases (MAPKs), and transcription factor families such as NF-κB and AP-1 (Flora 2011). Tyrosine phosphorylation system modulates and participates in various cell physiological processes and signal transduction pathways. Activation of enzyme protein tyrosine kinases and/or inhibition of tyrosine phosphatases induces tyrosine phosphorylation reaction. These kinases are either receptor mediated, i.e., receptor tyrosine kinases (RTKs) or nonreceptor tyrosine kinases (NTKs). RTKs include several growth factors such as epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), vascular endothelial growth factor receptor (VEGFR). Activation of RTKs either by ligand binding or receptor cross-linking leads to autophosphorylation on tyrosine residue, inducing conformational change that initiates cascade of reactions. Arsenic causes increased total cellular tyrosine phosphorylation, mainly affecting EGFR. Arsenic either binds directly with EGFR or causes dimerization for its activation. Tyrosine phosphorylation–induced conformation change in the EGFR relays the signal to downstream cascade pathway. EGFR tyrosine phosphorylation induced by arsenic is also responsible for activation of MAPK via Ras protein–mediated signaling. Arsenic induces EGFR also via NTKs such as Src in human epithelial cell line either by direct interaction with its vicinal thiol groups or integrin rearrangements, or indirectly via ROS. Arsenic-induced Src causes tyrosine phosphorylation of EGFR at tyrosine residues that are unique from its autophosphorylation sites. Src also activates MAPKs which is mediated by two parallel pathways, i.e., EGFR-dependent or EGFR-independent pathways.

MAPKs induction by arsenic is largely dependent on arsenic species, dose, duration, and cell type exposed. MAPK is a family of serine/threonine phosphorylating proteins that in response to various extracellular stress–mediated response regulates gene expression. Three major classes of MAPK, namely, extracellular signal regulated kinases (ERKs), c-Jun N-terminal kinases (JNKs), and p38 kinases, are differentially affected by arsenic exposure. ERKs are responsible for cell differentiation, transformation, and proliferation, while JNKs and p38 kinase are involved in cell growth arrest and apoptosis stimulated in response to stress. Thus, arsenic-induced carcinogenic effect involves the activation of ERKs, while apoptotic and anticarcinogenic effects are mediated through JNKs and p38 kinases. Ras/Raf/Mek pathway mediates ERK and p38 kinases activation and Rac, Rho, and MEKK3-4 activate the JNKs. Arsenic-induced ERK activation is mediated by various signal pathways most defined through RTKs such as EGFR. ERK in turn activates AP-1 (c-Jun + c-Fos) and ATF-2 transcription factors, resulting in its proliferating response. Mechanism for arsenic-induced JNK-mediated apoptosis is unclear. JNK upon activation is translocated to mitochondria and interacts with Bcl-$_{XL}$ and Bcl-2 where it phosphorylates and inactivates Bcl-2, ultimately resulting in release of cytochrome *c* and apoptosis proteases (Fig. 2).

MAPKs activation by arsenic influences the activation of transcription factors such as NK-κB and AP-1. The transcriptional pathways closely are involved in cell transformation and apoptosis. NK-κB is a dimeric transcription factor(s) belonging to Rel family. This is activated rapidly in response to stress and functions as a transcription factor for gene controlling cytokines, growth factors, and acute response proteins. Arsenic may pose differential and dose-dependent effect on NK-κB, i.e., at low,



Arsenic, Free Radical and Oxidative Stress, Fig. 2 Effect of arsenic on cellular signaling pathways

non-cytotoxic dose it activates NK-κB while at higher dose inhibits it. Activation of NK-κB generally involves arsenic-induced ROS or via degradation of its inhibitor IκB. Additionally, arsenic-induced NK-κB activation involves cross talk with ERKs and JNKs. Inhibition of NK-κB involves repression of IκB and inhibition of phosphorylation and degradation of IκB. Arsenic induces AP-1 transactivation and expression of c-jun and c-fos gene (Qian et al. 2003; Kitchin and Ahmad 2003).

Arsenic on Cellular Pathways: Protein Interface

As discussed in the previous section iAs^{III} has high affinity for sulfhydryl groups, and iAs^{V} mimics phosphate and is an uncoupler of oxidative phosphorylation. Thus, both iAs^{III} and iAs^{V} have potential to produce oxidative stress through uncoupling of mitochondrial oxidative phosphorylation, and/or increased cellular production of H_2O_2. Arsenic-induced oxidative stress leads to cell damage and death through activation of oxidative sensitive signaling pathways that affects a large number of genes and proteins involved in different physiologic and pathologic pathways.

Proteins involved with oxidative stress, such as heat shock proteins, metallothionein, heme oxygenase, ubiquitin, aldose reductase, and ferritin light chain, are induced by arsenic. Arsenic induces DNA damage, lipid peroxidation, and protein modification and apoptosis, through ROS-mediated reactions. In addition, the expression of genes encoding for several drug-metabolizing enzymes, such as CYP1B1, CYP2B9, CYP7A1, CYP7B1, CYP3A11, is also affected/altered (suppressed) during acute arsenic toxicity.

Heat Shock or Stress Proteins

Heat shock or stress proteins (HSPs/SPs) are constitutively expressed redox-sensitive proteins, that are synthesized in almost all organisms following induction due to exposed to a range of stressors, including oxidative stress, heat shock, free radicals, UV radiation, and heavy metals. Physiologically present in low concentrations, these function as "molecular chaperones" contributing to maintenance of the cellular homeostasis and defense. These support

the proper folding, assembly, and distribution during protein synthesis. These are also involved in modulation of immune system. HSPs through epigenetic repair also called heat shock response protect and repair vulnerable protein targets and DNA repair. HSPs are classified as metalloproteins (MT), ubiquitin (Ub), proteins of 15–40kDa such as HO, proteins of 60, 70, 90, and 110kDa. Additionally, stress-induced synthesis of HSP compared to heat shock-induced proteins is classified as analogous, subset, and specific induction. The specific induction causes synthesis of proteins exclusively by metals but not by heat such as metallothionein (MT). These metalloproteins are involved directly in the cellular detoxification of metal or protection and repair of metal-induced metabolic dysfunction. HSP induction by arsenic constitutes a defensive mechanism of cells against oxidative stress–induced damage. iAs^{III} is an effective inducer of a number of stress-related proteins including HSP27, HSP32, HSP60, HSP70, and HSP90 (Razo et al. 2001).

HSP27 belongs to small heat shock or stress proteins (sHSPs) family. Under stress condition such as arsenic exposure, these large oligomers dissociate into small oligomers as a result of phosphorylation by kinases. iAs^{III} increases the HSP27 phosphorylation by inhibiting protein phosphatase activity. HSP27 prevents apoptosis by regulating the activation of the phosphoinositide 3-kinase/protein kinase B pathway and inhibiting cytochrome *c*-dependent activation of procaspase 9. It can also decrease the intracellular level of ROS by modulating the metabolism of glutathione to maintain it in a reduced state. Thus, these have the potential to protect cell against iAs^{III} toxicity.

The Hsp60 synthesis, during stress, in cells is rapidly upregulated. Hsp65 is involved in autoimmunity by stimulating antibodies directed against it. Arsenic modulates cytokine secretion and increases the production of many cytokines including the tumor necrosis factor-a (TNFα), the proinflammatory cytokine interleukin 1a (IL-1a), the neurotrophic cytokine IL-6, the neutrophil chemotactic cytokine IL-8 as well as cellular growth factors such as the transforming growth factor-a (TGFα) and the granulocyte macrophage-colony stimulating factor (GM-CSF) as immunopathological response. HSP 60 and HSP 70 mediate inflammatory responses to arsenic as the expression of IL-1b, IL-6, TNF-a, and IFN-d mRNAs has been associated with the expression of Hsp60, Hsp47, Hsp70, and Hsp70/Hsp86 mRNAs. These proteins are involved in the induction/progression of hypertension, atherosclerosis and are markers for early cardiovascular disease associated with chronic arsenic exposure. Arsenic (iAs^{III}) also induces proteins that are functional homologue of HSP60 such as TCP1 involved with microfilaments.

HSP70 physiologically functions as chaperone by assisting in the membrane translocation of new synthesized structural or secretory proteins. These also prevent the misfolding and translocation during new protein synthesis by binding to them until process completion. The Hsp70 family of proteins plays a key role in reproductive physiology, particularly in gametogenesis and embryogenesis, and encodes the major inducible Hsp expressed in a wide range of tissues. Trivalent arsenic induces HSP70 differentially in various tissues. DMA exposure in vivo, for example, causes higher accumulation of HSP72 in lung compared to kidney. Presence of the stress-inducible *Hsp70–1* and *Hsp70–3* helps protect embryos from the effects of As^{III}-induced neural tube defects and related toxicity.

Hsp 90 is induced in stress responses that result in glucose depletion, intracisternal calcium, or other disruption of glycoprotein trafficking. iAs^{III}-induced stress response causes induction of HSP90 and HSP70 which is mediated through increased progesterone receptor (PR) activity. Homologous to HSP70 and HSP90 are "glucose-regulated proteins" (Grps) (74–78 and 94 kDa), another class of stress proteins which are involved in glucose-related stress. iAs^{III} induces Grp74 and Grp94; however, elevation of Grp94 in arsenic-induced diabetes needs to be investigated.

Metallothionein (MT)

MT is a sulfhydryl-rich, low-molecular-weight (6–7-kDa) metal-binding protein that provides protection against metal-induced oxidative cellular injury. Induction of metallothioneins (MT I and II) represents a major means of metal detoxification in the body to maintain trace elements within a physiological range or to protect the body from the damage by metal overload. Arsenic-induced MT greatly depends upon the arsenic species. iAs^{III} is the strongest inducer which is followed by its pentavalent forms, iAs^{V}, $MMAs^{V}$, and $DMAs^{V}$. Arsenic-induced MT induction is mediated by ROS, signaling pathways such as inflammatory response and induction of transcription factors such as MTF-1, rather than iAs chelation.

MTs regulate metal homeostasis by sequestering metals in protein-bound forms, providing a zinc reserve, and serving as a scavenger to quench ROS and other free radicals. The increased cellular tolerance to arsenic may result from an increase in the efficiency of transcription through an activation of the involved regulatory factors.

Heme Oxygenase (HO)

HO, an enzyme of 32 kDa, is responsible for the physiological breakdown of heme into equimolar amounts of biliverdin, carbon monoxide, and Fe using O_2 and electrons donated by NADPH-cytochrome P450 reductase. Induction of HO-1both in vivo and in vitro is a hallmark of arsenic-induced oxidative stress. HO-1 induction is dependent on the level of arsenic, its redox status, and target organ. Trivalent arsenicals (iAs^{III}) form the most effective inducers, while pentavalent methylated forms ($MMAs^{V}$ or $DMAs^{V}$) are ineffective. Since distribution and biotransformation of arsenic is organ specific, the kinetics and dynamics of arsenicals dictate the induction. For example, liver shows highest HO-1 induction due to predominance of iAs form. After arsenic exposure, induction of HO-1 may confer cytoprotection from oxidative damage by promoting the conversion of pro-oxidant metalloporphyrins, such as heme, to bile pigments (biliverdin and bilirubin) with free radical scavenging capabilities. The levels of the *HO-1* gene are transcriptionally controlled. The expression of HO-1 is under the regulation of AP-1 and NF-kB transcription factors and is mediated through activation of MAP kinase pathway.

Ubiquitin (Ub)

Ub is a low-molecular-weight (7–8-kDa) protein involved in the nonlysosomal degradation of intracellular proteins. Under stressful conditions, Ub acts as the cofactor for cytosolic degradation of abnormal proteins to maintain chromosome structure by a set of proteins induced under different environmental stress factors. iAs^{III} at high concentrations causes its inhibition while following low level exposures results in an increase in the Ub-conjugation enzymes, inducing the accumulation of Ub-conjugated proteins. Therefore, when As^{III} inhibits the deubiquitination, it contributes to increase in its toxicity by proteolysis of aged, damaged, or dysfunctional proteins. In contrast, when As^{III} increases the level of Ub, the protein allows the cell to enhance the repair process of tagged proteins; it also targets selective molecules for degradation, repair of accumulated proteins and damaged DNA, and provides transient elevation of cellular defenses.

Aldose Reductase (AR)

Aldose reductase is a member of the aldoketoreductase family that acts as an antioxidative stress protein. Following arsenite-induced oxidative insult, these metabolize several aldehyde product compounds including 4-hydroxy *trans*-2-nonenal, a major toxic product of lipid peroxidation. Arsenite upregulates AR in vitro.

Arsenic-Mediated ROS-Induced DNA Damage

Arsenic-induced oxidative stress leads to DNA damage, DNA hypo- or hyper-methylation forming an important mechanism underlying its apoptotic, necrotic, or carcinogenic effects. Acute inorganic arsenic exposure in vivo results in induction of DNA damage-inducible proteins GADD45 and GADD153 and the DNA excision repair protein ERCC1, as well as other DNA damaging-repair genes. Enzymes involved in nucleotide excision repair (NER) and base excision repair (BER) are also affected by arsenic. Trivalent arsenicals inhibit BER and NER activity by interacting with zinc finger motifs of proteins in these two DNA repair systems. Arsenic unlike other carcinogens does not induce mutations. It does not directly covalently bind to DNA but causes damage indirectly through ROS, leading to DNA adducts, strand break, cross-link, and chromosomal aberrations. Arsenic-induced hydroxyl radicals are mainly responsible for DNA damage through oxidation of any of the four bases especially thymidine. DNA methylation state is also altered by arsenic exposure by modifying the activity of DNA methylation enzymes. Mechanisms involved include either direct interaction of arsenic-thiol enzyme or indirectly through ROS-enzyme interaction. Depletion of S-adenosyl-methionine(s) required for DNA methylation via arsenic may also contribute to DNA effects. Arsenic also inhibits DNA ligase, thus inhibiting DNA repair. Arsenic also causes strand break via superoxide radicals and related secondary radicals by activation of NADH oxidase.

Arsenic-Mediated ROS-Induced Apoptosis

Apoptosis is a critical cellular response to maintain normal cell development and proper function of multicellular organisms. There are two major signaling pathways of apoptosis: the death receptor pathway (extrinsic) and the mitochondrial pathway (intrinsic). Arsenic-induced ROS-mediated apoptosis is well documented in the literature, in which the mitochondria-driven apoptotic pathway is probably a more favorable mechanism for arsenic-induced cell death. Excess ROS increases the mitochondrial membrane permeability and damages the respiratory chain, resulting in increased ROS production. The disruption in the mitochondrial membrane causes the release of cytochrome c from the mitochondria. The release of cytochrome c then activates caspase-9, which initiates the activation of caspases-3, -6, and -7, leading to apoptosis. Caspases, which are aspartate-specific cysteine proteases, are cytoplasmic proenzymes that play an important role in initiation and effector phases of apoptosis. A large percentage of the altered genes and proteins are known to be regulated by redox-sensitive transcription factors, (SP1, NF-κB, AP-1), suggesting that, at environmentally relevant levels of chronic exposure, arsenic may be acting through alteration of cellular redox status.

Role of Antioxidants in Arsenic-Induced Oxidative Stress

Various synthetic and natural antioxidants show beneficiary effects in arsenic-induced toxicity. Although chelation therapy forms the mainstay in therapy against arsenic toxicity, antioxidants are recommended as adjuvant for the additional benefits. There is no therapy defined yet for arsenic toxicity post chronic exposure. Since oxidative stress forms the basis for arsenic-induced toxicity, antioxidant therapy is recommended as the primary and immediate strategy to combat toxicity in clinical cases. Antioxidants have shown promising results both as monotherapy and as adjuvant to the primary chelation under investigation. Conventionally known antioxidants, such as vitamin C, E, N-acetyl cysteine (NAC), etc., provide significant benefit in arsenic toxicity. NAC is a precursor of L-cysteine and reduced glutathione. It is a thiol-containing mucolytic agent. Thus, mechanisms involved in NAC-mediated protection against arsenic include ROS scavenging, elevate cellular antioxidant, GSH, and mild chelation by virtue of sulfhydryl-containing group. Additionally antioxidants of natural origin and crude forms like the plant extracts have also gained success in lowering arsenic-induced oxidative stress. Some of the most promising candidates include *Curcumin, Hippophae rhamnoides, Aloe Vera barbadensis, Centella asiatica* and *Allium sativum*. Certain antioxidants like NAC or α-lipoic acid are associated with chelation ability and show specific protection against arsenic toxicity. *Moringa oleifera* is one such natural antioxidant that protects against arsenic-induced oxidative stress additionally by lowering the body arsenic burden. The chelation property of *Moringa oleifera* is attributed to the interaction between cysteine- and methionine-rich proteins that are present in high amount in its seeds. Similarly, organosulfur compounds present in garlic form the active agents effective in reducing the clastogenic effects of arsenic with possible role of p53 and heat shock proteins. Arsenic chelation ability of garlic is due to its thiosulfur components which act as Lewis acids and interact with Lewis base (arsenic) to form stable components. Natural polyphenols such as flavonoids are another class of compounds that have shown beneficiary effects. α-Lipoic acid (1,2-dithiolane-3-pentanoic acid, 1,2-dithiolane-3-valeric acid or thioctic acid, LA,) and its reduced form, the dihydrolipoic acid (6,8-dimercaptooctanoic acid or 6,8-thioctic acid, DHLA), are established synthetic antioxidants which also possess chelation property due to its thiol moiety. Lipoic acid protects against arsenic toxicity by its dual ability of lowering free radicals and arsenic from the system (Flora 2011).

Cross-References

- ▶ Arsenic
- ▶ Arsenic and Aquaporins
- ▶ Arsenic in Nature
- ▶ Arsenic in Pathological Conditions
- ▶ Arsenic in Therapy
- ▶ Arsenic in Tissues, Organs, and Cells
- ▶ Arsenic Methyltransferases
- ▶ Arsenic, Mechanisms of Cellular Detoxification
- ▶ Arsenic-Induced Diabetes Mellitus
- ▶ Arsenicosis
- ▶ As

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Arsenic, Mechanisms of Cellular Detoxification

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Synonyms

As

Definition

Arsenic (As) is a naturally occurring metalloid, found in water, air, and food; a frequent source of human exposure to inorganic As is from groundwater contamination. As has several oxidation states but those of greatest toxicologic importance are the inorganic arsenite (III) and arsenate (V) states.

As in the Environment

Arsenic (As) is a naturally occurring metalloid found in the Earth's crust and present in the environment. It can leach into the groundwater and its presence is the result of industrial emissions and natural sources such as surrounding sediment especially in places with volcanic tuff. The highest natural levels of As in groundwater are found in the West, Midwest, and Northeast regions of the United States and in India, China, Chile, Taiwan, and Bangladesh. Arsenic and arsenic compounds have been used in many insecticide sprays, weed killers, fungicides, wood preservatives, waste incineration, disinfectant compounds, paints, drugs, dyes, soaps, semiconductors, agricultural products, prints, and enamels. Its use has also been documented in homeopathic remedies and most notably historically as a homicide and suicidal agent. It is found in rice, poultry, and seafood and arsenic trioxide (ATO) has been used in the treatment of acute promyelocytic leukemia (APL) relapse.

Human Health Impact

A common source of human exposure to inorganic As is through groundwater and drinking water contamination. The maximum contaminant level (MCL) of As in drinking water is regulated by the US Environmental Protection Agency (EPA) and the World Health Organization (WHO) at 10 parts per billion (ppb 0.01mg/L) (Lievremont et al. 2009). Due to its widespread nature, As poses a significant risk to human health. Inorganic As has been associated with skin, lung and bladder cancers, vascular diseases, hypertension, genotoxicity, cellular disruption, developmental anomalies, decreased intellectual function, and diabetes (NRC, 2001). Arsenic is considered a Class I human

carcinogen by the International Agency for Research on Cancer due to its permissiveness to skin, lung and bladder cancers.

Elemental and Inorganic As

Organic arsenic is formed by the combination of arsenic with carbon and hydrogen. As can form inorganic complexes with oxygen, chlorine, and sulfur. It has several oxidation states but those of greatest toxicologic importance are inorganic trivalent arsenite (AsIII) and pentavalent arsenate (AsV), the two forms that are also most commonly found in drinking water (Smedley and Kinniburgh 2002).

Fate of As in the Human Body

Inorganic As species are well absorbed by both the oral and inhalation routes but can also enter the body through dermal exposure and injection. Once absorbed by the body As can be distributed to all systems. Arsenic is stored in the liver, kidney, intestines, spleen, lymph nodes, and bones and can pass both the blood-brain and placental barriers. The rate of absorption following oral and inhalational routes ranges from ~75% to 95%, with the rate of As sulfide, lead arsenate and other insoluble forms is much lower than the soluble forms. Several studies indicate that the absorption of As in ingested dust or soil is likely to be less than absorption of As from ingested salts. If there are differences in absorption rates in children versus adults these have not been documented (U.S. Agency for Toxic Substances and Diseases Registry 2005).

Transport of As is efficient due to its increased mobility resulting from its enzymatic methylation. The primary detoxification pathway of As is through its biomethylation, although it is well established that many of the metabolites exert their own toxicities. Inorganic arsenite (III) is more toxic than arsenate (V). Inorganic arsenic is primarily biotransformed in the liver by oxidation/reduction, followed by a series of methylation steps with S-adenosylmethionine (SAM) as the donor of methyl groups and reduced glutathione as a cofactor and excretion in the urine and feces as either monomethylarsonate (MMA) or dimethylarsinate (DMA). Metabolism of As does not vary by route of exposure, although there is tissue variation in the rate of methylation and the availability of methyl groups such as methionine, and cysteine is not a rate limiting step. There is some evidence for differences in methylation capacity among individuals. The main route of excretion is urinary, accounting for ~55–87% of daily oral or inhalation intakes of arsenate or arsenite. There also appears to be an upper-dose limit to excretion in the urine, which can account for the toxicity of As in tissues. Fecal excretion accounted for less than 5% of eliminated As. As is cleared from the blood within a few hours and most As absorbed from the lungs or the gastrointestinal tract is excreted within 1–2 days. An alternative metabolism pathway consists of nonenzymatic formation of glutathione complexes with arsenite resulting in the formation of As triglutathione followed by a series of methylation steps (U.S. Agency for Toxic Substances and Diseases Registry 2005).

Diagnosis of As exposure is difficult as renal excretion and blood levels may not give an accurate picture of total exposure. Renal excretion levels may be indicative of As exposure within 24 h with a half-life of 4 days of exposure. In cases where intoxication may have occurred weeks prior to medical evaluation, hair and fingernail samples can be used to determine levels for up to 3–6 months. The use of chelating agents for treatment of As poisoning has been advantageous if administered within hours of As absorption, their efficacy is probably due to the sulfhydryl groups they contain. Emetics, cathartics, lavages, and activated charcoal have also been used to remove As from the gastrointestinal tract (Flora and Pachauri 2010).

Cellular Handling of As

The gamut of health effects produced by As exposure can be attributed to whole body organ distribution of arsenic. Several mechanisms of As-induced damage have been proposed including oxidative stress, induction of chromosomal abnormalities through indirect effects on DNA, genotoxic damage, epigenetic mechanisms resulting in altered DNA methylation status, and disruption of protein activity by binding to and inactivation of sulfhydryl groups, especially disruption of metalloproteins, altered gene expression, and/or cell signaling. As generally does not induce point mutations.

Because arsenic is a nonessential metal it does not have a designated transport system, therefore cellular uptake of inorganic arsenite is mediated by the aquaglyceroporins (AQPs) which are normally responsible for transporting small uncharged molecules across cell membranes. AQP9, the major subtype found in liver and astrocytes, has been shown to handle both inorganic arsenite and its intermediate MMA. The major kidney, testis, and adipose tissue aquaglyceroporin, AQP7, has been implicated in uptake of arsenite. Inorganic arsenate is a phosphate analogue and therefore can be taken into the cell via phosphate transporters. Glucose transporter isoform 1 (GLUT1) is a glucose permease found in erythrocytes and epithelial cells forming the blood-brain barrier that has also been implicated in arsenite and MMA uptake (Martinez-Finley et al. 2012).

Arsenite has a high affinity for thiol (–SH) groups and in cells can form a complex with the amino acid cysteine, which contains many thiol groups. Toxic effects of arsenic are a result of its binding to vicinal sulfhydryl groups in key enzymes and interfereing with biological processes such as gluconeogenesis and DNA repair. The molecular mechanism(s) through which As causes oxidative stress in cells is related to binding of As to the thiol proteins, with some of these targets being metalloproteins. It is hypothesized that As binds thiol groups of enzymes that are stabilized by Zn^{2+} and displaces Zn from the protein binding site. Arsenate must be reduced in the body to arsenite so it is thought that it interferes with proteins by binding sulfhydryl groups, however, it may also act as a phosphate analogue, affecting other processes such as ATP production, bone formation, and DNA synthesis.

Arsenic Health Effects

Skin cancer and skin lesions are a major concern following exposure to As. These can include hyperkeratosis, hyperpigmentation, and depigmentation. Most often the presence of As in the lung leads to lung cancer as an endpoint. Additionally, effects of As on the cardiovascular system include cardiac arrhythmias, hypertrophy of the ventricular wall, Blackfoot disease, and altered myocardial depolarization. Other associations include cardiovascular effects due to chronic consumption of As-contaminated drinking water in pregnant mothers, leading to congenital heart disease in children, as well occurrence of hypertension (U.S. Agency for Toxic Substances and Diseases Registry 2005).

Spontaneous abortion, stillbirth, reduced birth weight, and infant mortality are reportedly associated with As exposure. Following fetal exposure, other studies have reported a slight increase in cancers overall, increased chronic obstructive pulmonary disease, increased cognitive deficits, increased acute myocardial infarction deaths, increased liver cancer mortality, and an increase in adult diseases following early life exposure. Experimental animal models have shown teratogenic effects including cleft palate, delayed bone hardening, and neural tube defects following in utero exposure to As. Arsenic levels in breast milk are negligible even when maternal blood As levels are high.

Inorganic As might act as an estrogen-like chemical in vivo and induce tumors in mice according to experimental animal and cell culture data which also shows that As is a potent endocrine disruptor, altering gene regulation by the closely related glucocorticoid, mineralocorticoid, progesterone, and androgen steroid receptors. Arsenic has shown positive correlation with diabetes. Deficits in cognitive function following As exposure have been reported and neurological symptoms following exposure include neuropathy, hallucinations, agitation, emotional changes, and memory loss (U.S. Agency for Toxic Substances and Diseases Registry 2005).

Conclusions

As is an environmental toxicant that affects many of the body's systems and has a high affinity for thiol groups, making it particularly detrimental to many cellular proteins.

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Cross-References

► As

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Arsenic, Physical and Chemical Properties

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Arsenic is a bright silver-gray metalloid; its outermost electrons are not free to move in the crystal structure because they are fixed in position in a covalent bond. It has metallic luster but is brittle with no useful mechanical properties. In addition to the metallic-like form, there are other modifications which change into the metallic-like form above 270°C:

- Black arsenic, formed as a mirror when arsenic hydride is passed through an incandescent glass tube and also when the vapor is rapidly cooled.
- Yellow arsenic, formed by the sudden cooling of arsenic vapor and consists of transparent, waxy crystals. It is unstable and changes into metallic-like arsenic on exposure to light or on gentle heating.
- Brown arsenic, is either a special modification or simply a more finely divided form is obtained in the reduction of solutions of arsenic trioxide in hydrochloric acid with tin(II) chloride or hypophosphorous acid.

Physical Properties

Atomic number	33
Atomic weight	24.92
Relative abundance in the Earth's crust, %	5×10^{-4}
Density, g/cm^3 at 20°C	
Metallic arsenic	5.72
Yellow arsenic	2.03
Brown arsenic	3.7–4.1
Melting point at 3.7 MPa, °C	817
Sublimation point at 0.1 MPa, °C	613
Linear coefficient of thermal expansion: K^{-1}	ca. 5×10^6
Specific heat capacity, J g^{-1} K^{-1}	
At 18°C	0.329
0–100°C	0.344
Electrical resistivity at 0°C, Ω · cm	24×10^{-6}
Hardness, Mohs scale	3–4
Crystalline form	Triagonal
Potential with respect to the normal hydrogen electrode, V	ca. 0.24

Chemical Properties

The surface of elemental arsenic tarnishes in humid air. When heated in air, it burns with a bluish-white flame, forming dense vapors of arsenic trioxide. In compounds, it has oxidation states of +3, +5, and −3. Concentrated nitric acid and aqua regia oxidize arsenic to arsenic acid; arsenic is oxidized to the +3 state by dilute nitric acid or concentrated sulfuric acid and by boiling alkali hydroxides in air. Hydrochloric acid has little effect on arsenic. Chlorine combines fierily with arsenic to form arsenic trichloride. When the metal is heated with sulfur, AsS, As_2S_3, or As_2S_5 is obtained, depending on the ratios used.

Arsenic combines with metals to form arsenides. When subjected to oxidizing roasting, arsenides give partly metal oxide and arsenous acid and partly basic arsenates. With oxygen, arsenic forms three oxides: arsenic trioxide, As_2O_3, arsenic pentoxide, As_2O_5, and As_2O_4, which apparently contains trivalent and pentavalent arsenic alongside. Arsenous acid is derived from arsenic trioxide and can only exist in aqueous solution. Its salts are the arsenates(III) (formerly arsenites). Arsenic acid is derived from arsenic pentoxide. The highly poisonous arsenic hydride, AsH_3, is formed from arsenic compounds in acidic solution in the presence of

strong reducing agents, e.g., zinc or from suitable arsenides, e.g., As_2Zn_3 and acids:

$$As_2Zn_3 + 6HCl \rightarrow 2AsH_3 + 3ZnCl_2$$

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Arsenical Compounds

▶ Arsenic and Primary Human Cells

Arsenicals

▶ Arsenic in Nature
▶ Arsenic in Pathological Conditions
▶ Arsenic, Free Radical and Oxidative Stress

Arsenic-Induced Diabetes Mellitus

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Synonyms

Chronic arsenic intoxication impairs glucose homeostasis; Environmental arsenic exposure and diabetes

Definition

Human beings can be exposed to arsenic from a variety of sources. Chronic arsenic exposure can cause skin lesions, neuropathy, cardiovascular disease, and cancer. Since the mid-1990s, many epidemiologic studies have shown a potential link between arsenic exposure and diabetes mellitus. Studies from animals and cell cultures have provided a possibility of diabetogenic effects of arsenic by mechanisms resulting in impaired insulin secretion, induction of insulin resistance and reduced cellular glucose transport. Most of these effects may be explained by the basic biochemical properties of arsenic including (1) functional similarity between arsenate and phosphate, (2) high affinity of arsenite to sulfhydryl groups, (3) increased oxidative stress, and (4) interference with gene expression. Factors related to individual susceptibility to arsenic-induced diabetes mellitus may include arsenic exposure dosage, nutritional status, genetic predisposition, antioxidant supplementation, and interaction with other trace elements.

Introduction

Arsenic can occur in the +5, +3, 0, and −3 valence states and can form alloys with metals. It is ubiquitous and can be found in soil, air, water, food, or some medications. Inorganic forms of arsenic usually present in well water are much more toxic than organic forms found in crustacean seafood. Inorganic arsenic is readily absorbed through the gastrointestinal tract, and mainly metabolized in the liver to organic forms of methylated arsenic, which can be excreted in the urine. It is also now known that both arsenite (inorganic trivalent form) and arsenate (inorganic pentavalent form) can be actively transported into cells via aquaglyceroporins and phosphate transporters, respectively (Tseng 2004, 2009).

Arsenic is well known for its acute toxicity and has been used for hundreds of years as a poisonous substance. With chronic exposure to arsenic, skin lesions, neuropathy, and cancer originating from the lung, liver, kidney, urinary bladder, and skin can develop. Arsenic is also atherogenic and may increase the risk of hypertension, ischemic heart disease, stroke, and peripheral arterial disease (Tseng 2008). In recent years, epidemiologic studies have demonstrated that

exposure to arsenic may induce diabetes mellitus in humans; and many studies have been done on animals and tissue cultures to investigate the potential mechanisms.

This entry first briefly reviews the pathophysiology of diabetes and the epidemiologic evidence linking arsenic exposure and diabetes; and finally provides potential biological explanations for the pathogenic links based on the biochemical properties of arsenic and evidence from current literature.

Key Proteins and Pathways Related to Glucose Homeostasis

Insulin, a hormone secreted by the islet β cells of the pancreas, is the principal hormone to lower blood glucose by suppressing gluconeogenesis and glycogenolysis in the liver and by stimulating the uptake of glucose into skeletal muscle and fat. Insulin molecule contains two peptide chains referred to as the A chain and B chain. Two disulfide bonds link these two chains together and insulin exerts its physiological actions by binding to its receptor on cell membrane.

There are at least 14 glucose transporters (GLUT) in the body. Among them, GLUT2 is expressed highly in pancreatic β cells, which is not insulin-dependent and functions as a glucose sensor for the glucose-stimulated insulin secretion. The molecular mechanisms of glucose-stimulated insulin secretion include the following steps which occur in the pancreatic β cells: (1) glucose entry into β cells via GLUT2, (2) phosphorylation of glucose by glucokinase and activation of glycolysis, (3) production of adenosine triphosphate (ATP) with reduced adenosine diphosphate (ADP) concentration leads to a rise in the ATP: ADP ratio, (4) closure of ATP-sensitive K-channel (Kir6.2) leads to cell membrane depolarization, (5) opening of the voltage-sensitive L-type Ca^{2+} channel, and (6) influx of calcium ions leading to insulin secretion (Polakof et al. 2011).

GLUT4 is insulin-dependent and is the major transporter for glucose to enter the cells of the adipose, muscle, and cardiac tissues (Augustin 2010). The insulin receptor complex contains two α- and two β-subunits, linked together by interchain disulfide bridges. Insulin binding to the α-subunits causes a cascade of signaling events including autophosphorylation of tyrosine residues on the β-subunits, tyrosine phosphorylation of the insulin receptor substrates, and activation of the phosphatidylinositol 3-kinase (PI 3-kinase) pathway, which triggers a series of downstream events involving protein kinase C (PKC), leading to insulin-stimulated translocation of GLUT4 to the plasma membrane and subsequent glucose transport via the transporters (Choi and Kim 2010).

Some other pathways not related to insulin signaling may also lead to the expression of GLUT4 and its translocation to the cell membrane. For examples, exercise may be associated with an increase in GLUT4 expression (Hussey et al. 2012) and an amplified signal transduction which is independent of proximal insulin signaling and leads to GLUT4 translocation to the cell membrane, probably mediated by atypical PKC (Maarbjerg et al. 2011). The mitogen-activated protein kinases (serine/threonine protein kinases), which are activated in response to a variety of external stimuli, may also be involved in exercise-induced glucose transport (Widegren et al. 2001). Metabolic stress can induce glucose transport via the 5′ adenosine monophosphate-activated protein kinase pathway, which is also independent of insulin-activated PI-3 kinase.

The disulfide bonds of insulin and insulin receptors are essential for maintaining the normal function and structure of the proteins. The exofacial sulfhydryl groups of the GLUT4 also play an important role in the maximal activity of the transporters, and are crucial for the regulation of transport rates by insulin.

Pathophysiology of Type 2 Diabetes Mellitus

The pathophysiology leading to hyperglycemia in T2DM is very complicated. Any gene mutation or metabolic disturbance leading to a defect in insulin secretion, insulin transport, insulin action, glucose transport, or enzymes associated with glucose metabolism can theoretically result in hyperglycemia or clinical diabetes.

Overexpression of tumor necrosis factor α (TNFα) and interleukin-6 (IL-6) has been found to play an important role on the induction of insulin resistance leading to the development of diabetes. On the other hand, activation of peroxisome proliferator-activated receptor γ (PPARγ) plays an important role in glucose

homeostasis by increasing insulin sensitivity. PPARγ is downregulated in adipocytes with the treatment of IL-6, and activation of PPARγ reduces TNFα expression. The expression of TNFα and IL-6 are regulated by nuclear factor-κB (NF-κB), and reactive oxygen species (ROS) resulting from hyperglycemia or other stress stimuli can lead to insulin resistance through its interactions with cytokines and other mediators involving the activation of NF-κB pathway (Henriksen et al. 2011).

Another mechanism leading to hyperglycemia in patients with T2DM involves the inability of insulin to inhibit hepatic glucose production. Enhanced phosphoenolpyruvate carboxykinase (an enzyme catalyzing the rate-limiting step in gluconeogenesis) activity leading to increased gluconeogenesis has been shown to be a major source of increased hepatic glucose production in patients with diabetes.

Pancreatic β-cell dysfunction has also been demonstrated in patients with diabetes. Progressive formation of amyloidosis with loss of β cells is always a major pathological change found in patients with diabetes (Westermark et al. 2011). ROS can induce rapid polymerization of monomeric pancreatic islet amyloid polypeptide into amylin-derived islet amyloid, which is extremely resistant to proteolysis.

Epidemiologic Evidence for Arsenic-Induced Diabetes

There are approximately 25 published papers in epidemiologic studies related to arsenic and diabetes mellitus (Chen et al. 2007; Del Razo et al. 2011; Huang et al. 2011; Navas-Acien et al. 2006, 2008; Tseng et al. 2000, 2002). The first studies were published in the mid-1990s and were mainly conducted in populations exposed to relatively high levels of inorganic arsenic from drinking water in Taiwan and Bangladesh. In these regions, a higher risk of diabetes mellitus associated with arsenic exposure can be demonstrated in a dose-responsive pattern. The evidence for such a link among residents with lower dose of arsenic exposure from drinking water in the USA and Mexico is not conclusive. Some studies conducted among copper smelters and art glass workers with arsenic exposure from the air in Sweden also suggest a potential role of arsenic on diabetes.

Animal Studies

There are approximately 25 animal studies conducted mainly in rats or mice aiming at investigating the effects of arsenic on diabetes mellitus or outcomes relevant to diabetes (Tseng 2004). Most studies used sodium arsenite or arsenic trioxide, administered via routes such as drinking water, intraperitoneal or subcutaneous injection, gavage, or oral intake from diet or as capsule. Hyperglycemia, impaired glucose tolerance, impaired insulin sensitivity, and pancreatic toxicity have been reported. Some studies also observe an attenuation of the toxic effects of arsenic on pancreas and on hyperglycemia when methyl donors or antioxidants are treated simultaneously. However, it should be noted that mice and rats may be less susceptible to arsenic toxicity than human beings.

Biochemical Properties of Arsenic That May Interfere with Glucose Metabolism

The following biochemical properties of arsenic may interfere with glucose metabolism and may be responsible for the mechanisms of arsenic-induced diabetes mellitus (Table 1) (Tseng 2004):

Substitution of Arsenate for Phosphate

Arsenate can replace phosphate in energy transfer phosphorylation reactions, resulting in the formation of ADP-arsenate instead of ATP. ADP-arsenate can serve as a substrate for hexokinase resulting in the formation of glucose-6-arsenate instead of glucose-6-phosphate. At high concentrations of arsenate, the activity of hexokinase is also inhibited.

Arsenite Reaction with Sulfhydryl Groups

Arsenite has high affinity for sulfhydryl groups of proteins and can form stable cyclic thioarsenite complexes with vicinal or paired sulfhydryl groups of cellular proteins. One example of such reaction is the complex formation of arsenite with dihydrolipoamide, a cofactor of the enzymes pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. This chemical change can cause aberration in structures of proteins and inactivate many enzymes and receptors. Inhibition of pyruvate dehydrogenase can impair the production of ATP by blocking the processing of citric acid cycle,

Arsenic-Induced Diabetes Mellitus, Table 1 Biochemical properties of arsenic related to its induction of diabetes

Biochemical property	Explanations
Functional similarity between arsenate and phosphate	Arsenate may substitute phosphate and form ADP-arsenate and glucose-6-arsenate, leading to impaired glucose metabolism and inefficient energy production.
High affinity of arsenite to sulfhydryl groups	Arsenite forms cyclic thioarsenite complex with paired sulfhydryl groups in proteins (insulin, insulin receptor, glucose transporters), and enzymes (pyruvate dehydrogenase and α-ketoglutarate dehydrogenase) could lead to impaired glucose transport and metabolism.
Increased oxidative stress	Oxidative stress can lead to formation of amyloid in pancreatic islet cells, leading to progressive β-cell loss. Superoxide may impair insulin secretion by interaction with uncoupling protein 2. Insulin resistance can also be induced by oxidative stress.
Interference with gene expression	Induction of insulin resistance by enhancing the expression of NF-κB, TNFα, and IL-6 and by inhibiting the expression of PPARγ.

ADP adenosine diphosphate, *NF-κB* nuclear factor-κB, *TNFα* tumor necrosis factor α, *IL-6* interleukin-6, *PPARγ* peroxisome proliferator-activated receptor γ

which is critical for providing reducing equivalents to the mitochondria needed for electron transport.

Although a high concentration of arsenite ($IC_{50} >$ 100 μM) is required for the inhibition of pyruvate dehydrogenase, recent studies suggest that trivalent methylated metabolites can exhibit inhibitory effect on pyruvate dehydrogenase with a two- to sixfold higher potency than arsenite.

Oxidative Stress

During the metabolism of arsenic, oxidative stress can be generated by the production of ROS and free radicals like hydrogen peroxide, hydroxyl radical species, nitric oxide, superoxide anion, dimethylarsinic peroxyl radical, and dimethylarsinic radical. A variety of normal physiological functions can be disrupted through the production of ROS and induction of oxidative stress.

Oxidative stress induced by arsenic was not only demonstrated in animal or cell biology studies, studies in human beings also disclosed an increased oxidative stress in subjects with chronic arsenic exposure. Therefore, oxidative stress can play an important role in the pathogenesis of atherosclerosis, cancer, and diabetes related to chronic arsenic exposure.

Gene Expression

Arsenic can influence the expression of a variety of proteins involving signal transduction and gene transcription. These include a variety of cytokines and growth factors associated with inflammation including IL-6. Arsenic has also been shown to upregulate TNFα and to inhibit the expression of PPARγ.

Arsenic Effects on Glucose Transport and Potential Link with Insulin Resistance

Studies suggest that arsenite or its methylated trivalent metabolites can cause insulin resistance by inhibiting insulin signaling, impairing the translocation of glucose transporters, and impeding glucose uptake into the cells.

Sulfhydryl groups on proteins are crucial for their maximal activity. Trivalent arsenicals may form stable cyclic thioarsenite complexes with sulfhydryl groups of cellular proteins including insulin, insulin receptors, and glucose transporters, thereby may impede the normal function of these proteins, leading to insulin resistance.

Arsenic may also induce a status of insulin resistance through its molecular effects on inducing gene expression of some cytokines including TNFα and IL-6. On the other hand, sodium arsenite at physiologically relevant concentration of 6 μM prevents adipocyte differentiation through a mechanism of inhibiting the expression of both PPARγ and CCAAT/enhancer-binding protein α. An interaction between PPARγ and its coactivator retinoid X receptor alpha is associated with an improvement in insulin sensitivity. Therefore, disruption of the expression of PPARγ will surely lead to a status of insulin resistance.

A growing number of reports in recent years have suggested a link between increased ROS production or oxidative stress and the development of insulin

resistance and β cell dysfunction in humans. This stress-sensitive pathway may involve the transcription factor NF-κB induced by arsenite, which may in turn induce the expression of TNFα and IL-6, followed by insulin resistance.

Evidence also suggests that arsenite may reduce the myogenic differentiation from myoblasts to myotubes by repressing the transcription factor myogenin. This may also lead to impairment in glucose uptake into the cells.

Therefore, arsenic effects on the functional groups of proteins and on the expression of genes involving ROS, cytokines, PPARγ, and NF-κB can work together in the induction of insulin resistance and diabetes mellitus associated with chronic exposure to arsenic (Tseng 2004).

Arsenic Effects on Glucose Metabolism and Energy Production

Because of its biochemical properties, arsenic may theoretically impair glucose metabolism by acting as an uncoupler of oxidative phosphorylation, as an inhibitor of sulfhydryl containing enzymes such as α-ketoglutarate dehydrogenase and pyruvate dehydrogenase, and as a competitor for phosphate-binding sites on glycolytic enzymes. The formation of ADP-arsenate instead of ATP can cause an inefficient production of energy and results in generalized inhibition of the metabolic pathways that require ATP. Glucose-6-phosphate is not only important as a mediator for glycolysis, gluconeogenesis, glycogenesis, and glycogenolysis, it is also important as an initiator for the pentose phosphate pathway, which generates nicotinamide adenine dinucleotide phosphate (NADPH, an important cofactor in the reduction of glutathione [GSH]) and provides the cell with ribose-5-phosphate for the synthesis of nucleotides and nucleic acids. Substitution of phosphate in the formation of glucose-6-phosphate by yielding glucose-6-arsenate may lead to an inefficient metabolism of glucose. Insufficient production of NADPH from the pentose phosphate pathway further disrupts the ability of the cells to deal with oxidative stress.

Therefore, a slowdown of the metabolic pathways induced by arsenic or its metabolites may contribute partly to an impairment in glucose metabolism (Tseng 2004).

Arsenic Effects on Insulin Biosynthesis and Secretion

Arsenic may theoretically cause impairment in insulin secretion by mechanisms involving functional or structural changes. Because arsenic may cause formation of ADP-arsenate instead of ATP, it is possible that the ATP-dependent insulin secretion could be impaired in the absence of sufficient energy supply.

Interaction between arsenite or its methylated metabolites and the sulfhydryl groups of insulin or proinsulin and competition with zinc in the formation of hexamers during the synthetic stages of insulin are also theoretically possible.

Arsenic may also indirectly cause functional impairment in insulin secretion through the generation of free radicals. Uncoupling protein 2 (UCP2) is a negative regulator of insulin secretion. It mediates proton leak across the inner mitochondrial membrane. A superoxide-UCP2 pathway has been suggested to cause impairment in insulin secretion in pancreatic β cells. Arsenic is well known for its ability to induce the production of superoxide. If excess superoxide is produced in the pancreatic β-cells, an impairment of insulin secretion is expected. Recent studies suggest that Nrf2-mediated antioxidant response may be responsible for the impairment in glucose-stimulated insulin secretion in pancreatic β cells induced by arsenic.

The increased oxidative stress induced by arsenic can theoretically cause structural damages to the pancreatic islets with the formation of amyloidosis, which not only prevents the release of insulin into the circulation, but also destroys the insulin-secreting β cells insidiously after prolonged exposure to arsenic.

Therefore, with prolonged arsenic exposure, insulin secretion may be impaired by either a disruption in insulin secretion function or a progressive loss of β cell mass (Tseng 2004).

Individual Susceptibility to Arsenic-Induced Diabetes Mellitus

Individual susceptibility is likely to vary based on duration and cumulative dosage of exposure, genetic variation, metabolic condition, nutritional status, health status, and other factors (Tseng 2004, 2007, 2009). The variation in individual susceptibility may influence the toxic effect of arsenic on target organs

and determine the clinical development of diabetes mellitus.

Nutritional status and supplement with antioxidants are important factors determining the chronic toxicity associated with arsenic exposure. Thus, good nutritional status with sufficient intake of antioxidants can reduce oxidative stress induced by arsenic and can possibly prevent the onset of arsenic-induced diabetes mellitus. GSH is essential for arsenic detoxification and is important for insulin action. Therefore, protein-restricted diets or deficiency in selenium, which is required in the biosynthesis of GSH, may aggravate the hyperglycemia induced by arsenic.

Most of the trace elements do not work in isolation and the interactions between arsenic and other trace elements from environmental co-contamination and oral intake could modulate the chronic toxicity and clinical manifestations associated with arsenic exposure. These interactions may include zinc, selenium, magnesium, and chromium. Deficiency of these trace elements per se may induce hyperglycemia. In low phosphate conditions, arsenate uptake will be accelerated and its toxicity will probably be promoted. Although there is not much published research, the interactions between arsenic and other trace elements that have an effect on glucose homeostasis, such as lithium and vanadium, can also contribute to the hyperglycemic effect of arsenic.

Summary

Epidemiologic studies suggest a potential link between arsenic exposure and diabetes mellitus in humans. Although the etiological links have not yet been fully elucidated, studies from experimental animals and cell biology can provide pathophysiological mechanisms for arsenic-induced diabetes mellitus. The mechanisms may involve one of the following biochemical properties of arsenic: (1) functional similarity between arsenate and phosphate, (2) high affinity of arsenite with sulfhydryl groups, (3) increased oxidative stress, and (4) effects on gene expression. As a result, insulin resistance, β cell dysfunction, and diabetes mellitus can be induced by chronic arsenic exposure. Individual variability in detoxification capability, nutritional status, and interactions with other trace elements may also influence the susceptibility of arsenic-exposed subjects to develop diabetes mellitus.

Cross-References

- ▶ Arsenic
- ▶ Arsenic and Alcohol, Combined Toxicity
- ▶ Arsenic and Primary Human Cells
- ▶ Arsenic and Vertebrate Aquaglyceroporins
- ▶ Arsenic in Pathological Conditions
- ▶ Arsenic Methyltransferases
- ▶ Arsenic, Free Radical and Oxidative Stress
- ▶ Arsenic, Mechanisms of Cellular Detoxification
- ▶ As

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Arsenic-Induced Stress Proteins

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Synonyms

Arsenic; Heat shock proteins; HSPs; Stress

Definition

Arsenic is a ubiquitous element in the Earth's crust. Exposure to this metalloid may induce a variety of responses including oxidative stress, metabolic alterations, growth inhibition, and eventually cell death. A complex enzyme network metabolizes arsenic, inducing the expression of stress proteins. Stress proteins are molecules produced after exposure to a physical or chemical agent. The most common stress proteins induced by arsenic exposure are metallotionein, ubiquitin, and heat shock proteins (HSPs).

Introduction

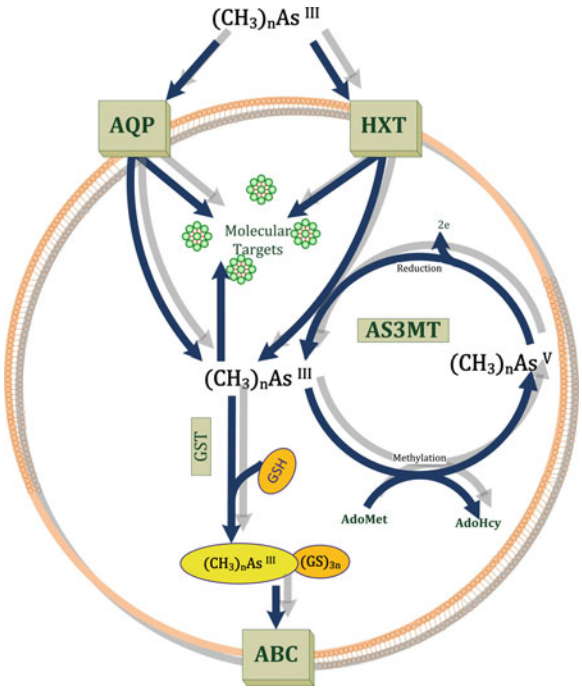
In nature, arsenic is found in several forms including oxides, chlorine, or sulfur compounds. Water is the most common medium for environmental distribution of arsenic (Del Razo et al. 2001). Exposure to arsenic produces a variety of stress responses in cells including morphological, physiological, biochemical, and molecular alterations. Arsenic (As) may enter an organism through epithelial exposure or ingestion, where consumption of contaminated drinking water is the most common mean among humans (ATSDR 2007). Although the World Health Organization (WHO) has determined As concentrations of 10 μg/L as acceptable, higher levels have been measured in parts of Argentina, Mexico, Bangladesh, India, China, Canada, Chile, and the USA, among others. Furthermore, since 2007 arsenic has been classified as a Class I human carcinogen by the International Agency for Research on Cancer (IARC), verifying the existence of substantial evidence for its carcinogenicity to humans. For this reason further investigation into arsenic toxicity and its carcinogenic effects should be a priority among health organizations.

Arsenic Exposure

Chronic exposure to arsenic is mainly connected with intake from natural sources, however, higher risk occupations such as mining and smelting may also be involved in disease development (ATSDR 2007). At present, millions of individuals worldwide are considered chronically exposed to arsenic via drinking water, with consequences ranging from acute toxicities, such as stomachache, nausea, vomiting, and diarrhea to the development of malignancies, such as skin, bladder, and lung cancer. Due to its indiscernible physical characteristics (no odor, color, or flavor), arsenic exposure often goes unnoticed, especially when ingested through contaminated food or drinking water. In this context, long-term effects are a major health concern in affected areas.

Metabolism of Arsenic

Arsenic enters the cells via transport through the membrane by aquaporin and hexosepermease transporters. In humans, as in many mammals, arsenic is readily absorbed and distributed to many organs, although the liver is the main target and the site of most of its biotransformation. The reduction of arsenate (iAsV)



Arsenic-Induced Stress Proteins, Fig. 1 *Model of arsenic metabolism.* Arsenic enters through membrane transporters such as aquaporin (AQP) or the hexose permease transporter (HXT) and may interact with specific molecules. Trivalent arsenic may be conjugated via glutathione-S-transferase (GST) and excreted through the ATP dependent transporter (ABC) or methylated via arsenic-3-methyl transferase (AS3MT) (Adapted from Thomas 2007)

to arsenite (iAsIII) is required for the enzymatic methylation of arsenic to yield mono-, di-, and possibly trimethylated metabolites. This process is catalyzed by an As^{3+} methyltransferase (As3MT), in which *S*-adenosylmethionine acts as the donor of methyl groups and produces trivalent and pentavalent methylated arsenicals (Thomas 2007). Arsenic is typically conjugated (Fig. 1) with glutathione via glutathione-*S*-transferase or with betaine, producing arsenobetaine and arsenocholine. In addition, arsenic generates ROS and free radicals such as hydrogen peroxide, nitric oxide, or hydroxyl radical (OH) species. This metabolic activity may lead to stress responses, growth inhibition, and cell death. Furthermore, As^{3+} may interact with proteins and inhibit DNA repairing processes. Stress responses that result from As exposure include the induction of certain proteins that maintain homeostasis in the cell.

Induction of Stress Proteins at mRNA and Protein Levels by Arsenic Exposure

Stress response is an adaptation event by prokaryotes and eukaryotes, regulated at the transcriptional, translational, and posttranslational levels. Altered patterns of protein synthesis induced by heat, UV radiation, or chemical exposure activate the expression of stress proteins (SPs) (Nriagu 2000). However, in normal conditions, several of the major SPs are constitutively expressed at low levels and work as "chaperones," facilitating the correct folding, assembly, and distribution of recently synthesized proteins (Kato et al. 1997). The presence of higher concentrations of As in the environment induces an expression of SPs that resembles that of the heat shock response (Ahsan et al. 2010). To date, the specific SPs related with exposure to As include: metallotioneins (MTs), ubiquitin (Ub), alpha B-crystallin, and heat shock proteins (HSPs) of various sizes including HSP25, HSP27, HSP30, HSP60, HSP70, HSP90, and HSP105 (Del Razo et al. 2001). MTs are small thiol-rich proteins typically related with Zn homeostasis; however, MTs are also induced in the presence of a wide range of metals, including As^{3+}. In mice, a lack of MTs increases toxicity of arsenic to the kidneys. However, the induction of MT by arsenic is probably a response to the generation of reactive species. Nevertheless, As modulation of MT expression may result from diverse interactions. For instance, As could act directly by an interaction with metal response elements (MRE) in the DNA such as MTF-1 or indirectly by glucocorticoids, the antioxidant response element, or through its effect on Zn regulation (Del Razo et al. 2001). The stress protein Ub, also expressed in the presence of As, binds to proteins and acts as a signal for degradation via proteosomes. Exposure to arsenite at low concentrations causes an increase in Ub-conjugation enzymes, resulting in a large number of Ub-conjugated proteins. Alpha B-crystallin, a major protein in the vertebrate lens, also has sequence similarity with small HSPs; arsenite exposure in lung cells induces this protein in response. Although alpha B-crystallin is constitutively expressed in cells, its expression increases in response to other

types of stress such as higher temperatures, drugs, and toxic metals (Wijeweera et al. 1995).

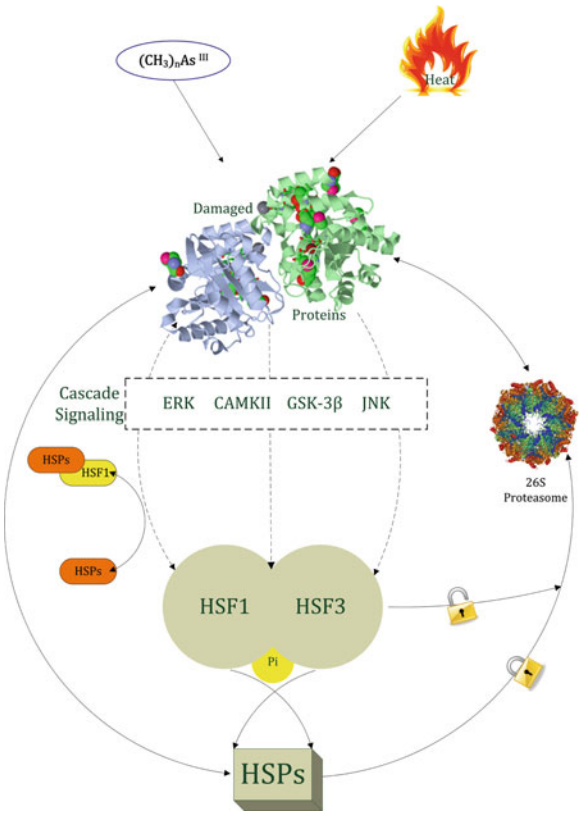
Under normal conditions HSPs are expressed at basal levels; however, As exposure is capable of triggering the oxidation of glutathione, leading to the activation of heat shock transcription factor (HSF). This transcription factor increases expression of HSPs such as Hsp70 and Hsp27. High concentrations of arsenite increase the phosphorylation level of Hsp27, inhibiting protein phosphatase activity and acting as a protective mechanism against oxidative damage. Hsp70 is a large, multigene family that includes both constitutively expressed Hsc70 and stress-inducible Hsp70 forms (Das et al. 2010). Cells that accumulate inducible Hsp70 after As exposure demonstrate protection, enhanced recovery, and resistance to subsequent exposures (Han et al. 2005). Hsp70 may guard cells from As-induced chromosome alterations through several mechanisms that facilitate repair of DNA damage (Barnes et al. 2002). Hsp70 and Hsp27 are associated with base excision repair enzymes that reduce alterations in DNA induced by As. Considering all stress proteins, Hsp70 is shown to be one of the most conserved stress proteins, whereby many studies have shown accumulation of Hsp70 in cells or tissues after As treatment. For this reason Hsp70 response may act as a biomarker to inorganic arsenic (iAs) exposure and damage. Hsp90 synthesis is also induced by arsenite in various cell types and although it is abundant in the cytosol of normal cells its expression increases with stress conditions (Lau et al. 2004). The Hsp90 chaperone is involved in several signal transduction pathways by stabilizing protein complexes. Specific inhibitors of Hsp90 increase the toxic effect of arsenic trioxide by inducing apoptosis, arresting the cell cycle, and blocking cell signaling. High molecular weight HSPs such as Hsp105 or Hsp110, are also expressed after arsenite treatment in different cells such as keratinocytes, embryonic, epithelial, and tumor-derived cells. These HSPs are suggested to play a protective role following stress induced by arsenite exposure (Liu et al. 2001).

Hemeoxygenase 1 (HO-1), an enzyme involved in heme degradation, is another protein that increases its expression after exposure to As. HO-1 is considered to be an inducible stress protein that confers protection against oxidative stress. The most useful HO-1 inducers are trivalent arsenicals, mainly iAsIII, in which neither MMAsV nor DMAsV are effective HO-1 inducers. Acute and subchronic exposure to iAs increases bilirubin excretion; HO-1 catabolizes heme into three products: carbon monoxide (CO), biliverdin (which is rapidly converted to bilirubin), and free iron radicals. For this reason, the inducibility of HO-1 by iAs in cells could be considered a potential biomarker (Lau et al. 2004).

Regulation of Stress Proteins Induced by Arsenic

It is well established that HSP may control a wide variety of cell processes including environmental response, adaptation, growth stimulation and inhibition, differentiation, and cell death. For this reason, As exposure may activate transcriptional responses that control the processes mentioned above (Pirkkala et al. 2001).

Arsenite stimulates the binding of transcriptional regulators to DNA such as the heat shock factor HSF1 through phosphorylation and subsequent activation of signaling pathways. Phosphorylation is an important determinant of the transactivating potency of HSF1. This protein is a member of the heat shock transcription factor family (the HSF family has been found in vertebrates (HSF1–4) and plants) and contains two distinct carboxyl-terminal activation domains, AD1 and AD2, which are controlled by a centrally located, heat-responsive regulatory domain (RD). Constitutive phosphorylation of two specific serine/proline motifs, S303 and S307, is important for the function of the RD and may be critical for the negative regulation of HSF1 (Fig. 2). These phosphorylation sites are targeted by glycogen synthase kinase 3ß (GSK-3ß) and extracellular signal-regulated kinase (ERK-1), whereas S363 may be a phosphorylation site by c-Jun N-terminal kinase (JNK) (Liu et al. 2010). The phosphorylation site S230 appears to be a suitable substrate for calcium/calmodulin-dependent protein kinase II (CaMK II). Protein-damaging stress induced by arsenic leads to the activation of HSF1, which then binds to upstream regulatory sequences in the promoters of heat shock genes that enhance heat shock gene expression. The activation of HSF1 proceeds through a multistep pathway, involving multiple protein modifications. HSF1 activity is modulated at different levels by the HSPs resulting in a regulatory feedback



Arsenic-Induced Stress Proteins, Fig. 2 *Regulation of stress protein by arsenic or heat.* Arsenic or heat induce changes that activate several signal pathways and result in the transcriptional activity of HSFs. HSFs activate the expression of HSPs thereby mediating regulation via a feedback loop

mechanism. However to date, regulation remains relatively unknown as do the phosphorylation sites and kinases/phosphatases responsible for HSF1 phosphorylation.

Another possible mechanism of regulation induced by As is through the glucocorticoid receptor (GR) where As simulates the effect of a hormone binding to the receptor. Glucocorticoids and other steroid hormones regulate many of the genes affected by low-dose As because As acts as a endocrine disruptor, altering gene regulation (Nriagu 2000).

Chronic Diseases Resulting from Arsenic Exposure

Ingestion of As may occur via food (mainly in fish, seafood, algae, and cereals), air (coal-fired power generation and smelting), or water. Long-term exposure of iAs has been associated with several human diseases (Goering et al. 1999). To date, iAs has only been conclusively demonstrated to be a carcinogen in humans although some reports suggest that As may act as a carcinogen in other animals in early life and with transplacental exposure (Tokar et al. 2010). Currently, sufficient evidence supports the observation that inhalation exposure to iAs leads to respiratory irritation, nausea, skin, and neurological effects, and an increased risk of developing lung cancer. Dermal exposure to iAs on the other hand, shows little risk of adverse effects with the exception of local irritation. In addition, hyperkeratinization, gangrene, cardiovascular effects, skin, lung, prostate, and bladder cancer have been associated with the ingestion of drinking water contaminated with As. From the various sources of As in the environment, long-term exposure of arsenic in drinking water likely poses the greatest threat to human health. The capacity of As^{3+} species to bind biologically important proteins and to alter DNA methylation profiles and oxidative stress account for the most plausible proposed mechanisms of arsenic carcinogenesis.

Summary

Study of heat shock proteins induced by arsenic may lead to the understanding of how cells respond to environmental stress and how chronic effects result in the development of diseases. It is obvious that the effects of arsenic exposure represent classical stress responses of cells with the participation of the "standard" heat shock mechanism. Arsenicals may activate the expression of the major HSP families and other stress response proteins such as metallotionein and hemoxygenase (OH-1). Among the different arsenic species, iAsIII has been shown to be the most powerful stress inducer. Additional work is necessary to completely uncover the general sequence of molecular events in the signal pathway induced by arsenic.

Cross-References

- ▶ Arsenic
- ▶ Arsenic and Aquaporins
- ▶ Arsenic in Nature
- ▶ Arsenic in Pathological Conditions
- ▶ Arsenic in Tissues, Organs, and Cells
- ▶ Arsenic Methyltransferases

- ▶ Arsenic, Biologically Active Compounds
- ▶ Arsenic, Free Radical and Oxidative Stress
- ▶ Arsenic, Mechanisms of Cellular Detoxification
- ▶ As
- ▶ Phosphatidylinositol 3-Kinases

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Arsenicism

- ▶ Arsenicosis

Arsenicosis

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Synonyms

Arseniasis; Arsenicism; Arsenism; Chronic arsenicosis ("arsenicosis" needs to be differentiated from "acute arsenic poisoning")

Definition

Arsenicosis is defined by the World Health Organization (WHO) working group as a "chronic health condition arising from prolonged ingestion (not less than 6 months) of arsenic above a safe dose, usually manifested by characteristic skin lesions, with or without involvement of internal organs" (WHO Regional Office for South-East Asia 2003). The *maximum permissible limit* recommended by WHO in groundwater is 10 μg/L; however, in India, Bangladesh, the accepted level is <50 μg/L in the absence of an alternative source of potable water in the affected area (Smedley and Kinniburgh 2002).

Magnitude of the Problem

Arsenicosis is a global problem with some local predilection. Asian countries are the worst affected with major disease burden reported from countries like *Bangladesh, India*, and *China*; however, people are also affected in parts of Mongolia, Hungary,

Arsenicosis, Table 1 Country profiles (in decreasing order of population at risk) in relation to arsenic contamination of groundwater

Countries	Area affected (sq. km)	Concentration range (μg/L)	Population at risk (in millions)	Sources of contamination	First reported case
Bangladesh	**1,18,849**	**<0.5–2,500**	**30[a]**	**Geogenic**	**1995**
India/West Bengal	**38,865**	**<10–3,200**	**6[a]**	**Geogenic + Anthropogenic**	**1983**
Argentina	10,00,000	1–7,500	2	Geogenic	1917
Chile	35,000	100–1,000	0.5	Geogenic + Anthropogenic	1962
Mexico	32,000	8–620	0.4	Geogenic + Anthropogenic	
China/Taiwan	4,000	10–1,820	0.1	Geogenic + Anthropogenic	1968 in Taiwan
Mongolia	4,300	1–2,400	0.1	Geogenic	
Thailand	100	1 to <5,000	0.015	Anthropogenic	1996

[a]Percentage of the total population at risk is about 25% in Bangladesh and 6% in West Bengal (India)
Source: Ghosh et al. (2008) (Permission obtained from editor-in-chief IJDVL)

Mexico, Argentina, Chile, Canada, and USA (Table 1) (Ghosh et al. 2008; Smith et al. 2000).

Approximately 40,000-sq. km area in West Bengal (12 out of 19 districts), 118,000-sq. km area in Bangladesh (50 out of 64 districts), and 4,000 sq. km in China (8 provinces and 37 counties) are reported to be affected (Ghosh et al. 2008). Apart from the Asian countries, reports from Hungary (Great Hungarian Plain) reveal that 4,263-sq. km area is having arsenic in groundwater in the range of 25–50 μg/L (Kapaj et al. 2006). With increasing scope of field testing and screening, these figures are on a rising trend, and increasing number of areas affected with arsenic contamination and at-risk population are identified with every new survey conducted. Reports from community-based surveys revealed that one in every five to ten persons in the endemic areas of south eastern Asian countries is actually manifesting the disease (Smith et al. 2000; Ghosh et al. 2008).

Epidemiological Determinants

Arsenicosis is a disease with multifactorial determinants, but the key factor is exposure occurring through naturally contaminated groundwater used for drinking or in food preparation. Sources such as mining and some pesticides and wood preservatives may contribute to human exposure and should be controlled in order to prevent environmental contamination.

Agent Factor

The metalloid arsenic is the obvious agent factor, and the trivalent (*arsenite*, As^{+3}) and pentavalent (*arsenate*, As^{+5}) states are principally implicated in arsenicosis. Arsenic is perhaps unique among the heavy metalloids and oxyanion-forming elements (e.g., arsenic, selenium, antimony, molybdenum, vanadium, chromium, uranium, rhenium) in its sensitivity to mobilization at the pH values typically found in groundwaters (pH 6.5–8.5) and under both oxidizing and reducing conditions (Smedley and Kinniburgh 2002).

Most oxyanions including arsenate tend to become less strongly sorbed as the pH increases. Under some conditions at least, these anions can persist in solution at relatively high concentrations (tens of μg/L) even at near-neutral pH values. Therefore, the oxyanion-forming elements are some of the most common trace contaminants in groundwaters. However, relative to the other oxyanion-forming elements, arsenic is among the most problematic in the environment because of its relative mobility over a wide range of redox conditions. It can be found at concentrations in the mg/L range when all other oxyanion-forming metals are present in the μg/L range (Smedley and Kinniburgh 2002).

Highest concentration of arsenic in water is found in groundwater, and range of concentration is also pretty wide, hence making it difficult to derive a typical or "usual" value. Moreover, majority of the researches focused on areas endemic for arsenicosis, resulting in extreme values, and these are possibly unrepresentative of global pattern.

Arsenicosis, Table 2 Postulated mechanisms of *geochemical processes* for leaching of arsenic in groundwater

Postulations	Principles and proposed mechanism
"Pyrite oxidation" hypothesis	*Mechanism*: Excessive withdrawal of groundwater for the purpose of irrigation ➔ The heavy groundwater usage leads to lowering of the water table ➔ gap created is being filled up by air (containing oxygen) ➔ subsequent oxidation of As-containing pyrites on the wall of the aquifer ➔ after rainfall, water table recharges ➔ as in its oxidized form, i.e., arsenate (As^{5+}) leaches out of the sediment into the aquifer
	Limitations: Fails to explain the presence of reduced form, arsenite (As^{3+}), in the groundwater
"Oxyhydroxide reduction" hypothesis	*Principle*: As in pore, water is controlled by the solubility of iron and manganese oxyhydroxides in the oxidized zone and metal sulfides in the reduced zone Digenetic sulfides act as an important sink for As in reducing sulfidic sediment
	Mechanism: In ground condition (with finely grained surface layers impeding the penetration of air into the aquifer and microbial oxidation of organic carbon depleting the dissolved oxygen), a greatly reduced environment is created ➔ under reducing condition, oxyhydroxides of iron and manganese dissolve ➔ arsenic sulfides precipitate and arsenite (As^{3+})
"Oxidation-reduction theory"	*Mechanism*: Arsenic originates from the arsenopyrite oxidation (as proposed in "pyrite oxidation" hypothesis) ➔ arsenic thus mobilized forms the minerals ➔ gets reduced to arsenite (As^{3+}) underground in favorable Eh conditions

Source: Ghosh et al. (2008) (Permission obtained from editor-in-chief IJDVL)

Baseline concentration of arsenic in atmospheric precipitation, river water, rainwater, or seawater is found to be quite low. The concentration of arsenic may increase to manifolds in river water or surface water where concentration is usually very low, if it gets contaminated by industrial pollution, by increased geothermal activities, or if water is flowing through bedrocks (with increased alkalinity and pH). Seasonal variation may influence geothermal activity or leaching of arsenic in surface water/river water. In low-flow period during summer months, it gets increased, and this may also be linked to temperature-controlled microbial reduction of As^{+5} to As^{+3} with consequent increased mobility of As^{+3} (Smedley and Kinniburgh 2002; Appelo 2006).

Host Factor

Propensity to develop arsenicosis in a community is variable. In large scale studies, it was noted that manifestation of arsenicosis was present among 11–20% of people living in arsenic contaminated areas of West Bengal, India, and Bangladesh (Ghosh et al. 2008). Understandably the susceptibility of individuals to develop full-blown disease varies in the same community using same water source.

The disease manifestation is strongly associated with *malnutrition*, especially with deficiency of proximate principle like protein or deficiency of trace element like *selenium*. People belonging to *lower socioeconomic strata* are more vulnerable to develop this disease. *Age* of the individual is also an important parameter which determines the expression of the disease and it usually takes *5–20 year of exposure* to develop the disease. Nevertheless, children with <10 year of exposure have developed this disease (Smith et al. 2000).

Genetic factors, viz., *decreased capacity of methylation of arsenic and genetic polymorphism of enzymes XPD and XRCC1*, having *null or variant genotype* of at least one of the three (M1/T1/P1) *glutathione S-transferases* are risk factors for carcinogenesis in arsenicosis patients (Ghosh et al. 2008).

Environmental Factors (Sources of Arsenic)

Arsenicosis is a disease, principally linked to environmental situation. People can get exposed to arsenic due to its presence in water (principal route of entry), soil, air, or less commonly through other miscellaneous agents as detailed below. Arsenic can enter these routes either though natural (geogenic) processes or by man-made (anthropogenic) activities, or both may contribute in certain situations (Ghosh et al. 2008).

Arsenic can gain entry in underground water as result of *geochemical processes* (vide Table 2), or it may be of anthropogenic origin, e.g., use of *pesticide, insecticide*, or *industrial pollution*. Presence of arsenic in soil is thought to be the result of use of *phosphate*

fertilizer which could result increased phosphate concentration in soil and consequently increase in sediment biota leading to increased resorption of arsenic from the sediment (Ghosh et al. 2008).

Volcanic eruption contributes to the majority of atmospheric flux of arsenic (as $As_2 0_3$). Natural low-temperature bio-methylation and microbial reduction are the other natural sources of arsenic in air. Of the anthropogenic causes, *smelting of nonferrous metal* and *combustion of fossil fuels* are important. The arsenic returns to the earth by wet or dry precipitation and contributes the arsenic load of water and soil (Ghosh et al. 2008).

Organic form of arsenic is primarily found in marine organisms in the form of *arsenobetaine* in marine animals and *dimethyl arsenoyl ribosides* in marine algae and can serve as important arsenic source for individuals consuming seafood (Ghosh et al. 2008).

Exposure to arsenic through medications (e.g., *Fowler's solutions* or 1% potassium arsenite) containing arsenic is of historical importance, but even in present days, *homeopathic medicine* containing arsenic is still being used in some countries. Recently, *arsenic trioxide* (Trisenox) has obtained FDA approval for use in acute promyelocytic leukemia, but its impact in causing arsenicosis is yet to be evaluated.

Gallium arsenide, used as a silicone substitute in computer microchips, poses an emerging threat arising due to the expanding opto-electric and micro-electronic industries (Ghosh et al. 2008).

Bioaccumulation of arsenic is still not an established threat, but case reports and studies of presence of arsenic in crops (particularly rice) irrigated with arsenic contaminated water or milk and meat sample obtained from animal husbandry of arsenic contaminated areas point toward this phenomenon (Ghosh et al. 2008).

Pathogenesis

Arsenic gaining entry in the body undergoes sequential reduction and oxidative methylation and is converted to less toxic methylated metabolite, monomethyl arsenic acid (MMA), and dimethyl arsenic acid (DMA). This *bio-methylation* is considered as *bio-inactivation* process, and persons differing in this capability suffer due to accumulation of As^{+3} and As^{+5} (Sengupta et al. 2008; Gomez-Caminero et al. 2001a).

As^{+3} has the ability to bind with the sulfhydryl groups present in various essential compounds (e.g., glutathione, cysteine) and can lead to inactivation of many thiol group-containing enzymes (e.g., pyruvate dehydrogenase) and other functional alteration (e.g., prevention of binding of steroid to glucocorticoid receptors). As^{+5}, on the other hand, mostly mediates its toxicity after conversion to the trivalent state but in certain situation, can render direct toxicity by replacing phosphate during glycolysis (phenomenon known as "*arsenolysis*") leading to ineffective adenosine triphosphate (ATP) production (Sengupta et al. 2008; Gomez-Caminero et al. 2001a).

Inorganic arsenic is capable of causing *teratogenic* effects, and animal studies have demonstrated that it can lead to neural tube defect, inhibition of development of limb-buds, pharyngeal arch defect, anophthalmia, etc. Arsenic can also induce *genotoxicity* by inhibiting DNA excision repair of thymine dimer, DNA ligase, and tubulin polymerization and altering the activity of tumor suppressor gene p53 by DNA methylation. Arsenic is also thought to build up oxidative stress by the production of oxygen free radical, which in turn can cause chromatid exchange and chromosomal aberration. The genotoxicity in turn is responsible for carcinogenicity. The free radicals generation also leads to increased *apoptosis* (programmed cell death) seen in arsenicosis (Sengupta et al. 2008; Gomez-Caminero et al. 2001b).

The cutaneous toxicity seen with arsenicosis is influenced by various growth factors and transcription factors. Expression of keratin 16 (marker for hyperproliferation) and keratin 8 and 18 (marker for less-differentiated epithelial cells) is found to be increased by arsenic exposure. Arsenic has shown to reduce transcription factor AP1 and AP2 which in turn reduces keratinocyte differentiation marker, involucrin. Arsenic is shown to trigger production of interleukin (IL)-8 and also stimulate the secretion of IL-1, tumor necrosis factor-alpha (TNFα), transforming growth factor (TGF) beta, and granulocyte monocyte colony-stimulating factor (GM-CSF) by the keratinocytes. The changed cytokine milieu is believed to exert the cutaneous changes (Sengupta et al. 2008; Gomez-Caminero et al. 2001a). The pathogenesis of other systemic manifestations is highlighted in Table 3.

Arsenicosis, Table 3 Proposed mechanism of pathogenesis for systemic manifestations in arsenicosis

Systemic involvements	Proposed mechanism of pathogenesis
1. Atherosclerosis	• Monoclonal expansion of smooth muscle cell
	• Production of reactive oxygen species (H_2O_2 & –OH radical) ➔ endothelial cell proliferation and apoptosis
	• Upregulation of inflammatory signal ➔ release of TNFα from mononuclear cells or stimulates cyclooxygenase II pathway
	• Enhances arterial thrombosis and platelets aggregation
2. Hepatotoxicity (fibrosis)	• Predominant lesion of hepatic fibrosis appears to be induced by oxystress and elevation of cytokines (TNFα and IL-6) associated with increasing level of collagen in the liver
	• Reduction/weakening of hepatic glutathione and enzymes of antioxidant defense system of liver ➔ free radical accumulation ➔ peroxidative damage of lipid membrane
3. Respiratory system	• Pathophysiology is not well understood, but oxidative damage is thought to play a role
	• Arsenic is potent respiratory toxicant, and ingested arsenic can reach respiratory tract to damage lung tissue
4. Neurological	• Predominantly sensory with distal axonopathy due to axonal degeneration
5. Genitourinary	• Renal tubular necrosis, nephritis, and nephrosis
6. Diabetes mellitus	• As^{3+} suppress insulin stimulated glucose uptake by interfering the mobilization of glucose transporters in adipose cell
	• Interfering the transcription factor involved in insulin-related gene expression

Source: Sengupta et al. (2008) (Permission obtained from editor-in-chief IJDVL)

Clinical Features

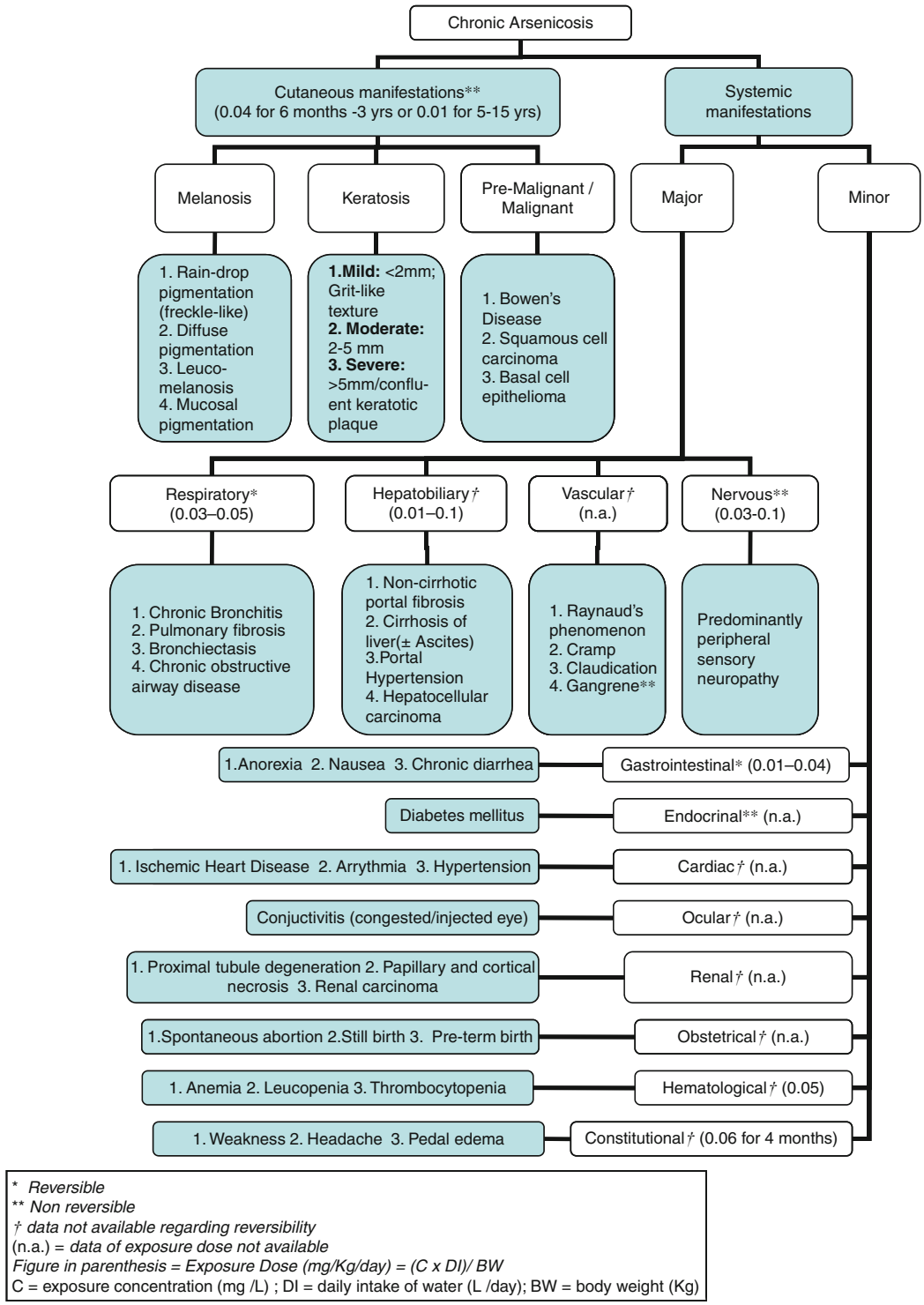
Arsenicosis is a multisystem disorder, but the hallmark clinical manifestations are its cutaneous changes. The cutaneous changes are so pathognomonic that the condition can be clinically confirmed by looking at the skin changes. The skin lesions can take the form of *melanosis* (pigmentary changes/dyspigmentation), *keratosis* (thickening of skin), or *malignant/premalignant lesions*. Respiratory, hepatobiliary, vascular, and nervous systems are among the major systems affected by arsenicosis, apart from constitutional symptoms, including weakness, headache, pedal edema, etc. (Sengupta et al. 2008). Though the systemic features of arsenicosis are nonspecific, but at times they may serve as taletell signs especially in early suspected cases. The cutaneous changes and neurological defects produced by arsenicosis are irreversible, but respiratory, hepatobiliary, gastrointestinal, and vascular effects can be reversed (World Bank technical report vol II). The manifestations of arsenicosis vary according to the cumulative exposure of arsenic, and the description of the exposure dose for different manifestations is detailed in Fig. 1.

Cutaneous Manifestations

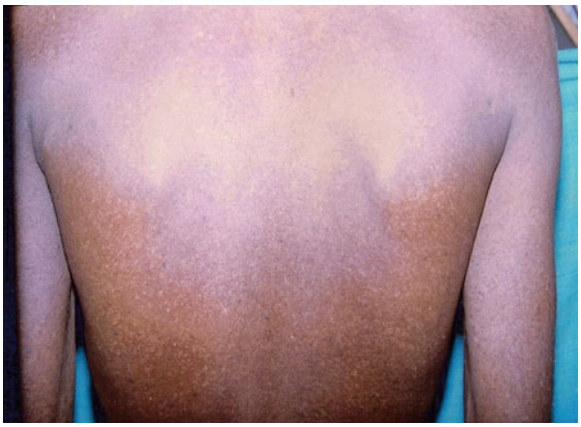
The cutaneous lesions are typically bilateral and are the earliest and commonest changes seen in arsenicosis patients. It is generally agreed that there is latency of approximately 10 years for development of skin lesion (i.e., exposure of arsenic and development of manifestations), though there are reports of arsenicosis in children less than 10 years (Smith et al. 2000).

Melanosis is found to be the earliest and commonest of all the cutaneous manifestation of arsenicosis and is most pronounced on trunk. The most characteristic arsenical dyspigmentation takes the form of guttate hypopigmented macule on hyperpigmented background, referred to as *leucomelanosis* (Fig. 2). In some cases, it can also take the form of freckle-like spotty pigmentation, quoted as *raindrop pigmentation* by some authors (Fig. 3). There can also be appearance of *diffuse pigmentation* of skin and/or *pigmentation of mucosa* (e.g., on tongue, buccal mucosa) (Sengupta et al. 2008; GuhaMajumder 2008).

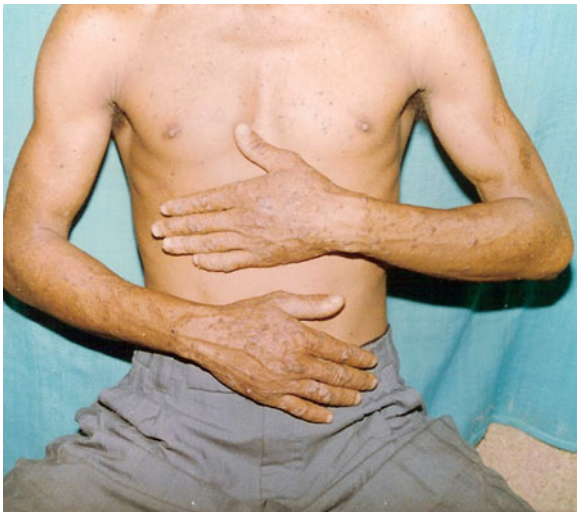
Arsenical keratosis mostly affects the palms and soles, though in long standing cases can affect other areas, including trunk and dorsa of feet or hands (Fig. 3). The keratosis is symmetric and graded



Arsenicosis, Fig. 1 Clinical manifestations in arsenicosis (Source: Sengupta et al. (2008) (Permission obtained from editor-in-chief IJDVL))



Arsenicosis, Fig. 2 Leucomelanosis affecting the trunk (Source: Sengupta et al. (2008) (Permission obtained from editor-in-chief IJDVL))



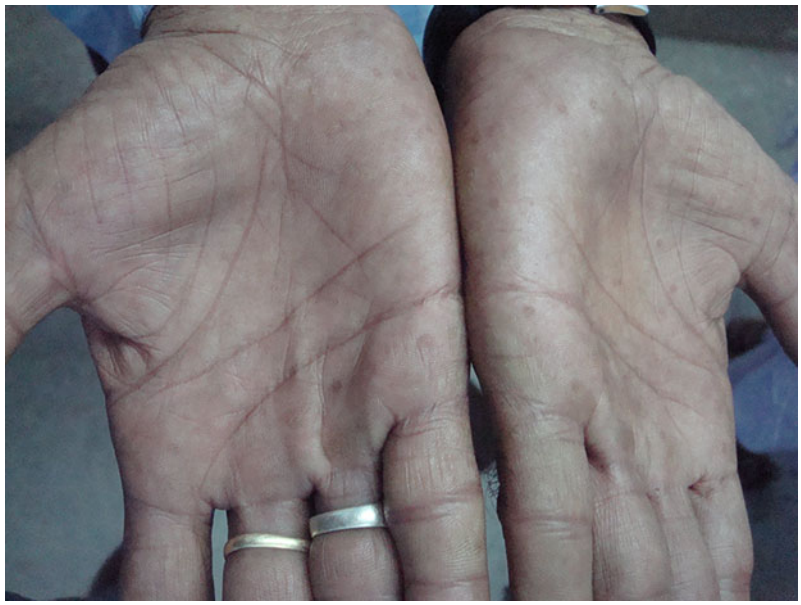
Arsenicosis, Fig. 3 Raindrop pigmentation on the trunk, along with arsenical hyperkeratosis affecting the dorsa of hands, fingers, and forearm (Source: Das and Sengupta (2008) (Permission obtained from editor-in-chief IJDVL))

according to its severity and extent into mild (grit-like texture or papule less than 2 mm) (Fig. 4), moderate (wart-like excrescence of size 2–5 mm) (Fig. 5), or severe (keratotic elevation of more than 5 mm or diffuse and confluent keratosis) (Fig. 6). Arsenical keratosis is often the forerunner of cutaneous malignancy and sometimes regarded as early clinical marker of carcinogenicity of internal organs (especially carcinoma of lungs and bladder) (Sengupta et al. 2008; GuhaMajumder 2008).

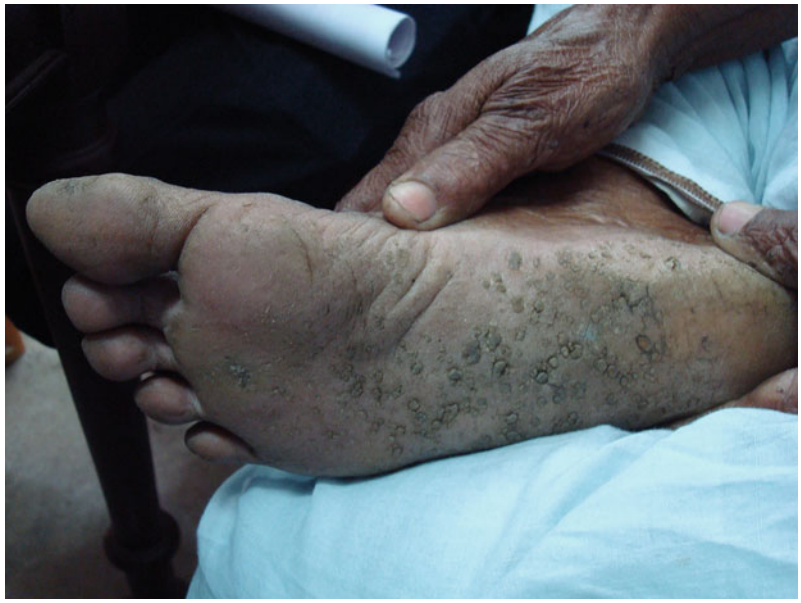
Carcinogenicity

Arsenic-induced carcinogenicity mostly affects the skin arising usually on keratotic lesions, though it may arise on apparently normal skin too. There is a dose-response relationship between skin cancers and arsenic consumption with a lifetime risk of developing skin cancer ranging from 1 to 2 per 1,000 with the intake of 1-μg/Kg body weight/day (approximately 1 l of water with arsenic concentration of 50 μg/L) (Smith et al. 2000). Bowen's disease is the most common form of precancerous lesions, and any hyperkeratotic plaque in arsenicosis suspect showing fissures and crusts at places should raise the suspicion for this premalignant form. *Multicentric Bowen's disease* affecting both covered and sun-exposed skin is a characteristic feature of arsenicosis (Fig. 7). Untreated Bowen's disease can transform into *squamous cell carcinoma* (Fig. 7) and can contribute significantly to the morbidity and mortality associated with the condition. *Basal-cell carcinoma* (Fig. 8) may also appear as a result of arsenicosis as well as other less-reported malignancies including *Merkel cell carcinoma* and *melanoma* (Sengupta et al. 2008; GuhaMajumder 2008).

Cancer risk for arsenicosis patients is not limited to skin malignancies, and there are reports of *visceral malignancies* including carcinoma of *liver, lungs, kidney, and urinary bladder*. There are also reports of malignancies arising from prostrate, uterus, and lymphatic tissues in cases of arsenicosis (Sengupta et al. 2008). It has been estimated that lifetime risk of dying from internal malignancy is high (around 13 per 1,000 on consumption of 1 l of water with arsenic concentration of 50 μg/L) than that of skin cancers because the later is detected early and not fatal if treated adequately (Smith, Lingus and Rahman, Smith et al. 2000). Based on the dose-response relationship, it is suggestive that if the patients with skin manifestations continue to drink arsenic contaminated water, the risk of mortality due to internal malignancies would increase when sufficient latency has been reached. Arsenic is regarded as class I human carcinogen by the International Agency for Research on Cancer (International Agency for Research on Cancer 2004).



Arsenicosis, Fig. 4 Mild variety of arsenical keratosis producing grit-like texture



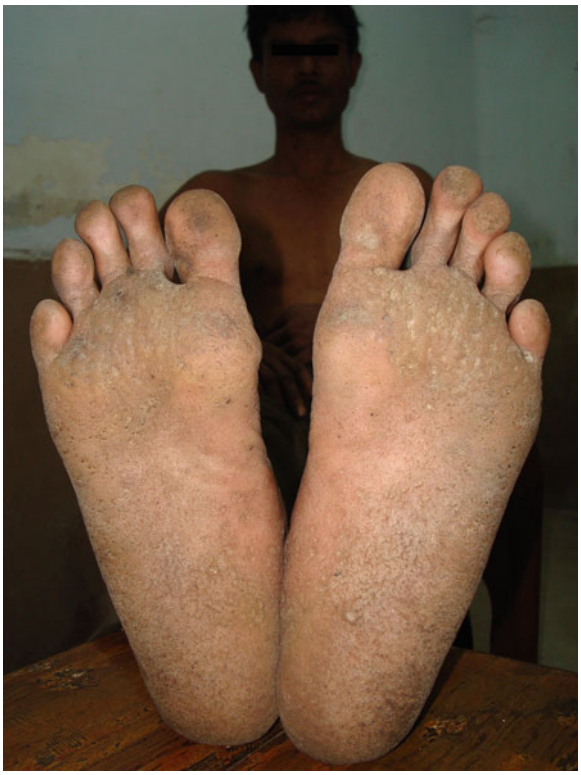
Arsenicosis, Fig. 5 Moderate variety of arsenical keratosis

Respiratory System

Respiratory symptoms include *chronic cough* and *respiratory distress*, which may result from *restrictive lung disease, obstructive lung disease, combination of both, interstitial lung disease, bronchitis*, or *bronchiectasis*. Crepitation and rhonchi are found on auscultation, and the pulmonary function test shows reduced forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) (Sengupta et al. 2008; GuhaMajumder 2008).

Hepatobiliary System

Arsenicosis produces hepatocellular toxicity mostly the form of *non-cirrhotic portal fibrosis* associated with *portal hypertension*, though *cirrhosis of liver* is rarely reported too. The portal fibrosis is characterized



Arsenicosis, Fig. 6 Severe variety of arsenical keratosis (Source: Sengupta et al. (2008) (Permission obtained from editor-in-chief IJDVL))

by expansion of portal zones with streak fibrosis containing leash of blood vessels. Prevalence of *hepatomegaly* is found to be significantly higher in patients with arsenicosis, and degree of liver enlargement shows a dose-response relationship (Sengupta et al. 2008; GuhaMajumder 2008).

Cardiovascular System

Vascular effects are mostly seen in southwest coast of Taiwan, where *Blackfoot disease* is endemic. This is characterized by dry gangrene and spontaneous amputation resulting from severe systemic arteriosclerosis. Signs of ischemia, including intermittent claudication, rest pain, and ischemic neuropathy, along with diminished or absent arterial pulsation, pallor on elevation, and rubor on dependency, are diagnostic of blackfoot disease. Histology shows either arteriosclerosis obliterans or thromboangiitis obliterans in this unique peripheral vascular disease.

Apart from peripheral vasculature, chronic arsenic poisoning can also affect cardio- and cerebrovascular systems, leading to *hypertension, ischemic heart disease, cerebrovascular disease*, and *carotid atherosclerosis* (Sengupta et al. 2008; GuhaMajumder 2008).

Neurological System

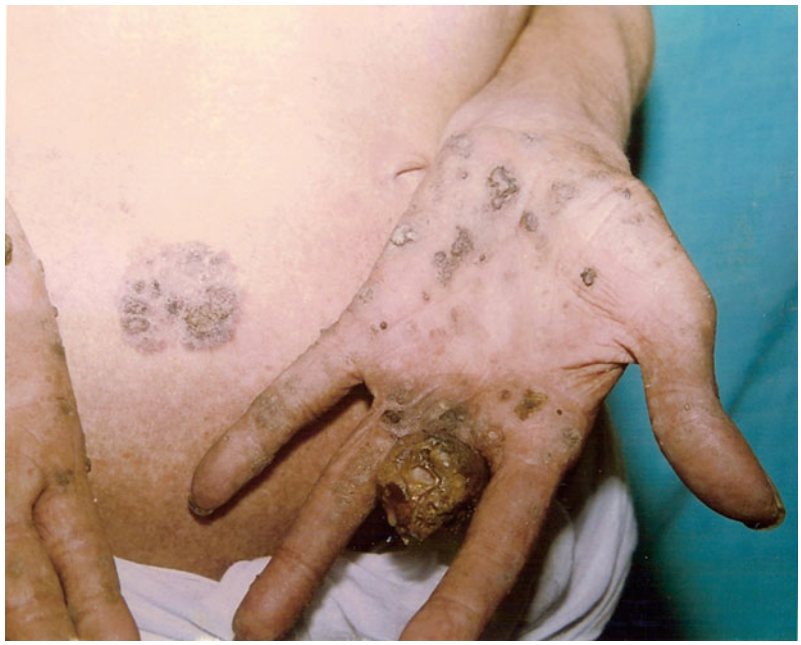
Peripheral sensory neuropathy is characteristic of arsenicosis and is manifested by paraesthesia (e.g., burning and tingling sensation), numbness, pain, etc. Motor involvement is rare and if occurs, can result in distal limb weakness and atrophy and diminished or absent tendon reflexes. The neuropathy is due to distal axonopathy with axonal degeneration, especially the large myelinated fibers. Decreased nerve conduction amplitude is found in arsenic-induced sensory neuropathy with little/no change in nerve conduction velocity and visual/auditory evoked potential (Sengupta et al. 2008; GuhaMajumder 2008).

Other Systems

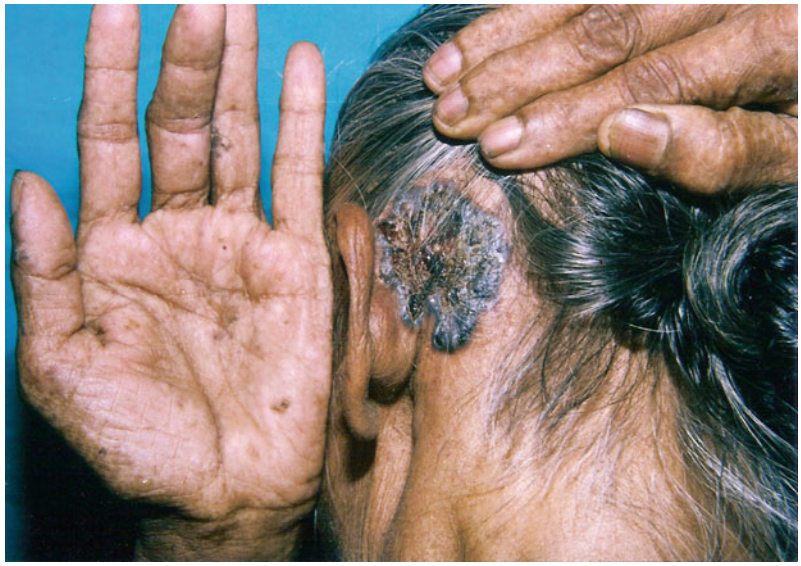
Arsenicosis can give rise to gastrointestinal symptoms in the form of *dyspepsia, anorexia, nausea, vomiting, diarrhea*, and *abdominal pain*. Anuria and dysuria are also reported in arsenicosis, arising due to *renal tubular necrosis*. Potential of arsenic to cross the placental barrier is thought to be the reason behind bad obstetrical outcome with increased incidence of *spontaneous abortion, still birth, preterm birth*, and *infant mortality* that are reported with arsenicosis. *Anemia, leucopenia*, and *thrombocytopenia* are usual accompaniment of arsenicosis and are the result of decreased erythropoiesis. There are also reports of *diabetes mellitus, conjunctival congestion, and non-pitting edema of feet* in arsenicosis, apart from constitutional symptoms including *headache and weakness* (Sengupta et al. 2008; GuhaMajumder 2008).

Diagnosis

Diagnosis of arsenicosis relies both on clinical manifestations (as detailed above) and laboratory estimation of arsenic content in drinking water and biological samples (Table 4). In countries where the laboratory backup cannot be made available in field situation, the cases can be clinically confirmed by virtue of its characteristic clinical features. WHO has proposed an algorithmic approach for diagnosing arsenicosis (Fig. 9) with the locally available resources. This approach has shown to have both the sensitivity and specificity of



Arsenicosis, Fig. 7 Squamous cell carcinoma affecting the hands, with Bowen's disease on the abdomen same patient with arsenical hyperkeratosis affecting the palm and forearm (Source: Sengupta et al. (2008) (Permission obtained from editor-in-chief IJDVL))



Arsenicosis, Fig. 8 Basal-cell carcinoma behind the ear, with arsenical keratosis on the palms (Source: Sengupta et al. (2008) (Permission obtained from editor-in-chief IJDVL))

more than 80% (WHO Regional Office for South-East Asia 2003; Das and Sengupta 2008).

High index of suspicion for arsenicosis is important while assessing persons hailing from areas documented to have high-arsenic content in drinking water, especially if history of similar skin lesions in persons (family members or neighbors) drinking water from the same source. At the same time, it is also important to thoroughly examine the suspected/probable cases to rule out clinical mimickers of arsenicosis (Table 5) (Das and Sengupta 2008).

Determination of Arsenic Content in Water and Biological Samples (Hair, Nail, and Urine)

Atomic absorption spectrometry is considered as the standard reference methods ("gold standard") for determination of arsenic content due to its high

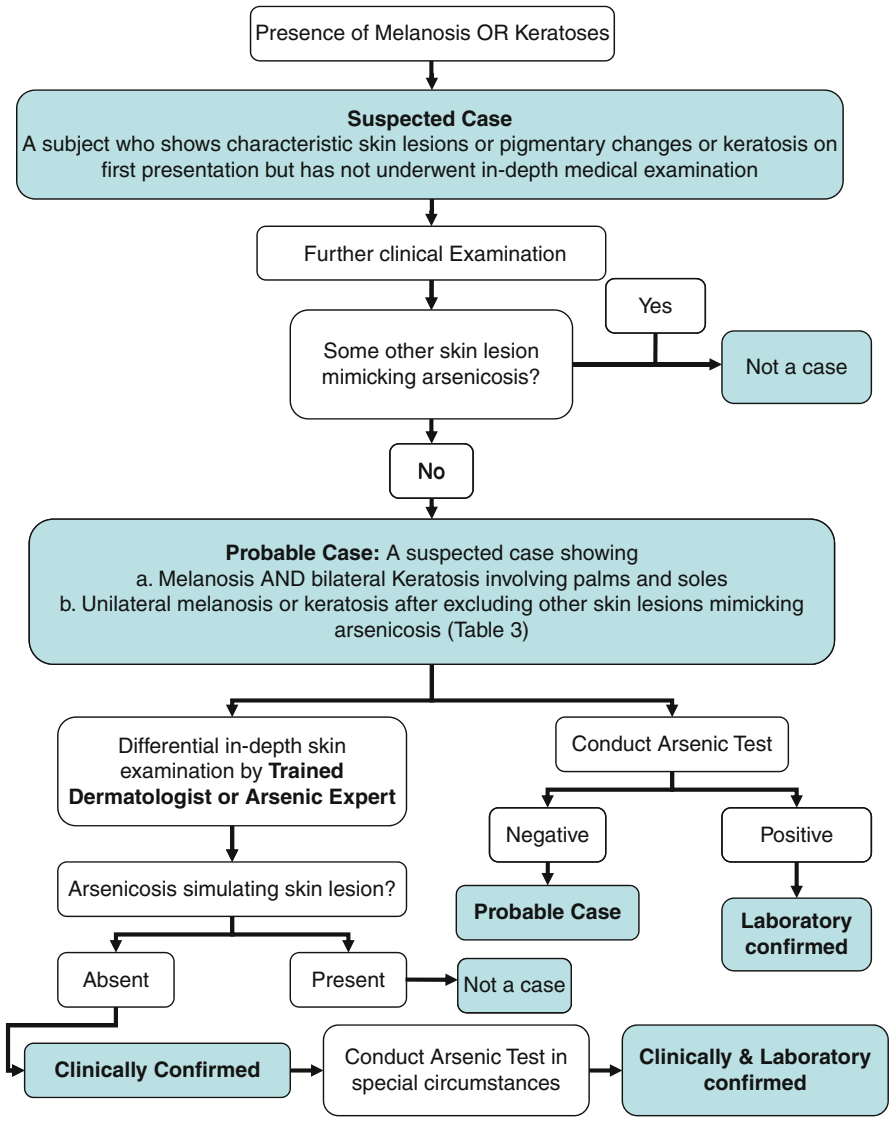
Arsenicosis, Table 4 Characteristic clinical and laboratory criteria for diagnosis of arsenicosis

Clinical (cutaneous) manifestations	
1. Melanosis	(a) Fine-freckled or spotted pigmentation (raindrop pigmentation)
	(b) Diffuse or generalized hyperpigmentation
	(c) Guttate hypopigmentation on normal or hyperpigmented background (leucomelanosis)
	(d) Mucosal pigmentation (esp. oral mucosa)
2. Keratosis	(a) Mild: Minute papules (<2 mm) with slight thickening of palms and soles associated with grit-like texture detected primarily by palpation
	(b) Moderate: Multiple keratotic papules (2–5 mm) present symmetrically on palms and soles
	(c) Severe: Large discrete papule (>5 mm) or confluent keratotic elevation with nodular, wart-like, or horny appearance
3. Malignant/ premalignant lesions	(a) Bowen's disease (squamous cell carcinoma in situ): multicentric Bowen's disease in non sun-exposed areas
	(b) Squamous cell carcinoma and basal-cell epithelioma
Laboratory criteria and collection method for establishment of exposure to arsenic	
1. Water (for at least 6 months)	(a) WHO guideline: >10 μg/L (also expressed as 10 parts per billion)
	(b) Indian standard: >50 μg/L(also expressed as 50 parts per billion)
	Collection method:
	50 ml of sample in acid washed plastic container, which is completely filled to avoid oxidation from air in the bottle
	Samples thus collected can be stored in room temperature for a week, at −20°C for 6 months and at −80°C for longer periods
2. Hair & Nail (evidence of past exposure within last 9 months)	(a) Hair: >1 mg/kg of dry hair
	Collection method:
	Collected after washing with arsenic-free shampoo and ensuring that it is free of coloring agent containing arsenic
	For females, 30 hairs of at least 6-cm length and for males, 60 short hairs from the base
	(b) Nail: >1.5 mg/kg of nail
	Collection method:
	Clipping every finger and toenail after they have grown for 1 month
	Both hair and nail shipped at room temperature to the laboratory, where it can be stored at 4°C till tested
3. Urine (evidence of recent exposure)	>50 μg/L in subjects who has not consumed seafood in the last 4 days
	Collection method:
	Same as for water. Furthermore, concentrated hydrochloric acid (1 in 100 ml of urine) is added to prevent the bacterial growth in urine, to it

Source: Das and Sengupta (2008) (Permission obtained from editor-in-chief IJDVL)

sensitivity and specificity. Other methods of arsenic estimation including colorimetric, inductive-coupled plasma, radiochemical methods, voltammetry, x-ray spectroscopy, hyphenated techniques, etc. are also described, but they suffer the inadequacy of being semiquantitative methods with low sensitivity. The development for *test kits for arsenic detection* is a much felt need for use in field situations, but unfortunately, none of the presently available test kit has proven reliable (Das and Sengupta 2008).

Maximum permissible limit for arsenic content in drinking water was reduced to 10 from 50 μg/L in 1993 by WHO. It has to be kept in mind that this limit is set depending on the analytical capability of available infrastructure to detect arsenic, and this limit would have been much lower if standard basis of risk assessment for industrial chemical is followed for arsenic. Though many countries, like United States and Japan, have adopted the new standard, developing nations which are worst affected by arsenicosis, like India and Bangladesh, are still operating under the national water quality standard of 50 μg/L due to inadequate testing facility (Smedley and Kinniburgh, 2002). This is making the residents more vulnerable to



Arsenicosis,
Fig. 9 Algorithmic approach toward diagnosis of arsenicosis (Adopted with modification from World Health Organization, regional office of Southeast Asia, A Field Guide for Detection, Management and Surveillance of Arsenicosis Cases; Editor, Deoraj Caussy) (Source: Das and Sengupta (2008) (Permission obtained from editor-in-chief IJDVL))

develop the disease by making them drink unsafe water under the false impression of safe water.

Newer Biomarkers of Arsenic Exposure

Biomarkers that reflect the exposure to arsenic in preclinical stage are being explored to take remedial measures before full-blown expression of disease has occurred. In this respect, raised *urinary uroporphyrin-III* and *coproporphyrin-III* have shown promise to detect arsenic exposure. Lowered *metallothionein* (metal-binding protein that protects against metal intoxication) in blood and tissue has also shown association in arsenic-exposed group and can be utilized in future (Das and Sengupta 2008).

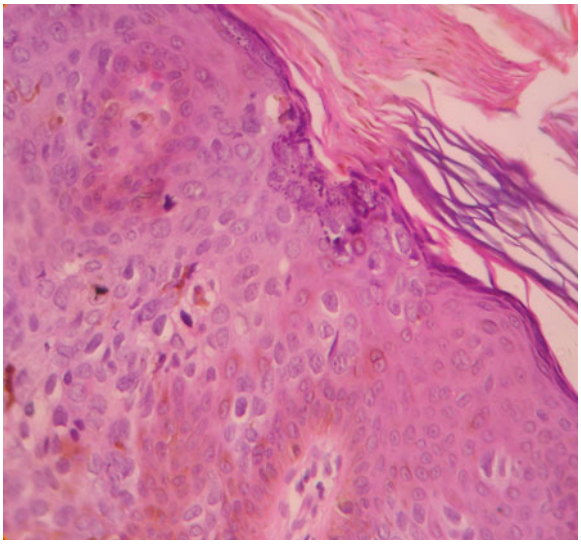
Histology as Diagnostic Clues

Histology is invaluable in diagnosing the malignant changes and for early detection of premalignant lesions. *Dysplastic keratinocytes*, with presence of *anisocytosis* and *mitotic figures*, and *dyskeratosis* are important histological features of malignant changes (Fig. 10). Loss of cellular architecture and polarity with wind-blown appearance and intact basement membrane suggests Bowen's disease. *Hyperkeratosis, parakeratosis, acanthosis, and papillomatosis* are features of keratotic lesions but are not specific for arsenical keratosis. It is important to evaluate the histology of keratotic lesions because of their malignant potential. Histology of pigmented lesions has fallen out of

Arsenicosis, Table 5 Clinical mimickers of arsenicosis

Clinical types	Other dermatological conditions to be differentiated
Melanosis	
Spotted(raindrop)	Pityriasis versicolor, freckels, lentigines, telangiectasia macularis eruptiva perstans
Diffuse/generalized	Photopigmentation, ashy-grey dermatosis, lichen planus pigmentosus, Addison's disease, hemochromatosis
Leucomelanosis	Pityriasis versicolor, epidermodysplasia verruciformis, pityriasis lichenoides chronica, xeroderma pigmentosa, pigmented xerodermoid, idiopathic guttate hypomelanosis, leprosy, PKDL, salt and pepper pigmentation in scleroderma
Mucosal	Drug induced, Peutz-Jeghers syndrome, racial pigmentation, pigmented nevus, Addison's disease
Keratoses	
Punctate keratoses (on palms, soles, as well as on body)	Verruca vulgaris, corn/calluses, pitted keratolysis, occupational keratoses, seborrheic keratoses, lichen amyloidoses, lichen planus hypertrophicus
Diffuse keratoses (on palms and soles)	Psoriasis, pityriasis rubra pilaris, occupational keratoses, hyperkeratotic eczema, hereditary keratoderma, tinea pedis, mechanic's hand sign in dermatomyositis

Source: Das and Sengupta (2008) (Permission obtained from editor-in-chief IJDVL)



Arsenicosis, Fig. 10 Parakeratotic hyperkeratosis with focal hypergranulosis, increased basal layer pigmentation, and early dysplastic changes (H&E stain, × 400) (Source: Sengupta et al. (2008) (Permission obtained from editor-in-chief IJDVL))

grace because of their nonmalignant potential (Sengupta et al. 2008).

Strategies for Control of Arsenicosis

Basic principle for arsenic mitigation program should include the following (WHO Regional consultation meeting 1997):

- Identification of the affected people
- Provision of symptomatic management including nutritional supplement
- Medical management of seriously ill patients in health centers
- Improved and rapid diagnostic facility in the regional centers
- Provision of safe drinking water
- Capacity building of health care providers
- Awareness generation in susceptible community
- Implement comprehensive and integrated studies for better alternatives

In addition to these, comprehensive program implementation plan, evaluation of existing technologies, research and development for newer approaches, networking with stakeholders, and better convergence should be part of long-term policy.

Provision of Safe Drinking Water

Of all sources of arsenic exposure to human beings, drinking water remains the most crucial one. Safe drinking water sources either can be identified (*rainwater harvesting*/*surface water*/*safe aquifers*, etc.) or engineered (*arsenic treatment units*/*arsenic removal plants*) (Table 6) (Ghosh et al. 2008; Yamamura et al. 2000).

It is technically feasible to achieve arsenic concentrations of 5 μg/L or lower using any of several possible treatment methods. However, this requires

Arsenicosis, Table 6 Details of the methods for obtaining arsenic-free safe water

Source of water	Methods	Technical details	Countries adopting the method
Surface water	Pond sand filter	Uses bed of fine sand (porous medium) through which water slowly percolates downward	Myanmar
		The pond should not be used for other purposes, e.g., bathing, washing, and fishing	
	Rainwater harvesting	Rainwater collected using sheet material rooftop or plastic sheet ➔ then diverted to a storage container	Bangladesh, Cambodia, and Taiwan
		Cannot be used in areas with a prolonged dry season	
	Piped water supply of surface water after simple treatment	Treatment needed to reduce turbidity, chlorination to protect against bacteriological contamination of surface water	Bangladesh and India
Groundwater	Dug well	Wells are excavated below the water table till the incoming water exceeds the digger's bailing rate ➔ lined with stones, bricks, or tiles to prevent collapse or contamination	Bangladesh, Nepal, and Myanmar
	Deep tube well (from safe aquifer)	Groundwater is mapped to identify at which depth arsenic-safe groundwater is located, i.e., shallow groundwater is separated from the deep groundwater by clay layer	Bangladesh, India, and Nepal
		Monitoring is important because arsenic may percolate into deep aquifer in future	
	Arsenic removal technologies	Basic principles of arsenic removal from water are based on conventional techniques of oxidation, coprecipitation and adsorption on coagulated flocs, adsorption onto sorptive media, ion exchange, and membrane filtration	1. Community arsenic removal plants: Bangladesh, India, Vietnam, and Taiwan
		Routine monitoring of arsenic safety of the water is needed	2. Household arsenic removal plants: Bangladesh, Nepal, Vietnam, and India
		If the source is surface water, bacteriological checkup also needs to be performed	

Source: Ghosh et al. (2008) (Permission obtained from editor-in-chief IJDVL)

careful process optimization and control, and a more reasonable expectation is that 10 μg/L should be achievable by conventional treatment (e.g., coagulation) (Ghosh et al. 2008; Yamamura et al. 2000).

The ideal solution is to use alternative sources of water that are low in arsenic. However, it is important that this does not result in *risk substitution*. Especially tropical countries most affected by arsenicosis area also bear the major burden of gastrointestinal disease. A large group of population is exposed to water source contaminated with enteric pathogen. Water safety frameworks should be used during planning, installation, and management of all new water points, especially ones based on surface water and very shallow groundwater, to minimize risks from fecal and other non-arsenic contamination. Screening for arsenic and other possible chemical contaminants of concern that can cause problems with health or acceptability, including fluoride, nitrate, iron, and manganese, is also important to ensure that new sources are acceptable. Periodic screening may also be required after a source is established to ensure that it remains safe (Ghosh et al. 2008; Yamamura et al. 2000; Johnston et al. 2001).

Many of the major problems lie in rural areas, where there are many small supplies, sometimes to the household level. At this level, water availability and financial and technical resources are all limited. There are several available approaches, but a basic requirement for education can never be overemphasized. In particular, there is a need to understand the risks of high-arsenic exposure and the sources of arsenic exposure.

To achieve safe arsenic concentration in water for human consumption, a number of approaches have been successfully used in rural areas, including *source substitution* and the use of both high- and low-arsenic sources blended together. *Pond water supply, piped water supply of surface water, rainwater harvesting, deep tube well from safe aquifers, and community-/household-based arsenic removal plants* are there as alternatives. Best suited one or combination of these methods has been tried in different parts of the world with variable success. These sources may be used to

provide drinking water and cooking water or to provide water for irrigation. High-arsenic water can still be used for bathing and clothes washing or other requirements that do not result in contamination of food, in situation where no better alternative is available (Ghosh et al. 2008; Ahmed et al. n.d.).

Arsenic removal process can be of four basic types: *Oxidation sedimentation, coagulation filtration, sorption techniques*, and *membrane techniques*. Decision for selecting the most suitable alternative depends upon effectiveness of the process in removing arsenic, installation and running cost, water output, etc. A report revealed that most cost effective method for long-term solution is piped water supply, whereas for medium-term solution at community level, oxidation and filtration or coagulation filtration are better alternatives. For household purpose, iron-filings/iron-coated sand filters are preferable choices for getting arsenic-free water (Ghosh et al. 2008; Ahmed et al. n.d.).

Health Communication

General community should be made aware of relevant health effects of arsenic poisoning, available alternatives for health protection, safe water options, testing facilities for detection of arsenic contamination, dispelling myths, and misconception about the disease. Communication should be a continuous process, and ultimate aim is to involve the community in planning, implementing, and evaluating arsenicosis control program at the local level to make it a sustainable one. Translational research should be carried out to bring the fruits of scientific research and development to the doorsteps of those who need it most (Galway n.d.).

Treatment of Arsenicosis

The treatment of arsenicosis is essentially symptomatic since no effective remedial measures are known till date. The symptomatic relief that can be offered is also very limited because most of the changes are irreversible and for some clinical manifestations even supportive therapy are not available.

Hyperkeratosis can be treated to some extent by using different keratolytic agents. Five to ten percent salicylic acid or 10–20% urea is recommended by WHO for treatment of arsenical keratosis. Retinoids are also tried in arsenicosis, but their use is limited by their cost and side effect profile. The *malignant and premalignant lesions* are to be treated by surgical excision at the earliest (Das and Sengupta 2008).

Dyspeptic symptoms are treated by H_2 blockers or proton pump inhibitors along with prokinetic agents. For manifestations of portal hypertension, like *esophageal varices*, sclerotherapy or banding may be done. *Peripheral vascular diseases* can be managed by vasodilator drugs (e.g., pentoxifylline or calcium channel blocker) with limited success. Tricyclic antidepressant (e.g., amitriptyline) is sometimes used for relieving the painful dysesthesia of arsenical *peripheral neuropathy*. Bronchodilators are used for controlling symptoms of *obstructive lung diseases*; also, it is important to avoid smoking and dusty environment (Das and Sengupta 2008).

Role of *chelation therapy* using dimercaptosuccinic acid (DMSA), dimercaptopropane succinate (DMPS), or D-penicillamine has not shown any favorable reports in recent studies. Presently, they are not considered for the treatment in spite of early success stories.

In arsenicosis, it is important to be vigilant to detect malignancies (either cutaneous or internal) at the earliest so as to offer the benefit of early intervention. Hence, constant follow-up and surveillance is needed for all who reside in arsenic contaminated zone.

Cross-References

- Arsenic in Nature
- Arsenic in Therapy
- Arsenic Methyltransferases
- Arsenic, Free Radical and Oxidative Stress
- Arsenic-Induced Diabetes Mellitus

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Arsenism

► Arsenicosis

Arsenite Methyltransferase

► Arsenic Methyltransferases

Arsenite S-Adenosylmethionine Methyltransferase

► Arsenic Methyltransferases

Artificial Metalloenzyme

► Palladium, Coordination of Organometallic Complexes in Apoferritin

Artificial Metalloprotein

► Palladium, Coordination of Organometallic Complexes in Apoferritin

Artificial Selenoproteins

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Synonyms

Incorporation of selenium into protein; Selenium-containing protein mimic

Definition

Through incorporation of selenium into existing protein scaffolds, artificial selenoproteins can be generated by chemical or biological strategies to mimic the behaviors of selenoproteins in natural processes. The implementation of biomimetic functions in these

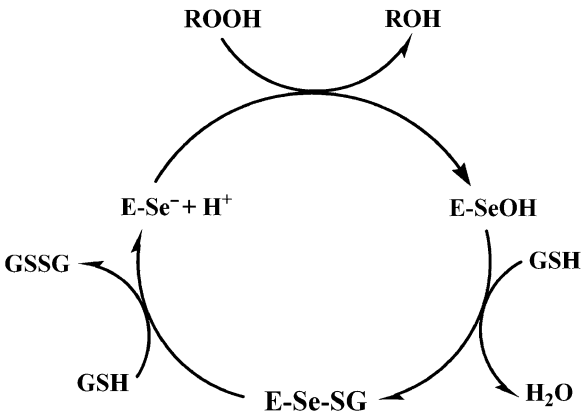
artificial systems will help to clarify the biological and biomedical effects of selenium and increase the understanding for the structures and functions of selenoproteins, which may lead to their valuable applications in human health.

Introduction

Selenium was discovered by the Swedish chemist Jöns Jacob Berzelius in 1817 and has been recognized as an essential micronutrient since 1957. However, selenium biochemistry really emerged after the bacterial enzymes formate dehydrogenase and glycine reductase were reported to contain selenium in 1973. At the same time, the biochemical role of selenium began to be established when it was verified as a vital part of the active site of the antioxidant enzyme glutathione peroxidase (Rotruck et al. 1973).

Selenium exerts its physiological functions in vivo mainly due to its presence in proteins as the 21st amino acid, selenocysteine (Sec, U). Unlike the other amino acids, Sec is encoded by the opal stop codon UGA and undergoes a very complicated biosynthetic pathway to be incorporated into protein by using its own $tRNA^{Sec}$ to recognize UGA. Both cis- and trans-acting factors are involved in decoding UGA for Sec insertion rather than translation termination (Gladyshev and Hatfield 2010). Sec is structurally similar to cysteine (Cys), except that it contains selenium instead of sulfur. However, the lower p*K*a value (5.2) and stronger nucleophilicity of Sec make it much more reactive, resulting in the unique redox behavior.

Selenoproteins, a class of proteins that contain one or more Sec residues, exist in archaea, bacteria, and eukaryotes. So far, more than 40 selenoprotein families have been identified using genomic and bioinformatic approaches, however, with about half of them still having unknown functions. The majority of the characterized selenoproteins are involved in various redox reactions, and their Sec residue is a key functional group for biological functions by reversibly changing its redox state. Selenoproteins can be classified into three major groups (Gladyshev 2006). The largest selenoprotein group, including glutathione peroxidases (GPxs); thyroid hormone deiodinases (DIs); selenoproteins H, M, N, T, V, and W; Sep15; and selenophosphate synthetase 2 (SPS2), contains Sec in the N-terminal regions and in most cases employs

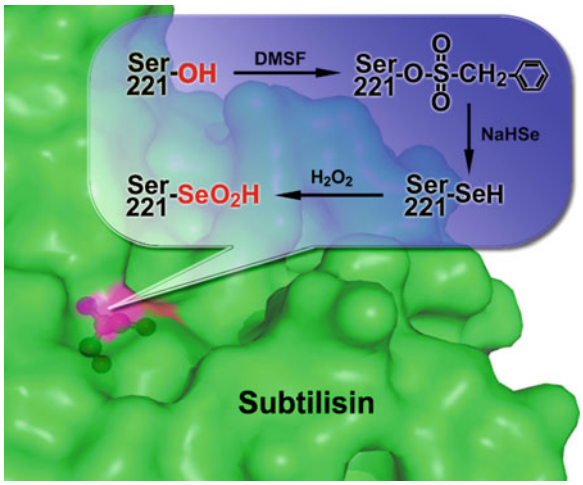


Artificial Selenoproteins, Fig. 1 The catalytic mechanism of GPx

a similar thioredoxin folds. The second selenoprotein group has Sec located in C-terminal sequences, such as thioredoxin reductases (TRs) and selenoproteins S, R, O, I, and K. Selenoproteins in the third group utilize Sec to coordinate a redox-active metal in the active sites. Hydrogenase, formate dehydrogenase (FDH), and formylmethanofuran dehydrogenase are examples of such proteins, which are found only in prokaryotes. Among these selenoproteins, the former two groups of mammalian selenoproteins are of particular interest and importance. In vitro and in vivo studies indicate that selenoproteins in mammals are important members of enzymes, and their expression or activity can influence the risk of a range of diseases, such as cancer, Keshan disease, virus infection, male infertility, impaired immunity, and so on (Lu and Holmgren 2009).

GPx is the first known mammalian selenoprotein and also perhaps the best studied. It is a highly efficient antioxidant enzyme that catalyzes the reduction of hydroperoxides, including hydrogen peroxides, by reducing glutathione (GSH) and functions to protect the cell from oxidative damage (Fig. 1). Over the past few decades, considerable effort has been devoted to mimic the behaviors of selenium in GPx, and a great number of artificial GPx models (e.g., ebselen, ebselen analogs, selenenamides, diselenides, α-phenylselenoketones, selenium-containing cyclodextrins, polymers, proteins) were reported to exhibit GPx-like functions (Huang et al. 2011; Liu et al. 2012). This has promoted researches in the well-known field, selenoprotein biomimetic chemistry, through the reasonable design and synthesis of a series of

selenoprotein mimics, ranging from small molecular compounds to macromolecular ones. As a new development in this regard, the protein-based GPx mimics have attracted much attention. Various approaches have been employed to create such molecules by incorporation of Sec into proteins. These artificial selenoproteins show structural and functional properties similar to GPxs and exhibit excellent catalytic efficiencies and specificities rivaling natural ones. All studies aim at exploring the structure-function relationships of selenoproteins and finding their valuable applications in the treatment of human diseases. In this chapter, we summarize recent advances in artificial selenoproteins prepared by chemical or biological strategies, including semisynthetic selenoproteins, selenium-containing catalytic antibodies, bioimprinted selenoproteins, and genetically engineered selenoproteins. How to improve the properties of these artificial systems has also been described.



Artificial Selenoproteins, Fig. 2 The preparation of selenosubtilisin by site-specific chemical modification of the active site Ser221 of subtilisin to Sec

Semisynthetic Selenoproteins

Naturally existing proteins are ideal precursor molecules for the preparation of artificial selenoproteins, whereas the incorporation of Sec into proteins remains a challenging problem for protein engineering. In nature, this process is highly regulated, with the UGA stop codon being used to specify the insertion of Sec. Therefore, it is difficult to obtain selenoproteins by using traditional recombinant DNA technology. As an alternative strategy, semisynthesis is an attractive technology that utilizes well-defined proteins as preformed intermediates for the resynthesis of the protein to avoid much synthetic effort, and Sec incorporation can be achieved by the chemical modification of protein scaffolds at the desired position.

Site-specific modification of proteins was developed by the groups of Polgar and Bender, who created a new, active enzyme by chemical transformation of the active site serine residue of subtilisin to cysteine. In subsequent work, Yokosawa et al. successfully prepared a thiol-trypsin using a similar chemical method, and Clark et al. converted the active site thiol of papain into a hydroxyl group to obtain the hydroxyl-papain, as a complementary for the work mentioned above. Despite these semisynthetic enzymes proved to retain only limited catalytic activity with respect to the typical substrates of the wild-type enzymes, these studies are significant because the method that can be used to produce new semisynthetic enzymes with novel catalytic activities was established (Qi et al. 2001).

Inspired by earlier work on thiol-subtilisin, the first artificial selenoprotein was prepared by selective activation of Ser^{221} with phenylmethanesulfonyl fluoride (PMSF) to form a sulfonylated subtilisin, which was reacted with NaSeH to produce selenosubtilisin by nucleophilic substitution (Fig. 2) (Wu and Hilvert 1990). The alteration of the active site of subtilisin yielded meaningful results that the semisynthetic selenoenzyme exhibited significant GPx-like redox activity and can catalyze the reduction of a variety of hydroperoxides by an aryl thiol. The rate for the reduction of *tert*-butyl hydroperoxide (*t*-BuOOH) in the presence of selenosubtilisin is over 70,000 times higher than that for diphenyl diselenide (PhSeSePh, a well-studied antioxidant) when using 3-carboxy-4-nitrobenzenthiol (TNB) as substrate. Kinetic investigation revealed that the selenosubtilisin-catalyzed reaction is a typical ping-pong mechanism, analogous to that described for natural GPx. These studies indicated that the redox activity and kinetics of selenosubtilisin can be attributed to the effect of an active site S/Se substitution. In addition, crystallographic analysis and substrate screening with selenosubtilisin suggested that this chemical modification did not disturb the overall structural integrity of the protein and the substrate selectivity of

selenosubtilisin was similar to that of wild-type subtilisin, leading to numerous possibilities in the design and even prediction of its substrate selectivity. As a result, an enrichment of novel racemic alkyl aryl hydroperoxides were chemically synthesized and reported to be catalyzed by selenosubtilisin with high efficiency and selectivity, which may complement the set of naturally available biocatalysts for enantioselective synthesis.

Along a similar line, trypsin was chemically converted into selenotrypsin by replacement of the active serine with Sec. The modified enzyme exhibited considerable GPx activity and was found to have a second-order rate constant about 10^3-fold lower than GPx. This indicated that the chemical approach can be also applied in the site-specific modification of other serine proteases, and GSH is not a suitable substrate for selenotrypsin.

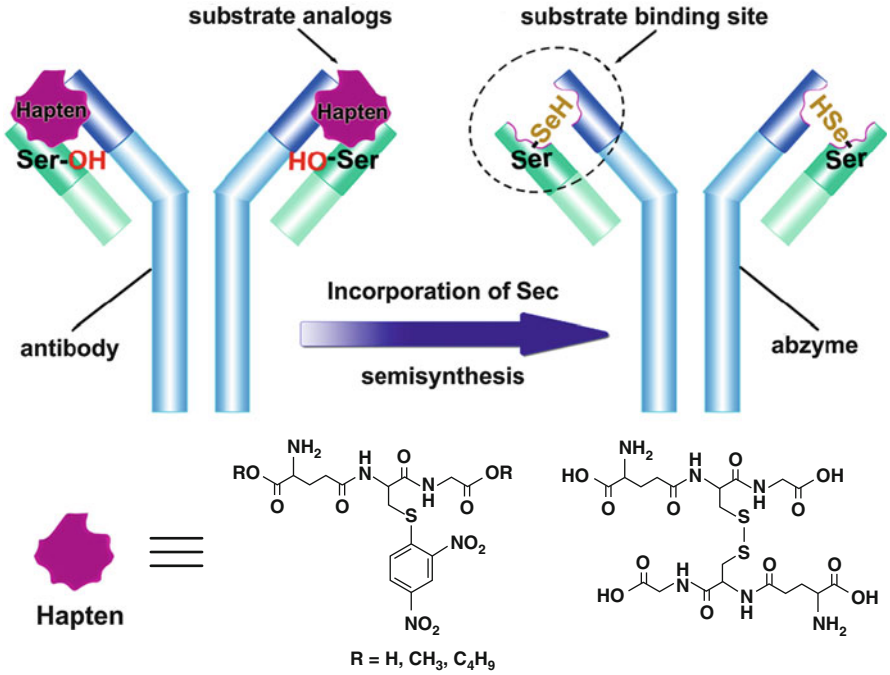
Although chemical modification of active-site residue dramatically changes the function of subtilisin and trypsin, the novel artificial selenoenzymes generated in this way are usually not optimally efficient when using GSH as the thiol substrate. To improve this problem, the active site of protein scaffolds should be modified and regulated to accommodate GSH. Recent years have seen some progress in the design of the artificial selenoenzymes following this routine. Luo and coworkers enhanced the GSH specificity of the semisynthetic selenoezyme by chemically modifying naturally occurring glutathione transferases (GSTs) (Huang et al. 2011). Taking advantage of the structure similarities between seleno-GST and GPx in the specific GSH-binding sites and the perfect location of Sec in the correct vicinity to reactive group of GSH, the resulting selenoenzymes displayed a significantly high efficiency for catalyzing the reduction of H_2O_2 by GSH. As examples, the GPx activity of rat theta-class-derived seleno-GST (Se-rGST T2-2) was found to be 102 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ that surpasses the activities of some natural GPxs (e.g., rabbit liver GPx, bovine liver GPx, human hepatoma HepG 2 cell giGPx, and human plasma pGPx), and the GPx activity of a new modified human glutathione transferase zeta1-1 (Se-hGSTZ1-1) was 8,602 ± 32 U/μM, about 1.5 times higher than that of rabbit liver GPx (5,780 U/μM). These results demonstrated that the general principle of combining a functional group involved in catalysis with a specific binding site for the substrate could be applied to the generation of other efficient semisynthetic biocatalysts.

Selenium-Containing Catalytic Antibodies

Antibodies are immune system-related proteins that selectively recognize a large number of structurally diverse ligands, called antigens, with the goal of eliciting an immune response. Each antibody recognizes a specific antigen, similar to that of enzymes in specifically binding substrate molecules. The possibility of the induction of catalytic antibodies (abzyme) was initially suggested from Pauling's enzyme hypothesis (Nevinsky et al. 2000). He hypothesized that enzymes achieve catalysis by stabilizing the transition state through binding interactions. According to this hypothesis, if the antigen is a transition state analog (hapten) of a corresponding reaction, the binding energy provided by the antibody could stabilize the transition state and facilitate the transformation. This prediction was subsequently confirmed by Lerner and Schultz's groups when the first anti-hapten monoclonal catalytic antibodies were obtained. At present, the technology that induces antibody production by haptens was widely applied in the design of abzymes.

The first known attempt to produce a selenium-containing catalytic antibody (seleno-abzymes) was made by Ding et al. The commercially available anti-human IgM (Fcμ) (rabbit immunoglobulin fraction) was adopted for incorporation of the catalytic group via selective chemical modification of Ser into Sec (Ding et al. 1994). Due to the lack of substrate binding site, the modified antibody was confirmed to have relatively low GPx activity as compared with natural enzymes.

Subsequently, Luo et al. succeeded in the preparation of monoclonal antibodies (McAbs) with GSH-specific binding sites using a series of hydrophobically modified GSH and GSSG as haptens, and the catalytic group Sec was then incorporated into the McAb by the semisynthetic method mentioned above to generate GPx mimics (Fig. 3) (Huang et al. 2011). In this process, substrate analogs rather than transition state analogs were used as haptens, because the hapten design based on transition state structure does not usually work well, especially for complex chemical reactions with unknown transition states (such as GPx). Intriguingly, these catalytic antibodies exhibited remarkably high catalytic efficiency. The GPx activity of seleno-abzyme Se-4A4 was 1,097 U/μM, approaching the magnitude level of native enzyme activity. Moreover, three new selenium-containing murine catalytic antibodies, including



Artificial Selenoproteins, Fig. 3 The combination of monoclonal antibody preparation technique and semisynthetic method to generate selenium-containing catalytic antibodies with GPx activities

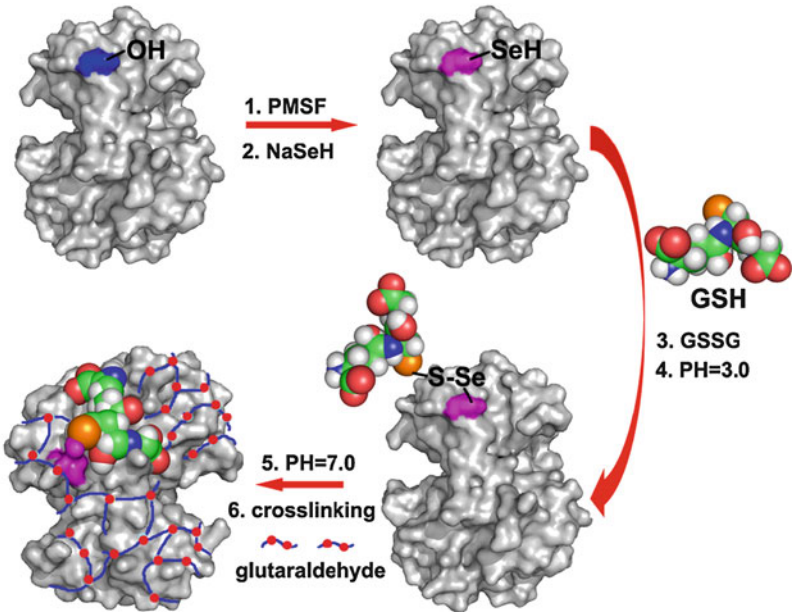
Se-3G5, Se-2F3, and Se-5C9, were reported to display remarkable activities (12,900, 24,300, and 21,900 U/μM, respectively) rivaling natural GPx from rabbit liver (Huang et al. 2011). These results demonstrated that an extremely high degree of precision in the match between the complementary structures of antibody binding sites and substrates is crucial for the efficient catalytic activities of artificial seleno-abzymes.

Recently, instead of an immunization protocol, the traditional genetic engineering technology has been explored as an efficient and more manipulative strategy to acquire single-chain variable region fragments of antibodies (scFv) for pharmacological use. Compared to McAb, scFv has a smaller size and less immunogenicity, without sacrificing its ability to bind antigen. Therefore, Ren et al. prepared the first selenium-containing single-chain abzyme (Se-2F3-scFv) from the 2F3 monoclonal antibody by successful construction of a high-level expression vector, and the produced seleno-abzyme shows GPx activity of 3,394 U/μM, which approaches that of rabbit liver GPx but was lower than that of Se-2F3 (Huang et al. 2011). The investigation of mitochondrial damage model induced by reactive oxygen species (ROS) indicated that Se-2F3-scFv can effectively inhibit the swelling of mitochondria, leading to new treatments for oxidative stress-related diseases.

To further improve the immunogenicity of seleno-abzyme, in vitro evolutionary method using a phage display system has been applied for protein engineering of human antibody fragments. As a result, several human antibodies B8, H6, C1, and B9 with the GSH-binding site were screened out for direct incorporation of Sec to form the corresponding seleno-abzymes (Liu et al. 2012), and most of them were detected to reach the level of native enzyme activity. Moreover, some human antibodies were also explored to enhance the GPx activity by the combination of computer-assisted modeling and site-directed mutagenesis. On the basis of modeling analysis, an Ala/Ser180 substitution at the binding site of Se-B3-scFv could greatly increase the enzyme-substrate interaction, resulting in 2.16 times enhancement in the GPx activity (Liu et al. 2012). It is suggested that attractive noncovalent interactions between enzyme and substrate can also play a key role in catalysis through the stabilization of substrate conformation within the active site.

Bioimprinted Selenoproteins

Molecular recognition is an important feature for enzyme-mediated catalysis, and the binding specificity



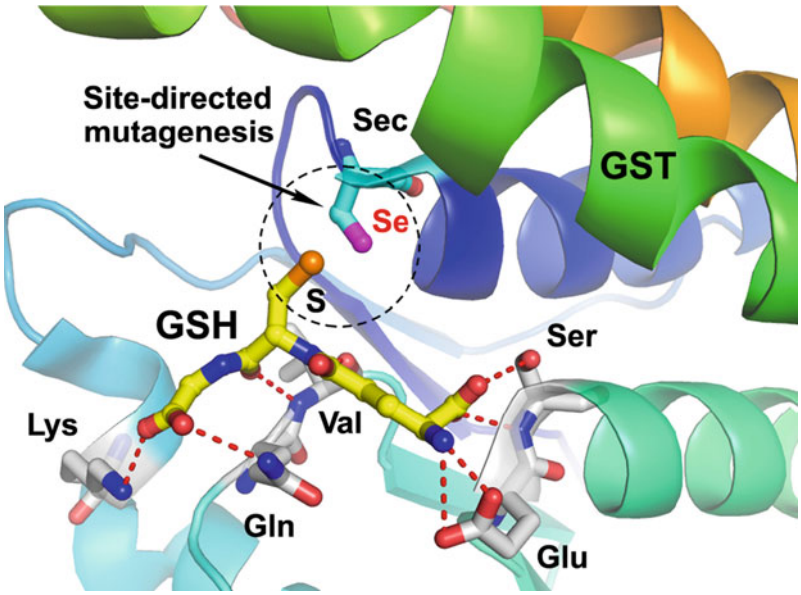
Artificial Selenoproteins, Fig. 4 Schematic representation of the production process of the GSH-imprinted selenoprotein

depends on attractive forces and complementary structures between enzyme and substrate. In the preparation of enzyme mimics, a number of recognition elements, both natural (e.g., antibodies) and synthetic (e.g., modified cyclodextrins), have been described to mimic the catalytic behaviors of enzymes, whereas these receptor molecules can only selectively bind their respective type of substrates. This means that each recognition problem requires a novel solution.

As an alternative strategy for the development of new and efficient catalysts, molecular imprinting is a more generic method that allows for the preparation of receptor scaffolds with specific binding sites for a target molecule through the copolymerization of functional and cross-linking monomers in the presence of a template molecule and followed by the removal of the template to yield a binding site with size and shape complementary to the target molecule in the cross-linked network polymer. Based on the same principle, the technique of bioimprinting has been developed using biopolymers for this imprinting procedure.

The design of bioimprinted proteins with GPx activity was proposed by Liu et al. *N*,*S*-bis-2,4-dinitrophenyl-glutathione (GSH-2DNP), a GSH derivative, was synthesized to imprint egg albumin (Liu et al. 2004). In this process, the structural change of egg albumin was induced by the noncovalent interactions (such as hydrogen bonds, ion pairing, and hydrophobic interactions) between protein and template molecule in mild denaturing condition. The new conformation was then fixed using a biofunctional cross-linker glutaraldehyde. After removal of the template by dialysis, an imprinted GPx enzyme model with template-shaped cavity capable of binding GSH was produced by incorporation of Sec using semisynthetic method, and its GPx activity was found to be 101–817 U/μM, approaching the level of selenium-containing catalytic antibody Se-4A4. These results proved the feasibility of bioimprinting to prepare an artificial recognition system with high selectivity toward target molecule for the design of selenoenzyme.

Later, the imprinting procedure was optimized by the same group using a covalent Se-S bond to link the template molecule GSH and semisynthesized selenosubtilisin to form GSH-selenosubtilisin (Fig. 4). The advantage of this improvement is that the catalytic group Sec is located in close proximity to the reactive thiol of the bound substrate GSH, a perfect position inside the active site to catalyze the reaction. Thus, the second-order rate constant of bioimprinted selenosubtilisin for H_2O_2 is larger than that of bioimprinted selenoalbumin (Liu et al. 2008). Moreover, the imprinted selenosubtilisin was found to have no activity against aromatic substrate TNB, suggesting the bioimprinting technique completely



Artificial Selenoproteins, Fig. 5 Schematic representation of genetically engineered seleno-GST with an active site Sec and a specific GSH-binding site

rearranged the active site of subtilisin to accommodate the native GPx substrate GSH.

Genetically Engineered Selenoproteins

Genetic engineering of proteins that contain noncanonical amino acid residues is a powerful tool for the redesign of natural proteins with novel properties. It utilizes the endogenous cellular machinery to translate the target protein, in which the unnatural amino acid can be selectively incorporated with high translation fidelity and efficiency. Cowie and Cohen pioneered the use of an auxotrophic bacterial host that starved for the natural amino acid and supplemented with a close structural analog to incorporate selenomethionine (SeMet) instead of Met. Budisa et al. extended the idea to create a series of atomic mutations ($-CH_2-$ → –S– → –Se– → –Te–) in proteins by substitution of Met by its noncanonical isosteres norleucine, selenomethionine, and telluromethionine (Beatty and Tirrell 2009). Several decades later, this strategy was successfully used to solve the problem of Sec biosynthesis.

The possibility of using a cysteine auxotrophic strain for the efficient substitution of cysteine residues in a protein by selenocysteine was first reported by Bőck et al. (Muller et al. 1994). The similar method for incorporation of Sec was then exploited to substitute Cys149 residue in gene-phosphorylating glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The obtained seleno-GAPDH exhibits high GPx activity against the aromatic substrate, which is similar to that of the first semisynthetic selenosubtilisins but remains low compared to selenoglutathione peroxidase.

Following this work, GST was chosen as protein scaffold to address the low GPx activity occurring on seleno-GAPDH toward GSH (Fig. 5). It is well known that GST belongs to the thioredoxin superfamily and shares a similar structural fold to GPx, known as thioredoxin fold, which is an ideal scaffold to create GPx mimics with high affinity for GSH. After the catalytically essential residue selenocysteine was bioincorporated into GSH-specific binding scaffold by replacing the active site serine 9 with a cysteine and then substituting it with selenocysteine in the cysteine auxotrophic system, the genetically engineered seleno-GST exhibited a remarkable activity of 2,957 U/μM rivaling native GPx. Furthermore, another member of the thioredoxin superfamily, glutaredoxin (Grx), was reported to offer a smaller protein scaffold to endow GPx properties by engineering a catalytic Sec residue (Huang et al. 2011). The novel seleno-Grx showed a GPx activity of 2,723 U/μM for the reduction of H_2O_2 with GSH, displaying obvious advantage for potential medical applications. These studies indicated that the redesign of evolutionarily related enzymes may be a common idea to design efficient biocatalysts.

Summary

The field of artificial selenoprotein is a rapidly evolving subject, especially for the antioxidant selenoprotein GPx. So far, several strategies that combine expertise from chemistry and biology have been developed to design the protein-based GPx mimics by site-specific incorporation of Sec. These studies do not simply reproduce naturally occurring selenoproteins, but offer an alternative approach to explore the biological role of selenium and the structure-function relationships of selenoprotein. Characterization of these artificial selenoprotein systems has provided valuable insight into the nature of molecular recognition and catalysis, which may promote the ability to create more efficient artificial selenoproteins for application in chemistry, biology, and medicine.

Cross-References

► Selenium, Biologically Active Compounds

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As

► Arsenic, Mechanisms of Cellular Detoxification

Ascorbate Oxidase

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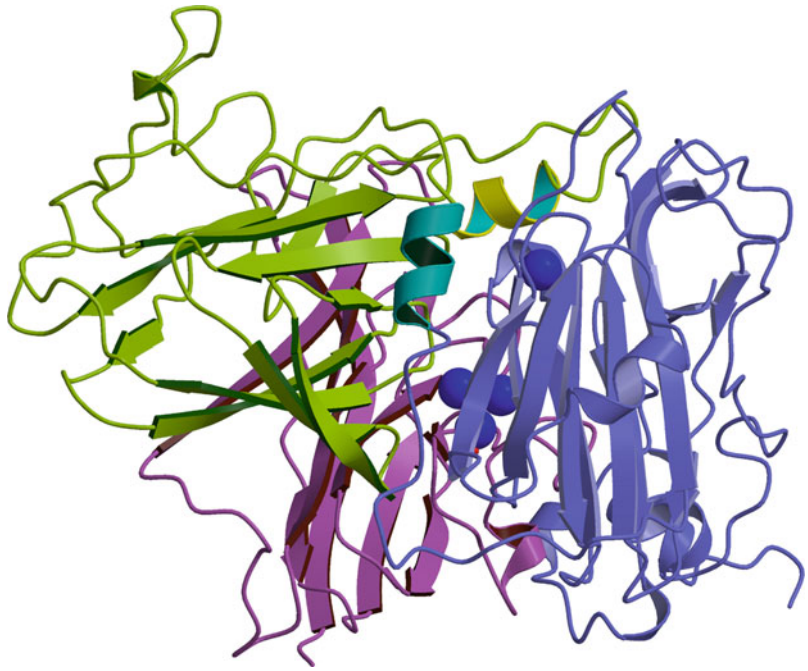
Synonyms

EC 1.10.3; L-ascorbate; Oxygen oxidoreductase

Definition

Ascorbate oxidase is a multicopper oxidase that catalyses the four-electron reduction of dioxygen with concomitant one-electron oxidation of the substrate.

Ascorbate oxidase, laccase, and ceruloplasmin known as blue oxidases are typical members of the multicopper oxidase (MCO) family (Messerschmidt 1997). They contain at least one type-1 copper center and a trinuclear copper site. Ascorbate oxidase has been reviewed several times (Messerschmidt 1994, 1997, 2001, 2010). It occurs in higher plants. The main source for investigations has been zucchini squash. The immunohistochemical localization of



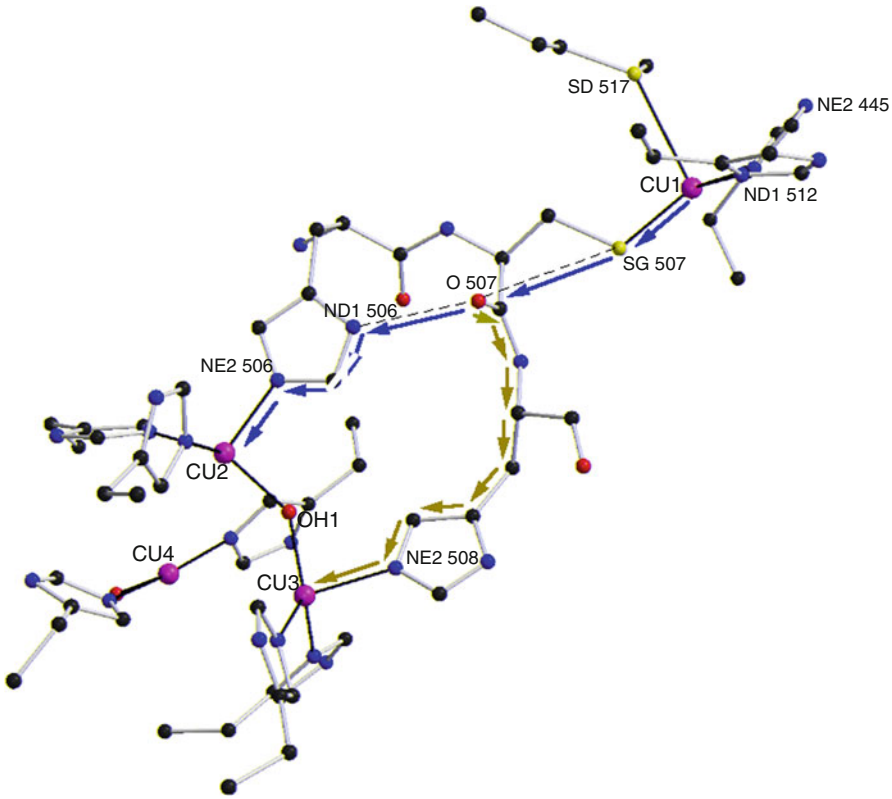
Ascorbate Oxidase, Fig. 1 Ribbon diagram of the monomer structure of ascorbate oxidase (PDB-code: 1AOZ), domain 1 in *turquoise*, domain 2 in *green*, and domain 3 in *pink* (Reproduced from Messerschmidt 2010, with permission from Elsevier Inc.)

ascorbate oxidase in green zucchini shows that it is distributed ubiquitously over vegetative and reproductive organs. Its in vivo role has not been fully elucidated. It might be involved in processes such as fruit ripening, growth promotion, or in susceptibility to disease. The best and probably the physiological substrate is ascorbate, but catechols and polyphenols are also substrates in vivo.

The spectral properties of the blue oxidases like ascorbate oxidase, laccase, and ceruloplasmin are very similar. The copper ions of copper proteins have been classified according to their spectroscopic properties. Type-1 Cu^{2+} shows high absorption in the visible region (generally >3,000 M^{-1} cm^{-1} at 600 nm) and an EPR spectrum with $A_{\|} < 95 \times 10^{-4}$ cm^{-1}. Type-2, or normal, Cu^{2+} has undetectable absorption and the EPR line shape of the low-molecular-mass copper complexes ($A_{\|} > 140 \times 10^{-4}$ cm^{-1}). Type-3 (Cu^{2+}) is characterized by strong absorption in the near-ultraviolet region ($\lambda = 330$ nm) and by the absence of an EPR signal. The type-3 center consists of a pair of copper ions that are antiferromagnetically coupled. The above-mentioned signals disappear upon reduction.

Ascorbate oxidases have eight coppers with two type-1, two type-2, and two type-3 copper centers per homotetramer. The X-ray structure (Messerschmidt et al. 1992) indicated that the non-blue EPR-active type-2 copper together with the type-3 copper pair form an integral part of the trinuclear active copper center. There has been experimental evidence in earlier studies that the type-2 copper is close to the type-3 copper. Solomon and coworkers (Spira-Solomon et al. 1986), based on absorption, EPR, and low-temperature magnetic CD spectroscopy of azide binding to laccase, classified this metal-binding site as a trinuclear copper site.

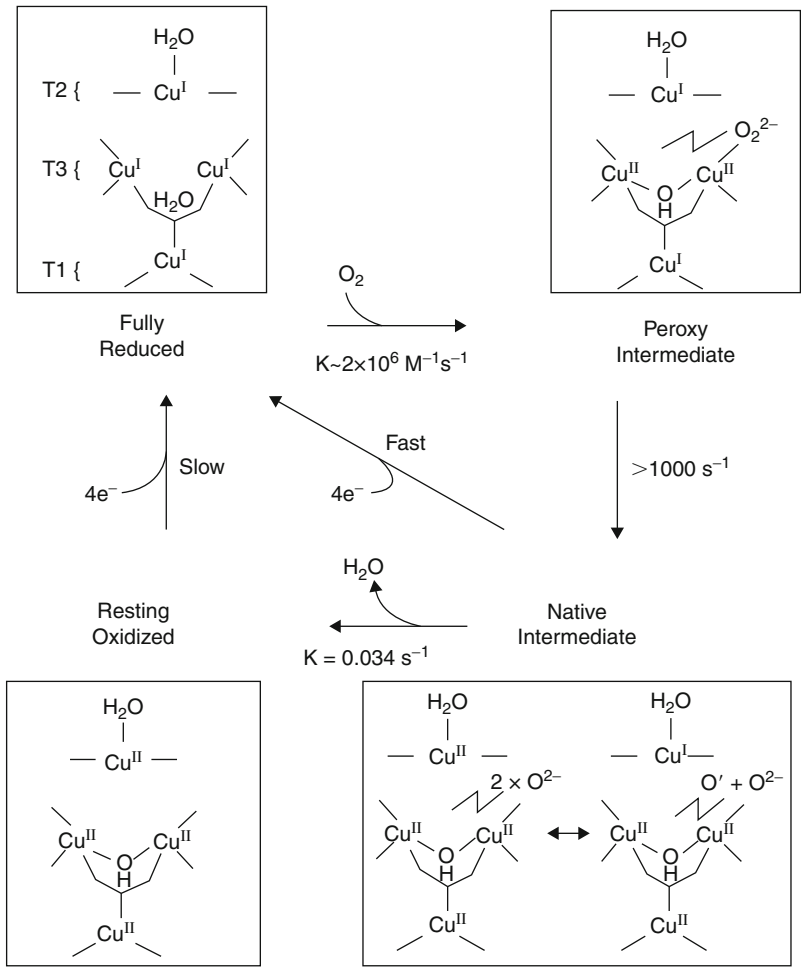
Ascorbate oxidase was the first blue oxidase whose crystal structure was determined (Messerschmidt et al. 1992). It is a homodimeric enzyme with a molecular mass of 70 kDa and 552 amino acid residues per subunit (zucchini). The three-domain structure and the location of the type-1 and trinuclear copper centers in the ascorbate oxidase monomer as derived from the crystal structure are shown in Fig. 1. The folding of all three domains is of a similar β-barrel type. The structure is distantly related to the small blue copper proteins like plastocyanin or azurin. The mononuclear type-1 copper site is located in domain 3 and has the four canonical type-1 copper ligands (His, Cys, His, Met) also found in the small blue copper proteins plastocyanin and azurin. The trinuclear copper species is bound between domains 1 and 3 and has eight histidine ligands symmetrically supplied by both



Ascorbate Oxidase,
Fig. 2 Drawing of the region in the ascorbate oxidase monomer between the type-1 copper center and the trinuclear copper site (PDB-code: 1AOZ) (Reproduced from Messerschmidt (2010), with permission from Elsevier Inc.)

domains and two oxygen atoms. Seven histidines are coordinated by their NE2 atoms to the copper ions, whereas His62 is ligated to CU3 by its ND1 atom. The region of the molecule between the two centers is depicted in Fig. 2 showing the respective copper coordinations. The distances between the type-1 copper and the three coppers of the trinuclear center are 12.20, 12.69, and 14.87 Å, respectively. A binding pocket for the reducing substrate that is complementary to an ascorbate molecule is located near the type-1 copper site and accessible from solvent. A broad channel providing access from solvent to the trinuclear copper species, which is the binding and reaction site for the dioxygen, is present in ascorbate oxidase. During catalysis, an intramolecular electron transfer between the type-1 copper and the trinuclear copper cluster must occur. Intramolecular electron transfer from the type-1 copper to the type-3 copper pair of the trinuclear copper site may be through-bond, through-space, or a combination of both. A through-bond pathway is available for both branches each with 11 bonds (see Fig. 2). The alternative combined through-bond and through-space pathway from the type-1 copper Cu1 to Cu2 of the trinuclear center that involves a transfer from the SG atom of Cys507 to the main-chain carbonyl of Cys507 and through the hydrogen bond of this carbonyl to the ND1 atom of His506. Electron transfer processes in blue oxidases have been discussed in detail (Farver and Pecht 1997).

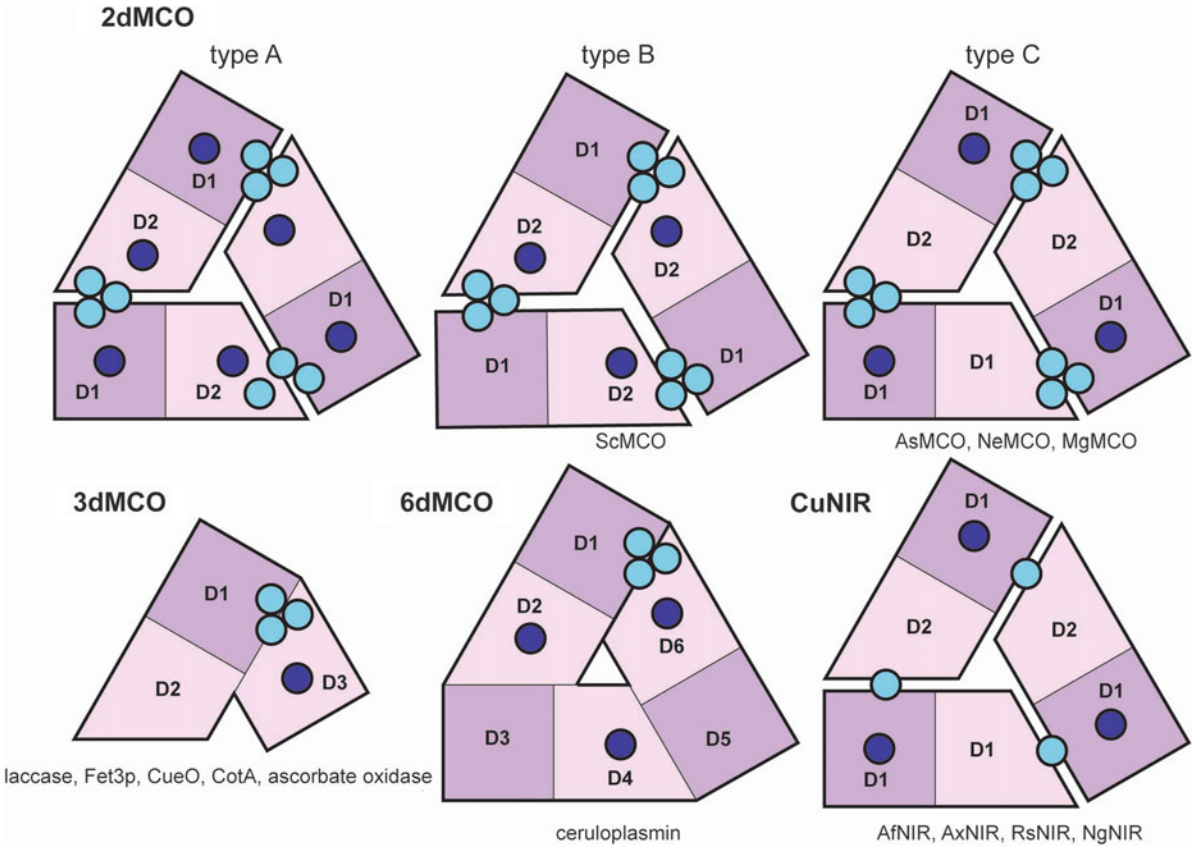
The crystal structure of the reduced form of ascorbate oxidase shows the type-1 copper site geometry virtually unchanged whereas the trinuclear site displays considerable structural changes (Messerschmidt et al. 1993). The bridging oxygen ligand OH1 is released, and the two coppers, Cu2 and Cu3, move toward their respective histidines and become three-coordinated in a trigonal-planar arrangement. The copper–copper distances increase from an average of 3.7–5.1 Å for Cu2–Cu3, 4.4 Å for Cu2–Cu4, and 4.1 Å for Cu3–Cu4. In the crystal structure of the peroxide form (Messerschmidt et al. 1993), the bridging oxygen ligand OH1 is released as well, and the peroxide is bound end on to the copper Cu2 of the trinuclear copper cluster. Solomon and coworkers (see e.g., Solomon et al. 1997) concluded from their spectroscopic data obtained from ascorbate oxidase



Ascorbate Oxidase, Fig. 3 Reaction scheme for laccase and ascorbate oxidase as proposed by Solomon and coworkers (Lee et al. 2002) (Reproduced from Lee et al. (2002), with permission from American Chemical Society)

and laccase that their reoxidation intermediate binds as 1,1-μ hydroperoxide between either Cu2 and Cu4 or Cu3 and Cu4.

A "two-site ping-pong bi bi" mechanism has been deduced for tree laccase from steady-state kinetics. This will be valid for ascorbate oxidase as well because both enzymes are structurally and mechanistically closely related. A reaction scheme for ascorbate oxidase has been proposed based on the available spectroscopic, kinetic, and structural information (Messerschmidt 2001) that should also be valid for laccase or ceruloplasmin in the main features. A somewhat different view of the catalytic mechanism has been presented by Lee et al. (2002) based on spectroscopic and computational chemical techniques. This reaction scheme is shown in Fig. 3. The reaction starts from the resting oxidized state (lower left panel in Fig. 3). This state has been observed in the X-ray structure of fully oxidized ascorbate oxidase and is also the starting point in the scheme proposed by Messerschmidt (2001). The addition of four reduction equivalents leads to the fully reduced state (upper left panel in Fig. 3). The main distinction between both schemes is the presence of a bridging oxygen ligand between the type-3 copper pair, which is a water in the fully reduced state and a hydroxyl in the other three states. This central oxygen species could not be observed in the various ascorbate oxidase X-ray structures and is therefore not part of the mechanism reported in Messerschmidt (2001). The native intermediate state is split up into two substates. The right one is similar to the state of release of the first water molecule and formation of an $O^{\bullet -}$ radical in the scheme reported in Messerschmidt (2001) with the difference that the



Ascorbate Oxidase, Fig. 4 MCO classification scheme. The *cyan* circles indicate the trinuclear copper site. Single *dark blue* circles represent the type-1 copper site. The CiNIR domain structure is shown for comparison (Reproduced from Lawton and Rosenzweig 2011, with permission from John Wiley and Sons)

type-2 copper is reduced instead of one copper from the type-3 copper pair. After transfer of the fourth electron to the oxygen radical, the second water molecule is released (left side in the lower right panel of Fig. 3) and returns to the oxidized resting form. In the scheme reported in Messerschmidt (2001), the enzyme moves back to the fully reduced state without traversing the resting state. However, in the main features, both reaction schemes are very similar and reflect the spectroscopic, kinetic, and structural known data. Bento et al. (2006) have presented a reaction scheme that does not involve the fully reduced state in the catalytic cycle and does not contain the spectroscopically proven oxygen radical intermediate. As it is very unlikely that dioxygen binds to the fully oxidized enzyme and the oxygen radical intermediate had not been included, this scheme seems to be very implausible.

According to the current state of knowledge, the MCO family comprises now members that consist of two domains (2dMCO), three domains (3dMCO), and six domains (6dMCO).

Although not being an oxidase, copper-dependent nitrite reductase (CuNIR) is closely related to the MCO family. 2dMCOs have recently been reviewed, and their relationship among each other and to CuNIR is discussed (Lawton and Rosenzweig 2011). The corresponding MCO classification scheme is shown in Fig. 4. The most surprising finding is that 2dMCOs occur as active enzymes in a trimeric form resembling CuNIRs. The trinuclear copper site is located between domains 1 and 2 of symmetry-related molecules. This is similar to CuNIRs where the type-2 copper site is also situated between domains 1 and 2 of symmetry-related molecules. 2dMCOs can be subdivided into three different types as deduced from the amino acid

sequences. Type A has type-1 copper centers in both domains, type B in domain 2, and type C in domain 1. X-ray structures are known from type B (*Streptomyces coelicolor* MCO, ScMCO) and type C (*Arthrobacter* sp. MCO, AsMCO; *Nitrosomonas europaea* MCO, NeMCO; *Uncultured bacteria* MCO, MgMCO). Despite the similarities between 2dMCOs and CuNIRs, there are significant differences at the termini. In all currently characterized CuNIRs, the N-terminal residues extend from the first cupredoxin domain into the crevice between domains 1 and 2 whereas the C-terminal b-strand hydrogen bonds with domain 1 of the adjacent monomer. In contrast, the C-termini of 2dMCOs either occupy the space between domains 1 and 2 (NeMCO and MgMCO) or do not extend into the neighboring monomer (AsMCO). In AsMCO, the domain–domain interface is filled by the N-terminus, similar to CuNIRs, but in a slightly different fashion. It is important to note that a significant portion of the N-terminus of AsMCO is not modeled.

The trimeric structure of 2dMCOs results in a domain arrangement that is surprisingly similar to the domain arrangement of 3dMCOs. Structural conservation is especially evident at the 2dMCO monomer–monomer interface and the analogous interface between domains 1 and 3 in 3dMCOs. This similarity results in the trinuclear copper site being coordinated in the same way in 2dMCOs and 3dMCOs and suggests that formation of the trinuclear copper site is dependent on precise positioning of the domains. Thermal denaturation studies on ferrous transport 3 protein (Fet3p), a 3dMCO involved in iron metabolism, show that binding of the trinuclear copper ions decreases the stability of domains 1 and 3. It was suggested that domain 2 compensates for this effect by reducing the degrees of freedom for domains 1 and 2. Thus, domain 2 likely plays a large role in positioning and stabilizing the interface between domains 1 and 3 in 3dMCOs. In 2dMCOs, this interface forms as a result of trimerization. Therefore, oligomerization in 2dMCOs and the presence of an additional domain in 3dMCOs likely serve a similar purpose.

Ceruloplasmin has all six domains on a single amino acid sequence chain, but its domains are similarly arranged to the 2dMCOs and CuNIRs. The trinuclear copper site is located between domains 1 and 6 and domains 2, 4, and 6 each hold a type-1 copper.

Models for MCO evolution are all consistent that the evolution began with a single domain duplication, followed by a trimerization event and metal binding at the monomer–monomer interfaces. The existence of functional 2dMCOs confirms the plausibility of these models. In light of the conserved domain placement between 2dMCOs and 3d/6dMCOs, it is extremely likely that modern day 2dMCOs and 3d/6dMCOs share a common 2dMCO ancestor.

In addition, the 2dMCO structures highlight their similarities to CuNIRs. Strikingly, the only major difference between CuNIR and the type-C 2dMCOs, NeMCO and ScMCO, is the presence of the type-3 copper ions. Two pathways for CuNIR evolution have been proposed: The first involves evolution of CuNIR following the initial domain duplication event, and the second involves evolution of CuNIR from a type-C 2dMCO. Current structural and sequence analysis cannot exclude either of these possibilities, and it is possible that both pathways may be relevant to multicopper blue protein evolution.

Cross-References

- ▶ Biological Copper Transport
- ▶ Catechol Oxidase and Tyrosinase
- ▶ Copper-Binding Proteins
- ▶ Copper, Biological Functions
- ▶ Laccases
- ▶ Monocopper Blue Proteins

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Ascorbic Acid, Vitamin C

▶ Chromium(VI), Oxidative Cell Damage

Aspartic Acid: Aspartate, Asp, D

▶ Magnesium Binding Sites in Proteins

Aspirin, Acetylsalicylic Acid

▶ Chromium(VI), Oxidative Cell Damage

Assimilation of Cadmium

▶ Cadmium Absorption

Association/Binding Constant

▶ Calcium Ion Selectivity in Biological Systems

Astacin

▶ Zinc-Astacins

Astacus Protease

▶ Zinc-Astacins

Asymmetric Allylic Alkylation/Amination

▶ Palladium-catalysed Allylic Nucleophilic Substitution Reactions, Artificial Metalloenzymes

Atomic Number 38

▶ Strontium, Physical and Chemical Properties

Atomic Number 56

▶ Barium, Physical and Chemical Properties

ATP Phosphohydrolase (Na^+,K^+, Exchanging)

▶ Sodium/Potassium-ATPase Structure and Function, Overview

ATP2A1-3 (Human Genes for SERCA1-3)

▶ Calcium ATPases

ATP2B1-4 (PMCA1-4)

▶ Calcium ATPases

ATR1 Paralogs in Yeast

▶ Boron Stress Tolerance, YMR279c and YOR378w

Atr1, Boron Exporter in Yeast

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Synonyms

Boric acid transport; Boron efflux pump; Boron toxicity; Boron transport in yeast

Definition

Boron is a micronutrient which is essential for plants and beneficial for animals. However, excess amount of it is toxic for organisms. Yeast Atr1 is a multidrug efflux pump and its major function is to export boron to outside of the cell. This entry will basically focus on boron transport and the role of Atr1 protein in boron stress response paths.

Boron in Living Organisms

In biological systems, boron is found mostly as boric acid [$B(OH)_3$] especially at neutral pH. Boric acid is a small molecule and is known as a weak Lewis acid which has a pKa of 9.24. Boric acid and borate can both bind and make strong interactions with biological compounds containing hydroxyl groups in *cis* position. These compounds include nucleic acids, nicotinamide, phosphoinositides, and sugars such as ribose. The finding of ribose stabilization achieved by borate puts forward a new vision to the "RNA world" hypothesis.

Boron is an essential metalloid for many organisms. Boron essentiality for plants was first established in 1923 by the finding that the growth of the plant *Vicia faba* was inhibited when boron was not present in the medium, however, when boron was supplied the growth was rescued (Warington 1923). Since that time boron has been known to be a necessary micronutrient for all vascular plants. Boron is also important for the life cycle of diatoms and algae, for the stabilization of heterocyst and nitrogen fixation in cyanobacteria and in some actinomycetes (*Frankia, Anabaena*). These organisms were dominant in ancient times, thus, boron may have special roles in the evolution of early life (Tanaka and Fujiwara 2008). Newly, a bacterial species *Bacillus boroniphilus* was isolated from soils of Kütahya, Turkey. The bacterium has a need for boron for its growth and can tolerate more than 450 mM boron (Ahmed et al. 2007). Also animals such as zebra fish (*Danio rerio*), rainbow trout (*Oncorhynchus mykiss*), and frogs (*Xenopus laevis*) required boron for their embryonic development. In addition, an interesting role of boron was found in the year 2002. It is involved in bacterial quorum sensing mechanism via appearing in the conserved structure of autoinducer AI-2 which is produced by various bacteria (Chen et al. 2002). Another important study related to one of the primary functions of boron comes from the year 1996 and revealed that boron acts as a crosslinker in plant cell walls. This crosslinking of rhamnogalacturonan II by borate cis-diol ester bonds is crucial for plants. Rhamnogalacturonan II is pectin and the formation of its network was found to be important especially for normal leaf expansion (Kobayashi et al. 1996).

When boron is present at high concentrations it becomes toxic. The optimum amount of boron differs from one organism to another. While 10 mM boric acid can cause lethality in *Arabidopsis*, the yeast *Saccharomyces cerevisiae* can grow in media containing 80 mM boric acid (Kaya et al. 2009).

Both deficiency and toxicity of boron can have some unwanted side effects on organisms. In plants, for example, usually expanding organs are firstly affected by boron deficiency. Abnormal cell wall formations, altered cytoskeleton polymerization, inhibition of leaf expansion and root elongation, as well as flower and fruit development are other examples of the results of boron deprivation (Tanaka and Fujiwara 2008). Boron toxicity symptoms occur mostly in the margins of leaves and other observed consequences of boron toxicity in plants are reduced growth of shoots and roots, lower photosynthetic rates, chlorosis, and necrosis of leaves. There are also several studies about the consequences of boron deprivation in human and animals including impairment of growth, abnormal

bone development, the decrease in blood steroid hormones, and the increase in calcium excretion. In animals, excessive boron causes neurological effects, weight loss, diarrhea, anorexia, testicular atrophy, cardiovascular defects, and skeletal malformation. However, the molecular mechanisms behind these observed defects caused by boron deficiency or toxicity remain unresolved.

Boron Transporters

Basically three mechanisms have been proposed for the detoxification of the metals and metalloids. First mechanism includes export of the metal or metalloid from the cytosol; second mechanism includes the compartmentalization of metal or metalloid from the cytosol to the vacuole via the transporters located on the vacuolar membrane; and third mechanism involves the chelation (Wysocki and Tamas 2010).

Boron toxicity is a worldwide problem and causes a decrease in agricultural yield and concomitant increase in economical loss. Since plants are directly exposed to boron in the soil, many of the studies come from the plant systems. However, the yeast *Saccharomyces cerevisiae* is also one of the widely used model organisms for the characterization of boron tolerance genes in plants.

There are three ways of boron uptake and transport; passive diffusion, active transport by boron transporters, and facilitated transport by NIP channels (Tanaka and Fujiwara 2008). Boron has a very high permeability coefficient and it is an uncharged molecule so that it can easily pass the lipid bilayer. The first boron efflux transporter *BOR1* was found in *Arabidopsis* in 2002. This transporter is localized to plasma membrane and responsible for the xylem loading against concentration gradient under low boron (Takano et al. 2002). However, when plants are exposed to high amounts of boron, *BOR1* is degraded in the vacuole. *BOR1* paralogs and homologs were found in many organisms, except prokaryotes. For instance, *Arabidopsis thaliana* genome carries seven homologs of *BOR1* which are probably expressed in different tissues or have different structural properties. *YNL275W* is the only homolog of *A.thaliana BOR1* in the yeast *Saccharomyces cerevisiae*. It was found that yeast Bor1 located on the plasma membrane and responsible for the boron export (Takano et al. 2002). In addition to yeast *BOR1* gene, there are two other genes *FPS1* (glycerol channel) and *DUR3* (urea transporter) suggested to influence the boron tolerance of yeast cells (Nozawa et al. 2006). Human *BOR1* homolog is the *HsNaBC1* which stands for human Na^+-coupled borate transporter providing the borate uptake into the cell coupled with the Na^+ (Park et al. 2004). In mammals, boron is rapidly excreted in the urine therefore it will not surprising if mammals would have sophisticated systems for boron efflux. The regulation of these transporters and channels is important to avoid the deficiency or toxicity of boron.

Yeast Atr1 Function as a Boron Exporter

In a 2009 study, a yeast genomic DNA library was screened for the genes that provide resistance to yeast cells and *ATR1* isolated as a boron resistance gene (Kaya et al. 2009). The deletion of *ATR1* was shown to cause sensitivity to boron. When boron tolerance of *atr1Δ* deletion mutant was compared to the other gene deletion mutants that have been known as boron tolerance genes in yeast, such as *BOR1*, *DUR3*, and *FPS1* (Nozawa et al. 2006), *atr1Δ* mutant was found to be more sensitive to boron than other mutants. It cannot tolerate even 50 mM boric acid, whereas other mutants showed a wildtype-like growth phenotype in the presence of boric acid. However, it was also shown that overexpression of *ATR1* gene both in wild-type and *atr1Δ* mutant provided strong boron resistance to these cells. *ATR1*-overexpressing cells could grow in media containing 225 mM boric acid and this is the highest boron concentration that can be tolerated by a eukaryote.

To find out where Atr1 is localized in the cell, the researchers created an *ATR1-GFP* fusion protein. By using a confocal microscope it was shown that the fusion protein is localized specifically to the plasma membrane and vacuole.

In order to determine how Atr1 provides boron resistance to the cells, intracellular boron measurements were performed both in wild-type and *atr1Δ* mutants. The *atr1Δ* cells were found to accumulate more boron than wild-type cells; however, when *ATR1* gene was overexpressed in wild-type and *atr1Δ* cells, intracellular boron levels decreased by 25% and 47%, respectively.

It was shown that Gcn4 transcription factor is required for *ATR1* expression in response to boron. It has been also found that boron shows its toxicity especially by inhibiting protein synthesis (Uluisik et al. 2011). Gcn2 is known as a protein kinase which is activated in response to starvation and stress conditions and phosphorylates eIF2α, leading the induction of GCN4 and general amino acid control (GAAC) pathway. Boron stress induces Gcn2-dependent phosphorylation of eIF2α and inhibits general protein synthesis (Uluisik et al. 2011).

Cross-References

► Boron Stress Tolerance, YMR279c and YOR378w

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[Au(C^N^C)(IMe)]CF_3SO_3

► Gold(III), Cyclometalated Compound, Inhibition of Human DNA Topoisomerase IB

AuNPs

► Gold Nanoparticle Platform for Protein-Protein Interactions and Drug Discovery

Autoimmune Diseases as the Complication of Silicosis

► Silica, Immunological Effects

Avian Thymic Hormone

► Parvalbumin