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TRENDS

Epigenetics: an important challenge for ICP-MS in metallomics studies

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Abstract Trace metal analysis has been long regarded as one of the principle tasks in areas of chemical analysis. At the early stage of instrumental development, total concentration was assessed in a variety of samples, yielding results, among others, for environmental, biological, and clinical samples. With the power of newer analytical techniques, such as inductively coupled plasma mass spectrometry (ICP-MS), accurate quantitative results can now be obtained at ultra-trace levels not only for metals, but also for metalloids and several non-metals. Even though the importance of trace elements in many biological processes is widely accepted, the elucidation of their biological pathways, understanding specific biological functions, or possible toxicological aspects is still a challenge and a driving force to further develop analytical methodology. Over the past decades, the scientific interest has moved from total element determination to include speciation analysis, which provides quantitative information of one or more individual element species in a sample. More recently, metallomics has been introduced as a more expanded concept, in which the global role of all metal/metalloids in a given system is considered. Owing to the multi-elemental focus of metallomics research, the use of ICP-MS becomes indispensable. Furthermore, considering the biological role of metals/metalloids and the use of elements as internal or external molecular tags, epigenetics should be considered as an important emerging application for metallomics studies and approaches. Among a variety of epigenetic factors, essential nutrients, but also environmental toxins, have been shown to affect DNA methylation, modification of histone proteins, and RNA interference, all of them being implicated in cancer, cardiovascular disease, and several inherited conditions. Recent studies suggest that epigenetics may be a critical pathway by which metals produce health effects. In this Trends article, the basic epigenetic concepts are introduced, followed by the early applications of ICP-MS classified as: (i) detection of ^{31}P as a natural element tag for DNA, (ii) analysis of DNA adducts with metal-based drugs, (iii) element species as epigenetic factors.

Keywords Mass spectrometry/ICP-MS · Trace elements · Speciation

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Introduction

Trace metal and metalloid determination has been long regarded as one of the principle tasks in areas of chemical analysis. As a result of the joint research effort undertaken in different areas of life sciences, environment-related studies, and in developing analytical methodology, the importance of these elements in the living organisms has been recognized. However, the elucidation of biological pathways of heteroelements, understanding their specific biological functions, or possible toxicological aspects is still a challenge and a driving force to further develop analytical methodology.

Over the past decades, the scientific interest has moved from total element determination to include speciation analysis, which provides quantitative information on one or more individual element species in a sample [1]. More recently, metallomics has emerged as a more expanded concept, which focuses on the study of metals and metal species, their interactions, transformations, and functions in biological systems [2]. Inductively coupled plasma mass spectrometry (ICP-MS) is an important analytical tool in trace element determination, speciation, and also in metallomics studies, since it provides exceptional detection power (low parts per billion to even sub parts per trillion range), multi-elemental and isotopic capabilities, wide dynamic range (>9 orders of magnitude), high sample throughput, relatively few chemical interferences, and easy coupling with different separation techniques. Furthermore, current state-of-the-art in ICP-MS instrumentation enables ultra-trace level determination of few non-metals (P, I, Br, S) that are important in biological systems and also in environmental studies. However, the identification of unknown forms and/or ultimate confirmation of unexpected compounds observed in the sample requires the use of molecular mass spectrometric techniques; hence the combination of elemental and molecular mass spectrometries markedly enhances speciation and metallomics approaches [3].

Basic concepts and recent advances in metallomics studies have been discussed in several comprehensive reviews [3–5]. Accordingly, the term metallomics is used in reference to the entirety of metal and metalloid species within a given system. The importance of metallomics studies relies on their contribution to better understanding the specific biological effects of these elements. As already mentioned, cellular processes involving heteroelements have not been fully elucidated yet and the research field of metallomics is still being defined. Since an important aspect of metal/metalloid roles in living organisms is their participation in epigenetic events [6], we propose here that such studies would fall within the metallomics domain.

In this Trends article, basic epigenetic concepts are introduced, followed by the preliminary ICP-MS applications of potential epigenetic relevance. The selected applications are classified as: (i) detection of ^{31}P as a natural elemental tag for DNA nucleotides, (ii) analysis of DNA adducts with metal-based drugs, and (iii) element species as epigenetic factors.

Epigenetics and its association with metallomics approach

Within any living organism, the instructions on how to grow, multiply, and function are contained in genes and each organism follows these instructions. Epigenetics is the study of factors that can change the way the genes are read without changing the genetic code itself. In other words, epigenetic refers to alternate phenotypic states that are not based in genotype differences. These states are potentially reversible, yet relatively stable during cell division and also appear to be heritable [7]. Since epigenetic factors regulate the gene activity, they influence the growth and appearance of an organism. It is not surprising though that epigenetics literally means “on top of or in addition to genetics”. Epigenetic events contribute to the normal process of cell differentiation and phenotype development, but have also been implicated in cancer biology, cardiovascular disease, and several inherited conditions [8].

A primary molecular mechanism underlying epigenetics is the alteration of chromatin structure by covalent DNA modification, covalent histone modifications, and nucleosome reorganization [6]. The best characterized epigenetic changes are DNA methylation and histone acetylation (Fig. 1). As shown in Fig. 2, the above processes regulate actual structural organization of chromatin in such a way that genes are inactivated when the chromatin is condensed and they are expressed when the chromatin is opened [9]. Consequently, analytical studies focusing chemical modifications of DNA nucleotides and DNA-related proteins (without affecting gene sequence) would be of interest in the field of epigenetics.

In mammals, DNA methylation occurs almost exclusively at cytosine (methyl tag attached to carbon 5 position), which is immediately followed in the chain by guanine base (CpG dinucleotide), a combination often found in the promoter region of genes. In normal cells, promoter regions are mostly free of methylation, whereas CpG islands outside the promoter region are almost always methylated. As already mentioned, epigenetic factors may contribute to aberrant methylation, which plays a prominent role in carcinogenesis in a manner that gene-specific hypermethylation generally is

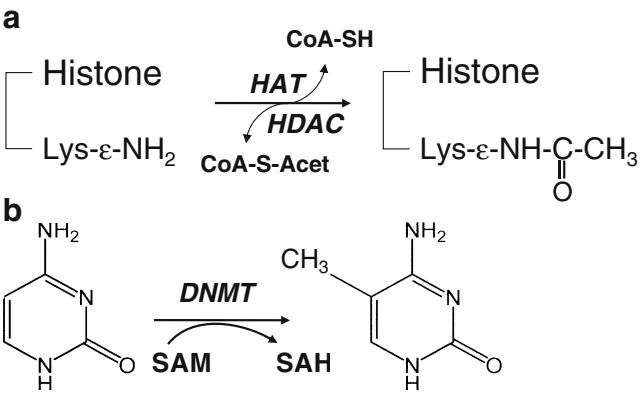

Fig. 1 Reaction schemes of **a** histone acetylation and **b** cytosine methylation (*HAT* histone acetyltransferase, *HDAC* histone deacetylase, *CoA-SH* coenzyme A, *CoA-S-Acet* acetylcoenzyme A, *DNMT* DNA methyltransferase, *SAM S*-adenosylmethionine, *SAH* S-adenosylhomocysteine)

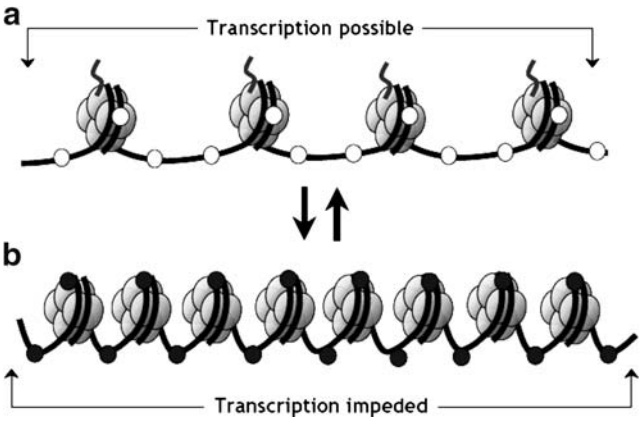

Fig. 2 Schematic of the epigenetic changes in chromatin organization that influence gene expression [14]. **a** Genes are expressed (switched on) when the chromatin is open: (○) cytosine unmethylated, (≀) histones acetylated. **b** Genes are inactivated (switched off) when the chromatin is condensed: (●) cytosine methylated, histones deacetylated

involved in deactivating tumor suppressor genes and global DNA hypomethylation leads to activation of genes important for cancer development [10, 11]. The analysis of actual DNA methylation status is gaining strength in the fields of epigenetics, cancer risk assessment, diagnosis, and therapy monitoring [12]. Regarding ICP-MS potential, phosphorus can be used as a natural elemental tag in quantitative evaluation of actual nucleotide modification status.

Perhaps the most interesting and important feature of epigenetics is the fact that, unlike the genome, epigenetic events can be modified by the environment, by diet, or by pharmacological intervention [13]. This in turn opens new possibilities for the development of therapeutic strategies, based on the modification of the epigenetic mechanism, for example, by controlling the amount of methyl group donors or by regulating the activity of DNA methyltransferases [14].

In particular, changes in DNA methylation have been associated with the exposure to some heavy metals and metalloids [6, 8]. Within this context, several elements such as arsenic, cadmium, nickel, and chromium have been recognized as epigenetic factors [15]. Histone deacetylation and aberrant methylation patterns of DNA have been observed in the presence of these elements. Furthermore, the results of epidemiological studies point to the causative role of metals/metalloids contained in dietary products and in drinking water, in epigenetic changes of DNA methylation status in the exposed populations [14, 16]. Changes in DNA methylation patterns were also reported in the presence of selenite or selenomethionine, and this effect was ascribed to a direct interaction between Se species and DNA methyltransferases. Furthermore, in vivo methylation of arsenic was shown to affect cellular levels of methylating agents that are important in regulation of DNA methylation [14]. It should be stressed that the above epigenetic studies have mainly focused the dose–response relation between exposure to the metal/metalloid chemical form and biological effects observed. The application of speciation and metallomics analytical schemes would be helpful in understanding the molecular mechanisms responsible for heteroelement-derived epigenetic effects.

ICP-MS applications of potential relevance to epigenetic studies

The exceptional features of ICP-MS make this technique an interesting, complementary tool in epigenetic studies. It has been extensively used as an element-specific detector, and a number of studies have provided analytical information of potential interest in the field of epigenetics, even though this term has not been mentioned in analytical literature yet. Thus, liquid chromatography or electrophoretic separations coupled with ICP-MS detection of phosphorus (^{31}P) have been used to evaluate different types of DNA modification [5]. ICP-MS has also been the primary analytical tool in studies focusing on the biological pathways of metal-based antitumor drugs and their specific binding to biomolecules [17–19]. Finally, the quantification of different elements and their species has been undertaken approaching their possible role as epigenetic factors [20–23].

Specific detection of phosphorus (^{31}P) as a natural elemental tag in studies focusing on characterization of DNA nucleotides

Phosphorus is a heteroatom associated with a variety of biomolecules, including nucleic acids, proteins, and lipids. Specific advantages of ICP-MS in quantification of phosphorus as an elemental tag have been summarized by Sanz-Medel et al. [24] as follows: (i) specificity to the heteroatom isotope (^{31}P), (ii) high and compound-independent sensitivity plus low detection limits, (iii) sample preparation and purity requirements comparatively low, (iv) versatility for coupling with separation techniques typically used in biological research.

The epigenetic relevance of phosphorus quantification relies on its presence in DNA nucleotides. Siethoff et al. [25] were first to explore phosphorus as a natural elemental tag for quantitative analysis of DNA adducts with styrene oxide obtained in vitro. Enzymatic digestion of DNA (Nuclease P1) was performed yielding alkylated and native monophosphorylated deoxynucleotides. The reversed-phase liquid chromatographic separation was achieved in a methanol gradient and online detection was carried out by high resolution ICP-MS and electrospray mass spectrometry (ESI-MS). Phosphoric acid was used as internal standard, and the loss of sensitivity at ^{31}P due to LC gradient was

corrected by applying a mathematical algorithm. Later, these same authors reported quantitative analysis of mephalan (a chemotherapeutic agent) adducts with DNA [26]. The determination of deoxynucleosides in calf thymus DNA digests was carried out by coupling HPLC or a capillary electrophoresis separation to ICP-MS with octopole collision/reaction cell [27]. The identification of 2′-deoxycytidine-5′-monophosphate (dCMP), 2′-deoxythymidine-5′-monophosphate (dTMP), 2′-deoxyguanosine-5′-monophosphate (dGMP), and 2′-deoxyadenosine-5′-monophosphate (dAMP) was achieved by HPLC–ESI-MS using the identical separation conditions as for HPLC–ICP-MS. More recently, ^{31}P and ^{195}Pt ICP-MS detection were used to study dAMP, dCMP, dGMP, and dTMP DNA adducts with cisplatin in experiments with the individual monophosphorylated deoxynucleotides, the customized oligonucleotide (5′-TCCGGTCC-3′), and calf thymus DNA [28]. The complex formed between pure dGMP and cisplatin, $(NH_3)_2Pt(dGMP)_2$, was structurally characterized by ESI-Q-TOF-MS.

Analysis of metal-based drugs with relevance to epigenetics

The chemotherapeutic metal-based drugs form intra- and interstrand DNA adducts that inhibit replication and transcription, leading to the induction of programmed death of rapidly dividing tumor cells. In particular, antitumor activity of cisplatin is mediated by the recognition of its DNA adducts by cellular proteins, such as repair enzymes, transcription factors, and histones [29]. A major clinical obstacle to the successful treatment of cancer is the development of cancer cells that are resistant to chemotherapeutic agents. The elucidation of mechanisms responsible for intrinsic and acquired cancer drug resistance is an actual research topic alone. According to epigenetic hypothesis, an important feature of cancer cells with acquired drug-resistant phenotypes are the changes in chromatin structure, which may be a factor contributing to enhanced DNA repair mechanisms and increased DNA damage tolerance [30].

The results of analytical speciation of metal-based drugs from in vitro and in vivo experiments are needed to evaluate their access to DNA, possible intracellular binding patterns, their effectiveness as well as the development of drug resistance [17, 18]. On the other hand, such studies might be helpful in the design of combination therapies based on the administration of a chemotherapeutic agent together with drugs targeting the epigenome in order to re-establish the gene transcription [30, 31].

Chronologically, platinum complexes were the first in use as metal-based anticancer drugs. Due to their limited spectrum, considerable toxicity, and development of drug resistance, there has been a considerable progress in the design of new formulas containing platinum and other metals (ruthenium, gallium, titanium, iron, cobalt, gold, arsenic) [18, 32]. The use of ICP-MS for the analysis of different metallodrugs has been reviewed by several authors, with emphasis on the total metal quantification in target cell fractions, the pharmacokinetics of metal complexes, and their binding to biomolecules (proteins, DNA) [17, 32, 33]

Potential of arsenic and selenium as epigenetic factors

Numerous studies link the exposure to inorganic arsenic (iAs) with the development of human cancers occurring in different types of tissue (prostate, bladder, skin, liver, lung), and there has been an increasing amount of experimental evidence pointing to the contribution of epigenetic processes [15]. The most important epigenetic events observed in exposure to inorganic arsenic have been: (i) loss of global DNA methylation, (iii) hypermethylation of DNA gene promoters, and (iii) alteration of global histone H3 methylation [13, 15, 20]. Furthermore, prenatal and early life exposure to low inorganic As levels have been shown to alter the DNA methylation pattern and to increase the risk of adverse health effects later in life [16].

As already mentioned, the specific molecular bases of arsenic action as an epigenetic factor have not been elucidated yet, but the results of analytical speciation studies provide potentially relevant information. In particular, the analytical results have contributed to better understanding of species-dependent uptake and metabolic pathways of arsenic [34]. Dopp et al. [35] investigated the cellular uptake of inorganic arsenic forms (arsenate, As(V); arsenite, As(III)) and the methylated arsenic species (monomethylarsonic acid, MMAs(V); monomethylarsonous acid, MMAs(III); dimethylarsinic acid, DMAs(V); dimethylarsinous acid,

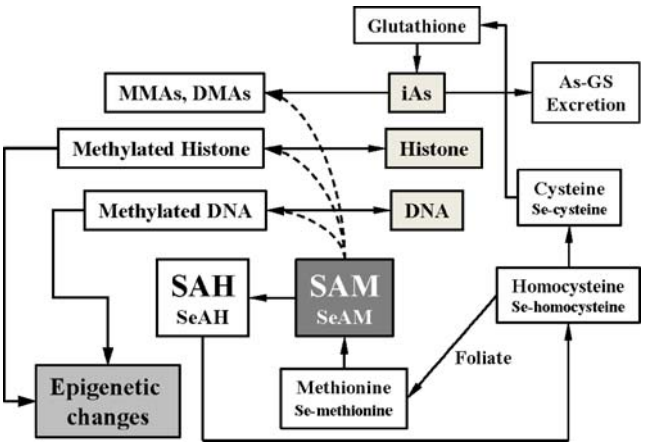

Fig. 3 Major cellular interactions of inorganic arsenic (iAs) and simplified route of selenium compounds in transmethylation reactions involved in epigenetic modifications of DNA and histones as well as arsenic metabolism [14, 15]

DMAs(III); trimethylarsenic oxide, TMAO(V)) in Chinese hamster ovary cells. Intracellular arsenic concentrations were determined by ICP-MS, and it was reported that the uptake rate for iAs and for MMAs(V) were 2% and 0.03%, respectively. On the other hand, MMA(III) and DMA(III) induced cytotoxic and genotoxic effects to a greater extent than MMA(V) or DMA(V). The use of different separation schemes combined with ICP-MS detection and structural characterization by molecular mass spectrometry enabled detection and identification of about 60 As species in a variety of biological systems [3, 34]. Valuable information relevant to As metabolism in mammals has been obtained by determination of element species in urine [3] with the methylated As species and thiol compounds of special interest [36, 37].

Figure 3 illustrates the major cellular interactions of inorganic arsenic [14, 15]. As can be observed, inorganic arsenic is metabolized by a series of reduction and methylation reactions, assisted by conjugation with glutathione and by *S*-adenosylmethionine (SAM) as methyl donor. SAM is the most important methyl donor used by a variety of methyltransferases to modify DNA, RNA, histones, and other proteins. It is regenerated from *S*-adenosylhomocysteine (SAH) via the methionine cycle. Intracellular SAM levels can be affected by dietary intake of methionine, cysteine, glutathione, folate, etc. [6, 14]. It is relevant that SAH itself is a potent competitive inhibitor of most methylation reactions, and the ratio of SAM/SAH is often referred to as the methylation capacity: the lower the value of this parameter, the higher the methylation capacity of SAM in a given system [23]. From the point of view of epigenetic effects of As, dose-dependent decrease of intracellular glutathione levels and depletion of methyl group stores have been observed and associated with aberrant DNA and histone methylation [15, 16, 20].

Selenium has received much attention both in cell biology research and in analytical chemistry, mainly because of its bioactivity in cancer prevention. In the context of epigenetics, the role of selenium in biomethylation processes appears important. It was demonstrated that Se-methionine may enter the metabolic pathway of methionine (transmethylation and polyamine synthesis) [38]. More recently, analytical speciation procedures were developed enabling the identification of Se-adenosylmethionine (SeAM) and Se-adenosylhomocysteine (SeAH), as two primary low molecular mass metabolites of Se-methionine in biological samples [23]. In the analysis of yeast extracts it was observed that the ratio between SeAM and SeAH (about 1: 20 based on the measurement of peak area) was lower than the ratio between SAM to SAH (about 1: 1), which suggests a higher methylation capacity of the Se analog [23]. Other authors observed the inverse proportion of selenium status to homocysteine levels in elderly human subjects, which means that selenium might influence methylation reactions by modifying the SAM/SAH ratio [8]. Furthermore, epidemiological studies suggest that there could be a link between global DNA hypomethylation and low selenium diet [14]. In Fig. 3, the simplified route of selenium compounds in transmethylation reactions involved in epigenetic modifications of DNA and histones as well as arsenic metabolism is marked. Future metallomics studies focused on intracellular transformations and interactions of these two elements may contribute to and advance the knowledge of their participation in epigenetic events.

Outlook

The current state-of-the-art in ICP-MS instrumentation and analytical methodology makes this technique a powerful and reliable tool to be used in different research fields that involve quantification of trace elements. In particular, metallomics has evolved in the life science as the study of the global role of metal/metalloids in a given biological system. Despite considerable progress in methodological development and real-life applications, the area of metallomics is still in the process of being fully defined. We propose here that the application of ICP-MS, as a complementary technique, to study the epigenetic events could be an interesting area of future development. Even though epigenetics has not been considered in the context of metallomics yet, several studies have focused on different types of DNA modification, specific binding of antitumor drugs to DNA and to proteins, as well as the quantification of certain elements and their species within the context of their possible role as epigenetic factors.

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