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The bacterial metallome: composition and stability with specific reference to the anaerobic bacterium *Desulfovibrio desulfuricans*

Larry L. Barton · Florence Goulhen · Mireille Bruschi · Nicole A. Woodards · Richard M. Plunkett · Frans J. M. Rietmeijer

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Abstract In bacteria, the intracellular metal content or metallome reflects the metabolic requirements of the cell. When comparing the composition of metals in phytoplankton and bacteria that make up the macronutrients and the trace elements, we have determined that the content of trace elements in both of these microorganisms is markedly similar. The trace metals consisting of transition metals plus zinc are present in a stoichometric molar formula that we have calculated to be as follows: $Fe_1Mn_{0.3}Zn_{0.26}Cu_{0.03}Co_{0.03}Mo_{0.03}$. Under conditions of routine cultivation, trace metal homeostasis may be maintained by a series of transporter systems that are energized by the cell. In specific environments where heavy metals are present at toxic levels, some bacteria have developed a detoxification strategy where the metallic ion is reduced outside of the cell. The result of this extracellular metabolism is that the bacterial metallome specific for trace metals is not disrupted. One of the microorganisms that reduces toxic metals outside of the cell is the sulfate-reducing bacterium *Desulfovibrio desulfuricans*. While *D. desulfuricans* reduces metals by enzymatic processes involving polyhemic cytochromes c_3 and hydrogenases, which are all present inside the cell; we report the presence of chain B cytochrome *c* nitrite reductase, NrfA, in the outer membrane fraction of *D. desulfuricans* ATCC 27774 and discuss its activity as a metal reductase.

Keywords Metallome · Chromium · Trace metals · Transition metals

L. L. Barton (✉) · N. A. Woodards · R. M. Plunkett
Department of Biology, Laboratory of Microbial Chemistry, University of New Mexico, MSC03 2020, Albuquerque, NM 87131-0001, USA
e-mail: lbarton@unm.edu

N. A. Woodards
e-mail: woodards8@hotmail.com

R. M. Plunkett
e-mail: richard_plunkett@sfu.ca

F. Goulhen · M. Bruschi
Institut de Biologie Structurale et Microbiologie, Unité de Bioénergétique et Ingénierie des Protéines, CNRS, 31 chemin Joseph Aiguier, Marseille cedex 20 13402, France

F. Goulhen
e-mail: goulhen@ibsm.cnrs-mrs.fr

M. Bruschi
e-mail: bruschi@ibsm.cnrs-mrs.fr

F. J. M. Rietmeijer
Department of Earth and Planetary Sciences, University of New Mexico, Albuquerque, NM 87131-0001, USA
e-mail: fransjmr@unm.edu

Introduction

Microbial mineral formation and dissolution converged to produce a new field of research: the bacterial-metal interactions or geomicrobiology. The research on microorganisms-metal interactions provides the basis of improved models of metal cycling and of its environmental impact. The composition of microorganisms has been described to reflect the materials present and these include in particular the genome, the proteome and the metallome (Fraústo da Silva and Williams 2001). While the genome and proteome refer to the DNA content and the protein content in cells, respectively, the metallome refers to the presence of metallic elements in the cell. The bacterial metallome consists of metals required in appreciable quantities as well as those metals required in trace amounts. In this paper, we discuss essential metals as macronutrients (K, Mg, Ca, and Na) and trace metals (the transition metals plus Zn).

Essential metals

K^+ and Na^+ are known as alkali metals that have distinct biological activities and do not bind strongly to ligands but remain highly mobile in the cell. The binding of K^+ and Na^+ to oxygen-donor ligands is size specific with a smaller binding cavity required for Na^+ than for K^+ because Na^+ has a smaller hydrated ionic radius than K^+. Improper size of cavity for binding of these cations results in either a weak interaction or no binding. In most bacteria, K^+ has an intracellular concentration of about 250 mM and some of its activities are associated with contributing to osmotic balance or maintaining a constant intracellular pH level. Na^+ rarely accumulates inside cells but is, never the less, an important ion with significant roles in cell energetic and solute transport.

Mg^{+2} and Ca^{+2} are alkaline earth metals that have highly specific activity in cellular systems. For nonspore-forming bacteria, intracellular concentrations of Ca^{2+} are generally limited (0.1–0.09 μM). On the other hand for all bacteria, the intracellular concentration of Mg^{2+} is appreciable (10–20 mM) (Barton 2005; Hughes and Pool 1998). The interaction between Ca^{2+} and an organic constituent containing an oxygen ligand produces an insoluble compound especially at high concentrations of Ca^{2+} where the conditions are neutral pH or slightly alkaline environment. Neutralization of negative charged phosphate groups on ATP is attributed to Mg^{2+} but not to Ca^{2+}. Mg^{2+}, as a hard acid, readily dissociates to permit phosphate transfer from ATP to an appropriate substrate. Ca^{2+}, classified as intermediately hard acid, will dissociate from organophosphates at a markedly slower rate than Mg^{2+} and, therefore, if Ca^{2+} binds to organophosphates instead of Mg^{2+} the rate of metabolism would be reduced. In a few enzymes, Ca^{2+} functions as metal cofactor because it binds tightly into the enzyme to produce the appropriate structure for activity. Membrane stability is attributed to the binding attributed to alkaline earth metals. While the interior of the membrane structure is lipophilic, the inner and outer surfaces contain hydrophilic phosphate molecules. Metals stabilize cell membranes and principally, Mg^{+2} and Ca^{+2} are found to neutralize the negative charges on ionized phosphate molecules on the cell membrane surface. The only metal that has a large variation in algae especially is calcium because in some marine algae, calcium may be deposited in the cell wall. For this reason, research methods exclude calcium in the hard structures when calculating the values for metals in algal cells.

Trace metals

The trace metal content in cells consists of Zn (a nonredox active metal) and Fe, Mn, Co, Ni, Cu, Mo, W, and V (the redox active metals). These trace metals, in contrast to the alkali metals and alkaline earth metals are not free ions inside the cell but are bound into specific sites of proteins, enzymes and related compounds. Due to the toxicity of trace metals at high levels, it is of paramount importance that metal homeostasis be maintained and this is accomplished through a series of highly regulated import and export transporter systems. Bacteria growing in environments where mineralization activities are high

and where toxic metals exist avoid intracellular accumulation of metals by a series of highly regulated exporter systems (Silver and Phung 1996).

Elemental composition of microbial cells

An important correlation can be constructed using the elemental composition in cells which reflects their fundamental metabolic activities. The mole content of major nutrients in phytoplankton with respect to phosphorus content is described as the Redfield stoichometry and this expression has been especially useful when assessing limiting nutrients for growth of organisms in the environment. From the analysis of marine eukaryotic phytoplankton, the elemental composition for marine phytoplankton has been reported (Ho et al. 2003) and they have expressed it as follows:

$$(C_{124}N_{16}P_1S_{1.3}K_{1.7}Mg_{0.56}Ca_{0.5})_{1000}\,Sr_{5.0}\,Fe_{7.5}Mn_{4.0}Zn_{0.8}Cu_{0.38}Co_{0.19}Cd_{0.21}Mo_{0.03}$$

This evaluation did not include the presence of hydrogen or oxygen in these algae. Ho et al. (2003) indicate that for most elements this average stochiometry remains relatively constant for these 15 algal species examined. The molar ratio of elements in phytoplankton and bacteria is given in Table 1 with P as the element for comparison. From the analysis of the composition of bacterial and algal cells, it is apparent that trace metals account for a fraction of the total biological cell mass. However, metals are essential for cells to carry out enzymatic activities for catabolism, biosynthesis, energetics, maintaining charges on the membranes and managing intracellular buffering activity.

The bacterial metallome

The trace metals and their oxidation states for bacteria are similar to those required by other biological systems and include Zn, Ni, Co, Cu, Mo, Fe, Mn, V, and W. Of these, V and W are associated with only a few enzymes and their

Table 1 Stochiometric formula for cells based on moles present in cell with comparisons make to phosphorus

Element	(g/100 g dry weight)		Element abundance compared to moles of P	
	Algae[a]	Bacteria[b]	Algae	Bacteria
Non-metallome				
C	33.6	50	108	41
H	7.2	8	277	78
O	48.3	20	118	12
N	6.2	50	17	34
P	0.8	3.2	1	1
S	1.1	1.1	1.3	0.3
Metallome				
Essential metals				
K	1.81	1.7	1.78	0.42
Ca	0.55	0.1	0.53	0.02
Mg	0.35	0.25	0.54	0.1
Na	–	–	–	–
Trace metals				
Fe	0.011	0.015	0.0075	0.003
Mn	0.006	0.005	0.0042	0.001
Zn	0.001	0.005	0.0006	0.0008
Cu	0.0007	0.001	0.0004	0.0001
Ni	–	–	–	–
Mo	0.0001	0.001	0.00004	0.00001
Co	0.0003	0.001	0.0002	0.00002

Data from the following references:

[a] Ho et al. (2003) and Strumm and Morgan (1996)

[b] Barton (2005) and Hughes and Poole (1998)

requirement may be highly dependent on environmental conditions by specific bacterial species. On the other hand, iron is required by all bacteria, except the lactobacilli, and the concentration of iron in bacterial cells is at the level of 10–100 ηM. Free intracellular iron would be expected to be low because this metal is bound into specific sites on the proteins (Wackett et al. 1989) and the toxicity of free cationic iron (Hughes and Poole 1998) is well known. In bacteria, the intracellular concentrations of trace metal ions are expected to be very low and Outten and O'Halloran (2001) have established that for zinc there is no intracellular pool of free cationic Zn.

The origin of life is connected to the ability of iron, the most abundant metal in the earth's crust, to readily cycle between Fe(III) and Fe(II) states. One of the first geochemical signals of earth life is the conversion of Fe(II) dissolved in the archaeon seas to deposits of Fe(III) oxides. The microbial

dissimilatory Fe(III) reduction is considered as the early form of microbial respiration and Fe(III) reduction has been highly conserved during evolution. We propose that the expression of the trace metal ratio in cells is to use iron as the basis of comparison. This selection of iron is based on the consideration that iron is present in cells for the synthesis of heme in cytochrome and the presence of iron in iron-sulfur clusters in redox active proteins. Cysteine is the amino acid binding heme to the cytochrome protein in *c*-type cytochromes and to iron in the Fe-S clusters. A recent genomic analysis by Bragg et al. (2005) reveals that the abundance of genes coding for cysteine is similar for the various bacterial species; thereby, suggesting that the abundance of potential metal binding amino acids is conserved throughout the bacteria.

Due to the ubiquitous presence of iron in microorganisms and the involvement of iron in cellular energetics, we suggest that iron should be the reference metal for trace elements. When comparing molar abundance of trace metals the following stoichometric formula for trace metals in bacteria is as follows:

$$Fe_1Mn_{0.3}Zn_{0.26}Cu_{0.03}Co_{0.03}Mo_{0.03}$$

The abundance of the trace metals in bacteria with respect to iron is similar to that found in algae, see Table 2, and is consistent with the elemental analysis by Outten and O'Halloran (2001). This consistency across species reflects a specific requirement of the trace metals in the metabolism and suggests that even with differences in cellular activities due to variations in environmental settings there are only minimal changes in trace metal content. Not only does this reflect the relative use of these metals by organisms for common physiological processes but also implies that the intracellular metallome is highly regulated.

Table 2 Expression of stochiometric molar abundance for cellular metallome based on iron concentration

	Algae[a]	Bacteria[b]
Essential metals		
K	230	143
Ca	69	7
Mg	71	33
Trace metals		
Fe	1	1
Mn	0.53	0.3
Zn	0.08	0.26
Cu	0.05	0.03
Co	0.03	0.03
Mo	0.005	0.03

Calculations are from data in the following references:

[a] Ho et al. (2003) and Strumm and Morgan (1996)

[b] Barton (2005) and Hughes and Poole (1989)

Homeostasis of the *Desulfovibrio* metallome

Over the years numerous studies have provided an understanding of the transporter systems in bacteria for transition metals. The homeostasis of Ca, Mg, Zn, Cu, Mn, Ni, Mo, and Fe in bacteria is attributed to a balance between uptake transporters and exporters (Hantke 2001; Cellier 2001; Solioz 2001; Eitinger 2001; Pau et al. 1997; Smith 1995; Braun and Hantke 2001). While most of the metal transport systems have been studied in aerobic bacteria, metal transport systems would be expected to be present in anaerobes also. Several of these transporter systems would appear to be present in the genome of *D. vulgaris* and of *D. desulfuricans*, see Table 3. While the transporter proteins to maintain appropriate intracellular concentration of trace metals are known, the sensory systems that regulate these transport activities remain to be established.

To prevent intracellular iron toxicity, bacteria utilize specific uptake systems for the transport and storage of iron. As presented in Table 4, *Desulfovibrio* have a high affinity system iron transport system (feoB) and it may be similar to that reported for other bacteria (Cartron et al. 2006). This ferrous uptake system would enable sulfate-reducing bacteria to acquire iron in an environment where most of the iron is precipitated as FeS. *Desulfovibrio* and other sulfate-reducing bacteria have considerable demand for iron because they have numerous multiheme containing cytochromes. The putative genes for both ferritin and bacterioferritin are present in both *D. desulfuricans* and *D. vulgaris*; however, when isolated, the bacterioferritin protein is

Table 3 Putative genes in *Desulfovibrio* associated with trace element metallome[a]

Element transported	Common name (Gene symbol)	Gene locus *D. vulgaris* Hildenborough	Gene locus *D. desulfuricans* G20
Cobalt	Magnesium and cobalt transport CorA (corA)		NT01DS0450
	Cobalt ABC transporter, permease protein CbiQ (cbiQ)	DVU_1057	NT01DS1249
	Cobalt ABC transporter, ATP- binding protein, putative	DVU_1056 DVU_2888	NT01DS1250
Copper	Copper-transporting ATPase 2		NT01DS2439
Nickel	Peptide/opine/nickel uptake family ABC transporter, periplasmic substrate-binding protein, putative		NT01DS0383
	ATPase component ABC-type didpetide/olgiopeptide/nickel transport system		NT01DS3823
Manganese	Mn^{2+}/Zn^{2+} ABC transporter, permease protein		NT01DS2267
	Molybdate ABC transporter, periplasmic molybdate-binding protein (modA)	DVU_0177	NT01DS0150 NT01DS3568
	Molybdenum ABC transporter, permease protein (modB)	DVU_0181	NT01DS0149 NT01DS3569
	Molybdenum ABC transporter, ATP-binding protein (modC)	DVU_0180	
Zinc	Zinc transporter (zupT)	DVU_A0136	NT01DS3605
	ZIP zinc transporter family protein	DVU_0079	

[a] From The Institute for Genomic Research Comprehensive Microbial Database at www.tigr.org

Table 4 Putative genes in *Desulfovibrio* associated with iron transport and storage[a]

Activity	Common name (Gene symbol)	Gene locus *D. vulgaris* Hildenborough	Gene locus *D. desulfuricans* G20
Iron	Ferrous iron transport protein B		NT01DS1661
	Ferrous iron transport protein B (feoB)	DVU_2571	NT01DS2749
	Ferrous iron transport protein A, putative	DVU_2572	NT01DS1662
	Ferrous iron transport protein, putative	DVU_2574	NT01DS2753
Ferritin	Ferritin (ftnA)	DVU_1568	NT01DS1851
Bacterioferritin	Bacterioferritin (bfr)	DVU_1397	NT01DS0128
	Bacterioferritin comigratory protein, putative	DVU_0814	NT01DS1067

[a] From The Institute for Genomic Research Comprehensive Microbial Database at www.tigr.org

devoid of iron (Romao et al. 2000). Perhaps the Fe(II) pools in *D. desulfuricans* are established by binding activity of a phosphorylated sugar derivative previously found in *Escherichia coli* (Bohnke and Matzanke 1995). Not only is it important to learn how this iron homeostasis is maintained in *Desulfovibrio* but also the mechanisms of coordinating trace metal homeostasis with other elemental requirements must be examined.

Response to toxic metals

Metals can support growth of various microorganisms by serving as either electron donors or electron acceptors. In *Geobacter, Desulfuromonas* and several other chemolithotrophic bacteria, growth is coupled to the reduction of Fe(III) or Mn(IV). In some bacteria, Se and As can be used to support growth but other heavy oxidized metals are toxic and lethal for bacteria. Several species of anaerobic bacteria can grow in environments containing toxic levels of oxyanions of various metals and this would suggest that these bacteria have acquired a detoxification strategy that would be associated with trace metal homeostasis.

Several mechanisms of metal reduction for detoxification of oxidized soluble metals by anaerobic bacteria have been suggested. Under

appropriate conditions cationic metals readily bind to acidic capsular material of bacteria (Volesky 1990); however, bacterial structures rarely have positive charges needed for binding of metals that are oxyanions. Recently, there has been the observation that small hair-like structures termed 'nanowires' conduct electrons from the surface of the bacterial cell into the medium (Reguera et al. 2005) and while this report would be another example of extracellular metabolism, direct evidence for reduction of soluble toxic anions remains to be established. Although numerous possibilities for metal reduction have been raised, there have been few specifics to support the system of metallome homeostasis in bacteria in the presence of toxic metals.

Metal reduction by *Desulfovibrio*

A common means of detoxifying the environment from redox metals is to employ bacteria to reduce the metal ion and the resulting product has low solubility and, therefore, reduced toxicity. The metal reduction appears to be a detoxification strategy that helps the bacteria to keep the environment favorable for growth and, additionally, may contribute to the lowering of the redox potential of the medium. Recent reviews of metal and metalloid reduction by *Desulfovibrio, Shewanella, Geobacter* and *Cupriavidus* enumerates Fe, Mn, Ag, V, Cr, Co, Cu, Pd, Tc, Mo, Re, Au, As, Se, Te, and Pu as electron acceptors in dissimilatory reduction even though most of these elements do not support bacterial growth (Barton et al. 2003; Ledrich et al. 2005). Extracellular accumulation of reduced Mo, Se, U, Cr and Re have been studied in *D. desulfuricans* (Xu et al. 2000; Tucker et al. 1996, 1997, 1998; Tomei et al. 1995).

Another proposal for the metabolism of toxic cationic metals is the reduction of internalized metals followed by export through the general secretion pathway (Marshall et al. 2004). As revealed from examination of the published genomes, several different metabolic systems for chromate and arsenic are present in *D. vulgaris* and *D. desulfuricans* (Table 5). These systems merit evaluation because anaerobic bacteria may be found in areas where metal oxides are present. Unlike metabolic systems where each substrate has a specific enzyme, various different proteins have been implicated in metal reduction. Recently, Goulhen et al. (2006) used microscopy analysis and Energy Electron Loss Spectroscopy (EELS) to demonstrate that when *D. vulgaris* Hildenborough is stressed by high levels of chromate (CrVI), chromium (CrIII) phosphate accumulates in the extracellular environment and on the membrane surfaces. These findings imply that under specific conditions soluble chromate enters the *Desulfovibrio* cells where it is reduced before being exported by an unknown system. In the *Pseudomonas fluorescens* system, it has been demonstrated that chromate will enter cells on the sulfate transporter system (Ohtake et al. 1987). As reviewed by Bruschi et al. (2006), the reduction of Mn(IV), Fe(III), Cr(VI), Tc(VII), Pd(II), U(VI), and Se(VI) has been attributed to various molecular forms of *c*-type cytochrome, hydrogenase, and ferredoxin isolated from species of *Desulfovibrio* (Chardin et al. 2003; Lojou et al. 1998a, 1998b; Lloyd et al. 1998, 1999, 2001; De Luca et al. 2001; Michel et al. 2001; Lovley et al. 1993b; Lovley and Philips 1994). The diversity of molecules for metal reduction suggests that molecules containing specific motifs may function as nonspecific metal reductases. With hydrogenases and ferredoxin, metal reduction may be associated with iron-sulfur centers because they have a

Table 5 Putative genes in *Desulfovibrio* associated with toxic oxy-anion metals

Transporter activity[a]	Common name	Gene locus *D. vulgaris* Hildenborough	Gene locus *D. desulfuricans* G20
Chromate transport family protein		DVU0426 DVUA0093	
Arsenical-resistance protein	acr3		NT01DS2878

[a] From The Institute for Genomic Research Comprehensive Microbial Database at www.tigr.org

Table 6 Cytochromes reported in the outer membranes of bacteria

Organism	Cytochrome characteristics [proposed or demonstrated function]	Reference
Hildenborough	HMC: 77.5 kDa and 62.5 kDa [oxidation of Fe^0]	Van Ommen Kloeke et al. (1995)
Geobacter sulfurreducens	c-type, 41 kDa, [reduction of Fe(III)]	Gaspard et al. 1998; Lovley (2000)
Neisseria gonorrhea	c': c-type, 16 kDa [protects against NO stress]	Turner et al. (2005)
Shewanella oneidensis MR-1	OmcA: c-type, decaheme, 83 kDa OmcB: c-type, decaheme, 75.7 kDa [reduction of Mn(IV) oxide, Fe(III), and V(V) oxide]	Myers and Myers (1997, 2001, 2003) and Myers et al. (2004)
Shewanella frigidimarina	CymA: c-type tetraheme, 11.78 kDa [reduction of Fe(III)]	Gordon et al. (2000) Field et al. (2000)

low redox potential and readily donate electrons to an oxidized substrate. For cytochromes it appears that the key characteristics for metal reduction would include a heme with low (–100 mV to –400 mV), a *bis*-histidinyl coordination of heme, and a slightly positive charge on the protein around the heme to facilitate interfacing of the heme and redox metals. Studies on the cytochrome c_3 family described by Bruschi (1994) and Florens and Bruschi (1994) have demonstrated that the metal reduction activity of these multihemic proteins is linked to the low redox potential of the heme moieties of the cytochrome.

Metal stress response

Bencheikh-Latmani et al. (2005) observed a global response when *Shewanella oneidensis* MR-1 was exposed to Cr(VI) and U(VI). Using DNA microarrays, *S. oneidensis* was found to up regulate 83 genes at the time of Cr(VI) reduction and 121 genes were upregulated when U(VI) was being reduced. Genes encoding for membrane stability and electron transport were up regulated by both Cr(VI) and U(VI) while enhanced gene expression for efflux pumps were seen only with Cr(VI) stress. Down regulation of genes for general metabolism, energy production, transcription and translation were observed with both Cr(VI) and U(VI) exposure. Up regulation of Fe(III) citrate reduction was confirmed through the use of mutants. The increased production of activity for the heavy metal efflux pump, CzcA gene family, was seen only as a response to Cr(VI) stress.

When *D. vulgaris* Hildenborough was exposed to Cr(VI) and examined by transcriptional analysis, the expression of 337 genes was modified (Wall et al. 2006). About half of the genes upregulated and the other half were diminished in expression. It could not be determined if there was a universal stress protein family functioning in *D. vulgaris*, but increased Fur regulon was suggested to occur with chemical stress response in *D. vulgaris*. These experiments suggest an active metabolic response by growing bacteria stressed by Cr(VI) or U(VI) but a single protein function as a metal reductase was not revealed.

Cell surface electron transfer in bacteria

There are numerous minerals metabolized by bacteria involving electron donor or electron acceptor activity with mineral formation and dissolution frequently requires the interaction of bacterial cells with insoluble materials (Ehrlich 1996). Cytochromes associated with the membrane may contribute to the extracellular reduction of metals and several reports are listed in Table 6. For electron flow coupled to bacterial respiration with Fe(III) and Mn(IV), it is well established that polyheme cytochrome *c* in the outer membrane is important in *Shewanella* and *Geobacter*. Silver resistance in *Cupriavidus metallidurans* is attributed to a mega plasmid and while these cells deposit elemental silver in the outer membranes (Ledrich et al. 2005), it remains to be establish if silver reduction is attributed to the presence of cytochromes.

Another demonstration of cell surface electron transfer is with the bacteria involved with the biological fuel cell systems. Electrochemically active bacteria transfer electrons from their cell surface directly to an electrode without a mediator chemical. It is suggested that the source of electrons for this process is the bacterial electron transport system and the specific mechanism for this transfer would involve electron carriers on the cell surface. In the biofuel system, the donor bacteria are placed in an anaerobic chamber and electrons are transferred to the anode. Electron flow in a closed circuit proceeds from the anode to the cathode. In the case of *Shewanella oneidensis* (Kim et al. 1999, 2002) and *Geobacter sulfurreducens*, (Bond and Lovley 2003) electron flow from the bacterial cell could be mediated by cytochrome *c* in the outer membrane. However, with *Aeromonas hydrophila, Clostridium butyricum, Desulfobulbus propionicus*, *Enterococcus gallinarum* and *Rhodoferax ferrireducens* the mediator of electron transfer from the cell surface to the anode remains to be characterized (Chang et al. 2006). Thus, the flow of electrons at the surface of bacterial cells would appear to occur in numerous unrelated bacterial species.

Cytochrome isolated with the outer membrane of *Desulfovibrio*

In the biocorrosion of ferrous metals by *Desulfovibrio*, it has also been proposed that electrons flow from the metallic surface to the cell (Laishley and Bryant 2003). In fact, a cytochrome was reported to be present in the outer membrane of *D. vulgaris* Hildenborough by Van Ommen Kloeke et al. (1995). Their research provided an impetus for us to extend the implications of cytochrome *c* in the outer membrane of *D. desulfuricans* to facilitate metal reduction.

D. desulfuricans ATCC 27774 was grown in a lactate-nitrate medium and outer membranes were prepared according to the procedure described by Van Ommen Kloeke et al. (1995). When the outer membrane fraction was reduced with dithionate, spectral peaks characteristic of cytochrome *c* were observed (Fig. 1). Proteins in this outer membrane fraction were solubilized in

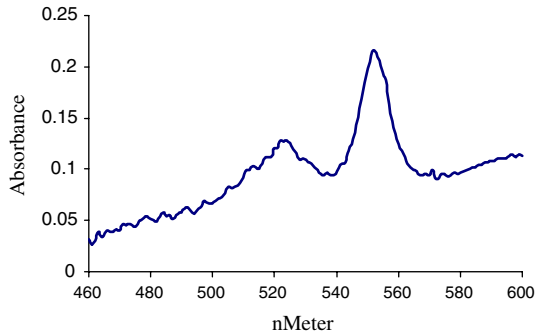

Fig. 1 Difference spectra of outer membrane fraction from *D. desulfuricans*. Peaks at 555 and 525 nm are characteristic of cytochrome *c*. The 1 ml cuvette contained 0.13 mg of protein

0.6% sodium deoxycholate and were separated on SDS-PAGE. We detected several protein bands and these are presented in Fig. 2. Using a specific reagent for the specific coloration of proteins containing hemes, two bands were

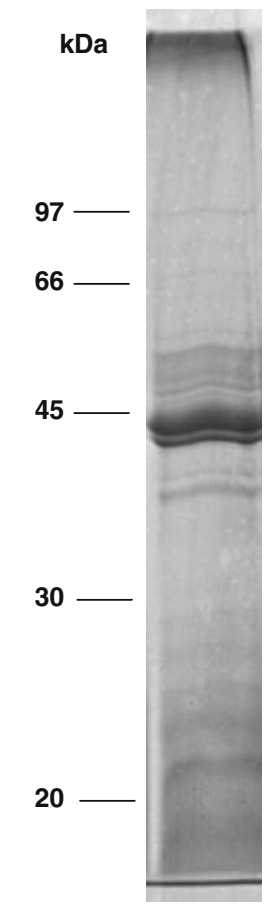

Fig. 2 Membrane protein profile of *Desulfovibrio desulfuricans* strain 27774 by 10% SDS PAGE and coomassie blue staining

apparent: one of 50–60 kDa and another of more than 200 kDa since it is localized in the stacking gel. Using proteomic analysis of these two bands, we have demonstrated that the protein band of 50–60 kDa contains chain B cytochrome *c* nitrite reductase, NrfA, from *D. desulfuricans* (accession number 31615784, score 100.4, coverage sequence 14.9%). Additionally, there was homology of this protein with chain D [NiFe] hydrogenase characteristic of *D. desulfuricans* (accession number 14719753, score 30.1, coverage sequence 7.6%). The cytochrome *c* nitrite reductase of *D. desulfuricans* is an heterooligomeric complex of two subunits of 61 kDa containing 5hemes and 19 kDa containing 4 hemes (Almeida et al. 2003; Rodrigues et al. 2006, Moura et al. 2006). The multihaem nitrite reductase is associated with the dissimilative ammonification process catalyzing the reduction of nitrite to ammonia, and is located in the periplasmic side of the plasma membrane (Moura et al. 2006). By analogy, nitrite reductase is characterized by a large content of low potential hemes and this enzyme is located also in the region of the membranes. We propose that NrfA is isolated along with the outer membrane of *D. desulfuricans* and could be responsible for metal reductase activity. Since several other anaerobic bacteria have nitrite reductase in the periplasm, this enzyme may account for metal reduction by other bacteria growing in anaerobic environments.

Concluding remarks

Iron and other transition metals are required by biological systems and the cellular concentrations of these trace metals appear to be similar for plants, algae and bacteria. Two different mechanisms (e.g. metal uptake and metal transport) must be working in concert to maintain the optimal concentration for cellular metabolism and growth. Many of the features associated with homeostasis of the bacterial metallome would be found in chloroplasts and other biological systems (Shcolnick and Keren 2006). Several bacterial species grow in the presence of toxic metals and it would be expected that these toxic metal ions would provide considerable stress on metal homeostasis because toxic ions may mimic the structure of essential metal ions and enter the metabolizing cell (Hughes and Poole 1998). A mechanism for reduction of redox active toxic metals has evolved in bacteria to exclude metal ions from the cytoplasmic region of the cell by reduction of the metal at the surface of the bacterial cell. The consequence of this activity is avoiding perturbation of the metallome. Several different bacterial species have been reported to have cytochromes in the outer membrane or near the surface of the cells and these cytochromes function as nonspecific metal reductases. Chromium may be one of the elements driving the evolution of toxic stress response to metals because chromium is in high abundance in meteoric infall (Anders and Grevesse 1989). We report that a heme protein that can be isolated with the outer membrane fraction of *D. desulfuricans* is NrfA, a subunit of nitrite reductase. Many of the bacteria reported to contain cytochrome in the outer membrane also have the metabolic capability of reducing nitrite. Thus, in future experiments, it will be important to learn if the nitrite reductase subunit, NrfA, is capable of functioning as a general metal reductase. This respiratory enzyme may be critical for homeostasis of trace elements in bacteria and important in metal stress response in anaerobic bacteria.

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