Biotransformations of Toxic Metals

Stuart Strand

Background

Metals in the environment predate the biosphere. Volcanic activity brings metals to theg

surface in high enough concentration to be toxic to microorganisms. Microbes have evolved various systems to detoxify, resist, or avoid toxic metals.

Metals may serve as electron donors or acceptors. Metal redox pairs (reduced/oxidized) include

Ferrous/Ferric iron (Fe^{+2}/FE^{3+})

Elemental/Mercuric mercury (Hg^{0}/Hg^{+2})

Arsenite/Arsenate (As^{+3}/As^{+5})

Chromate/Dichromate (Cr^{3+}/Cr^{+6}, CrO_4^{2-}/$Cr2O_7^{2-}$)

Selenate/Selenite (Se^{+6}/Se^{+4}) reduced beyond (to insol. red ppt organoselenium)

Mercury

Mercuric Reductase: Most studied of all metallic transformations is the action of the mercuric reductase:

Elemental mercury is volatile and thus escapes from the aquatic environment. Note that mercury in the environment is increasing, probably due to anthropogenic processes, including reduction of mercury deposited in anaerobic aquatic environments.[1]

Mercuric reductase has been isolated from several bacteria. It is an FAD-containing flavoprotein, requiring an excess of sulfhydryl reagents for activity *in vitro*. NADPH is preferred, but NADH can also serve as the electron donor. Evidence exists for reductases as monomers, dimers, trimers, tetramers, and octamers, but these forms are apparently related forms of the same enzymes. Mercuric reductase has significant structural homology to mammalian and bacterial glutathione reductases, including the FAD and NADPH binding regions and the active sites. A mercuric reductase system (not glutathione reductase) exists in mammalian tissue, but Hg^0 is usually reoxidized by catalase.

Mercuric ion resistance is typically carried on extrachromosomal plasmids in many species, including *Thiobacillus ferrooxidans, Caulobacter*. The ability to select bacterial strains by their ability to grow on media treated with mercury has greatly facilitated study. The mercuric resistance operon consists of a regulatory gene *merR*, followed by the binding site of the *merR* regulatory protein. The system is off (repressed) in the absence of the *merR* regulatory protein or Hg^{+2}. Four structural genes are expressed when operon is on: *merT* (a transport protein), *merC* (cryptic), *merA* (subunit of mercuric reductase), and *merD* (cryptic). The *merT* codes for a highly hydrophobic polypeptide with two cysteine pairs that are probably involved in binding and passage of Hg^{+2} across the cell membrane.

In heavily polluted sites, other metal ions may inhibit mercuric reductases: Ag^+, Cu^{+2}, and Cd^{+2}. Small amounts of colloidal elemental gold and silver produced by resistant cells induced for mercuric reductase.

Organomercurial lyase: Some Hg^{+2}-resistant bacteria can also detoxify the highly toxic organomercurial compounds. This activity is always plasmid encoded, due to organomercurial lyase, a soluble enzyme which cleaves the Hg-C bond forming Hg^{+2} under reducing conditions.

First isolated from *Pseudomonas*. The organomercurial lyases differ in their specificity for the various organomercurials. Zn^{2+}, Cu^{2+}, Cu^+, and Hg^{2+} inhibited lyase activity. Strong product inhibition by Hg^{+2}.

Oxidation of Hg^0 and mercury methylation: The oxidation of Hg^0 to Hg^{+2} increases its toxicity. This probably occurs due to the activity of the widespread catalase enzyme. Demonstrated as the mechanism for the mammalian toxicity of the non-toxic Hg^0 vapor (oxidation by catalase in the blood to Hg^{+2}).

Bacterial methylation of mercury is common in sediments. Methyl mercury is the dominant form of mercury in fish and human tissue. Some methylation may be due to the action of methylcobalamin (vitamin B_{12}) which is synthesized by cells but which does not require live cells to be active.

Methylation of mercury in sediments is inhibited by molybdate, a sulfate reducer inhibitor, but not by 2-bromoethanosulfonic acid (BESA), an inhibitor of methanogenesis.[2]

Applications: A bench-scale system was used to reduce Hg^{+2} to volatile Hg^0 at concentrations up to 70 mg/L. The acclimated system was dominated by *Pseudomonas,* although *E. coli* strain was used as the inoculum. However, without the inoculum there was little Hg reduction.

Arsenic

Trivalent arsenical ions (arsenite, As^{+3}) are hundreds of times more toxic than pentavalent ions (arsenate, As^{+5}). Oxidation may be a useful detoxification mechanism. Arsenate can be precipitated frm water using $FeCl_3$.

As^{+3}-resistant *Bacillus* and *Alcaligenes* strains have been isolated. The **arsenite oxidase** appears to be a soluble enzyme, without heme or flavin, but with Fe and acid-labile sulfur. Oxygen is the terminal electron acceptor.

The dominant inorganic arsenic species in neutral waters are $As(OH)_3$ for arsenite and $H_2AsO_4^-$ and $HAsO_4^{2-}$. Other As compounds include arsine (AsH_3), monomethylarsonate ($CH_3AsO_2OH^-$), dimethylarsenate ($(CH_3)_2AsOO^-$), dimethylarsine ($(CH_3)_2AsH$), trimethylarsine oxide ($(CH_3)_3AsO$), and trimethylarsine ($(CH_3)_3As$). Some complex organics also observed in cultures, including arsenobetaines, cholines, ribosides, and phospholipids.

Methylated arsenic species are present in lake and estuarine waters, comprising a significant percentage of total As. These compounds are apparently produced by various bacteria, fungi, and algal species. Dimethylarsinic acid found to dominate in the upper lake water late in the growing season.[3]

Chromium

Chromates (CrO_4^{2-}) and dichromates ($Cr_2O_7^{2-}$) contain Cr^{+6} and are very toxic with evidence for carcinogenicity in animals. Cr^{3+} compounds are 1000x less toxic and also less, so reduction of chromates is of interest.

Chromate-resistant *Pseudomonas fluorescens* strain is able to reduce chromate by action of a constitutive, membrane-associated, metallo-enzyme. The reaction requires NADH. A Pseudomonas utida uses a soluble chromate reductase.[4]

Selenium

Selenium is toxic to wildlife, accumulates in irrigation waters due to salt accumulation. The soluble species, selenate (SeO_4^{2-}, Se^{+6}), which is highly mobile and toxic, can be reduced to selenite (SeO_3^{2-}, Se^{+4}), which is strongly adsorbed, or to organoselenium species (such as dimethylselenium), which may be volatilized. The most reduced form of selenium is selenide
(Se^{-2}), a gas, highly toxic, rapidly oxidized.

Biotransformation of selenate by reduction beyond selenite to an insoluble, red precipitate organoselenium (valence between zero and 4).[5]

Se-oxyanions (SeO_4^{2-}, SeO_3^{2-}) were reduced by dissimilatory mechanisms at surface of sediment. SeO_4^{2-} reduction increased by adding H_2 or acetate. Inhibited by adding O_2, NO_3^-, or MnO_2, but not inhibited by sulfate, Fe^{+3}, tungstate, chromate, or MoO_4.[6]

Pseudomonas sp AX isolate respires selenate and selenite to insoluble selenium anaerobically. Another isolate is an obligate anaerobe (strain E) that produces elemental Se, stain E.[7]

An anaerobic, freshwater enrichment grew with either nitrate or selenate as an electron acceptor. Selenate reduction repressed by presence of nitrate. An isolate from the enrichment grew on either ion, but the presence of nitrate prevented selenate reduction.[8]

Selenium methylation in pond water was inhibited by bactericides, but not by fungicides. Adding carbon substrate and aerobic conditions increased Se methylation. SeO_{32-} and SeO_{42-} were reduced equally. NO_{3-} and NO_{2-} inhibited dimethylselenium release.[9]

Uranium

The mobile form of uranium, U^{6+} or U(VI), is reduced to the insoluble form, U^{4+} or U(IV), by sulfate-reducing *Desulfovibrio desulfuricans*. Reaction requires electron donor, such as lactate or hydrogen. U(IV) not further reduced, forming uraninite mineral by extracellular precipitation. No inhibition by air, sulfate, or azide. biological mechanism for accumulation of U(IV) in sulfidogenic environments, previously thought to be abiological.[10]

Dissimilative U reduction by iron-reducing bacteria, *Alteromonas putrefaciens* and GS-15 isolate, which used U as e acceptor while oxidizing acetate to CO_2. Note plutonium and technitium are also insoluble in reduced form.[11]

Adsorption of metals into biomass

Cells of a *Citrobacter* sp took up cadmium due to action of cell phosphatase. Cells were immobilized in a polyacrylamide gel. Required pH to be greater than 6.5. Problems with trace CN, which complexed Cd^{2+}, preventing precipitation.[12]

Cd^{2+}, Ag^{+}, Cu^{2+}, La^{3+} were sorbed from solution by four bacteria strains in batch cultures. Ag^{+} removed best, 89% by all bacteria, precipitated as discrete particles, collected at the cell surface and in cytoplasm. *E. coli* best at Cd^{2+} removal (only 12%), *B. subtilis* best for Cu^{2+} (29%), La^{3+} removed 27% (ppt as needle crystals). All metals were present at 1 mM.[13]

Organic matter itself can sorb heavy metals. Soil columns treated with digested sewage sludge were incubated for five months. Organically-bound metals were extracted with pyrophosphate following incubation. Over half of the cadmium was in the organic phase, compared to less than 20% of the lead. Sorption of lead was unaffected by the rate of sludge addition and nickel and zinc sorption decreased, but copper sorption increased with increasing sludge application. Organic complexes of copper and cadmium were stronger than for the other metals, and the binding capacity of the organic matter form metals was greatest for copper and nickel.[14]

Copper-resistant strains of *Pseudomonas syringae* carrying the cop operon produce periplasmic copper-binding proteins. *P. syringae* strain PS61 carrying the cloned cop operon accumulated excess cellular copper. Two highly-resistant species accumulated up to 115 to 120 mg of copper per g (dry weight) of cells. Several metals were accumulated by these bacteria, but when copper was added to induce the cop operon, there was generally no increase of accumulation of the other metals, suggesting that the cop operon does not contribute to their accumulation. The exceptions were aluminum for PT23 and iron for *P. putida*, which accumulated to higher levels when copper was added to the cultures.[15]

Sorbed metals are labile in acidic environments and thus may be mobilized by pH changes. *Thiobacillus ferrooxidans* has been used to acidify sludges (pH from 7.8 to 2.0) and solubilize metals. After 2 d solubilization was as follows: Cu 47-80%; Mn 81-89%; Ni 42-60%. Chromium was the only metal in this study that could not be solubilized.[16]

Tin

Tin is important in the environment due to its use as an antifouling toxicant in boat paints, wood preservative, molluscide and insecticide. Dibutyl and monobutyltin may also leach out of polyvinyl chloride plastics. It is toxic to fish and plankton and to the human population.

Tributyltin is biodegraded by a series of successive dealkylations to the inorganic tin salt:

Biodegradation proceeds to dibutyltin with a half-life of about 6-19 d, faster for polluted sites (e.g., yacht harbors). Complete mineralization to CO2 takes about 50-75 d half-life [17]

Municipal wastewater and sewage sludge are contaminated by organotins. Wastewater contained about 10 mg/L and about 7 mg/L after biotreatment. Sludge contained 0.28 to 0.83 mg/kg tributyltin, dibutyltin, and monobutyltin. No significant degradation of tributyltin in anaerobic sludge digestion.[18]

Tin has been shown to be volatilized by the reduction to stannane (SnH_4) in anaerobic environments.[19]

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Review

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