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# Selenium and Mercury

## Their Interactions and Roles in Living Organisms

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**Abstract**

The essential or toxic character of the elements depends not only on their concentration, but also on the chemical form in which they occur. This is the case of arsenobetaine, which has limited biological activity compared to the highly toxic inorganic arsenic. Some elements, however, can counteract the toxic action of others through cooperative, competitive, or availability mechanisms. A good example of this is the protective effect of some chemical forms of selenium (Se) against mercury (Hg) toxicity. The range between deficient and essential dose of selenium is nonetheless narrow, and some chemical forms of selenium are toxic (e.g., selenocystathionine, which is very abundant in *Lecythis minor* and causes hair loss).

Cadmium causes the conversion of xanthine dehydrogenase into xanthine oxidase and abnormalities in urate transporters (hyperuricemia), observed in rats under oxidative stress, but cadmium does not have redox properties. This is because cadmium replaces other metals with redox properties. Another example is that toxicity caused by the presence of arsenic in drinking water in countries like Bangladesh is increased through selenium and zinc (Zn) deficiency detected in the soil.

Clearly, the essentiality or toxic character of trace elements cannot be considered in isolation, since it can be modulated by their interaction with the particular organism (its genome), with other elements, and with biomolecules, and is dependent on dose and chemical form.

### Introduction

Metals play an essential role in the metabolism of living organisms and, in contrast to metabolites, cannot be produced or consumed through biochemical reactions. For this reason, the study of the metabolism of metals lies at an important interface between chemistry and biology. The availability of elements and their specific chemical properties are the promoter forces for the evolution

of life on Earth (Thiele and Gitlin 2008). This explains the extremophile organisms and variety of organisms that can live under different geochemical conditions. It is important to consider systems biology in relation to the metabolism of trace metals, in which the metalome is defined as "the distribution of elements, concentration at equilibrium of free metallic ions or free elements in a cellular compartment, cell or organism and refers to the identity and/or quantity of metals/metalloids and their species" (Williams 2001). The systematic study of trace metals requires information about how the organisms detect, incorporate, and use these metals (Thiele and Gitlin 2008). Metallic ions are strongly bound to proteins, which overcomes steric problems, electrostatics repulsions, and other noncovalent interactions that prevent the association with monomeric proteins. The importance of metals in biological systems is revealed by their influence on more than 50% of the proteins as well as by the fact that metalloproteins represent about 30% of the known proteins (Mounicou et al. 2009).

Metalloproteins use the singular properties of metals present in living organisms to develop their function, making life possible. In this sense, the ability of eukaryotic cells to detect and interact with metals is performed through three mechanisms: affinity, allosterism, and accessibility (Waldron and Robinson 2009). Another important factor is the lability of the metal–biomolecule link that promotes the rapid assembly and disassembly of the metal cores as well as rapid association and dissociation of substrates. In this way, metalloproteins consist of kinetically labile and thermodynamically stable units (Lippard and Berg 1994). In addition, the study of complex interactions in cells, where the viscous medium contains high concentrations of other molecules, which stimulate competitive reactions, is mandatory to understand the biological role of metalloproteins in their native environments. Moreover, the regulation of metals in cells differs considerably from that observed *in vitro*, since the metal–biomolecule union is completely modulated by biological molecular filters (Maret 2010). In this way, metallochaperones guide the metals and distribute them among the different enzymes and biomolecules, thus requiring them, at a precise moment, to develop their function and contribute to the metals traffic, homeostasis, signaling, detoxification, and metabolism (O'Halloran and Culotta 2000).

However, elements interact not only with biomolecules but also with other elements or chemical species (García-Barrera et al. 2012). In this sense, some elements or their species can counteract the action of others through cooperative, competitive, or availability mechanisms. A good example of this is the antagonistic effect of selenium on Hg toxicity, first reported in 1967 in an experiment with rats treated with mercury chloride and selenite (Parizek and Ostadalova 1967). Since living organisms are usually exposed to a complex environment in which different elements and their species are present together, these types of interactions complicate even more the panorama of

"metallomics." Thus the metabolism of trace elements cannot be considered in isolation.

## The Essentiality of Trace Elements

The classification of essential elements is not absolute because some elements historically considered to be toxic are now classified as essential, such as selenium, chromium (Cocho et al. 1998; Maret and Copsey 2012), or tungsten, which was recently added to the list of metals found in biology (Lippard and Berg 1994). In addition, certain elements have a dual character: they are either essential or toxic depending on their concentration and/or chemical form, which in turn depends on their chemical properties (i.e., selenium or chromium).

The ligands in bioinorganic chemistry are commonly amino acid side chains or constituents of nucleic acids. The coordination depends critically on the three-dimensional folding of proteins and tertiary structures of nucleic acids (Lippard and Berg 1994). Metals, however, can also be bound to prosthetic groups of metalloproteins (i.e., iron-protoporphyrin IX, magnesium-chlorophyll), bleomycin, siderophores, coenzymes (i.e., cobalamin-cobalt), and methylcobalamin. The latter can transfer a $CH_3^-$ ion to Hg, Pb, and Sn salts in aqueous solution, a biomethylation reaction that probably contributes to the toxicity of these elements. Finally, metals can be bound to complex assemblies such as cell membranes, viruses, and intracellular compartments (i.e., ribosome, the mitochondrion and endoplasmatic reticulum) (Lippard and Berg 1994). Another example is the structure defined as "zinc fingers," which is a small protein structural motif characterized by the coordination of one or more Zn ions to stabilize the fold. In this way, some elements (e.g., copper, zinc, cadmium, mercury, and silver) coordinate by proteins through a sulfur atom whereas others (e.g., molybdenum, manganese, iron, cobalt, nickel, copper, and zinc) do so through nitrogen or oxygen atoms. Metabolites of arsenic, selenium, and iodine have a metalloid-carbon covalent bond. Other elements (e.g., aluminum, nickel, and iron) coordinate by small organic ligands. Magnesium, vanadium, iron, cobalt, and nickel coordinate by tetrapyrol ligands; calcium, strontium, barium, lanthanum, and lead form complexes with polysaccharides; and, finally, platinum, ruthenium, chromium, and nickel are coordinated by nucleic acids and their constituents (Schaumlöffel et al. 2005). In selenoproteins (i.e., glutathione peroxidase, selenoprotein P), selenium is strongly bound to the organic moiety since selenocysteine is genetically encoded in these selenoproteins, and thus it is an integral protein constituent.

### Selenium Essentiality and Toxicity

Selenium has antioxidant properties and is used in cancer chemoprevention. It is needed to produce triiodothyronine, which is required for healthy brain and

bone development, normal growth, and thermoregulation. At correct levels, selenium has been found to be necessary for proper immune system functioning and has been shown to inhibit the progression of human immunodeficiency virus to acquired inmunodeficiency syndrome (Gergely et al. 2006). Selenium can be incorporated into the diet from several food sources (e.g., yeast, garlic, onions). The daily recommended selenium dose is 21 and 16 μg per day for men and women, respectively (WHO/FAO/IAEA 1996). Due to the narrow range between beneficial and toxic levels of selenium, however, both effects can be elicited. Lower levels can increase the risk of cardiovascular disease and other degenerative diseases, whereas high intake can induce hair loss, nail brittleness and loss, gastrointestinal disturbances, skin rash, garlic breath, fatigue, irritability, and nervous system abnormalities (Gergely et al. 2006). There is, however, some controversy related to the upper limits for Se intake, and while some authors do not report any adverse level for an intake of < 800 μg/day for adults, others report selenosis when Se intakes are ≥850 μg/day (Roman et al. 2014). In this context, the U.S. Environmental Protection Agency has defined an intake limit of 1262 μg/day as the reference at which clinical selenosis appears, whereas the tolerable upper intake level established by the European Community has been set at 300 μg/day (Rayman and British 2004; Roman et al. 2014). For a discussion of the recommended dietary allowance (RDA) and tolerable upper intake levels (UILs) for selenium, as reported by the World Health Organization, see Wang and Zhang (this volume).

It is also well known that inorganic forms of selenium are more acutely toxic than organic forms, such as Se-enriched yeast (Se-yeast), in which selenomethionine (SeMet) is the main species, accounting for 54–74% of total selenium (Rayman and British 2004). The LD50 in rats for Se-yeast has been established by several authors at 37.3 mg/kg, compared with 12.7 mg/kg for sodium selenite, demonstrating that Se-yeast is considerably less acutely toxic than sodium selenite. Histological examination of livers of animals fed Se-yeast revealed up to 50% greater deposition of selenium; however, no corresponding toxicity (e.g., severe hepatotoxicity, cardiotoxicity, splenomegaly) was found in rats fed selenite at levels of 16 μg/day over an eight-week period (Rayman and British 2004). It has been suggested that organic forms of selenium may be more toxic during long-term consumption due to its rapid incorporation into tissue proteins rather than its excretion. In 2002, the EC Scientific Committee on Food expressed concern that organic selenium (e.g., SeMet or Se-yeast) could accumulate in body tissues to toxic levels. However, a large number of studies have shown that Se-yeast can be administered in doses as high as 800 μg per day for lengthy periods without any toxic effects. This lead to the conclusion that there cannot be a continuing rise in tissue selenium and that organic selenium is no more hazardous than supplementation with inorganic selenium. In this way, the conversion of organic Se into $H_2Se$ may be an important regulator of Se bioavailability; this may protect against excessive incorporation of selenium into proteins and prevent toxicity mediated through

**Table 14.1** Lethal doses of the main Se species for $LD_{50}$ mice or rats by intraperitoneal (I), oral (O), or respiratory (R) absorption.

| Compound | Formula | Lethal Dose | Reference |
|---|---|---|---|
| Elemental selenium | Se | 6,700 mg/kg (O) | Cummins and Kimura (1971) |
| Dimethylselenide (–2) | $(CH_3)_2Se$ | 1,600 mg/kg (I) | Al Bayati et al. (1992) |
| Hydrogen selenide (–2) | $H_2Se$ | 0.02 mg/L (R) | Wilber (1980) |
| Trimethylselenonium (–2) | $(CH_3)_3Se^+$ | 49 mg/kg (I) | Wilber (1980) |
| Selenocystine (–1) | $(HO_2CCH(NH_2)CH_2Se)_2$ | 35.8 mg/kg (O) | Sayato et al. (1993) |
| Selenomethionine (–2) | $CH_3Se(CH_2)_2CH(NH_2)CO_2H$ | 4.3 mg/kg (I)<br>37.3 mg/kg (O, Se-yeast) | Wilber (1980) |
| Selenite (+4) | $SeO_3^{2-}$ | 3.5 mg/kg (I)<br>12.7 mg/kg (O) | WHO (1987)<br>Vinson and Bose (1987) |
| Selenate (+6) | $SeO_4^{2-}$ | 5.8 mg/kg (I) | WHO (1987) |

reactive oxygen species from excessive intake (Rayman and British 2004). Table 14.1 summarizes the lethal doses of the main Se species for mice or rats by intraperitoneal, oral, or respiratory absorption.

## Mercury Toxicity

Mercury species in the environment are well-established toxicants to human and other organisms. Found in different industrial settings, air, soil, drinking water and food, humans are continuously exposed to Hg species. Divalent inorganic mercury ($Hg^{2+}$) has a high affinity for thiol groups of endogenous proteins and metabolites; thus, it is invariably found in cells, tissues, and biological fluids bound to thiol-containing proteins and metabolites, such as reduced glutathione or cysteine (Andrew et al. 2007). Most likely, $Hg^{2+}$ and $CH_3Hg^+$ bound to cysteine and/or glutathione play important roles in the intracellular transport and disposition of these toxic metal species. Mercury can also produce free radicals that induce protein, lipid, and DNA oxidation (Clarkson 1997). In addition, it is well known that inorganic mercury is linked to some alteration in metabolic pathways, such as energy metabolism, amino acid metabolism, and gut microflora (Wei et al. 2008). Nevertheless, the mechanisms responsible for toxic effects of inorganic mercury in organisms are still not known.

The organic forms of mercury are especially toxic since alkyl mercury is not well metabolized. Due to its high solubility in lipids, organic mercury bioaccumulates and becomes biomagnified through the food chain, thus affecting human health. The World Health Organization sets a maximum tolerable weekly intake of 5 µg/kg of body weight for total mercury, of which no more than 3.3 µg/kg should be present as $MeHg^+$. The guideline level for total mercury in drinking water has been established at 1 µg/l (WHO 1993:51). The European

Commission Regulation 466/2001/EC (amended by Regulation 221/2002/EC) came into effect on April 2002 and sets maximum levels for mercury in bivalve mollusks at 0.5 mg/kg of their wet weight (Moreno et al. 2010).

## Interaction of Mercury and Selenium Species through Biological Mechanisms

### Effects on Toxicity

In general, the simultaneous administration of selenite counteracts the negative impacts of exposure to inorganic mercury, particularly in regard to neurotoxicity, fetotoxicity, and cardiovascular diseases. In humans, a selenite and SeMet-dependent protection against mercury-induced apoptosis and growth inhibition in human cells has been observed. However, other studies reveal that inorganic selenium is ineffective in preventing most of the $MeHg^+$-induced brain biochemical alterations and that alone it is also toxic. The Se:Hg molar ratio is very important since mercury in molar excess over selenium is a stronger inducer of metallothionein levels in some animals (García-Barrera et al. 2012).

As stated previously, selenium can counteract the toxicity of methylmercury, as demonstrated by an *in utero* study of mice exposed to $MeHg^+$ and selenium. The group that was given the lowest amount of selenium and highest dose of $MeHg^+$ was mostly adversely affected in neurobehavioral outcome. In rodents, antioxidant nutrients such as dietary selenium and vitamin E may alter $MeHg^+$ reproductive and developmental toxicity (García-Barrera et al. 2012).

A great number of studies have been carried out related to the protective influence of the selenocompounds against $MeHg^+$ toxicity, especially SeMet. Exposition of $MeHg^+$ in rats resulted in a significant increase in urinary porphyrins and a decrease in motor activity that was counteracted by SeMet (García-Barrera et al. 2012).

### Mechanisms

The following mechanisms have been proposed to explain the interaction between selenium and mercury:

- Selenium provokes the redistribution of mercury to less sensitive organs.
- They compete for the same cleavages.
- Both combine to form Hg-Se complexes.
- Highly toxic Hg species are converted by selenium into less toxic forms.
- Selenium prevents the oxidative stress caused by mercury.

It is believed that a 1:1 Hg-Se compound of low biological availability and activity is formed inside cells, and that cell damage is quite low, even in the presence of very high Hg concentrations, if both elements are present in an

equimolecular ratio. This has been stated in studies with marine mammals and humans exposed to high levels of inorganic mercury. In 1978, experiments with marine organisms suggested a direct Hg-Se linkage, and the 1:1 molar ratio of mercury and selenium increment holds true in several species, including humans (García-Barrera et al. 2012). A tissue with a Se:Hg molar ratio higher than 1 is suggested as a threshold for the protective action against Hg toxicity.

The possibility that Hg-Se formation is responsible for the 1:1 molar ratio is supported by experiments which have shown that (a) enzimatically digested liver and plasma fractions with a 1:1 molar ratio release mercury and selenium in insoluble forms and (b) binding to the same plasma protein is preceded with the conversion of selenite to $H_2Se$ in red blood cells (García-Barrera et al. 2012). Selenium can also affect the activities of enzymes cleaving the C-Hg bond in organic mercury compounds. In this way, experiments with rats show an enhancement of PMA (phorbol myristate acetate) cleavage enzymes in liver when sodium selenite is supplemented in drinking water. It has also been observed that $MeHg^+$ exposure exerts an inhibitory effect on paronaxe 1 activity in humans that can be counteracted by selenium. Other hypotheses are that selenium can promote a redistribution of mercury from more sensitive organs (kidney, central nervous system) to less sensitive ones (muscle), that selenium competes for the same receptors, that complexes such as tiemannite or Se-Hg-S are formed, and that $MeHg^+$ conversion into less toxic forms is promoted and oxidative damage prevented. Yang et al. (2002) propose that selenium is involved in the demethylation of $MeHg^+$ in the liver, which results in the formation of inorganic and less toxic Hg compounds (see also García-Barrera et al. 2012).

## Biochemical Interactions

The ability of different Se compounds and selenium incorporated *in vivo* into liver tissue (biological selenium) to form an Hg-Se compound varies and increases in the following order: biological Se < SeMet < selenite. The protective effect of the Se compounds against mercury toxicity might, therefore, follow the same order. Mercury ions can react with thiols (–SH) and selenols (–SeH), which constitute a part of cysteine and selenocysteine. As a consequence, they can be incorporated to proteins, prosthetic groups of enzymes, and peptides. Mercury ions can also react with selenides ($Se^{2-}$) and, with hydrogen selenide, they can form complexes together with glutathione that can be finally bound to selenoprotein P (García-Barrera et al. 2012).

Similar complexes can be formed in other cells with active Se metabolism or during degradation of metal-bonded proteins and metallo(selenoproteins) in lysosomes (biomineralization processes), representing the last step of detoxification. A direct interaction between $MeHg^+$ and the selenol group of glutathione peroxidase has also been reported. However, to explain the reduced activity of the enzyme after $MeHg^+$ exposure, another molecular mechanism

has been proposed: cultured cells showed that $MeHg^{+}$ induced a "Se-deficient-like" condition, which affected the synthesis of glutathione peroxidase thought to be a posttranscriptional effect (García-Barrera et al. 2012).

Mercury vapor shows similar behavior to $MeHg^{+}$ in relation to its ability to penetrate cell membranes, where it is oxidized in the biological active form ($Hg^{2+}$) by catalase. Such *in situ*-generated ions can react with endogenously generated highly reactive Se metabolites, like $HSe^{-}$, and consequently a part of the selenium is unavailable for selenoprotein synthesis. Mercury can also provoke the increase of free radicals that induce lipid, protein, and DNA oxidation (García-Barrera et al. 2012).

## Other Interactions

Although the Hg-Se interaction has been widely studied, there are other well-known interactions and other less studied but sufficiently confirmed. One well-studied element is arsenic, which can interact in an antagonistic fashion with zinc and phosphorous and synergistically with cadmium. Likewise, it has been demonstrated in rats that zinc prevents As-induced tissue oxidative stress (Modi et al. 2006), while there is evidence in bacterial systems of arsenic in macromolecules that normally contain phosphorous (Rosen et al. 2011). Otherwise, arsenic and cadmium provoke a more pronounced renal toxicity than exposure to each of the agents alone; they induce lipid peroxidation, expression of glutathione and metallothionein, and redistribution of essential elements.

Zinc, on the other hand, antagonizes testicular damage induced in mice through low doses of mercury (Orisakwe et al. 2001), the teratogenic and embryophatic effects (Gale 1973), and replaces mercury in metallothionein (Day et al. 1984). In rats, zinc also counteracts the hepathotoxicity of cadmium (Rogalska et al. 2011), reduces the Cd-induced metallothionein synthesis (Scheuhammer et al. 1985) and alterations in lipid metabolism (Rogalska et al. 2009), and, in mice, protects against Cd effects on pre-implantation of embryos (Belmonte et al. 1989).

## Analytical Strategies to Study Interactions between Elements

During the last decade, various "-omics" technologies have provided massive information-generating methods to allow comprehensive description of nearly all components within the cell. Genomics has revealed the characteristics of the information contained in the cellular core, which determines cell function and behavior; transcriptomics allows gene expression to be examined; and proteomics analyzes protein synthesis and cell signaling. Nicholson et al. (1999) defined metabonomics as "the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli

or genetic modification." Metabolomics can also be defined as the measurement of all the metabolites in a specified biological sample (Fiehn et al. 2000). Whereas metabonomics provides a means for understanding the variation in low molecular mass metabolites in complex multicellular organisms and their response to change, the "-omics" sciences are concerned with cellular macromolecules. To understand a cell, tissue, or living organism behavior, it is also necessary to consider low molecular mass molecules since they represent the last action mechanism of the organisms. Although metabolomics and metabonomics are the most common strategies for metabolomic analysis, other important approaches (Ogra and Annan 2012) include:

- Metabolite profiling: the identification and quantification of a selected number of pre-defined metabolites, generally related to a specific metabolic pathway.
- Metabolic fingerprinting: high throughput, rapid, global analysis of samples to provide sample classification, usually without quantification and metabolic identification.
- Metabolite target analysis: qualitative and quantitative analysis of one or few metabolites related to a specific metabolic reaction.
- Metabolite footprinting: the study of metabolites in extracellular fluids.
- Metal-metabolomics: the study of metal or metalloid containing metabolites (i.e., selenometabolomics).
- Environmental metabolomics: the comprehensive metabolome analysis of living organisms for the characterization of their interactions with the habitat (see Maret et al., this volume).

As discussed earlier, it is necessary to remember that approximately one-third of all proteins require the presence of metals as cofactors to develop their function (Lobinski et al. 2010). These metals are responsible for catalytic properties or structure of proteins, and their presence in molecules is determined in many cases by the genome (Tainer et al. 1991). Metallomics considers that the identification of a metal cofactor into a protein can greatly assist its functional assignment and help place it in the context of known cellular pathways (González-Fernández et al. 2008). Since chemical species are the specific forms of an element defined to isotopic composition, electronic or oxidation state, and/or complex or molecular structure (Templeton et al. 2000), the defining line between metallomics, metal metabolomics, and chemical speciation is very thin.

In general, genomics can explain "what could happen" in living organisms; transcriptomics can address "what seems to happen"; proteomics looks at "what is provoked so that it does happen"; and metabolomics can explain "what happened in the past and what is happening at the moment." Although metabolomics can be very instrumental in understanding biological systems, other "-omics" are also useful and should be used as well. Under such scenario,

metals play essential roles due to their interaction with proteins, metabolites, and other biomolecules (for further discussion, see Maret et al., this volume).

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