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Review

Reactive selenium metabolites as targets of toxic metals/metalloids in mammals: a molecular toxicological perspective

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Human activities have been contaminating the environment with toxic heavy metal and metalloid compounds. Since the toxicity of one metal or metalloid can be dramatically modulated by the simultaneous ingestion of another, studies addressing the molecular basis of chemical interactions between toxic and essential elements are increasingly important. The intravenous injection of rabbits with selenite and arsenite or with selenite and mercuric mercury resulted in the *in vivo* formation of the seleno-bis (*S*-glutathionyl) arsinium ion, $[(GS)_2AsSe]^-$, or a glutathione-coated mercuric selenide, $(GS)_5(HgSe)_{core}$, in blood. The formation of these species (and the formation of a cadmium–selenium species in blood after the exposure of rats to selenite and cadmium) critically involves reactive selenite metabolites, such as $GS–Se^-$ and/or HSe^-, which indicates that these physiologically important metabolites are molecular targets of ingested toxic metals and metalloids. The fate and stability of $[(GS)_2AsSe]^-$ and $(GS)_5(HgSe)_{core}$ *in vivo* imply that the chronic exposure of mammals to inorganic arsenic and mercury will cumulatively affect the bioavailability of selenium, which could lead to selenium deficiency. Since selenium deficiency is significantly associated with the etiology of cancer in humans, the GSH-driven *in vivo* formation of selenium-containing metal and metalloid species provides a likely molecular mechanism for the chronic toxicity of environmentally persistent inorganic arsenic, mercury and cadmium. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: selenite; arsenite; mercury(II); rabbits; cadmium; glutathione

INTRODUCTION

Approximately 20 inorganic elements are consistently found in living organisms and have to be regularly ingested by them for their maintenance and propagation.[1] To keep the tissue concentrations of these essential elements constant throughout life,[2] all higher forms of life have evolved ingenious homeostatic regulation mechanisms, such as iron regulatory protein 1.[3] Investigations aimed at understanding the molecular basis of these mechanisms revealed that their disruption will lead to diseases. Mutations in genes coding for proteins involved in a particular homeostatic regulation mechanism represent 'genetic' factors that cause diseases, such as Wilson's disease[4] and haemochromatosis.[5,6] On the other hand, the chronic ingestion of a diet that is deficient in a particular essential element represents an 'environmental' factor that can also lead to disease. Selenium deficiency, for instance, significantly increases the risk of cancer mortality in humans.[7] Another 'environmental' factor that adversely effects homeostatic regulation mechanisms is the ingestion of dietary constituents that form complexes with essential elements in the gut and, therefore, decrease their intestinal absorption.[8,9] Dietary phytic acid, for example, greatly

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diminishes the intestinal absorption of the essential element zinc and can thus result in zinc deficiency even though enough dietary zinc is ingested.[9] Yet another 'environmental' factor that significantly effects the metabolism of an essential element is the *in vivo* formation of compounds between essential elements (or their metabolites) and simultaneously ingested toxic metals or toxic metalloid compounds. Even though direct experimental evidence in favour of such 'interactions' was reported between the essential trace element selenium (given as selenite) and arsenite,[10] inorganic mercury,[11] methylmercury,[12] or cadmium[13] in rats long ago, the underlying molecular mechanisms are just now being unravelled.[14–18] Since a new role of selenium in the detoxification of toxic metals and metalloids is emerging, this article aims to provide a perspective on the relevance of molecular interactions between this essential trace element and inorganic pollutants with regard to human health and disease.

TOXIC METALS AND METALLOIDS IN THE ENVIRONMENT

Apart from the elements that are essential for living organisms, the Earth's crust also contains toxic elements, such as arsenic, cadmium, lead and mercury. Even though these elements are locked up in the lithosphere, mostly in the form of insoluble sulfidic ores, chemical hydrolysis, along with biotic/abiotic oxidation and/or reduction reactions, constantly releases compounds of these elements to natural waters.[19] Therefore, life on Earth has always been exposed to background concentrations of toxic metals and metalloid compounds. It is believed that the resulting enhanced cellular lipid peroxidation and related peroxidative processes[20,21] eventually led to the evolution of heavy metal binding proteins, such as selenoprotein P,[22] metallothionein[23–25] and phytochelatins.[26] Since other detoxification mechanisms are likely to exist that protect mammalian cells from the toxic effects of natural background concentrations of heavy metals and metalloid compounds, their elucidation will help to better understand their molecular toxicology. This is particularly relevant if one considers the fact that human activities release quantities of toxic metals and metalloids into the environment that rival or exceed their natural inputs[27,28] and significantly contaminate the biosphere.[29–33] Since the ingestion of methylmercury,[34] selenium,[35] inorganic arsenic,[36] cadmium[37] and lead[38] has, in some locations, led to widespread death and disease, the exposure of various human populations to increasing concentrations of environmentally persistent pollutants is of much public concern.[33,39] Even though toxicological studies involving the exposure of animals to various doses of *single* metal or metalloid compounds have yielded valuable information, such as the acute toxic dose (LD_{50}), the mechanism of acute toxicity (*e.g.* enzyme inhibition) and their metabolism (*e.g.* biomethylation), strikingly little is known about the molecular form(s) of toxic metals and metalloids inside cells. It is therefore not surprising that many biochemical mechanisms of chronic metal and metalloid toxicity, such as the mechanism of arsenite-induced carcinogenesis in humans, are still unknown.[40,41]

One way to gain insight into the molecular mechanism(s) of chronic metal and metalloid toxicity is to structurally characterize all metabolites that are formed *in vivo* after the exposure of a model organism to a particular toxin. The subsequent assessment of the biological activity and fate of these metabolites could then reveal the active carcinogenic species. This approach, however, must take into account that, in reality, organisms are *simultaneously* exposed to essential elements and several toxic elements (metals and metalloid compounds) and that the combined exposure of organisms to individual toxic compounds (essential elements are also toxic when large doses are administered) can result in antagonistic, additive or synergistic effects which are mediated by the biological system itself.[42,43] These 'interactions' can cause nonlinearities in the overall dose–response relationship of an environmental toxin and represent a dilemma for risk assessment.[42] In spite of the *simultaneous* exposure of the general population to increasing concentrations of toxic heavy metals and metalloid compounds, compared with the days before the industrial revolution,[29] the necessity to elucidate the molecular mechanism of interactions between essential and toxic elements is increasingly recognized.[44–49] Figure 1 depicts a possible metabolic interaction between an essential element (A) and a toxic element (metal or metalloid compound) (B) *in vivo*. The formation of a compound containing the essential and the toxic element (A–B) in blood will have several important biochemical consequences. On the one hand, the intestinal uptake of the toxic element and the formation of A–B in the bloodstream will decrease the amount of the essential element that is able to reach biological target sites, such as storage proteins and protein-active sites and, therefore, decrease the bioavailability of the essential element. On the other hand, and probably more important, is the fact that A–B in itself represents a novel chemical entity that will have a different toxicity than A and B and could profoundly effect signal transduction pathways and gene expression (Fig. 1). Consequently, the overall biological effect of an interaction between an essential and a toxic element will be the sum of the individual effects (bioavailability + toxicity/signal transduction/gene expression). Hence, all toxic metals or metalloid compounds that form compounds with essential elements *in vivo* could be implicated in human diseases and must be identified. In view of the large number of possible essential element/toxic element combinations, however, a focus on those essential elements that have to be ingested only in minute quantities for optimum health seems most appropriate. As will be outlined below, selenium appears to be an interesting element to start with, since its

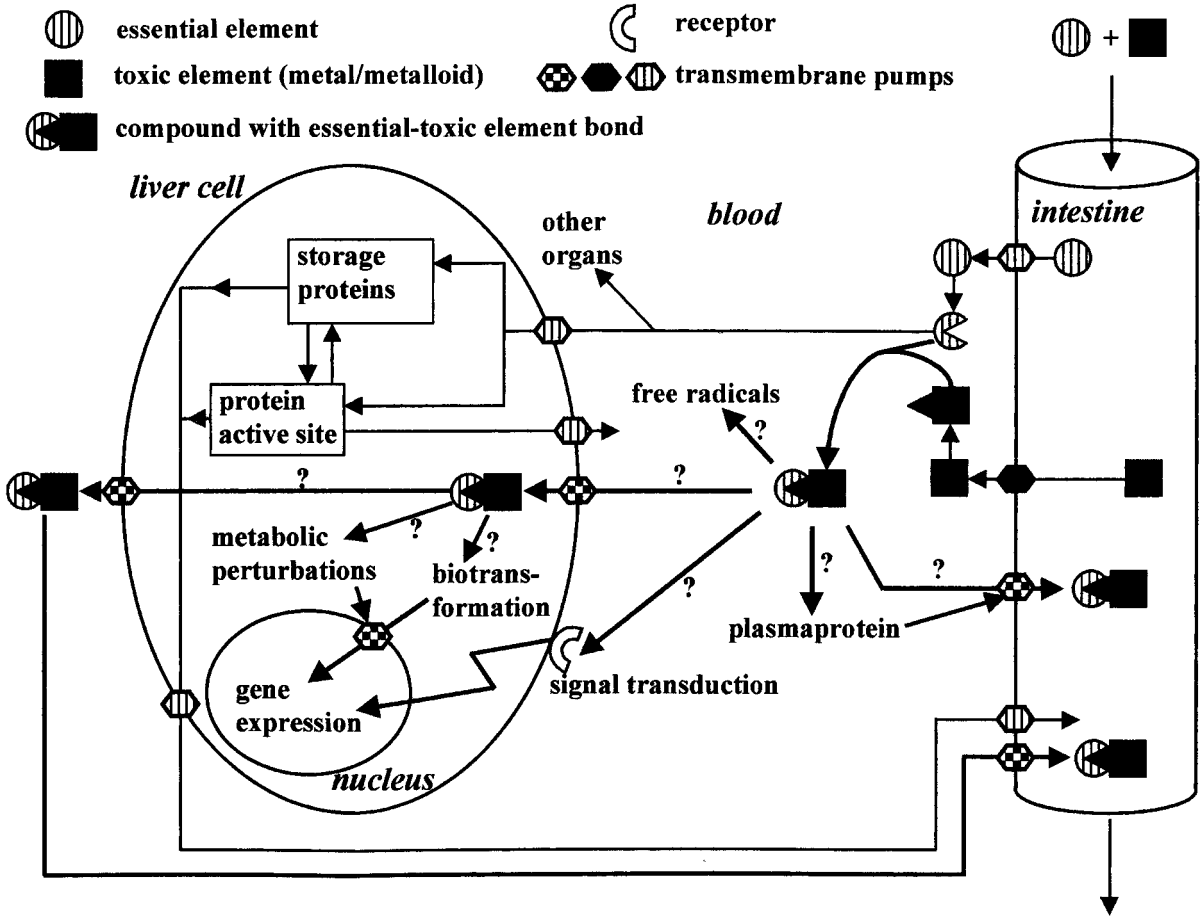


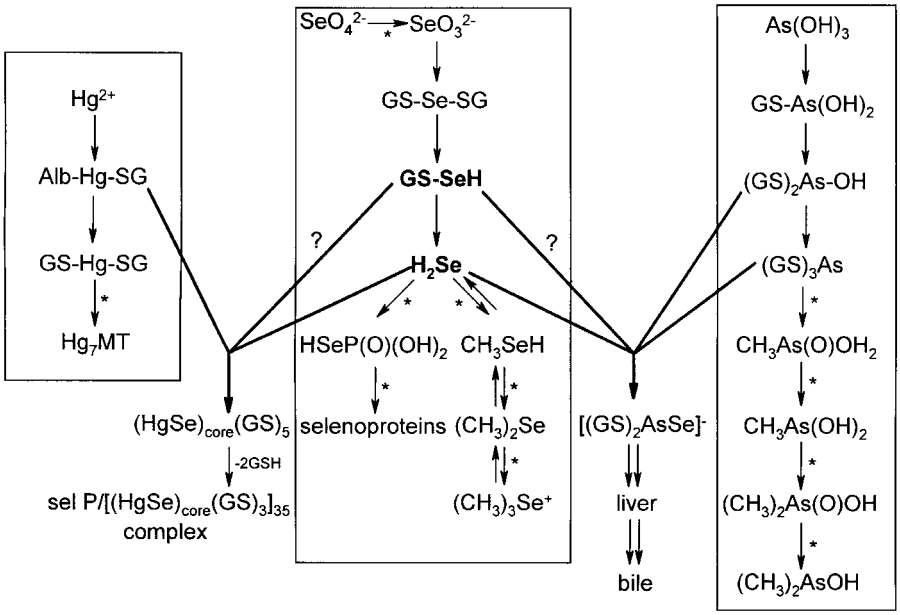
Figure 1. Metabolic interaction and formation of a compound between an ingested essential element and a toxic element (metal or metalloid compound) in blood. The ingestion of the toxc element will decrease the bioavailability of the essential element and the structural and chemical properties of the compound formed will determine its biological activity in/on mammalian cells.

dietary requirement for humans is estimated at only 50–200 μg per day.[50]

EFFECT OF TOXIC METALS AND METALLOIDS ON THE METABOLISM OF SELENIUM

The essential trace element selenium[51] may be the most important antioxidant element in the human body[52] and it exerts its biochemical functions mostly as an integral constituent of several key antioxidant selenoproteins.[53,54] In addition, selenium compounds play an important role in intracellular[55] and cell death signalling.[56] In mammals, ingested inorganic and organic selenium compounds are metabolized to selenide (Scheme 1).[57] Selenide is then used either for selenoprotein biosynthesis (via selenophosphate)[58] or for biomethylation to methylselenol, dimethylselenide or the trimethylselenonium cation,[59] and thus represents a key intermediate in selenium metabolism (Scheme 1).[53,57,59]

The observation of a causal relationship between selenium deficiency and cancer in humans[7,60–62] and subsequent human intervention trials[63,64] eventually established a cancer protective effect of dietary selenium compounds.[53,65–67] This fact, together with the aforementioned small dietary requirement of selenium (compared with other essential elements) makes studies of interactions between this trace element and environmentally abundant toxic metals and metalloids particularly relevant, since the ingestion of small doses of toxins that disrupt the metabolism of selenium will have dramatic effects on human health. In fact, experiments involving the simultaneous chronic exposure of animals to sodium selenite (or high selenium yeast essentially containing selenomethionine) and cadmium, lead or inorganic arsenic demonstrated that the latter compounds counteracted the anticarcinogenic effect of the administered selenium compounds.[65,68] Thus, an eluci-



Scheme 1. Scheme of the individual mammalian metabolism of mercuric mercury (Hg^{2+}), inorganic selenium (SeO_3^{2-}, SeO_4^{2-}) and arsenite [$As(OH)_3$] and molecular interactions between H_2Se and GS—SeH with mercuric mercury and arsenite metabolites *in vivo* (abbreviations: GS, glutathione; Alb, albumin; MT, metallothionein; sel P, selenoprotein P). Thick and thin arrows correspond to reactions that occur in blood and liver; reactions that occur in liver only are labelled with an asterisk; double arrows symbolize transport across a biomembrane.

dation of the underlying molecular basis of these *in vivo* interactions could potentially uncover the biochemical mechanisms that are involved in the chronic toxicity of heavy metals and metalloid compounds.

Following the discovery of a remarkable ability of arsenite to protect rats from selenium poisoning,[10] further studies revealed that arsenite inhibited the pulmonary excretion of dimethylselenide in rats also receiving selenite.[69] Since selenite, in turn, inhibited the biomethylation of arsenite,[70] these results indicated that the metabolism of these metalloid compounds is intertwined.[70] The lack of appropriate physico-chemical methods to probe the structure of a potentially formed arsenic–selenium detoxification compound *in vivo*, however, severely hampered the elucidation of the underlying molecular detoxification mechanism for a long time. Similarly, investigations into the striking protective effect of selenite against mercuric chloride toxicity in mammals[11] revealed that the coadministration of mercuric chloride (with sodium selenite) dramatically decreased the pulmonary excretion of the volatile selenite metabolite dimethylselenide.[69] Even though subsequent studies revealed that the mutual detoxification between selenite and mercuric mercury is based on their binding to selenoprotein P[71] and critically involves glutathione,[72] the structural basis of the mercury–selenium interaction remained elusive.

Using X-ray absorption spectroscopy (XAS) and size-exclusion chromatography (SEC) with simultaneous multi-element-specific detection by inductively coupled plasma atomic emission spectroscopy (ICP-AES), both antagonistic interactions could be finally placed on a molecular basis. The intravenous injection of rabbits with selenite followed by arsenite (or mercuric chloride) and the subsequent analysis of bile and plasma samples with XAS and SEC–ICP-AES revealed that both interactions are based on the *in vivo* formation of compounds with selenium–metal or selenium–metalloid bonds. The mutual detoxification between arsenite and selenite can be rationalized in terms of the *in vivo* formation and subsequent biliary excretion of the seleno-bis (*S*-glutathionyl) arsinium ion, $[(GS)_2AsSe]^-$ (Scheme 1).[14,16,17] *In vitro* studies revealed that this species is rapidly formed in blood[73] and most likely assembled inside erythrocytes[74] after individual import of arsenite and selenite into these cells.[75] Intracellular metabolism of arsenite and selenite in erythrocytes to $(GS)_2As$–OH/$(GS)_3As$[76] and selenide[59,70,77,78] would then allow nucleophilic attack of the latter on the arsenic atom of $(GS)_2As$–OH or $(GS)_3As$ to give $[(GS)_2AsSe]^-$.[18]

The combined application of XAS and SEC–ICP-AES also revealed the structural basis of the mutual detoxification between selenite and mercuric chloride in mammals. It is based on the *in vivo* formation of a glutathionyl-coated mercuric selenide core, $(GS)_5(HgSe)_{core}$,[15] which essentially

is non-toxic.[79] The formation of this detoxification species in blood most likely involves the reaction of albumin-bound mercury(II) (Hg^{2+}) with an erythrocyte-derived selenite metabolite, possibly HSe^-.[77,80] In view of our previously reported selenium EXAFS data of a synthetic model (of the *in vivo* formed mercury- and selenium-containing detoxification compound) in which five GS moieties are ligated to the $(HgSe)_{core}$ via S–Se bonds, however, it cannot be excluded that GS–Se^- is the selenite metabolite that is effluxed from the erythrocytes to plasma. Since the mutual detoxification between cadmium and selenite in mammals is mechanistically closely related to that of mercuric chloride and selenite,[22] reactive selenite metabolites, such as HSe^- and GS–Se^-, emerge as important molecular targets of ingested arsenite, mercuric mercury, and cadmium (Scheme 1).

IMPLICATIONS

The observed *in vivo* formation and subsequent biliary excretion of $[(GS)_2AsSe]^-$ in rabbits demonstrates that the simultaneous administration of arsenite severely disrupts the mammalian metabolism of selenite[14] and that the arsenic–selenium bond in $[(GS)_2AsSe]^-$ is stronger than the labile arsenic–sulfur bond in another arsenite metabolite, $(GS)_3As$.[76,81] The rapid formation of $[(GS)_2AsSe]^-$ in blood,[73] and its equally rapid biliary excretion (and thus the removal of highly toxic trivalent arsenic from the organism itself), suggests that the formation and excretion of $[(GS)_2AsSe]^-$ represents an important mammalian detoxification mechanism. Any excess arsenite that is left over after this step in blood will be shuttled to the liver to undergo biomethylation (Scheme 1).[82] The simultaneous exposure of animals to selenite and cadmium[22] or to selenite and mercuric mercury[15] also resulted in the *in vivo* formation of heavy-metal- and selenium-containing compounds in blood (only the mercury–selenium compound has so far been demonstrated to be a detoxification compound), which provides direct evidence for the interference of these heavy metals with the metabolism of selenite and precedes the binding of cadmium and mercury to metallothionein in the liver (Scheme 1).[25]

Combined, these results imply that the mammalian biochemistry of arsenite, mercuric mercury and cadmium is not only driven by interactions with endogenous thiols, such as GSH,[83] but will also be determined by interactions with *in vivo* generated, reactive selenium metabolites, such as GS–Se^- and/or HSe^- in blood (Scheme 1)[15,73] and liver.[84] This provides a conceptually new perspective on the molecular toxicology of environmentally persistent toxic heavy metals and metalloid compounds. The identification of selenite in blood following the oral administration of rats with selenite, selenate or selenomethionine[85] and the detection of free selenite in human plasma[86] suggests that selenium-containing metal and metalloid compounds will also be formed under physiological conditions and that their formation represents the first mechanism by which mammals detoxify ingested inorganic arsenic, mercury and cadmium.

At the same time, however, the *in vivo* formation of selenium-containing heavy metal and metalloid compounds provides a molecular mechanism for the chronic toxicity of heavy metals and metalloids, since the ingestion of these will inevitably decrease the bioavailability of ingested dietary selenium compounds (Scheme 1). In fact, experiments involving the simultaneous chronic exposure of rats to seleniferous wheat and sodium arsenite in drinking water demonstrated a significant depletion of total selenium in liver by the administered arsenite.[87] In addition, the prolonged exposure of humans to inorganic arsenic in drinking water significantly reduced tissue selenium concentrations.[88] These results, which are most likely based on the *in vivo* formation and rapid biliary excretion of $[(GS)_2AsSe]^-$, together with the chemical stability of $(GS)_5(HgSe)_{core}$ in plasma,[89,90] suggest that the chronic ingestion of inorganic arsenic, mercury and cadmium could cumulatively effect the bioavailability of selenium and result in selenium deficiency (Scheme 1). Since selenium deficiency significantly increases the risk of cancer in humans,[7,60–62] and is also associated with a variety of other pathologies,[91,92] the *in vivo* formation of selenium-containing metal and metalloid compounds in blood[15,73] and liver[84] represents a likely molecular mechanism for the chronic toxicity of ingested inorganic arsenic, mercury and cadmium in mammals.

CONCLUSION

Studies investigating the molecular basis of the antagonistic interactions between selenite and arsenite, mercuric chloride or cadmium in mammals revealed the *in vivo* formation of compounds with distinct selenium–metal/metalloid bonds. The formation of these compounds in blood is driven by erythrocytes and critically involves reactive selenite metabolites, such as HSe^- and GS–Se^- (and possibly others). The stability of these selenium-containing species and their fate *in vivo* suggests that the simultaneous exposure of mammals to arsenite, mercuric chloride and cadmium will cumulatively effect the bioavailability and the metabolism of selenium. Since other interactions are known to exist between selenium and methylmercury,[12] dimethylarsinic acid,[93,94] lead,[95,96] copper[97] and zinc,[98] studies involving the simultaneous exposure of whole animals (rather than cell culture experiments) to the interacting species and the subsequent analysis of biological samples by XAS and SEC–ICP-AES will reveal the structural basis of other toxicologically important mechanisms. Assessing the biological activity and fate of the underlying *in vivo* formed selenium-containing compounds will provide exciting new insights into the chronic toxicity of metals (and metalloids) and their individual mechanisms of carcinogenicity, which

will ultimately allow us to define better the effect of multiple metal and metalloid exposures on human health. Extending this approach to other essential elements[47,99] could reveal the molecular basis of diseases that are caused by the chronic exposure of the general population to metals and metalloids[39,100,101] and may lead to practical applications, such as the treatment of chronic metal or metalloid-based toxicity,[102,103] the development of novel metal- or metalloid-based anticancer agents[104–106] and the development of molecular-based methodologies for risk assessment purposes.[42] R.J.P. Williams concluded his presentation at the last International Conference for Bioinorganic Chemistry in Florence 2001 with the statement "Living organisms cannot be understood by studying extracted (dead) molecules. We have to study flow systems". Following this basic concept, the emerging new science of environmental bioinorganic chemistry will uncover other toxicologically important species that may be critically involved in numerous human diseases including cancer.

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