A Metabolic Link between Arsenite and Selenite: The Seleno-bis(*S*-glutathionyl) Arsinium Ion

Jürgen Gailer,*,†,△ Graham N. George,*,‡ Ingrid J. Pickering,‡ Roger C. Prince,§ Steven C. Ringwald,¶ Jeanne E. Pemberton,¶ Richard S. Glass,¶ Husam S. Younis,⊥ Donald W. DeYoung,|| and H. Vasken Aposhian†

Contribution from the Departments of Molecular and Cellular Biology, Chemistry, Pharmacology and Toxicology, and University Animal Care, University of Arizona, Tucson, Arizona 85721, the Stanford Synchrotron Radiation Laboratory, P.O. Box 4349, MS 69, Stanford, California 94309, and the ExxonMobil Research and Engineering Company, Annandale, New Jersey 08801

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Abstract: Among the most startling observations in mammalian toxicology is that a lethal dose of selenium can be overcome by an otherwise lethal dose of arsenic. We report the molecular basis of this antagonism. Using X-ray absorption spectroscopy we have identified a new arsenic−selenium compound in the bile of rabbits injected with aqueous selenite and arsenite solutions. This compound contains equimolar arsenic and selenium and exhibits X-ray absorption spectra which are essentially identical with those of a synthetic species in solution which we have identified spectroscopically as the seleno-bis(*S*-glutathionyl) arsinium ion. The in vivo detection of this compound links the mammalian metabolism of arsenite, selenite, and sulfur. It provides a molecular basis for the antagonistic interaction between these metalloid compounds, and a potential explanation of the association of cancer with prolonged intake of inorganic arsenic in humans.

Both arsenic and selenium compounds are known for their toxicity, although selenium, and possibly also arsenic, is an essential trace element. Natural and anthropogenic processes release As and Se compounds to the environment,[1,2] sometimes leading to significant contamination of freshwater resources and to an accumulation in the food chain.[3] Additionally, the unintended consequence of a "safe-water" program in Bangladesh has provided a public water supply contaminated with low levels of arsenic on a massive scale.[4] Typically, the most toxic As and Se compounds in natural waters are the oxy-anions arsenite and selenite.[1,5] A surprising antagonism between arsenite and selenite was first reported in the late 1930s when drinking water containing arsenite completely protected rats against the otherwise lethal liver damage caused by ingestion of seleniferous wheat or selenite.[6] Subsequent experiments revealed that arsenite can also overcome the toxicity of selenite in dogs, swine, and cattle.[7] Arsenite inhibited pulmonary excretion of $(CH_3)_2Se$ in rats also receiving selenite,[8] but biliary excretion of Se was dramatically increased.[9] Similarly, selenite stimulated gastrointestinal excretion of arsenic.[10] In vitro, greater than stoichiometric arsenite prevents $(CH_3)_2Se$ formation from selenite in the presence of glutathione (GSH) and GSH-reductase, suggesting the formation of an As−Se compound,[11] which may be excreted in vivo from the liver to bile.[9] To investigate this possibility, we collected bile from rabbits injected with arsenite, selenite, or both.[12] We report a new As−Se compound in rabbit bile, its structural identification with an As/Se-model compound, and its significance for mammalian toxicology.

X-ray fluorescence spectrometry[13] was used to quantify As and Se (Figure 1). Bile from rabbits injected with As or Se contained 1.7 ± 0.3 ppm As, or <0.1 ppm Se, respectively.

† Department of Molecular and Cellular Biology, University of Arizona.
△ Present address: Department of Nutritional Sciences, University of Arizona, Tucson, Arizona 85721.
‡ Stanford Synchrotron Radiation Laboratory.
§ ExxonMobil Research and Engineering Company.
¶ Department of Chemistry, University of Arizona.
⊥ Department of Pharmacology and Toxicology, University of Arizona.
|| University Animal Care, University of Arizona.

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(12) We used New Zealand white rabbits because humans and rabbits have a very similar As(III) metabolism [Vahter, M.; Marafante, E. *Chem.-Biol. Interact.* **1983**, *47*, 29−44]. Animals were deprived of food overnight, and following halothane anesthesia, midline abdominal incision, and gallbladder ligation, the common bile duct cannulated. Intravenous lactated Ringer's solution was given via the marginal ear vein and a tracheal tube ensured free airways. After constant bile-flow was established, either selenite, arsenite (0.63 and 0.60 $mg \cdot kg^{-1}$ body weight, respectively), or both (selenite followed 3 min later by arsenite) were injected (20 mM in phosphate buffered saline, pH 7.4.) through the marginal ear vein (3 rabbits per experiment). Bile was collected for 25 min after injection, mixed with 40% v/v glycerol (taking precautions against air exposure), and frozen in liquid nitrogen.
(13) X-ray absorption measurements were carried out at the Stanford Synchrotron Radiation Laboratory (SSRL) as previously described [George, G. N.; Garrett, R. M.; Graf, T.; Prince, R. C.; Rajagopalan, K. V. *J. Am. Chem. Soc.* **1998**, *120*, 4522−4523]. Energy calibration assumed lowest inflection of elemental As and hexagonal Se to be 11867 and 12658 eV, respectively. Fluorescence quantification used pseudo-Voigt fitting to estimate peak areas relative to 10 ppm As and Se standards in control bile.

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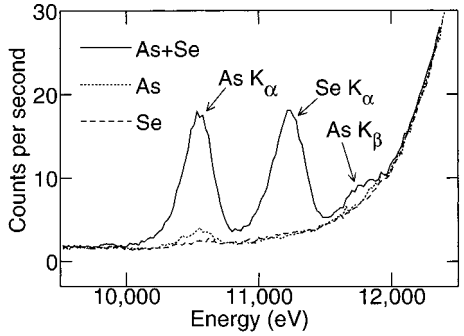

Figure 1. As and Se quantification in rabbit bile by X-ray fluorescence emission. Representative spectra are shown from animals treated with As, Se, and both As and Se. The rising background (from left to right) is the tail of the intense X-ray scatter peak from the 13400 eV excitation.

However, when the two were injected almost simultaneously, a substantial increase of both metalloids was detected: 20.9 ± 5.4 ppm As and 21.6 ± 5.9 ppm Se. As/Se molar ratios in individual rabbit bile samples were 0.97 ± 0.02, suggesting that a 1:1 As−Se compound was excreted to bile. Since selenite and arsenite both have a high propensity to react with thiols,[11,14] and because GSH is the most prevalent intracellular thiol (5 mM in rat hepatocyte cytoplasm),[15] the abiotic reaction of an aqueous solution containing equimolar arsenite and selenite with increasing GSH was investigated.[16] Seven to eight mole equivalents of GSH were required to form a water-soluble As−Se species.

Figure 2 shows the As and Se K-edge extended X-ray absorption fine structure (EXAFS)[13] of the in vitro arsenic−selenium−glutathione compound. The Se data could not be fitted with both As and S ligands; instead only a single As at 2.31 ± 0.02 Å was indicated (Figure 2). Similarly, inclusion of a Se−O interaction did not improve the fit, although two outer-shell Se−S at 3.03 ± 0.08 Å improved the fit marginally in the low-*k* region. The As K-edge data showed two As−S bonds at 2.25 ± 0.02 Å and a single As−Se bond at 2.32 ± 0.01 Å (Figure 2). A search of the Cambridge Structural Database indicated typical As−Se bond lengths of 2.26−2.33 and 2.39−2.47 Å for double and single bonds, respectively. Our data thus imply the structure shown in the inset to Figure 2, the selenobis(*S*-glutathionyl) arsinium ion, which we will refer to as $[(GS)_2AsSe]^-$.[17] The As−Se distance of 2.32 Å suggests significant contribution from both resonance forms:

$$(GS)_2As{-}Se^- \leftrightarrow (GS)_2As^-{=}Se$$
1a **1b**

^{77}Se-NMR shows a chemical shift of −5.7 ppm, versus 144−317 ppm for terminal (exocyclic) As−Se entities in cyclic selenoarsenates,[18] consistent with contribution of **1a**. The Raman

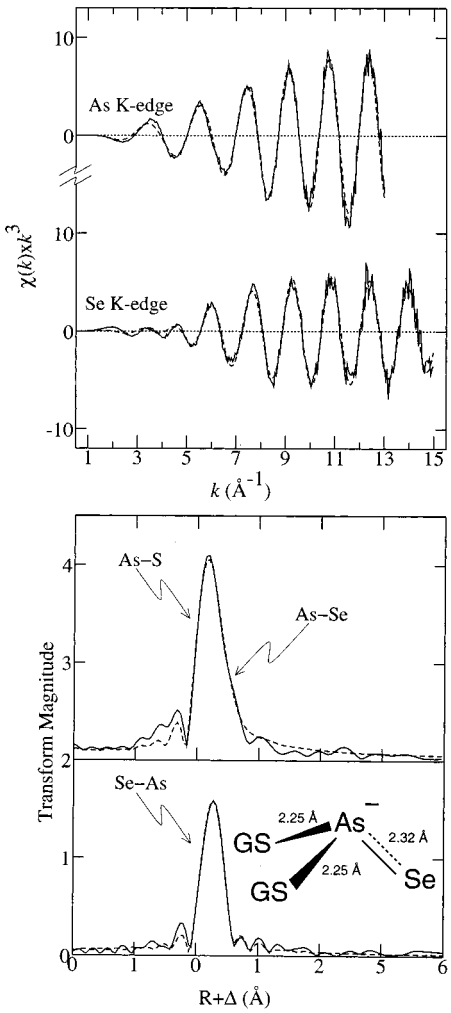

Figure 2. As and Se K-edge EXAFS (A) and corresponding EXAFS Fourier transforms (B) of $[(GS)_2AsSe]^-$, showing data (solid lines) and best fits (broken lines). The As and Se Fourier transforms were phase-corrected for S and As backscattering, respectively. The inset shows the postulated structure for $[(GS)_2AsSe]^-$.

spectrum of $[(GS)_2AsSe]^-$ contains a peak at 290 cm^{-1}, assigned as the ν(As−Se) mode. This frequency is lower than previously reported for As=Se bonds (325−370 cm^{-1}),[19] again consistent with an As−Se bond order of slightly less than two. Collectively, the EXAFS, ^{77}Se-NMR, and Raman data strongly support the $[(GS)_2AsSe]^-$ structure depicted in Figure 2.

Representative bile As and Se near-edge spectra are shown in Figure 3, together with model compound spectra. The Se spectrum of bile is almost identical with that of $[(GS)_2AsSe]^-$ (Figure 3), which is unique among the many Se species we have investigated. Thus the bile Se near-edge spectrum clearly indicates the presence of a species $[(RS)_2AsSe]^-$, where R is an organic donor; the near-edge cannot explicitly identify GSH as the sulfur donor, although the metabolic roles and the high GSH levels in vivo make this likely. The As near-edge spectrum of the bile is similar to both $[(GS)_2AsSe]^-$ and $(GS)_3As$, although with a somewhat broader peak (Figure 3).

The As and Se K-edge EXAFS spectra from the bile of a single animal dosed with twice the amount of As and Se clearly indicate 2.32 Å As=Se coordination in the naturally produced compound. Quantitative analysis of both near-edge and EXAFS by total curve-fitting to a linear combination of the spectra of

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(16) Reactions were carried out at 37 °C. A red-brown precipitate was formed with 1 to 6 mol equiv of GSH. With 7 to 10 mol equiv of GSH, a clear solution was formed after initial precipitate formation. In the absence of arsenite, α-Se was formed for all stoichiometries. Samples for spectroscopy were prepared by adding equimolar sodium arsenite and selenite to 9 mol equiv of GSH. X-ray absorption samples were 10 mM metalloid with 40% v/v glycerol.

(17) For simplicity we neglect charges from amino and carboxylates of glutathione. In support of our postulated structure, $[(GS)_2AsSe]^-$ has recently been chromatographically separated and the charge verified [Gailer J. G.; Madden, S.; Burke, M. F.; Denton, M. B.; Aposhian, H. V. Unpublished].

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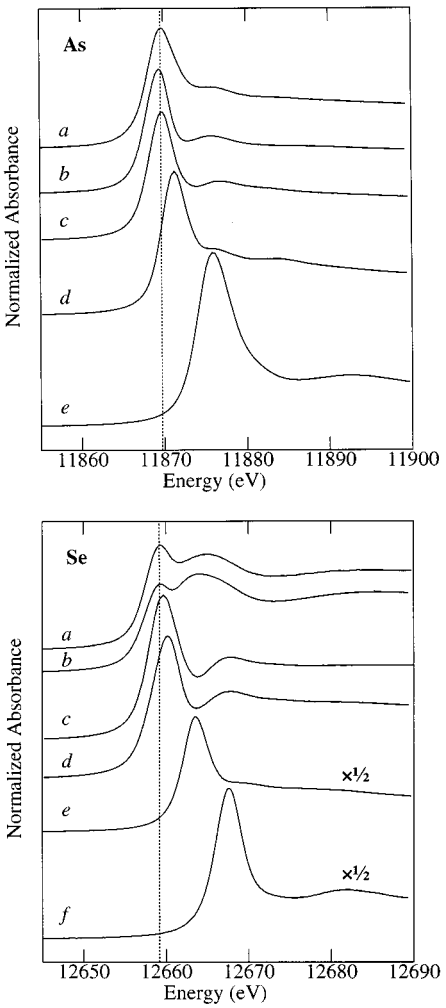

Figure 3. As (upper) and Se (lower) K X-ray absorption near-edge spectra of $[(GS)_2AsSe]^-$, bile, and relevant model compounds, all 5−10 mM in aqueous solution: (upper and lower) (a) the As + Se bile sample of Figure 1 and (b) $[(GS)_2AsSe]^-$; (upper) (c) $As(GS)_3$, (d) arsenite, and (e) arsenate; (lower) (c) elemental α-Se, (d) $Se(GS)_2$, (e) selenite, and (f) selenate.

$[(GS)_2AsSe]^-$, and arsenite or α-Se, for As or Se data, respectively, indicate that the bile contained at least 60% $[(GS)_2AsSe]^-$. Some loss of $[(GS)_2AsSe]^-$ might have occurred during collection of the bile, since samples were briefly exposed to air, and might have undergone partial oxidation. We noted that both bile samples and synthetic $[(GS)_2AsSe]^-$ [16] developed a precipitate of α-Se upon prolonged air exposure.

The reversal of Se toxicity by arsenite can thus be explained by the formation and subsequent excretion of $[(GS)_2AsSe]^-$ by hepatocytes to bile (although we have no evidence, as yet, on whether $[(GS)_2AsSe]^-$ enters the enterohepatic circulation). The chemistry reported herein has important consequences for the mammalian toxicology of both arsenite and selenite. When not co-administered, both are enzymatically methylated in the liver.[20,21] On co-administration, a mutual inhibition of the individual methylation pathways occurs,[8,11,20] and this can now be explained by the formation of $[(GS)_2AsSe]^-$.

The biliary excretion of $[(GS)_2AsSe]^-$ may be especially important in view of the fact that Se is an essential trace element. Prolonged exposure to inorganic arsenic in drinking water significantly reduces tissue selenium concentrations.[22] Since a chronic daily inorganic arsenic intake of ∼200 μg is significantly associated with the development of various cancers,[23] it is conceivable that this consumption causes the formation and excretion of $[(GS)_2AsSe]^-$, thereby leading to Se-deficiency, which has been linked with cancer.[24] Indeed, the chronic ingestion of arsenite abolishes the anticarcinogenic effect of selenium in rats,[25] providing a potential explanation of the association of cancer with prolonged As intake in humans.[26] Consequently, in cases of chronic exposure to inorganic arsenic, for example, the well-publicized "safe water" problems in Bangladesh,[4] an increased daily intake of selenium leading to the formation and excretion of $[(GS)_2AsSe]^-$ might protect against the pathological effects of inorganic arsenic in drinking water.

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Supporting Information Available: Tables of EXAFS curve-fitting results and Raman spectral frequencies, plus figures of ^{77}Se-NMR, Raman, and X-ray absorption spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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