Observation of the seleno *bis*-(S-glutathionyl) arsinium anion in rat bile

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ABSTRACT: Certain arsenic and selenium compounds show a remarkable mutual cancellation of toxicities, where a lethal dose of one can be voided by an equimolar and otherwise lethal dose of the other. It is now well established that the molecular basis of this antagonism is the formation and biliary excretion of seleno *bis*-(S-glutathionyl) arsinium anion $[(GS)_2AsSe]^-$. Previous work has definitively demonstrated the presence of $[(GS)_2AsSe]^-$ in rabbit bile, but only in the presence of other arsenic and selenium species. Rabbits have a gall bladder, which concentrates bile and lowers its pH; it seems likely that this may be responsible for the breakdown of biliary $[(GS)_2AsSe]^-$. Since rats have no gall bladder, the bile proceeds directly through the bile duct from the hepatobiliary tree. In the present work we have shown that the primary product of biliary co-excretion of arsenic and selenium in rats is $[(GS)_2AsSe]^-$, with essentially 100% of the arsenic and selenium present as this species. The chemical plausibility of the XAS-derived structural conclusions of this novel arsenic and selenium co-excretion product are supported by density functional theory calculations. These results establish the biomolecular basis to further explore the use of selenium dietary supplements as a possible palliative for chronic low-level arsenic poisoning of human populations.

1. Introduction

Arsenic is well known for the toxicity of some of its compounds, several of which have been used since antiquity either as poisons or as medicines [1,2]. In modern times arsenic contaminated drinking water poses enormous problems for close to 100 million people worldwide [3,4]. In Bangladesh alone, approximately 57 million people are affected in what has been called the world's worst mass poisoning [5]. In the worst affected areas chronic low-level arsenic poisoning, called arsenicosis [3-6], is now the leading cause of death and is responsible for a quarter of all deaths [6].

The surprising antagonism between arsenic and selenium compounds has been known since the ground-breaking study of Moxon in 1938 [7]. Subsequently, this observation has been confirmed and extended in a number of animal studies [8-12] that showed that a lethal dose of selenite can eliminate the toxic effects of a similar, and otherwise lethal, dose of arsenite. In prior work with rabbits, we have used X-ray absorption spectroscopy (XAS) to show the *in vivo* formation of the seleno *bis*-(S-glutathionyl) arsinium anion $[(GS)_2AsSe]^-$ (Fig. 1) in which arsenic is covalently bound to selenium [8]. At physiological pH, the compound is likely predominantly the tri-anion as both glutathione carboxylates and amines will be expected to be charged (Fig. 1), but for simplicity we include only the mono-anionic charge on the As—Se core in our abbreviation $[(GS)_2AsSe]^-$. The discovery of this novel excretory product explains many of previous observations involving arsenic-selenium antagonism. In particular, the well-known enzymatic methylation pathways for both As and Se [13-15] are observed to be inhibited when both As and Se are present [16,17], which can be rationalized by the *in vivo* formation of $[(GS)_2AsSe]^-$ [8,15,18]. The species $[(GS)_2AsSe]^-$ forms in hepatocytes [8,10], and probably in erythrocytes [11], is rapidly excreted into bile [8-12] and *in vitro* is transported by the multidrug

resistance protein 2 (MRP2) [19]. While there is evidence of considerable specificity in the transport of $[(GS)_2AsSe]^-$ [19], to date there is no evidence of the involvement any specific gene product in the biosynthesis of $[(GS)_2AsSe]^-$ [12,18]. A synthesis of $[(GS)_2AsSe]^-$ has been reported from sodium selenide, arsenite and glutathione [20], and the molecule has been previously studied by computational chemistry [20], but only with semi-empirical methods that are less rigorous than more modern approaches.

The initial suggestion that selenium supplements might be used as a palliative for arsenicosis in human populations arose from the discovery of $[(GS)_2AsSe]^-$ [8] and was reinforced by the realization that the Bangladeshi diet was poor in selenium [22]. Selenium is an essential trace element with roles in anti-oxidant metabolism [23], whereas arsenic has no confirmed role in humans and is not known to be required at any level. The selenium-dependant arsenic excretion through formation of $[(GS)_2AsSe]^-$ has given rise to the hypothesis that arsenicosis may be an arsenic-induced chronic selenium deficiency [8]. These and other studies have led to a number of clinical trials to test roles for selenium supplements in the treatment of arsenicosis, some of which are ongoing [24-26]. The molecular-level foundation of all of these studies is the formation of $[(GS)_2AsSe]^-$ and the subsequent biliary co-excretion of arsenic and selenium in the form of this species.

In our previous studies, $[(GS)_2AsSe]^-$ was identified in the bile of rabbits dosed with both arsenite and selenite [8]. While arsenic and selenium in the bile were present in an exactly equimolar ratio [8], only about half of the arsenic and selenium were present as $[(GS)_2AsSe]^-$, with the balance being predominantly arsenite $[As(OH)_3]$ and elemental α -selenium $[Se_8]$, respectively [8]. This finding was attributed to the reported sensitivity of $[(GS)_2AsSe]^-$ to oxygen [8, 20], and the exposure of the collected bile to air during aerobic sampling after the surgical

cannulation of the bile duct [8]. We have also previously observed that although $[(GS)_2AsSe]^-$ is stable under anaerobic conditions at neutral pH for hours, it tends to break down at lower pH. Rabbits, like humans, possess a gall bladder which allows the bile to pool and concentrate. Since the pH of the bile also tends to decrease in the gall bladder [21], unstable $[(GS)_2AsSe]^-$ might possibly degrade there before flowing through the bile duct. Rats, on the other hand, have no gall bladder with the bile proceeding directly through the bile duct from the hepatobiliary tree, which might improve the chance of observation of intact molecular $[(GS)_2AsSe]^-$. We present herein an XAS study of rat bile from animals dosed with arsenite and selenite, together with density functional theory calculations of $[(GS)_2AsSe]^-$. We show that in rat bile arsenic and selenium are present in essentially a single species, the seleno *bis*-(S-glutathionyl) arsinium anion.

2. Materials and Methods

2.1 Chemicals

All chemicals and reagents were purchased from Sigma Aldrich (Oakville, ON) and were of the highest quality available. Commercially unavailable arsenic and selenium species were prepared as previously described [8,20,27].

2.2 Animal care and sample collection

Adult male Wistar rats were obtained from Charles River (Wilmington, MA USA) and were handled in compliance with animal ethics regulations. Animals were deprived of food overnight, and were maintained under halothane anesthesia throughout all subsequent procedures. A tracheal tube was inserted to ensure free airways and midline abdominal incision carried out and the common bile duct was cannulated. After constant bile-flow was established, animals were dosed with equimolar selenite and arsenite (0.63 and 0.60 mg/kg body weight, respectively, in phosphate buffered saline, pH 7.4) through injection in the tail vein. Bile was collected for 25 minutes post-injection, immediately mixed with 40% v/v glycerol and loaded into 2 mm × 3 mm × 22 mm bromine-free acetal homopolymer Delrin® cuvettes closed with metal and bromine-free mylar adhesive tape windows and frozen in liquid nitrogen. Samples were stored and transported at liquid nitrogen temperatures until XAS data acquisition.

2.3 X-ray Absorption Spectroscopy

Arsenic and selenium K-edge XAS spectra were measured on the structural molecular biology beamline 7-3 at the Stanford Synchrotron Radiation Lightsource (SSRL) using the data acquisition program XAS Collect [28]. A Si(220) double-crystal monochromator was used with harmonic rejection from an upstream Rh-coated mirror set at an angle of incidence to obtain a cutoff energy of ~15 keV. Incident X-ray intensities were monitored using nitrogen-filled gas

ionization chambers and X-ray absorption of samples was measured as the X-ray fluorescence excitation spectrum using a Canberra 30-element germanium detector array [29] (Canberra Ltd. Meriden, CT, USA) with fast analog electronics employing a Gaussian shaping amplifier shaping time of 0.125 μ s. Soller slits and Z-1 X-ray filters (Ge and As for As and Se, respectively) were used to preferentially reject unwanted scattered radiation and to limit detector count rates to the pseudo-linear regime [29]. Samples were mounted in a liquid helium flow cryostat (Oxford instruments, Abingdon, UK) to maintain an approximate temperature of 10K during data collection. Simultaneous absorption of downstream standard foils of elemental arsenic and hexagonal elemental selenium, were measured by transmittance as an internal energy standard. X-ray energy calibration used the lowest energy K-edge inflection point of the standard foils, assumed to be 11,867.0 and 12,658.0 eV for arsenic and selenium, respectively.

Extended X-ray absorption fine structure (EXAFS) oscillations $\chi(k)$ were analyzed using the EXAFSPAK program suite [30], as previously described [31] and assuming As and Se K-edge threshold energies (E_0) of 11,885 and 12,675 eV, respectively. FEFF version 8.45 was utilized to compute theoretical phase and amplitude functions.

2.4 Density functional theory (DFT) calculations

DFT calculations used the program Dmol³ and Biovia Accelrys Materials Studio V7.0 for geometry optimization [32,33]. Geometry optimization used the Perdew-Burke-Ernzerhof functionals [34] for both the potential during the self-consistent field procedure and the energy, and employing all-electron relativistic core treatment for all atoms. Double numerical basis sets included polarization functions for all atoms, and dispersion corrections used the Tkatchenko-Scheffler scheme [35]. Solvent effects were used only where stated, and in these cases were

simulated by using the Conductor-like Screening Model (COSMO) with a dielectric value representing water ($\epsilon = 78.39$) [36].

3. Results and Discussion

3.1 X-ray Absorption Spectroscopy

X-ray absorption spectra arise from photo-excitation of a core electron such as a 1s electron for a K-edge. The spectrum can be arbitrarily divided into two overlapping regions – the near-edge spectrum which is the structured region within approximately 50 eV of the absorption edge, and the extended X-ray absorption fine structure (EXAFS) which are oscillations in the absorption on the high-energy side of the absorption edge. The EXAFS arises from photoelectron backscattering by nearby atoms and can be accurately interpreted in terms of a local radial structure [37]. Intense absorptions in the near-edge spectrum arise from Laporte-allowed ($\Delta l = \pm 1$) transitions of the core electron to bound states. The near-edge spectrum, sometimes referred to as the X-ray absorption near-edge fine structure or XANES, provides a sensitive probe of electronic structure, and is most often used in conjunction with standard compounds in a spectroscopic matching or fingerprint type of analysis.

Fig. 2 shows the As and Se K near-edge spectra of rat bile, compared with the near-edge spectra of relevant standard compounds. The spectra show clear identity with that of synthetic $[(GS)_2AsSe]^-$ and, for selenium, the spectrum of $[(GS)_2AsSe]^-$ is quite distinctive among the model compounds. Linear combination analyses of the As and Se near-edge data, employing the standards of Fig. 2, indicates that the rat bile data fits to 91% and 97% $[(GS)_2AsSe]^-$ at the arsenic and selenium K-edge, respectively (not illustrated). The remaining of 9% for As fits to a single additional component of $[As(GS)_3]$ with an estimated standard deviation of 0.8%, while the 3% for Se fits to selenite with an estimated standard deviation of 0.3%. The latter is close to

our established rejection criteria in the refinements [38] and may not in fact be present in the bile. Comparison of the edge jumps with standard solutions indicates an As:Se ratio of 1.1:1.0, with slightly more As than Se in the bile sample. This is consistent with the fitting results, showing the presence of a small quantity (9%) of As(GS)₃ in the arsenic bile data. The arsenic spectrum of [(GS)₂AsSe][−] shows some similarities with other relevant model compounds, such as [As(GS)₃] and [(GS)₂AsCH₃] and while the broad post-edge feature at ~11876 eV shows some variability, these would be hard to distinguish based on their most intense near-edge spectrum peak alone. However, the presence of subtle energy shifts of the major peak, shown in the inset in Fig. 2a, means that with care the spectra can be distinguished, with shifts relative to the peak of [(GS)₂AsSe][−] of 0.35 and 0.25 eV for (GS)₃As and (GS)₂AsCH₃, respectively.

Fig. 3 shows the arsenic and selenium K-edge EXAFS spectra and corresponding Fourier transforms of rat bile, together with the best fits. The As K-edge EXAFS data is truncated at $k=14 \text{ \AA}^{-1}$ because of the selenium K-edge which occurs at just above this energy. The As K-edge EXAFS is dominated by strong backscattering arising both from As—S and As—Se coordination, with overlapping contributions giving a broader Fourier transform peak than would be expected for a single scattering type. The Se K-edge EXAFS data is dominated by Se—As backscattering, with a higher frequency component visible at low k (Fig. 3). Curve-fitting analysis gives interatomic distances consistent with those previously reported (Table 1), and as with the near-edge data, the EXAFS data are consistent with close to 100% of [(GS)₂AsSe][−]. The As—Se bond-lengths obtained from independent As and Se K-edge EXAFS analysis gives very consistent results with refined As—Se and Se—As bond-lengths of (2.320 ± 0.001) and $(2.321 \pm 0.002) \text{ \AA}$, for As and Se EXAFS data, respectively, with both Debye-Waller factors equivalent

within the respective errors. The best-fit As—S distance is 2.25 Å, in excellent agreement with previous analyses of the EXAFS of synthetic [(GS)₂AsSe][−] [8].

The high frequency Se EXAFS, visible at low k , is consistent with an outer shell contribution from a distant backscatterer. As discussed below, this could be oxygen from water molecules hydrogen bonded to the partially negatively charged selenium. The overall fit index improves slightly on inclusion of three such Se...O interactions (Table 1) with an interatomic distance of 3.47 Å, although the match between the fit and the experimental data at low- k is substantially improved upon their inclusion (Fig. 3). These interactions would correspond to water hydrogen bonded to the negatively charged selenium, with an arrangement Se...H—O—H, where the Se...H—O arrangement is nearly linear.

3.2 Density Functional Theory (DFT)

Because modelling large flexible structures such as [(GS)₂AsSe][−] with DFT tends to be problematic due to the large number of degrees of conformational freedom, we first examined the DFT of more compact model structures, both to gauge accuracy and to gain initial insights into [(GS)₂AsSe][−]. It is well known that DFT tends to over-estimate bond-lengths [39]. For example, DFT of arsenic(III)-*tris*-thiolates such as [(CH₃S)₃As] gives geometry optimized bond-lengths of around 2.28 Å, whereas the Cambridge Structural Database (CSD) [40] gives an average As—S bond-length of 2.24 Å for all arsenic(III)-*tris*-thiolates in which the thiolates are aliphatic; a DFT over-estimation of 0.04 Å. Terminal As—Se bonds are found in *bis*(μ₂-diselenido)-*bis*(selenido)-di-arsenic(III), [Se-As(Se₂)₂As-Se]^{2−} species, which contain two terminal selenides bound to each of the two arsenic atoms, which are themselves bridged by two diselenides [Se₂]^{2−} forming a core with a six-membered [As₂Se₄] ring with a chair conformation

[40]. DFT, constrained to C_{2h} point group symmetry, estimates the terminal As—Se bond-lengths as 2.37 Å, whereas the CSD gives a mean experimental value of 2.28 Å [40]; a DFT over-estimation of 0.09 Å. The ring As—Se(Se) bonds are also over-estimated with DFT giving 2.53 Å, compared with a database value of 2.43 Å [40]; an over-estimation of 0.10 Å. In general, over estimation of computed bond-lengths tend to be smaller for terminal ligands than for non-terminal ligands. Thus, $[\text{As}_3\text{S}_6]^{3-}$ anions (2,4,6-trisulfido-1,3,5,2,4,6-trithiatriarsinane) and the analogous selenium species $[\text{As}_3\text{Se}_6]^{3-}$ have six-membered chairs with alternating arsenic and chalcogenide plus terminal chalcogenides on each arsenic. Their computed terminal chalcogenide bond-lengths are over-estimated by less than 0.05 Å and 0.07 Å, for S and Se respectively, while the ring As—S/Se bond-lengths are over-estimated by 0.12 Å. We have previously observed similar over-estimates for the bond-lengths in the dimethyldiselenoarsinate anion [41]. The structure of the seleno *bis*-phenyl arsinium anion $[(\text{C}_6\text{H}_5)_2\text{AsSe}]^-$ has been reported [42] with As—C and As—Se bond-lengths of 1.97 and 2.34 Å, respectively, which DFT over-estimates by 0.06 and 0.02 Å, respectively. In our case the use of relativistic core potentials changes the results only slightly, improving the DFT match to crystallography by only about 0.02 Å; this is expected because relativistic effects are not anticipated to be large for any atoms in our calculations. Also as expected, omitting dispersion corrections introduces even larger discrepancies with bond-length over-estimations as large as 0.25 Å. Thus, we conclude that DFT consistently over-estimates bond-lengths in the systems studied, in some cases by more than 0.1 Å, and still larger discrepancies may be anticipated because of the narrow range of the systems we have evaluated.

Fig. 4 shows the energy minimized geometry optimized DFT structure of the hypothetical but chemically plausible species seleno *bis*-(S-methylthio) arsinium anion $[(\text{CH}_3\text{S})_2\text{AsSe}]^-$. The

molecule was constrained to C_s point group symmetry, with computations carried out without inclusion of solvent effects. The geometry optimized As—S and As—Se bond-lengths were 2.41 and 2.28 Å, respectively; the latter agrees reasonably well with the EXAFS value of 2.32 Å, while the former differs by 0.16 Å, which is large, but close to the expected bounds derived from our calculations of known structures, discussed above. The DFT computed partial atomic charge for the terminal selenium is -0.58, and for the arsenic is +0.12. Thus, the As—Se bonding in this species and by analogy in $[(GS)_2AsSe]^-$ is correctly formulated as a partial double bond, As====Se, as we have previously argued, based on EXAFS-derived bond-length, and Raman and ^{77}Se nuclear magnetic resonance spectroscopies [8]. Intermolecular hydrogen bonding has been previously observed between N-H groups and an arsenic-bound terminal selenide [42], and we have previously suggested that the hydrogen bonding to the protonated amines of the glutathione might act to stabilize $[(GS)_2AsSe]^-$ [20]. Such interactions might partly account for the weak long-range Se EXAFS, discussed above. We sought to use DFT to investigate whether hydrogen-bonded water might also contribute to the weak long-range observed interactions in the Se K-edge EXAFS of $[(GS)_2AsSe]^-$. Inclusion of a single water in proximity to the selenium of $[(CH_3S)_2AsSe]^-$ gives a geometry optimized Se····O interatomic distance of 3.47 Å, consistent with our EXAFS-derived distance. We find that this species can readily accommodate three such water molecules with similar Se····O interatomic distances, hydrogen bonded via an intervening water hydrogen to the negatively charged selenium, indicating that it is very plausible that the weak low k EXAFS discussed above may indeed come from waters associated with the negatively charged terminal selenide group in $[(GS)_2AsSe]^-$.

Fig. 5 shows the DFT energy minimized geometry optimized structure for the entire 72-atom entity $[(GS)_2AsSe]^-$. The most stable refinements included water molecules adjacent to the

selenium, or used the COSMO field to simulate water. As expected, the DFT core structure is very similar to that of $[(\text{CH}_3\text{S})_2\text{AsSe}]^-$, with bond-lengths for As—Se and As—S of 2.41 and 2.29 Å, which, allowing for DFT over-estimation are consistent with the EXAFS-derived bond-lengths (Table 1). In both $[(\text{CH}_3\text{S})_2\text{AsSe}]^-$ and $[(\text{GS})_2\text{AsSe}]^-$ the lowest unoccupied molecular orbital is a π^* orbital involving selenium and arsenic p-orbitals, and the distinctive pre-edge peak in the Se K-edge near-edge spectrum at 12659.3 eV can be assigned to a $\text{Se}(1s) \rightarrow \pi^*$ transition. The reported similarity of the selenium K-edge spectrum of the formally arsenic(V) species $[(\text{CH}_3)_2\text{AsSe}_2]^-$ [41] is consistent with this assignment, as the As—Se bonding in both species is expected to be similar.

In conclusion, we have shown that the primary product of biliary co-excretion of arsenic and selenium in rats is the seleno *bis*-(S-glutathionyl) arsinium anion $[(\text{GS})_2\text{AsSe}]^-$, and have shown that the XAS-derived conclusions about the solution structure of this biliary excretion are supported by density functional theory calculations. Unlike previous work using rabbit bile [8], the arsenic and selenium of rat bile are essentially entirely present as $[(\text{GS})_2\text{AsSe}]^-$ with less than 10% of arsenic and selenium present as other chemical forms. This difference between mammalian species is probably due to the presence of a gall bladder in the rabbit but not in the rat, which will concentrate the bile and decrease the pH [21], possibly causing partial breakdown of $[(\text{GS})_2\text{AsSe}]^-$.

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References

1. D. M. Jolliffe, J. R. Soc. Med. 86 (1993) 287-289.
2. D. Doyle, Brit. J. Haematol. 145 (2009) 309-317.
3. M. F. Ahmed, S. Ahuja, M. Alauddin, S. J. Hug, J. R. Lloyd, A. Pfaff, T. Pichler, C. Saltikov, M. Stute, A. van Geen, Science 314 (2006) 1687-1688.
4. D. G. Kinniburgh, P. L. Smedley, Eds., vol. 2 of Arsenic Contamination of Ground Water in Bangladesh, Final Report (BGS Technical Report WC/00/19, British Geological Survey, Keyworth, UK, 2001).
5. A. A. Meharg, (2005) "Venomous earth: How arsenic caused the world's worst mass poisoning" McMillan, Basingstoke UK, New York, NY USA.
6. M. Argos, T. Kalra, P. J. Rathouz, Y. Chen, B. Pierce, F. Parvez, I. Islam, A. Ahmed, M. Rakibuz-Zaman, R. Hasan, G. Sarwar, V. Slavkovich, A. van Geen, J. Graziano, H. Ahsan Lancet 376 (2010) 252-258.
7. A. L. Moxon, Science 88 (1938) 81.
8. J. Gailer, G. N. George, I. J. Pickering, R. C. Prince, S. C. Ringwald, J. C. Pemberton, R. S. Glass, H. S. Younis, D. W. DeYoung, H. V. Aposhian, J. Am. Chem. Soc. 122 (2000) 4637-4639.
9. J. Gailer, G. N. George, I. J. Pickering, R. C. Prince, H. S. Younis, J. Winzerling. Chem. Res. Toxicol. 15 (2002) 1466-1471.
10. J. Gailer, L. Ruprecht, P. Reitmeir, B. Benker, P. Schramel, Appl. Organomet. Chem. 18 (2004) 670-675.

11. S. A. Manley, G. N. George, I. J. Pickering, R. S. Glass, E. J. Prenner, R. Yamdagni, Q. Wu, J. Gailer, *Chem. Res. Toxicol.* 19 (2006) 601-607.
12. J. Gailer, *Biochimie* 91 (2009) 1268-1272.
13. H. E. Ganther, *J. Am. Coll. Toxicol.* 5 (1986) 1-5.
14. R. Zakharyan, Y. Wu, G. Bogdan, H. V. Aposhian, *Chem. Res. Toxicol.* 8 (1995) 1029-1038.
15. H.-J. Sun, B. Rathinasabapathi, B. Wu, J. Luo, L.-P. Pu, L. Q. Ma, *Environ. Int.* 69 (2014) 148-158.
16. O. A. Levander, L. C. Argrett, *Toxicol. Appl. Pharmacol.* 14 (1969) 308-314.
17. H. S. Hsieh, H. E. Ganther, *Biochemistry* 14 (1975) 1632-1636.
18. J. Gailer, *Coord. Chem. Rev.* 251 (2007) 234-254.
19. M. W. Carew, E. M. Leslie, *Carcinogenesis* 31 (2010) 1450-1455.
20. J. Gailer, G. N. George, I. J. Pickering, G. A. Buttigieg, M. B. Denton, R. S. Glass, J. Organomet. Chem. 650 (2002) 108-113.
21. D. Gleeson, K. A. Hood, G. M. Murphy, R. H. Dowling, *Gastroenterology*, 102 (1992) 1707-1716.
22. J. E. Spallholz, L. Mallory Boylan, M. M. Rhaman, *Sci. Total. Environ.* 323 (2004) 21-32.
23. V. K. Sharma, T. J. McDonald, M. Sohn, G. A. K. Anquandah, M. Pettine, R. Zboril, *Environ. Chem. Lett.* 13 (2015) 49-58.
24. <https://clinicaltrials.gov/ct2/show/NCT00392561>

25. <https://clinicaltrials.gov/ct2/show/NCT01442727>
26. <https://clinicaltrials.gov/ct2/show/NCT02377635>
27. I. J. Pickering, G. N. George, V. van Fleet-Stalder, T. G. Chasteen, R. C. Prince, *J. Biol. Inorg. Chem.* 4 (1999) 791-794.
28. M. J. George, *J. Synchrotron Radiat.* 7 (2000) 283-286.
29. S. P. Cramer, O. Tench, M. Yocum, G. N. George, *Nucl. Instrum. Meth. A*, 266 (1988) 586–591.
30. G. N. George, (2000) <http://ssrl.slac.stanford.edu/exafpak.html>
31. G. N. George, R. M. Garrett, R. C. Prince, K. V. Rajagopalan, *J. Am. Chem. Soc.* 118 (1996) 8588–8592.
32. B. J. Delley, *Chem. Phys.* 92 (1990) 508–517.
33. B. J. Delley, *Chem. Phys.* 113 (2000) 7756–7764.
34. J. P. Perdew, K. Burke, M. Ernzerhof, *Phys. Rev. Lett.* 77 (1996) 3865–3868; Erratum: 78 (1997) 1396.
35. A. Tkatchenko, M. Scheffler, *Phys. Rev. Lett.* 102 (2009) 073005.
36. A. Klamt, G. Schüürmann, *Chem. Soc. Perkin. Trans. 2* (1993) 799–805.
37. J. J. H. Cotelesage, M. J. Pushie, P. Grochulski, I. J. Pickering, G. N. George, *J. Inorg. Biochem.* 115 (2012) 127–137.
38. G. N. George, S. P. Singh, R. C. Prince, I. J. Pickering, *Chem. Res. Toxicol.* 21 (2008) 2106-2110.
39. Y. Minenkov, Å. Singstad, G. Occhipinti, V. R. Jensen, *Dalton Trans.* 41 (2012) 5526-5541.
40. F. H. Allen, *Acta Cryst. B*, 58 (2002) 380-388.

41. J. Gailer, G. N. George, H. H. Harris, I. J. Pickering, R. C. Prince, A. Somogyi, G. A. Buttigieg, R. S. Glass, M. B. Denton, *Inorg. Chem.* 41 (2002) 5426-5432.
42. T. Kanda, K. Mizoguchi, S. Kagohashi, S. Kato, *Organometallics*, 17 (1998) 1487-1491.

Table 1. $[(GS)_2AsSe]^-$ Extended X-ray absorption fine structure (EXAFS) curve-fitting results ^a

	Interaction	<i>N</i>	<i>R</i>	σ^2	ΔE_0	<i>F</i>
As K-edge	As—Se	1	2.320(1)	0.0026(4)	-14(2)	0.2817
	As—S	2	2.247(1)	0.0039(9)		
Se K-edge	Se—As	1	2.321(2)	0.0021(2)	-8.0(7)	0.2608
	Se···O (H ₂ O)	3	3.47(1)	0.009(2)		

a. Coordination number *N*, interatomic distances *R* (Å), Debye-Waller factors σ^2 (Å²), and threshold energy shift ΔE_0 (eV). Values in parentheses represent the standard uncertainties between the curve fitting parameters and spectroscopy data, expressed as the last digit of each value, obtained from the diagonals of the variance-covariance matrices. Goodness of fit function *F* is defined as $F = \sqrt{\sum k^6 (\chi(k)_{calc} - \chi(k)_{expt})^2 / \sum k^6 \chi(k)_{expt}^2}$, where $\chi(k)_{calc}$ and $\chi(k)_{expt}$ are the calculated and experimental EXAFS, respectively, with *k* (Å⁻¹) being the photoelectron wave vector and the sum being over all data points in the spectrum.

Fig. 1. Schematic structure of the seleno *bis*-(S-glutathionyl) arsinium anion $[(GS)_2AsSe]^-$. The charge states of the carboxylates and amines in the structure are those expected at physiological pH (7.4) and at the slightly acidic pH of bile (~6).

Fig. 2. X-ray absorption near-edge spectra for rat bile and relevant standard compounds, at the As K-edge (*a*) and the Se K-edge (*b*). All standard compounds were prepared at 1mM As or Se in buffered aqueous solution (50 mM HEPES, pH 7.4) mixed with 30% v/v glycerol. The inset in *a* shows detail of the peaks for selected spectra, showing subtle shifts. Vertical lines are included in both the main figure and the inset in order to guide the eye, indicating the peak positions of $[(GS)_2AsSe]^-$ (broken line), $(GS)_3As$ and $(GS)_2AsCH_3$, at 11869.45, 11869.80 and 11869.65 eV, respectively. In *b* *Se-Met* refers to seleno-L-methionine and *Sec-Cys*, refers to the selenyl sulfide created on reaction of L-selenocysteine with L-cysteine.

Fig. 3. Arsenic and selenium K-edge EXAFS of rat bile together with best fits (*a*) and corresponding Fourier transforms (*b*). Experimental data is shown using blue lines and the best fits using a red line. The green line in (*a*) shows the selenium EXAFS fit including only the Se—As interaction, with somewhat worse match in the low-*k* region, as discussed in the text. Parameters derived from the curve-fitting analysis are summarized in Table 1.

Fig. 4. Density functional theory energy minimized geometry optimized structure for $[(CH_3)_2AsSe]^-$, including three waters hydrogen bonded to the Se atom, as described in the text. In order to speed up the geometry optimization the structure was constrained to C_s point group symmetry.

Fig. 5. Density functional theory energy minimized geometry optimized structure for $[(GS)_2AsSe]^-$. When the expected ionization of the caboxylate and amine groups of the

glutathione moieties are accounted for (as CO_2^- and NH_3^+ , respectively), the species is overall a tri-anion.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5