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Simultaneous arsenic- and selenium-specific detection of the dimethyldiselenoarsinate anion by high-performance liquid chromatography–inductively coupled plasma atomic emission spectrometry

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The seleno-bis (*S*-glutathionyl) arsinium ion, $[(GS)_2AsSe]^-$, which can be synthesized from arsenite, selenite and glutathione (GSH) at physiological pH, fundamentally links the mammalian metabolism of arsenite with that of selenite and is potentially involved in the chronic toxicity/carcinogenicity of inorganic arsenic. A mammalian metabolite of inorganic arsenic, dimethylarsinic acid, reacts with selenite and GSH in a similar manner to form the dimethyldiselenoarsinate anion, $[(CH_3)_2As(Se)_2]^-$. Since dimethylarsinic acid is an environmentally abundant arsenic compound that could interfere with the mammalian metabolism of the essential trace element selenium via the *in vivo* formation of $[(CH_3)_2As(Se)_2]^-$, a chromatographic method was developed to rapidly identify this compound in aqueous samples. Using an inductively coupled plasma atomic emission spectrometer (ICP-AES) as the simultaneous arsenic- and selenium-specific detector, the chromatographic retention behaviour of $[(CH_3)_2As(Se)_2]^-$ was investigated on styrene–divinylbenzene-based high-performance liquid chromatography (HPLC) columns. With a Hamilton PRP-1 column as the stationary phase (250 × 4.1 mm ID, equipped with a guard column) and a phosphate-buffered saline buffer (0.01 mol dm^{-3}, pH 7.4) as the mobile phase, $[(CH_3)_2As(Se)_2]^-$ was identified in the column effluent according to its arsenic : selenium molar ratio of 1 : 2. With this stationary phase/mobile phase combination, $[(CH_3)_2As(Se)_2]^-$ was baseline-separated from arsenite, selenite, dimethylarsinate, methylarsonate and low molecular weight thiols (GSH, oxidized GSH) that are frequently encountered in biological samples. Thus, the HPLC–ICP-AES method developed should be useful for rapid identification and quantification of $[(CH_3)_2As(Se)_2]^-$ in biological fluids.

KEYWORDS: dimethyldiselenoarsinate anion; HPLC; ICP-AES

INTRODUCTION

Among the environmentally abundant arsenic and selenium compounds, arsenite and selenite are most acutely toxic and teratogenic for mammals.[1] Once ingested, the biochemistry of both metalloid compounds is driven by interactions with endogenous thiols, the most abundant of which is glutathione (GSH).[2] In contrast to arsenite, which can react with up to 3 mol equivalents of GSH to $(GS)_3As$,[3,4] selenite is reduced by 6 mol equivalents of GSH to HSe^-.[5] Equimolar arsenite and selenite, however, react with 8 mol equivalents of GSH to form the seleno-bis (*S*-glutathionyl) arsinium ion, $[(GS)_2AsSe]^-$,[6] which has been detected in rabbit bile by X-ray absorption spectroscopy (XAS)[7] and size-exclusion chromatography (SEC) coupled on-line to an inductively coupled plasma atomic emission spectrometer (ICP-AES).[8] Since inorganic pollutants which individually react with *in vivo*-generated highly reactive selenium metabolites (to form

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compounds with selenium–metal bonds) will cumulatively effect the metabolism of selenium,[9] all environmentally abundant metals or metalloid compounds that behave in this manner should be identified.

To this end, a systematic investigation of the reaction between dimethylarsinate, selenite and GSH at physiological pH revealed that 8 mol equivalents of GSH are required for the formation of the dimethyldiselenoarsinate anion, $[(CH_3)_2As(Se)_2]^-$.[10] Dimethylarsinate is a natural mammalian metabolite of inorganic arsenic,[11] it is frequently found in fresh and saline waters,[11,12] it occurs in seafood,[13] and it is also used as an insecticide, pesticide and herbicide.[14,15] The simultaneous exposure of the general population to dimethylarsinate and dietary selenium compounds could, therefore, lead to the *in vivo* formation of $[(CH_3)_2As(Se)_2]^-$. Consequently, analytical methods must be developed for the rapid identification of $[(CH_3)_2As(Se)_2]^-$ in biological samples. Since $[(CH_3)_2As(Se)_2]^-$ and $[(GS)_2AsSe]^-$ have very similar selenium XAS K near-edge spectra,[10] the development of a high-performance liquid chromatography (HPLC) method for the unambiguous identification of $[(CH_3)_2As(Se)_2]^-$ in biological samples is highly desirable. In view of the successful identification of $[(GS)_2AsSe]^-$ in rabbit bile by SEC coupled on-line with ICP-AES,[8] and since $[(CH_3)_2As(Se)_2]^-$ also contains selenium and arsenic, ICP-AES is a suitable spectroscopic technique for the detection of $[(CH_3)_2As(Se)_2]^-$ in biological samples. Additionally, ICP-mass spectrometry (ICP-MS) must also be considered useful for the identification of $[(CH_3)_2As(Se)_2]^-$ in biological fluids after HPLC separation.

During the development of an SEC–ICP-AES method for the identification of $[(GS)_2AsSe]^-$ in aqueous solutions, we previously noticed that the stationary phase must be chosen carefully in order to prevent the decomposition of this species during the chromatographic separation.[6] In view of these findings, and with regard to the similar chemical properties of $[(GS)_2AsSe]^-$ and $[(CH_3)_2As(Se)_2]^-$ (both are oxygen sensitive and decompose below pH 7.0),[6,10] the development of an HPLC method for the identification of $[(CH_3)_2As(Se)_2]^-$ requires that this species can be passed through the stationary phase unchanged and structurally intact. $[(CH_3)_2As(Se)_2]^-$ contains two As–C and two As–Se single bonds. Since As–C bonds are known to be stable covalent bonds,[16] the structural element $(CH_3)_2As$ in $[(CH_3)_2As(Se)_2]^-$ is expected to remain unchanged during the migration of this compound through a chromatographic bed at ambient temperature. Even though the stability of the two As–Se single bonds in $[(CH_3)_2As(Se)_2]^-$ during a chromatographic separation cannot be predicted, the detection of a peak in the column effluent with an arsenic : selenium molar ratio of 1 : 2 would provide an elegant means to prove that $[(CH_3)_2As(Se)_2]^-$ did not decompose or rearrange during the chromatographic separation process. Dimerization appears chemically unlikely, due to the negative charge on the species.

To develop an HPLC method for the separation and identification of $[(CH_3)_2As(Se)_2]^-$, we investigated numerous aqueous mobile phase/stationary phase combinations using ICP-AES for simultaneous arsenic-, selenium- and sulfur-specific detection. Since $[(CH_3)_2As(Se)_2]^-$ contains a negative charge and two methyl groups, a Hamilton PRP-X100 anion-exchange column and a Hamilton PRP-1 HPLC column were investigated.

EXPERIMENTAL

Chemicals and solutions

Sodium selenite pentahydrate ($Na_2SeO_3 \cdot 5H_2O$) and methanol were purchased from Merck (Darmstadt, Germany) and sodium arsenite ($NaAsO_2$) was obtained from GFS Chemicals (Columbus, OH, USA). Sodium cacodylate [$(CH_3)_2As(O)ONa \cdot 2.5H_2O$] and reduced glutathione (GSH, 98%) were purchased from Sigma Chemicals (St Louis, MO, USA). Disodium hydrogen phosphate ($Na_2HPO_4 \cdot 2H_2O$) and sodium dihydrogen phosphate ($NaH_2PO_4 \cdot H_2O$) were purchased from Merck. Sodium phosphate buffer (0.03 mol dm^{-3}) of pH 7.4 was prepared by mixing solutions of 0.03 mol dm^{-3} disodium hydrogen phosphate and 0.03 mol dm^{-3} sodium dihydrogen phosphate. Phosphate-buffered saline (PBS, 0.01 mol dm^{-3} phosphate; 0.0027 mol dm^{-3} KCl and 0.137 mol dm^{-3} NaCl) was prepared by dissolving PBS tablets (Sigma) in the appropriate volume of doubly distilled water. Aqueous solutions containing arsenic : selenium molar ratios of 1 : 1, 1 : 2 and 1 : 3 were prepared by dissolving the appropriate amounts of $NaAsO_2$ and $Na_2SeO_3 \cdot 5H_2O$ in distilled water. $[(CH_3)_2As(Se)_2]^-$ was synthesized as previously reported[10] and was used for all chromatographic investigations. Next to $[(CH_3)_2As(Se)_2]^-$, this solution also contained oxidized glutathione (GSSG, a by-product from the reaction), free GSH (to protect the oxygen-sensitive species) and unreacted dimethylarsinic acid (possibly bound to GSH).

Chromatography

The HPLC system consisted of a Beckman 110 B solvent delivery module, a Rheodyne 6-port injection valve (50 µl loop) and either a Hamilton PRP-1 column (Reno, NV, USA; 250 × 4.1 mm ID; spherical, 10 µm particles of a styrene–divinylbenzene copolymer) or a Hamilton PRP-X100 column (Reno, NV, USA; 250 × 4.1 mm ID; spherical 10 µm particles of a styrene–divinylbenzene copolymer with trimethylammonium exchange sites, exchange capacity 0.19 meq g^{-1}). Both columns were operated with guard cartridges filled with the same stationary phase. The effect of methanol on the retention behaviour of $[(CH_3)_2As(Se)_2]^-$ was studied on a Hamilton PRP-1 column (Reno, NV, USA; 100 × 4.1 mm ID). Each column was equilibrated with the corresponding mobile phase at a flow rate of 1.0 cm^3 min^{-1} for at least 1 h. All separations were carried out at 25 °C and a flow rate of 1.0 cm^3 min^{-1} was maintained throughout the study. The void volume of the PRP-1 column was determined

with aqueous arsenite ([As] = 50 mg dm^{-3}) and was 1.90 min. The retention times of methylarsonate, sodium selenite, GSH and dimethylarsinate were 2.03 min, 2.06 min, 2.41 min and 2.78 min respectively.

ICP-AES detection

Simultaneous arsenic-, selenium- and sulfur-specific detection was achieved with a Thermo Jarrel Ash IRIS HR radial-view ICP atomic emission spectrometer (Franklin, MA, USA). A ThermoSPEC/CID software (version 2.2.1.c1) provided the necessary time-scan functions and the multitasking controller allowed the processing of one atomic emission line every 0.02 s. The integral, proprietory data files were read using custom-written software, which also provided the ability to perform the derivative as well as third-order polynomial smoothing. The nebulization gas-flow was maintained at 2 dm^3 min^{-1}, the plasma forward power at 1150 W, and the CID temperature at −85 °C. The column exit was coupled to the Meinhardt TR-30-K3 concentric glass tube nebulizer (J.E. Meinhardt Assoc. Inc., Santa Ana, CA, USA) with a polyethylene tube (length 12 cm). Accumulating charge on the detector of the ICP atomic emission spectrometer from arsenic was monitored at 228.812 nm (order 147), from sulfur at 182.624 nm (order 184) and from selenium at 206.279 nm (order 164).

RESULTS AND DISCUSSION

To develop an HPLC method for the identification of $[(CH_3)_2As(Se)_2]^-$ in aqueous solutions, two retention mechanisms appear chemically feasible. The negative charge on $[(CH_3)_2As(Se)_2]^-$ could be exploited to retain this species on an anion-exchange column, provided that the compound can be passed through the column intact. On the other hand, the methyl groups on $[(CH_3)_2As(Se)_2]^-$ should render this molecule sufficiently hydrophobic to be retained on a hydrophobic stationary phase. Additionally, the latter retention strategy would most likely also minimize a possible decomposition of $[(CH_3)_2As(Se)_2]^-$ during the chromatographic separation process, as was previously observed with the structurally related species $[(GS)_2AsSe]^-$ on anion-exchange columns.[6] Since the ultimate goal was to develop a chromatographic method for the identification of $[(CH_3)_2As(Se)_2]^-$ in biological samples, PBS buffers (0.01 mol dm^{-3}) were investigated as mobile phases (the utilization of other mobile phases could lead to the decomposition of $[(CH_3)_2As(Se)_2]^-$ on the chromatographic system). The decomposition of $[(CH_3)_2As(Se)_2]^-$ below pH 7.0, however, required a mobile phase with pH > 7.0.

Since a Hamilton PRP-X100 anion-exchange column had already been used successfully to retain and chromatographically separate arsenic-containing anions, such as arsenate, methylarsonate and dimethylarsinate,[17] the retention behaviour of $[(CH_3)_2As(Se)_2]^-$ was investigated on this stationary phase. However, neither a sodium phosphate buffer of pH 7.4 nor of pH 8.5 (both 0.03 mol dm^{-3} and degassed with nitrogen) allowed $[(CH_3)_2As(Se)_2]^-$ to pass through the column intact. This was indicated by the fact that only an arsenic peak eluted from the column. Owing to the apparent decomposition of $[(CH_3)_2As(Se)_2]^-$ on this stationary phase at the mobile phase pH values investigated, 0.01 mol dm^{-3} GSH was added to the sodium phosphate buffer (the pH was readjusted to pH 7.4 after the addition of GSH) in an attempt to stabilize $[(CH_3)_2As(Se)_2]^-$ during the chromatographic separation process. The results were similar to the experiments with phosphate buffer alone. Even though the specific mechanism for the on-column decomposition of $[(CH_3)_2As(Se)_2]^-$ on this particular stationary phase is unknown, the positively charged trimethylammonium exchange sites are likely involved (see below).

Using a Hamilton PRP-1 column and a PBS buffer (0.01 mol dm^{-3}, pH 7.4) as the mobile phase, a peak containing arsenic and selenium eluted from the column (Fig. 1). The sulfur-specific chromatogram revealed a rather broad sulfur-containing double peak that essentially eluted in the void volume of the column and corresponds to GSSG and unreacted GSH, which may be complexed to unreacted dimethylarsinate. The arsenic-specific chromatogram revealed two major peaks. The first had a retention time of 2.24 min and accounted for 43% of the arsenic that eluted from the column. Its peak shape clearly indicated the presence of more than one arsenic-containing compound and most likely corresponds to a mixture between free dimethylarsinate (retention time 2.78 min) and GSH-complexed dimethylarsinate (GSH alone had a retention time of 2.41 min). The second peak (which eluted simultaneously with selenium) had a retention time of 4.65 min, accounted for the remaining 57% of total arsenic and was extremely broad in comparison with chromatographic peaks that were previously obtained with this column using a comparable flow rate but a flame atomic absorption spectrometer as the detector.[18] Even though the reason for this behaviour is not known with certainty, we have reason to believe that it is due to instrumental memory effects (related to the nebulizer used) and is not caused by the HPLC column itself. Nevertheless, the peak shape indicates a single arsenic-containing compound. The selenium-specific chromatogram revealed two peaks with retention times of 2.62 and 4.65 min. The first peak accounted for only 5% of the total selenium that eluted from the column, and the second accounted for the remaining 95%. This second peak also co-eluted with the second arsenic peak which suggests that this peak corresponds to $[(CH_3)_2As(Se)_2]^-$. Direct experimental evidence in favour of the elution of unchanged $[(CH_3)_2As(Se)_2]^-$ could be obtained by calculating the selenium : arsenic emission intensity ratio at the peak maximum from this chromatogram. The comparison of this ratio with ratios that were obtained for standard solutions with different molar selenium : arsenic ratios (1 : 1, 2 : 1 and 3 : 1) clearly demonstrated that the selenium : arsenic molar ratio of this chromatographic peak was 2 : 1 and that $[(CH_3)_2As(Se)_2]^-$ had therefore migrated through the chromatographic bed structurally unchanged. Since the retention

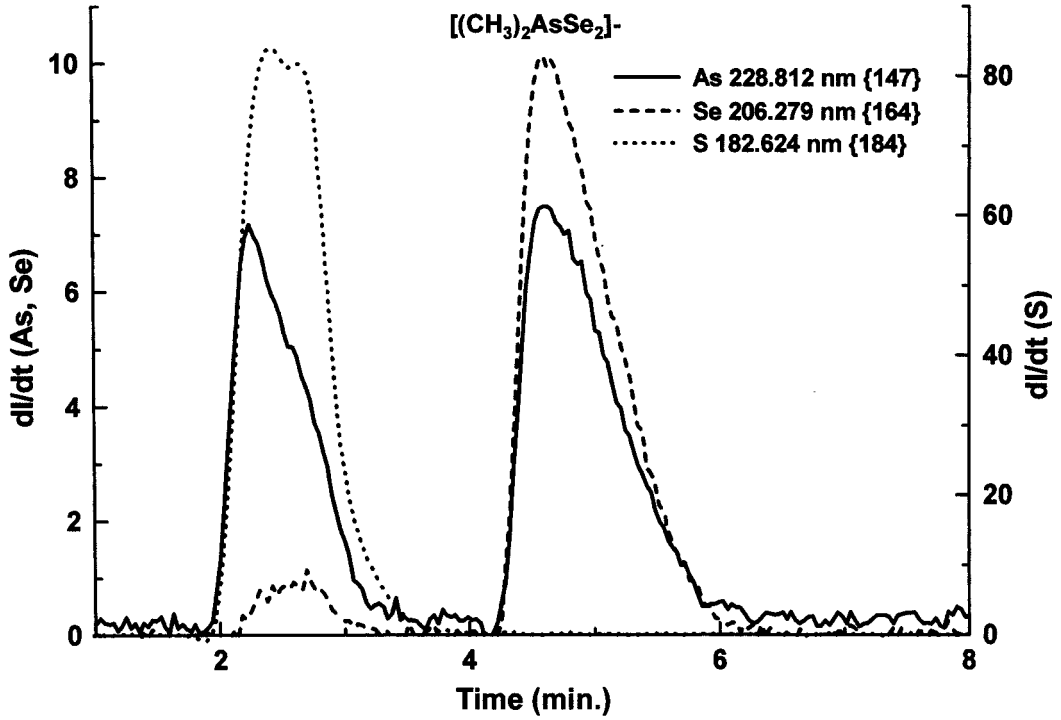

Figure 1. Arsenic-, selenium- and sulfur-specific chromatogram of a solution containing $[(CH_3)_2As(Se)_2]^-$ (0.038 mol dm^{-3}), GSSG and dimethylarsinate by HPLC–ICP-AES. Column: Hamilton PRP-1 column (250 × 4.1 mm ID with guard column); mobile phase: PBS buffer (0.01 mol dm^{-3}, pH 7.4); flow rate: 1.0 cm^3 min^{-1}; loop: 50 µl.

times of arsenite, selenite, dimethylarsinic acid, methylarsonic acid, GSH and GSSG (Fig. 1) were all less than 2.8 min, the HPLC–ICP-AES method developed should be capable of identifying $[(CH_3)_2As(Se)_2]^-$ in biological samples.

In order to gain insight into the chromatographic retention mechanism, the effect of methanol on the retention time of $[(CH_3)_2As(Se)_2]^-$ was investigated. Increasing the concentration of methanol in the pH 7.4 PBS buffer mobile phase in 5% increments up to 15% brought about a pronounced decrease of the retention time of $[(CH_3)_2As(Se)_2]^-$ on a 100 × 4.1 mm ID PRP-1 column (the k' decreased from 1.45 with PBS buffer alone to 0.82 with 15% methanol). This indicates that hydrophobic interactions between the methyl groups of $[(CH_3)_2As(Se)_2]^-$ and the hydrophobic backbone of the stationary phase are involved in the retention mechanism.

Using a calibration curve in which the concentration of $[(CH_3)_2As(Se)_2]^-$ (in the solution injected into the sample loop) was plotted against the selenium emission intensity (the 206.279 nm emission line) of the peak corresponding to $[(CH_3)_2As(Se)_2]^-$, the absolute detection limit of this HPLC–ICP-AES method (50 µl loop; flow-rate: 1.0 cm^3 min^{-1}) was 29 µg $[(CH_3)_2As(Se)_2]^-$ (5 σ of background). The utilization of a 250 µl loop would decrease the absolute detection limit to approximately 6 µg $[(CH_3)_2As(Se)_2]^-$ or [Se] = 14 mg dm^{-3}. Compared with the arsenic and selenium concentrations of approximately 20 mg dm^{-3} that were previously measured in bile after the intravenous injection of rabbits with selenite and arsenite (~0.6 mg of arsenic and selenium per kilogram body weight),[7,8] the detection limit of the HPLC–ICP-AES method developed (250 µl loop) should be sufficient to identify $[(CH_3)_2As(Se)_2]^-$ in biological fluids from animals treated with similar doses of the particular metalloid compounds (dimethylarsinic acid and selenite).

CONCLUSIONS

We report herein the first HPLC method for the rapid identification of $[(CH_3)_2As(Se)_2]^-$ in aqueous solutions using ICP-AES for simultaneous arsenic-, selenium- and sulfur-specific detection. With a Hamilton PRP-1 column and using a PBS buffer (0.01 mol dm^{-3}, pH 7.4) as the mobile phase, $[(CH_3)_2As(Se)_2]^-$ was identified in the column effluent by its arsenic : selenium molar ratio. Interactions between the methyl groups of $[(CH_3)_2As(Se)_2]^-$ and the hydrophobic stationary phase retained this compound to allow its baseline-separation from arsenite, dimethylarsinate, methylarsonate, selenite, GSH and GSSG. The element specificity of the ICP-AES and the demonstrated chromatographic removal of biological matrix molecules (GSH and GSSG) should render this HPLC–ICP-AES

method suitable for the rapid identification and quantification of $[(CH_3)_2As(Se)_2]^-$ in biological samples, such as bile.[8]

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