

Chemical reactions of As complexation by glutathione: an XAFS study

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Abstract. In this study, the chemical reactions between As(III) and As(V) with glutathione, which is a target compound in As biochemistry due to its primordial role in As immobilization and intracellular reduction, in various molar ratios were investigated using As K-edge XAFS spectroscopy. Results showed a gradual substitution of As-O bonds in the coordination of aqueous As(III) and As(V) for three As-S bonds in the As+GSH complex. Moreover, the data showed reduction of As(V) to As(III) prior or concomitant to the As+GSH complex formation.

1. Introduction

Inorganic As oxyanions are known for their high toxicity and represent an environmental concern in many countries [1]. Chemical reactions between organic compounds and inorganic arsenic have been under investigation in an attempt to better understand the mechanisms by which arsenic may be intracellularly immobilized by living organisms or their environment. Once absorbed by living cells, As can be immobilized in peptides containing sulfhydryl groups or methylated to produce organic As compounds. The reduced tripeptide glutathione (γ -glutamylcysteinylglycine; GSH) plays a critical role in the cell homeostasis as the most abundant reducing agents in various biochemical pathways [2]. Complexation of As with thiol functional groups like those in GSH has been studied using various techniques [3-8] but, to our knowledge, a systematic XAFS study is still lacking.

X-ray absorption fine structure (XAFS) spectroscopy is element specific and allows to discriminate the oxidation state of a target atom in combination with its local structure (coordination). This study focused on the investigation of the chemical reaction between arsenite [As(III)] and arsenate [As(V)] and GSH using XAFS. Contributions to understand As behavior in covalent bond with sulfhydryl groups are also provided in this study.

2. Materials and Methods

As(III)+GSH and As(V)+GSH solutions (pH = 4.3) were prepared in 1:0.5, 1:1, 1:2, 1:4 (for As(III)) and 1:0.5, 1:2, 1:4, 1:6 (for As(V)) molar ratios, with GSH concentration of 0.16 M. Arsenic K-edge (11868 eV) XAFS data were collected at the National Synchrotron Light Laboratory (LNLS) in



Campinas, Brazil, in transmission mode using a double crystal monochromator equipped with Si (111) crystals and ionization chambers operating with air at atmospheric pressure. Energy calibration was monitored by acquiring simultaneous reference spectra from an Au foil at the Au L1-edge (11918 eV). Theoretical phase shift and scattering amplitude functions were calculated by FEFF 6.0 [9] and the data were analyzed as described elsewhere [10] using software (Athena and Artemis) included in the IFEFFIT computer package [11]. Theoretical scattering paths were calculated based on the following crystalline compounds: As^{III}-O path from NaAsO₂, As^{III}-S path from As₂S₃ and As^V-O from As₂O₅. The theoretical paths were tested by fitting data obtained from aqueous As(III) and As(V) and crystalline standards. A value of 0.87 ± 0.05 for S_0^2 was obtained from fits to crystalline NaAsO₂ and used in all fits. A k-range of 3.0 to 14.5 Å⁻¹ and a R-range of 1.0 to 3.0 Å were used for Fourier transform and fit the data, respectively. XANES (X-ray absorption near-edge spectroscopy) spectra for aqueous As(III) and As(V) and As(III)+GSH in the 1:4 ratio were used as basis functions for linear combination fits.

3. Results and discussion

The As(III) XANES spectra (Fig. 1A) show a shift in absorption edge as the GSH concentration increased. This is consistent with formation of As bonds with the less electronegative S replacing O in the aqueous complex. As(V) data (Fig. 1B) show the appearance of what looks like a 2nd edge positioned at As(III) energy values, indicating reduction of As(V) to As(III) prior or concomitant to As-S bond formation. The reactions saturate at 1:4 and 1:6 molar ratios for As(III) and As(V), respectively, indicating that extra GSH is necessary to reduce As(V).

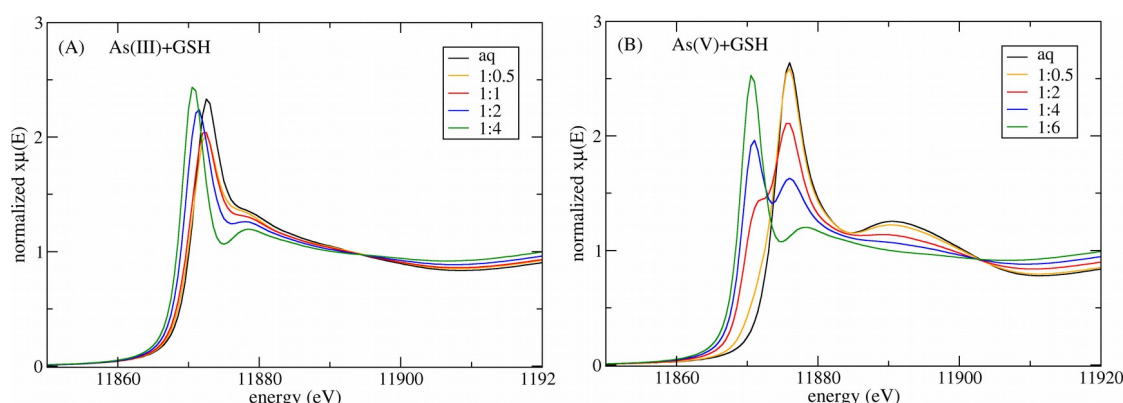


Figure 1. Normalized As K-edge XANES spectra for (A) As(III)+GSH and (B) As(V)+GSH at decreasing As:GSH molar ratios. Aqueous As(III) and As(V) are represented by the label aq.

Linear combination fits were applied to the XANES data (Fig. 2) to determine the chemical species present in the samples with intermediate As:GSH ratios (in which saturation was not reached.) The fits show that decreasing As(III):GSH ratio leads to an increase in the proportion of As^{III}-S bonds at the expense of As^{III}-O bonds. Fits for the As(V)+GSH system required the inclusion of three possible bonds As^V-O, As^{III}-O, and As^{III}-S. The As^{III}-S bond was observed in the 1:2 and 1:4 samples but not in the 1:0.5 sample. These samples show the presence of the three possible bonds in different proportions in a fashion consistent with the proposed mechanism of As(V) reduction to As(III) prior or concomitant to the formation of As-S bonds in the As(GS)₃ complex.

The changes in As coordination are confirmed by the magnitude of the Fourier transform of the EXAFS (extended X-ray absorption fine structure) oscillations (Fig. 3). The As-O signal gradually decreases accompanied by a gradual increase of the As-S signal (see arrows). The signal of the As^{III}-S bond is observed from 1:0.5 up to 1:4 for As(III) and from 1:2 up to 1:6 for As(V). The formation of As^{III}-S from As^V-O at 1:2 and above is consistent with the consumption of GSH in the As(V) to As(III) reduction process prior or concomitant to As(GS)₃ formation. Indeed, this reduction has previously

been demonstrated by ^1H and ^{13}C NMR from studies with bench-prepared solutions [4]. Our findings are also consistent with a recent report [5] which showed that the reduction of organoarsenic(V) compounds in the presence of GSH is slower than the complexation. Thus, the reduction step is rate-limiting. Here, the As(V) reduction was complete at a As(V):GSH ratio of 1:6.

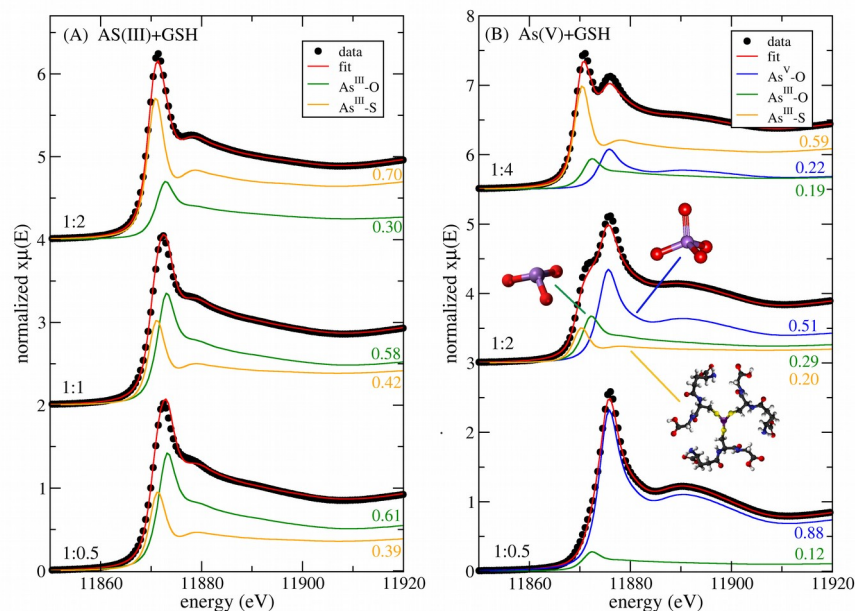


Figure 2. Linear combination fits of XANES spectra obtained for (A) As(III)+GSH and (B) As(V)+GSH at decreasing As:GSH molar ratios. Fractions of each As coordination are shown in matching colors with the legend.

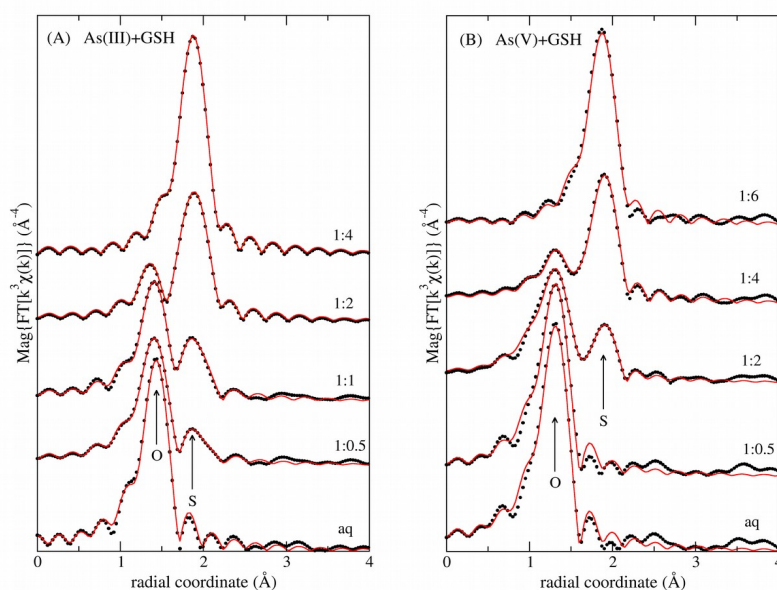


Figure 3. Magnitude of Fourier-transformed EXAFS data for (A) As(III)+GSH and (B) As(V)+GSH at decreasing As:GSH molar ratios.

The best-fit results to the EXAFS data show the gradual substitution of As-O bonds in the coordination of As(III) and As(V), formed respectively by approximately 3 to 4 oxygen atoms, towards the formation of the three As-S bonds in the GSH complex (Table 1). As-O bond lengths and σ^2 values for the As(V)+GSH samples confirm the presence of both As^{III}-O and As^V-O bonds in the intermediate reaction steps.

These fit results are consistent with previous studies on aqueous As(III) characterization [12] and As(GS)₃ complex formation [13]. It is important to note that EXAFS data provide an averaged picture of the local coordination of As and cannot rule out the possibility of oxygen and sulfur binding simultaneously to As ions. However, the formation of organometallic complex bearing oxygen and sulfur bound simultaneously to As in living organisms is still to be demonstrated [14,15].

Table 1. Best-fit results to EXAFS data \pm uncertainty values. N = coordination number; R = bond length; σ^2 = Debye-Waller factor; relative misfit (R-factor) $\leq 0.7\%$ (for all fits).

Samples	As-O			As-S		
	N	R (Å)	σ^2 (10^{-3} Å ²)	N	R (Å)	σ^2 (10^{-3} Å ²)
As(III) aqueous	3.1 \pm 0.1	1.791 \pm 0.007	2.1 \pm 0.4	-	-	-
As(III)+GSH 1:0.5	2.4 \pm 0.1	1.789 \pm 0.002	2.9 \pm 0.2	0.3 \pm 0.1	2.282 \pm 0.011	2.3 \pm 1.2
As(III)+GSH 1:1	2.0 \pm 0.1	1.785 \pm 0.004	1.7 \pm 0.4	0.7 \pm 0.1	2.272 \pm 0.007	2.2 \pm 0.9
As(III)+GSH 1:2	0.8 \pm 0.1	1.790 \pm 0.012	1.5 \pm 0.9	1.9 \pm 0.2	2.266 \pm 0.008	2.7 \pm 0.8
As(III)+GSH 1:4	-	-	-	3.1 \pm 0.2	2.254 \pm 0.007	2.8 \pm 0.7
As(V) aqueous	4.2 \pm 0.3	1.691 \pm 0.006	3.4 \pm 0.7	-	-	-
As(V)+GSH 1:0.5	3.9 \pm 0.3	1.695 \pm 0.005	3.6 \pm 0.8	-	-	-
As(V)+GSH 1:2	3.3 \pm 0.2	1.713 \pm 0.006	6.0 \pm 0.8	1.2 \pm 0.2	2.244 \pm 0.009	4.4 \pm 1.3
As(V)+GSH 1:4	1.4 \pm 0.2	1.728 \pm 0.010	4.9 \pm 1.5	2.1 \pm 0.2	2.258 \pm 0.005	3.1 \pm 0.6
As(V)+GSH 1:6	-	-	-	3.2 \pm 0.2	2.254 \pm 0.006	3.1 \pm 0.7

4. Conclusion

Arsenic complexation by GSH was studied by XAFS spectroscopy. It has been shown that in the presence of GSH, As-O bonds from aqueous arsenate and arsenite are replaced by As-S bonds to form As+GSH complexes. If arsenic is initially in arsenate form (As(V)), then it is reduced into arsenite (As(III)) prior to the formation of the As+GSH complex. Information about the molecular mechanisms of As bonding can help clarify the complex biochemistry of this element providing means to develop efficient remediation processes and methods.

Acknowledgments: Financial support was provided by the National Institute of Science and Technology on Mineral Resources, Water and Biodiversity (INCT-Acqua). The Synchrotron Light Laboratory (LNLS) in Campinas, Brazil, provided lodging and logistic support to the first two authors for the measurements.

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