

A XAFS study on Arsenic and GSH molecular interactions

Maione W. Franco¹, Igor F. Vasconcelos², Luzia V. Modolo³, Francisco A. R. Barbosa^{1*}

¹Laboratory of limnology, ecotoxicology and aquatic ecology, Institute of Biology and Science. Universidade Federal de Minas Gerais, Belo Horizonte, MG, 31270-901, Brasil.

²Department of Metallurgical and Materials Engineering, Universidade Federal do Ceará, Campus do Pici Bloco 714, CEP 60455-760, Fortaleza, CE, Brasil

³Department of Botany, Universidade Federal de Minas Gerais. Belo Horizonte, MG, 31.270-901, Brasil

Corresponding author: barbosa@icb.ufmg.br – Av. Antônio Carlos, 6627, Zip code 30.270-901, Belo Horizonte, MG, Brazil.

The chemical reactions between organic compounds and inorganic arsenic (As) have been investigated in an attempt to better understand the mechanisms by which arsenic may be immobilized by living organisms. The tripeptide glutathione (γ -glutamylcysteinylglycine; GSH) plays a critical role in the cell homeostasis as the most abundant reducing agents in various biochemical pathways (Monostori et al., 2009). This tripeptide has been used as a model molecule to study As binding to reduced sulfhydryl groups.

X-ray Absorption Spectroscopy (XAFS) provides data in atomic scale, is element specific and allows to discriminate the oxidation state of a target atom in combination with its local structure (coordination). In this study we investigated the chemical reactions between As(III) or As(V) with GSH using X-ray absorption spectroscopy (XAFS). XAFS measurements were performed in freshly prepared solutions in the molar ratios of 1: 0.5, 1: 1, 1: 2, 1: 4 As(III)+GSH and 1: 0.5 ; 1: 2, 1: 4, 1: 6 As(V)+GSH. The measurements were performed at the National Synchrotron Light Laboratory (LNLS) in Campinas, Brazil. The data were analyzed by using software (Athena, Artemis and FEFF 6.0) (Ravel and Newville, 2005). The passive electrons reduction factor (S_0^2) of 0.87 ± 0.05 was obtained from a fit to a crystalline standard (NaAsO_2) and used in all fits to the data. The XANES spectra for As(III)+GSH showed a clear shift in absorption edge towards lower energy values as the As:GSH ratio changes from 1:0 (aqueous As(III)) to 1:4 (Fig. 1A), the reduction in the energy absorption edge is remarkable in As(V)+GSH samples (Figure 1B). A saturation is observed (in green) in the higher proportion of GSH regardless of the initial As oxidation state (Fig. 1 A and B). This

phenomena is a result of changes in electron density of As atom which was initially coordinated by oxygen and became coordinated by the less electronegative sulfur atom in the reaction product: the complex $\text{As}(\text{GS})_3$. The fit of the Extended X-Ray Absorption Fine Structure (EXAFS) spectra showed a phase shift due to differences in As coordination (Figure 1 C). The magnitude of the Fourier transform of the EXAFS data (Figure 2) shows evident changes in the As coordination with increasing proportions of GSH as indicated by arrows, the signal provided by the As-O bond gradually decreases accompanied by a gradual increase in the signal provided by the As-S bond which became evident in the 1:2 proportion. This is consistent with the consumption of GSH in the As(V) to As(III) reduction process as a precondition for the complex formation.

Best fit results to EXAFS data (Table 1) shows the gradual substitution of As-O bonds in the coordination of As(III) or As(V) formed respectively by approximately 3 or 4 oxygen atoms, towards the formation of the three As-S bonds in the GSH complex. This previous reduction of As(V) to As(III) before complexation with GSH was also demonstrated in prepared solutions using proton and ^{13}C NMR (Delnomdedieu et al., 1994). EXAFS specially provides information on atomic environment. In the present study, the higher σ^2 was obtained for the intermediate proportions of As(V)+GSH, indicating more disorder in the local atomic environment that may be a consequence of the reaction steps, in which the intermediary As(III) is formed before complexation. As-S bond lengths do not significantly changed between samples confirming the robustness of the fits.

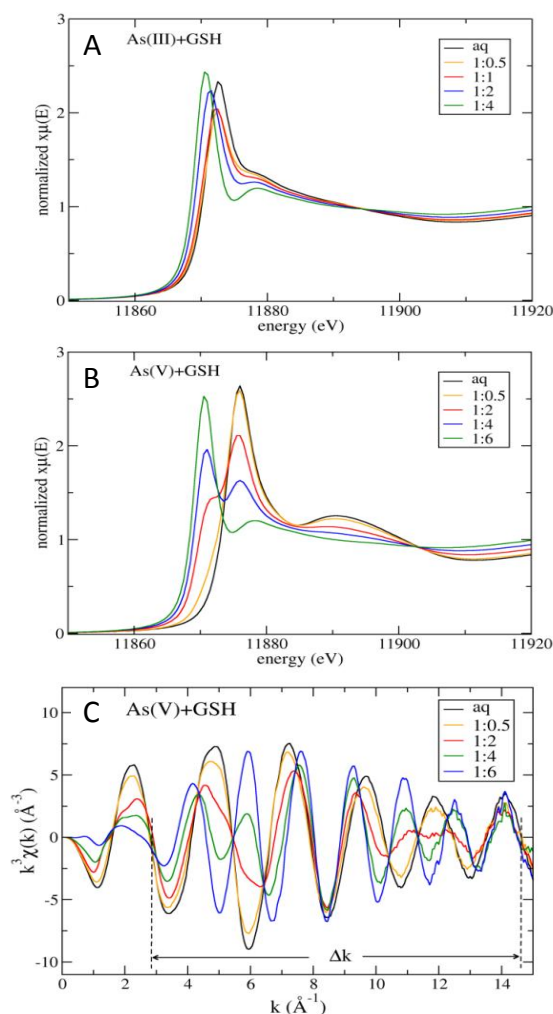


Figure 1. (A) Normalized As K-edge XANES spectra for As(III)+GSH and (B) As(V)+GSH at increasing As: GSH molar ratios. (C) EXAFS oscillations (multiplied by k^3) extracted from As(V)+GSH XAFS spectra.

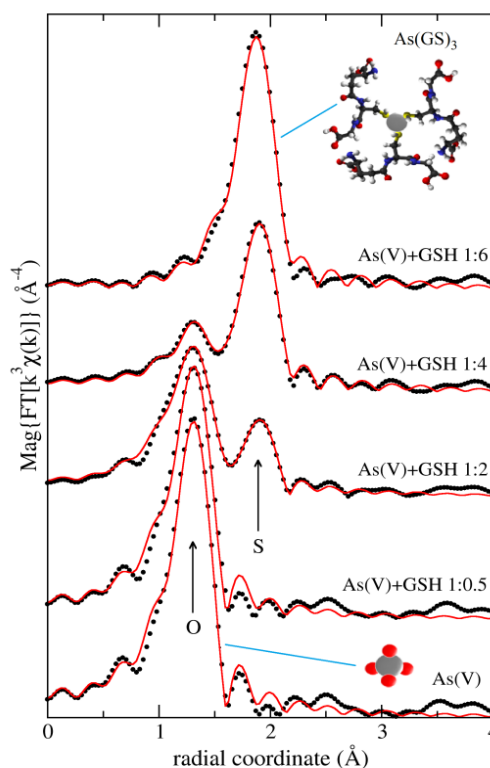


Figure 2. Magnitude of Fourier- EXAFS data for As(V)+GSH at increasing As: GSH molar ratios.

The complexation behavior of As(III) and As(V) with GSH was analyzed by XAFS point of view. After the reduction of As(V) to As(III) in freshly prepared solutions, the compound $\text{As}(\text{GS})_3$ is formed with a higher electron density around As atom compared with the oxyanions of As(III) and As(V). Information about the molecular mechanisms of As biological immobilization can help to clarify the complex biochemistry of As.

Table 1. Best fit results to EXAFS data

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

N = coordination number; R = bond length; σ^2 = Debye-Waller factor; relative misfit (R-factor) of at most 0.7% for all fits. Uncertainty values in brackets.

References

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