

## Interactions of arsenic(III) with metallothionein and a model oligopeptide

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Metallothioneins, small metal-rich proteins, appear to play a role in the homeostasis of essential metal ions and in the detoxification of non-essential ones. Among the 61 amino acids of its structure, 20 are cysteine residues. It is well known that arsenic(III) has a high affinity for sulfur-containing molecules. Previously we showed that As(III) forms  $AsL_3$  species with cysteine and glutathione, which are the predominant species from acid to neutral medium<sup>1</sup>.

In this study, as a model for metallothionein (MT), we have used a peptide containing three cysteinyl residues, which corresponds to residues 56-61 of mouse MT(I)<sup>2</sup>. Rabbit liver  $Zn_2Cd_5MT(I)$  and  $H_2N-Lys-Cys-Thr-Cys-Cys-Ala-OH$  were purchased from Sigma. NMR spectra were obtained in  $D_2O$  solutions containing different metal-to-ligand molar ratios at pH 7.0. The stability constant was calculated from UV-Vis titration curves by means of the SQUAD program.

In the  $^1H$  NMR MT spectrum, the two  $\beta$  methylene protons of Cys and the  $\epsilon$  methylene protons of Lys appear overlapped at  $\delta$  3.02 and after addition of arsenic an upfield shift and a broadening of these signals occur. A high excess of arsenic over MT was necessary to displace zinc and cadmium from MT. Even in the presence of an As(III)-to-protein molar ratio equal to 100, saturation was not attained. In the oligopeptide  $^1H$  NMR spectrum, the addition of arsenic also induces a broadening of the signals; the most affected being those of Cys and Lys residues at  $\delta$  3.41, 3.26, 3.07, 2.29, and 2.13. The broadening of the peaks probably results from the quadrupole moment of the  $^{75}As$  nucleus ( $I = 3/2$ ). In the UV-vis region, the addition of arsenic leads to the appearance of a shoulder centered at 280 nm, which can be assigned to a sulfur-to-arsenic charge transfer transition. The best fit between the experimental and calculated curves was obtained assuming the formation of an ML species with an apparent constant  $\log \beta = 6.76 \pm 0.04$ . We propose that As(III) forms a complex with the oligopeptide via the three sulfur atoms of cysteines in a pyramidal geometry.

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