

**Interactions of peptides and proteins with arsenic species and metal ions: investigations by means of electrospray ionization mass spectrometry (ESIMS)**

Anne-Christine Schmidt and Matthias Otto

Institute of Analytical Chemistry, Technical University Bergakademie Freiberg, Leipziger Straße 29, D-09599 Freiberg, Germany

The various arsenic species identified in living organisms show a different toxicity. However, the influence of arsenic species on proteins and especially the mechanism of interaction are not well understood. Inorganic arsenic species arsenite and arsenate as well as heavy metal ions are assumed to induce peptides with a high cysteine content like metallothioneins and phytochelatinins as a metabolic response preventing cell toxicity [1]. For the elucidation of biochemical effects of arsenic at the molecular level in dependence on the chemical species, extraction, enrichment, and detection methods are to be developed that preserve the native form of such complexes. Electrospray ionization mass spectrometry is suited for the investigation of intact protein-ligand-complexes [2].

In the presented mass spectrometric binding studies for arsenic species and the divalent metal cations  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  with thiol-reactive biomolecules characterized by growing molecular masses, the amino acid cysteine ( $121 \text{ g mol}^{-1}$ ), the tripeptide glutathione ( $307 \text{ g mol}^{-1}$ ), the nonapeptide isotocin ( $966 \text{ g mol}^{-1}$ ), and the protein thioredoxin ( $11688 \text{ g mol}^{-1}$ ) were used. In addition, lysozyme (14.3 kDa) was chosen as a model protein containing four structure-determining disulfide linkages. Trivalent and pentavalent arsenic was incubated with amino acid, peptide and protein solutions both as organic compound (phenylarsine oxide, phenylarsonic acid, dimethylarsinic acid) and as inorganic compound (arsenite and arsenate). After incubation of phenylarsine oxide with cysteine, glutathione, isotocin, and thioredoxin the mass spectra showed a covalent binding between arsenic and sulfur, which was stable at acidic pH values as well as in neutral solution [3]. Furthermore, the influence of the solvent composition particularly with regard to the water concentration on the intensity of the mass signals for the arsenic-containing reaction products became evident owing to the release of water as second product [4]. Interestingly, under the same conditions no interactions of inorganic arsenite or arsenate could be measured. In presence of added  $\text{Cu}^{2+}$  ions all mass signals caused by a covalent reaction of phenylarsine oxide and glutathione disappeared. In these mass spectra only the oxidized form of glutathione was found because of the catalytic activity of  $\text{Cu(II)}$ . Regarding the model protein lysozyme, no interactions with arsenic could be detected, whereas definite  $\text{Cu}$ - and  $\text{Zn}$ -lysozyme complexes with a stoichiometry of 1:1 and 2:1 for  $\text{Zn}^{2+}$  ions and  $\text{Cu}^{2+}$  ions, respectively, were observed. For these metal complexes a pronounced pH dependency was ascertained. Another kind of non-covalent complexes was found for solutions containing pentavalent organic arsenic species and glutathione. From the presented results a different mechanism of interaction of metal cations and the metalloid arsenic with thiol-functions in amino acids and peptides as well as with sulfur-containing proteins can be concluded. This behaviour proved by ESI-MS investigation poses the question if the detoxification of metal ions and arsenic, which is often mentioned in the literature, can be really unified concerning the role of thiol reactivity. Regarding the arsenic compounds tested a strongly dependence of stable thiol interactions on the chemical species not only in terms of arsenic's redox-state but also in the presence of a phenyl ring can be deduced.

## References:

- [1] H. Goenaga Infante, F. Cuyckens, K. Van Campenhout, R. Blust, M. Claeys, L. Van Vaeck, F.C. Adams; *J. Anal. At. Spectrom.*, 19 (2004) 159-166.
- [2] A. Tjernberg, S. Carnö, K. Benckestock, P.O. Edlund, W.J. Griffiths, Hallén; *Anal. Chem.*, 76 (2004) 4325-4331.
- [3] A.C. Schmidt, J. Koppelt, M. Neustadt, M. Otto, *Rapid Commun. Mass Spectrom.*, 21: (2007) 153-163.
- [4] A.C. Schmidt, M. Neustadt, M. Otto; *J. Mass Spectrom.*, 42: in press, 2007