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Multi-instrumental studying of interaction between heavy metal ions and free aminoacids

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Abstract

The main contribution of this work is in use of different techniques for the study of interactions in the field of metallomics. Interactions of free amino acids with heavy metals are subjects, which have only partial scientific interest. However, both the transport of metals and metal binding with free amino acids and/or small molecules have been still only poorly understood. We developed few methods spectrophotometric, flow injection analysis and chromatographic both with various type of electrochemical detection, which all were successfully used for studying of creation of complexes between cadmium or platinum derivatives *in vitro*.

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Introduction

Biologically active metals can be included in two groups - essential and toxic with respect to used physiological criteria. Free amino acids (AA) are AA, which are not bounded in proteins or in other biochemically active structures. Furthermore, we know also biogenic amines, which are originated from biogenic AA or they are their precursors. In the case that toxic metals can occur in free ionic state in the organism, they are able to generate reactive oxygen species (ROS). ROS can subsequently oxidatively damage number of biomolecules and thus disrupt the homeostasis. In first line of defence against ROS – their detoxification, small biological active molecules, which are able to scavenge ROS, play crucial role. Cysteine with thiol group the most importantly participates on ROS scavenging, together with nucleophile centres of side chains of amino acids methionine, histidine of tryptophan.

Materials and methods

Aim of this work consisted in *in vitro* monitoring of interactions between metals and AA by use of different techniques. Experiments were carried out on several levels. Interaction between free sulphur containing AA and cadmium, between cysteine-rich peptides and cadmium and platinum based cytostatics and between protein metallothionein (MT) and metal were investigated.

Results and discussion

Observing of interaction between AA and cadmium was carried out thanks to monitoring of absorption maximum of Murexide (Leverrier et al. 2007). Furthermore there was observed creation of complex due to factorial analysis with change of concentrations of both reagents and time of interaction. Samples prepared for studying of intensity of interaction were carried out using automated preparation of sample with robotic station and so detection by Ellman reaction in automatic analyser. This high throughput approach was designed because many of parameters (concentration of components, various temperature or various time of interaction) had to be studied. For determination of intensity of interaction

between peptides and metals a flow injection analysis with electrochemical detection (FIA-ED) was employed. FIA-ED method was optimized for detection of cysteine-rich peptides from MT and Platinum cytostatics by amperometric detection. For this method a glassy carbon (GC) electrode as working was employed and applied potential 500mV was used with regards to interference of cisplatin. After we obtained the best parameters (flow rate applied potential, electrolyte) we determined the intensity of interaction by determination of decreasing of free thiol groups which peptides contained. After high complex creation we detected fast decreasing slopes and that shows which of tested peptides was the best for interaction. Than the same method was coupled with coulometric detection instead of amperometric and used for detection of peptides in presence of cadmium. The most effective applied potential was 900mV. Obtained results after correlation gave information about creation of complex (Zitka et al. 2007). Same as in previous case the slopes of decreasing of signals of thiol groups defined of complex creation by all studied peptides.

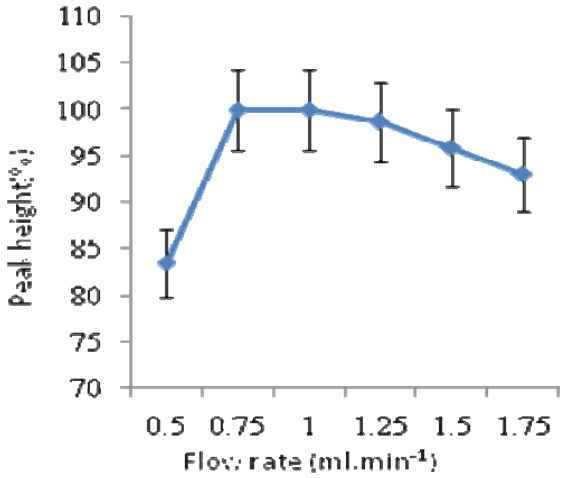

Figure 1: influence of flow rate on peak height of separately analyzed fragments of MT.

After connection of chromatographic column Kinetex C-18 (150 × 4.6; 2,5 µm beads, Phenomenex, USA) into the gradient system (mobile phase: A: 80mM TFA, B: Methanol) with coulometric detection we developed effective separation of peptides for further studies of interactions between them and strong ligands. Gradient was linearly increasing from 3%B to 50%B in 20 minutes and 20µl of the sample was injected to the system where flow rate was 1ml/min. Optimal detection was under applied potential 900mV. We used this method might be very useful for basic competitive studying of complex creation metal could be added to the mixture and after incubation and subsequent separation the chromatogram compared to the untreated control variant can provide information as decreasing which is associated with complex creation or complex could change the retention time also. Finally for observing of complex of MT with metals we used the QCM system in optimised conditions pH 8 and time of interaction 180s. MT was immobilised on gold QCM crystal and than analyzed by electron microscopy. There were visible bright regions which represented metal occurrence in MT domains (Adam et al. 2007).

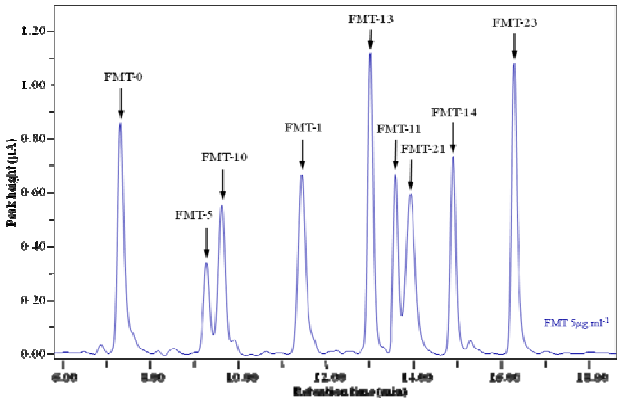

Figure 2: Chromatogram of various peptide fragments of MT acquired by HPLC-ED.

Conclusion

Methods developed and used in this work can be very useful in solving of presented questions and represent alternative to more costly analytical techniques, such as NMR or X-ray analysis. In addition to application possibilities of UV-Vis spectroscopy and advanced flow electrochemical detectors, this work covers field of proteomics. Our approach demonstrates complementarily of proteomics and metallomics. The protein sequence of the specific protein can be used *in vitro* for advanced studies, which are the aim of interest of metallomics. *In vitro* studies of metallothionein protein fragments can provide also reference data that could confirm or disconfirm computational analysis of complex formation.

Acknowledgements

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