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Protein based biosensors in analysis of heavy metal ions

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Population explosion and rapid development of various technologies bring continuing pollution of environment by toxic substances (pesticides resides, toxic metabolites, heavy metal ions). Their elevating concentration in the environment can be concern in human health protection, foodstuff productions and conservancy. At both plant and animal organisms the heavy metal ions entering to a cell can be bound by compounds rich in cysteine residues, plant phytochelatins and animal metallothioneins.

Accordingly, methods for easy and quick detection of heavy metals at very low concentrations in environmental and biological samples are highly appreciated for assurance of disposals against acute intoxications and, first of all, against long-time exposure that may lead to many diseases or even death. Several analytical methods, such as atomic absorption spectrometry, inductively coupled plasma with mass spectrometry and others have been developed for these purposes. In addition biosensors represent tools used for simple, quick and low-cost detection of heavy metals in the environment.

Peptides and proteins, which are able to bind heavy metals, can be used for proposing simple electrochemical biosensors. The beginning of electrochemical study of protein behaviour can be dated to thirties of the past century, when Prof. Rudolf Brdička investigated behaviour various cobalt salts. He discovered that after introducing of bovine

serum to a supporting electrolyte interesting polarographic signals appeared. These signals can be used in fabrication of new sensors and biosensors, where the surface of sensors can be modified by monoclonal antibodies, enzymes and nucleic acids. The aim of this work was to suggest new heavy metal biosensors based on interaction of metals (cadmium and zinc) with heavy metal binding biological compounds (phytochelatin – PC2 and/or metallothionein – MT), using adsorptive transfer stripping (AdTS) differential pulse voltammetry (DPV). Suggestion of biosensors could be very suitable for these purposes instead of robust analytical techniques, because biosensors have the advantages of specificity, low cost, ease of use, portability and the ability to furnish continuous real time signals. Primarily, we utilized PC2 biosensor to determine cadmium, zinc and cisplatin. The quantification limits (3 S/N) of Cd(II), Zn(II) and cisplatin were about 0.6, 8 and 1 pmole in 5 μl, respectively. After that we did proved that we were able to suggest biosensor through modification of hanging mercury drop electrode, we attempted to utilize metallothionein instead of PC2 for the same purposes. We studied the electrochemical behaviour of MT on the surface of hanging mercury drop electrode by AdTS DPV. The quantification limits of the analysed heavy metals (cadmium(II) and zinc(II)), which were analysed in the presence of 0.5 M NaCl (pH 6.4), were 160 and 220 fmole in 5 μl drop, respectively. The recovery of the signal was about 90 %. In addition, we applied the MT biosensor to analyse heavy metals in human body liquids (human blood serum and human urine) and to compare with differential pulse anodic stripping voltammetry.

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