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USING OF ELECTROCHEMICAL METHODS FOR STUDYING OF METALLOTHIONEIN CONTENT IN THE HUMAN BLOOD SERUM OF A PATIENT POISONED BY LEAD AND TREATED BY PLATINUM

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Metallothioneins belong to the group of intracellular, high molecular and cysteine-rich proteins whose content increase with increasing concentration of a heavy metal. Here we applied the adsorptive transfer stripping differential pulse voltammetry Brdicka reaction for the determination of metallothionein in human blood serum of patient poisoned by lead and/or treated by platinum. The increased metallothionein concentrations in both cases were observed.

INTRODUCTION

Metallothionein (MT) belongs to the group of intracellular, high molecular and cysteine-rich proteins with molecular weight from 6 to 10 kDa (ref.[1]). The MT was discovered in 1957, when Margoshes and Valee isolated it from horse kidney[2]. It is known that aromatic amino acids are not present in the MT molecule and twenty cysteines usually occur in its primary sequence at these repetitions: Cys-X-Cys, Cys-Cys-X-Cys-Cys, Cys-X-Cys-Cys, where X represents other aminoacid than cysteine[1,3]. MTs consist of two binding domains (α, β) that are assembled from cysteine clusters. Cysteine sulfhydryl groups participate in covalent bindings with heavy metals. The N-terminal part of the protein is marked as α-domain, which has three binding places for divalent ions. β-Domain (C-terminal part) has the ability to bind four divalent ions of heavy metals. Moreover a group of human metallothioneins such as MT1, MT2, MT3 and MT4 has been described (Table 1).

Number of analytical techniques including electrochemistry[4–8] is used for the determination of MT. Many authors have been giving attention to electrochemical determination and study of MT for more than 20 years[9–12]. In addition, more than 70 years ago Brdicka discovered the catalytic evolution of hydrogen in the presence of cobalt(III) solution and of proteins[13-16]. This reaction has been intensively using for electrochemical determination of proteins and has been modified by number of authors[17–19].

The aim of this work was to use adsorptive transfer stripping (AdTS) differential pulse voltammetry (DPV) Brdicka reaction for studying of metallothionein content in the human blood serum of a patient poisoned by lead and treated by platinum.

MATERIAL AND METHODS

Chemicals

Sodium chloride and other used chemicals were purchased from Sigma Aldrich. The stock standard solutions of MT at 10 μg/ml was prepared by ACS water (Sigma-Aldrich, USA) and stored in the dark at –20 °C. Working standard solutions were prepared daily by dilution of the stock solutions. The pH value was measured using WTW inoLab Level 3 with terminal Level 3 (Weilheim, Germany), controlled by personal computer program (MultiLab Pilot; Weilheim, Germany). The pH-electrode (SenTix- H, pH 0–14/3M KCl) was regularly calibrated by set of WTW buffers (Weilheim, Germany).

Human blood serum samples

Human blood serum samples were obtained from the Department of Clinical Biochemistry and Pathobiochemistry, 2nd Faculty of Medicine Charles University, Czech Republic. The samples were prepared by heat treatment and solvent precipitation. Briefly, the samples were kept at 99 °C in a thermomixer (Eppendorf 5430, USA) for 15 min with occasional stirring, and then cooled to

4 °C. The denatured homogenates were centrifuged at 4 °C, 15 000 g for 30 min. (Eppendorf 5402, USA). Heat treatment and solvent precipitation effectively denature and remove high molecular weight proteins out from samples[19,20]. Determination of MT in the human blood serum samples was performed by optimised AdTS DPV Brdicka reaction. Analysed sample volume was 5 µl.

Electrochemical measurements

Electrochemical measurements were performed with AUTOLAB Analyser (EcoChemie, Netherlands) connected to VA-Stand 663 (Metrohm, Switzerland), using a standard cell with three electrodes. The working electrode was a hanging mercury drop electrode (HMDE) with a drop area of 0.4 mm^2. The reference electrode was an Ag/AgCl/3M KCl electrode and the auxiliary electrode was a graphite electrode. The supporting electrolyte was prepared by mixing buffer components except sodium chloride. For smoothing and baseline correction the software GPES 4.4 supplied by EcoChemie was employed.

AdTS DPV Brdicka reaction of MT

The Brdicka supporting electrolyte containing 1 mM $Co(NH_3)_6Cl_3$ and 1 M ammonia buffer (NH_3(aq) + NH_4Cl, pH = 9.6) was used; surface-active agent was not added. AdTS DPV Brdicka reaction parameters were as follows: an initial potential of –0.35 V, an end potential –1.6 V, a modulation time 0.057 s, a time interval 0.2 s, a step potential of 1.05 mV/s, a modulation amplitude of 250 mV, E_{ads} = 0 V. Temperature of supporting electrolyte was tested[20].

RESULTS AND DISCUSSION

Brdicka discovered protein catalysed hydrogen evolution signals more than 70 years ago[21]. In addition Brdicka found out that the hydrogen evolution could be used for diagnostic of tumour diseases[22,23]. On the other hand this technique has been replaced by new analytical techniques such electrophoresis, chromatography coupled with different kind of detectors in clinical medicine. Therefore we were interested in the issue if Brdicka reaction could be used in clinical lab nowadays. Recently new approaches such as adsorptive transfer stripping technique or modification of a surface of working electrode by a high- and/or low-molecular compound (Fig. 1) used for improvement of selectivity of Brdicka reaction has been described[24,25,26]. If the authors used the adsorptive transfer stripping technique in combination with Brdicka reaction, the sensitive determination of proteins was reached[27,20].

Here we wanted to analyse the human blood serum samples of a patient poisoned by lead. To be specific, a 11-year-old girl was referred to the hospital by her general practitioner because of abdominal pain, vomiting, dark colour of tongue, low intake of fluid and food, and abnormal laboratory results (bilirubin 55 µmol/l, AST 2.94 µkat/l, ALT 3.46 µkat/l, haemoglobin 86 g/l). Blood film, which

Table 1. The biochemical properties of human metallothioneins

Metallothionein name[1]	*Number of amino acids*	*Molecular weight*	*Theoretical pI*	*Swiss-Prot number*[2]
MT1A	61	6133.2	8.38	P04731
MT1B	61	6115.3	8.47	P07438
MT1E	61	6014.1	8.38	P04732
MT1F	61	6086.2	8.23	P04733
MT1G	61	6070.2	8.38	P13640
MT1H	61	6039.2	8.49	P80294
MT1I	61	6040.2	8.38	P80295
MT1K	62	6141.3	8.38	P80296
MT1L	61	6068.2	8.38	P80297
MT1R	61	6062.2	8.38	Q93083
MT2	61	6042.1	8.23	P02795
MT3	68	6926.9	4.79	P25713
MT4	62	6418.7	8.38	P47944

ExPASy Proteomics Server – [1]http://www.expasy.ch; [2]http://www.expasy.org/sprot/

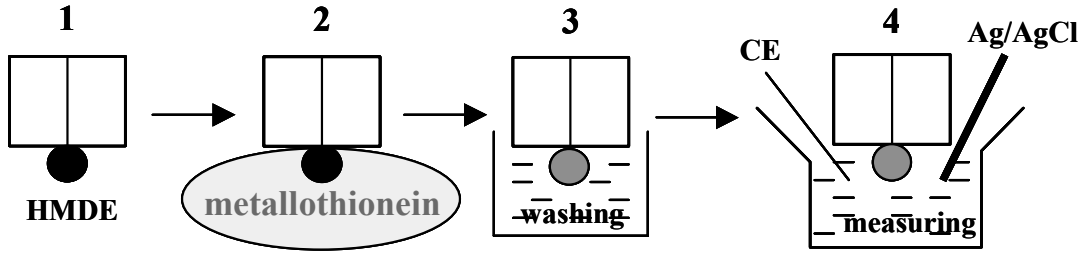

Fig. 1. Scheme of adsorptive transfer stripping technique; (**1**) renewing of the hanging mercury drop electrode (HMDE) surface; (**2**) adsorbing of MT in a drop solution onto the HMDE surface; (**3**) washing electrode in sodium chloride (0.5 M, pH 6.4) at open circuit; (**4**) measurement of MT by DPV Brdicka reaction.

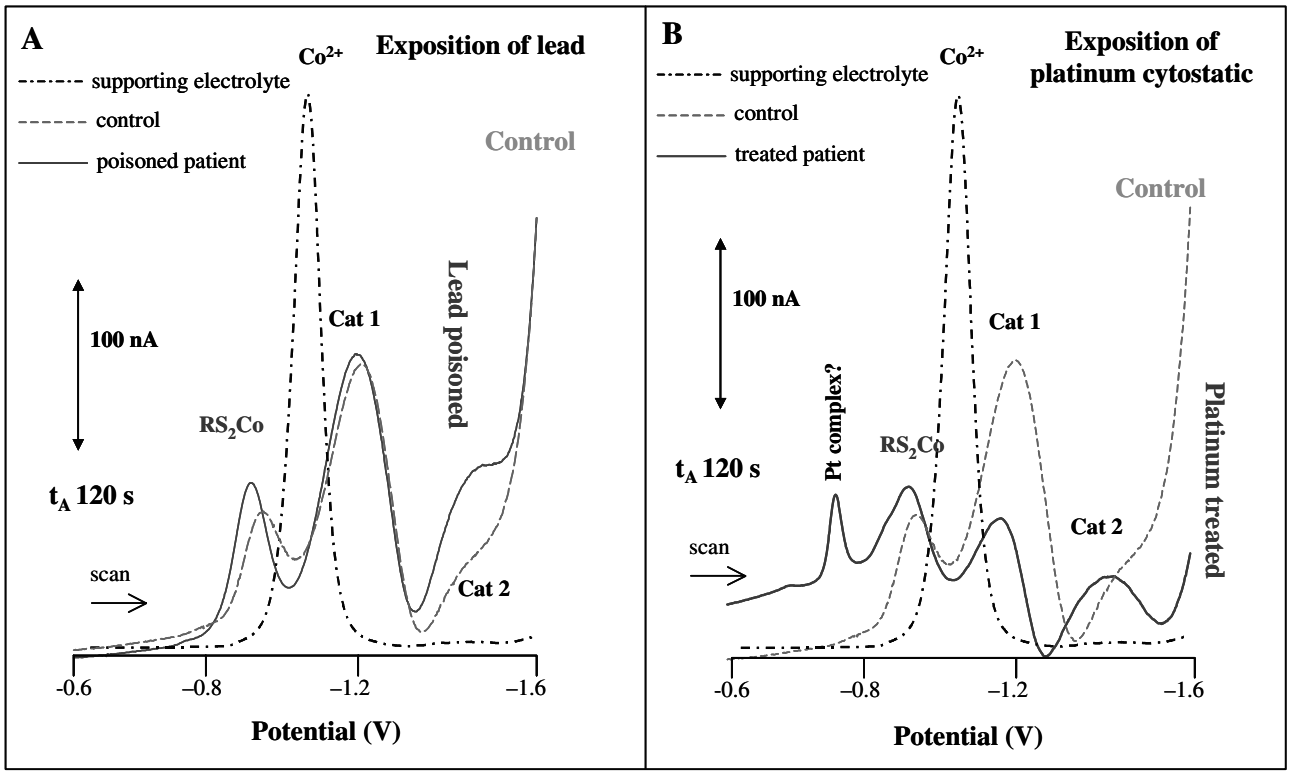

Fig. 2. AdTS DPV Brdicka reaction of blood serum of lead poisoned patient (**A**) and of platinum cytostatic treated patient (**B**). AdTS DPV Brdicka reaction parameters were as follows: an initial potential of –0.35 V, an end potential –1.8 V, a modulation time 0.057 s, a time interval 0.2 s, a step potential of 1.05 mV/s, a modulation amplitude of 250 mV, E_{ads} = 0 V, the temperature of supporting electrolyte is 5 °C, the time of accumulation of MT is 120 s, the concentration of $Co(NH_3)_6Cl_3$ is 1 mM. The AdTS DPV signals i) of reduction of Co(II) contained in supporting electrolyte (dot-and-dash line); ii) of control (dashed line) and iii) of human blood serum of poisoned patient – first day of hospitalisation (continuous line) are shown.

exhibited basophilic stippling, is prompting for investigation of lead poisoning. The source of 6 months lead exposure was identified as tea from a ceramic tea pot with insufficient glassing (lead concentration was 45332 μg/l in tea after 30 minutes). It is common knowledge that heavy metals induce a synthesis of MT. Therefore we assumed that concentration of MT increased with rising amounts of lead in blood serum of the poisoned patient. The AdTS DPV signals i) of reduction of Co(II) contained in supporting electrolyte (dot-and-dash line); ii) of control (dashed line) and iii) of human blood serum of poisoned patient – first day of hospitalisation (continuous line) are shown in Fig. 2A. We observed three MT signals – RS_2Co (complex of MT with Co(II)) and two catalytic signals (Cat1 and Cat2). We estimated that amount of MT in a blood serum of a healthy patient varies between 1 and 10 μM (n = 5). It clearly follows from the figure that Cat2 signal increased according to lead poisoning. Finally, it was found out that amount of MT in the human blood serum of the lead poisoned patient was more than 200 μM.

Moreover MT concentration increase during treating of tumour diseases by platinum based cytostatics[28]. Therefore we determined the MT amounts in human blood serum of a patient, which has been treated by platinum complexes, by AdTS DPV Brdicka reaction. The increase in Cat 2 signal height of the treated blood serum corresponds to MT concentration of 150 μM (Fig. 2B). That is why it is possible to assume that content of metallothionein was increased due to treatment by platinum based cytostatic. Moreover we observed a signal at potential of –0.7 V, which probably corresponds to complex between MT and platinum cytostatic. The experimental data indicate that it would be suitable to monitor the content of MT at patients treated by platinum based cytostatics for better recognising of platinum cytostatic resistance[29].

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REFERENCES

1. Kagi JHR, Schaffer A. (1988) Biochemistry of Metallothionein. Biochemistry *27*, 8509–15.
2. Margoshes M, Vallee BLA. (1957) A cadmium protein from equine kidney cortex. J Am Chem Soc *79*, 4813–4.
3. Palmiter RD. (1994) Regulation of metallothionein gense by heavy metals appears to be mediated by a zinc-sensitive inhibitor that interacts with a constitutively active transcription fakctor, MTF-1. Proc Natl Acad Sci USA *91*, 1219–23.
4. Kizek R, Trnkova L, Palecek E. (2001) Determination of metallothionein at the femtomole level by constant current stripping chronopotentiometry. Anal Chem *73*, 4801–7.
5. Dabrio M, Rodríguez AR, Bordin G, Bebianno MJ, De Ley M, Šestáková I, Vašák M, Nordberg M. (2002) Recent developments in quantification methods for metallothionein. J Inorg Biochem *88*, 123–34.
6. Trnkova L, Kizek R, Vacek J. (2002) Catalytic signal of rabbit liver metallothionein on a mercury electrode: a combination of derivative chronopotentiometry with adsorptive transfer stripping. Bioelectrochemistry *56*, 57–61.
7. Strouhal M, Kizek R, Vacek J, Trnkova L, Nemec M. (2003) Electrochemical study of heavy metals and metallothionein in yeast *Yarrowia lipolytica*. Bioelectrochemistry *60*, 29–36.
8. Prusa R, Kizek R, Vacek J, Trnkova L, Zehnalek J. (2004) Study of relationship betwenn metallothionein and heavy metals by CPSA method. Clin Chem *50*, A28–9.
9. Olafson RW, Sim RG. (1979) Electrochemical approach to quantitation and characterization of metallothioneins. Anal Biochem *100*, 343–51.
10. Olafson RW. (1988) Electrochemical characterization of metallothionein metal mercaptide complexes – Application of cyclic voltammetry to investigation of metalloproteins. Bioelectrochem Bioenerg *19*, 111–25.
11. Olafson RW, Olsson PE. (1991) Electochemical detection of metallothionein. Methods Enzymol *205*, 205–13.
12. Heyrovsky M. (2004) Early polarographic studies on proteins. Electroanalysis *16*, 1067–73.
13. Brdicka R. (1933) Polarographic studies with the dropping mercury kathode. – Part XXXII. – activation of hydrogen in sulphydryl group of some thio-acids in cobalt salts solutions. Coll. Czech. Chem. Commun. *5*, 148–64.
14. Brdicka R. (1933) Polarographic studies with the dropping mercury kathode. -Part XXXI. – a new test for proteins in the presence of cobalt salts in ammoniacal solutions of ammonium chloride. Coll Czech Chem Commun *5*, 112–28.
15. Brdicka R. (1936) Polarographic studies with the dropping mercury kathode. -Part LXI. – the effect of buffer solutions on the reaction of proteins. Coll Czech Chem Commun. *8*, 366–76.
16. Kolthoff IM, Yamashita K, Hie TB. (1975) Brdicka currents observed with bovine serum albumin and completely reduced bovine serum albumin in the presence of urea. Proc Natl Acad Sci USA *72*, 2044–8.
17. Mader P, Kolthoff IM, Vesela V. (1982) Catalytic hydrogen activity of the low-molecular weight thiols – kinetic control of the Brdicka catalytic current. Electrochim Acta *27*, 1393–401.
18. Heyrovsky M. (2000) New aspects of the Brdicka reaction of low-molecular thiols; Voltammetry at low rate of potential scan. Electroanalysis *12*, 935–9.
19. Erk M, Ivanković D, Raspor B, Pavičić J. (2002) Evaluation of different purification procedures for the electrochemical quantification of mussel metallothioneins. Talanta *57*, 1211–8.
20. Petrlova J, Potesil D, Mikelova R, Blastik O, Adam V, Trnkova L, Jelen F, Prusa R, Kukacka J, Kizek R. (2005) Attomole voltammetric determination of metallothionein. Electrochim Acta *submitted*.
21. Kalous V. (2004) 70 let od objevu Brdičkovy polarografické reakce bílkovin. Klin. Biochem. Metab. *12*, 265–7.
22. Brdicka R. (1937) Polarographic investigation in serological cancer diagnosis. Nature *139*, 1020–1.
23. Brdicka R. (1937) Application of the polarographic effect of proteins in cancer diagnosis. Nature *139*, 330.
24. Adam V, Zehnalek J, Petrlova J, Potesil D, Sures B, Trnkova L, Jelen F, Vitecek J, Kizek R. (2005) Phytochelatin modified electrode surface as a sensitive heavy-metal ion biosensor. Sensors *5*, 70–84.
25. Palecek E, Postbieglova I. (1986) Adsorptive stripping voltammetry of biomacromolecules with transfer of the adsorbed layer. J Electroanal Chem *214*, 359–71.
26. Palecek E, Jelen F, Postbieglova I. (1989) Adsorptive transfer stripping voltammetry offers new possiblities in DNA research. Stud Biophys *130*, 51–4.
27. Kizek R, Vacek J, Trnková L, Klejdus B, Havel L. (2004) Application of catalytic reactions on a mercury electrode for metallothionein electrochemical detection. Chem Listy *98*, 160–7.
28. Zelená J, Potešil D, Vacek J, Adam V, Hradecký J, Prusa R, Kizek R, Vojtěšek B. (2004) Metalothionein jako prognostický marker nádorového onemocnění. Klinická onkologie *17*, 190–5.
29. Kizek R, Vacek J, Adam V, Vojtěšek B. (2004) Vztah metalothioneinu k rakovině a protinádorové léčbě. Klin Biochem Metab *12*, 72–8.