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Brdička-type processes of cysteine and cysteine-containing peptides on silver amalgam electrodes

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Abstract

Silver solid amalgam electrode (AgSAE) was used for differential pulse voltammetric (DPV) measurements of cysteine and cysteine-containing peptides, glutathione, γ-Glu-Cys-Gly and phytochelatin $(\gamma\text{-Glu-Cys})_3$-Gly (PC3), in the presence of Co(II) ions. It had been established earlier that cysteine-containing peptides and proteins catalyze hydrogen evolution at mercury electrodes in presence of cobalt salts; these processes are known as the Brdička reaction. DPV signals measured with the AgSAE, the surfaces of which had been modified by mercury meniscus or mercury film, were qualitatively the same as those obtained with the hanging mercury drop electrode (HMDE). With these electrodes the number and the intensity of Brdička signals of cysteine, glutathione and PC3 differed, making a distinction among them possible. On the other hand, with the polished silver solid amalgam electrode (the surface of which was completely free of liquid mercury) all three compounds produced only one but strikingly intense peak in the region of Brdička reaction. Using this signal, cysteine, glutathione as well as PC3 could be determined at 10^{-8} M level, representing sensitivity up to 2 orders of magnitude better than attained with the mercury-modified AgSAEs or HMDE.

Keywords: Silver solid amalgam electrode; Hanging mercury drop electrode; Brdička reaction; Catalytic hydrogen evolution; Cysteine; Glutathione; Phytochelatin

1. Introduction

The hanging mercury drop electrode (HMDE) has been the superior electrode in electrochemical analysis due to its well known favorable properties [1]. When compared with solid electrodes, the limited mechanical stability of HMDE is its certain disadvantage; it can cause problems when working with portable analyzers, especially directly "in field", when flow-through techniques are applied, or in measurements in adsorptive transfer (medium exchange, *ex situ*) modes. The use of liquid mercury can also be undesirable in some specific analyses. For these reasons, the development of solid electrodes has been one of the long-term trends in the area of electroanalytical research. During the last few years several new types of solid or modified electrodes have been introduced [2–8] to achieve wider working windows, better performance in field or other desirable properties. Silver solid amalgam electrodes (AgSAE), introduced by Mikkelsen and Schroder [9,10] and independently by Yosypchuk and Novotny [11], represent an intermediate between the mercury electrode and usual solid electrodes; it combines advantages of both. These electrodes can work with actually solid amalgam surface [5,9,10,12–21] or can easily be modified with mercury meniscus [21–26] or thin electrolytically deposited mercury film [20]. Such electrodes have recently been used for determination of various inorganic [13–16,27–30] as well as organic [30,31] compounds, for electrochemical analysis of nucleic acids [23,25], including detection of DNA damage [20,24].

As described above, the amalgam electrodes have found a variety of applications related to environmental monitoring [32,33] and protection. In addition to direct electrochemical determination of the pollutants, another approach can be based on monitoring changes induced by these species in biomolecules such as DNA. Up to now, a number of electrochemical techniques suitable for detecting damage to the genetic material have been proposed [34], including those utilizing the AgSAE as working electrode [20,24]. Besides nucleic acids, also other

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biomolecules can be employed as biomarkers of environmental pollution. Exposure of living organisms to heavy metals (which represent one of the major environmental problems) results in spontaneous synthesis of cysteine-rich peptides or small proteins called metallothioneins (MT, in animals) or phytochelatins (PC, in plants) [35–37]. The PCs, $(\gamma\text{-Glu-Cys})_n$-Gly where $n = 2–11$, are synthesized via glutathione, γ-Glu-Cys-Gly, an abundant peptide taking part in controlling the *in vivo* redox balance [35]. PCs bind heavy metals in complexes involving coordination of the metal ions by thiol groups of the cysteine units, thus preventing their toxic effects during their transport across the cytoplasm inside the vacuoles [38–40]. For analysis of cysteine and cysteine-containing peptides, several electrochemical techniques have been proposed. Complexation of metal ions by cysteine [19,31] as well as by MTs [41–44] or PCs [26,45] was studied by voltammetric or potentiometric methods. A specific approach utilizes the Brdička procedure [46]. In the presence of cobalt ions and the cysteine-containing peptides (proteins), several electrochemical processes (including catalytic hydrogen evolution) giving rise to characteristic electrochemical signals can be observed at mercury electrodes. Recently it has been shown that using dc voltammetry in solutions with Co(II), the PCs can be easily distinguished from glutathione or its fragments [47] which has been utilized in development of a constant current chronopotentiometric technique of PC determination in plant cell lysates [48]. In the present work, voltammetric signals of cysteine, glutathione and $(\gamma\text{-Glu-Cys})_3$-Gly (PC3) were measured in Co(II)-containing solution using the silver amalgam electrodes with different surface modifications. We show that with the m-AgSAE and MF-AgSAE, the voltammetric responses of all substances are qualitatively similar to those observed with the HMDE. On the other hand, responses of these species measured with the p-AgSAE differed from those measured by electrodes with mercury surface. Among the electrodes tested with the –SH compounds, the p-AgSAE offered the best sensitivity of their determination.

2. Experimental

2.1. Reagents

All chemicals used for preparing the supporting electrolyte, standard solutions and other stock solutions were of p.a. purity. Ammonium buffer of pH 9.75 was prepared from ammonium chloride and ammonia solution (Lachema, Brno, Czech Republic). Stock solution of Co(II) was prepared from $Co(NO_3)_2{\cdot}6H_2O$ (Sigma–Aldrich). Cysteine and oxidized glutathione were obtained from Sigma–Aldrich. Phytochelatin $(\gamma\text{-Glu-Cys})_3$-Gly (PC3) synthesized at the Institute of Organic Chemistry and Biochemistry, ASCR (Prague) was kindly provided by RNDr. Ivana Šestáková, CSc. (J. Heyrovský Institute of Physical Chemistry, ASCR, Prague).

2.2. Instrumentation

Voltammetric measurements were performed with the computer controlled Eco-Tribo Polarograph PC-ETP (Eco Trend Plus, Prague, Czech Republic) [35], equipped by POLAR.PRO software for Windows XP v. 5.1. HMDE or AgSAEs (Eco Trend Plus, Prague, Czech Republic) served as working electrodes, Ag/AgCl/1 M KCl as reference, and platinum wire as auxiliary electrode (both from Monokrystaly, Turnov, Czech Republic). The measurements were performed at laboratory temperature ($20 \pm 2\,^{\circ}C$).

2.3. Procedures

2.3.1. Preparation and pretreatment of the amalgam electrodes

Silver solid amalgam electrode (AgSAE), prepared as described [29], was first ground on a soft emery paper and then polished using polishing kit (Electrochemical Detectors, Turnov, Czech Republic) consisting of polishing polyurethane stocks, Al_2O_3 suspension (particle size 1.1 μm), and soft polishing Al_2O_3 powder (particle size 0.3 μm). Before starting measurements with the *polished* mercury-unmodified *AgSAE* (p-AgSAE), as well as after every pause longer than 1 h, the electrode surface was activated in the solution of 0.2 M KCl by applying −2.2 V for 300 s while stirring the solution (as it was recommended in [28]). In addition to the p-AgSAE, two types of mercury-modified AgSAE were prepared. *Mercury meniscus-modified* AgSAE (m-AgSAE) was prepared by immersion of the p-AgSAE into liquid mercury for 15 s (while swirling the flask with mercury). At the daily used m-AgSAE, the meniscus was renewed once a week by re-dipping the electrode in liquid mercury. *Mercury film-modified* AgSAE (MF-AgSAE) was prepared via electrolytic deposition of the mercury film onto the p-AgSAE surface (300 s at −0.8 V in solution of 0.01 M $HgCl_2$). The activation process of the m-AgSAE or MF-AgSAE surfaces was the same as that used for the p-AgSAE (see above). The computer program for regeneration of the amalgam electrode surfaces (polished amalgam, mercury meniscus or mercury film) after each measurement was inserted into the software design of individual measuring methods implemented under POLAR.PRO 5.1. It consisted of 30 electrochemical potential jumps between −0.1 and −1.7 V (usually directly in the analyzed solutions).

2.3.2. Voltammetric measurements

Differential pulse voltammetry (DPV) was used for most measurements with the following settings: pulse width 80 ms, pulse amplitude −50 mV, initial potential $E_{in} = -0.1$ V, final potential $E_{fin} = -1.7$ V, $E_{quiescent} = -0.1$ V, $t_{quiescent} = 2$ s, scan rate 25 mV s^{-1}, 0.1 M ammonium buffer, pH 9.75 (prepared by 1:1 mixing of 2 M NH_3 and 1 M HCl, followed by 10-fold dilution), containing 1 mM Co(II) was used as the base electrolyte. All measurements were performed on air. Peak heights were measured from linear baseline (tangent to the curve joining beginning and end of the given peak). Limits of detection (LD) were calculated using the ADSTAT software [49].

3. Results and discussion

Characteristic polarographic signals yielded by proteins in solutions of cobalt salts were for the first time reported by