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Bioelectrochemistry 65 (2004) 69–76

Bioelectrochemistry

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Redox kinetic measurements of glutathione at the mercury electrode by means of square-wave voltammetry. The role of copper, cadmium and zinc ions

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Received 2 January 2004; received in revised form 2 April 2004; accepted 24 May 2004
Available online 25 August 2004

Abstract

The electrode reaction of glutathione (GSH) at the hanging mercury drop electrode is studied by means of square-wave voltammetry (SWV). At potentials more positive than −0.350 V (vs. Ag/AgCl (3 mol/l KCl)) the oxidation of the mercury electrode in the presence of GSH leads to creation of a sparingly soluble mercury–GSH complex that deposits onto the electrode surface. Under cathodic potential scan, the deposited complex acts as a reducible reactant, giving raise to a well-defined cathodic stripping reversible SW voltammetric response. The electrode reaction can be described by the scheme: $Hg(SG)_{2(s)}+e^{-}+2H^{+}_{(aq)}=Hg_{(l)}+2GSH_{(aq)}$. Thus, the electrode reaction provides information on both thermodynamics and kinetics of the chemical interactions of GSH with mercury. An experimental methodology for measuring the kinetics of the electrode reactions, based on the property known as ''quasireversible maximum'', is developed. The standard redox rate constant is 5.09, 5.75 and 5.22 cm s^{-1} in a phosphate buffer at pH 5.6, 7.0 and 8.5, respectively, with a precision of ± 10%. The high rate of the electrode reaction reflects the strong affinity of GSH towards chemical interaction with mercury. The electrode reaction is particularly sensitive to the presence of heavy metal ions such as Cu^{2+}, Cd^{2+}, and Zn^{2+}. The rate of the electrode reaction decreases significantly in the presence of these ions due to simultaneous interactions of GSH with the respective ion and mercury.

Keywords: Glutathione; Square-wave voltammetry; Redox kinetics; Quasireversible maximum

1. Introduction

Three peptide glutathione (GSH) is of particular importance for all mammalian cells performing a variety of physiological and metabolic functions, including the detoxification of free radicals, metals, and other electrofilic compounds [1]. One important detoxification mechanism involves the binding of GSH to electrophilic chemicals through SH group to form S-conjugates that are exported out of the cell. It is well established that GSH binds endogeneous metal ions, such as copper, selenium, chromium, and zinc, via nonenzymatic reactions. The binding of GSH to these metal ions serves important roles: (i) it serves to limit and regulate the reactivity of metal ions, (ii) it facilitates their membrane transport and elimination from the cell and organism; and (iii) in some cases, it leads to the formation of essential biological mediators.

Voltammetric techniques lend themselves as a powerful tool to study the interactions between metal ions and divers ligands, including simple monomeric or macromolecular ligands [2–7]. Particularly appealing are the electrode reactions of thiol-containing compounds, such as GSH, at the mercury electrode [8–11]. Over the last several decades significant scientific efforts have been paid on understanding the electrode mechanisms of these compounds at the mercury electrode. It is already well established that the electrochemical activity of the thiol-containing compounds at the mercury electrode are mainly due to chemical interactions of the thiol with the electrode material, rather than the redox transformation of the thiol itself. Indeed, the thiol group has a strong chemical activity towards heavy metal ions resulting in creation of sparingly soluble complexes that are deposited onto the electrode surface. Using a single

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doi:10.1016/j.bioelechem.2004.05.007

mercury drop as a working electrode, the insoluble complex is formed under anodic polarization of the electrode in the presence of the reactive thiol. Afterwards, the insoluble complex plays a role of an electroactive reducible reactant. Applying a cathodic potential scan, the complex can be reduced resulting in a stripping off the reactive thiol from the electrode surface. The electrode reaction can be thus described by the scheme:

$$Hg_aL_{2(s)} + 2e^- + 2H^+_{(aq)} = aHg_{(l)} + 2HL_{(aq)} \quad (I)$$

where a equals either 1 or 2. The redox active centre in the deposited complex is the mercury ion, whereas the thiol containing compound L plays a role of a complexing ligand only. The properties of this specific electrode reaction are of particular importance since they provide information on both thermodynamics and kinetics of the chemical interactions of the reactive thiol containing ligand L with heavy metal such as mercury.

In a series of previous publications, a theoretical basis has been provided for studying the electrode reactions of the type (I) by means of square-wave voltammetry (SWV) [12–15]. The latter technique is one of the most advanced voltammetric techniques unifying the advantages of cyclic voltammetry and pulse voltammetric techniques [16]. In particular, it is a powerful tool for measuring the redox kinetics of electrode reactions of an immobilized reactant, such as reaction (I).

In the present paper, an experimental methodology for redox kinetic measurements of the reaction (I) is presented. GSH is chosen as a reacting ligand due to its remarkable physiological significance. The experimental methodology is based on the property known as "quasireversible maximum" for which the theoretical basis has been established in the recent studies [14,17–20]. In addition, the redox kinetics of the reaction (I) is measured in the presence of Cu^{2+}, Cd^{2+}, and Zn^{2+} ions. It is demonstrated that the kinetics of the reaction (I) is dramatically sensitive to the presence of these ions, revealing that the reaction can serve as a sensitive indicator for the simultaneous interactions of GSH with mercury and other heavy metal ions.

2. Experimental

All used chemicals where of analytical reagent grade. GSH is a product of Sigma, whereas all the other chemicals were purchased from "Merck". Doubly distilled water was used in all experiments. The stock solution of GSH was prepared by dissolving in a doubly distilled water. Phosphate buffers (0.10 mol/l), prepared from K_3PO_4 and KOH, were used as supporting electrolytes.

Pure nitrogen was used for purging the electrolyte solutions for 8 min prior to each measurement. A nitrogen blanket, over the electrolyte solution, was maintained thereafter.

All voltammograms were recorded using μAutolab multimode potentiostat/galvanostat (ECO Chemie, Utrecht, Netherlands), which was connected to the static mercury drop electrode (SMDE), Model 663 VA, Metrohm (Switzerland). A platinum wire was used as an auxiliary electrode and Ag|AgCl|3 mol/l KCl as the reference electrode.

3. Results and discussion

3.1. General voltammetric properties of GSH at the hanging mercury drop electrode

A typical SW voltammetric response of GSH at a mercury electrode, recorded in a phosphate buffer at pH 7, is represented in Fig. 1. The response consists of a forward (reduction, curve 1 in Fig. 1) and a backward (oxidation, curve 2 in Fig. 1) component, indicating that electrode reaction is chemically reversible. The peak potentials of the reduction and oxidation components are -0.441 and -0.455 V, respectively. Interestingly, the reduction component is located at more positive potential than the oxidation one, which is opposite to the voltammetric behavior of a common dissolved redox couple. The relative position of the reduction and oxidation components of the SW voltammogram of GSH is typical for a cathodic stripping electrode reaction of a completely immobilized reactant [13–15]. The ratio of the reduction and oxidation peak currents is $I_{p,c}/I_{p,a} = 0.625$. The net SW component (curve 3 in Fig. 1), calculated as a difference between the cathodic and anodic currents, provides information on both reduction and oxidation half electrode reactions. As can be seen, the net SW component is a single well developed bell-shaped curve with a peak potential of $E_p = -0.439$ and half-peak width of $\Delta E_{p/2} = 0.118$.

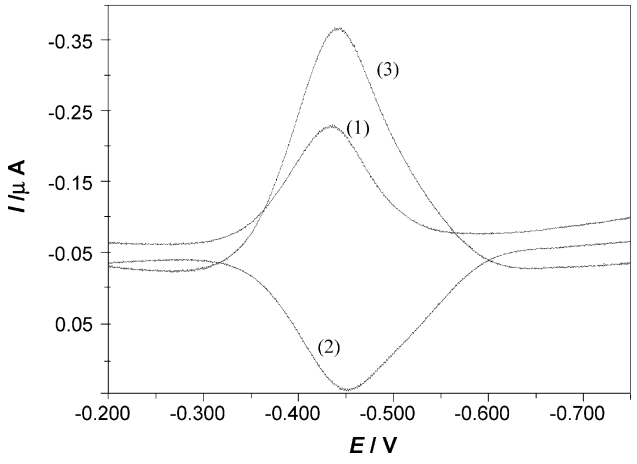

Fig. 1. SW voltammogram of 5×10^{-6} mol/l GSH solution recorded in 0.1 mol/l phosphate buffer at pH 7. The components of the SW response are: forward (reduction) (1); backward (oxidation) (2); and net (3) current. The other experimental conditions were: SW frequency $f = 150$ Hz, SW amplitude $E_{sw} = 50$ mV, step potential $dE = 0.45$ mV, accumulation potential $E_{acc} = -0.2$ V, and accumulation time $t_{acc} = 15$ s.

As previously mentioned, the electrochemical activity of GSH at the mercury electrode is a consequence of its strong chemical affinity towards the electrode material, rather than due to redox transformation of GSH itself. In the course of the accumulation period, a complex is created between GSH and mercury ions. The mercury electrode serves as virtually inexhaustible source of mercury ions through the following equilibria: $Hg_{(l)} \leftrightarrows Hg_{(aq)}^{2+} + 2e^-$, and $2Hg_{(l)} \leftrightarrows Hg_{2(aq)}^{2+} + 2e$. In the absence of compounds capable to react chemically with mercuric ions, the position of the latter redox equilibria is predominantly fixed by the applied potential at the mercury electrode. However, in the presence of GSH, mercury ions are bounded through the thiol group to form a sparingly soluble complex. The formed compound deposits onto the electrode surface due to its low solubility. As assumed by Heyrovsky et al. [9], the most hydrophobic part of the complex is the mercury atom. Thus the complex is adsorbed predominantly through the mercury atom on the mercury electrode. Once the complex is adsorbed, the mercury atom incorporates into the bulk structure of the mercury electrode. Therefore, after the deposition step, the mercury electrode surface is covered by a film of GSH molecules (or more precise GS-ions) chemically bounded to the mercury, via the sulfur atom.

In the course of the stripping step, the deposited complex at the electrode surface plays a role of an electrochemically reducible reactant. Thus, the forward (reduction) component of the SW response reflects the reduction of the GS–Hg complex, resulting in stripping off the GS^- ions from the electrode surface. Accordingly, the backward (oxidation) component of the SW response reflects the creation of the GS–Hg complex, which involves an oxidation of the mercury electrode to mercury ions and bonding the ions to the GSH molecules. Thus, the overall electrode mechanism can be described by the following scheme:

$$Hg(SG)_{2(s)} + 2e^- + 2H^+_{(aq)} \leftrightarrows Hg_{(l)} + 2GSH_{(aq)} \qquad \text{(II)}$$

Note that an analogous reaction scheme could be written considering the complex of the type $Hg_2(GS)_2$. However, by analogy with cysteine [9], we assume that if ever the complex of the type $Hg_2(GS)_2$ was formed during the deposition step, it transforms simultaneously into the more stable form $Hg(GS)_2$.

It has to be emphasized that electrode mechanisms of the type (II) are frequently accompanied by adsorption of the thiol, i.e. GSH, at potential more negative than the formal potential of the reaction (II). It means, after stripping off the deposited complex, the reacting ligand remains adsorbed on the electrode surface. The theory for cathodic stripping electrode reaction accompanied by the adsorption of the reacting ligand has been recently developed under conditions of SWV, and simple diagnostic criteria for recognition of the ligand adsorption have been established [13].

By analogy with cysteine that adsorbs significantly on the mercury electrode [9], one can expect a similar effect in the cathodic stripping electrode mechanism of GSH. Nevertheless, in the current study it seems that the adsorption of GSH does not play a significant role under the selected experimental conditions. This assumption has been supported by voltammetric experiments in which GSH was accumulated at potential of $E_{acc} = -0.750$ V and the SW voltammograms have been recorded in anodic direction. In a phosphate buffer at pH = 5.6 and GSH concentration of 1×10^{-6} mol/l, the accumulation of GSH from 0 to 45 s resulted in a constant net peak current of $I_p = 9 \pm 1.05$ nA, and peak potential of $E_p = -0.412 \pm 0.003$ V.

Furthermore, a careful comparison of the shape of the experimental voltammograms of GSH with the theoretically calculated voltammograms for the simple cathodic stripping mechanism [14] and the adsorption coupled cathodic stripping mechanism [13], does not indicate significant adsorption of GSH. The overall properties of the experimental response fit better with the theoretical response of the simple cathodic stripping reaction [14]. It is finally worth noting that the SW voltammetric response of the adsorption coupled cathodic stripping reaction [13] is attributed with a unique feature under the large amplitude of the potential modulation, i.e. the splitting of the net SW peak. The splitting, as an intrinsic feature of the latter mechanism, serves as a qualitative diagnostic criterion for recognition of the mechanism type as well as for redox kinetic measurements [15,21]. However, in the case of GSH, the variation of the amplitude over the wide interval from 2 to 100 mV, for a series of constant frequencies, did not result in splitting of the net SW peak, indicating the absence of significant adsorption of GSH. Accordingly, for the purpose of this study, the electrode reaction of GSH will be treated as a simple cathodic stripping mechanism of the type (II).

A thermodynamic treatment of the electrode reaction (II) leads to the following formal potential of the redox system:

$$E^{\emptyset'} = E^{\emptyset} - 2.303\frac{RT}{F}\mathrm{pH} - 2.303\frac{RT}{2F}\log(a_{GSH}) \qquad (1)$$

where $E^{\emptyset'}$ and $E^{\emptyset}$ are the formal and standard potential of the system, respectively, a_{GSH} is the activity of GSH, R is the gas constant, F is the Faraday constant, and T is the thermodynamic temperature. For a reversible electrode reaction, the peak potential of the net SW response is equivalent to the formal potential of the redox system. In the present case, the net peak potential depends linearly on pH of the phosphate buffers with a slope of $(\Delta E_p/\Delta\mathrm{pH}) = -54.8$ mV, which is close to -59 mV, predicted by Eq. (1). Moreover, the net SW peak potential is also a linear function of the logarithm of the GSH concentration with a slope of $\Delta E_p/\Delta\log(c_{GSH}) = -24.5$ mV, which is

close to the theoretical value of 29 mV, supporting strongly the proposed electrode mechanism (II).

As the electrode mechanism involves formation of a sparingly soluble complex deposited on the electrode surface, the accumulation time plays a significant role in the overall electrode reaction. The typical effect observed at prolonged accumulation time is represented in Fig. 2. For GSH concentration of 5×10^{-6} mol/l, the net SW peak current is proportional to the accumulation time over the interval $t_{acc.} < 5$ s, whereas the peak potential and half-peak width are virtually independent on the accumulation (see peak I in Fig. 2). Within this accumulation interval, the increase of the net SW peak is proportional to the enhancement of the surface concentration of the first monomolecular deposited layer. However, for $t_{acc.} > 5$ s, the half-peak width begins to increase, and for $t_{acc.} = 10$ s, a new SW peak emerges at more positive potential (see peak II in Fig. 2). The further increasing of the accumulation time causes the new peak II to increase, whereas the first peak I remains at a constant height. In addition, it was found that the critical value of the accumulation time, above which the splitting of the response appears, depends on the GSH concentration. The higher the GSH concentration, the shorter is the critical accumulation time causing the splitting. Furthermore, the maximal height of the first peak I is solely determined by the electrode surface area of the hanging mercury drop electrode. On the base of these results we assume that the first peak I is ascribed to the stripping off the first deposited layer at the electrode surface. Its height is determined by the surface concentration, whilst its maximal height corresponds to the complete saturation of the electrode surface by a monolayer film. The second peak II, positioned at more positive potential, is not limited by the electrode surface area, thus it is ascribed to the reduction of the subsequent higher deposited layers of the insoluble complex. The first deposited layer is firmly bounded to the electrode surface and its electrochemical stripping requires higher energy than the stripping of the higher layers. For these reasons the peak potential of the first peak is more negative than the second one. A similar effect of the deposition time was observed in the electrode reactions of 1-isobutyl-tetrazole-5-thiole [10] and 1-benzyl-tetrazole-5-thiole [11].

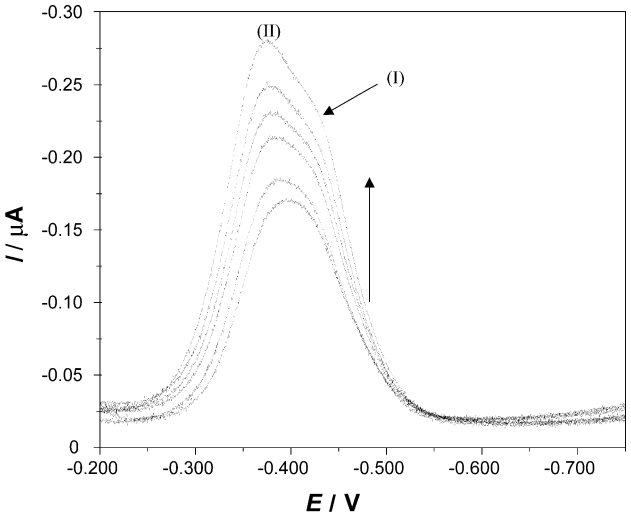

Fig. 2. The effect of the accumulation time on the net SW response of GSH recorded in a phosphate buffer at pH 5.6. The accumulation time increases in the direction of the arrow from 0, 2, 4, 6, 8, to 10 s. The frequency of the potential modulation was $f = 100$ Hz. The other conditions were same as in the caption of Fig. 1.

3.2. Redox kinetic measurements

We have recently demonstrated that SWV is particularly convenient and powerful technique for redox kinetic measurements of electrode reactions of the general type (I) [14,15]. The methodology for estimation of the standard redox rate constant of electrode reaction (I) is based on the property known as "quasireversible maximum" [12,14,15,21]. According to the theory [14], the apparent reversibility of the electrode reaction (I) is determined by the single complex kinetic parameter defined as

$$\kappa = k_s D^{-1/4} f^{-3/4} r_s^{-1/2} \quad (2)$$

where k_s (cm s^{-1}) is the standard redox rate constant of the reaction (II), D (cm^2 s^{-1}) is the diffusion coefficient of the reacting ligand (here it is GSH), f (s^{-1}) is the frequency, and $r = 1$ cm is an auxiliary constant. The dimensionless kinetic parameter κ, controlling the reversibility of the electrode reaction, unifies all relevant phenomena affecting the electrode reaction, such as diffusion mass transport, kinetics of the electron transfer, and the time window of the voltammetric experiment. The ratio of the net peak current and the frequency of the potential modulation, I_p/f, depends non-linearly on the kinetic parameter κ, forming a well-developed maximum within the quasireversible kinetic region [14–21]. Hence, this type of the dependency, typical for electrode reactions of an immobilized reactant, is called "quasireversible maximum". The position of the quasireversible maximum is associated with a strictly defined critical value of the kinetic parameter κ_{max}. The critical kinetic parameter is a function of the SW amplitude and the electron transfer coefficient of the electrode reaction. For the electron transfer coefficient $\alpha = 0.5$ and the SW amplitude of $E_{sw} = 50$ mV, the critical parameter is $\kappa_{max} = 0.47$. Thus, if κ_{max} is theoretically calculated, the frequency associated with the quasireversible maximum, f_{max}, experimentally measured, the standard redox rate constant can be calculated through the simple formula:

$$k_s = \kappa_{max} D^{1/4} f_{max}^{3/4} r_s^{1/2} \quad (3)$$

In the experimental study, one analyze the net SW peak current as a function of the frequency over the wide

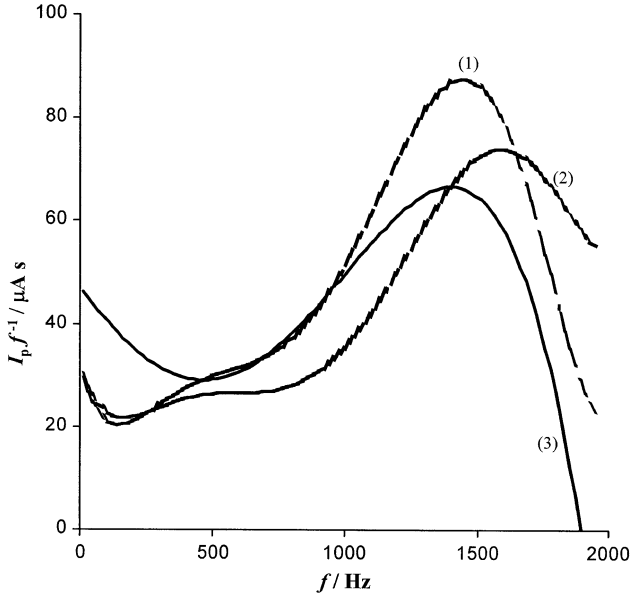

Fig. 3. Quasireversible maxima of GSH recorded in a phosphate buffer at pH 5.6 (1); 7(2) and 8.5 (3). The other conditions were same as in the caption of Fig. 1.

frequency interval, i.e. $8 < f/\mathrm{Hz} < 2000$. Plotting the ratio I_p/f as a function of f, one obtains a parabola-like dependence. Note that the variation of the frequency in the experiment corresponds to the variation of the kinetic parameter κ in the theoretical study. The position of the experimentally constructed quasireversible maximum reveals the critical frequency f_{max} that is used for estimation of the standard redox rate constant with the aid of Eq. (3).

Fig. 3 depicts the quasireversible maxima of GSH measured in a phosphate buffer at three pH values. The pH values are close to that encountered in physiological fluids. Table 1 summarized the critical frequencies together with the estimated standard redox rate constants. For these calculations, the diffusion coefficient of GSH and the electron transfer coefficient of the electrode reaction were assumed to be $D = 5 \times 10^{-6}$ cm^2 s^{-1}, and $\alpha = 0.5$, respectively. As pointed out in Ref. [14], the accuracy of the estimated values is $\pm$ 10%. The high values of k_s reflect the high chemical affinity of GSH towards formation of compounds with heavy metal ions such as the mercury ions.

The quasireversible maxima have been measured at different accumulation times and different concentrations of GSH. For concentration of $c(\mathrm{GSH}) \leq 5 \times 10^{-6}$ mol/l and the accumulation time of $t_{acc.} \leq 15$ s, the position of the quasireversible maximum is virtually identical. It points out that under these conditions the electrode reaction proceeds without significant interactions between the deposited species, which is the main prerequisite for correct estimation of the standard redox rate constant utilizing the quasireversible maximum [14].

3.3. Redox kinetic measurements in the presence of Cu^{2+}, Cd^{2+}, and Zn^{2+} ions

In order to study the interactions of GSH with Cu^{2+}, Cd^{2+} and Zn^{2+} ions, one can either inspect the voltammetric response of these ions at different concentrations of GSH, or alternatively, one can inspect the influence of these ions on the cathodic stripping response of GSH. The former methodology is virtually inapplicable for Cu^{2+} since its voltammetric response is strongly overlapped by the mercury oxidation current in the presence of GSH. In addition, for both Cd^{2+} and Zn^{2+}, the voltammetric response is appreciably complex in the presence of GSH [2–7] and hence deriving unambiguous conclusions on the type and stability of the formed complexes is very difficult. This is particularly true for a medium at physiological pH values ($7 < \mathrm{pH} < 8$) due to the formation of interfering hydroxo complexes.

Cathodic stripping voltammetry appears to be an alternative approach to cope with all drawbacks previously mentioned. Similar to the methodology of self-assembled monolayer on a gold electrode, in cathodic stripping voltammetry the thiol molecules bounded via the sulfur atom are well organized on the mercury electrode surface. Although the thiol group is already engaged in the interaction with the electrode, the rest of the molecule remains free for additional covalent, or even electrostatic interactions with other ions present in the electrolyte solution. Furthermore, once the thiol is stripped off from the electrode surface under cathodic potential pulses, the kinetics of the reestablishing of the film under anodic potential pulses will be affected by the presence of other heavy metal ions that exhibit affinity towards engaging the thiol group in complexes formation. Accordingly, it is reasonable to expect that the kinetics of the overall electrode reaction (II) will be sensitive to the presence of heavy metal ions affecting either the morphology of the film or kinetics of its formation, or both.

Fig. 4 shows the influence of Cu^{2+} ions on the shape of the SW voltammetric response of GSH. It is evident that both

Table 1
Quasireversible maxima of GSH measured in a phosphate buffer at different pH

pH	GSH		Cu^{2+}		Cd^{2+}		Zn^{2+}	
	f_{max}/s^{-1}	k_s/cm s^{-1}	f_{max}/s^{-1}	k_s/cm s^{-1}	f_{max}/s^{-1}	k_s/cm s^{-1}	f_{max}/s^{-1}	k_s/cm s^{-1}
5.6	1400	5.09	350	1.80	800	3.34	< 8	< 0.11
7	1650	5.75	200	1.18	850	3.5	< 8	< 0.11
8.5	1450	5.22	< 8	< 0.11	1100	4.24	< 8	< 0.11

The parameters of the excitation potential modulation were: SW amplitude $E_{sw} = 50$ mV and step potential d$E = 0.45$ mV. The concentration of GSH, as well as the concentration of metal ions, was 5×10^{-6} mol/l.

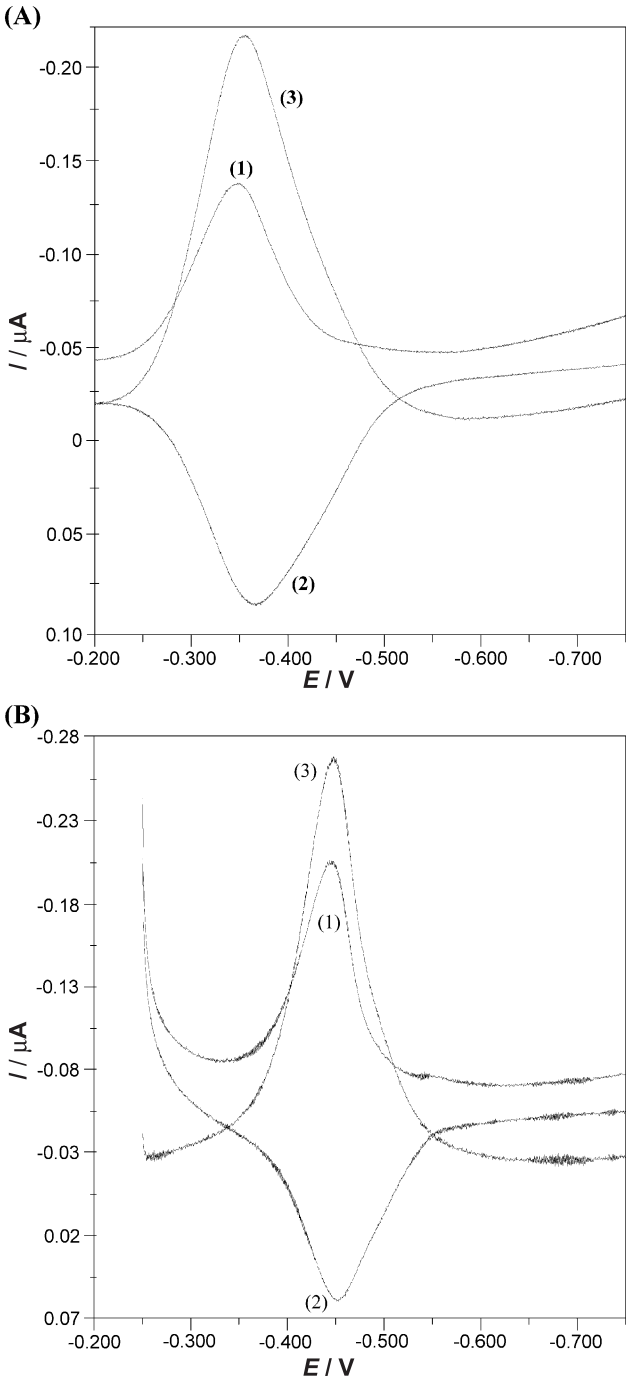

Fig. 4. SW voltammograms of 5×10^{-6} mol/l GSH solution (A) and 5×10^{-6} mol/l GSH + 4×10^{-6} mol/l Cu^{2+} ions (B) recorded in a phosphate buffer at pH 5.6. The numbers (1), (2), and (3) refer to the forward, backward, and net SW components, respectively. The other conditions were same as in the caption of Fig. 1.

forward and backward components become sharper in the presence of copper, whereas their peak potential separation decreases by increasing the content of copper. Being shifted towards more negative potentials, the net SW peak enhances in proportion to copper concentration. The shift of the net SW peak potential with the logarithm of the copper concentrations is linear ($R^2=0.9975$) with a slope of $\Delta E_p/\Delta\log(c_{Cu^{2+}}) = -40$ mV. At the same time, the half-peak width decreases by increasing copper content.

Obviously, the electrode reaction (II) is significantly modified in the presence of copper. The morphology of the monomolecular deposited film becomes more uniform, resulting in a sharper forward (reduction) component in the presence of copper ions. The sharpness of the backward (oxidation) component is even more evident. Its shape implies that GS^- ions (or, after protonation, GSH molecules) remain immobilized on the electrode surface soon after the reduction of the $Hg(GS)_2$ complex due to additional interactions with copper ions. All these results indicate strongly that copper creates a stable hydrophobic complex with GSH that adsorbs to the electrode surface. Nevertheless, the creation of copper complex does not prevent the creation of the mercury complex. On contrary, it seems that a synergetic effect occurs, i.e. copper complex serves as an additional source for GSH molecules that react chemically with the electrode surface. This phenomenon could be possible only by assuming that the thiol group, which plays a crucial role in the chemical interactions between GSH and mercury, is still free, i.e. it is not engaged in coordination of copper ion. This assumption is in accordance with the pronounced affinity of copper towards nitrogen as a coordinating atom. Thus, on the electrode surface, the film of GSH molecules is stabilized by lateral interactions (coordinations) with the copper ions.

The behavior of the present electrochemical system is in a fairly good correlation with the physiological role of GSH in the copper metabolism. Namely, it has been assumed that copper is complexed by GSH soon after entering the living cell [22]. The complexed metal is than transferred to metallothionien where it is stored. The question is however what is the driving force transferring the copper complex to the metallothionien. In our voltammetric experiment an analogous effect was observed. It is the hydrophobicity of the copper complex that forces it towards the hydrophobic medium, i.e. the electrode, where the complex adsorbs. Thus copper ions serve as an additional supplier of the electrode surface with GSH molecules that are still capable to react with the electrode surface. For the same reasons, the copper complex in the living cell is forced towards the hydrophobic medium, i.e. metallothioniens.

The quasireversible maxima of GSH measured in the presence of copper were associated with the following critical frequencies: 350, 200, and <8 Hz, for pH = 5.6, 7.0, and 8.5, respectively (see Table 1). Thus, the estimated standard redox rate constants of the electrode reaction (II) in the presence of copper are 1.80, 1.18, and <0.11 cm s^{-1}, for pH = 5.6, 7.0, and 8.5, respectively. The decreased rate of the reaction (II) reflects the simultaneous interaction of GSH molecules with mercury and copper ions. The rate of the electrode reaction decreases by increasing pH. In a basic medium, the dissociation of the carboxylic group and deprotonation of the amino groups of GSH occur, enabling

stronger coordination with copper ions that causes a decrease of the electrode reaction rate.

In the presence of Cd^{2+} ions, the shape of the forward and backward components of the SW response is slightly modified (see Fig. 5). The morphology of the deposited film is not changed appreciably. Contrary to the copper ions, the film is not stabilized by simultaneous interactions with the mercury electrode and cadmium ions. It is however evident that the backward component decreases (see Fig. 5), indicating a competition between the mercury and cadmium ions towards creation of complexes through the same atomic group, i.e. the thiol group. Bonding the thiol group, cadmium ions prevent the formation of the mercury complex and hinder the rate of the electrode reaction. The redox kinetic measurements of the GSH in the presence of Cd^{2+} clearly support these assumptions showing that the reversibility of the electrode reaction is significantly decreased (see Table 1).

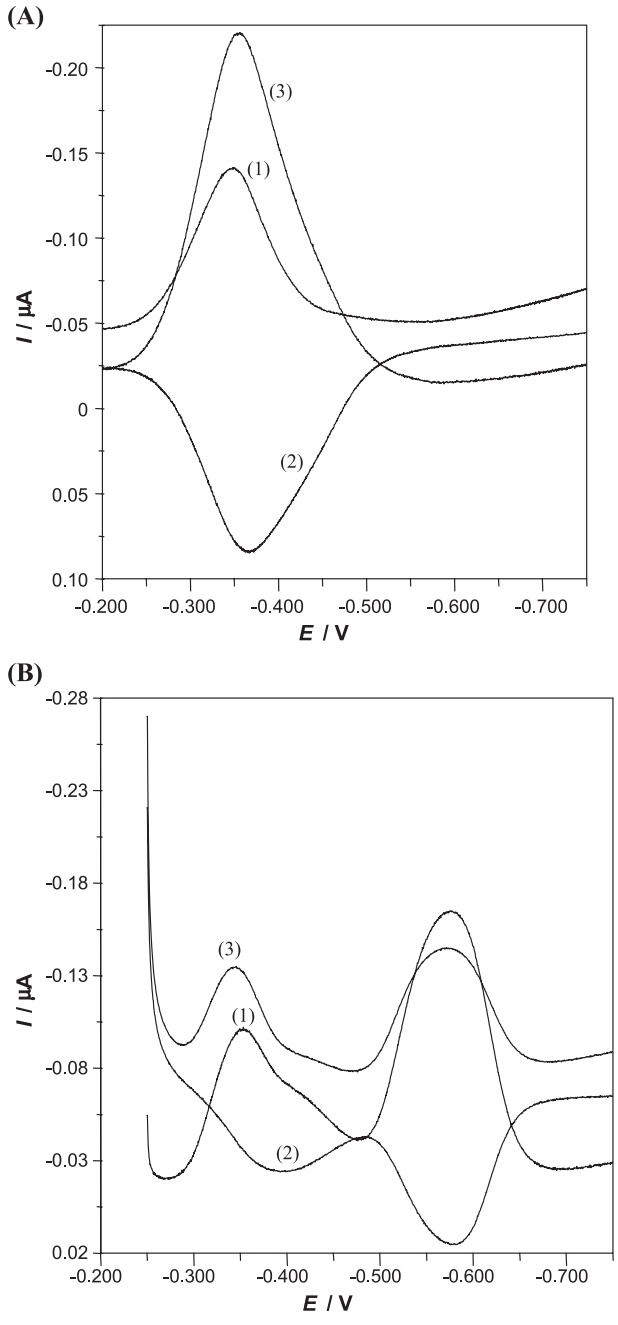

Fig. 5. SW voltammograms of 5×10^{-6} mol/l GSH solution (A) and 5×10^{-6} mol/l GSH + 5×10^{-6} mol/l Cd^{2+} ions (B) recorded in a phosphate buffer at pH 5.6. The numbers (1), (2), and (3) refer to the forward, backward, and net SW components, respectively. The frequency of the potential modulation was $f = 100$ Hz. The other conditions were same as in the caption of Fig. 1.

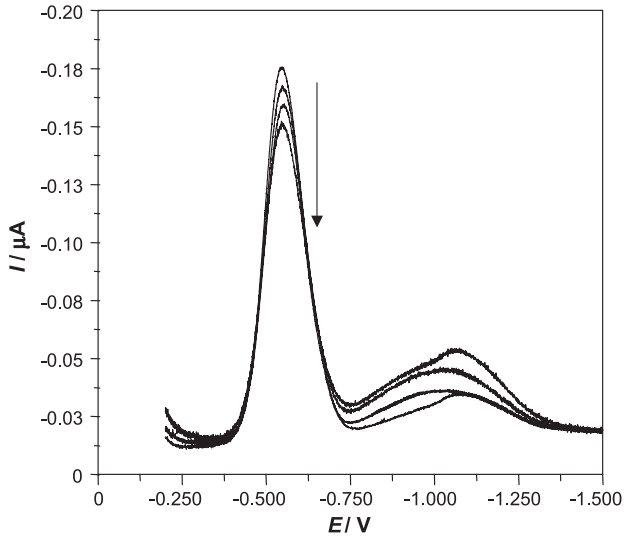

Fig. 6. The effect of Zn^{2+} ions on the net SW response of GSH recorded in a phosphate buffer at pH 8.5. The concentration of GSH was 5×10^{-6} mol/l GSH. The concentration of Zn^{2+} ions increases in the direction of the arrow from 0; 1×10^{-6}; 3×10^{-6} to 5×10^{-6} mol/l. The voltammograms were recorded without accumulation time. The other conditions were same as in the caption of Fig. 1.

The influence of Zn^{2+} ions is completely different than those of Cu^{2+} and Cd^{2+} ions. The shape of all components is unaffected by Zn^{2+} ions, as well as the net SW peak potential remains the same. Only the net SW peak current decreases in proportion to the concentration of Zn^{2+}, over the concentration interval $1 \times 10^{-6} \leq c(Zn^{2+})/\text{mol/l} \leq 5 \times 10^{-6}$ (see Fig. 6). The overall effect of Zn^{2+} is equivalent to the decreasing of GSH concentration in the electrolyte solution. Although the shape of the voltammetric response does not indicate simultaneous interactions of GSH with the mercury electrode and zinc ions, the redox kinetic measurements revealed a dramatic decrease of the rate of the electrode reaction in all three buffer solutions (see Table 1).

This effect could be rationalized by assuming a creation of a zinc complex with GSH in which the thiol group is occupied in the coordination of zinc ion. It has been recently proposed that Zn^{2+} ions create a complex with GSH with a stoichiometry 2:2. Diaz-Cruz et al. [2,3] assumed that the zinc complex is a binuclear cluster linked by bridging cysteine thiolate ligands, in which the two Zn^{2+} ions are tetrahedrally coordinated by two α-amino groups and the two glycil carboxylic acids. Therefore, the

decreased rate of the cathodic stripping reaction of GSH in the presence of Zn^{2+} ions is most probably a consequence of the slow dissociation rate of the stable Zn–GSH complex.

Acknowledgements

V.M. gratefully acknowledges the financial support from both A. v. Humboldt-Stiftung and the Ministry of Education of Macedonia.

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