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Homocysteine Electrochemistry at a Mercury Electrode in the Presence of Nickel Ions

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Abstract

Homocysteine (Hcy) gives rise to a series of voltammetric peaks in the presence of Ni^{2+} and under the conditions of cathodic stripping voltammetry, as follows: A) cathodic reaction of mercury thiolate (with a Ni^{2+}-dependent peak potential); B and C) catalytic Ni^{2+} reduction peaks; E) catalytic hydrogen evolution; G) Hcy-assisted anodic reaction of mercury; F) Hcy-catalyzed anodic reaction of Ni(0).This paper reveals some differences between homocysteine and cysteine behaviour under these conditions and points out possible applications for Hcy determination in the presence of Cys.

Key words: Homocysteine, Cysteine, Cathodic stripping voltammetry, Nickel ion, Electrocatalysis, Mercury thiolates.

Introduction

Homocysteine (Hcy, Chart 1 a) is a sulfur-containing, nonproteinogenic amino acid biosynthesized from methionine. Hcy takes a key place between the folate and activated methyl cycles, and has three main metabolic fates: to be remethylated to methionine, to enter the cysteine (Cys) biosynthetic pathway, or to be released into the extracellular medium. Mild

hyperhomocysteinemia has been suggested as a new, independent risk factor for cardiovascular disease. This fact has produced a new, increased interest in the study of Hcy metabolism and its relation to pathogenesis [1]. Hcy is rapidly oxidized in plasma to the disulfides homocystine and Cys-Hcy and also is bound to proteins by disulfide bridges [2].

Plasma/serum total Hcy (also termed homocyst(e)ine), is the sum of Hcy in all 3 above mentioned components. Normal levels of plasma homocyst(e)ine are considered to be between 5 and 15 μmol/L. Moderate, intermediate, and severe hyperhomocyst(e)inemia refer to concentrations between 16 and 30, between 31 and 100, and >100 μmol/L, respectively [3]. For analytical purposes, Hcy is first converted to a free thiol species by reduction from its disulfide forms before total Hcy being determined by a suitable method like gas chromatography, liquid chromatography, capillary electrophoresis or immunoassay [4], [5], [6], [7].

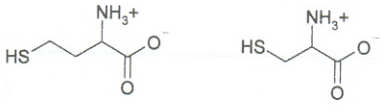

Chart 1. Structure of homocysteine (Hcy, a) and cysteine (Cys, b).

Due to the advantages of electrochemical Hcy detection in liquid chromatography [5], electrochemical reactivity of Hcy at various electrodes deserved a particular attention [5], [8]. As detection at mercury covered gold electrodes proved particularly versatile, Hcy behavior at mercury electrodes was explored and compared with that of Cys, (Chart 1 b) that is also present in biological sample and may interfere due to structure similarity [9], [10]. No major differences between Hcy and Cys were actually noticed and, at a first glance, electrochemistry at mercury electrodes is not able to distinguish one of the above compounds in the presence of the other one.

The single structure difference between Hcy and Cys is the length of the thiol-containing side chain. (Chart 1). Consequently, Cys and Hcy are expected to behave differently when interacting with a metal ion (like Ni^{2+}) to form coordination compounds. Ni^{2+} interaction with Cys gives rise to several chelate species hat are extremely stable and include five-member rings with the Ni^{2+} ion coordinated by both $-S^-$ and $-NH_2$ groups [11]. No data on Hcy-Ni^{2+} complexes are available, but a 6-membered chelate involving thiol and amino groups in Hcy molecule should by much less stable than the Cys analogue. We did select Ni^{2+} as a representative metal ion because catalytic electrochemical processes in the

Ni^{2+}-Cys system are well documented (refs. [12,13] and references therein). Electrochemical investigations of Hcy in the presence of other metal ions like copper or cobalt were already reported by Heyrovský and Vavřička [10].

This paper presents first an overview of the electrochemical processes occurring in the presence of Hcy and Ni^{2+} at a hanging mercury drop electrode (HMDE) under the conditions of cathodic stripping voltammetry (CSV). Then, more details on electrochemical reactions susceptible of distinguishing between Hcy and Cys are presented.

Experimental

Hcy and Cys were Sigma products (98%) and were used as received. Fresh solutions were prepared daily. Other reagents were of the p.a. grade. The supporting electrolyte was a 0.05 M Na_2HPO_4 solution with the pH adjusted by 4 M $HClO_4$.

Staircase voltammetric investigations (50 mV/s scan rate) were performed by means of an Autolab PGSTAT 30 (Eco Chemie) instrument fitted with a Metrohm 663 VA stand. The cell included, in addition to the HMDE, an Ag|AgCl (3.0 M KCl) reference (connected via a salt bridge filled with the supporting electrolyte) and a glassy carbon auxiliary electrode separated from the test solution by means of a porous glass plug. The deposition step occurred in a stirred solution and the voltammetric run followed after 15 sec equilibration time.

Results and discussion

Electrochemical processes in the Ni^{2+}-Hcy system

In the experiments here reported, every voltammetric run was preceded by a pre-deposition step when mercury thiolate [$Hg(RS)_2$] forms. A subsequent CV scan reveals a series of electrochemical processes as emphasized in Fig. 1.

Peak A is caused by the reduction of mercury ion in $Hg(RS)_2$. More details on this process are presented in next Section.

Peaks B and C are due to Ni^{2+} reduction catalyzed by Hcy. The peak B occurs at the same potential than the similar peak produced by Cys [13] and was therefore ascribed to a similar reaction catalyzed by adsorbed Hcy. The process C is alike to the catalytic nickel reduction catalyzed by non-adsorbable amino acids like selenomethionine [14], arginine,

lysine and ornithine [15]. It was consequently ascribed to a similar process induced by non-adsorbed Hcy.

Peak D is due to the diffusion controlled Ni^{2+} reduction. The amount of Ni^{2+} reduced in the peaks B on C region is negligible and the peak D current is the same in the absence (curve 1) and in the presence of Hcy (curve 2-4).

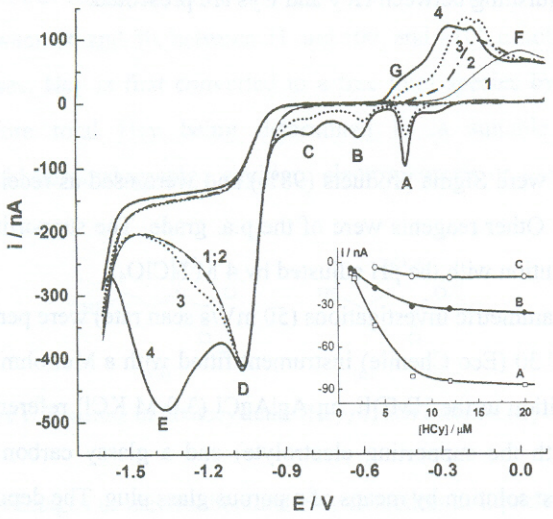

Figure 1. Electrochemical reactions at the HMDE in the presence of Hcy and Ni^{2+} ($4 \cdot 10^{-4}$M). pH 6.5. Hcy concentration (M): (1) 0; (2) $6 \cdot 10^{-7}$; (3) $4 \cdot 10^{-6}$ (4) $1.2 \cdot 10^{-5}$. E_{dep}, +0.1 V; t_{dep}, 30 s. Inset: effect of Hcy concentration on the peaks A, B and C.

Peak E may be ascribed to the catalytic evolution of hydrogen. In this connection, a marked difference between Cys and Hcy should be pointed out. In this potential region, Cys induces a sigmoid voltammetric pattern. The shape of the current-voltage curve is in this case the consequence of the kinetic control by a parallel chemical reaction [13]. Conversely, Hcy generates the peak–shaped pattern E which reminds the peak produced by hydrogen evolution catalyzed by cobalt sulfide [16]. Moreover, the intensity of the peak E produced by Hcy is much higher than that measured with Cys at a similar concentration. Such dissimilarity was also noticed with Cys or Hcy in the presence of Co^{2+} [10]. We infer therefore that a small amount of Hcy gets decomposed during the deposition step and produces nickel sulfide that acts as a catalyst in the peak E process.

In order to check this hypothesis, CSV was performed under similar conditions with thiourea and Ni^{2+}- containing solutions. Under these conditions, mercury sulfide (HgS) forms under anodic polarization at +0.1 V. On the cathodic stripping scan, mercury ion reduction gives rise to the specific peak that is alike to that recorded with a hydrogen sulfide containing solution. Nickel sulfide also form if the deposition step is performed in the presence of Ni^{2+}. Nickel sulfide (or its reduction product) generates a cathodic peak that is alike to the peak E produced by Hcy. These findings confirm the above hypothesis on the nature of the peak E recorded in the Ni^{2+}-Hcy system.

Peak G is an anodic peak that occurs at approximately the same potential than the peak A and was consequently ascribed to mercury oxidation resulting in mercury thiolate.

Peak F is due to the anodic reaction of Ni(0) that forms during the prior cathodic scan. Similarly to Cys [13], Hcy causes the overvoltage of this process to decrease due to the formation of an intermediate nickel-thiolate complex.

All that processes depend in a similar way on the deposition time proving that the accumulation is a critical step for each of them.

When performing this investigation we have been looking for differences between Hcy and Cys. According to preliminary investigations, such differences occur in the peak A and E processes. That is why these processes will be further dealt with in a more detailed way.

Mercury ion reduction in the surface adsorbed thiolate (peak A)

As already mentioned, results here reported were obtained under the CSV conditions at a HMDE. The deposition was performed at a rather anodic potential for allowing mercury thiolate to from according to the following reaction:

$$Hg + 2\,RSH = Hg(RS)_2 + 2e^- + 2H^+$$

where RSH stands for either Hcy or Cys.

In accord with literature data [9, 10], we noticed that the peak A occurs at the same potential with either Hcy or Cys and is not suitable for Hcy determination in the presence of Cys. However, as already pointed out, some differentiation could occur if a metal ion is available for complexation by the amino acid

That is why we did investigate the effect of Ni^{2+} concentration on the CSV peak A produced by either Cys or Hcy. Fig. 2 displays this effect in the case of Cys and proves that

Ni^{2+} in a rather high excess causes the peak A to vanish (compare curves 1 and 5 in Fig. 2). At the same time, the background (capacity) current on each side of the peak A increases with the increase of the Ni^{2+} concentration, proving that the surface adsorbed compound is, under these circumstances, very different of the mercury thiolate formed in the absence of Ni^{2+}. Data in Fig. 2 suggested that a mixed layer of mercury and nickel cysteinates forms at a moderate Ni^{2+} excess (curves 1 to 4) causing the peak A current to decrease with the rise in Ni^{2+} concentration.

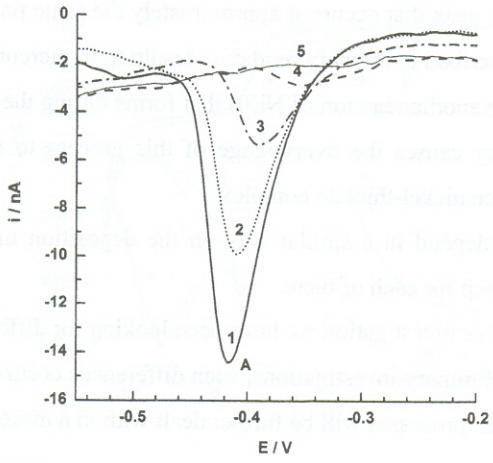

Figure 2. Effect of Ni^{2+} ion on the cathodic reaction of mercury cysteinate. Cys, $1 \cdot 10^{-6}$ M. Ni^{2+} (M): (1) 0; (2) $1 \cdot 10^{-5}$; (3) $5 \cdot 10^{-5}$; (4) $2 \cdot 10^{-4}$; (5) $4 \cdot 10^{-4}$. E_{dep}, +0.1 V; t_{dep}, 30 s.

At a high Ni^{2+} excess, the nickel complex is the single surface accumulated compound. Ni^{2+} reduction in this one occurs at a more cathodic potential (peak B in Fig. 1) and no peak A results under these conditions. Adsorption of bis-cysteinatonickelate ion is enhanced by its negative charge.

As shown in Fig. 3, Ni^{2+} effect is very different in the case of Hcy. In the presence of Ni^{2+}, a new peak (A') appears at more cathodic potentials. Peak A current decreases with the rise of Ni^{2+} concentration and, at the same time, peak A' current increases until the cathodic process is totally shifted to the region at this peak (curve 5 in Fig. 3). This behavior can be accounted for by assuming that the thiol group in Hcy is not involved in a strong coordination bond with Ni^{2+}, as it occurs with Cys. Instead, Hcy thiol group is free to bind to the mercury surface

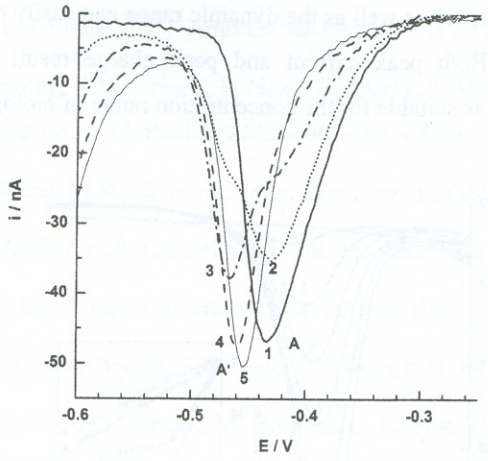

Figure 3. Effect of Ni^{2+} ion on the cathodic reaction of the Hg(II)-Hcy compound. $4 \cdot 10^{-6}$ M Hcy. Ni^{2+} (M): (1) 0; (2) $1 \cdot 10^{-5}$; (3) $5 \cdot 10^{-5}$; (4) $2 \cdot 10^{-4}$; (5) $4 \cdot 10^{-4}$. Other conditions are as in Fig. 2.

and forms a mercury thiolate which is further reduced in the peak A potential region. Ni^{2+} ions can instead bind to carboxyl and amino groups of the surface-attached Hcy molecules and give rise to intramolecular bridges. This brings about a higher stability at the surface layer and the noticed shift of the cathodic reaction from the peak A to A'.

It is interesting at this point to compare the Hcy behavior with that of sulfide ion in the presence of a transition metal ion, as described in refs. [16,17]. The metal ion (M^{2+}) facilitates the reduction of Hg(II) in the surface-accumulated HgS by an exchange reaction that precedes Hg(II) reduction, according to the following scheme:

$$HgS + M^{2+} = MS + Hg^{2+}$$
$$Hg^{2+} + 2e^{-} = Hg$$

Consequently, the cathodic reaction of HgS shifts to a less cathodic potential in the presence of M^{2+}. Data in Fig. 3 demonstrate that no such an effect occurs in the case of Hcy because Ni^{2+} shifts the process in the opposite direction.

It is possible therefore, to work out a CSV method for Hcy determination in the presence of Cys starting for the following premise: an excess of Ni^{2+} inhibits the participation of Cys in the peak A process but has only a minor effect on Hcy reactivity in this process. As

shown in Fig. 4, both peak current and peak charge are proportional to Hcy concentration in the presence of Cys. Sensitivity as well as the dynamic range can easily be adjusted by means of the deposition time. Both peak current and peak charge result in similar accuracy performance. This method is suitable for the concentration range of biological samples.

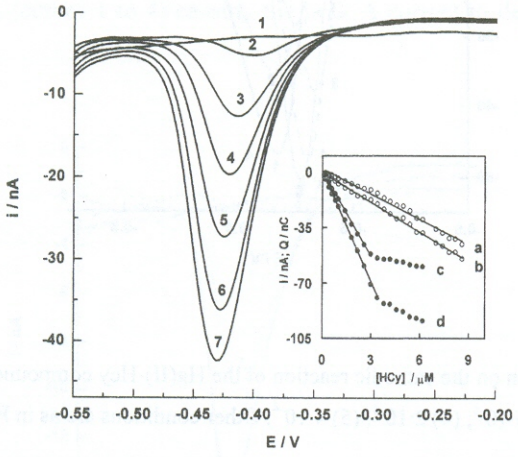

Figure 4. Effect of Hcy concentration on the peak A current and charge in the presence of Cys (1 mM) and Ni^{2+} ($4 \cdot 10^{-4}$ M) at pH 6.5. Hcy (mM): 1) 0; 2) 0.2; 3) 0.7; 4) 1.0; 5) 1.4; 6) 1.8; 7) 2.2. E_{dep}, +0.1 V; t_{dep}, 60 s. Inset: Calibration graph for Hcy determination in the presence of Cys. Lines a and b: t_{dep}, 10 s; a) peak current; b) peak charge. Lines c and d: t_{dep}, 60 s; a) peak current; b) peak charge.

Conclusions

Hcy gives rise to a series of voltammetric peaks in the presence of Ni^{2+} and under the conditions of CSV. This paper reveals some differences between Hcy and Cys behavior and point out possible applications for Hcy determination in the presence of Cys.

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References

[1] M. A. Medina, J. L. Urdiales, M. I. Amores-Sanchez, *Eur. J. Biochem.*, 268 (2001) 3871.

[2] H. Refsum, A.D. Smith, P.M. Ueland, E. Nexo, R. Clarke, J. McPartlin, C. Johnston, F. Engbaek, J. Schneede, C. McPartlin, J.M. Scott, *Clin. Chem.*, 50 (2004) 3.

[3] S.S. Kang, P.W. Wong, M.R. Malinow, *Annu. Rev. Nutr.* 12 (1992) 279.

[4] K. Rasmunsen, J. Møller, in R. Carmel and D. W. Jacobsen (Eds.), Homocysteine in health and disease, Cambridge University Press, 2001, p.199.

[5] O. Nekrassova, N.S. Lawrence, R.G. Compton, *Talanta*, 60 (2003) 1085.

[6] V. Ducros, K. Demuth, M.P. Sauvant, M. Quillard, E. Causse, M. Candito, M.H. Read, J. Drai, I. Garcia, M.F. Gerhardt, *J. Chromat.* B, 781 (2002) 207.

[7] C. Bayle, E. Causse, F. Couderc, *Electrophoresis*, 25 (2004) 1457.

[8] P.C. White, N.S. Lawrence, J. Davis, R.G. Compton, *Electroanalysis*, 14 (2002) 89.

[9] A.M. Bond, S.B. Thomson, D.J. Tucker, *Anal. Chim. Acta*, 156 (1984) 33.

[10] M. Heyrovský, S. Vavřička, *Bioelectrochem. Bioenerg.*, 48 (1999) 43.

[11] D. D. Perrin, J. G. Sayce, *J. Chem. Soc. A,* 1968, 53.

[12] F. G. Banica, A. Calusaru, *J. Electroanal. Chem.,* 145 (1983) 389.

[13] F. G. Banica, E. Diacu, *Collect. Czech. Chem. Commun.*, 56 (1991) 140.

[14] F.G. Banica, B. Kafar, S. Skrzypek,W. Ciesielski, *Electroanalysis*, 18 (2006) 2269.

[15] F.G. Banica, D. Guziejewski, S. Skrzypek, W. Ciesielski, D. Kaźmierczak, *manuscript in preparation.*

[16] F. G. Banica, N. Spataru, T. Spataru, *Electroanalysis*, 9 (1997) 1342.

[17] J.H. Carney, H.A. Laitinen, *Anal. Chem,*. 42 (1970) 473.