Single-use metal ion voltammetric sensors. An assessment of amino acids and peptides as receptors for the copper ion

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Abstract: Thiol-containing amino acids and peptides were attached to the gold surface by covalent binding via the sulfur end cap. Copper ions can be co-ordinated by carboxyl and amino groups in the receptor molecule and further determined by their electrochemical reaction. Surface layer stability as well as the surface coverage were assessed by the anodic reaction of the adsorbate whereas the amount of bound copper ion resulted from the electrical charge passed during the reduction of surface–confined Cu(II) ions. The binding capacity of the surface layer was defined as the ratio between the amount of accumulated copper to the receptor surface coverage (both in mole cm^{-2}). Sensitivity and binding capacity are discussed in connection with the receptor structure.

Keywords: Single-use sensors; Copper determination; Modified electrodes, Gold electrodes, Amino acids; Peptides.

Introduction

Modification of the solid surfaces by chemisorption of various organic compounds in the form of an organized monolayer is a valuable tool in the field of sensors science [1, 2, 3] and also shows promising applications related to molecular electronics [4] and nanotechnology [5]. In addition, electrode-solution interfaces with a special design offer good opportunities for the experimental validation of theoretical approaches in the field of surface chemistry [6].

A convenient method for surface modification is represented by irreversible adsorption of thiols or other organic sulfur derivatives to metals like gold and silver [7]. The most typical example is represented by alkane thiols that can form organized monolayers due, on one side, to the template effect of the metal substrate and, on the other side, to intermolecular van der Waals interactions. Metal ion pre-concentration can be performed if the adsorbate contains one or more functional groups which are directed towards the solution phase and are able to combine with the ion of interest. Subsequently, the metal ion will be detected by means of an electrochemical reaction, alike to the stripping voltammetry determination. Thus, mercaptocarboxylate films were proposed for determining lead [8], copper [8,9] or cadmium [10] ions. Surface immobilized chelators bring about more selectivity and are suitable for electrochemical detection after ion accumulation. Thus, copper ion was determined by means of 2,2'-thiobisethyl acetoacetate-octanedecanethiol mixed surface layer [11]. A nitrilotriacetic-modified thioalkane monolayer enabled determining metal ions by electrochemical impedance measurements, [12] whereas dithizone monolayers were proposed for detecting copper and lead ions [13].

A promising method in this field is represented by the application of amino acids and peptides as metal-binding receptors at the metal surface [14], an approach that was previously widely used in stripping voltammetry at mercury electrodes [15]. Metal ion binding properties of such compounds have been widely investigated [16] but their application for electrochemical sensor purpose is somewhat limited by the possibility of immobilizing them at the metal surface. In this connection, a direct approach consists of performing chemisorption of thiol derivatives like cysteine [17], gluthathione [18] or N-acetyl-L-cysteine [19]. The thiol function attaches the adsorbate to the gold surface whereas the remaining functions confine metal ions like Cu^{2+} and make possible its accumulation. Alternatively, a functionalized thiol (like 3-mercaptopropionic acid) was first attached to the gold surface via the sulfur end-group and the chelator was subsequently attached to the carboxyl by

carbodiimide coupling. This procedure was tested for Cu^{2+} determination under the ppb level by means of either Gly-Gly-His [20] or polyaspartate [21], whereas the hexapeptide His-Ser-Gln-Lys-Val-Phe proved to be an efficient chelator for sensitive detection of the cadmium ion [22]. In a different approach, screen-printed carbon electrodes were fabricated with amino acid functionality by using in situ co-deposition of mercury and cysteine and employed for trace Cd^{2+} determination [23].

Although a very low detection limit is often sought when designing a chemical sensor, many applications require determinations to be performed within a narrow concentration range with no preliminary sample dilution. From this standpoint, tuning of the detection limit by a suitable selection of the surface modifier/chelator represents a convenient approach. This is why we endeavored to investigate various cysteine derivatives in order to assess their ability to serve as gold surface modifier in view of Cu^{2+} determination. The investigated modifiers where the amino acids cysteine (Cys), homocysteine (Hcy), and penicillamine (Pen) and the dipeptide cysteinyl-glycine (Cys-Gly) (Fig. 1). We investigated (i) the irreversible adsorption of the above compounds from aqueous solutions to the gold surface, (ii) the anodic

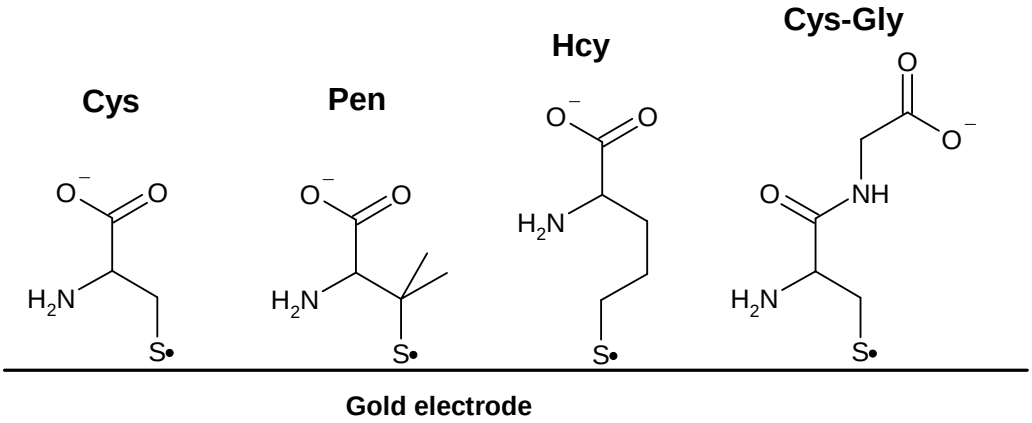

Figure. 1. Gold surface modifiers as copper ion receptors. The binding site was figurate in the non-protonated form which is suitable for copper ion complexation. Under the experimental conditions in this paper (0.1 M KNO_3), the amino group in the free receptor is present in the protonated form ($-NH_3^+$).

(oxidative) reaction of thiol group in the adsorbed receptor in order to assess the stability of the surface layer, and (iii) copper ion accumulation at modified surfaces. We propose a method for assessing modifier efficiency in copper ion accumulation and report pertinent figures for the investigated modifiers. The above compounds were also investigated as facilitators for cytochrome *c* reaction at gold electrodes [24].

Experimental

L-cysteine ((R)-2-amino-3-mercaptopropionic acid), D- penicillamine (D(-)-2-amino-3-mercapto-3-methylbutanoic acid), D,L-homocysteine (2-amino-4-mercaptobutiric acid) and cysteinyl-glycine were supplied by Sigma and used as received. All other reagents were of analytical-reagent grade. The water (18 MΩ cm resistivity) was obtained from a Millipore purification system.

The working electrode was a polycrystalline disk electrode (0.5 mm diameter; 0.20 mm^2 geometric area) which was fabricated by sealing with epoxy resin a gold wire (Aldrich, 99.999 %) into a glass tube. The gold electrode surface was prepared by mechanical polishing followed by a series of CV scans between 0.00 and 1.45 V in a 0.01 M perchloric acid solution until a typical and reproducible voltammogram for gold oxide formation/reduction was obtained. The electrode was then rinsed with pure water and immersed in a 0.01 M $HClO_4$ solution containing 0.01 M modifier. In order to investigate the anodic reaction of the adsorbate, the modified electrode was further rinsed with pure water and transferred to a 0.01 M perchloric acid solution in order to perform CV runs between 0.0 and 1.45 V at 20 mVs^{-1} scan speed. Cu^{2+} binding to the modified surface was achieved by placing the modified electrode into a 1 mM Cu^{2+} and 0.1 M KNO_3 solution for a selected time interval. Then, the electrode was removed from the Cu^{2+} solution, rinsed with water and transferred to the 0.1 M KNO_3 containing cell in order to investigate the electrochemical reactions of the surface-confined copper ion by cyclic voltammetry.

The electrochemical cell was fitted with an Ag|AgCl, KCl (1M) reference electrode (via a 1 M KNO_3 salt bridge) and a Pt wire as auxiliary electrode. Potentials are reported with reference to the saturated calomel electrode. Oxygen was removed from the test-solution by a stream of nitrogen which was directed above the solution during the CV scans.

Voltammetric experiments were performed with a BAS CV-1B instrument connected to a personal computer via a general-purpose data logger (ADC-212, Pico Technology Ltd., UK). Data processing was carried out by the Origin® software. The positive sign was assigned to cathodic currents.

Results and Discussion

Cysteine

Anodic oxidation of thiols and other organic sulfur derivatives is widely used for electrochemical detection in liquid chromatography [25] and capillary electrophoresis [26]. The reaction involves molecules that are adsorbed in the form of radicals27 (Fig. 1) and occurs as follows [27, 28]:

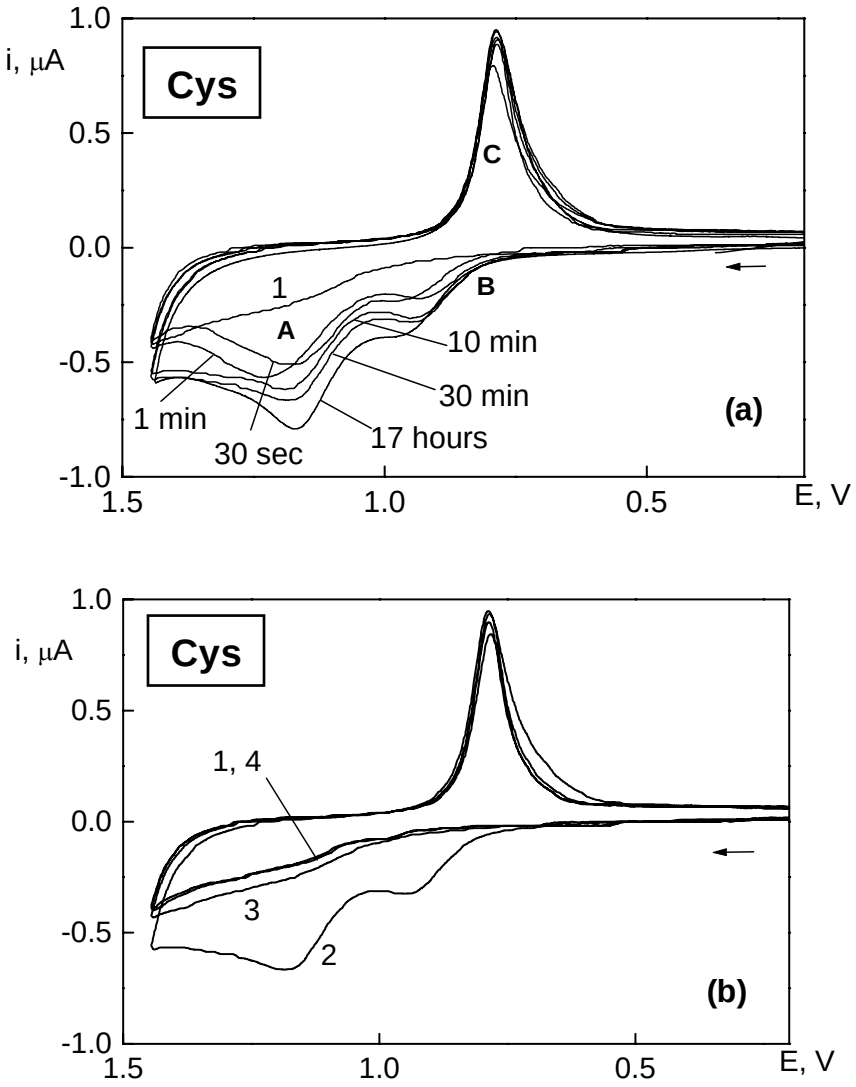

Figure 2. a) Anodic reaction of adsorbed Cys. a) Effect of the modification time (shown on each curve). Curve 1: plain gold electrode. b) Evolution of Cys desorption during a multi-scan CV run. Curve 1: plain gold electrode; curves 2 to 4: succesive CV runs. Modification time, 30 min.

$$RS\cdot_{(ads)} + 3H_2O \rightarrow RSO_3^- + 6H^+ + 5e^- \quad (1)$$

This process is an oxide-catalyzed reaction. Anodic oxygen transfer to the adsorbate reaction is promoted by various gold oxide species [29].

Anodic reactions of the Cys surface layer are presented in Fig. 2a for various coverage degree values that were adjusted by the adsorption time. Curve 1 demonstrates the reactions of the plain gold surface and, for the purpose of further comments it is important to define three characteristic regions. Wave A region corresponds to formation of a gold oxide monolayer, which can be reduced on the reverse (cathodic) scan in the peak C region. The region B on the direct (anodic) scan is the pre-oxide region where a sub-monolayer of hydrated gold oxide may form if water access to the metal surface is not prevented by an adsorbed organic compound. CV records with the Cys modified electrode (Fig. 2a)

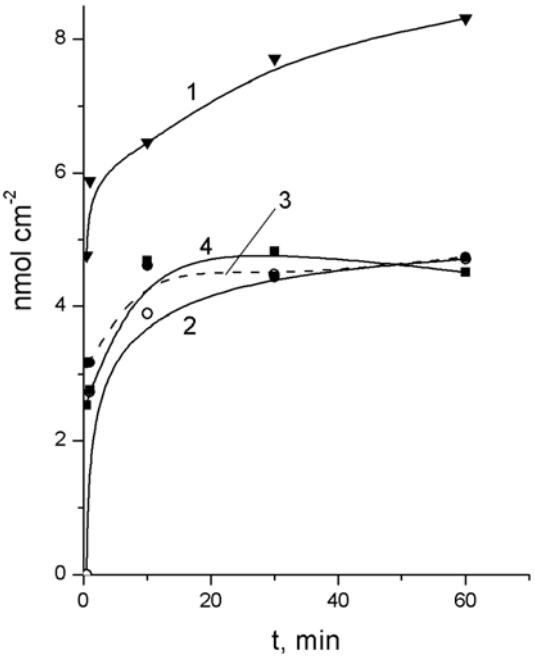

Figure 3. Effect of the modification time on the coverage degree. 1) Cys; 2) Pen; 3) Hcy; 4) Cys-Gly. The coverage degree in this Figure agrees satisfactorily with the figures reported in ref. [29b] for various Cys related compounds.

demonstrate that Cys oxidation starts in the pre-oxide region (B) and proceeds further in the oxide region (A). This proves that adsorbed Cys does not preclude water access to the surface in the pre-oxide region. Consequently, hydrated gold oxide can form and this one functions as

a catalyst in the oxidation of the thiol group according to reaction (1). The oxide monolayer which forms in the region A inhibits this reaction [30] and in this region the reaction occurs only on the free metal as long as no total coverage by oxide was achieved. Thiol oxidation is accompanied by desorption because the product cannot form strong covalent bonds with gold. The degree of completion for the desorption process was assessed by a multiple-scan CV run with a Cys modified electrode (adsorption time, 30 min, Fig. 2b). Here curve 2 represents the first scan which, as expected, is very far from the plain gold electrode curve (curve 1). Subsequent scans with the modified electrode (curves 3 and 4) are very close to curve 1 and demonstrate that the desorption was almost complete after the first anodic scan. Consequently, the net charge for thiol oxidation (Q_S) can be estimated as follows:

$$Q_S = Q_T - Q_C \qquad (2)$$

where Q_T is the charge for the overall anodic reaction (i.e. thiol oxidation and gold oxide formation in the region A) and Q_C stands for the charge passed during the reduction of the gold oxide (peak C). Q_C (in absolute value) is equal with the charge consumed for gold oxidation in the region A. Actually, Q_S results from the integration of current along the single-sweep CV scan with the modified electrode. This procedure is alike to that used for the electrochemical detection of thiols in liquid chromatography.[25] The surface coverage with respect to the geometric surface area was further estimated by means of reaction (1) stoichiometry and was plotted in Fig. 3 as curve 1. This one demonstrates that a modification time of 30 minutes was enough for approaching the limiting coverage degree under the selected experimental conditions.

Fig. 4 exhibits the electrochemical reactions of copper ions accumulated at a Cys modified gold electrode. On the forward scan Cu^{2+} reduction to Cu^{+} occurs, whereas the opposite reaction proceeds on the backward scan. In a multiscan record, the cathodic peak on the first scan (Fig. 4a) is shifted with respect to the following scans, which are very reproducible. That is why in further experiments the first CV scan was always disregarded. The difference in peak potentials (about 300 mV) is indicative of high activation energy for the electrochemical reaction, probably due to the slow redistribution of counter-ions around the copper site. The effect of the scan rate is displayed in Fig. 4b and proves the expected behavior for a surface-confined reactant.

Penicillamine

As shown by Fig. 5a, Pen anodic reaction occurs in the oxide region but not in the pre-oxide

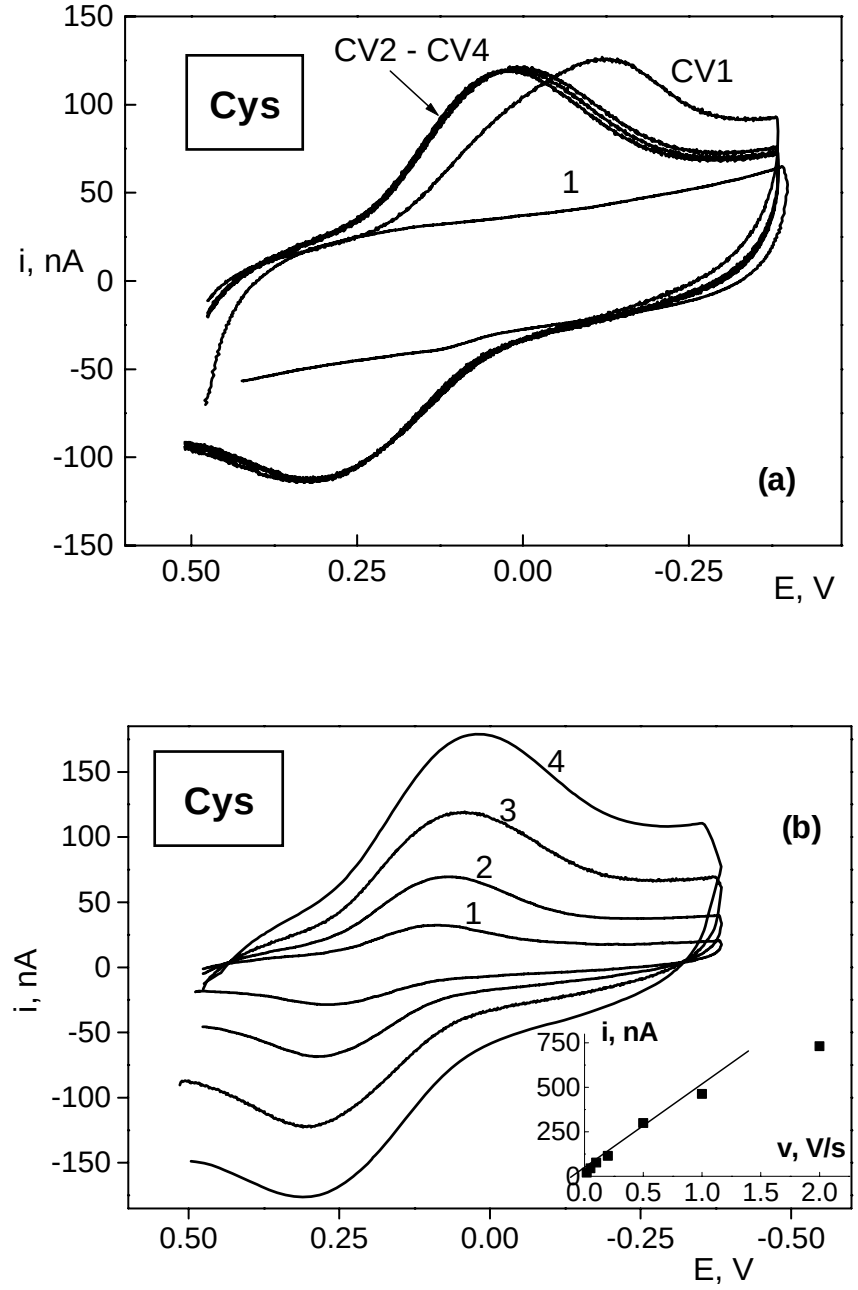

Figure 4. Electrochemical reactions of the copper ion accumulated at a Cys modified electrode. a) Effect of successive scans (indicated on each curve); curve 1: CV at a copper-free Cys modified electrode. Modification time, 10 min; Cu^{2+} pre-concentration time, 30 min; scan rate, 100 mV s^{-1}. b) Scan rate effect on the cathodic current. Scan rate (mV s^{-1}): (1) 20; (2) 50; (3) 100; (4) 200.

range, as it happens with Cys (Fig. 2). This is due to the additional methyl substituents in Pen molecule (Fig. 1) that prevents water penetration to the metal surface and formation of the hydrated gold oxide layer which is required as a catalyst in this potential region. However, the high overpotential in the region A stimulates the formation of sub-stoichiometric gold oxides which catalyses the oxidation of the thiol group. It appears therefore that the Pen surface layer

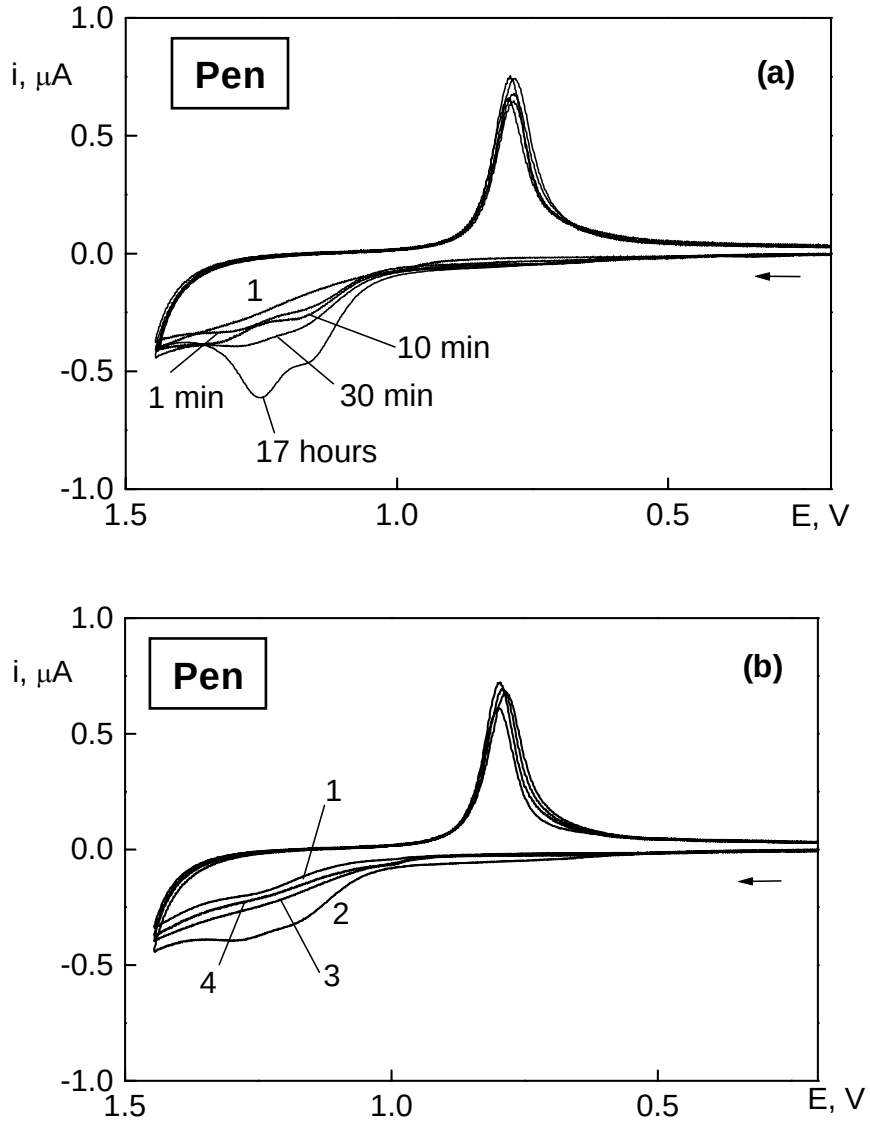

Figure 5. a) Anodic reaction of adsorbed Pen. a) Effect of the modification time (shown on each curve). Curve 1: plain gold electrode. b) Evolution of Pen desorption during a multi-scan CV run. Curve 1: plain gold electrode; curves 2 to 4: succesive CV runs. Modification time, 30 min.

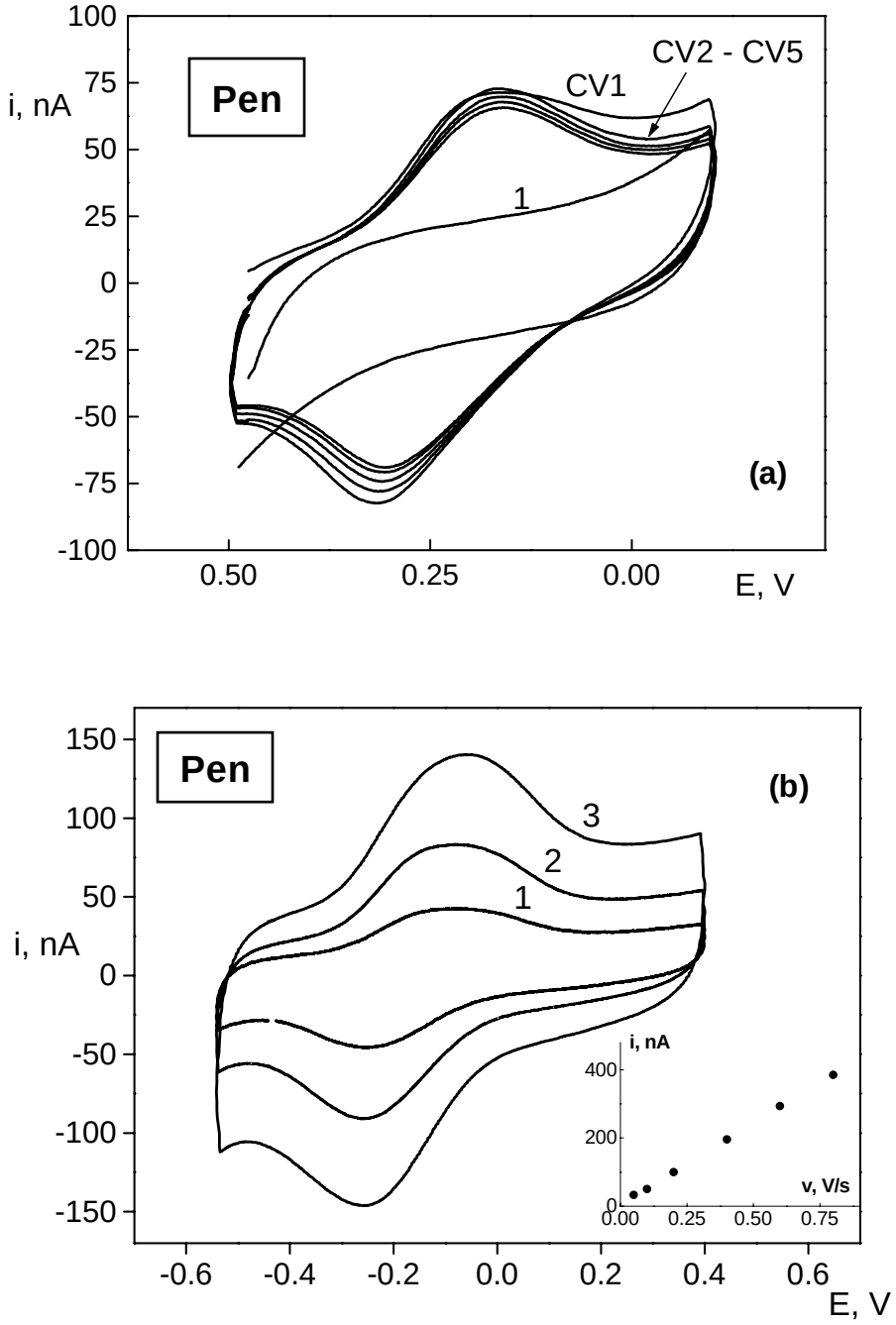

Figure 6. Electrochemical reactions of the copper ion accumulated at a Pen modified electrode. a) Effect of successive scans (indicated on each curve); curve 1: CV at a copper-free Pen modified electrode. Modification time, 30 min; Cu^{2+} pre-concentration time, 10 min; scan rate, 100 mV s^{-1}. b) Scan rate effect on the cathodic current. Scan rate (mV s^{-1}): (1) 50; (2) 100; (3) 200.

is stable over a broader potential range as compared with Cys. Curve 2 in Fig. 3 displays the Pen surface coverage as a function of the adsorption time and demonstrates that this compound generates a surface layer with a lower package degree than that produced by Cys. Fig. 5b proves that the Pen surface layer was almost completely depleted after the first scan (curve 2) as the next scans (curves 3 and 4) are nearly alike to curve 1 which was recorded with the bare gold electrode.

Electrochemical behavior of copper ions attached to the Pen surface layer is illustrated by Fig. 6. Successive CV scans are quite reproducible, except the first cathodic peak which is quite different from the next ones and was therefore always disregarded in further trials. The cathodic peak potential is alike to that for a Cys covered surface but the anodic peak lies at a less negative potential (-0.26 V at 200 mV s^{-1} scan rate; compare with -0.30 V for Cys under the same conditions). As in the case of Cys, the effect of the scan rate (Fig. 6b) is typical of an electrochemical reaction involving a surface-immobilized reactant.

Homocysteine

According to Fig. 7a, Hcy anodic reaction occurs to a high extent in the pre-oxide region but the remaining surface compound is oxidized in the gold oxide potential range as it happens with Cys. Fig. 3, curve 3 demonstrates that the coverage degree for Hcy is much lower than that for Cys but not too far from that for Pen. No steric factors are expected to dictate the lower Hcy coverage degree, as in the case of Pen. Rather, the reason seems to be the length of the Hcy side chain. A high coverage degree is achieved when the adsorbate molecules orient themselves in a nearly perpendicular direction to the metal surface. Apparently, the longer Hcy molecules need a longer time to organize themselves in a more compact layer. As in the cases of the previous amino acids, the Hcy surface layer is almost completely stripped off throughout the first anodic scan (Fig. 7b).

Fig. 8a presents a series of CV successive scans with an Hcy modified electrode after performing Cu^{2+} accumulation. It demonstrates that the cathodic peak on the first scan is shifted with respect to the subsequent scans but these are very stable and reproducible. The effect of the scan rate on copper ion peak currents is presented in Fig. 8b which demonstrates the expected behavior for a difussionless electrode reaction, as in the case of the previous amino acids.

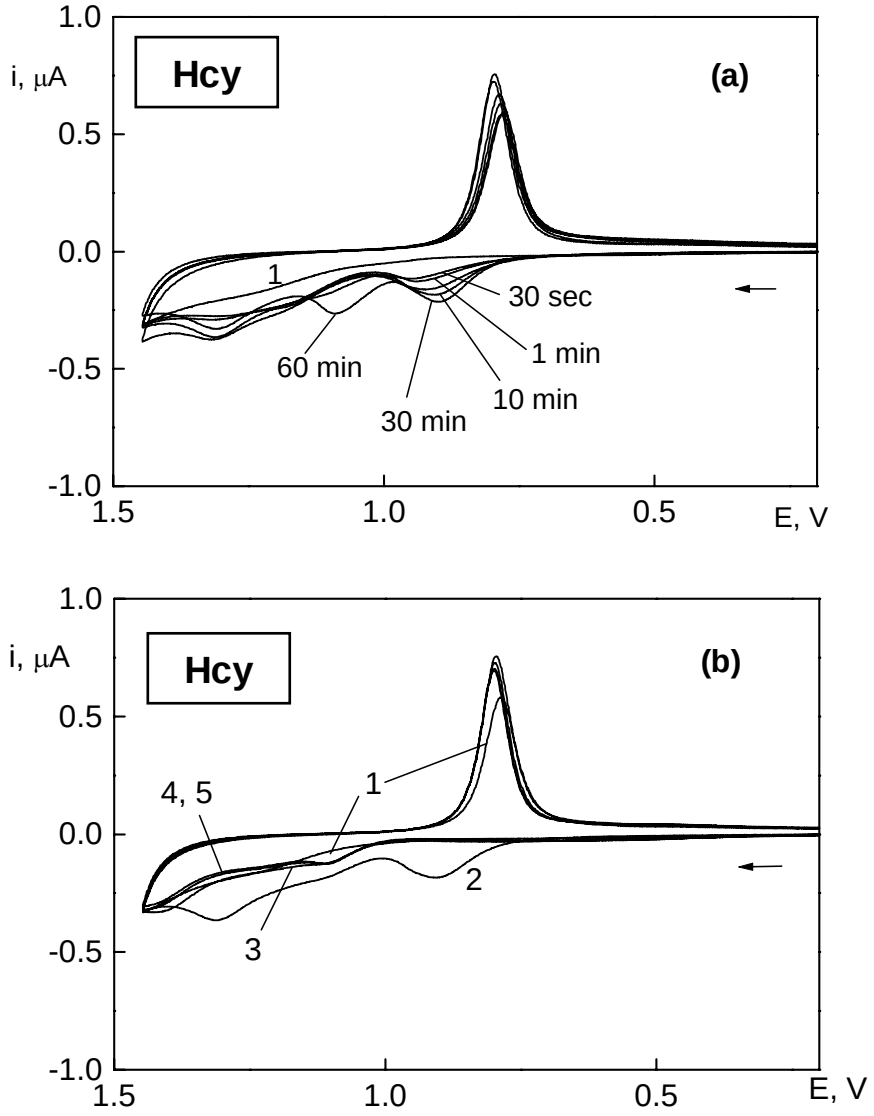

Figure 7. a) Anodic reaction of adsorbed Hcy. a) Effect of the modification time (shown on each curve). Curve 1: plain gold electrode. b) Evolution of Hcy desorption during a multi-scan CV run. Curve 1: plain gold electrode; curves 2 to 5: succesive CV runs. Modification time, 30 min.

Cysteinyl-Glycine

According to Fig. 9a, the anodic reaction of Cys-Gly occurs mostly in the gold oxide region and no characteristic peak forms in the pre-oxide region. From this point of view, it behaves like Pen and it may be supposed that the Cys-Gly prevents water access to gold surface and formation of a hydrated oxide monolayer.

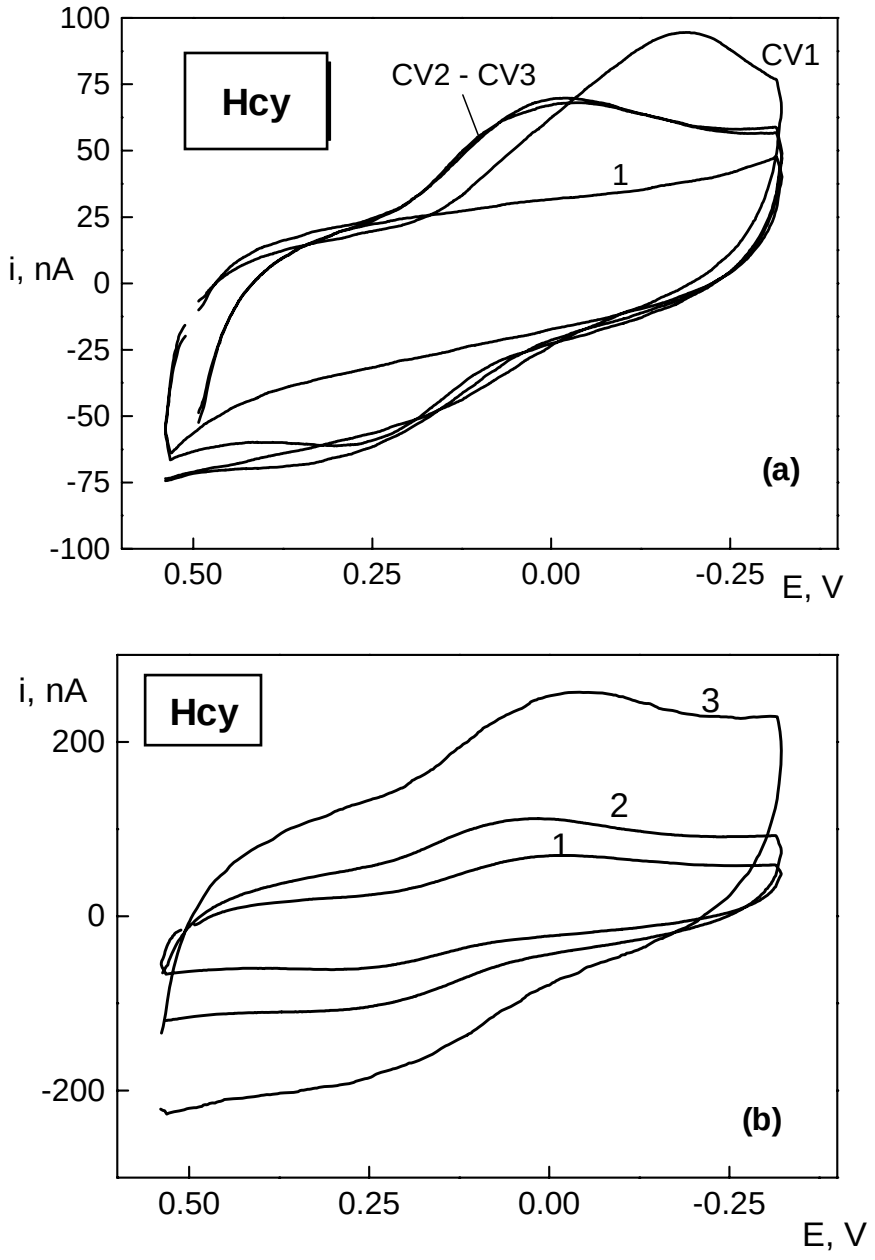

Figure 8. Electrochemical reactions of the copper ion accumulated at a Hcy modified electrode. a) Effect of successive scans (scan number indicated on each curve); curve 1: CV at a copper-free Hcy modified electrode. Modification time, 10 min; Cu^{2+} pre-concentration time, 10 min; scan rate, 100 mV s^{-1}. b) Scan speed effect. Scan rate (mV s^{-1}): (1) 100; (2) 200; (3) 500.

The Cys-Gly coverage degree (Fig. 3, curve 4) is roughly alike to that obtained with Pen and not particularly higher than that with Hcy, but much lower than that with Cys.

As in the case of Hcy, the low coverage degree by Cys-Gly may be a consequence of the slow organization of the longer adsorbate molecules. Fig. 10a dispalys a series of successive CV scans for copper ion accumulated at a Cys-Gly modified electrode. As with the previous modifiers, the cathodic peak shifts gradually to les negative potential values with the progress of the multiple scan but attains a stable position

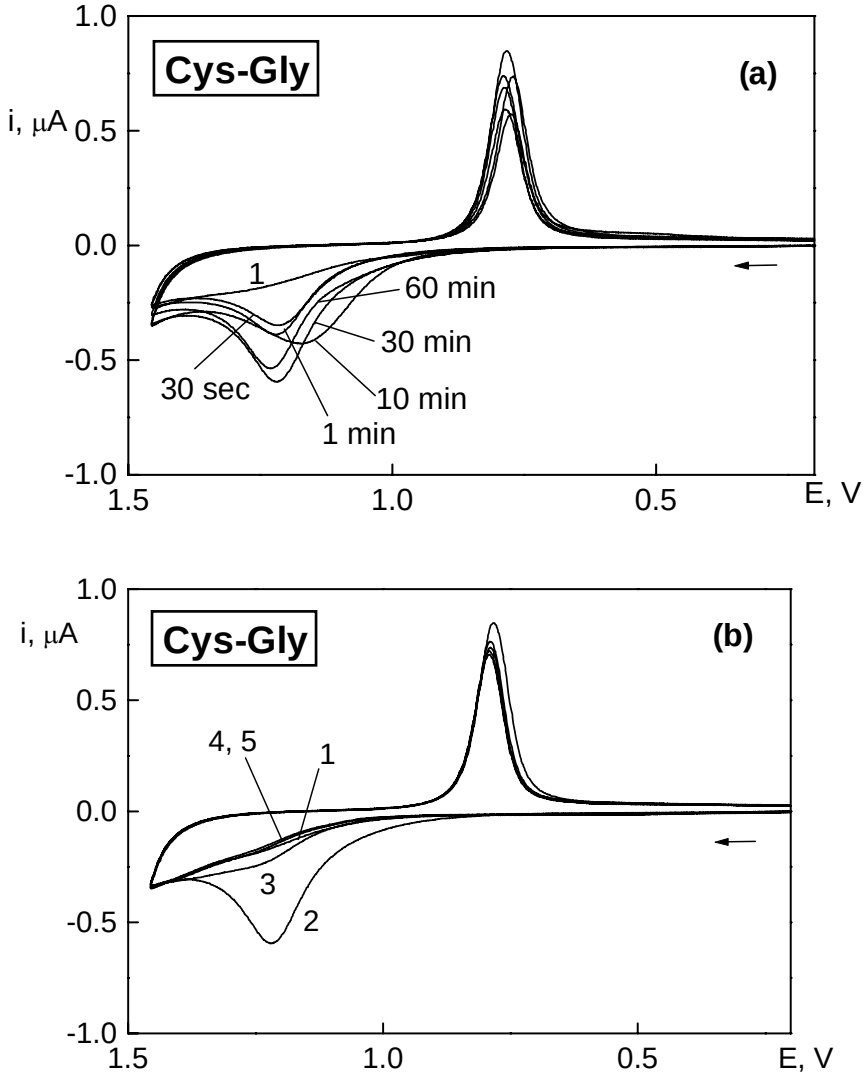

Figure 9. a) Anodic reaction of adsorbed Cys-Gly. a) Effect of the modification time (shown on each curve). Curve 1: plain gold electrode. b) Evolution of Cys-Gly desorption during a multi-scan CV run. Curve 1: plain gold electrode; curves 2 to 5: succesive CV runs. Modification time, 30 min.

after a few scans. It is clear that reorganization of copper binding sites occurs when the charge of the copper ion swings between +2 and +1 and this is a common feature of all the receptors

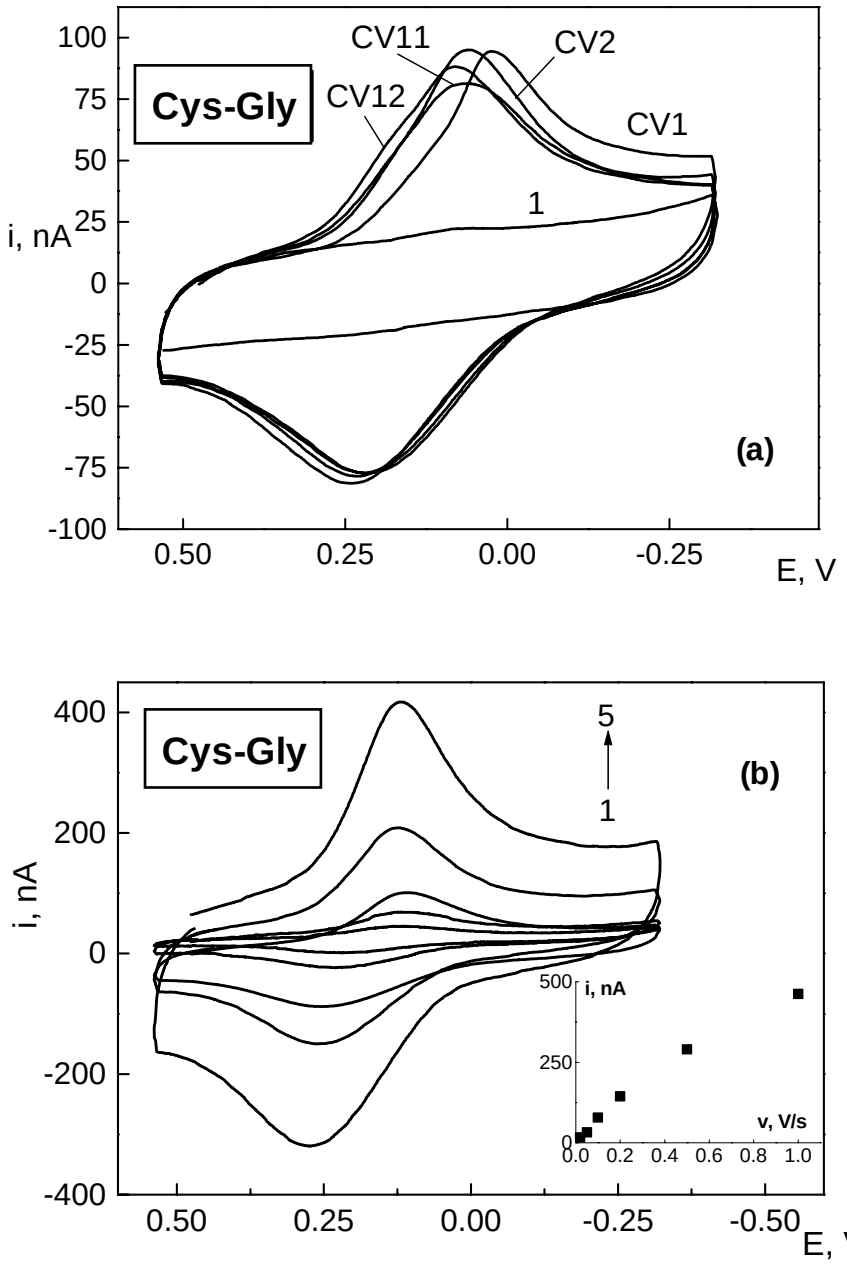

Figure 10. Electrochemical reactions of the copper ion accumulated at a Cys-Gly modified electrode. a) Effect of successive scans (scan number indicated on each curve); curve 1: CV at a copper-free Cys-Gly modified electrode. Modification time, 10 min; Cu^{2+} pre-concentration time, 10 min; scan rate, 100 mV s^{-1}.b) Scan speed effect. Scan rate (mV s^{-1}): (1) 20; (2) 50; (3) 100; (4) 200; (5) 500.

here investigated. Fig. 10b confirms that the cathodic peak current is proportional to the scan speed, in agreement with the characteristics of an electrochemical reaction involving a surface-attached reactant.

Copper binding capacity

For convenience, copper binding capacity is here defined as follows:

$$r = N_{Cu} / N_R \qquad (3)$$

where N_R represents the coverage degree for the receptor whereas N_{Cu} represents the amount of copper ions (in mole cm^{-2}) attached to the surface of the modified electrode. N_{Cu} was calculated from the charge for Cu^{2+} reduction in the surface confined form. In other words, the binding capacity represents the amount of Cu^{2+} ions attached to the surface relative to the amount of surface confined receptor (both in moles). This is an empirical definition and, consequently, r values cannot be considered as combining ratios. A higher r reflects a higher binding capacity. The ideal r value for a 1/1 complex would be 1.

r values for various modifiers are summarized in Table 1 along with the amount of attached copper (N_{Cu}). The last parameter is an indicator of the sensitivity for Cu^{2+} determination under specific conditions. The parameter r characterizes the modifier itself, whereas N_{Cu} is an overall characteristic of the modified electrode.

Table 1. Characteristics of the Cu^{2+} pre-concentration process at gold electrodes modified with amino acids or peptides.

Surface modifier	Modification time / min	Cu^{2+} pre-concentration time / min	N_{Cu} nanomoles cm^{-2}	Binding capacity (r)
Cys	30	10	1.04	0.22
Cys	10	30	1.49	0.37
Pen	10	10	0.34	0.11
Hcy	10	20	0.62	0.33
Cys-Gly	10	10	0.65	0.25

The amount of attached Cu^{2+} depends on both the modifier surface coverage and binding capacity. According to Fig. 3, a modification time of 10 to 20 minutes was enough for approaching the limiting coverage degree. Consequently, variations in the modification time for a given modifier have only minor effects on the N_{Cu} values.

It is obvious that the best sensitivity is achieved with the Cys modified electrode. Hcy and Cys-Gly lie on the second place, but with sensitivity values of about half the figure for Cys. The lowest sensitivity results with Pen. As far as the binding capacity is concerned, the highest figures resulted for Cys whereas Hcy and Cys-Gly figures are only slightly lower. Pen however displays the lowest r value.

In order to make an interpretation of the above results, it is important to point out the factors that control the binding capacity. First of all, it depends on the molecular properties of the modifier, i.e. the nature of the co-ordinating sites, their relative position and the stability of the copper-modifier complex. From this standpoint, the amino acids here investigated are similar and may bind to the copper ion via the carboxyl and amino groups located in vicinal positions and, therefore, able to generate chelate complexes. Despite the lower coverage degree, Hcy displays almost the same binding capacity as Cys. Probably, the above amino acids are not uniformly distributed over the surface but form closely packed island that are able to bind copper ions. Packing of adsorbate molecules within such islands is promoted by intramolecular hydrogen bonds. At the same time, the width of the copper ion reduction peak is very large, something which suggest a broad dispersion in the properties of the binding sites. This behavior is not consistent with the occurrence of a single binding type of the chelate complex form. Rather, copper ion binding seems to involve two or more modifier molecules, each of them contributing with one or two complexing groups according to a random distribution. In the frame of this model, the particular behavior of Pen can be assigned to the steric hindrance of the methyl substituents that hamper the participation of more adsorbate molecules to the binding of one single copper ion. The same binding groups are also present in the Cys-Gly molecule but at distant position and the formation of chelates is less probable. The shape of the CV curves for the Cys-Gly bound copper (Fig. 10) is also somewhat different from those recorded with amino-acids. Namely, Cys-Gly produces sharper cathodic copper peaks, something which suggests a lower dispersion in the copper binding sites properties.

Conclusions

All the modifiers here investigated adsorb to the gold surface via the thiol group. Cys gives rise to the highest density of surface confined receptor sites (as expressed in terms of surface coverage). Surface layer stability in the anodic potential region is dictated by the adsorbed layer permeability to water molecules. For compounds like Cys and Hcy, water permeability is higher than for Pen and Cys-Gly. Consequently, the most stable layers are generated by the last two compounds.

All the investigated compounds can act as copper ion receptors in the adsorbed form. Binding of a copper ion involves one or more receptor molecules and this characteristics leads to the occurrence of relatively broad CV peaks for copper electron transfer reactions. It is worth noting that copper peak potential are almost the same with any of the receptors here investigated, something that suggest that no essential differences in the copper–receptor interaction strength occurs. However, in contrast to the amino-acids, Cys-Gly leads to narrower CV peaks, in accord with the particular disposition of the binding sites in this compound.

Reorganization of copper binding sites occurs when the charge of the copper ion swings between +2 and +1 in a multiple scan experiment, but the state gets stable after a few scans. The initial instability can be associated with the electrostatic repulsion between Cu^{2+} ions whose charge is not completely neutralized by the negative carboxyl groups.

Among the thiol-containing amino acids and peptides that were tested in this work, Cys displays the best sensitivity and binding capacity for copper ion determination. The binding capacity for Hcy and Cys-Gly is close to that of Cys, but these compounds display a lower sensitivity which is mostly due to a lower coverage degree. The lowest parameters occur with Pen which cannot form tightly-packed molecular assemblies at the surface due to the steric hindrance induced by methyl substituents.

Proper selection of the receptor nature and coverage degree allows one to design copper ion sensor with a convenient sensitivity, according to the constraints imposed by samples of various origins.

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