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Electrochemical behavior of unitary or binary self-assembled monolayers with thiol-derivatized cobaltous porphyrin

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Abstract A new thiol-derivatized metalloporphyrin, 5-{3-methoxyl-4-(4-mercaptobutoxy)}phenyl-10,15,20-triphenylporphyrincobalt (MBPPCo), has been synthesized. The electrochemical behavior of unitary or binary self-assembled monolayers (SAMs) of MBPPCo and thiols with carboxylic terminal groups was investigated using $\text{Fe}(\text{CN})_6^{3-/4-}$ and ascorbic acid (AA) as probe species. The binary modified electrode showed a small increase in peak current but a large decrease in overpotential. However, in anionic electroactive species [$\text{Fe}(\text{CN})_6^{3-/4-}$ or AA], either positively charged MBPPCo or negatively charged thiol SAMs solely, slow electron transfer kinetics was obtained and the possible reasons for the discrepancy are discussed.

Keywords Ascorbate · Cobaltous porphyrin · Dopamine · Electroanalysis · Self-assembled monolayers

Introduction

Electrochemical techniques have proved to be significantly advantageous to the biosciences [1, 2, 3, 4, 5, 6, 7]. A sensor based on an electrochemical method has many advantages over other methods. For example, the electrodes can be made extremely small, can be implanted conveniently in living organisms, and the response time is very fast so that the neurotransmitters can be easily

monitored in real time. However, a major problem of electrochemical analyses at unmodified electrodes is the lack of selectivity due to the presence of interfering compounds that overlap with the signal of the compound of interest. For example, ascorbic acid oxidizes at nearly the same potential as dopamine [2]. Moreover, the bare electrodes very often suffer from the fouling effect due to the accumulation of oxidized product on the electrode surface, which results in rather poor selectivity and sensitivity. To overcome these difficulties, a variety of approaches have been developed to increase the sensitivity and selectivity of the electrode process [1, 2, 3, 4, 5, 6].

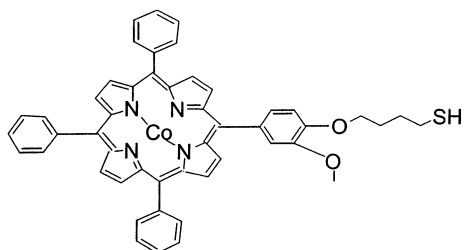
Functionalized self-assembled monolayers (SAMs) of thiols, sulfides and disulfides on gold surfaces offer a unique strategy for constructing stable, well-defined structures on electrodes with controlled chemical features [7, 8, 9, 10, 11, 12]. Also the SAMs serve as a challenging interface for constructing fast responding amperometric biosensors with a low working potential [5, 8, 13]. The possibility of achieving an ordered molecular structure oriented specifically and consistently makes it possible to optimize complexation or other supramolecular interactions at the electrode/solution interface [7, 8]. The molecular dimensions of SAMs avoids slow diffusion of electroactive species towards the electrode surface and reduces the undesirable accumulation of species on or close to the electrode surface, which may lead to electrode fouling. Fast electron transfer, good selectivity and high sensitivity can be easily achieved at the functionalized SAM electrode [1, 2, 3, 4, 5]. However, the amelioration is often achieved at the expense of slowing down the electrode response time. Malem and Mandler [6] have shown that SAMs of mercaptocarboxylic acids on gold electrodes can improve the selectivity of the electrodes for detection of dopamine.

Among the many possible candidates for the fabricated center, metalloporphyrin is most attractive because of its well-known functions in biological and biomimetic systems [14, 15, 16, 17, 18, 19, 20]. However,

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owing to the difficulty of synthesis and separation, thiol-derivatized metalloporphyrins have not been applied for the determination of dopamine and ascorbic acid. Very recently, we used a gold electrode modified with a SAM of a thiol-derivatized cobalt porphyrin for the determination of ascorbic acid [21, 22]. The significant results obtained at the porphyrin monolayer-modified electrode, which contains a positively charged terminal group, encouraged us to examine further the performances of electrodes modified with a SAM having positively (metallic porphyrins) or negatively (carboxylate anions) charged terminal moieties. For this purpose, in the present work we chose a gold electrode modified with a cationic SAM of the positively charged 5-{3-methoxyl-4-(4-mercaptobutoxy)}phenyl-10,15,20-triphenylporphyrincobalt (MBPPCo) (Scheme 1) and



Scheme 1 The proposed structure of MBPPCo

anionic SAMs of thioglycolic acid, cysteine and thioacetic acid. We wish to report the influence of the electrostatic interaction on the electrochemical properties and the correlation between the performance and the structure.

Experimental

Instrumentation and materials

The thiol-derivatized cobalt porphyrin MBPPCo was synthesized as described below. Dopamine (DA) and ascorbic acid (AA) were purchased from Fluka and Beijing Chemical Laboratory, respectively, and were used as received without further purification. All other chemicals used in this investigation were of reagent grade and were used without further purification. The phosphate buffer solution (PBS) was prepared with 0.1 mol L⁻¹ sodium monobasic phosphate and potassium dibasic phosphate (pH 7.3). Solutions of dopamine and ascorbic acid were prepared fresh every day using doubly distilled water. Prior to each set of measurements, oxygen was removed by purging with nitrogen gas for 15 min.

Electrochemical studies were performed using a one-compartment three-electrode cell with a gold disc working electrode (2.0 mm in diameter; CH Instruments, Austin, Tex., USA), a Pt wire counter electrode and a Ag/AgCl/KCl_{sat} reference electrode (all potentials are given with respect to this electrode). The cyclic voltammetry and differential pulse voltammetry were carried out using a computer-controlled electrochemical analyzer (CHI 660, CH Instruments). The following instrumental parameters were used to record the differential pulse voltammetry: pulse amplitude, 50 mV; pulse width, 0.04 s; quiet time, 2 s. The working electrodes were polished with aqueous slurries of alumina (1.0, 0.3 and 0.05 μm, respectively) and were sonicated for 10 min in water. The polished electrodes were then exposed to aqua regia for 1 min. The substrate was treated in piranha solution (7:3 v/v, concentrated

H₂SO₄/30% H₂O₂) at 90 °C for 10 min to oxidize any impurities. After the piranha treatment, the gold electrode was etched electrochemically by scanning the electrode between -0.4 to 1.5 V at 10 mV s⁻¹ in 0.1 mol L⁻¹ H₂SO₄ solution for 10 min or until the characteristic CV for a clean Au electrode was obtained.

The chemicals used for monolayer modification were MBPPCo, thioglycolic acid (TGA), cysteine (CYST) and lipoate (LIP). Modification of the gold electrode was carried out in solutions that consisted of 1 mmol L⁻¹ thiol solutions of dichloromethane (for MBPPCo) or ethanol (for TGA and LIP) or water (for CYST) for at least 20 h. The modified gold electrodes were then washed with homologous solvent copiously to remove any thiols in the bulk before measurements were made.

Synthesis of 5-{3-methoxyl-4-(4-mercaptobutoxy)}phenyl-10,15,20-triphenylporphyrincobalt (MBPPCo)

The monothiolated cobalt porphyrin (see Scheme 1) was synthesized according to the modified procedure [23].

5-{3-Methoxyl-4-(4-hydroxy)}phenyl-10,15,20-triphenylporphyrin (1)

A 250-mL, three-necked flask fitted with a magnetic stirrer, dropping funnel and condenser was charged with benzaldehyde (7.93 g, 75 mmol), vanillin (3.82 g, 25 mmol) and propionic acid (100 mL; redistilled from *p*-toluenesulfonic acid and then from potassium permanganate). Freshly distilled pyrrole (6.8 mL, 0.1 mol) was added dropwise to the solution, which was heated to reflux. The mixture was heated at reflux for 3 h, then allowed to cool to room temperature. Anhydrous alcohol (30 mL) then was added to the resultant dark solution, which was kept at 4 °C for 8 h. The mixture was filtered and the purple precipitate washed with anhydrous alcohol until the washings were colorless. After drying, the crude product was purified by silica gel column chromatography (CHCl₃), affording a brown-purple solid as a monosubstituted porphyrin (1.23 g).

5-{3-Methoxyl-4-(4-bromobutoxy)}phenyl-10,15,20-triphenylporphyrin (2)

1,4-Dibromobutane (0.5 mL) in *N,N*-dimethylformamide (DMF, 10 mL) was added dropwise to a magnetic stirred solution of **1** (0.31 g, 0.46 mmol) and potassium carbonate (2.0 g) in DMF (20 mL). After being heated at reflux for 8 h, the solvent was removed by high vacuum. The resulting residue was dissolved in a minimum amount of CHCl₃ and purified on a silica gel column (CHCl₃) to yield a dark purple solid (0.19 g).

5-{3-Methoxyl-4-(4-mercaptobutoxy)}phenyl-10,15,20-triphenylporphyrin (3)

To a magnetically stirred solution of **2** (174 mg, 200 μmol), anhydrous potassium carbonate (0.34 g) and a catalytic amount of potassium iodide (0.1 g) in dichloromethane (20 mL), thioacetic acid (0.15 mL, 2.1 mmol) was slowly added. Then the mixture was heated at 80 °C for 1.5 h and cooled to room temperature. Distilled water (20 mL) was added to the solution to remove K₂CO₃ and the solution was then extracted with dichloromethane. The organic phase was washed with brine, dried (MgSO₄), filtered, evaporated to dryness, and the residue dried at high vacuum to yield 5-{3-methoxyl-4-(4-thioacetylbutoxy)}phenyl-10,15,20-triphenylporphyrin as a dark brown solid. To a solution of the solid in chloroform (10 mL) and methanol (10 mL; degassed with a stream of nitrogen gas) was added a solution of potassium hydroxide (1 g) in ethanol (5 mL) and water (3 mL), and magnetic stirred under N₂ at ambient temperature for 1.5 h. The mixture was extracted with chloroform. The combined extracts were evaporated to dryness at

< 35 °C to give **3** as a dark purple solid (110 mg). UV-vis (Toshiba U-3400 UV-vis spectrometer): 412.1, 516.1, 551.6, 590.6, 646.3 nm. ¹H NMR (Avance DRX 200, CDCl₃): δ 8.90 (m, 6H, β-pyrrole), 8.58 (s, 2H, β-pyrrole), 8.22 (d, 6H, phenyl rings), 8.01 (m, 2H, phenyl rings), 7.77 (d, 8H, phenyl rings), 7.26 (s, 2H, phenyl rings), 4.18 (s, 3H, OCH₃), 3.98 (t, 2H, ArOCH₂), 2.12 (m, 2H, CH₂SH), 1.26 (m, 4H, -CH₂-), -2.77 (s, 2H, NH).

5-{3-Methoxy-4-(4-mercaptobutoxy)}phenyl-10,15,20-triphenylporphyrincobalt(II) (**4**)

Cobalt(II) acetate tetrahydrate (595 mg, 3.4 mmol) was added to a solution of **3** (20 mg, 26 μmol) in DMF (20 mL) bubbled with nitrogen gas and the solution heated at reflux for a further 3 h under N₂. The mixture was allowed to cool and poured into cold water (50 mL) and then filtered. The resultant solid was recrystallized from hexane/dichloromethane to afford the cobalt(II) porphyrin (**4**) (40 mg). UV-vis: 409.8, 529.2 nm.

Results and discussion

Electrochemical behavior of modified electrodes

Figure 1 shows the cyclic voltammograms of (a) a bare gold electrode and (b) MBPPCo on Au in 0.1 mol L⁻¹ phosphate buffer (pH 7.3). It is clearly seen that there was no reduction or oxidation peak on the bare Au electrode within the potential range examined. In contrast, a pair of reduction and oxidation peaks appeared for the gold electrode after immersion in a chloroform solution of MBPPCo for 24 h, suggesting formation of a thiol-derivatized metalloporphyrin on Au. The reduction and oxidation peak potentials are 0.029 V and 0.185 V, respectively. The peak potentials were found to remain unaltered with an increasing scan rate of the potential, and the peak currents are proportional to the scan rate over the range 10–120 mV/s, indicative of a surface electrochemical reaction. The cyclic voltammograms of the MBPPCo/Au remained stable after 100 scans, suggesting electrochemical stability of the porphyrin-coated layer.

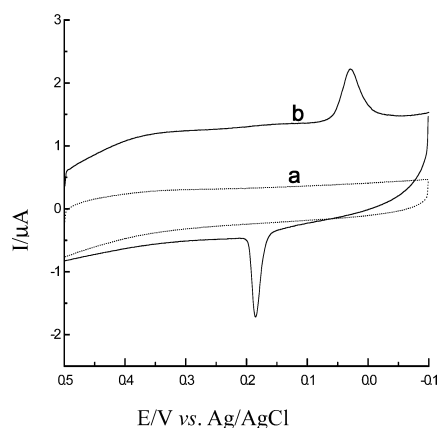


Fig. 1 Cyclic voltammograms (CVs) for (a) the bare electrode and (b) the MBPPCo-modified electrode in 0.1 mol L⁻¹ phosphate buffer solution, pH 7.3

The cyclic voltammograms recorded for 0.1 mmol L⁻¹ potassium ferricyanide on bare gold, MBPPCo/Au, TGA/Au and the binary component electrode of MBPPCo and TGA (where / denotes the interface) are shown in Fig. 2. On a bare gold electrode the peaks for potassium ferricyanide occur at 0.31 V and 0.19 V (Fig. 2b). After coating the gold electrode with a TGA monolayer, we found that the oxidation and reduction of Fe(CN)₆^{3-/4-} are completely irreversible and no obvious redox peak was observed (Fig. 2d). Although the pK_a value of TGA has not been obtained, the carboxylic group is expected to be negatively charged in 0.1 mol L⁻¹ phosphate buffer (pH 7.3). The above case may be attributed to the repulsive interaction between Fe(CN)₆^{3-/4-} and RCOO⁻ which affect the potassium ferricyanide redox and an increased distance between the molecules and the electrode [1, 2, 3]. It is a reasonable inference that after being modified with the positively charged Co²⁺ active center of MBPPCo/Au, the redox of Fe(CN)₆^{3-/4-} would be more reversibly accompanied by a large enhancement in peak currents and it would favor the oxidation and reduction of K₃Fe(CN)₆. However, as can be readily seen from Fig. 2(c), compared with the direct potassium ferricyanide redox at the bare electrode, broader peaks (0.34 V and 0.22 V) and slower electron transfer kinetics were obtained. However, the binary component self-assembled monolayers of MBPPCo and TGA show a dramatic enhancement in the peak currents with no significant effect on the peak position and width being observed (Fig. 2a). Analogous experimental results also were observed on bare and modified electrodes for the oxidation of AA. The reason for the observed differences with the various modified electrodes will be discussed below.

Electrochemical oxidation of AA and DA

Figure 3 depicts typical cyclic voltammograms (CVs) for 2 mmol L⁻¹ AA in phosphate buffer (pH 7.3) on the

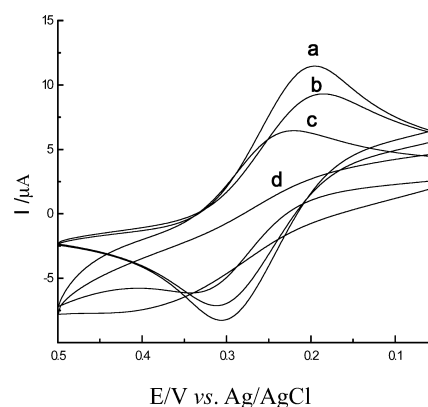


Fig. 2 CVs for 0.1 mol L⁻¹ Fe(CN)₆^{3-/4-} on binary MBPPCo+TGA/Au (a), bare Au (b), MBPPCo/Au (c) and TGA/Au (d). Scan rate: 0.08 V s⁻¹; sensitivity: 10⁻⁶

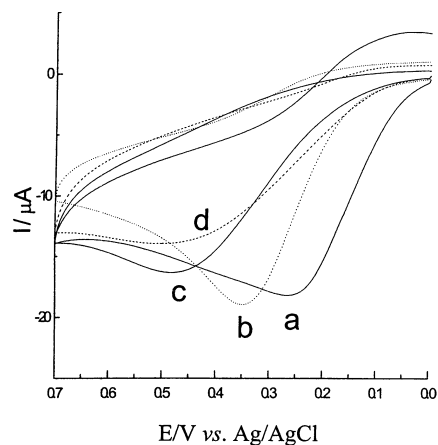
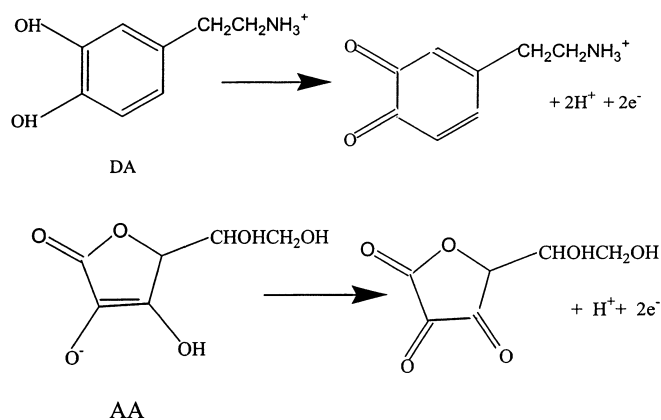


Fig. 3 CVs for 2 mmol L⁻¹ AA in phosphate buffer solution (pH 7.3) on the bare electrode (b) and on electrodes modified with MBPPCo+TGA (a), MBPPCo (c) and TGA (d). Scan rate: 0.1 V s⁻¹; sensitivity: 10⁻⁵

bare Au electrode and on the modified electrodes with MBPPCo and TGA, respectively. On the bare electrode, the oxidation of AA occurs at 0.35 V and is irreversible (Fig. 3b). The electron transfer kinetics for the oxidation of AA on bare Au are rather sluggish owing to the fouling of the electrode surface by the AA oxidation products [3, 4]. It has been established that the oxidation of AA involves two electrons and two protons in acidic solution [6]. However, in phosphate buffer, the pH value is higher than the first pK_a value (4.2 [1, 3, 6]) of AA, and here the oxidation of AA involves one proton and



Scheme 2 The oxidation of DA and AA in phosphate buffer solution

two electrons due to the fact that AA exists as an anion (Scheme 2). From Fig. 3c and Fig. 3d, a large increase in the overpotentials of AA (around 110 mV) accompanied by a decrease in peak currents were obtained with both electrodes modified with the positively charged Co²⁺ center of the MBPPCo layer and the negatively charged RCOO⁻ of the TGA layer compared to the bare electrode. As can be readily seen from Fig. 3a, with the gold electrode modified with the binary component

SAM of the positively charged Co²⁺ active center of MBPPCo and a carboxylate terminal group of TGA, a small enhancement in the peak current at 0.26 V with a large decrease in the overpotential (90 mV) for the oxidation of AA was observed. The possible explanation for the large negative shift in the AA oxidation potential could be the electrostatic attraction between the negatively charged AA in neutral aqueous solution and the positively charged Co²⁺ redox center of the MBPPCo layer [1, 2, 3, 6, 9], whereas the positive monolayer attracts the anionic AA towards the gold electrode surface and it consequently prevents adsorption of the AA oxidation products on the surface [2, 3]. However, this result has not been observed at a MBPPCo layer-modified electrode which also has a cationic center.

This paradox encouraged us to further investigate the possible reasons for the properties of the binary component monolayer-modified electrode. There are many references which state that phase-separated structures occur for mixed self-assembled thiols of different lengths [24, 25]. These results suggest that the degree of difference in the lengths of the thiols comprising the binary SAMs influences the spatial distribution of the thiols. In our studies, TGA (two carbons) and MBPPCo were chosen as a short and a long thiol, respectively. In mixed SAMs of MBPPCo and TGA, although there exists a negatively charged carboxylate terminal group, there may be some defects or loosely packed sites due to a small van de Waals' force between the TGA molecules [25, 26, 27, 28]. Therefore, the relative disordered structure of the TGA/Au surface provides a mechanism for rapid electron transfer between the electrode and the AA electroactive species [6]. In addition, the electrostatic attraction between the MBPPCo cationic center in the phase-separated two-component SAM and the anionic AA urges the AA towards the electrode and prevents the adsorption of the AA oxidation product on the electrode surface. Of more importance may be the existence of the electrocatalytic action of MBPPCo. The available evidence supports the idea that the oxidation of AA is



Scheme 3 Mechanism for the oxidation of AA catalyzed by MBPPCo

catalyzed by MBPPCo in the form of metallic porphyrin SAMs that provide a flexible means of anchoring the electrocatalyst, with the SAMs blocking access of the target redox couple to the electrode. The catalytic oxidation of AA by MBPPCo is illustrated in Scheme 3.

Figure 4 shows the CVs for 1 mmol L⁻¹ DA in phosphate buffer on the bare Au electrode (Fig. 4c) and on the electrodes modified with MBPPCo (Fig. 4e) and various chain length thiols with terminal carboxylate groups (Fig. 4a, b and d for TGA, LIP and CYST, respectively). Cyclic voltammetric experiments on Au

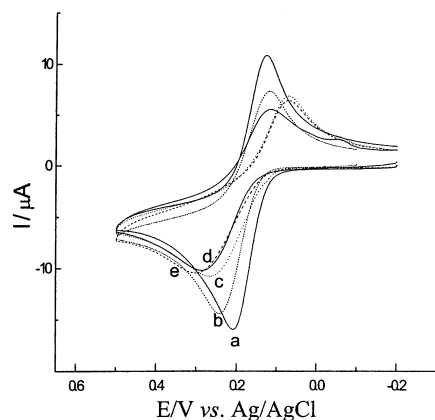


Fig. 4 CVs for 1 mmol L⁻¹ DA in phosphate buffer solution (pH 7.3) on TGA/Au (a), LIP/Au (b), bare Au (c), CYST/Au (d) and MBPPCo/Au (e); scan rate: 0.1 V s⁻¹; sensitivity: 10⁻⁵

electrodes derivatized by various chain length and terminally substituted alkanethiol monolayers were performed in order to demonstrate the above conclusion. On the bare electrode, the peak potentials for the DA oxidation–reduction system occur at 0.267 V and 0.072 V and the process is irreversible (see Scheme 2). However, on the cationic monolayer-modified MBPPCo and CYST electrodes (pI 5.02) in phosphate buffer solution, the electron transfer kinetics for the oxidation were found to be rather sluggish owing to the repulsion between the positively charged DA (pK_a 8.9 [2, 3]) and the monolayers. For example, with the MBPPCo-modified electrode the potentials at 0.301 V and 0.073 V were assigned to the oxidation and reduction of DA, respectively. The separation between anodic and cathodic peak potentials on the bare electrode was found to be 195 mV, which became 228 mV on the MBPPCo-modified electrode. In contrast, owing to the electrostatic attraction between DA and the anionic carboxylate end group of alkanethiol monolayers, the oxidation of DA appears at 0.209 V and is more reversible for the TGA-modified electrode (Fig. 4a). Compared with TGA (two carbons), the LIP-modified electrode (eight carbons) for the oxidation of DA is shifted to a more positive potential and the peak current is significantly smaller. It has been shown that the electron transfer kinetics at the monolayer become slower as the chain length of the monolayer increases. In the present investigation, although both TGA and LIP show a similar structure, the slower electron transfer kinetics of the DA electroactive species on the LIP monolayer is due to the more compact nature of the monolayer and the longer distance for the electron transfer compared with the TGA monolayer [6, 26, 27, 28].

Conclusions

The electrochemical behavior of electrodes modified with one- or two-component SAMs of the positively

charged Co²⁺ center of MBPPCo and the negatively charged COO⁻ of thiols has been investigated by cyclic voltammetry using Fe(CN)₆^{3-/4-} and AA as probes. As can be clearly seen from the CVs, the electrostatic repulsion between anionic species and monolayers modified with negatively charged RCOO⁻ slows down the electron transfer between the electroactive species and the electrode. The MBPPCo-modified monolayer with the cationic center did not show the expected result, i.e. the electrostatic attraction of the monolayer with the anionic species was not enhanced by the electrode reaction. In sharp contrast to the unitary SAMs of MBPPCo or TGA, the binary SAM of MBPPCo and TGA largely accelerated the oxidation of electroactive species [both Fe(CN)₆^{3-/4-} and AA] and the possible reasons for the difference were discussed.

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