Full Paper

Electrochemical Behavior of Dopamine at a Penicillamine Self-Assembled Gold Electrode and its Analytical Application

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The fabrication and electrochemical characteristics of penicillamine (PCA) self-assembled monolayer modified gold electrode were investigated. The self-assembled electrode shows an obvious electrocatalytic activity for the oxidation of dopamine (DA). In phosphate buffer (pH 7.0), the peak current is proportional to the concentration of DA in the range of $8.0 \times 10^{-6} \sim 1.0 \times 10^{-3}$ M by the cyclic voltammetry methods with the detection limits of 4.5×10^{-7} M. The PCA self-assembled monolayer modified gold electrode can be applied to the determination of DA in practical injection samples with simplicity, rapidness, and accurate results.

Keywords: Dopamine / Penicillamine / Self-assembled monolayer

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Introduction

Dopamine (DA) is the important substance for the message transfer in the mammalian central nervous system. Extreme abnormalities of DA concentration levels may lead to several diseases such as Parkinson's [1]. DA is now the focus of intense research of neuroscientists and chemists, and it is essential to develop rapid and simple methods for the determination of the concentration of DA [2]. Dopamine has been determined using various methods: spectrophotometry [3], mass spectrometry [4], fluorimetry [5], chemiluminescence [6], capillary electrophoresis [7], chromatography [8]. On the other hand, DA can be determined with electrochemical methods because of its electrochemical activity [1, 2, 9].

The self-assembled monolayer approach is a good way to control the surface of electrodes at the molecular level [10, 11]. It has grown enormously in recent years, primarily due to the easiness of fabrication, characterization, and manipulation. Therefore, the self-assembled monolayers have been used to study electron transfer, proteins, and other biomolecules and to fabricate biosensors [12,

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13]. In this paper, the compound used to modify the gold electrode is penicillamine (PCA). It is the main product of penicillin decomposition [14]. Since PCA contains a mercapto group, it can be self-assembled on a gold electrode surface and thus can be used as a new chemically modified electrode to study the electrochemical properties of biomolecules. In this paper, we have studied the electrochemical behavior of DA at the penicillamine (PCA) selfassembled monolayer (SAM) modified gold electrode (PCA-Au electrode) in phosphate buffer (pH 7.0), which pH value is much closer to the physiological condition in humans. Some electrochemical parameters, such as the diffusion coefficient D, proton transfer number m, and rate constant k_s have also been determined. The proposed PCA SAM modified gold electrode has been applied to the determination of DA in practical medicine with satisfactory results.

Results and discussion

Characterization of the PCA SAM in a Fe(CN) $_6^{-}$ / Fe(CN) $_6^{+}$ solution

The redox behavior of a reversible ion couple can be used to probe the packing structure of the monolayer [15, 16]. Figure 1 shows the cyclic voltammograms of the bare gold electrode and the PCA-Au electrode in 1.0×10^{-3} M K₃[Fe(CN)₆]³⁻ solution containing 0.1 M KCl. For a bare gold electrode, a couple of well-defined waves of

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Figure 1. Cyclic voltammograms of 1.0×10^{-3} M Fe(CN) $^{8-}$ /Fe(CN) $^{4-}_{6-}$ at a bare gold electrode (a) and PCA-Au electrode (b) 0.1 M KCI; scan rate: 100 mVs⁻¹.

Fe(CN)₆³⁻/Fe(CN)₆⁴⁻ should appear and the separation between two peaks should be 60 mV. However, it can be seen that the peak current was decreased and the ΔE_p , the difference between the anodic peak potential and the cathodic peak potential, was increased for the PCA-Au electrode. Because PCA is a short mercaptan molecule, there are many pinhole defects and collapsed sites in the PCA monolayer and the electron transfer rate constant at pinhole defects is the same as that at the bare gold electrode, *i.e.*, the redox couples can reach the gold surface through pinhole defects in the PCA monolayer.

Characterization for the PCA SAM by complex impedance analysis in the presence of Fe(CN) $_6^{3-}$ / Fe(CN) $_6^{4-}$

The impedance method is based upon the measurement of the response of the electrochemical cell to a small amplitude alternating potential. The response is often shown in the complex impedance presentation and results are interpreted in terms of an equivalent electrical circuit. The change in faradic impedance can be used to prove the presence of electroactive species at the electrode [17].

Figure 2 shows complex impedance plots of the bare gold electrode and PCA-modified gold electrode. A comparison of complex impedance plots of the bare gold electrode and the monolayer-covered gold electrode shows the effect of the absorbed PCA monolayer on the AC response. For the monolayer-covered electrode, R_{ct} , which is the diameter of the semicircle at high frequency, is clearly greater than that of the bare gold electrode due to an inhibition of the PCA SAM to electrode transfer. This proves the presence of PCA on the gold electrode.



Figure 2. Complex impedance plots measured in 1.0×10^{-2} M Fe(CN) $^{8-}_{6}$ /Fe(CN) $^{4-}_{6}$ + 0.1 M KCl + phosphate buffer (pH 7.0) at the formal potential of 0.22 V for (a) bare gold electrode; (b) PCA-Au electrode. The frequency range is between $10^{-2} \sim 10^{6}$ Hz with signal amplitude of 50 mV.

Electrochemical response of DA at the PCA SAM electrode

Cyclic voltammograms (CV) of DA under the potential windows between -0.2 and 0.6 V at the bare gold electrode (Fig. 3a) and at the PCA-Au electrode (Fig. 3b) were obtained in phosphate buffer of pH 7.0. As shown in Fig. 3, at the PCA-Au electrode, DA showed a well defined reversible wave with the anodic potential 0.149 V which was more negative than that at bare electrode and the peak current increased greatly. The above mentioned results suggest that the electron diffusion was slow at the bare gold electrode, while PCA accelerated the electron transfer rate between DA and electrode at the PCA-Au



Figure 3. Cyclic voltammograms at bare gold electrode (a) and PCA-Au electrode (b, c) in the presence (a, b) and absence (c) of 2.0×10^{-4} M DA. Phosphate buffer of pH 7.0; scan rate: 0.05 Vs⁻¹.



Figure 4. Effects of pH on the anodic peak potential and peak current of DA in phosphate buffer. DA concentration: 2.0×10^{-4} M; scan rate: 0.03 V.

electrode. Therefore, the PCA-Au electrode promoted the electrochemical reaction of DA.

Effects of pH on the peak current and peak potential of DA

The effects of the pH value of phosphate buffer on the anodic potential and peak current were investigated and the results are shown in Fig. 4. It can be seen that the potential of DA shows a decrease with the increase in solution pH and the potential of DA vs. pH in phosphate buffer has a good linear relation in the range of pH 5.7~8.5. As the solution pH increases, the anodic peak potential shifts to the negative, and a linear regression equation $E_p = 0.5937 - 0.0642$ pH (E_p : V; correlation coefficient r = 0.9909) was obtained, which showed that the uptake of electrons is accompanied by an equal number of protons. Figure 4 also shows the dependence of the anodic peak current on the pH of the solution. It was found that the anodic peak current increased with increasing the pH up to 7.0; beyond that the peak current decreased. Therefore, the pH value of 7.0 was selected for further studies.

Effect of the scan rate on the peak current

As show in Fig. 5, the anodic peak current i_p of DA increased with the increase of the scan rate on PCA SAM in pH 7.0 phosphate buffer, and exhibited a linear relation to the square root of the scan rate, $v^{1/2}$, with the linear regression equation $i_p = -0.2769 + 4.4996 v^{1/2}$ (i_p : 10^{-6} A, v: Vs⁻¹), and a correlation coefficient of 0.9987. The result indicates that the electron transfer reaction is controlled by the diffusion of DA.

The relationship between $\ln i_p$ and $\ln v$ was also obtained, and the linear regression equation was $\ln i_p = -12.2950 + 0.6056 \ln v (i_p: A, v: V s^{-1}, r = 0.9989)$. The linear



Figure 5. Cyclic voltammograms of 2.0×10^{-4} M DA at PCA-Au electrode in phosphate buffer (pH 7.0) at different scan rates. (1) 0.03 Vs⁻¹; (2) 0.05 Vs⁻¹; (3) 0.08 Vs⁻¹; (4) 0.12 Vs⁻¹; (5) 0.15 Vs⁻¹; (6) 0.18 Vs⁻¹; (7) 0.22 Vs⁻¹; (8) 0.25 Vs⁻¹. The insert shows the dependency of the anodic peak current of DA on the square root of the scan rate.

regression equation of anodic peak potential vs. ln v was $E_p = 0.1669 + 0.0160 \ln v (E_p: V, v: V s^{-1})$ with a correlation coefficient of 0.9977.

The reaction mechanism of DA at the PCA modified electrode

Diffusion coefficient

The diffusion coefficient can be calculated according to following equation (1) [18]:

$$i_{\rm p} = 2.69 \times 10^5 \,An^{3/2} \,D^{1/2} C \,v^{1/2} \tag{1}$$

where i_p is peak current (Ampere), A is the surface area of the electrode (cm²), n is the electron transfer number, D is the diffusion coefficient (cm² s⁻¹), C is the bulk concentration of DA (mol cm⁻³), v is the scan rate (V s⁻¹). From the slopes of i_p vs. $v^{1/2}$, D can be obtained if the parameters of A, n, and C are known. Here, n = 2 [1] and from the slopes of the plot between i_p and $v^{1/2}$ the diffusion coefficient D, was calculated as 8.9×10^{-7} cm² s⁻¹.

Standard rate constant and electron transfer coefficient The standard rate constant (k_s) and electron transfer coefficient (a) can be obtained according to following equations [19]:

$$i_{\rm p} = 0.227 \, nFAC_0 \, k_{\rm s} \times \exp \left[(-anF/RT) \, (E_{\rm p} - E^0) \right]$$

$$E_{\rm p} = E^0 + RT/(anF) \left[0.780 + \ln (Db)^{1/2} - \ln k_{\rm s} \right], b = anFv/RT$$
(3)

From the intercept of $\ln i_p$ vs. $\ln v$ and E_p vs. $\ln v$ which is described before, $k_s = 1.8 \times 10^{-2}$ cm s⁻¹ and a = 0.4 can be calculated.

Protons transfer number

As shown in Fig. 4, with the pH increasing, the anodic peak potential E_p linearly shifts to the negative, which showed that protons took part in the electrode reaction. Based on Nernst equation, the relationship between E_p and pH can be expressed in the following equation [20]:

$$E_{\rm p} = E^0 + (0.0591/n) \log \left[(O_{\rm x})^{\rm a} / (R)^{\rm b} \right] - (0.0591 \ m/n) \ \rm pH$$
 (4)

where a and b are coefficients of oxidant and reducer in the reaction equation and *m* is the number of protons transfered, *n* have the significance mentioned above. The slope of E_p vs. pH was -0.0642 obtained from Fig. 4. Therefore, the proton transfer number was calculated as 2.2. That is, $m \approx 2$, which indicates there are two protons and two electrons transferred in the reaction.

Kinetic mechanism for the electrocatalytic process

Since PCA contains the undissociated COOH group, which has a much higher affinity for DA under experimental conditions, and the electroactive group in DA is the OH group, which presents in much closer vicinity to the carboxylic acid modified electrode [21], this might lead to much faster kinetics of DA oxidation at the PCA-Au SAMs. Our experimental results were in accordance with the literature for SAM with COOH head groups [22]. Mandler et al. reported improved kinetics of DA at HS(CH₂)_nCOOH monolayers compared to that on a bare Au electrode [22]. According to our experimental results, the probable catalytic process may be expressed as follows:

Calibration plots

Under the optimum analytical conditions, the linear range of DA at the PCA-Au electrode has been studied. As shown in Fig. 6, the linear range is from 8.0×10^{-6} to 1.0×10^{-3} M, and linear regression equation is $i_p = 0.2234 + 0.2412 c$ (i_p : 10^{-6} A, *C*: 10^{-4} M), correlation coefficient is 0.9938, and the detection limit is 4.5×10^{-7} M.



Figure 6. CVs of different concentrations of DA in phosphate buffer of pH 7.0. Concentration of DA, (1) 8.0×10^{-6} M; (2) 8.0×10^{-5} M; (3) 1.0×10^{-4} M; (4) 2.0×10^{-4} M; (5) 4.0×10^{-4} M; (6) 6.0×10^{-4} M; (7) 8.0×10^{-4} M; (8) 1.0×10^{-3} M. scan rate: 0.05 Vs⁻¹. Insert: the calibration curve of DA concentrations at the PCA-Au modified electrode with CV method.

Tolerance of foreign substances

The influence of various foreign species on the determination of 2.0×10^{-4} M DA was investigated. The tolerance limit was taken as the maximum concentration of the foreign substances which caused an approximately ±5% relative error in the determination. The tolerated ratio of foreign substances to 2.0×10^{-4} M DA was 200 for glucose, 155 for chitosan, 125 for cystine, 50 for Cl⁻, K⁺, 10 for glycin and *L*-asparagine, respectively.

Analytical application

The concentration of DA in an injection sample purchased from Jiangsu Yabang Pharmaceutical Co. Ltd., Branch was determined by the proposed methods. DA sample solutions of Differential concentrations were obtained by diluting two DA injections (2 mg: 2 mL) respectively. The results are listed in Table 1. The recoveries were 104 and 102% by CV.



Scheme 1. Proposed mechanism for the electrocatalytic process.

DA sample	DA specified $(mg mL^{-1})$	Found ^{a)} (mg mL ⁻¹)	Recovery (%)
1	2.00	2.08	104
2	2.00	2.04	102

^{a)} Mean of three determinations.

Experimental

Reagents

Penicillamine (Sigma) and dopamine (Huamei Reagent Co., Ltd) were used as received. Penicillamine (PCA) solution (0.01 M) was prepared by dissolving 0.0746 g of PCA in water and diluting to the mark in a 50 mL calibrated flask. Dopamine (DA) stock solution (0.001 M) was prepared by weighing 0.0095 g of DA and dissolving with water and then diluting to the mark in a 50 mL calibrated flask. A phosphate buffer solution was used to control the pH. Dopamine hydrochloride injection was purchased from Jiangsu Yabang Pharmaceutical Co. Ltd Branch. All other chemicals were of analytical reagent grade and were used without further purification. Doubly distilled water was used throughout. All the experiments were carried out at room temperature (approx. 25°C).

Instruments

Electrochemical measurements were carried out on a CHI 660 electrochemical analyzer (CH Instruments, USA). Electrochemical impedance measurement was carried out on a Model IM 6e (Zahner Elektrik Co., Germany). A three-electrode system used in the measurements consists of a gold electrode (d = 2 mm) or PCA-Au electrode as the working electrode, Pt as the counter electrode, and a Ag-AgCl electrode as the reference electrode. All potentials are given with respect to the Ag-AgCl electrode.

Preparation of PCA-Au electrode

The bare gold electrode was polished to a mirror-like surface with 0.3 µm and 0.05 µm Al₂O₃ powder, respectively and immersed in Piranha solution (a mixed solution of 30% H₂O₂ and concentrated H₂SO₄, v/v 1:3), then rinsed ultrasonically with water and absolute ethanol for 3 min. This electrode was voltammetrically cycled in 0.1 M H₂SO₄ until a stable cyclic voltammogram was obtained. The potential range is between 0.4 and 1.2 V. After washed with sonication, the electrode was immersed in an aqueous solution of 1.0×10^{-2} M PCA for approximately 30 h at room temperature. Upon removal from the PCA solution, the electrode was thoroughly rinsed with doubly distilled water to remove the physically adsorbed species.

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