Electrocatalytic Oxidation of Dopamine by Ferrocene in Lipid Film Cast on a Glassy Carbon Electrode

WANG, Jian-Guo[a,b](王建国) WU, Zheng-Yan[a](吴正岩) TANG, Ji-Lin[a](唐纪琳)
TENG, Ren-Rui[b](滕人瑞) WANG, Er-Kang*,[a](汪尔康)
[a]*State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, China*
[b]*Institute of Modern Physics, Chinese Academy of Sciences, Lanzhou, Gansu 730000, China*

The ferrocene-lipid film electrode was successfully prepared by means of casting the solution of ferrocene and lipid in chloroform onto a glassy carbon (GC) electrode surface. Ferrocene saved in the biological membrane gave a couple of quasi-reversible peaks of cyclic voltammogram. The electrode displays a preferential electrocatalytic oxidation of dopamine (DA). The effect of electrocatalytic oxidation of DA depends on the solution pH and the negative charge lipid is in favor of catalytic oxidation of DA. The characteristic was employed for separating the electrochemical responses of DA and ascorbic acid (AA). The electrode was assessed for the voltammetric differentiation of DA and AA. The measurement of DA can be achieved with differential pulse voltammetry in the presence of high concentration of AA. The catalytic peak current was proportional to the concentration of DA in the range of 1×10^{-4}—3×10^{-3} mol/L.

Keywords ascorbic acid, dopamine, ferrocene, dimyristorylphosphatidylcholine, lipid, cast film.

Introduction

DA is one of essential participants in the neurotransmission process in mammalian central nervous system. A loss of DA-containing neurons may result in some serious disease such as Parkinsonism.[1] Since its discovery in the 1950s, DA has been of interest to neuroscientists and chemists. It possesses very strong electrochemical activity and is one of the main objects of study in the electroanalytical chemistry of neurotransmitters. Several factors make the electrochemical detection of DA difficult. The main problem is that the basal DA concentration is very low (down to the nanomolar level[2]), while the concentration of interfering anions, such as AA, is much higher (about 0.1 mmol/L[3]). At a bare GC electrode the two species were electrooxidized at similar potential,[4,5] preventing reliable measurement of the DA response. A further difficulty is that oxidation of DA in the presence of AA results in a homogeneous catalytic oxidation of AA which may enhance the current due to DA by an amount related to the AA concentration.[6,7]

In recent years, some methods have been developed to overcome the problems. Franck and Daniel used self-assembled monolayers of ω-mercapto carboxylic acid,[8] $HS(CH_2)_nCOOH$ ($n = 2, 5, 10$), on a gold electrode as a means to induce electrochemical differentiation between DA and AA. Wang and Walcarius reported zeolite-Y-modified carbon paste electrodes which displayed a preferential incorporation of DA while rejecting the anionic ascorbate species.[9] A greater separation of DA and AA could be obtained when the electrode is pretreated electrochemically at high potentials.[10] One common route to impart higher selectivity onto the chemically modified electrode is to cover the surface with an appropriate permselective coating, such as the Nafion,[11-14] which is known to incorporate positively charged molecules and repel anionic ones due to its ion-exchange properties. Inorganic inomers, such as zeolites[9] and nontronit clays[15]

* E-mail: ekwang@ns.ciac.jl.cn; Fax: 86-431-5689711.
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are extensively investigated. Kunitake and co-workers described an ordered film of water-insoluble surfactant cast onto a solid surface,[16] which is similar to biological membranes formed by lipid in living organisms and could incorporate electroactive substance to facilitate electocatalysis. This technique provides a method to deposit multiple bilayers of surfactant onto a solid electrode surface.

In the present work, we reported a different approach to achieve electrochemical separation between DA and AA. A modification of the electrode with ferrocene-multiple bilayers of lipid was described. The modified electrode shows up electrocatalytic activity for the oxidation of DA and AA. The lipid film was used to control the access of ionic species to the electrode surface. Three types of lipid cast films were studied. Under proper pH and l-α-phosphatidylcholine dipalmitoyl (DPPC) cast film, DA could be determined in the presence of AA.

Experimental

Reagents

l-α-Phosphatidyl-*dl*-glycerol dimyristoyl (DMPG), dimethyldioctadecylammonium bromide (DODAB), DPPC, L-AA (purity 99.0%), and DA were purchased from Sigma (USA) without further purification. Ferrocene (purity 98%) was purchased from Fluka (Germany). Chloroform was of analytical grade and was distilled before the experiments. All other chemicals were also of analytical grade. Obtained by means of a Millipore Q (USA) water purification set, pure water was throughout used. DA solutions were prepared daily using glycine buffer or phosphate buffer.

Apparatus

All voltammetric experiments were carried out with a model CS-1087. Cypress Systems (Inc., Lawrence, KS, USA) connected a PC for control and data storage. A standard three-electrode cell was employed for the electrochemical experiment. When DA is electrochemically oxidized at a metal electrode, it may polymerize readily to melanin-like products.[17] The measurement of DA is usually carried out with carbon electrodes. A GC electrode was used as the substrate electrode and the counter electrode was a platinum wire. All potentials reported in the paper are referenced to a Ag/AgCl (KCl saturated) electrode.

All experiments were carried out at room temperature. The buffer and sample solution were deaerated with purified nitrogen for 5 min at least for removing oxygen prior to the beginning of a series of experiments.

Electrode Preparation

The modification of the multiple bilayer lipid cast on a GC electrode was proceeded by the following way. A GC electrode (diameter 4.5 mm) was first polished with sand paper, followed by alumina slurry of 1.0, 0.3, 0.05 μm on polishing cloth with water as the lubricant, respectively, then sonicated in pure water both for 2 min, rinsed with pure water again, lastly the GC electrode was put in the air for drying.

Lipid which represents DPPC, DODAB and DMPG respectively and ferrocene were dissolved in chloroform, giving a final concentration of 2.5 mg/mL lipid and 1.2 mg/mL ferrocene. The thickness of the film could be controlled through adjusting the volume of lipid solution. An aliquot of 6.0 μL of the lipid solution was dropped onto the surface of the GC electrode by a microsyringe. A small bottle was tightly fixed over the electrode for serving as a closed evaporation chamber. Chloroform on the GC electrode surface was being evaporated gradually for 30 min, then, the electrode was serving as working electrode.

Results and discussion

Electrochemical behavior of the GC electrode cast by lipid film containing ferrocene

When the ferrocene-DPPC film electrode was immersed in phosphate buffer solution and was scanned in a potential range from − 100 mV to 750 mV, a couple of quasi-reversible redox peaks at lower scan rates can be observed from Fig. 1. The difference between the redox peaks is about 100 mV at scan rate 80 mV/s. This couple of peaks corresponds to the ferrocene/ferricinium redox system. In the first few scans, the anodic peak was high and decreased swiftly by further scanning, then, it kept nearly constant. The fact suggested that some ferrocene had been remained in the DPPC films. There is a

hydrophobic region in the bilayer lipid membrane and ferrocene is hydrophobic, so the hydrophobic interaction could be thought for a primary factor of keeping ferrocene to stay in the cast film. The electroactivity of the modified electrode is independent of pH in the range of 4.0—8.0 because protons are not involved in the electrode reaction. These results are consistent with the results in the references 15 and 18.

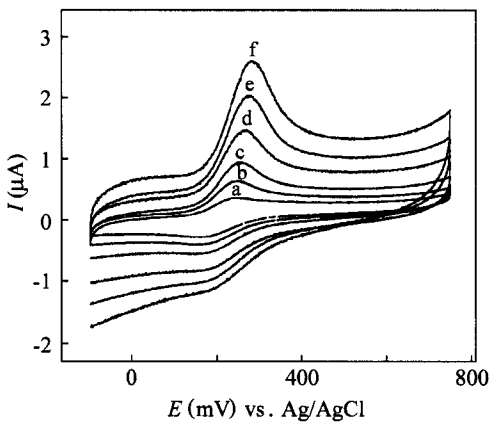

Fig. 1 Cyclic voltammograms of ferrocene staying in DPPC film in 0.1 mol/L KCl and 0.1 mol/L phosphate buffer (pH 6.7). Scan rate (mV/s): (a) 10, (b) 20, (c) 40, (d) 80, (e) 160 (f) 250.

The oxidation of ferrocene in DMPG and DODAB were also investigated in the absence of DA. Fig. 2 shows that the electroactivity of ferrocene in DPPC and DMPG is similar, while the oxidation of ferrocene in DODAB is different from them. When ferrocene is oxidized at the GC electrode, there must be some sort of charge exchange between the lipid film and the bulk in order to maintain charge neutrality in the film. It means that the oxidation of ferrocene is accompanied by the transfer of a cation from the film to the solution or the transfer of a negative charge into the film. In the case of the neutral and negatively charged lipid there is no apparent possibility to charge transfer from the lipid film to the bulk solution, while positively charged lipid possesses the possibility. It prevents ferricenium in DODAB from dissolving in the water phase. In the case of the negatively charged lipid, efficient ion pairing might also prevent ferricenum from dissolving in the water phase. So it is observed in Fig. 2 that the anodic and cathodic current of ferrocene in DODAB and DMPG are higher than that in DPPC.

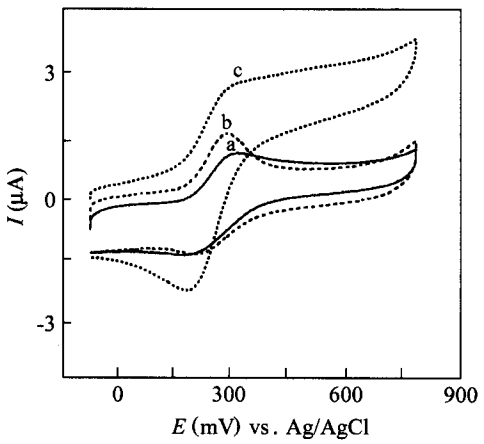

Fig. 2 Cyclic voltammograms of ferrocene in lipid film in 0.1 mol/L NaCl and 0.1 mol/L phosphate buffer (pH 6.7). Scan rate 50 mV/s; (a) DPPC, (b) DMPG, (c) DODAB.

Electrocatalytic oxidation of DA *at a ferrocene-*DMPG *film electrode*

To test the elctrocatalytic activity of the ferrocene-DMPG film electrode toward DA oxidation, the cyclic voltammetric responses in pH 6.64 phosphate buffer solution in the absence and presence of DA was investigated. Fig. 3b shows the cyclic voltammograms of ferrocene-DMPG film electrode in 6.25×10^{-4} mol/L DA solution. There is a drastic enhancement of the anodic current, while the cathodic current is very low in revered direction. Fig. 3c shows that the anodic peak potential of DA at bare GC electrode is at 410 mV vs. Ag/AgCl, while the peak potential of DA at ferrocene-DMPG film electrode is 330 mV under the same conditions. The overpotential of oxidation of DA decreases approximate 80 mV. The behavior shows that the ferrocene-DMPG film electrode has a strong electrocatalytic effect. This increasing oxidation current is due to the fact that DA in solution diffuses toward the electrode surface and reacts with ferricinium ion.[11,19] The electrochemical process could be expressed as follows:

$$CpFeCp \rightleftharpoons CpFe^{+}Cp + e$$

$$2CpFe^{+}Cp + QH_2 \longrightarrow 2CpFeCp + Q + 2H^{+}$$

(CpFeCp and $CpFe^{+}Cp$ represent ferrocene and ferricinium ion, respectively.)

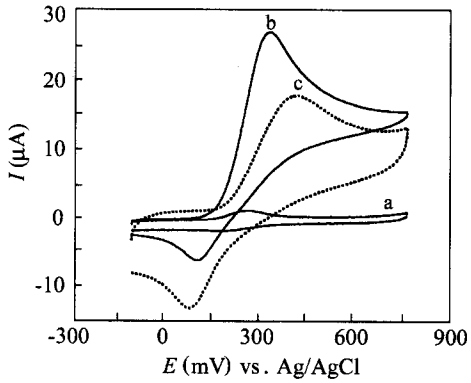

Fig. 3 Cyclic voltammograms of DA in 0.1 mol/L KCl and 0.1 mol/L phosphate buffer (pH 6.7). Scan rate 50 mV /s; (a) no DA at a ferrocene-DMPG GC electrode, (b) 6.25×10^{-4} mol/L DA at a ferrocene-DMPG GC electrode, (c) 6.25×10^{-4} mol/L DA at a bare GC electrode.

Influence of pH *on voltammetry*

Fig. 4 shows the cyclic voltammograms of DA at a ferrocene-DPPC film electrode at different pH. It is known that DA contains amine groups and is positively charged at neutral pH (pK = 8.87).[8] It means that the

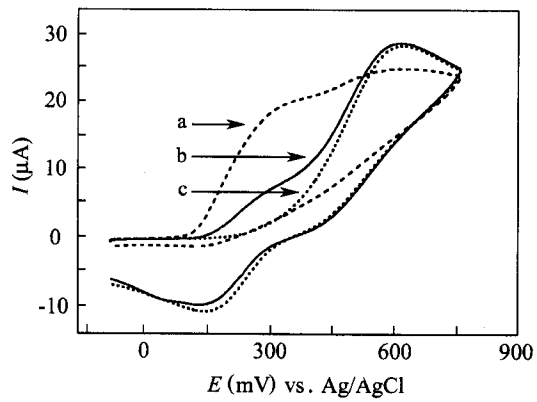

Fig. 4 Cyclic voltammograms of 1.43×10^{-3} mol/L DA in glycine buffer solution at different pH. Scan rate 50 mV/s at a ferrocene-DMPC GC electrode; (a) pH 8.0, (b) pH 7.0 (phosphate), (c) pH 5.0.

degree of positive charge depends on the pH of the solution and further the pH influences the diffusion of DA around the multiple bilayers of lipid. For DA, it is known to be oxidized via a two-electron process,[20] the reaction sequence can be written as

$$QH_2 = R + H^+ + e = Q + 2H^+ + 2e$$

(QH_2, Q and R represent DA, the oxidized forms and intermediates of DA, respectively.)

The electrochemical oxidation of DA includes the proton and electron transfers at GC electrode,[18] so the oxidation of DA depends on the solution of pH. Thus it could be expected that the catalytic efficiency of DA also depended on the pH of the solution. We observed that at pH 8.0 the electrocatalytic oxidation of DA is the most effective, as the anodic peak potential is lower than that at pH 7.0 or 5.0 and the cathodic peak almost could not be observed.

Influence of the types of lipid on voltammetry

The influence of different types of lipid has also been investigated. Fig. 5 shows cyclic voltammograms recorded for 6.25×10^{-4} mol/L DA in a 0.1 mol/L phosphate buffer solution (pH 6.7) using the lipid modified electrode. Lipid is amphipathic with a polar headgroup and two long nonpolar hydrocarbon chains. The polar headgroups of DPPC, DMPG and DODAB are expected to have neutral, negative and positive charges respectively, under the experimental conditions examined. By taking these natures of the polar headgroups into account, the following two factors can be accounted for by the effect of the polar headgroups on the electrocatalytic oxidation process of DA in solution: (a) the change of the concentration of DA at the electrode surface[21] and (b) the influence of electrocatalytic oxidation of DA.

The contributions of the above two factors on the electrode process at the modified electrode can be explained as follows: (a) The charge of the polar headgroups will also affect the distribution of cationic DA in the vicinity of the electrode surface. The negatively charged surface will attract the DA and the opposite effect will be expected at the positively charged electrode surface.[22](b) It was presumed that the negatively charged polar headgroups would attract some cationic DA and ferricinium ions in lipid film, so that it makes electron transfer between DA and ferricinium ions easy and anodic peak potential shift negatively. It will also be presumed that the positively charged polar headgroup causes the opposite effect.

It is observed that the electrochemical responses change depending on the charged headgroup of lipid from

Fig. 5. The first kind was DPPC modified electrode, which shows an electrocatalytic oxidation of DA (Fig. 5b). The second type is DODAB modified electrode, which does not show effective elecctrocatalytic oxidation of DA as DPPC in Fig. 5c. The polar headgroup of DODAB is positively charged, so the catalytic current decreases and anodic peak potential turns to positive direction. The third type is DMPG modified electrode. Its cyclic voltammograms is given in Fig. 5a. The catalytic current increases drastically and the anodic potential shifts negativly at 330 mV. From these results, it could be inferred that the negatively charged polar headgroups enhances the effect of electrocatalytic oxidation of DA at the electrode.

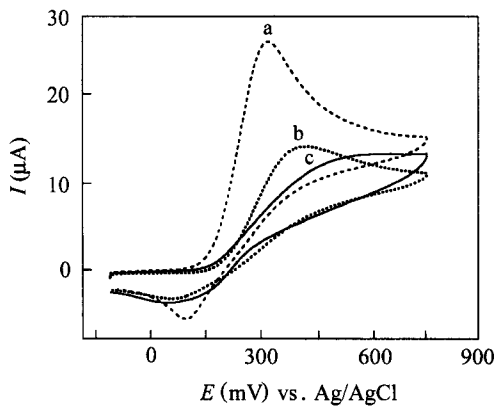

Fig. 5 Cyclic voltammograms of 6.25×10^{-4} mol/L DA in 0.1 mol/L KCl and 0.1 mol/L phosphate buffer (pH 6.7). Scan rate 50 mV/s at a ferrocene-lipid GC electrode, lipid: (a) DMPG, (b) DPPC, (c) DODAB.

Thus, from the cyclic voltammogram measurement of the lipid with different charge headgroups, we can conclude that the charge headgroup of lipid influences the concentration of DA at the electrode surface and further influences the effect of electrocatalytic oxidation of DA.

Determination of DA *in the presence of* AA

The separation of electrochemical response of DA and AA at the ferrocene-DPPC electrode was successfully achieved by differential pulse voltammetry as shown in Fig. 6. The electrocatalytic behavior of ferrocene-DPPC modified electrode toward AA has been investigated. The result indicated that ferrocene could catalyze AA efficiently in the DPPC film (figure not shown). It was observed that at pH 8.0 the electrocatalytic oxidation of DA is the most effective, and the anodic peak potential is lower than that at pH 5.0 and the cathodic peak almost could not be observed. So DA can be determined in the presence of 1×10^{-3} mol/L AA in 0.1 mol/L NaCl and 0.1 mol/L glycine buffer (pH 5.0). The differential pulse voltammetry peak currents for DA is proportional to its concentration between 1×10^{-4} and 3×10^{-3} mol/L.

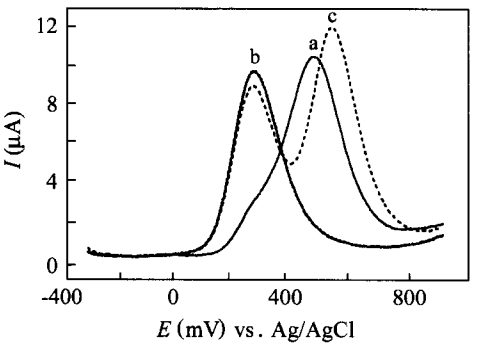

Fig. 6 Differential pulse voltammograms of DA and AA in 0.1 mol/L NaCl and 0.1 mol/L glycine buffer (pH 5.0). Scan rate 10 mV/s, pulse height 50 mV, pulse width 40 ms. (a) 1.43×10^{-3} mol/L DA (b) 1.43×10^{-3} mol/L AA (c) 1.25×10^{-3} mol/L DA and 1.25×10^{-3} mol/L AA.

Conclusion

In this article, a ferrocene-lipid film electrode was performed to investigate the oxidation of DA. It was observed that the effect of electrocatalytic oxidation of DA depended on the solution pH. The electrocatalysis of DA is effective in the high pH solution. And also we found that the negatively charged polar headgroup of lipid was in favor of electrocatalytic oxidation of DA. These characters can be applied for analytical purpose and biological study. Finally the electrode was used to determine DA in the presence of AA.

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