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Multi-step reduction of nitric oxide by cytochrome *c* entrapped in phosphatidylcholine films

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Abstract

By entrapping cytochrome *c* (Cyt *c*) in phosphatidylcholine (PC) film, we have obtained the direct electrochemistry of the protein. Meanwhile, the catalytic reduction of nitric oxide (NO) has been investigated. Besides the pair of peaks corresponding to the redox reactions of Cyt *c*, two new cathodic peaks can be observed after the addition of NO into the test solution. One, located at −0.510 V, is proposed to come from the formation of hydroxylamine. The other, at −0.690 V, is assigned to the electrochemical reduction of NO. These observations are very different from the previous reports, because only one peak can be obtained for the other studies. The difference is related to the electron transfer rate and the escape rate of the products in the NO reduction process. This study might bring clearer insight into the reduction mechanism of NO.

Keywords: Cytochrome *c*; Electrochemistry; Nitric oxide; Phosphatidylcholine; Catalytic reduction

1. Introduction

Nitric oxide (NO) is a molecule that has drawn a lot of attention in the last decade. It is an endogenously free radical synthesized from arginine by nitric oxide synthase. It plays an important role in many physiological processes [1]. Since it was proved to be the endothelium-derived relaxing factor (EDRF) in the cardiovascular system [2,3], the study on NO has been the focus of many scientists' interest. And lots of studies are based on the catalytic reduction by proteins or enzymes [4–10]. However, the mechanism of the NO reduction process is still unclear till now.

Electrochemical method, such as protein film voltammetry [11–15], is an efficient method for the characterization of electron-transfer process. This method can be sensitive to the amperometric response of NO reduction [16–26]. Some researchers have reported the different products for the NO reduction process [16,23]. In the meantime, Mimica et al. [27] have reported two NO reduction peaks (located at −0.6 and −1.0 V) in the electrocatalytic process by hemoglobin entrapped in surfactant films. But in this report the second peak is contributed to the reduction of Fe in the nitroxyl adducts.

Phosphatidylcholine (PC) has been a good membrane material for embedding proteins [5,28]. It is a component of a biological membrane, while can provide a mimic environment for the functioning of proteins and enzymes [11,29,30]. So, we have used PC in this work to embed cytochrome *c* (Cyt *c*) and to study the electrocatalytic activity of the protein towards NO. Interestingly, two reduction peaks rather than one can be observed. The reduction process has been discussed accordingly. This study might provide a new and clearer insight into the mechanism of NO reduction process.

2. Experimental

2.1. Materials

Cyt *c* was obtained from Sigma and used as received. PC was obtained from the Chemical Plant of Huadong Normal University in Shanghai (China). Other reagents were of ana-

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lytical grade. Water was purified with a Milli-Q purification system and was used to prepare all solutions. The saturated solution of 2.0 mmol/L NO was prepared according to the previous report [31].

2.2. Electrochemical experiments

Electrochemical experiments were carried out with a PAR 283 Potentiostat/Galvanostat (EG&G, USA). A three-electrode system was employed with a modified pyrolytic graphite (PG) working electrode, a saturated calomel reference electrode (SCE) and a platinum wire auxiliary electrode. All the potentials reported in this paper are versus SCE. The PC vesicle dispersion was prepared by ultrasonicating a 1.0 mmol/L PC suspension in water for at least 2 h until it became clear. The Cyt *c*/PC modified PG electrode was made as following procedures. The substrate PG electrode was first polished using rough and fine aluminum oxide papers. Then it was polished to mirror smoothness with an aluminum oxide (particle size of about 0.05 μm)/water slurry on silk. Finally, the electrode was thoroughly washed with doubly distilled water and treated in an ultrasonic water bath for five minutes. A mixture of 10 μL 0.1 mmol/L Cyt *c* and 10 μL 1.0 mmol/L PC was spread on the PG electrode surface. The film was then dried overnight at room temperature. The modified electrode was thoroughly rinsed with nanopure water and was then ready for use. Electrochemical measurements were carried out under an anaerobic condition. The test solution was first bubbled thoroughly with high purity nitrogen for at least 10 min. Then a stream of nitrogen was blown gently across the surface of the solution in order to maintain the anaerobic condition throughout the experiments.

3. Results and discussion

As is well known, Cyt *c*, which acts as an electron shuttle in the respiratory chain, displays a slow electron transfer rate at an electrode surface. Therefore, numerous efforts have been made to enhance the electron transfer reactivity of the protein [32–38]. In this work, we have used protein film voltammetry technique to facilitate the electron transfer between Cyt *c* and the electrode. Fig. 1 (solid line) is the cyclic voltammograms (CVs) of Cyt *c* incorporated in a PC membrane. A pair of redox peaks can be observed attributing to the redox reaction of Cyt *c*. Alternatively, if an electrode is coated with PC alone, no peak occurs in the potential range of interest (Fig. 1 dot line). No corresponding peak can be observed either with the bare PG electrode (Fig. 1 dash line). These results clearly demonstrate that Cyt *c* can take redox reaction after being entrapped in PC membrane. The anodic and cathodic peaks are located at −0.170 and −0.305 V, respectively. And the peak currents are proportional to scan rate in the range from 50 to 1000 mV/s (Fig. 2), which implicates a thin-layer electrochemical behavior.

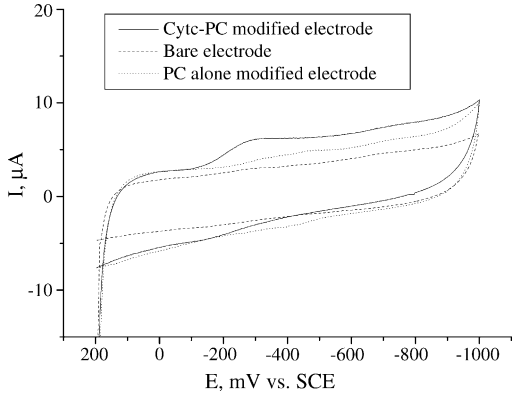

Fig. 1. Cyclic voltammograms obtained at (i) Cyt *c*/PC co-modified PG electrode; (ii) bare PG electrode; (iii) PC alone modified PG electrode in a pH 4.0 buffer solution.

Compared with the cathodic peak, the anodic peak of Cyt *c* is not obvious (Figs. 1 and 2), which suggests that the ferrous Cyt *c* (reduced form of Cyt *c*) on the electrode surface is only partially converted to ferric Cyt *c* (oxidized form of Cyt *c*). So, in the second cycle, the cathodic peak decreases remarkably (Fig. 3). These results clearly demonstrate that the electron transfer between Cyt *c* and electrode is not quick enough.

When NO is added to the test solution, besides the redox peaks of Cyt *c*, two new cathodic peaks appear. These two peaks are located at about −0.510 and −0.690 V, respectively (Fig. 4). It should be mentioned that the catalytic peak of NO can also be observed in the previous reports, but there is only one cathodic peak when NO is catalytically reduced by Cyt *c* [4,29]. So, the reductive process of NO in this system should be different from the previous models. We propose that these two peaks are related to two different products, which are formed via the catalytic reduction of NO by Cyt *c*.

Metalloporphyrins, phthalocyanines, schiff bases and related complexes have been reported in previous researches as electro-catalysts for NO reduction [16–27]. It has been proposed that NO first binds to the ferrous heme to form

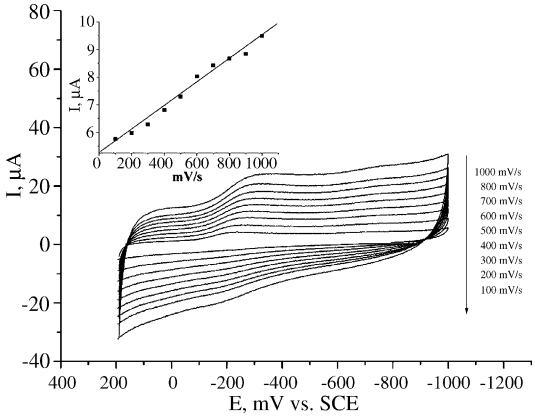

Fig. 2. Cyclic voltammograms obtained on the Cyt *c*/PC co-modified PG electrode in the pH 4.0 buffer solution with different scan rate. Inset is the relation of cathodic peak current and scan rate of Cyt *c*/PC modified electrode.