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Direct electrochemical behavior of cytochrome *c* on DNA modified glassy carbon electrode and its application to nitric oxide biosensor

Yun-Chun Liu, Jun Zhao, Wei-Li Wu, Zhou-Sheng Yang *

College of Chemistry and Materials Science, Anhui Key Laboratory of Functional Molecular Solids, Anhui Normal University, Wuhu 241000, PR China

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Abstract

Cytochrome *c*/DNA modified electrode was achieved by coating calf thymus DNA onto the surface of glassy carbon electrode firstly, then immobilizing cytochrome *c* on it by multi-cyclic voltammetric method and characterized by the electrochemical impedance. The electrochemical behavior of cytochrome *c* on DNA modified electrode was explored and showed a quasi-reversible electrochemical redox behavior with a formal potential of 0.045 ± 0.010 V (*versus* Ag/AgCl) in 0.10 M, pH 5.0, acetate buffer solution. The peak currents were linearly with the scan rate in the range of 20–200 mV/s. Cytochrome *c*/DNA modified electrode exhibited elegant catalytic activity for the electrochemical reduction of NO. The catalytic current is linear to the nitric oxide concentration in the range of 6.0×10^{-7} to 8.0×10^{-6} M and the detection limit was 1.0×10^{-7} M (three times the ratio of signal to noise, S/N = 3).

Keywords: Cytochrome *c*; DNA; Electrochemical behavior; Nitric oxide; Biosensor

1. Introduction

Direct electron transfer between a protein (the redox active group) and an electrode can serve as a model system to help understand electron transfer mechanisms in biological system. Cytochrome *c* is an important stable heme protein containing covalently bound heme. At the neutral pH, cytochrome *c* has a positively charge of +7/+8, and usually shows a short-lived and transient response on a metal surface [1,2]. Since the direct electron transfer of cytochrome *c* was first observed [3,4], the idea of an electrode's surface having a specific adsorption interaction that can be modified to enhance the electron transfer rate of cytochrome *c* has been proposed [5–8]. McNeil and co-workers have developed a superoxide sensor based on cytochrome *c* immobilized on short-chain thiol modified gold electrode. The sensor signal is proportional to the real $O_2^{\bullet -}$ concentration that fits well with the mathematical model *in vitro* studies [9]. Li and co-workers used phosphatidycholine (PC) to embed cytochrome *c* and to study the electro-catalytic activity of protein towards NO, found the modified electrode might offer an alternative to investigate enzyme biomimetic towards NO [10]. Oh et al. adopted two different methods to fabricate micro-pattern of self-assembled cytochrome *c* monolayer. The bioelectrochemical activity between cytochrome *c* molecular center and electrode interface for the self-assembled cytochrome *c* monolayer was investigated through the measurement of cyclic voltammetry [11]. Paolo et al. examined the incorporation and electrochemical behavior of cytochrome *c* at glassy carbon electrode modified with the polyestersulfonated ionomer Eastman AQ 55, and they found the modified electrode with incorporating cytochrome *c*, displayed electrocatalytic properties with respect to anionic substrates, such as $Fe[(CN)_6]^{3-}$ and acorbate, but does not with respect to a possible cationic substrate such as FA^{2+} [12].

It is recognized that DNA plays a determinative role in biological information transfer. Immobilized DNA on the surface of a substrate electrode can act as promoter, for example, they are applied to the direct electron transfer of heme protein (myoglobin) [13,14], metabolites generated *in situ* [15] and

* Corresponding author. Tel.: +86 553 3869303; fax: +86 553 3869303.
E-mail address: yzhoushe@mail.ahnu.edu.cn (Z.-S. Yang).

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horseradish peroxidase [16] on pyrolytic graphite electrodes. DNA can easily be immobilized with polycation [17]. Polyanionic DNA and polycation can make a water-insoluble polyion complex membrane.

In this paper, the glassy carbon electrode was modified with DNA molecules by adsorption action firstly, then, cytochrome *c* was immobilized to DNA modified electrode by multi-cyclic voltammetric method. The direct electrochemical behavior of cytochrome *c* on DNA modified electrode was achieved and their relative electrochemical parameters were obtained. Cytochrome *c*/DNA modified electrode exhibited elegant catalytic action for electrochemical reduction of NO, which could be applied as a biosensor to determine concentration of nitric oxide.

2. Experimental

2.1. Apparatus and chemicals

Horse heart cytochrome *c* (Cyt. *c*) was purchased from Roche (No. 103870) and deoxyribonucleic acid from calf thymus (DNA) was purchased from Sigma (Cat. No. D1501), they were used as received. NO solution was prepared as reference [18] typically containing NO ≈ 2.0 mM. Other chemicals were of analytical grade. Acetate buffer solutions were prepared by 0.10 M NaAc-HAc (pH 5.0), and the pH was adjusted with 0.10 M HAc and 0.10 M NaOH. All aqueous solutions were prepared with doubly distilled water.

Electrochemical experiments were performed with a CHI440a electrochemical analyzer (Shanghai Chenhua Apparatus, China) with conventional three-electrode cells. The working electrode was a Cyt. *c*/DNA modified glassy carbon electrode ($\Phi = 2$ mm). The reference electrode was an Ag/AgCl (saturated KCl) electrode and platinum electrode was used as the auxiliary electrode. Prior to each experiment, solutions were purged with purified nitrogen for 15 min to remove oxygen; all the measurements were performed at room temperature unless otherwise specified.

2.2. Electrode modification

The glassy carbon electrode was polished using a piece of 1500 diamond paper, followed by 0.2 μm alumina on chamois leather, and washed with absolute alcohol and doubly distilled water in an ultrasonic bath. Following that, 20 μL DNA solution (0.10 mg/mL) was dropped onto the surface of the clean glassy carbon electrode and solvent was evaporated at 4 °C for 12 h. By this means, a layer of DNA film was bound to the surface of the glassy carbon electrode.

Cyt. *c*/DNA modified electrode was prepared by following steps: Cyt. *c* was dissolved in 0.1 M (pH 5.0) acetate buffer solution and the above-modified glassy carbon electrode was transferred into Cyt. *c* solution. A successive cyclic scan was performed in the potential range from +0.3 to −0.2 V (*versus* Ag/AgCl) up to obtaining a stable voltammogram. Then, the electrode was removed from the solution, washed with double-distilled water gently and stored at about 4 °C until the solvent was dried.

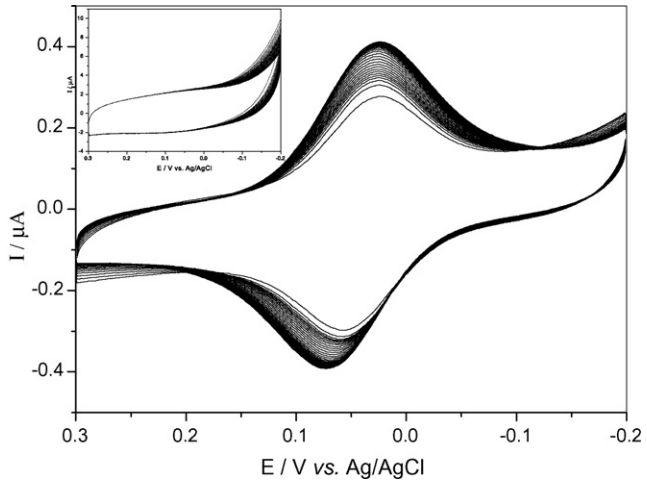

Fig. 1. Multi-cyclic voltammograms of a DNA modified electrode in 0.10 M acetate buffer (pH 5.0) containing 5.0×10^{-5} mol/L Cyt. *c*. Scan rate, 50 mV/s. *Inset*: Multi-cyclic voltammograms of bare carbon electrode under the same conditions.

3. Results and discussion

3.1. The adsorption of cytochrome c on DNA modified glassy carbon electrode

Fig. 1 shows the cyclic voltammograms of Cyt. *c* on DNA modified glassy carbon electrode in 0.10 M acetate buffer (pH 5.0) containing 5.0×10^{-5} mol/L Cyt. *c* under the condition of consecutive scans. A couple of a well-defined redox waves could be observed on cyclic voltammograms, the cathodic and anodic peak appear at potential of near +0.024 and +0.057 V, respectively. The more the cycles that the modified electrode swept in the Cyt. *c* solution was, the higher the redox peak was, demonstrating that Cyt. *c* could be adsorbed onto the surface of DNA modified glassy carbon electrode. When the cycles were above 40 circles, no obvious changes of peak current could be observed from the cyclic voltammogramms, indicating the adsorption of Cyt. *c* reached a saturated state [19].

The contrast experiment was performed with bare glassy carbon electrode under the same experimental conditions above mentioned. There were no redox peaks of Cyt. *c* occurring on bare glassy carbon electrode (Fig. 1, inset). It is difficult for Cyt. *c* to exhibit well direct electrochemical activeness on electrode because Fe(III)/Fe(II) redox center of Cyt. *c* could not contact with surface of electrode. Therefore, Cyt. *c* has no electrochemical behavior on bare glassy carbon electrode. However, DNA modified on surface of glassy carbon electrode can play a promoter role for the electron transfer reaction of Cyt. *c* on electrode. DNA could provide negatively charged sites and interact with the hydrophilic surface of Cyt. *c*, which has the positive charges. Therefore, Cyt. *c* could be absorbed to surface of the DNA modified glassy carbon electrode and possibly well arranged in proper orientation. Its Fe(III)/Fe(II) redox center of Cyt. *c* could undergoes direct electron transfer on surface of DNA modified electrode and improved electron transfer reaction [20].