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Direct Electrochemistry of Cytochrome C on the Glassy Carbon Electrode Modified with 1-Pyrenebutyric Acid/MWNTs

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Abstract: With 1-Pyrenebutyric acid (PBA) and multiwalled carbon nanotubes (MWNTs), glassy carbon electrode modified was successfully prepared. In phosphate buffer solution (pH 7.0), the direct electrochemistry of cytochrome C (Cyt C) was realized. In the cyclic voltammetry experiment two pairs of redox peaks of Cyt C were observed at 0.018 V and –0.314 V (*vs.* SCE), respectively. The redox reaction at 0.018 V was diffusion controlled, while the redox reaction at –0.314 V was adsorption controlled.

Keywords: Cytochrome C, carbon nanotubes, 1-pyrenebutyric acid, modified electrode.

The electrochemical study based on carbon nanotubes (CNTs) electrode has been widely carried out[1-3]. Direct electrochemistry of macro biomolecules including proteins, enzymes, and DNA on CNTs electrode has been demonstrated recently[3]. The sensor based on CNTs with high sensitivity also has attracted more attentions [3].

Cytochrome C (Cyt C) is an important electron transfer protein responsible for shuttling electron from cytochrome reductase to cytochrome oxidase. As a model for bioelectrochemical redox reaction system, the direct electrochemistry of Cyt C has been largely reported[4-5]. It has been demonstrated that electrostatic interaction between Cyt C and the electrode surface plays a key role in the redox reaction of Cyt C because in neutral condition the groups near the active redox center of Cyt C keep positive charge and the molecule Cyt C has positive charge[4,5]. However, by now only several papers reported the electrochemistry of Cyt C on CNTs electrode[6-9]. CNTs with carboxyl groups introduced by treating with nitric acid[6,9] or electrochemical activation[7] can promote the direct electron transfer of Cyt C. In this paper, 1-pyrenebutyric acid (PBA) and multiwalled carbon nanotubes (MWNTs) modified glassy carbon (GC) electrode was successfully prepared (denoted PBA/MWNTs/GC electrode), and carboxyl group was introduced on the sidewalls of the CNTs through noncovalent π-π stacking interaction[10,11]. The direct electrochemistry of Cyt C was realized on the modified electrode.

MWNTs were produced by catalytic chemical vapor deposition (CCVD) method, and the details of synthesis were reported elsewhere[12]. Horse heart Cyt C was purchased from Merck. Water was triply distilled with a quartz apparatus. Highly

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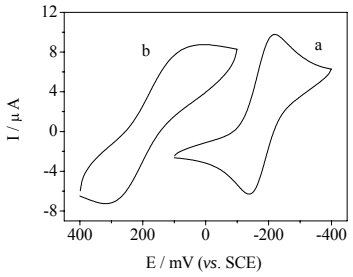
purity nitrogen was used for deaeration. All other reagents were of analytical grade. A BAS 100W (Bioanalytical Systems, Inc.) electrochemical workstation with a three-electrode cell was employed to perform cyclic voltammetry (CV). The working electrode was a GC electrode or a modified GC electrode, the auxiliary and reference electrodes were platinum wire and saturated calomel electrode (SCE), respectively. All experiments were carried out at room temperature (20 ± 1 ^{o}C).

The MWNTs modified GC electrode (called MWNTs/GC) was prepared as follows: 2 mg MWNTs was dispersed in 10 mL ethanol with the aid of sonication to give a black dispersion. The GC electrode was polished on the emery paper (No.1500), then polished alumina slurry, and then was washed ultrasonically in triply distilled water and ethanol, respectively. 15 μL of suspension of MWNTs was dropped on the GC electrode, and then was dried in air. The PBA modified electrode (called PBA/GC electrode) was prepared by casting 15 μL PBA solution in ethanol (2 mg/mL) on the polished GC electrode, and dried in air. The PBA/MWNTs/GC electrode was fabricated by casting 15 μL of MWNTs suspension in ethanol (0.2 mg/mL) in which PBA had been dissolved (2 mg/mL), and dried in air. PBA was adsorbed irreversibly on the sidewalls of the CNTs[10,11].

Figure 1 Cyclic voltammograms for the PBA/MWNTs/GC electrode



(a) 1 mmol/L $Ru(NH_3)_6Cl_3$+ 0.1 mol/L KCl solution and (b) 1 mmol/L $K_3Fe(CN)_6$ + 0.1 mol/L KCl solution. Scan rate: 50 mV/s.

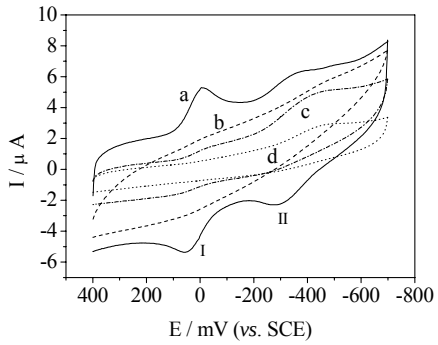
The PBA/MWNTs/GC electrode was examined in 1 mmol/L $Ru(NH_3)_6Cl_3$+ 0.1 mol/L KCl, and a pair of well defined redox peaks were observed. The result was shown in **Figure 1a**. However, in 1 mmol/L $K_3Fe(CN)_6$ + 0.1 mol/L KCl solution the electrode showed poor electrochemical behavior with larger peak separation (see **Figure 1b**). In contrast, on the MWNTs/GC electrode both $K_3Fe(CN)_6$ and $Ru(NH_3)_6Cl_3$ gave nearly electrochemical reversible redox peaks, which was in agreement with the nice electrochemical properties of CNTs[1-3]. The different electrochemical responses of $Ru(NH_3)_6Cl_3$ and $K_3Fe(CN)_6$ on PBA/MWNTs/GC electrode was attributed to adsorbed PBA on the CNTs[10,11]. PBA had negative charge in near neutral solution, so it repulsed $Fe(CN)_6^{3-}$ and attracted $Ru(NH_3)_6^{3+}$ by electrostatic interaction.

Figure 2 showed the cyclic voltammograms of Cyt C in 0.1 mol/L phosphate buffer solution (pH 7.0). On the PBA/MWNTs/GC electrode，a good pair of peaks of Cyt C was given at 0.018 V (peak I) and another pair of peaks was observed at –0.314 V (peak II) (see **Figure 2a**). In the blank phosphate buffer solution (pH 7.0), no peaks were

observed. The peak currents of peak I increased linearly with the square root of the scan rate. This showed that the electrochemical reaction of peak I was diffusion controlled. The diffusion coefficient was calculated to be 1.9×10^{-7} cm^2/s[14]. The separation of peak I was 64 mV at 50 mV/s. This indicated that the redox reaction was nearly reversible. The apparent standard rate constant was determined to be 6.6×10^{-3} cm/s according to Nicholson theory[14]. While the peak currents of peak II were proportional to the scan rate, which showed the reaction was controlled by adsorption. When the PBA/MWNTs/GC electrode that had been measured in the Cyt C solution was removed from the solution, washed in the blank phosphate buffer, and then the CV was recorded again in the blank phosphate buffer solution. The peak I disappeared, while the pair of peaks at –0.314 V was still observed, which also proved that the peak II corresponded to the adsorbed Cyt C redox reaction. The potential of peak I was in agreement with the reported data (+0.258 V *vs*. NHE, *i.e.* +0.017 V *vs.* SCE)[15]. The potential of peak II was more negative than the potential of native Cyt C in solution, but close to the reported value of adsorbed Cyt C[16]. The potential change might be resulted from the conformation change of the Cyt C due to its adsorption on the electrode surface.

In the 0.5 mmol/L Cyt C + 0.1 mol/L phosphate buffer solution, no peaks were observed on the PBA/GC electrode (see **Figure 2b**). But on the MWNTs/GC electrode, a weak pair of peaks at about 0.04V with a 170 mV peak separation and a pair of peaks with larger reduction peak at about –0.3 V were given. The result was shown in **Figure 2c**. On the bare GC electrode only an irreversible reduction peak was given in the 0.5 mmol/L Cyt C + 0.1 mol/L phosphate buffer solution (see **Figure 2d**). These showed that the CNTs without carboxylic group cannot facilitates the direct electrochemistry of Cyt C very well compared with PBA/MWNTs/GC electrode. The PBA modified GC electrode was not effective in the direct electrochemistry of Cyt C (see **Figure 2b**). It is obvious that the carboxylic group plays an important part in the realization of the direct electron transfer between the Cyt C and the electrode because molecule Cyt C has overall positive charge and kept positive charged near the electroactive center at pH 7 [4,5]. The favorable electrochemical properties of CNTs are also crucial.

Figure 2 Cyclic voltammograms in 0.5 mmol/L Cyt C + 0.1 mol/L phosphate buffer (pH 7.0)



(a) PBA/MWNTs/GC electrode, (b) PBA/GC electrode, (c) MWNTs/GC electrode, and (d) bare GC electrode. Scan rate: 50 mV/s.

In conclusion, we have reported the direct electrochemistry of Cyt C on the PBA and MWNTs modified GC electrode. The modified electrode might be used as a biosensor.

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