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A Reagentless Biosensor of Oxygen Based on the Direct Electrochemistry of Cytochrome c on the Surface of Multiwalled Carbon Nanotubes

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Direct electrochemical response of cytochrome c (Cyt c), which was adsorbed onto the surface of multiwalled carbon nanotubes (MWNTs) through a simple cyclic potential scan technique, was observed. Cyt c adsorbed on MWNTs showed an enzyme-like activity to catalyze the reduction of oxygen (O_2). Thus, a third generation O_2 reagentless biosensor was constructed.

Heme proteins are important proteins in living systems, which perform different physiological functions. Recent years, direct electrochemistry of metalloproteins and metalloenzymes arouse many scientists' interest because of its potential application in the study of the redox and electron-transfer properties of biomolecules,[1] and in fabricating mediator-free or the third generation biosensors.[2] Cytochrome c (Cyt c) is a biologically important redox protein that is involved in electron-transfer reactions in the mitochondrial respiratory chain. In electrochemistry, Cyt c is also an ideal model protein for the study of heme proteins or enzymes because of its commercial availability and a known structure. However, it is generally difficult for Cyt c to transfer electron with a conventional electrode. So, various modified electrodes were proposed to investigate its direct electrochemistry,[3–5] including carbon nanotube-modified electrode.[6,7] Carbon nanotube, a kind of inorganic material with a nanostructure, is an ideal electrode material owing to its high surface area, excellent electrical conductivity, significant mechanical strength, and good chemical stability.[8] The subtle electronic properties indicate that it can promote the electron-transfer reactions of biomolecules when used as an electrode material. In this letter, Cyt c was adsorbed on the surface of multiwalled carbon nanotubes (MWNTs) and the direct electron transfer between Cyt c and MWNTs modified electrode was observed. Further investigation suggested that Cyt c adsorbed on MWNTs still showed a bioactivity to catalyze the reduction of oxygen (O_2). Thus, a novel reagentless biosensor of oxygen can be constructed.

The Cyt c-modified electrode can be prepared as following. The glassy carbon electrode (3 mm in diameter) was polished sequentially with slurries of 0.3 and 0.05-μm alumina. Then, the electrode was washed with double distilled water and ethanol in an ultrasonic bath. 15 cyclic scans were carried out in the potential range of 2.0 to −2.0 V (vs SCE) in the solution of 1.0 mol/L H_2SO_4. The MWNTs, which were synthesized by catalytic decomposition of hydrocarbons using a floating catalyst, were provided by Chengdu Institute of Organic Chemistry of Academy of Sciences and the purity is more than 95%. Before used, they were further purified by a mixed acid according to the previous report.[9] 2.5 mg of acid treated MWNTs was dispersed in 10 mL of acetone to form a black solution. 25 μL of MWNTs solution was cast on the surface of GC electrode, and then the solvent acetone was evaporated in air to form a MWNTs-modified electrode. The effective area of the electrode, calculated from the cyclic voltammogram of 1 mM $K_3[Fe(CN)_6]$, is about 0.30 cm^2. Cyt c was directly dissolved in pH 7.0 phosphate buffer. The MWNTs electrode was placed into a Cyt c solution (0.09 mmol/L, pH 7.0 PBS) and a consecutive cyclic potential scans was performed in the potential range from 0.3 V to −0.8 V with a scan rate 50 mV/s up to obtain a stable CV curve. Then, the electrode was removed from the solution, washed with double-distilled water and stored in pH 7.0 PBS. The electrode was noted as Cyt c/MWNTs electrode.

When the MWNTs-modified electrode was placed in Cyt c solution and continuous cyclic scans were carried out, two couple redox peaks could be observed. One couple peaks at about 0 V, the peak currents decrease slightly with continuous cyclic scans, is corresponding to the redox of carboxylic groups on the surface of acid-treated MWNTs.[9] Another one at near −0.32 V, which peak currents increase with scanning, should be corresponding to the redox of Cyt c. The more the cycles that the modified electrode swept in the Cyt c solution was, the higher the redox peaks was, demonstrating that Cyt c could adsorb onto the surface of MWNTs. When the cycle was above 70 circles, no obvious changes of peak currents could be observed from the cyclic voltammograms, indicating that the adsorption of Cyt c on MWNTs reached a saturated state.

As shown in Figure 1, a couple of quasireversible redox peaks can be observed when the Cyt c/MWNTs electrode was

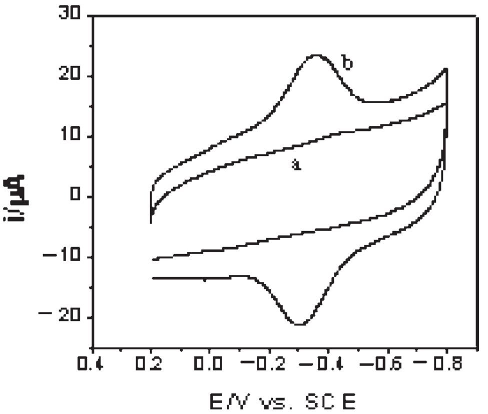

Figure 1. Cyclic voltammograms of Cyt c/MWNTs-modified electrode in 0.1 mol/L PBS (pH 7.0). (a) MWNTs-modified electrode, (b) Cyt c/MWNTs-modified electrode. Scan rate: 50 mV/s.

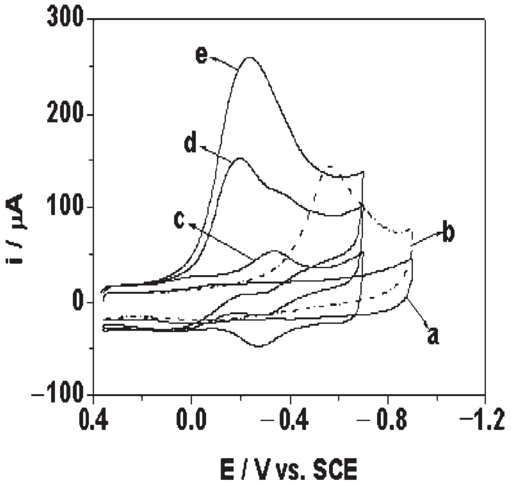

Figure 2. Cyclic voltammograms obtained at MWNTs electrode (a, b) and Cyt c/MWNTs electrode (c, d, e) in 0.1 mol/L phosphate buffer (pH 7.0) solution. The concentration of oxygen: (a) 0 mM, (b) 1.2 mM, (c) 0 mM, (d) 0.6 mM, (e) 1.2 mM. Scan rate: 200 mV/s.

transferred into a blank phosphate buffer solution. The anodic and cathodic peak potentials were located at −303 and −365 mV, respectively. The heights of the oxidative and reductive peaks were nearly equal, and the shapes of the redox peaks were symmetric, indicating that all electroactive Cyt c Fe(III) on the surface MWNTs is converted to Cyt c Fe(II) on the forward cathodic scan, and the reduced protein in their Cyt c Fe(II) forms are fully oxidized back to the Cyt c Fe(III) forms on the reversed anodic scan.

The effect of scan rate on peak current was investigated. The results showed that cathodic and anodic peak currents increased linearly with scan rates in the range of 20 to 800 mV/s. Integration of reduction peaks gave approximately constant charge (Q) values with different scan rates. According to the equation of $I_p = n^2F^2A\Gamma^*\nu/4RT$, an average surface concentration (Γ^*) of electroactive Cyt c adsorbed on MWNTs could be evaluated. In our experiments, it is about $(4.1 \pm 0.3) \times 10^{-10}$ mol/cm^2, showing an approximate monolayer adsorption. According to Laviron's equation,[10] the heterogeneous electron-transfer rate constant (K_s) was calculated to be $4.0 \pm 0.2\,\mathrm{s}^{-1}$, suggesting a fast electron-transfer kinetics process.

Cyt c/MWNTs-modified electrode showed a good electrocatalytic activity for the reduction of oxygen, as shown in Figure 2. In experiments, the saturated oxygen solution and various concentrations of oxygen solution were prepared according to previous literature.[10] In a pH 7.0 buffer solution, at a bare glassy carbon electrode, no obvious electrochemical response for oxygen could be observed in the potential range of 0.4–−0.8 V. At an MWNT-modified electrode, the reduction peak potential of oxygen appeared at about −0.53 V (as shown in Figure 2b). However, at a Cyt c/MWNTs electrode, the reduction peak potential of oxygen moved up to −0.25 V and the peak current increased obviously. Figures 2d and 2e are the cyclic voltammograms of Cyt c/MWNTs-modified electrode in pH 7.0 phosphate buffer solution containing different oxygen concentrations. Compared with MWNT-modified electrode, Cyt c/MWNTs-modified electrode decreased the reduction overpotential of O_2 about 280 mV.

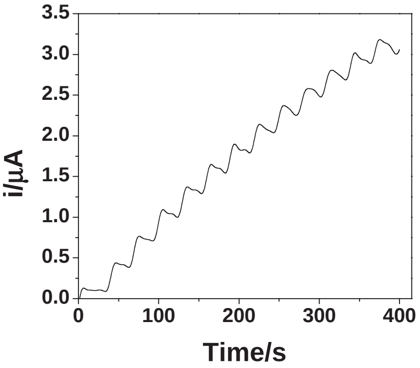

Figure 3. Amperometric response of Cyt c/MWNT electrode to oxygen in 0.1 M, pH 7.0 PBS. Conditions: constant potential of −0.29 V, each addition of 1.5 µM O_2.

In order to test the response of Cyt c/MWNTs electrode for oxygen, the amperometric experiments were carried out and the results were shown in Figure 3. The current signal of the Cyt c/MWNTs electrode was proportional to the O_2 concentration from 0 to 18 µM. The stability of the electrode was also tested. When the electrode was stored in pH 7.0 phosphate buffer solutions at 4 °C for 40 days, the CV peak currents and potentials were essentially stable. Even if the electrode was dried and stored in air, its CV peak only slightly decreased during one week.

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References

1 F. A. Armstrong, "Probing Metalloproteins by Voltammetry," Struct Bonging, Springer, Berlin (1990), Vol. 72, pp 137–230.
2 L. Gorton, A. Lindgren, T. Larsson, F. D. Munteanu, T. Ruzgas, and I. Gazaryan, *Anal. Chim. Acta*, **400**, 91 (1999).
3 Z. Q. Feng, I. Shinichiro, K. Takashi, and N. Katsumi, *J. Electroanal. Chem.*, **394**, 149 (1995).
4 Y. Sato and F. Mizutani, *Electrochim. Acta*, **45**, 2869 (2000).
5 B. Ge and F. Lisdat, *Anal. Chim. Acta*, **454**, 53 (2002).
6 J. X. Wang, M. X. Li, Z. J. Shi, N. Q. Li, and Z. N. Gu, *Anal. Chem.*, **74**, 1993 (2002).
7 G. Wang, J. J. Xu, and H. Y. Chen, *Electrochem. Commun.*, **4**, 506 (2002).
8 B. I. Yakabson and R. E. Smally, *Am. Sci.*, **85**, 324 (1997).
9 G.-C. Zhao, F.-H. Wu, and X.-W. Wei, *Chem. Lett.*, **2002**, 520.
10 J. V. O'Fallon, R. W. Wright, and R. E. Calza, *Anal. Biochem.*, **239**, 193 (1996).

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