Electroreduction of Oxygen by Myoglobin on Multi-walled Carbon Nanotube-Modified Glassy Carbon Electrode

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Direct electrochemical response of myoglobin (Myb) on multi-walled carbon nanotube (MWNT)-modified glassy carbon (GC) electrode was observed. Interestingly, Myb on the MWNT exhibited an elegant catalytic activity for electrochemical reduction of oxygen (O_2), based on which an unmediated biosensor for O_2 was developed.

In electrochemistry, myoglobin (Myb) is suggested to be an idea model protein for the study of heme proteins or enzymes because of its commercial availability and a known structure.[1] However, it is usually difficult for Myb to transfer electron to a conventional electrode. The reason is ascribed either to its 3-D structures hindering interaction with the electrode or to its adsorption onto and subsequent passivation of the electrode surfaces. To remedy this problem, many methods have been developed,[2–6] each has its own advantages and disadvantages. A relatively new method is to immobilize protein onto electrode surface by its incorporation into polymer films,[4] surfactants[5] or lipid.[6] So, it is still an important subject to find new promoters to promote the redox reaction of Myb, and based on which to construct new biosensors.

Carbon nanotube is a kind of inorganic material with an atomic structure which is promising as an immobilization substance because of its significant mechanical strength, high surface area, excellent electrical conductivity, and good chemical stability.[7] The subtle electronic properties suggest that carbon nanotubes will have the ability to promote electron transfer reaction when used as an electrode. They have been used as modified electrodes to catalyze the electrochemical reaction of some biomolecules, such as dopamine, NADH, cytochrome c etc.[8–10] In this letter, the direct electron transfer between Myb and MWNTs modified electrode is reported, which is extended to the preparation of a Myb/MWNT-modified GC electrode and the investigation of the catalytic activity of the electrode for the reduction of O_2.

25 μL of acid-treated MWNT solution[11] was cast on the surface of GC electrode and dried in air to form an MWNT-modified electrode. Then the MWNT-modified GC electrode was dipped into 0.24 mmol/L Myb solution over 72 h and removed from the solution, washed with twice distilled water and stored in an acetate buffer solution (pH = 5.6) at about 4 °C. The electrochemical impedance spectroscopy (EIS) measurements were performed in the presence of 5.0×10^{-3} mol/L $K_3[Fe(CN)_6]$/$K_4[Fe(CN)_6]$ by applying an AC voltage with 5 mV amplitude in a frequency range from 1 Hz–100 KHz under open circuit potential conditions.

The impedance measurement was used to observe the adsorption process of Myb. A typical Nyquist plot was shown in Figure 1. The semicircle corresponds to the electron-transfer limited process. Compared with MWNT-modified GC electrode (Figure 1a), adsorption of Myb resulted in an increase of semicircle part. The longer time that the MWNT-modified electrode soaked in Myb solution is, the larger the charge transfer resistance is (as shown in the curves b and c in Figure 1). When the soaking time in the Myb solution is over 72 h, there is no dramatically change of the interfacial electron-transfer resistance, which means the adsorption of Myb reaches a saturated state.

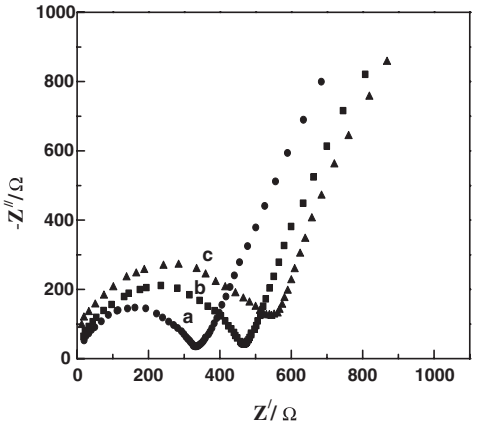

Figure 1. The Nyquist plots for MWNT-modified GC electrode measured at different adsorption time recorded in 5.0×10^{-3} mol/L $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$: a, 0 h; b, 48 h; c., 72 h.

The Myb molecules adsorbed on MWNT-modified GC electrode is subject to a direct electron transfer to and from the electrode. As shown in Figure 2a, a pair of well-defined peaks can be observed in phosphate buffer solution (pH 7.0). The cathodic and anodic peak potentials were −0.298 and −0.198 V vs SCE (saturated calomel electrode), respectively. The formal potential (an average midpoint potential of cathodic and anodic peak potential) is −0.248 V, which is very closed to the literature reported.[12] It can also be observed that the peak current (I_p) increased linearly with scan rate (ν) from 0.01 to 0.3 V/s. The slope of the plot of $\log(I_p)$ vs $\log(\nu)$ was 0.94 with a correlation coefficient of 0.999, which was closed to the theoretical slope of 1 for thin layer voltammetry.[13] According to the slope of the I_p–ν curve and the equation of $I_p = n^2F^2\nu\Gamma A/4RT$, the surface concentration (Γ) of Myb on the surface of MWNT-modified GC electrode was estimated. The surface area of the electrode was calculated according to the cyclic voltammogram obtained in 5.0×10^{-3} mol/L $K_3[Fe(CN)_6]$ solution, which is about 0.29 cm^2 for an MWNT-modified electrode. Under the condition of saturated adsorption, the average surface concentration (Γ) of Myb is about $4.2 \pm 0.4 \times 10^{-10}$ mol/cm^2, which means that the adsorbed Myb is an approximate monolayer. The formal potential of

Myb/MWNT-modified electrode shifts linearly to negative direction with increasing pH value with a slope of −56.4 mV/pH. This slope is reasonably close to the theoretical value of −58 mV/pH at 20 °C for reversible one-electron transfer coupled with single-proton transportation,[14] indicating that Myb on MWNT-electrode undergoes a single electron, single proton electrode reaction process:[14]

$$\text{MybFe(III)} + H^+ + e \rightarrow \text{MybFe(II)}$$

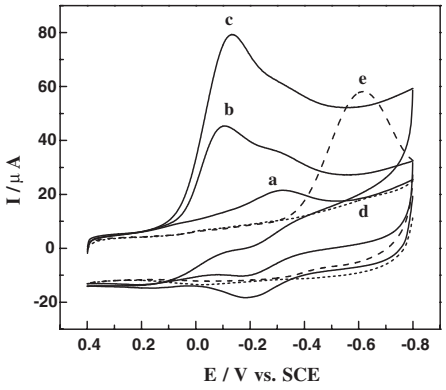

Figure 2. Cyclic voltammograms at an Myb/MWNT-modified electrode (curves a, b, and c) and at an MWNT-modified electrode (curves d and e). Concentrations of O_2 in the electrolyte solution: a and d (dotted line), 0 mM; b, 0.3 mM; c and e (dashed line), 0.6 mM. Scan rate: 50 mV/s.

Myb adsorbed on MWNT-modified GC electrode showed a good electrocatalytic property for the reduction of O_2. At a bare GC electrode, no obvious electrochemical response for oxygen was observed in the potential range of 0.4–−0.8 V, and at an MWNT-modified electrode, the reduction peak of oxygen appeared at −0.62 V (as shown in Figure 2e). However, at an Myb/MWNT electrode, the reduction peak potential moved up to −0.12 V and the peak current increased obviously. Figures 2b and 2c are the cyclic voltammograms of Myb/MWNT-modified electrode in pH 7.0 phosphate buffer solution containing different oxygen concentrations. Compared with MWNT-modified electrode, Myb/MWNT-modified electrode decreased the reduction overpotential of O_2 about 500 mV. According to the previous report, the possible electrode reaction process could be described as follows:[15]

$$\text{Myb Fe(II)} + O_2 \rightarrow \text{Myb Fe(II)–}O_2 \text{ (fast)}$$

$$\text{Myb Fe(II)–}O_2 + 2H^+ + 2e \rightarrow \text{Myb Fe(II)} + H_2O_2$$

In order to test the response of Myb/MWNT-electrode for oxygen, the amperometric experiments were carried out and the results were shown in Figure 3. The current signal of the Myb/MWNT-electrode was proportional to the O_2 concentration from 0 to 21.6 μM.

The stability of the electrode was also tested. When the electrode was stored in pH 5.6 acetate buffer solutions at 4 °C for one month, 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.

In conclusion, Myb can be strongly adsorbed on the surface of MWNT in an approximate monolayer to form a Myb/MWNT-modified electrode. The electrochemical behaviors of the electrode suggested that MWNT could promote the electron transfer between Myb and electrode. The further investigation showed that the Myb/MWNT-modified electrode could be used as a biosensor to catalyze the reduction of oxygen. These results maybe help us to develop a new potential application of carbon nanotubes in the field of biosensors.

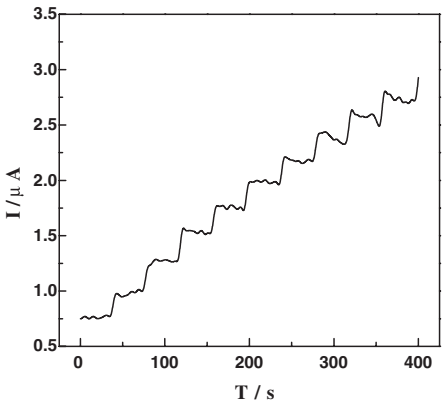

Figure 3. Amperogram obtained with successive addition of O_2 in 0.1 mol/L phosphate buffer solution. Constant potential of −0.12 V, each addition of 2 μmol/L O_2.

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References

1 I. Hamachi, S. Noda, and T. Kunitake, *J. Am. Chem. Soc.*, **113**, 9625 (1991).
2 I. Taniguchi, K. Watanabe, and M. Tominaga, *J. Electroanal. Chem.*, **333**, 331 (1992).
3 M. Tominaga, T. Kumagai, S. Takita, and I. Taniguchi, *Chem. Lett.*, **1993**, 1771.
4 P. Bianco, A. Taye, and J. Haladjian, *J. Electroanal. Chem.*, **377**, 299 (1994).
5 A. E. Nassar, Y. Narikiyo, T. Sagara, N. Nakashima, and J. F. Rusling, *J. Chem. Soc., Faraday Trans.*, **91**, 1775 (1995).
6 Z. Zhang, A. E. Nassar, Z. Lu, J. B. Schenkman, and J. F. Rusling, *J. Chem. Soc., Faraday Trans.*, **93**, 1769 (1997).
7 B. I. Yakabson and R. E. Smally, *Am. Sci.*, **85**, 324 (1997).
8 M. Musameh, J. Wang, A. Merkoci, and Y. Lin, *Electrochem. Commun.*, **4**, 743 (2002).
9 G. Wang, J.-J. Xu, and H.-Y. Chen, *Electrochem. Commun.*, **4**, 506 (2002).
10 J. Wang, M. Li, Z.-J. Shi, N.-Q. Li, and Z.-N. Gu, *Anal. Chem.*, **74**, 1993 (2002).
11 G.-C. Zhao, F.-H. Wu, and X.-W. Wei, *Chem. Lett.*, **2002**, 520.
12 A.-E. F. Nassar, Z. Zhang, N. Hu, J. F. Rusling, and T. F. Kumosinski, *J. Phys. Chem. B*, **101**, 2224 (1997).
13 R. W. Murray, in "Electroanal. Chem.," ed. by A. J. Bard, Marcel Dekker, New York (1984), Vol. 13, pp 191–368.
14 L. Meites, "Polarographic Techniques," 2nd ed., Wiley, New York (1965).
15 C. Onuoha and J. F. Rusling, *Langmuir*, **11**, 3296 (1995).

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