

Direct Electron Transfer between Glucose Oxidase and Multi-walled Carbon Nanotubes

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The direct electrochemical behavior between the glucose oxidase (GOD) and the multi-walled carbon nanotubes (MWNTs) has been studied. Two pairs of cyclic voltammetric peaks corresponding to the two different processes, *i. e.* mass-transport and surface reaction of GOD are observed on this MWNTs. The formal potentials with $E^{\circ} = -0.45$ V and $E^{\circ} = -0.55$ V were obtained respectively. The GOD film was observed on the carbon nanotube by the TEM.

Keywords carbon nanotube, glucose oxidase, direct electron transfer

Introduction

Carbon nanotubes are graphite sheets rolled up into cylinder-like structures that can be single- or multi-walled. Their diameters range from 1—2 nm for single-walled tubes to a few tens of nanometers for multi-walled tubes, and their lengths can exceed several μm .¹ Since the discovery of carbon nanotubes in 1991,² many experiments and theoretical researches have been directed toward their production, purification, mechanical, electronic properties and electrical conductivity.³

Depending on the tube diameter and symmetry of the two-dimensional carbon lattice of the cylindrical shells, carbon nanotubes behave electrically as a metal or as a chemical semiconductor.^{4,6} The subtle electronic properties suggest that carbon nanotubes have the ability to promote electron-transfer reaction when used as an electrode in reaction. As they are insoluble in most solvents, the MWNTs can be mixed with bromoform, mineral oil, or liquid paraffin and then packed into a glass capillary. Electrical attachment was made through a platinum or copper wire. The resulting carbon nanotube microelectrodes are used to probe bioelectrochemical reactions^{7,8} and the electrocatalysis of oxygen.⁹ Ajayan *et al.*¹⁰ have reported the fast electron transfer kinetics on multiwalled carbon nanotube microbundle electrode. Crooks *et al.*¹¹ have reported the fabrication and characterization of electrode con-

structed from single carbon nanotubes. A novel carbon nanotube modified GC electrode has been developed by Li *et al.*^{12,13} Their performance has been found to be superior to that of carbon electrodes.

The direct electron transfer between an electrode and the redox-active prosthetic group of enzyme is interesting both for the development of new types of biosensors or bio-fuel cells and for the fundamental understanding of biological processes. However, the electroactive center of the enzyme molecules is hidden in their interior, and it is difficult to realize the direct electron transfer between an enzyme and an electrode surface. Some results have been reported that the direct electrochemistry of glucose oxidase (GOD) can be carried out on the GC electrode and some modified GC electrodes.¹⁴⁻¹⁹ Other researches have been reported that the direct electron transfer may be carried out when the GOD molecules are adsorbed into the conducting polymer microtubules.²⁰⁻²² The properties of the carbon nanotube are hollow, big surface and good ability of electron transfer. The direct electrochemistry of the GOD on carbon nanotubes has not been reported to our knowledge.

The purpose of this work is to study the direct electrochemistry of glucose oxidase on multi-walled carbon nanotube modified GC electrode (MWNT/GC). The results show that the direct electron transfer occurs between the GOD and the MWNTs modified GC electrode.

Experimental

Reagents

Glucose oxidase (GOD) was obtained from Sigma and used as received. GOD solution of 2 mg/mL was stored at 4 °C. The MWNTs were produced by chemical vapor deposition (CVD),²³ and treated with nitric acid during the purification process. All other chemicals were of analytical grade. All solutions were prepared with doubly distilled

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water.

Fabrication of the MWNTs modified GC electrode

The glassy carbon electrode with a diameter 10 mm was polished with 1.0, 0.3 and 0.05 μm aluminum oxide, rinsed thoroughly with deionized water, sonicated in deionized water and ethanol, and then dried in air before use. 2 mg of MWNT was dispersed with the aid of ultrasonic agitation in tetrahydrofuran (THF) to give a black solution. The MWNTs film was prepared by dropping a solution of MWNT on the GC electrode surface, and was evaporated the solvent under an infrared heat lamp.

Apparatus and procedures

CHI660 Electrochemical workstation (CHI Co. USA) was used for electrochemical measurements. A three-electrode system incorporating this MWNTs-modified GC electrode as the working electrode, a saturated calomel reference electrode (SCE) and a platinum wire counter electrode were used for the measurement. Transmission electron microscopy (TEM) images were obtained by employing JEOL JEM-200CX (Japan) transmission electron microscope, using an accelerating voltage of 200 kV.

The sample of TEM was prepared as the following process. MWNTs were added into the GOD solution and dispersed by ultrasonic agitation, the solution was stored at 4 $^{\circ}\text{C}$ for 12 h, then the solid sample was separated by centrifugation and washed with distilled water. A part of the wet sample was renewedly dispersed into water for TEM.

Results and discussion

Cyclic voltammetry studies

When the MWNTs modified GC electrode was placed into pH = 7.0 phosphate buffer solution (PBS), cyclic voltammetric curve was observed in the range of -0.1 — $+0.1$ V. It accorded with the result on a SWNT modified GC electrode.¹² Fig. 1 shows the CV curves of the bare GC electrode (a) and MWNT/GC (b). No waves can be observed on the MWNT/GC in the range of -0.3 — -0.8 V. The background current of MWNT/GC electrode is apparently larger. This may be attributed to the increasing surface differential capacitance. The stability of the modified electrode was tested. The electrode was exposed to the air for different time, and then the same cyclic voltammetric experiment was performed again. The background current did not change. It means that there is a good adsorptive ability between the clean GC electrode surface and the MWNTs, and the MWNTs on the electrode surface are entwined each other. These can prevent the drop of MWNTs from the electrode and keep the stability.

When MWNT/GC electrode was immersed into the GOD solution for 2 h, two cyclic voltammetric peaks were

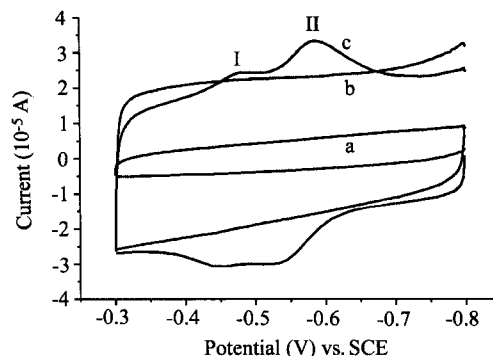


Fig. 1 Cyclic voltammograms at a bare GC electrode (a), MWNT/GC electrode (b) and GOD/MWNT/GC electrode (c) in PBS (pH = 7.0). I and II are used to mark different peaks of the curve c. The potential scan rate is 0.1 V/s.

observed. The pair of peaks I was at $E^{o'} = -0.45$ V (Fig. 1c) and the pair of peaks II was at $E^{o'} = -0.55$ V (Fig. 1c). However, no voltammetric responses were observed at bare GC electrode under the same condition. The results suggest direct electron transfer between GOD and MWNT/GC electrode. With the increasing scan time, these peak currents decrease. The peak I disappears after about 1 h, and the peak II tends to a stable value. But it was reported that only a pair of peaks were observed.¹⁶ In order to explore the reason, the relation between the peaks current and scan rate was studied. It is found that the cathodic peak current of peak I (Fig. 2a) is proportional to the square root of the scan rates, and the cathodic current of peak II (Fig. 2b) is linear to scan rate from 10—200 mV/s. This indicates that the pair of peak II is attributed to the GOD molecules adsorbed on the MWNTs surface. There are some GOD molecules in the MWNTs film on the electrode, but these molecules are not intensively adsorbed to the MWNTs. Because the MWNTs film has certain thickness and the MWNTs are entwined each other, the bigger GOD molecules can not diffuse quickly from the film to the solution. The peak I is attributed to the direct electrochemistry of the unadsorbed GOD molecules in the MWNTs film. In order to confirm the explanation, the CV curve is recorded immediately when a new MWNTs modified electrode is dipped into the GOD solution, Fig. 3a shows that only a pair of peaks are obtained at -0.45 V.

The quantity of GOD in MWNTs film is increased with the adsorption time. When time is up to 12 h, the quantity is saturated. Then, the electrode is put into the blank PBS for removing the unadsorbed GOD molecules. The Fig. 3b is the typical CV curves of the GOD immobilized on the MWNT/GC electrode in $0.1 \text{ mol} \cdot \text{L}^{-1}$ PBS. According to the Q — Γ relationship, the surface concentration (Γ) of GOD in the surface could be estimated.²⁴ Each molecule of GOD has two molecules of flavin adenine dinucleotide (FAD) tightly bound. If the stokes radius of the GOD is taken for 4.3 nm, the theoretical quantities of the monolayer on the bare electrode surface is $2.86 \times 10^{-12} \text{ mol/cm}^2$. The value of Γ on the MWNT/GC is

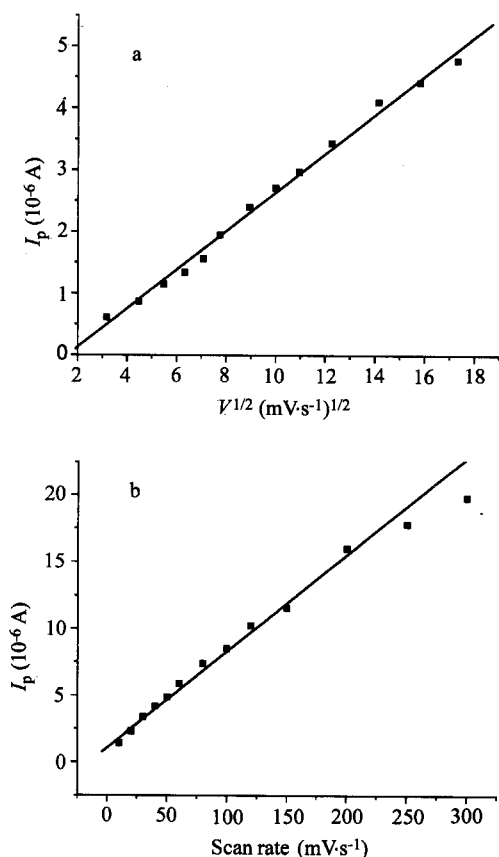


Fig. 2 Calibration plots between the peak current of the reduction peak at -0.45 V (a), -0.55 V (b). The different scan rates are from 0.01 to 0.3 V/s.

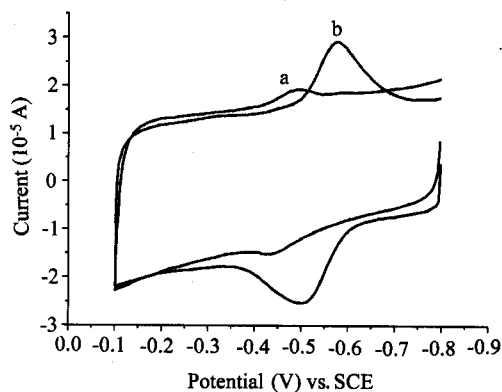


Fig. 3 Cyclic voltammograms of an MWNT/GC (a) in PBS solution with 2 mg/mL GOD and the GOD/MWNT/GC (b) in pH 7.0 PBS at scan rate of 0.1 V/s.

about 1.9×10^{-10} mol/cm² at a scan rate of 0.02 V/s. Here the area is the geometry area of the electrode. The result shows that the MWNT on the electrode surface can obviously raise the surface concentration. Because the real area is by far larger than the geometry area, the real surface concentration is lower than that of monolayer.

CVs of GOD/MWNT/GC show a strong dependence on pH. An increase of pH in solution leads a negative shift in peak potential for both reduction and oxidation processes. CV data were used to investigate the pH effect on the formal potential ($E^{o'}$). The $E^{o'}$ had linear relationship

with pH from pH 4.0 – 9.0 with a slop of -59 mV/pH (Fig. 4). This value is approximately close to the theoretical value of -57.6 mV/pH at 18 °C for a reversible, two-proton couples two electrons transfer. The result accords with that reported on the electrochemically pretreated GC electrode,¹⁶ and can be expressed as:



In order to detect the catalytic activity of the GOD on the MWNT, the glucose was added into the PBS solution. But no obviously catalytic response was observed in the solution. It indicates that the adsorption between the GOD and the MWNT is strongly adsorptive process. Wang *et al.*^{18,19} have studied the native and unfolded GOD structures on the highly ordered pyrolytic graphite (HOPG) by the scanning tunneling microscopy (STM). We think that the GOD molecule maybe extend to an unfolded structure on the MWNTs surface in adsorption process and the glucose can not be embedded into it.

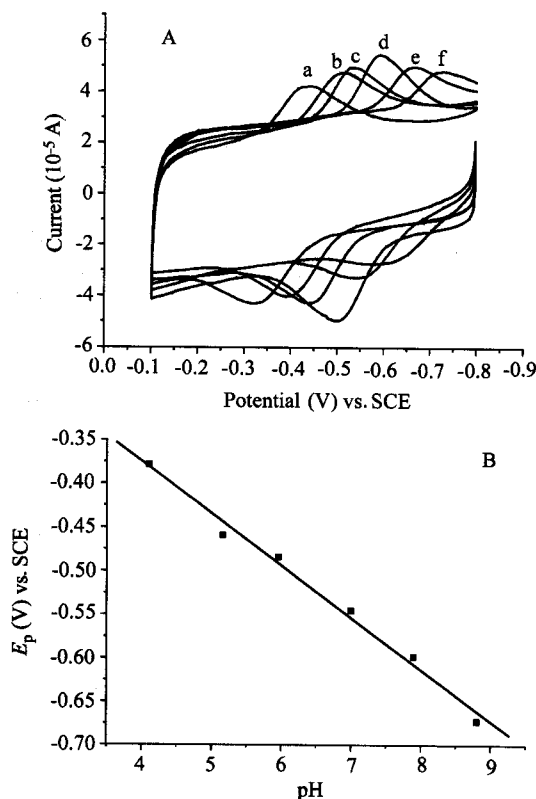


Fig. 4 Cyclic voltammograms of a GOD/MWNT/GC in different pH (A) from 4.0 (a) to 9.0 (f) at scan rate of 0.1 V/s and the plot relation (B) between the pH and the formal potentials.

TEM characterization

Hill *et al.*²⁵ directly got the image in which the protein and enzyme immobilized in carbon nanotubes have been observed by the high-resolution transmission electron microscopy. The interaction between the GOD and carbon

nanotube were confirmed by TEM. Fig. 5a shows the carbon nanotubes without treatment by GOD, and Fig. 5b is the carbon nanotubes treated with GOD. The increase of thickness of the tube-wall obviously implies the adsorption of GOD molecules. The shape of the GOD indicates that the GOD molecules are adsorbed close on the MWNTs surface.

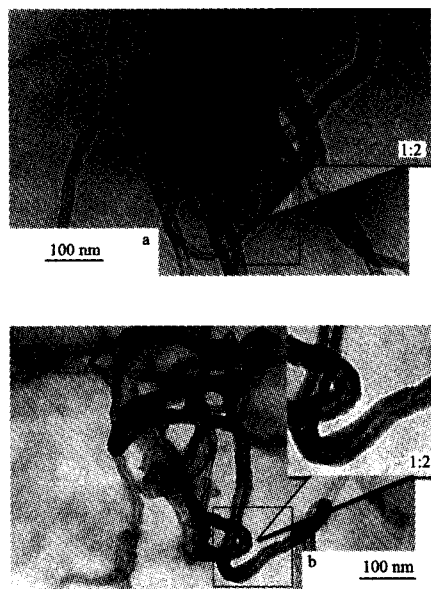


Fig. 5 Transmission electron microscopy photos of the MWNTs (a) and the MWNTs (b) adsorbed GOD molecules.

Conclusion

The GOD direct electrochemistry controlled by adsorption and diffusion process was observed on the MWNTs surface. The GOD molecules were observed on the MWNTs surface by the TEM. The further work is being carried out in our laboratory.

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