Chinese Chemical Letters Vol. 15, No. 7, pp 813-816, 2004 813
http://www.imm.ac.cn/journal/ccl.html

Promotion of the Direct Electron Transfer of Hemoglobin by the Carbon Nanotube

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Abstract: It is reported that the direct electron transfer of hemoglobin (Hb) can be effectively promoted by carbon nanotubes when Hb was immobilized on the surface of the carbon nanotubes modified electrode. The results indicated that the conversion of Hb-Fe(III)/Hb-Fe(II) is a one-electron coupled one-proton reaction process. The method presented can be easily extended to study the direct electrochemistry of other proteins or enzymes.

Keywords: Carbon nanotube, direct electrochemistry, modified electrode, hemoglobin.

The direct electrochemistry of redox proteins or enzymes has been received more and more attention[1-8] because one can obtain the valuable information on mechanisms of biological electron transfer reactions and find potential applications in biotechnology from these studies. For example, if a protein (or enzyme) immobilized on electrode surface is capable of the direct electron transfer without loss of bioactivities, it can be used in the biosensors to measure the substrates without the addition of mediator onto the electrode surface or into the solution. Unfortunately, it is difficult for a protein to carry out the direct electrochemical reaction due to several factors, such as denaturation when it was adsorbed on the electrode surface and deeply burying of its active sites in the molecules *etc*.. Therefore, the suitable electrode materials and immobilization methods are important for obtaining the direct electrochemical reaction of proteins (or enzymes).

Hemoglobin (Hb), functions physiologically in the storage and transport of molecular oxygen in mammalian blood, has four polypeptide subunits, each of them has an iron-bearing heme. Although Hb does not play a role as an electron carrier in biological systems, it is an ideal model molecule for study on electron transfer reactions of heme enzymes because of its commercial availability and a documented structure. The large three-dimensional structure, inaccessibility of the heme center and the electrode passivation due to the protein adsorption, it is generally difficult to obtain the direct electron transfer of Hb. Many efforts have been made to achieve its direct electron transfer[9-15]. Here, we report the promotion effect of carbon nanotubes (CNTs) on the direct electron transfer of Hb immobilized by adsorption on the surface of a CNTs modified glassy carbon (CNT/GC) electrode.

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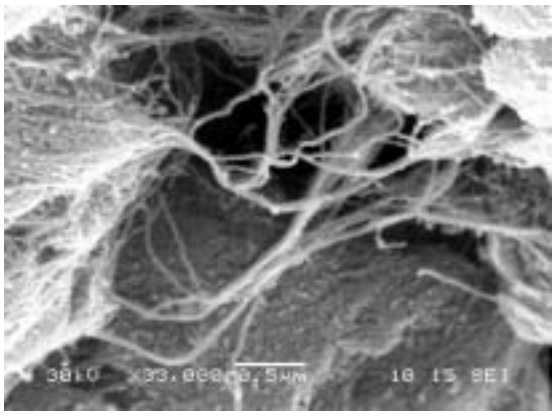
Figure 1 The SEM image of CNTs on the surface of GC electrode

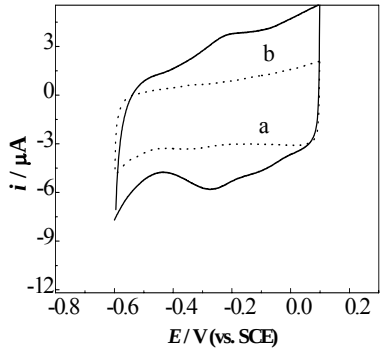


The CNT/GC electrode was prepared by casting 15 μL CNTs (purchased from Shenzhen Nanotech Port Co. Ltd., with diameter < 10 nm and the length in the range of 0.5 to 500 μm, used as received) suspension (0.5 mg/mL H_2O) on the surface of glassy carbon (GC, 4-mm in diameter) electrode. SEM image (**Figure 1**), which was obtained with a JEOL JSM-5610LV Scanning Electron Microscope (Japan), indicates that CNTs are formed as bundles with individual CNT arranged in parallel to each other on the electrode surface. Some bundles twisted together. The diameter of CNTs bundles is in the range of 25 to 60 nm, the length of the bundle could not be measured since both ends of the bundle were not visible at the same time. Hb (from Sigma, used as received) was immobilized by adsorption on the surface of CNT/GC electrode by incubating the electrode into the protein solution (5 mg/mL in 0.1 mol/L PBS solution, pH 5.5) for 1 h at 4 ˚C. The Hb immobilized CNT/GC electrode (Hb-CNT/GC) was then taken out from incubated solution, washed thoroughly with water and transferred into other solutions to perform the electrochemical measurements. If it was not used immediately, the electrode was stored at 4 ˚C in a refrigerator.

The electrochemical measurements were performed using a CHI 600A elecrochemical workstation (CH Instruments) in a conventional three-electrode cell. The working electrode was a Hb-CNT/GC or a CNT/GC electrode. The coiled Pt wire and saturated calomel electrode (SCE) were used as the counter electrode and the reference electrode, respectively. Before the electrochemical experiments, the solution was deaerated by passing through highly pure nitrogen for 20 min, and a continuous flow of nitrogen was maintained over the sample solution during the experiments.

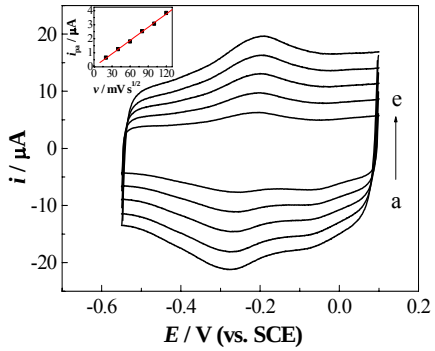
Figure 2 shows the cyclic voltammograms of a CNT/GC (curve a) and a Hb-CNT/GC electrode (curve b) in 0.1 mol/L PBS (pH 6.8) at a scan rate of 20 mV/s. No any redox peaks was observed at the CNT/GC electrode, but a pair of redox peaks was obtained at the HRP-C/GC electrode, suggesting that the redox peaks are ascribed to the electrochemical reaction of Hb immobilized on the surface of CNTs. The anodic peak and cathodic peak potential is -0.222 and -0.273 V, respectively, at a scan rate of 20 mV/s. The separation of peak potentials, ΔE_p, is 51 mV. The cathodic current is almost equal to

Figure 2 The cyclic voltammograms of Hb-CNT/GC electrode



Curve a for CNT/GC and curve b for Hb-CNT/GC electrode in 0.1 mol/L PBS (pH 6.8). The scan rate is 20 mV/s.

Figure 3 The background subtracted cyclic voltammograms of Hb-CNT/GC electrode at various scan rates



The scan rate for curve a to e is 40, 60, 80, 100, 120 mV/s, respectively. The inset shows the dependence of anodic peak current on the scan rates.

the anodic one. Thus, it can be concluded that Hb immobilized on the surface of the CNTs undergoes a quasi-reversible electrochemical reaction. Its formal potential, $E^{0'}$ (defined as average of anodic and cathodic peak potentials), is -0.248 V. The cyclic voltammetric results indicated that the value of $E^{0'}$ is almost independent on the scan rates. In addition, i_{pa} and i_{pc} are linearly proportional to the scan rate at least up to 120 mV/s (**Figure 3**), suggesting the reaction is not a diffusion controlled process but a surface controlled one, as expected for immobilized systems[16]. The $E^{0'}$ value is similar to that previously reported for Hb incorporated into the Eastman AQ 55, entrapped into dimyristoyl phosphatidylcholine (DMPC) film and immobilized into poly(vinyl sulfonate) film by Hu *et al.*[9-11] It is also similar to those of other heme containing proteins including myoglobin[17,18] and cyt. $P450_{cam}$[19] *etc*.. According to previous reports, the electrochemical reaction in **Figure 2**, curve b corresponds the conversion of Hb-Fe(III) and Hb-Fe(II).

The anodic and cathodic peak potentials are scan rate dependence. With the increasing of the scan rates, the anodic and cathodic peak potentials shift slightly to positive and negative direction, respectively. The value of $E^{0'}$, however, is almost independent on the scan rates. From the dependence of ΔE_p on the scan rate, one can calculate the apparent heterogeneous electron transfer rate constant, k_s, to be 1.67 s^{-1}, using the method developed by Laviron[20] for surface-controlled electrochemical system. The value of k_s obtained is much larger than that obtained by Gu *et al.*[14] for Hb immobilized on a nanometer sized Au colloid-cysteamine-modified gold electrode using the same method (0.49 s^{-1}), suggesting CNT is more effective in facilitating the direct electron transfer of Hb than the nanometer sized Au colloid. CNTs can facilitate the direct electron transfer of Hb partially may be due to some oxygen-contained groups, such as phenolic hydroxides[21] and quinone[22] *etc*, presented on the CNTs surface and also its small dimension, even though the exact reason is not fully understood. These oxygen contained groups can be introduced during CNTs preparation. The presence of oxygen contained groups on the

surface of CNTs can be verified by IR spectrum (not shown here). It was reported[23,24] that CNTs promote the direct electron transfer of cytochrome c and catecholamines is due to the presence of the oxygen contained groups on its surface.

The peak potentials are pH dependent. An increasing of solution pH leads to a negative shift in the value of $E^{0'}$ with a slope of -53.8 mV/pH. This value of the slope is close to the theoretical one of -59.2 mV/pH at 25˚C for a reversible, one-proton coupled one-electron reaction process, suggesting the conversion of Hb-Fe(III)/Hb-Fe(II) is one-electron transfer reaction process coupled with one-proton transfer.

In conclusion, CNTs can effectively facilitate the direct electron transfer of Hb after Hb was immobilized on the CNT/GC electrode and the method presented can easily be extended to study the direct electrochemistry of other proteins or enzymes.

Acknowledgments

The project is supported by the Natural Science Foundation of the Education Commission of Jiangsu Province (03KJA150055).

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Received 10 June, 2003