Direct Electrochemical Oxidation of NADPH at a Low Potential on the Carbon Nanotube Modified Glassy Carbon Electrode

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NADPH can be directly oxidized on a carbon nanotube modified glassy carbon (CNT/GC) electrode in phosphate buffer solution (pH＝6.0) with a diminution of the overpotential of more than 700 mV. The anodic peak currents increase linearly with the increase of concentration of NADPH in the range of 5×10^{-7} to 1×10^{-3} mol/L with a detection limit of about 1×10^{-7} mol/L. The CNT/GC electrode exhibits high sensitivity, low potential and stability in detecting NADPH and thus might be used in biosensors to study the electrocatalytic reaction of important dehydrogenase-based biological systems.

Keywords   carbon nanotube, chemically modified electrode, electrochemical oxidation, NADPH

Introduction

Because of its novel structural and electronic properties, high chemical stability, and extremely high mechanical strength and modulus,[1] carbon nanotube (CNT), which has become a major subject of many experimental and theoretical investigations, has a wide potential application from structural materials to nanoelectronic components[2-12] since its initial discovery by Iijima[13] in 1991 and the subsequent report about the synthesis of large quantities of CNT by Ebbesen and coworkers[14] in 1992. Theoretical calculations have shown that, depending on its symmetry and diameter, CNT can behave as either a metal or a semiconductor.[15-17] Furthermore, it has been proved that CNT has better conductivity than graphite.[18] Those results suggest that CNT can be used as an electrode material and has ability to promote electron transfer in electrochemical reactions. This is a new application of CNT and has received more and more attention. For example, CNT has been used to facilitate the direct electron transfer of enzymes and proteins, such as glucose oxidase,[19] cytochrome *c*,[20-23] azurin (a blue copper protein),[22] horseradish peroxidase,[24] *etc.*, to catalyze the oxidation of dopamine, ascorbic acid, norepinephrine, serotonin, NADH, *etc.*,[25-31] to detect nitrite and nitric oxide[32,33] and to catalyze the reduction of oxygen, hydrogen peroxide,[34,35] *etc.*

The direct electrochemical oxidation of NADH or NADPH is becoming more and more interesting, especially for the purpose of analysis, because many oxidoreductases, particularly dehydrogenases, need it as cofactor to catalyze redox reaction.[36,37] However, on a bare electrode the direct electrochemical oxidation of NADH or NADPH takes place at a high overpotential. Although much effort has been devoted toward the goal of finding the new material (mediator) for electrode modification, which will reduce the overpotential of the oxidation of NADH,[24,26,38-48] there are only a few examples of studying the direct electrochemical oxidation of NADPH.[49] In this paper the direct electrochemical oxidation of NADPH on CNT modified glassy carbon (CNT/GC) electrode at a low potential (at about 0 V vs. SCE) with high stability is reported. A diminution of overpotential of more than 700 mV is achieved, and the anodic peak current increases linearly with the increase of concentration of NADPH in the range of 5×10^{-7} to 1×10^{-3} mol/L with the detection limit of about 1×10^{-7} mol/L. It is suggested that the CNT/GC electrode can be used in dehydrogenase-based amperometric biosensors.

Experimental

Chemicals

β-Nicotinamide adenine dinucleotide phosphate (NADPH, reduced form, tetrasodium salt, ＞95% in purity, from Sino-American Biotech. Co., Ltd.) and multi-walled carbon nanotube (CNT, ＜10 nm in diameter and 0.5 to 500 μm in length with the purity of more than 95%, from Shenzhen Nanotech Port Co., Ltd., Shenzhen) were used as received. All other chemicals were of analytical grade. All the solutions were prepared with doubly distilled water. Phosphate buffer solution (PBS, 0.1 mol/L, pH＝6.0) which was made up from Na_2HPO_4 and NaH_2PO_4 was always employed as supporting electrolyte except that the pH-dependent

experiments were carried out in PBS with various pH values. The solution of NADPH was prepared freshly before each experiment using 0.1 mol/L PBS.

Fabrication of the CNT/GC electrode

0.5 mg of CNT was dispersed thoroughly in 1 mL of H_2O with aid of ultrasonication to give a 0.5 mg/mL black suspension, then 15 μL of the mixture was cast onto the surface of a glassy carbon (GC, 4 mm in diameter) electrode with a microsyringe to form a CNT/GC electrode. Before casting, the GC electrode was polished sequentially with metallographic abrasive paper (No. 6), slurries of 0.3 and 0.05 μm alumina to a mirror finish. After rinsed with doubly distilled water, the GC electrode was sonicated with absolute ethanol and doubly distilled water, respectively, for about 1 min. The CNT/GC electrode was allowed to dry at ambient temperature before use.

Apparatus

The scanning electron micrograph (SEM) of CNT was obtained using a JEOL JSM-5610LV Scanning Electron Microscope (Japan). Fourier transform infrared (FTIR) spectrum of CNT was recorded with a 670 FT-IR spectrophotometer (Nicolet Instrumental Co., USA) on KBr pellet. The resolution was 4 cm^{-1}.

The electrochemical measurements were performed using a CHI 600A electrochemical station (CH Instruments). The working electrode was a CNT/GC or a bare GC electrode. A 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 at least 20 min, and during experiments a flow of nitrogen was maintained over the sample solution. All the electrochemical experiments were performed at room temperature of (22 ± 2) ℃.

Results and discussion

Physical characterization of CNT

Figure 1 shows a SEM image of CNT on the surface of GC electrode. It can be seen that the CNT is formed as bundles with individual CNT arranged in parallel to each other. Some bundles twisted together. The diameter of CNT 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. Figure 2 is a FTIR spectrum of CNT. The appearance of the peaks corresponding to the carboxylic group at 1715 cm^{-1} and the carboxylate group at 1574 cm^{-1}, which are in accordance with the previous reports,[50,51] indicates that oxygen-containing groups present on the surface of the CNT. Those oxygen-containing groups might be introduced during purification using concentrated nitric acid and the carboxylate group might be attributed to the electro-ionization of the carboxylic group during washing with water after purifica-

tion. The peaks at 3426 and 1166 cm^{-1} are due to stretching vibration of ─OH and C─OH, respectively.
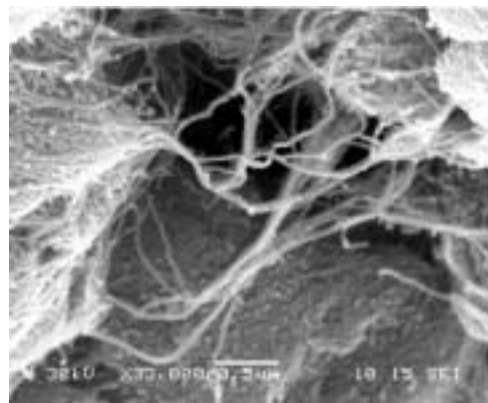

Figure 1 SEM image of carbon nanotube on the surface of GC electrode.
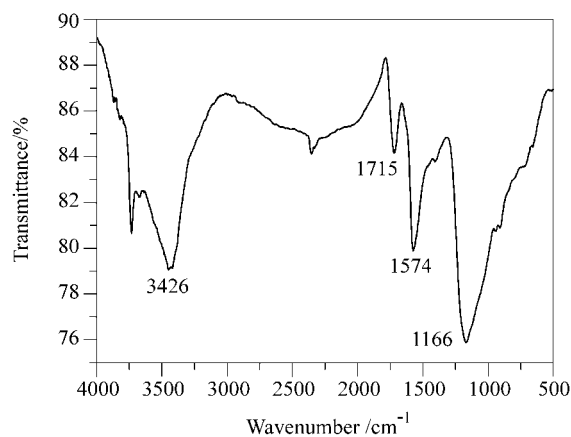

Figure 2 FTIR spectrum of CNT.

Electrochemical oxidation of NADPH on the CNT/GC electrode

In Figure 3, curves a and b are cyclic voltammograms of a bare GC and a CNT/GC electrode, respectively, in 0.1 mol/L PBS (pH=6.0) at a scan rate of 10 mV/s. No any electrochemical reactions can be observed from curve a, while a pair of broad redox peaks appears on the curve b at a potential of about ─50 mV, which can be ascribed to the electrochemical reaction of oxygen-containing groups on the surface of CNT.[51] The background currents of curve b are much larger than those of curve a, which might be attributed to the reason that the apparent surface area of a CNT/GC electrode is larger than that of a bare GC electrode. The CNT on the surface of GC electrode is fairly stable since the electrochemical response of CNT/GC electrode stored in a desiccator for several days does not almost change comparing with that of the electrode prepared immediately. Curve c is a cyclic voltammogram of a CNT/GC electrode in 0.1 mol/L PBS (pH=6.0) containing 1×10^{-3} mol/L NADPH at a scan rate of 10 mV/s. A large and sharp anodic peak, which corresponds to the potential of direct electrochemical oxidation of NADPH, ap-

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pears at about −4 mV. From the results, one can conclude that NADPH can be directly oxidized on the CNT/GC electrode at a very low potential. The oxidation process starts at *ca.* −150 mV with an anodic peak locating at about −4 mV (at a scan rate of 10 mV/s). There occurs an irreversible electrochemical reaction for NADPH with an anodic peak at 719 mV on a bare GC electrode in 0.1 mol/L PBS (pH=6.0, see curve d, the inset of Figure 3), hence, a decrease in overpotential of more than 720 mV is achieved on a CNT/GC electrode. The decrease of the overpotential is much larger than that obtained on an electrode modified with copolymerization of pyrrole and methylene blue (400 mV).[49] The anodic peak current of the oxidation of NADPH at bare GC electrode is much lower than that obtained on a CNT/GC electrode, suggesting that CNT is very effective in promoting the electrochemical oxidation of NADPH.
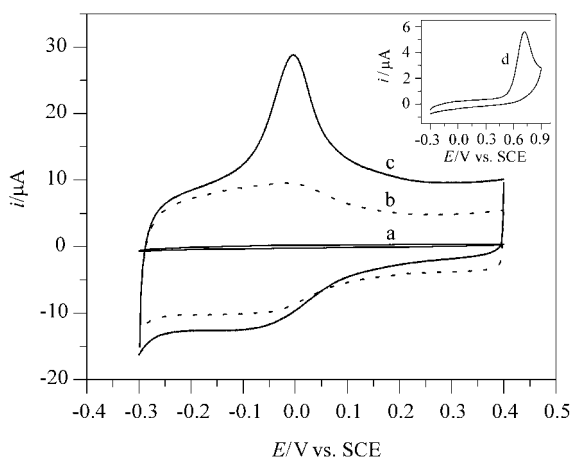

Figure 3 Cyclic voltammogramms of the bare GC (curves a and d) and the CNT/GC (curves b and c) electrodes in 0.1 mol/L PBS (pH=6.0) in the absence (curves a and b) and presence (curves c and d) of 1×10^{-3} mol/L NADPH. The scan rate was 10 mV/s.

The promotion effect of CNT on the direct oxidation of NADPH may be partially due to some oxygen-containing groups,[25,51] small dimension, particular electronic structure and high electric conductivity on the surface of CNT, but the exact reason is not fully understood at present time. These oxygen-containing groups could be introduced during the CNT preparation and purification and were verified by IR spectrum (Figure 2). It was reported[21,52] that CNT can promote the direct electron transfer of cytochrome *c* and catecholamines also due to the presence of the oxygen-containing groups on its surface.

The value of anodic peak potential of NADPH oxidation on a CNT/GC electrode (*ca.* 52 mV at a scan rate of 50 mV/s) obtained in present case is much more negative than that obtained by Musameh *et al.*[25] for the electrochemical oxidation of NADH at CNT modified GC electrode (0.33 and 0.36 V at multi-walled CNT and single-walled CNT modified GC electrodes, respectively, at a scan rate of 50 mV/s). The decrease in overpotential of the oxidation of NADH on CNT modified

GC electrode was about 490 mV,[25] which is much small compared with that obtained in present case (720 mV). These results indicate that CNT we used is much more effective for mediating electron transfer from NAD(P)H than that used by Musameh *et al.*[25]

The cyclic voltammetric results at various scan rates indicate that the anodic peak potentials of the electrochemical oxidation of NADPH on the CNT/GC electrode move to positive direction with the increase of the scan rates. For example, the anodic peak potential was −4 mV at a scan rate of 10 mV/s, however, this value changed to be 89 mV at a scan rate of 80 mV/s. Moreover, the peak currents increased linearly with the increasing of square root of the scan rates at least up to 100 mV/s, indicating that the currents are limited by the diffusion of NADPH in solution.

Effect of solution pH

The electrochemical oxidation of NADPH is a pH-dependent reaction and can be expressed as follows:

$$NADPH \rightarrow NADP^+ + H^+ + 2e \tag{1}$$
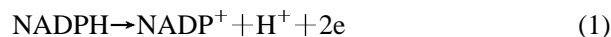
The effect of solution pH on the performance of the CNT/GC electrode in electrochemical oxidation of NADPH was studied by cyclic voltammetry in 0.1 mol/L PBS in the pH range of 4.5 to 9.3. The anodic peak potential shifted linearly to negative direction with the increasing of solution pH. The slope was found to be −34.2 mV/pH unit within the studied pH range and is very close to the theoretical value of −29.3 mV/pH unit (at 22 ℃) since the oxidation of NADPH is a two-electron and one-proton reaction [Eq. (1)]. Figure 4 shows the dependence of the anodic peak current on the solution pH. It can be seen that the largest anodic current appears at pH value of about 6.0. The anodic current increased with the increase of the solution pH when the solution pH was lower than 6.0, however, it decreased drastically when the solution pH was higher than 6.0. Hence, the solution pH of 6.0 was used in the following experiments.
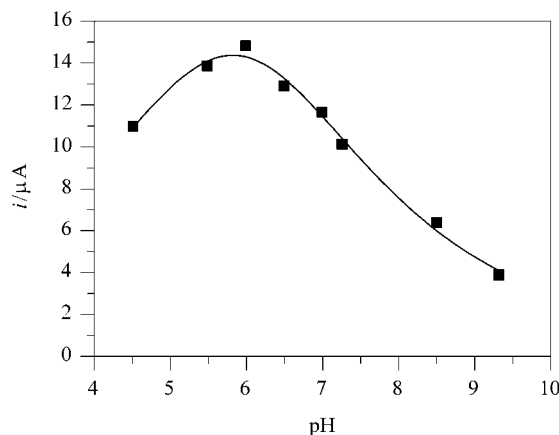

Figure 4 Dependence of anodic current for the electrochemical oxidation of 1×10^{-3} mol/L NADPH at the CNT/GC electrode on the solution pH (0.1 mol/L PBS).

Effect of NADPH concentration

The cyclic voltammetric responses of the CNT/GC electrode in 0.1 mol/L PBS (pH=6.0) containing various concentration of NADPH indicated that both the anodic currents and the anodic potentials depended on the concentration of NADPH. The anodic peak potential shifted to positive direction with the increasing of the concentration of NADPH. The anodic peak currents increased with the increasing of the concentration of NADPH (Figure 5A). In the range of 5×10^{-7} to 1×10^{-3} mol/L, the anodic peak currents increased linearly with the increase of concentration of NADPH (Figure 5B) with $r=0.99966$ (11 points), and the detection limit was about 1×10^{-7} mol/L. This linear range can be used as a calibration to determine the concentration of NADPH in sample.

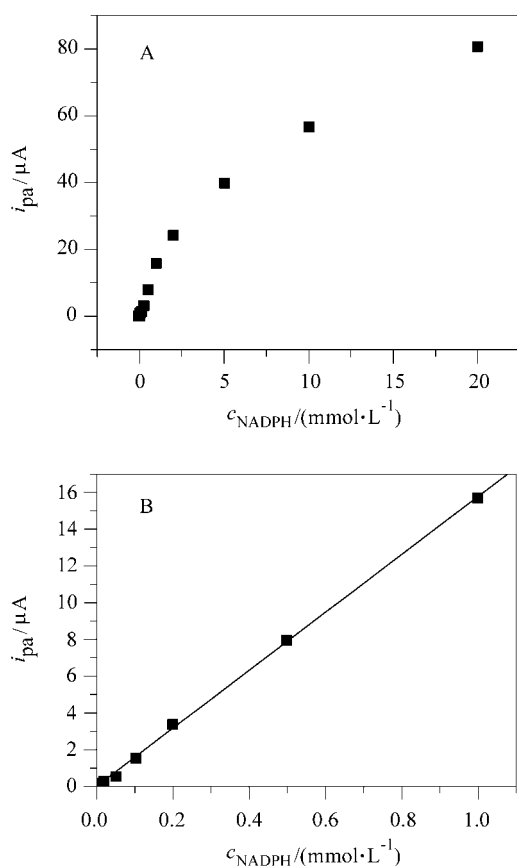**Figure 5**   Dependence of the anodic currents of the CNT/GC electrode on the concentration of NADPH (A) and the linear relationship between them (B).

Stability of the CNT/GC electrode toward oxidation of NADPH

The stability of the CNT/GC electrode was studied by repetitive cycling the electrode in a solution of NADPH. Although the cyclic voltammetric response of NADPH decreased rapidly in the initial several cycles (about 6 to 7 cycles), the electrode could still remain about 72% of the response of the first cycle after 40 repetitive scanning cycles (Figure 6). These results indicate that the CNT/GC electrode has a good stability to-

ward the electrochemical oxidation of NADPH. The decrease of response upon continuous cycling is not due to the NADPH depletion near the surface of electrode since the similar phenomenon was also found when the solution was stirred. It may be due to the decrease of the concentration of oxygen-containing groups on the surface of CNT/GC in the continuous cycling.[53] The dependence of the anodic current of the CNT/GC electrode to the electrochemical oxidation of NADPH on the storage time was also investigated by keeping one electrode in a desiccator, and each day the cyclic voltammogram was recorded and the peak current was measured using the same one. In the first several days (about 6 to 8 d), the peak current decreased rapidly, then, the peak current decreased slowly. For example, the response of the electrode remained only 84% of the initial one at the second day, however, it could still remain more than 60% of the initial at the 22nd day (the inset of Figure 6).
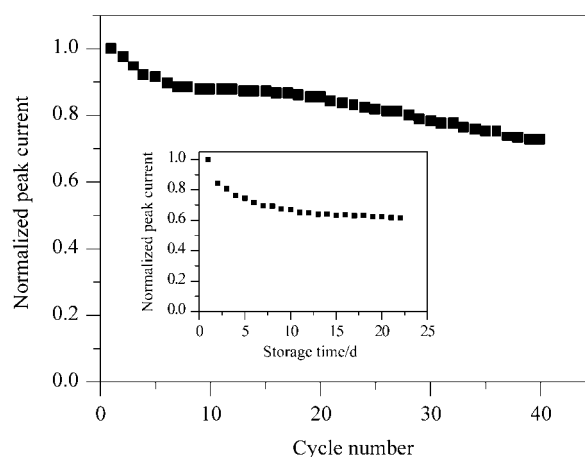

Figure 6   Dependence of the normalized anodic peak current of the CNT/GC electrode towards the electrochemical oxidation of NADPH on the continuously scanning (the main) and the storage time (the inset).

The reproducibility of the CNT/GC electrode toward the electrochemical oxidation of NADPH was also studied. The cyclic voltammetric response of oxidation of 1×10^{-3} mol/L NADPH was recorded at six CNT/GC electrodes. The results indicated that the responses of six CNT/GC electrodes are similar and the average anodic current is (15.84 ± 0.41) µA for oxidation of 1×10^{-3} mol/L NADPH, suggesting that the CNT/GC electrode has a good reproducibility toward the electrochemical oxidation of NADPH.

Conclusions

The results of this work have shown that NADPH can be directly oxidized at a very low potential (at about 0 V, at a scan rate of 10 mV/s) on the CNT/GC electrode in PBS (pH=6.0) with a diminution of the overpotential of more than 700 mV. The effects of scan rate, solution pH and the concentration of NADPH on the performance of the CNT/GC electrode were studied. The anodic

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peak currents increase linearly with the increasing of concentration of NADPH in the range of 5×10^{-7} to 1×10^{-3} mol/L with a detection limit of about 1×10^{-7} mol/L. The CNT/GC electrode might be used in biosensors to study the electrocatalytic reaction of important dehydrogenase-based biological systems because the method of preparation of the CNT/GC electrode is simple, and the electrochemical oxidation of NADPH on the electrode is effective and stable.

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