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ScienceDirect
Chinese Chemical Letters 19 (2008) 965–968
CHINESE CHEMICAL LETTERS
www.elsevier.com/locate/cclet

Third generation biosensor based on myoglobin-TiO_2/MWCNTs modified glassy carbon electrode

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Received 27 February 2008

Abstract

TiO_2 nanoparticles were homogeneously coated on multi-walled carbon nanotubes by hydrothermal deposition, this nanocomposite may be a promising material for myoglobin immobilization in view of its high biocompatibility and large surface. The glassy carbon electrode modified with Mb-TiO_2/MWCNTs films exhibited a pair of well defined, stable and nearly reversible cycle voltammetric peaks. The electron transfer between Mb and electrode surface, Ks of 3.08 s^{-1}, was greatly facilitated in the TiO_2/MWCNTs film. The electrocatalytic reductions of hydrogen peroxide were studied, the apparent Michaelis–Menten constant is calculated to be 83.10 μmol/L, which shows a large catalytic activity of Mb in the TiO_2/MWCNTs film to H_2O_2.

Keywords: Myoglobin; Multi-walled carbon nanotubes; TiO_2; Electrocatalysis; Electrochemistry

There has been increasing interest in studying protein in order to prepare the third generation biosensors. Myoglobin (Mb) and multi-walled carbon nanotubes (MWCNTs) have gained considerable attention in recent years for their remarkable electronic and mechanical properties [1,2]. On the other hand, TiO_2 nanoparticles were used as a film-forming material since they have high surface area, optical transparency, good biocompatibility, and relatively good conductivity. Various TiO_2 films were also used to immobilize proteins or enzymes on electrode surface for either mechanistic study of the proteins or fabricating electrochemical biosensors [3,4].

Herein, a novel Mb-TiO_2/MWCNTs film biosensor was fabricated to improve their electroactivity for H_2O_2. The TiO_2 nanoparticles were coupled with MWCNTs successfully via the vapor phase transfer (VPT) method, and Mb could be immobilized on the surface of TiO_2/MWCNTs film by adsorption. Electrochemical behavior of the sensor was studied in detail. Direct electron transfer between Mb and glassy carbon (GC) electrode and the electrocatalytic reduction of H_2O_2 at biosensor was observed. This Mb-TiO_2/MWCNT biosensor responds more sensitively to H_2O_2 than those modified by TiO_2 nanoparticles alone.

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1001-8417/$ – see front matter
doi:10.1016/j.cclet.2008.04.027

1. Experimental

100 mg of MWCNTs was oxidized in 45 mL of concentrated HNO_3 by refluxing for 6 h in a silicone oil bath at 140 °C. Acid-treated MWCNTs (5 mg) and tetrabutyltitanate (100 μL) were added into 4 mL of anhydrous ethanol, after sonicating for 10 min, this solution was transferred into the VPT instrument as a solid phase [5]. The liquid phase at the bottom of the VPT instrument consisted of 4 mL distilled water. Heat treatment on the sealed VPT instrument was conducted at 80 °C for 2.5 h in an oven, and then the solid phase was washed with distilled water three times. The precipitated solid collected by centrifugation, dried at 60 °C in vacuum for 6 h.

Fourier transform infrared (FT-IR) spectra were obtained on a NEXUS 670 (Nicolet) FT-IR instrument at room temperature. Transmission electron micrograph (TEM) images were recorded on a JEOL JEM 200CX transmission electron microscope, using an accelerating voltage of 200 keV.

The products were characterized by using an X-ray powder diffractometer (XRD, D/max 2550 V) with Cu-Ka radiation. The electrochemical response was measured in a conventional three-electrode system using a modified GC electrode as working electrode, a platinum wire as counter electrode, and a SEC (3.5 mol/L KCl) electrode as reference electrode. All potentials were reported in this context with respect to this reference. Prior to the electrochemical experiments, all phosphate buffer solution (PBS) was in thoroughly anaerobic conditions by bubbling with high-purity nitrogen.

2. Results and discussion

Fig. 1 shows the TEM images of MWCNTs with TiO_2 nanoparticles formed on their outer shell. The size of TiO_2 nanoparticles is in the range of 2–10 nm. The interface between MWCNTs and TiO_2 can clearly be observed, indicating that TiO_2 nanoparticles are well attached on the outermost shell of MWCNTs.

XRD patterns of the MWCNTs coated with TiO_2 nanoparticles (a) and the acid-treated MWCNTs (b) are presented in Fig. 2. The peaks of MWCNTs located at 26° (2θ). The crystal structure of MWCNTs may suffer some damage during the refluxing process in concentrated nitric acid to functionalize and shorten the MWCNTs. It is hard to elicit the characteristic peaks of MWCNTs from the spectrum of TiO_2 coated MWCNTs that shows the TiO_2 nanoparticles were fine coated on the surface of MWCNTs.

Fig. 3 shows the FT-IR spectra of Mb-TiO_2/MWCNTs (a), free Mb (b), TiO_2/MWCNTs (c) film. The amide I and II bands of Mb on the surface of TiO_2/MWCNTs (Fig. 3a) had the similar shapes to that of the free Mb (Fig. 3b), except that the bands had a slight shift (1654–1641 cm^{-1} and 1540–1541 cm^{-1} for amides I and II, respectively), which indicated the existence of interaction between Mb and TiO_2/MWCNTs.

Fig. 4A shows cyclic voltammograms (CVs) of the Mb-TiO_2/MWCNTs modified GC electrode and bare GC electrode in pH 7.0 PBS. A pair of well defined and nearly symmetrical redox peaks was obtained, this suggested that the redox peaks were ascribed to the electrochemical reaction of Mb immobilized on the surface of TiO_2/MWCNTs.

Fig. 4B shows the amperometric response for the reduction of hydrogen peroxide on Mb-TiO_2/MWCNTs modified GC electrode at different concentration range. The electrocatalytic current (i_{cat}) linearly increased with increasing concentration of H_2O_2 in the beginning and thereafter began to level off, suggesting that the immobilized enzyme responds to the presence of the substrate in a Michaelis–Menten model. The i_{cat} values are linear with increasing

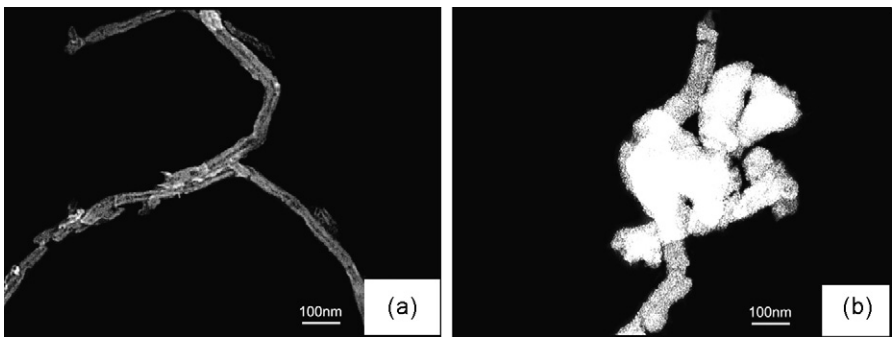

Fig. 1. TEM images of A-MWCNTs (a) and TiO_2/MWCNTs composites (b).

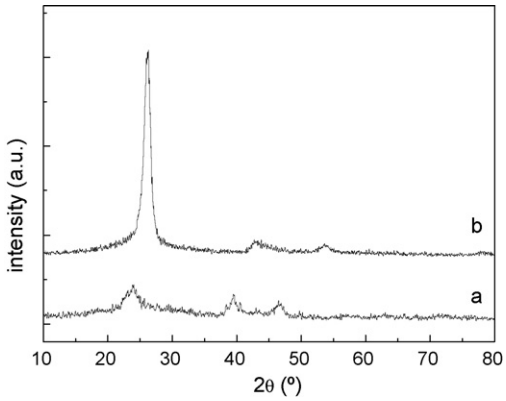

Fig. 2. XRD pattern of TiO_2/MWCNTs (a) and acid-treated MWCNTs (b).

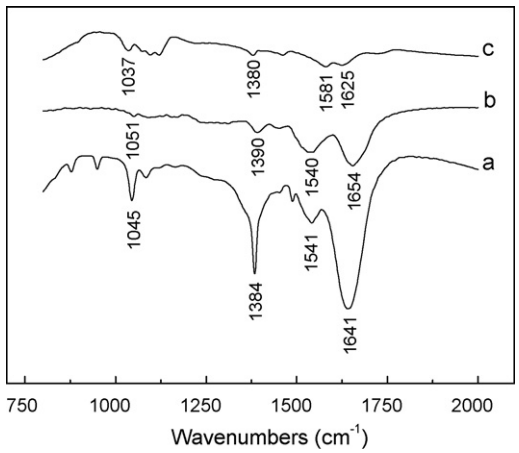

Fig. 3. FT-IR spectra of Mb-TiO_2/MWCNTs (a), free Mb (b), TiO_2/MWCNTs and film (c).

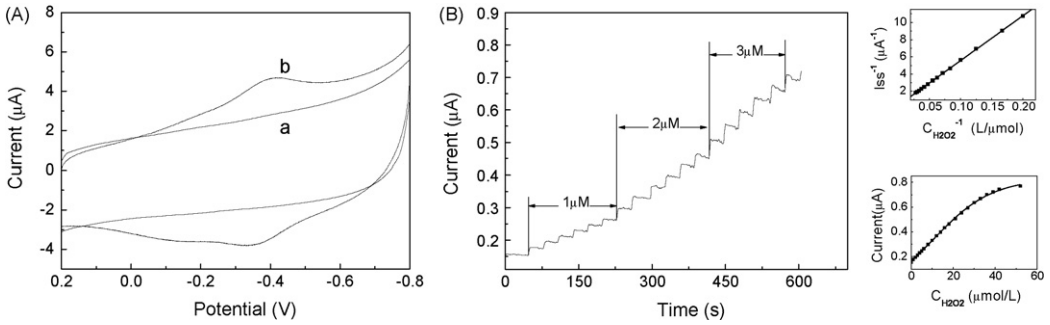

Fig. 4. (A) CVs of the bare GC electrode (a) and Mb-TiO_2/MWCNTs modified GC electrode (b) in pH 7.0 PBS. Scan rate 100 mV/s. (B) Amperometric response of Mb-TiO_2/MWCNTs modified GC electrode with successive addition of H_2O_2 to the 0.1 mol/L pH 7.0 PBS under string. The applied potential is −0.4 V.

concentration of H_2O_2 in the range of 1–42 μmol/L for the Mb-TiO_2/MWCNTs electrode. The detection limit is 0.41 μmol/L H_2O_2 (S/N = 3). The Lineweaver–Burke [6] plot shows an apparent Michaelis–Menten constant (K_M) of 83.10 μmol/L for the Mb-TiO_2/MWCNTs electrode. For comparison, other reported protein films were also listed in Table 1. The Mb-TiO_2/MWCNTs film demonstrates a smaller K_M value, indicating that Mb entrapped in TiO_2/MWCNTs film shows higher peroxidase-like activity.

Table 1
Peroxidase-like activity of some protein films

Film	Detection range (μmol/L)	Detection limit (μmol/L)	K_M (μmol/L)
Mb-TiO_2/MWCNTs[a]	1–42	0.41	83
Mb-titanate nanotubes [4]	2–160	0.6	140
Mb-nanocrystalline TiO_2 [4]	6–80	3.0	1300
Mb-ziroconium phosphonates [7]	–[na]	–[na]	440

na: not available.
[a] This work.

3. Conclusion

A Mb-TiO_2/MWCNTs modified electrode is fabricated. Direct electrochemistry of Mb was performed at this modified electrode. For H_2O_2, this electrode exhibits a low detection limit of 0.41 μmol/L, a linear range from 1 to 42 μmol/L, and an apparent K_M of 83.10 μmol/L. These parameters demonstrate that TiO_2/MWCNTs provide a favorable microenvironment for direct electron transfer of Mb. The immobilized Mb retained their biological activity and showed a good electrocatalytic response to hydrogen peroxide reduction.

Acknowledgment

We greatly appreciate the support of the National Natural Science Foundation of China (No. 20635020, 90606016).

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