Int. J. Electrochem. Sci., 3 **(2008)** 118 - 124

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Hydrogen Peroxide Biosensor Based on the Bioelectrocatalysis of Myoglobin Incorporated in Multi-Walled Carbon Nanotubes/Chitosan Composite Film

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Received: 9 November 2007 / *Accepted:* 29 November 2007 / *Online published:* 20 December 2007

Myoglobin/multi-walled carbon nanotubes/chitosan film (Mb/MWNTs/Cs) electrodes were fabricated based on cross-linking myoglobin (Mb) with multi-walled carbon nanotubes/chitosan (MWNTs/Cs) composite film coated on a glassy carbon electrode (GCE). Direct electrochemistry and electrocatalysis of Mb on the Mb/MWNTs/Cs/GCE were studied. The Mb/MWNTs/Cs/GCE exhibited excellent electrocatalytic activity and rapid response for H_2O_2 in the absence of a mediator. The linear range of detection towards H_2O_2 was from 3.79×10^{-5} to 5.50×10^{-4} mol L^{-1} by the method of amperometric *i-t* curve. The Mb/MWNTs/Cs/GCE had good repeatability and stability for the determination of H_2O_2.

Keywords: Direct electrochemistry, Electrocatalysis, Myoglobin, Chitosan, MWNTs

1. INTRODUCTION

Carbon nanotubes (CNTs) have attracted a great deal of interest because of their dimensions and structure-sensitive properties since CNTs were discovered by Iijima in 1991 [1]. CNTs show electrical properties as metal or semiconductors, depending on their size and lattice helicity [2]. The subtle electronic properties suggest that CNTs, when used as an electrode material in electrochemical reactions, have the ability to promote electron-transfer reactions. CNTs was broadly explored as a kind of material for electrode modification to investigate electrocatalytic activity for some biomolecules including cytochrome c [3,4], NADH [5], horseradish peroxidase [6], hydrogen peroxide [7, 8], and catecholamines such as dopamine [9] and ascorbic acid [10].

Mb, a kind of oxygen transportation protein with the function to store and transport oxygen, has an active heme redox center. Although there is an electroactivity center in the Mb, its large spatial structure makes the electroactivity unexposure. On the other hand, electrode surface could be passivated with Mb absorbing greatly, thus slows down the electron transference and could not obtain good current response. So it is important to construct a biofilm to keep the protein active and facilitate the electron transferring. In recent years, many studies focused on the direct electrochemistry of proteins which were incorporated into the composites to investigate the stability and biocompatibility of films. These films contained water-insoluble surfactants [11,12], hydrogel polymers [13,14], polyelectrolyte- or clay-surfactant composites [15], Cs [16] and MWNTs. Herein, we developed a composite biofilm, which contains MWNTs, Cs and Mb, based on the idea that the MWNTs could facilitate the electron transfer and Cs had good biocompatibility. The preparation of the modified electrodes is rather simple. The mixture of MWNTs and Cs makes the composite film posing the advantages of fast electron transfer and excellent bio-affinity on the modified electrode. The excellent activity and stability of myoglobin sensor was obtained. This film showed favorable electrocatalytic behavior toward H_2O_2.

2. EXPERIMENTAL PART

2.1. Chemicals and reagents

Horse heart Mb (MW 16.7 kDa) was from Sigma. Cs (MW 20, 0000) was purchased from Zhejiang Jinke biochemical limited company. MWNTs crude materials were purchased from Huazhong Normal University (Wuhan, China). The MWNTs crude materials were ultrasonic agitation in 3 mol L^{-1} HNO_3 for 1 h and refluxed in 5 mol L^{-1} HCl for 4 h at 110 ºC. After acid treatment, the samples were calcined in static air at 350 ^{o}C for 2 h. 5 mg purified MWNTs were dispersed with the aid of ultrasonic agitation in 1 mL of chitosan (7 mg mL^{-1}) to give a 5 mg mL^{-1} black suspension. Other reagents were of analytical grade. All solutions were prepared with double distilled water.

2.2. Apparatus

Cyclic voltammetry (CV) experiments were performed by using CHI 660A electrochemical workstation (CH Instrumental, USA) coupled with a conventional three-electrode cell. The working electrode was the Mb/MWNTs/Cs/GCE or a bare GCE, the auxiliary electrode was a platinum wire, and the reference electrode was a saturated calomel electrode (SCE). All the potentials in this paper were given against the SCE.

2.3. Preparation of Mb/MWNTs/Cs/GCE

The GCE was carefully abraded with emery paper, polished on chamois leather containing 0.05μm alumina slurry, and then washed ultrasonically in water, ethanol and water, respectively.

Mb was resolved in 100 mmol L^{-1} pH 7.0 PBS, then 20 μL Mb, 16 μL MWNTs/Cs mixture (c_{Cs}= 7 mg mL^{-1}, c_{MWNTs}= 5 mg mL^{-1}) were mixed. The cleaned GCE was coated by casting 30 μL of the complex and dried in the air for 24 h. Then the Mb/MWNTs/Cs/GCE was prepared.

3. RESULTS AND DISCUSSION

3.1. UV-Vis spectra characterization of Mb/MWNTs/Cs film

Shift of Fe^{III}/Fe^{II} Soret absorb band in Mb could provide whether protein was denatured or not [17]. When protein was denatured, the Soret absorb band would move or disappear. As shown in Fig. 1, the Soret absorb band of Mb, Mb + Cs, Mb + MWNTs and Mb + MWNTs + Cs almost kept consistently, which indicated that microenvironment was little changed around Mb.

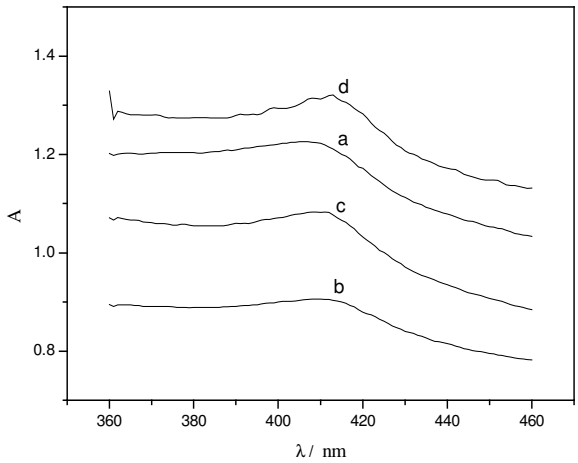

Figure 1. UV-Vis spectra of protein-films on glass slides (a) Mb (b) Mb + Cs (c) Mb + MWNTs (d) Mb + MWNTs + Cs.

3.2. Direct electrochemistry of Mb on Mb/MWNTs/Cs/GCE

Fig.2 was the CV of Mb on the Mb/MWNTs/Cs/GCE (Fig 2c) and the Mb/Cs/GCE (Fig 2a) in the presence of pH 7.0 PBS. At about -0.35 V a pair of stable and a quasi-reversible process was involved. As can be seen, at the same window, the electro-response current of Mb on the Mb/MWNTs/Cs/GCE was higher than that on the Mb/MWNTs/GCE or Mb/Cs/GCE. The reason for the better performance of the MWNTs-modified GCE may be due to the nanometer dimensions of the CNTs, the electronic structure and the topological defects present on the CNTs surfaces [18]. Meanwhile the CNTs increased the effective area of the electrode, so the peak current increased significantly. At the same time, Cs with good compatibility makes it possible to keep bioactivity of Mb.

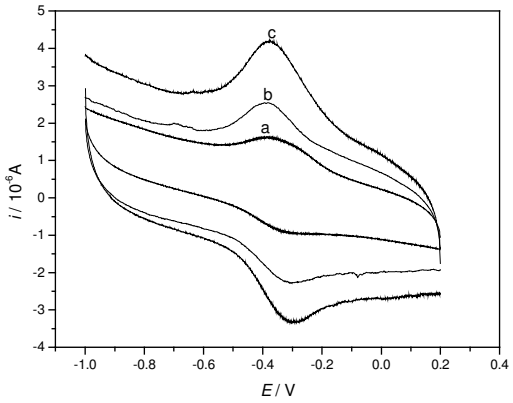

Figure 2. Cyclic voltammograms at 0.2 V s^{-1} pH 7.0 PBS for: (a) Mb/Cs/GCE, (b) Mb/MWNTs/GCE, (c) Mb/MWNTs/Cs/GCE.

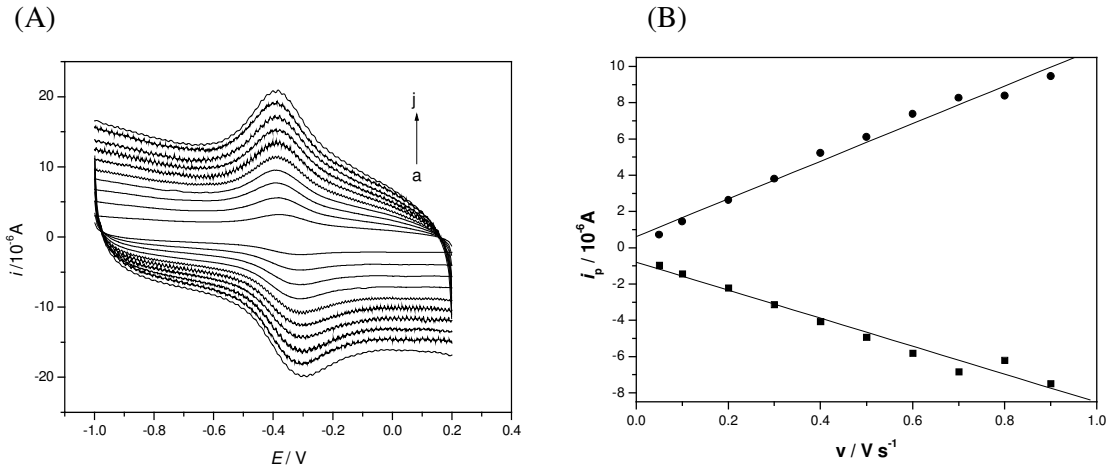

Figure 3. (A) Cyclic voltammograms of Mb/MWNTs/Cs/GCE in 0.1 mol L^{-1} PBS (pH 7.0) at scan rates of (from a to j): 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 V s^{-1}. (B) The relationship between the peak currents (a-i_{pc}, b-i_{pa}) and scan rates.

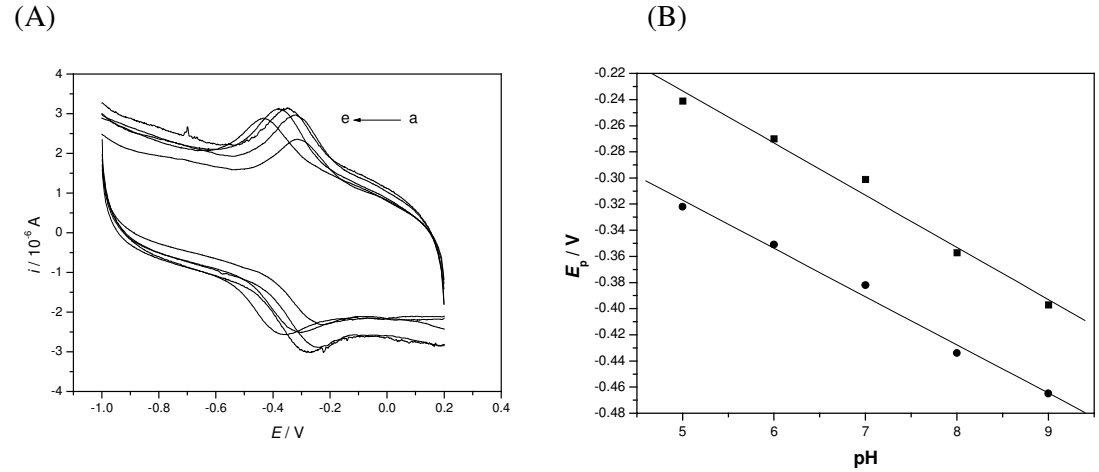

Figure 4. (A) CV of Mb /MWNTs/Cs/GCE at different pH. Scan rate: 0.1 mV s^{-1}. (B) Plots of *E* vs. pH (a to e: 5.0, 6.0, 7.0, 8.0, and 9.0).

From Fig. 3, it could be seen that the peak currents on modified electrode were linearly with the scan rates. This indicates that a surface controlled process is involved on the Mb/MWNTs/Cs/GCE.

Fig. 4 is the plot of peak currents versus pH on the Mb/MWNTs/Cs/GCE. In pH 5.0-9.0, Mb exhibited a pair of irreversible redox peak current on the Mb/MWNTs/Cs/GCE, and anodic and cathodic peak potentials shifted positively with the increase of pH, which indicated that the redox of Mb on the electrode was related with the combine or release of proton.

3.3. Electrochemical catalysis of H_2O_2 on Mb /MWNTs/Cs/GCE

Seen in CV (Fig. 5), with the H_2O_2 concentration increased, oxidation peak decreased gradually, and reduction one increased linearly with the H_2O_2 concentration. When H_2O_2 concentration reach to a certain value, oxidation peak disappeared, and reduction peak value started to departure the linear relationship. Higher H_2O_2 concentration would reduce bioactivity of enzyme, even denature it. In this condition, Michaelis-Menten response happened between electrocatalysis reduction currents and H_2O_2 concentrations. According to the Lineweaver-Burk equation, Michaelis-Menten constant was $K_m^{app}=1.07\times10^{-3}$ mol L^{-1}. Electrocatalytic redox mechanism was supposed to be follows [19]:

$$MbFe^{3+} + H_2O_2 \rightarrow \text{compound I} + H_2O$$
$$\text{compound I} + H^+ + e \rightarrow \text{compound II}$$
$$\text{compound II} + H^+ + e \rightarrow MbFe^+$$

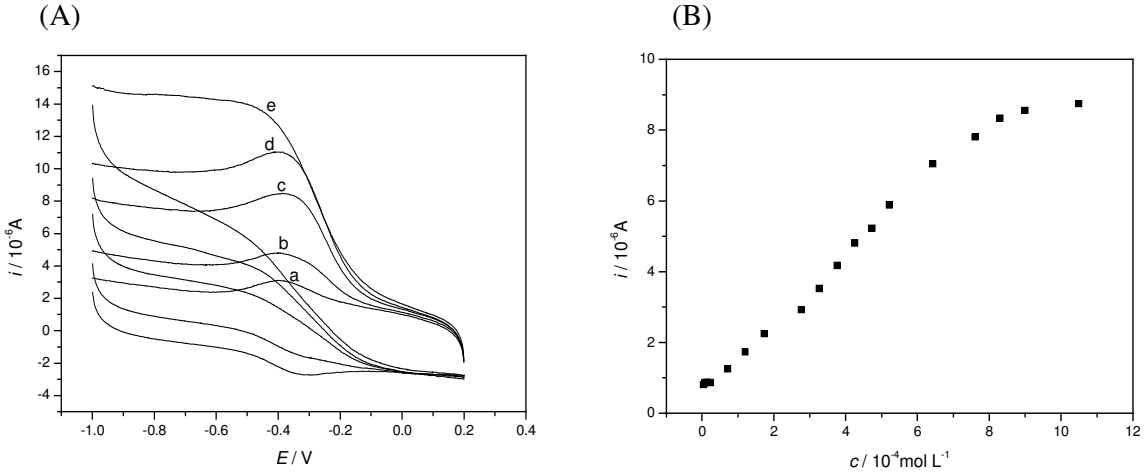

Figure 5. (A) Cyclic voltammograms at 0.1 V s^{-1} on Mb/MWNTs/Cs/GCE in pH 7.0 PBS containing a~e: 0, 1.73×10^{-4}, 4.74×10^{-4}, 6.43×10^{-4}, 1.05×10^{-4} mol L^{-1} H_2O_2. (B) The calibration plot of the catalytic peak current to different concentrations of H_2O_2.

3.4. Amperometry i-t curve

The electrocatalytic reduction of hydrogen peroxide at Mb/MWNTs/Cs/GCE was also studied by amperometry i-t curve. The potential dependence of amperometric signal was tested in the range

from 0 to -0.60 V. The steady-state reduction current increased as the applied potential decreased from 0 to -0.30 V, which was due to the increased driving force for the fast reduction of H_2O_2 at low potential. The response approached maximum at -0.30 V, so we selected this value as the working potential. Fig. 6 illustrates a typical amperometric response of the Mb sensor at -0.30 V on successive step changes of H_2O_2 concentration under stirring. When the same concentration and volume of H_2O_2 was added, the reductive current increased steeply to reach a stable value. The modified electrode achieved 95% of steady-state current within 4s. The current had a linear relationship with the concentration of H_2O_2 for the Mb/MWNTs/Cs/GCE. The reduction currents was linear with H_2O_2 concentrations in the range of 3.79×10^{-5} ~ 5.50×10^{-4} mol L^{-1}, the linear relationship is i (A) = $-6.46\times10^{-7} + 1.08\times10^{-2}c$ (mol L^{-1}), R= 0.999.

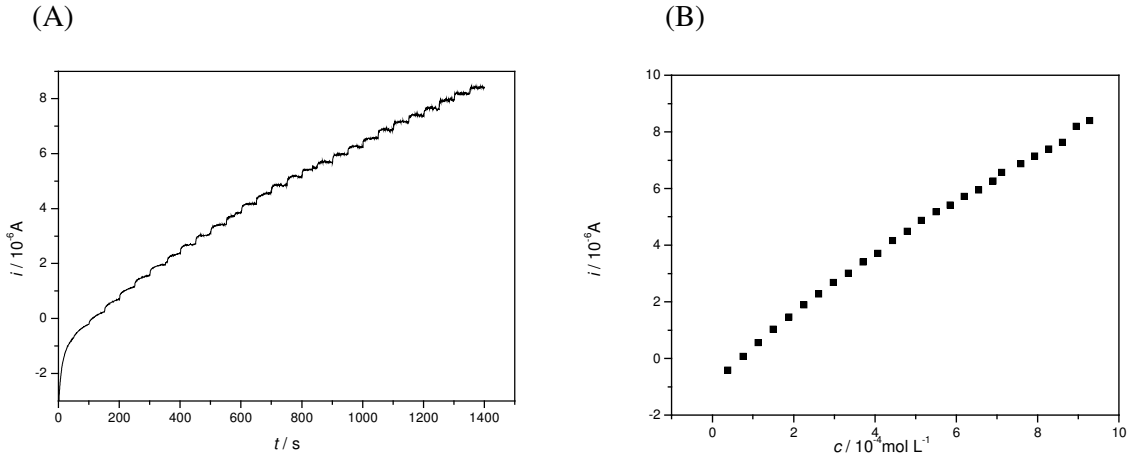

Figure 6. (A)Typical current-time response curve of the sensor upon successive additions of 10 μL every time 10×10^{-3} mol L^{-1} H_2O_2 to 7 mL pH 7.0 buffer at -0.3V. (B)The calibration plot of the catalytic peak current to different concentrations of H_2O_2.

The developed biosensor displayed good stabilization and reproducibility. The biosensor was stored in PBS in a refrigerator at 4 ^oC when not in use. It retained 92% of its initial current response after 6 days. In a series of 5 sensors independently made, a relative standard deviation (RSD) of 5.1 % was obtained for the individual current responses to the same sample.

4. CONCLUSIONS

A new amperometric biosensor for H_2O_2 was prepared based on cross–linking Mb and MWNTs/Cs composite film. Mb retained its original conformation well in MWNTs/Cs composite film which combined the utilities of MWNTs facilitating the electron transfer, and of Cs preserving bioactivity for enzyme on the modified electrode. The biosensor could be applied for amperometric determination of H_2O_2 in the absence of a mediator. The amperometric experiments showed excellent electrocatalytical activity of the biosensor for H_2O_2.

ACKNOWLEDGEMENT
This work was supported by the National Nature Science Foundation of China (No. 20575017).

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