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Direct Electrochemistry and Electrocatalysis of Hemoglobin in MWCNTs-TiO₂/Nafion Composite Matrix

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Abstract : Multiwalled carbon nanotubes-TiO₂ (MWCNTs-TiO₂) core-shell nanocomposites were synthesized by the vapor phase transfer method and dispersed in Nafion solution to form a composite matrix for the fabrication of hemoglobin (Hb) biosensor. The Hb/MWCNTs-TiO₂/Nafion film exhibited a pair of well-defined, quasi-reversible redox peaks. This biosensor showed excellent electrocatalytic activity to H₂O₂. The sensitivity and apparent Michaelis–Menten constant of this Hb biosensor to H₂O₂ were 0.78 A/M/cm² and 34.9 μmol/L, respectively, which indicated that Hb had high affinity to H₂O₂.

Keywords : Hemoglobin, TiO₂, Multi-walled carbon nanotubes, Nafion, Bioelectrochemistry

Hemoglobin (Hb) usually can not exhibit heterogeneous electron transfer processes at conventional electrodes owing to its large protein size and the inaccessibility of the electroactive centers as well as its denaturation caused by strong adsorption on the electrode surface. Nanotechnology has provided a novel way to enhance electron-transfer rate between protein and electrode [1, 2]. Many proteins immobilized on the multi-walled carbon nanotubes (MWCNTs) have realized direct electron transfer for the excellent electronic property of MWCNTs [1]. Nano TiO₂ has high specific surface, abundant surface-active groups, excellent biocompatibility and chemical stability, it has been reported that Ti atom on the surface of nano TiO₂ has coordinative affinity for the oxygen of the carboxylic group of the amino acids, and less denaturation of absorbed proteins were found [3]. Thus, the nanocomposite of multiwalled carbon nanotubes (MWCNTs) coated with TiO₂ may induce interesting charge transfer and enhance the electrocatalytic activity of enzymatic bioelectrode due to the excellent biocompatibility and electronic property of nano TiO₂ and MWCNTs.

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Since Nafion is biocompatible, the presence of moderately hydrophobic Nafion polymer in the biocomposite film not only makes the film uniform but also can maintain optimum enzyme activity [4]. In this work, MWCNTs-TiO₂ nanocomposite synthesized by the vapor phase transfer method and Nafion inorganic-organic hybrid film was used as the immobilization matrix for Hb. This composite matrix combined the advantages of inorganic composite (MWCNTs-TiO₂) and organic polymer (Nafion), which could promote the direct electron transfer of Hb. The direct electron transfer between Hb and glassy carbon electrode (GCE) was realized. This biosensor displayed high electroactivity and electrocatalytic response to H₂O₂.

1. Experimental : MWCNTs were oxidized in concentrated HNO₃ by refluxing for 10 h at 140 °C. This cut the MWCNTs into shorter tubes and also opens up carboxylic groups and hydroxyl groups on the sidewalls [5]. The acid-MWCNTs were dispersed in a 1 wt% sodium dodecyl sulfate (SDS) aqueous solution by ultrasonication for 3 h, in order to make SDS adsorb on the surface of MWCNTs. After rinsing repeatedly and drying, 5 mg of SDS adsorbed MWCNTs and 100 μL of tetrabutyltitanate were added into 4 mL of anhydrous ethanol, after being sonicated for 30 min, this solution was transferred into beaker A (10 mL, volume). Then the beaker A was put into beaker B (20 mL, volume) which contained 4 mL twice-distilled water. The instrument was sealed and heated for 3 h at 80 °C in an electronic oven. After the synthesis, the solid product recovered by centrifugation was washed several times with anhydrous ethanol and twice-distilled water, and dried at 60 °C in vacuum.

200 μL of MWCNTs-TiO₂ (1 mg/mL) suspension was mixed with 200 μL of 2 wt% Nafion solution and 200 μL of Hb solution (6 mg/mL, pH 6.5). The resulting suspension (5 μL) was deposited onto the electrode surface. The electrode was then left to dry and stored at 4 °C when not in use.

Electrochemical measurements were carried out in a conventional three-electrode system using a modified GCE as working electrode, a platinum wire as counter electrode, and a saturated calomel electrode (SCE) as reference electrode. Phosphate buffer solutions (PBS) were deoxygenated before and during measurements.

2. Results and discussion : Fig. 1 shows the TEM images of MWCNTs before (Fig. 1a) and after (Fig. 1b) coated with TiO₂ nanoparticles. When MWCNTs were coated with TiO₂, the diameters of MWCNTs were significantly larger than those of pristine acid-treated MWCNTs. The nanotube surface was homogeneously and compactly covered with TiO₂ nanoparticles. The interface between MWCNTs and TiO₂ could clearly be observed, indicating that TiO₂ nanoparticles were well attached on the outermost shell of MWCNTs.

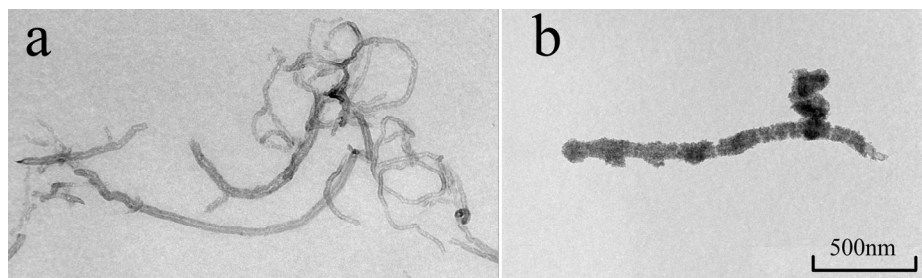


Fig. 1. TEM images of acid-MWCNTs (a) and MWCNTs-TiO₂ core-shell hybrid nanocomposite (b).

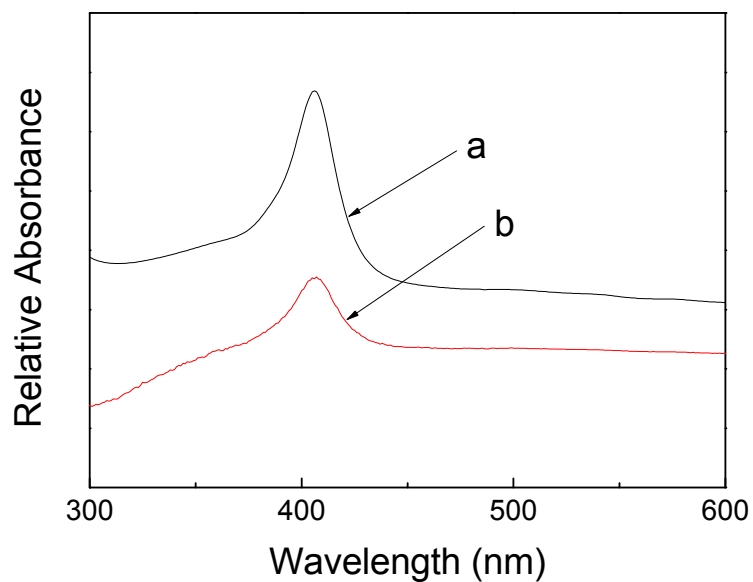


Fig. 2. UV-vis spectra of Hb in 7.0 PBS (a) and Hb in MWCNTs-TiO₂/Nafion biocomposites (b).

UV-vis spectroscopy is a useful tool for monitoring the possible change of the Soret adsorption band in the heme group region. As shown in Fig. 2, the Soret absorption of immobilized Hb was located at 407 nm, just 1 nm red shift compared with native Hb (i.e. 406 nm), suggesting that there was no significant denaturation for the immobilized Hb.

Fig. 3 shows typical cyclic voltammograms of different modified electrodes. A wide peak (Fig. 3e) was observed, which was attributed to the redox of carboxylic groups of some MWCNTs which were not coated with TiO₂ completely [6]. A pair of well-defined, quasi-reversible redox peaks of Hb for the Hb(FeIII)/Hb(FeII) redox couple transformation

were observed at the Hb/MWCNTs-TiO₂/Nafion/GCE (Fig. 3a). The cathodic and anodic peak potentials were located at -0.391 and -0.330 V, respectively. The peak to peak separation (ΔE_p) was 61 mV at a scan rate of 100 mV/s. Compared with Hb/MWCNTs-TiO₂/Nafion/GCE, the Hb/GCE only showed an irreversible and small reduction peak (Fig. 3c) and the redox peaks observed at the Hb/Nafion/GCE were smaller (Fig. 3b). It revealed that MWCNTs-TiO₂ played an important role in facilitating the direct electron transfer of Hb by the synergetic with Nafion.

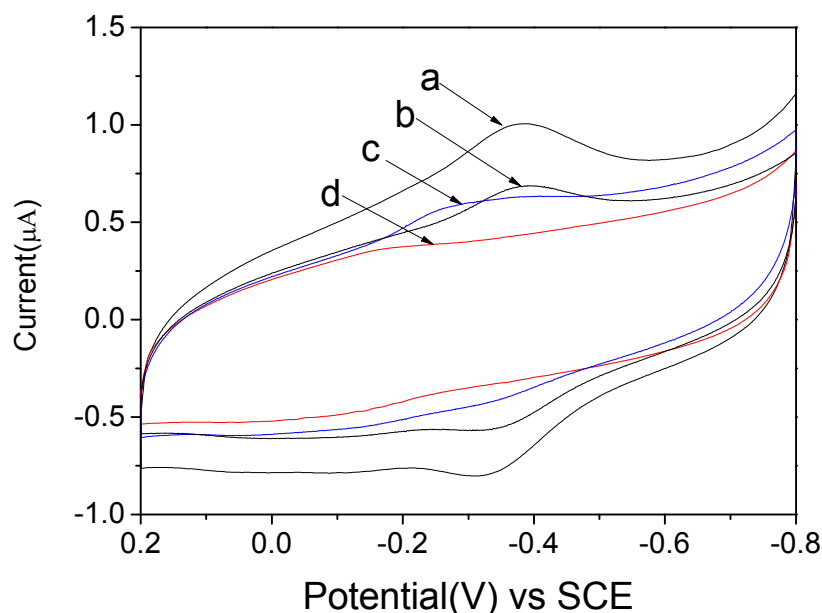


Fig. 3. Cyclic voltammograms of Hb/MWCNTs-TiO₂/Nafion/GCE (a), Hb/Nafion/GCE (b), Hb/GCE (c) and MWCNTs-TiO₂/Nafion/GCE (d) in 0.1 M pH 7.0 PBS at 100 mV/s.

Fig. 4 shows the amperometric response of Hb/MWCNTs-TiO₂/Nafion/GCE to H₂O₂. The electrocatalytic current (i_{cat}) linearly increased with increasing concentration of H₂O₂ in the beginning and thereafter began to level off, suggesting that the immobilized enzyme responded to the presence of the substrate in a Michaelis-Menten model. The i_{cat} had a good linear relationship with the concentration of H₂O₂ in the range of 2×10^{-7} to 2×10^{-5} mol/L and the detection limit (S/N=3) was 6.5×10^{-8} mol/L. The sensitivity of this biosensor was 0.78 A/M/cm², which was higher than those of 0.6 A/M/cm² [7] and 2.85×10^{-3} A/M/cm² [8]. The apparent Michaelis-Menten constant (K_m^{app}) calculated by the Lineweaver-Burk equation [9] was 34.9 µmol/L, which was smaller than those of Hb immobilized on cobalt oxide particles, 0.385 mM [10], Hb entrapped in chitosan and CaCO₃ nanoparticles, 0.75 mM [11]. The higher sensitivity and smaller K_m^{app} value implied that Hb in this composite film possessed higher enzymatic activity and this biosensor exhibited higher affinity towards H₂O₂ than other H₂O₂ biosensors. This could ascribe to that Hb molecules could be tightly absorbed on the MWCNTs-TiO₂ by the electrostatic force and had relative favorable orientation which

was beneficial for the realization of the whole activity of Hb. Since Nafion is biocompatible, the presence of Nafion in the biocomposite film not only makes the film uniform but also could lead to the increased activity of Hb [4]. In addition, three-dimension silica sol-gel network has good affinity to water, which provides an excellent microenvironment to maintain the catalytic activity of Hb [12].

This biosensor had good long-term storage stability, after storing it in refrigerator (4 °C) in a dry state for 20 days, it retained 97% of the initial current response.

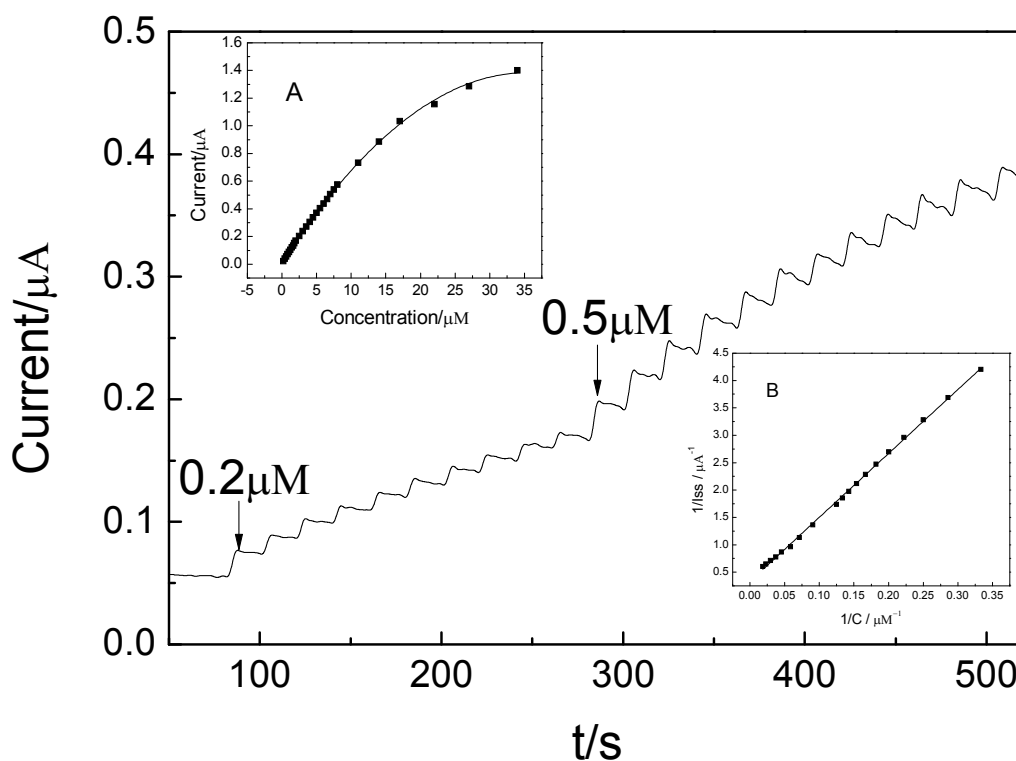


Fig.4. Amperometric response of Hb/MWCNTs-TiO₂/Nafion/GCE at -0.39 V upon successive injection of different concentration of H₂O₂ into 0.1 M pH 7.0 PBS while stirring. Inset: plot of catalytic current vs. H₂O₂ concentration (A), recorded plot for determination of K_m^{app} (B).

3. Conclusions

Hb was effectively immobilized in MWCNTs-TiO₂/Nafion inorganic-organic composite matrix. Direct electron transfer was realized between Hb and the GCE surface due to the synergic effect of MWCNTs-TiO₂ and Nafion. Hb in the three-dimension structure retained its near-native conformation. This biosensor showed highly catalytic activity to H₂O₂, the sensitivity and K_m^{app} of the immobilized Hb to H₂O₂ were 0.78 A/M/cm² and 34.9 μmol/L,

respectively. The hybrid material can provide a good electrochemical sensing platform for the applications in biosensor and biocatalysis.

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