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A novel hydrogen peroxide sensor based on hemoglobin immobilized PAn–SiO_2/DTAB composite film

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ABSTRACT

A novel hydrogen peroxide (H_2O_2) sensor based on hemoglobin (Hb) immobilized polyaniline–silica (PAn–SiO_2) and dodecyltrimethylammonium bromide (DTAB) composite film modified carbon paste electrode (CPE) was fabricated and the direct electron transfer of immobilized Hb was investigated. On Hb/PAn–SiO_2/DTAB/CPE, Hb displayed a fast electron transfer process accompanied with one proton participate in and its electron transfer rate constant which was estimated at 0.94 s^{-1}. Thus, the proposed biosensor exhibited a high sensitivity and excellent electrocatalytic activity to the reduction of H_2O_2. The catalytic reduction current of H_2O_2 was proportional to H_2O_2 concentration in the range of 0.09 to 2.8 mM with the detection limit of 0.03 mM. The apparent Michaelis–Menten constant (K_m^{app}) of the biosensor was calculated to be 0.275 mM, exhibiting high enzymatic activity and affinity. With the characteristics above, the sensor in this paper can be potentially applied in other reactive oxygen species as well.

1. Introduction

Highly sensitive and selective detection of H_2O_2 is of greatly practical importance in chemical, biological, clinical, environmental and many other fields. Many traditional techniques for this purpose have been employed, such as titrimetry [1], spectrometry [2], chemiluminescence [3] and electrochemistry [4–6]. Among these methods, electrochemistry biosensors [7,8] based on electrocatalysis of immobilized enzymes towards H_2O_2 are useful due to their simplicity, high sensitivity and selectivity. In recent years, because of its better electrocatalytic activity for H_2O_2, many electrochemical heme proteins and enzyme have been used to construct H_2O_2 sensor. Hemoglobin (Hb), one heme protein, is generally used as a representative to investigate the dynamic and thermodynamic mechanism of the redox reactions and develop biosensors for the determination of H_2O_2 due to its well-documented structure and intrinsic peroxidase activity. However, at the bare electrode, Hb does not display electroactivity due to the deep burying of the electroactive groups within the protein structure, unfavorable orientation [9]. Therefore, in order to prepare excellent biosensors, many materials were employed to improve the microenvironment around proteins, and accelerate the electron transfer between protein and the electrode surface. For example, when electrodes were modified with nanoparticles [10], conducting polymers [11], and ionic liquid [12], Hb showed direct electrochemical behavior and good electrocatalytic ability to the reduction of H_2O_2. Thus finding a new material and new system is the key of constructing H_2O_2 sensor.

Mesoporous materials are porous materials with the crystal structure, which can be used to immobilize proteins on the electrode surfaces due to their unique properties such as variable pore diameter, large surface area, excellent absorbability and excellent chemical stability. In recent years, a number of mesoporous materials such as mesoporous silicas [13,14], mesoporous titanium [15], molecular sieves MCM-41 [16], mesoporous Al_2O_3 [17] and mesoporous carbons [18] have been widely studied about the immobilization of Hb. It was shown that these materials enhanced the direct electron transfer between Hb and electrodes and Hb immobilized on the electrodes exhibited excellent electrocatalytic effect toward the reduction of H_2O_2. Furthermore, mesoporous silica materials have been used as template for the preparation of new nanocomposites. Among some composite materials, much attention has also been paid to conducting polymers, such as polypyrrole (PPy) and polyaniline (PAn) for their outstanding conductivity and chemical stability [19,20].

2. Experimental

2.1. Reagents and apparatus

Hb was obtained from Sigma and used without further purification. DTAB was obtained from Kermel Chemical Reagent Co., Ltd (Tianjin, China). Mesoporous silica and PAn–SiO_2 composite materials were prepared following the procedures described in literature [21,22]. Stock solutions of H_2O_2 were freshly diluted 30% solution (purchased from Xi'an Chemical Reagent Company China). Phosphate

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buffer solutions (PBS, 0.1 M) with various pH values were prepared by mixing stock standard solutions of K_2HPO_4 and KH_2PO_4 and adjusting the solution pH with H_3PO_4 and NaOH. All solutions were prepared with double-distilled water which purified in Millipore system (U.S. Millipore companies).

All electrochemical measurements were performed on a CHI660B electrochemical workstation (Shanghai Chenhua Co., China) in a conventional three-electrode system, equipped with a saturated calomel electrode (SCE) reference electrode, a platinum wire counter electrode and modified CPE working electrode. Prior to measurement, all solutions were deoxygenated by bubbling highly purity nitrogen for at least 20 min and maintained in nitrogen atmosphere during the experiments. UV–vis adsorption spectra were performed with Evolution 300 UV–vis Spectrophotometer (Thermo, USA). FT-IR spectrum (IR Prestige-21, Shimadzu, Japan) was obtained by forming transparent KBr pellet containing the sample. DL-180 ultrasonic apparatus (Haitian Electronic Apparatus Company, China) was applied in ultrasonic experiment. The morphologies of the resultant electrodes were observed with SEM (model: JEOL JSM-2000) operated at an accelerating voltage of 15 kV.

2.2. Preparations of the modified electrodes

The traditional CPE was prepared as followed: 4.0 g of graphite powder and 1 mL of paraffin oil were mixed by hand to a homogeneous carbon paste in an agate mortar. The carbon paste was packed into the cave of glass tube with the diameter of 3.0 mm. The electrical contact was got with a copper wire connected to the paste in the tube. Prior to use, the CPE was polished with weighing paper. 1 mg PAn–SiO_2 was dispersed into 1 mL of 0.3 mM DTAB solution with the deoxygenated 0.1 M PBS at pH 7.0 and ultrasonic dispersion 20 min. 10 μL of the obtained suspension (PAn–SiO_2/DTAB) was dropped on the bare electrodes and allowed to dry at room temperature. After drying, 5 μL of Hb solution (10 mg Hb was dissolved in 1 mL of the deoxygenated 0.1 M PBS at pH 7.0) was dropped on the PAn–SiO_2/DTAB/CPE surface and allowed to dry at 4 °C overnight to prepare the Hb/PAn–SiO_2/DTAB/CPE. By the similar way, DTAB/CPE and Hb/DTAB/CPE were prepared. All of these modified electrodes were stored at 4 °C in air when not in use.

3. Results and discussion

3.1. Spectroscopic analysis of Hb/PAn–SiO_2/DTAB composite film

UV–vis spectroscopy analysis is a useful technique for characterizing the structure of heme protein in modified electrodes. The shape and position of the Soret absorption band of heme iron can provide information about the possible denaturation of heme protein, especially the conformational change of the heme-group region. Fig. 1 shows the UV–vis spectra of PAn–SiO_2/DTAB, Hb/PAn–SiO_2/DTAB and Hb in 0.1 M pH 7.0 PBS. As can be seen, no adsorption band for PAn–SiO_2/DTAB (Fig. 1a) was observed while the Soret bands of Hb entrapped in the Hb/PAn–SiO_2/DTAB (Fig. 1b) and natural Hb (Fig. 1c) were located at 411 nm and 404 nm, respectively. The slight shift of the Soret band for Hb/PAn–SiO_2/DTAB in comparison with natural Hb may be due to the interaction between surfactant and Hb or adsorption of PAn–SiO_2 particles for Hb. The results suggested that such interactions neither destroyed the structure nor changed the fundamental microenvironment of Hb.

An FT-IR spectroscopy is also a sensitive method to probe into the structure and conformation of proteins. The infrared absorption band of amide I (1700–1600 cm^{-1}) and amide II (1620–1500 cm^{-1}) of proteins can provide detailed information on the secondary structure of the polypeptide chain [23]. The amide I band is attributed to the C═O stretching vibrations of peptide linkage in the backbone of protein. The amideIIband is caused by a combination of N – H bending and C – N stretching vibration of peptide groups. If protein molecules are denatured, the intensity and shape of the amide I and amide II bands will diminish or even disappear [24]. As can be seen in Fig. 2, FT-IR spectrum of native Hb (Fig. 2b) shows two obvious adsorption bands at 1631 cm^{-1} and 1592 cm^{-1} which are attributed to the amide I and amide II infrared absorbance of Hb. Fig. 2c shows the adsorption bands of PAn–SiO_2/DTAB at 1629 cm^{-1}. For Hb/PAn–SiO_2/DTAB system (Fig. 2a), its spectrum not only shows the corresponding peaks of PPy–SiO_2/DTAB, but also exhibits amides I (1630 cm^{-1}) and II (1594 cm^{-1}) of immobilized Hb, which are all essentially similar to that obtained for native Hb. The results suggested that Hb adsorbed on PAn–SiO_2/DTAB composite films retained its native secondary structure. The results suggested that Hb adsorbed on PAn–SiO_2/DTAB composite films retained its native secondary structure.

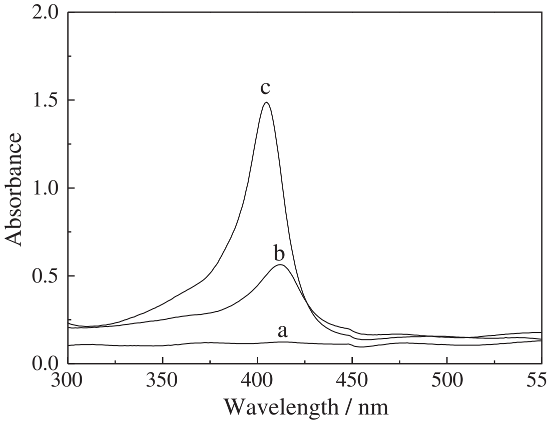

Fig. 1. UV–vis spectra of (a) PAn–SiO_2/DTAB, (b) Hb/PAn–SiO_2/DTAB and (c) Hb in 0.1 M pH 7.0 PBS.

Fig. 3 shows the scanning electron microscopy (SEM) of the PAn–SiO_2 film, the PAn–SiO_2/DTAB film and the PAn–SiO_2/DTAB/Hb film, respectively. PAn–SiO_2 film alone revealed the uneven massive granular and needle-like material on the surface of CPE (Fig. 3A), which may due to partial reunion of needle-like PAn–SiO_2. However, when DTAB was mixed with PAn–SiO_2, the appearance of the composite obviously changed from needle-like morphology to a smooth coral-like (Fig. 3B). When Hb is introduced, the morphology of Hb immobilized PAn–SiO_2/DTAB films is significantly changed (Fig. 3C). Hb deeply penetrates into the composite films and formed a layer of oxide film. The results indicated that Hb successfully adsorb to the surface of electrode.

3.2. Direct electrochemistry of Hb/PAn–SiO_2/DTAB modified electrode

Fig. 4 shows the cyclic voltammograms (CVs) of different modified electrodes in the 0.1 M pH 7.0 PBS at 0.1 V s^{-1}. In the potential range from −0.8 to 0.4 V, no obvious redox peaks occurred at DTAB/CPE (curve d), PAn–SiO_2/CPE (curve e), and CPE (curve g), which indicated no electroactive substance existed on the electrode surface. The CVs of the Hb/CPE (curve f) showed that only a small reduction peak appeared, indicating the direct electron transfer of Hb was not realized. While a couple of small redox peaks observed at Hb/DTAB/CPE (curve b) and Hb/PAn–SiO_2/CPE (curve c) indicated that the direct electron transfer reaction occurred between Hb and the modified electrode. However, a couple of well-defined quasi-reversible redox peaks appeared at the Hb/PAn–SiO_2/DTAB/CPE (curve a) and the electrochemical response of Hb was greatly enhanced. This demonstrated the presence of DTAB and PAn–SiO_2 nanomaterials could facilitate the electron transfer between electrode and redox protein. The anodic and cathodic peak potentials of redox peaks were at −0.184 V and −0.285 V, respectively. The peak potential