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A H_2O_2 electrochemical biosensor based on biocompatible PNIPAM-g-P (NIPAM-co-St) nanoparticles and multi-walled carbon nanotubes modified glass carbon electrode

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ABSTRACT

The poly(N-isopropylacrylamide)-g-poly(N-isopropylacrylamide-co-styrene) (PNIPAM-g-P (NIPAM-co-St), we denote as PNNS in the later content) nanoparticles were obtained by an emulsifier-free emulsion polymerization method. Hemoglobin (Hb), as a model enzyme, was immobilized on the film, which was mixed by multi-walled carbon nanotubes (MWCNTs) and PNNS nanoparticles to construct a novel H_2O_2 biosensor. The PNNS/MWCNTs films were examined by scanning electron microscopy (SEM) and Fourier transform infrared spectrophotometer (FTIR). The performances of the PNNS/MWCNTs/GCE were characterized with cyclic voltammetry (CV), electrochemical impedance spectra (EIS) and typical amperometric response (i-t) measurements. The immobilized Hb maintains its bioactivities and displays an excellent electrochemical behavior with a formal potential of −349 mV. The biosensor exhibited a good electrocatalytic activity to the reduction of hydrogen peroxide (H_2O_2). The linear response range of the H_2O_2 biosensor was from 1.0×10^{-7} to 5.9×10^{-4} M with a low detection limit of 2.9×10^{-8} M. The apparent Michaelis–Menten constant (K_{app}^{M}) of Hb on the PNNS/MWCNTs film was estimated to be 0.19 mM, showing its high affinity to H_2O_2 and good bioactivity of the Hb/PNNS/MWCNTs film toward H_2O_2 reduction. Good stability and repeatability were assessed for the biosensor.

1. Introduction

In recent years, direct electron transfer between biomolecules and electrode has been extensively studied because of its great importance in fundamental science in biological reactions, bioelectronics and biosensors [1–5]. The direct electrical communication between redox protein and electrode surface could particularly provide the way for superior mediator-free and sensitive biosensors, which cannot only work at a sensing potential closer to the potential of the redox protein to eliminate interference reactions, but also simplify the detection system for less reagent and better stability [6]. While great efforts have been made to facilitate the direct electron transfer of redox-active proteins at an electrode and, to this end, recent years have witnessed a rapid progress in the protein electrochemistry and thereby the development of so-called third-generation electrochemistry biosensors [7–11]. It remains a challenge to investigate the protein unfolding with the protein electron transfer properties. Because the proteins usually have large and complex structure, where the redox centers are deeply immersed in the bodies, and three-dimensional structures hinder onto electrodes and the unfavorable orientations at the electrode [12,13]. An approach to realize direct electrochemistry of proteins and enzymes is to incorporate them into films to fabricate a modified electrode surface. Thin films may provide a well-defined microenvironment for proteins, and enhanced the direct electron transfer between proteins and electrodes.

Hemoglobin (Hb) consisting of a four-polypeptide chain, each with one heme group, can store and transport oxygen in red blood cells. Studies of the electrochemical behavior of heme proteins are an important essential for a fundamental understanding of their biological activity. Hb is an ideal molecule for the study of electron transfer reactions of heme proteins because of its commercial availability, moderate cost, and its known and documented structure [14]. To explore the methods of increasing the electron transfer rate between Hb and the electrode, great efforts have been devoted to the characterization of the electrochemistry of Hb using electrodes modified with films such as Nafion–nano-$CaCO_3$ [15], sol–gel silica [16], TiO_2 nanotube [17], PEO–PPO–PEO triblock copolymer [18] and gold colloids [19].

Among the biocompatible and biodegradable polymers, poly (N-isopropylacrylamide) (PNIPAM) is certainly the most commonly

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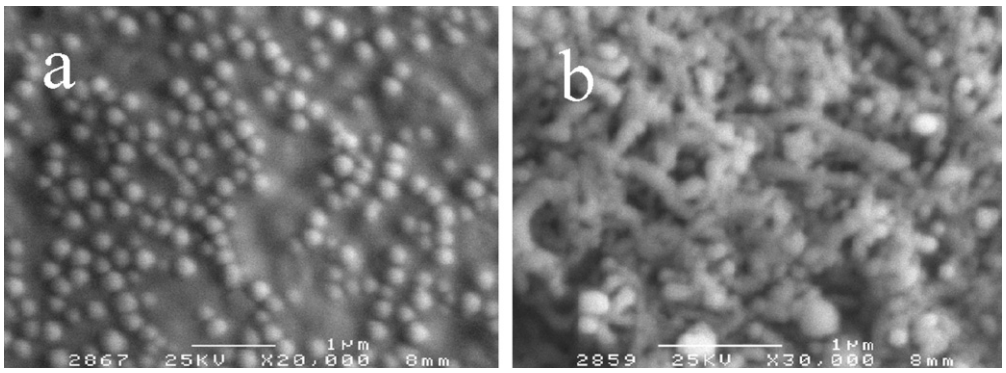

Fig. 1. SEM image of PNNS (a) and PNNS/MWCNTs films (b).

used thermoresponsive polymer. It exhibits a lower critical solution temperature (LCST) of 32 °C, which is very close to the physiologic temperature [20]. Below LCST, PNIPAM is hydrophilic, while it is hydrophobic above. The LCST can be shifted by copolymerization with hydrophilic or hydrophobic monomers, pH, co-solvents, or by additives such as salts [21]. It is well known that due to the size effect, nanomaterials exhibit novel properties compared with the bulk materials. Our group prepared the PNNS nanoparticles and explored their properties, such as good solubility and dispersibility in water, and satisfactory biocompatibility [22,23].

Carbon nanotubes (CNTs) have gained considerable attention in recent years for their remarkable electronic and mechanical properties. The closed topology and the tubular structure of CNTs make them unique among different carbon forms and provide useful pathways for chemical studies. The immobilization of proteins on carbon nanotubes has been proved to be an effective method for biosensing applications [12]. Basically, CNTs have two distinct types of structures: singlewalled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). SWCNTs are one-dimensional conductor with all electrons moving in an atomic layer having surface atoms. MWCNTs have a complex structure with each carbon layer having different chirality and electronic properties [24]. In this paper, MWCNTs were chosen to modify the electrodes.

Due to the good biocompatibility of PNNS and conductivity of MWCNTs, PNNS nanoparticles and MWCNTs were chosen to modify the GCEs for the retention of Hb's biological activity and the electron transfer between Hb and electrodes. The Hb/PNNS/MWCNTs film biosensor was fabricated to improve their electroactivity for H_2O_2. Electrochemical behavior of the sensor was studied in detail. Direct electron transfer between Hb and GCE and the electrocatalytic reduction of H_2O_2 at biosensor were observed.

2. Experiments

2.1. Reagents

NIPAM (99%, Aldrich Chemical Co. Inc., US) was used without further purification. Styrene (St) was distilled under reduced pressure and then stored in the refrigerator before use. Hb (Sigma–Aldrich Co., US) was dry power and dialyzed and purified before use. Phosphate buffer solution (PBS) was prepared by mixing stock standard solution of Na_2HPO_4 and NaH_2PO_4. Hydrophilic multi-walled carbon nanotubes (MWCNTs) were obtained from Shenzhen Nanotech Port Co. Ltd., and H_2O_2 (30%) was from Nanjing Chemical Plant. All solutions were made up with twice-distilled water. Other reagents were of analytical grade.

2.2. Preparation of PNNS nanoparticles

The preparation method of PNNS nanoparticles is described in Ref. [22].

2.3. Construction of the Hb/PNNS/MWCNTs modified electrode

Prior to the modification, the GCE was polished to mirror-like surface with 0.3 and 0.05 μm alumina slurry followed by rinsing thoroughly with double-distilled water, and was successively sonicated in ethanol and double-distilled water for 5 min, respectively, and then allowed to dry at room temperature. 2.5 mg MWCNTs were dispersed in 1.0 mL of dimethylformamide (DMF) solution by sonication for 60 min. Equivalent volume of 2.5 mg mL^{-1} MWCNTs solution and 0.125 mg mL^{-1} PNNS solution were hand-mixed thoroughly, and then 8.0 μL of the resulting mixture and equivalent volume of 5.0 mg mL^{-1} Hb solution in PBS (pH 7.0) were dropped onto the GCE surface successively and dried in room temperature. So that the Hb/PNNS/MWCNTs modified electrode was obtained, and the same modified method was used to get the Hb/PNNS/GCE. When not in use, the electrodes were stored at 4 °C in a refrigerator.

2.4. Apparatus and measurements

Scanning electron microscopy (SEM) image was recorded on a JSM Model 6300 scanning electronic microscopy. The FTIR spectra of PNNS nanoparticles, Hb, Hb/PNNS and Hb/PNNS/CNTs were measured using a Cary 5000 Fourier transform infrared (FTIR) spectrophotometer from VARIAN Company. All electrochemical experiments were performed on a CHI 760C electrochemical analyzer (CH Instruments, Inc., US), using a conventional three-electrode system with a glassy carbon electrode (GCE) (3 mm in diameter, Shanghai Chenhua, China) as the working electrode, a platinum wire as the auxiliary electrode and a saturated calomel electrode (SCE) as the reference electrode. Cyclic voltammogram (CV) experiments were carried out in quiescent solution at 100 mV s^{-1} in 5 mL of 0.1 M PBS, and the solution was purged with high purity nitrogen prior to and blanked with nitrogen during the electrochemical experiments. Electrochemical impedance measurements were performed in a 0.1 mM PBS containing 1 mM $K_3Fe(CN)_6$ and 1 mM $K_4Fe(CN)_6$ and plotted in the form of complex plane diagrams (Nyquist plots) with a frequency range of 0.01 Hz to 100 kHz. The amplitude of the applied sine wave potential is 5 mV, where the formal potential of the system was set at 188 mV. The current-time curves were recorded in a stirred cell with a successive addition of H_2O_2 standard solution to the cell at an operating potential of −350 mV.