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Hydrogen peroxide biosensor based on the bioelectrocatalysis of horseradish peroxidase incorporated in a new hydrogel film

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Abstract

Horseradish peroxidase was immobilized on a glass carbon electrode by poly (*N*-isopropylacyamide-*co*-3-methacryloxy-propyltrimethoxysilane) (PNM). Horseradish peroxidase entrapped in the PNM film exhibited a pair of well-defined, quasi-reversible cyclic voltammetric peaks at about −0.36 V versus a saturated calomel electrode (SCE) in pH 7.0 buffer solution, corresponding to $\text{heme}Fe^{III} + e \rightarrow \text{heme}Fe^{II}$. Some electrochemical parameters were calculated by performing nonlinear regression analysis of square wave voltammetry (SWV) experimental data. Fourier transform infrared (FTIR) spectra suggested that horseradish peroxidase entrapped in the PNM film retained the secondary structure. Hydrogen peroxide could be reduced by the catalysis of the entrapped horseradish peroxidase without any mediator. The reagentless hydrogen peroxide sensor had a fast response of less than 2.5 s with linear range of 0.19–1.35 μM with a detection of 4.75×10^{-8} mol L^{-1}. The sensitivity of the sensor for H_2O_2 was 0.62 A mol^{-1} cm^{-2}. The activation energy for enzyme reaction was calculated to be 14.84 kJ mol^{-1}.

Keywords: Horseradish peroxidase; PNM; Direct electrochemistry; Amperometric biosensor; Hydrogen peroxide

1. Introduction

Horseradish peroxidase (HRP) has long been a representative system for investigating the structure, dynamic and thermodynamic properties of peroxidases, especially in understanding the biological behavior of the catalyzed oxidation of substrates by H_2O_2. To facilitate the direct electron transfer between enzyme and electrode, HRP had been immobilized extensively onto colloidal Au [1], biomenbrane-like surfactant [2], kieselgubr [3], carbon nanotube [4,5], conducting polymer [6,7], Eastman AQ [8], polyethylene glycol [9], nano-Au [10], DNA [11], hydroxyethylcellulose [12] film and so on. So far, the immobilizing matrix is still an important factor in achieving the direct electrochemistry of proteins or enzymes.

It is well known that silica sol–gel material has emerged as one matrix well suited for the immobilization of proteins or enzymes [13]. This kind of inorganic silica sol–gel material can be prepared under ambient conditions and exhibits tunable porosity, high thermal stability, chemical inertness and negligible swelling in both aqueous and non-aqueous solutions. However, silica sol–gel derived matrix is fragile and easy to shrink, crack and desquamate from the electrode surface [14]; meanwhile, the silica sol–gel process is usually carried out in acidic condition, which is hostile to the activities of enzymes. The organic polymer components are rigorously introduced into the starting solution under low humidity conditions, and the substrates coated with sol–gel usually need to be calcined at high temperature [15] which is also not beneficial to the activities of enzymes or proteins. In our previous work [16], we reported the synthesis and characterization of linear poly(*N*-isopropylacyamide-*co*-3-methacryloxypropyltrimethoxy silane) (PNM), which is water-soluble polymer and becomes hydrogel under wild conditions. Compared to the sol–gel material above-mentioned, the polymer has several

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advantages such as good water-solubility, better biocompatibility, and better film-forming ability at room temperature in nearly moderate pH conditions. We thus expect that PNM act as a new and better matrix for immobilization of proteins or enzymes, and create a suitable microenvironment for the direct electron transfer of redox proteins.

In this work, measurements of the direct electron transfer between HRP immobilized in the PNM film and underlying glass carbon (GC) electrode have been reported, and the electrocatalytic reduction of H_2O_2 at the HRP-PNM/GC electrode has been investigated. One of the main advantages of this approach is that the gelatification process of PNM is under gentle conditions which help to maintain the biological activity of HRP to a large extent and thus enable the HRP-PNM/GC electrode to response immediately to H_2O_2 sensing and also improve the stability of biosensor.

2. Experimental

2.1. Reagents

Lyophilized horseradish peroxidase was from Dongfeng Biotechnology Co. Ltd., Shanghai, China. PNM was synthesized in our laboratory. The synthesis and characterization of the PNM was reported in our previous paper [14]. All other chemicals were reagent grade. All of the chemicals were used as received. H_2O_2 (30%) was from Beijing Chemical Engineering Plant. A stock solution of H_2O_2 (0.050 mol L^{-1}) was prepared. The solution was standardized by titration with potassium permanganate. Testing standard solutions were prepared daily by appropriate dilution of the stock solution. All the water used in the experiment was deionized water that was purified twice successively by ion exchange and distillation. Buffer solution for voltammetry all contained 100 mM KBr. Buffer solution were 25 mM citrate for pH 3.0–6.0, 25 mM phosphate for pH 7.0–8.0, and 25 mM borate for pH 9.0–10.0.

2.2. Apparatus

Electrochemical measurements were performed at a CHI660 electrochemistry workstation (CH Instruments Co., USA). The electrochemical cell consisted of a three-electrode system where the modified glass carbon electrode ($d = 3$ mm) was used as the working electrode, a platinum wire as a counter electrode and a SCE as the reference electrode. All measurements were carried out at a room temperature. All electrochemical experimental solutions were in nitrogen controlled atmosphere. FTIR spectrometer instrument was purchased from Co. Perkin-Elmer (U.S.A). All the spectra were obtained with an average of 100 scans and 4 cm^{-1} resolution.

2.3. Preparation of the modified electrodes

Prior to coating, GC electrodes were polished with 0.05 μm alumina and sonicated in deionized water three times. They were then treated in 1:1 (v/v) aqueous nitric acid for 10 min, followed by rinsing and sonication in water and methanol successively.

A PNM solution (0.1 g mL^{-1}) was prepared by dissolving the PNM in a aqueous solution at refrigerator (4 °C) for 30 min. The concentration of the HRP stock solutions, prepared by dissolving HRP in 0.05 M phosphate buffer solution (pH 7.0), was 18 g L^{-1}. A 15 μL volume of PNM and 10 μL of *N*,*N*-dimethylformamide (DMF) were mixed with 15 μL of the HRP stock solution, and then 20 μL of the mixture was pipetted onto the surface of the pretreated GC and spread gently over the entire surface. A small bottle was fit tightly over the electrode so that water evaporated slowly and more uniform films were formed. The films were then dried in air overnight. The PNM film modified electrode was prepared in the same way as described above but without HRP.

3. Results and discussion

3.1. FTIR spectroscopy

FTIR spectral band shapes can be used to monitor changes in protein conformation [17]. The shapes of amides I (1700–1600 cm^{-1}) and amide II (1600–1500 cm^{-1}) infrared absorption bands of proteins provide detailed information on the secondary structure of the polypeptide chain [18]. Fig. 1 showed the FTIR spectra of HRP (Fig. 1a), HRP-PNM (Fig. 1b) and PNM (Fig. 1c) films. The amide I and amide II bonds of HRP in the PNM film (1625.68 cm^{-1} and 1533.31 cm^{-1}) deviated slightly the values from those obtained for the HRP itself (1642.57 cm^{-1} and 1532.32 cm^{-1}), respectively. This indicated that HRP retained the essential feature of its secondary structure on the PNM films.

3.2. Electrochemical characteristics of HRP-PNM film electrodes

3.2.1. Cyclic voltammetry

HRP-PNM/GC were immersed into pH 7.0 buffer solution, a pair of stable, well-defined, quasi-reversible CV peaks at about −0.36 V were observed (Fig. 2). The peaks were located at the potentials characteristic of the hemeFe^{III}/Fe^{II} redox couples of the proteins [19]. No redox peaks were observed for the

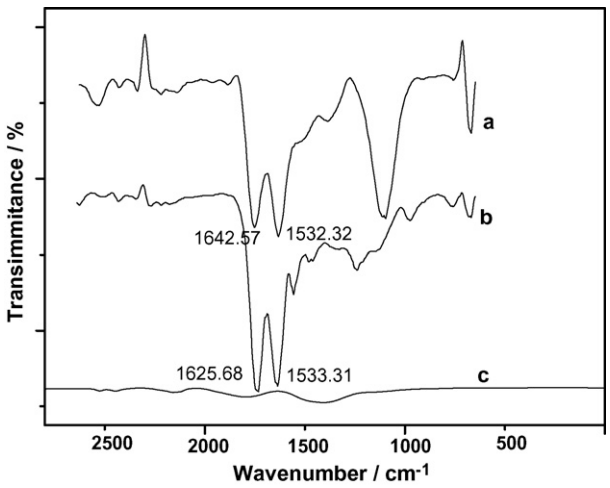

Fig. 1. FTIR spectra for (a) HRP film, (b) HRP-PNM film, and (c) PNM film.