Sensors and Actuators B 140 (2009) 267–272

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Direct electrochemistry and biosensing of hydrogen peroxide of horseradish peroxidase immobilized at multiwalled carbon nanotube/alumina-coated silica nanocomposite modified glassy carbon electrode

Jian-Lung Huang, Yu-Chen Tsai*

Department of Chemical Engineering, National Chung Hsing University, 250, Kuo Kuang Road, Taichung 402, Taiwan, ROC

ARTICLE INFO

Article history:
Received 14 March 2009
Received in revised form 20 April 2009
Accepted 21 April 2009
Available online 3 May 2009

Keywords:
Direct electron transfer
Horseradish peroxidase
Multiwalled carbon nanotubes
Alumina-coated silica

ABSTRACT

Direct electrochemistry and bioelectrocatalytic ability toward the reduction of hydrogen peroxide of horseradish peroxidase (HRP) were found at the multiwalled carbon nanotube/alumina-coated silica (MWCNT/ACS) nanocomposite modified glassy carbon electrode. The surface morphology of the HRP/MWCNT/ACS nanobiocomposite modified glassy carbon electrode was characterized by scanning electron microscopy. In cyclic voltammetric response, the immobilized HRP at the MWCNT/ACS nanocomposite modified glassy carbon electrode displayed a pair of well-defined and quasi-reversible redox peaks with a formal potential of −0.328 V versus Ag/AgCl at a scan rate of 200 mV s^{-1}. The formal potential of HRP varied linearly in the range of solution pH values from 6.0 to 8.0 with a slope of −56 mV pH^{-1}, which is close to the theoretical value of −59 mV pH^{-1} for a reversible one-electron coupled with one-proton electrochemical reaction process. Several important experimental variables were optimized. The optimized hydrogen peroxide biosensor exhibited a sensitivity of 0.157 A M^{-1} cm^{-2}, a detection limit of 0.63 μM, a linear range of 1–500 μM, and an apparent Michaelis–Menten constant of 0.39 mM. The performance of the prepared biosensor was evaluated with respect to four possible interferences.

1. Introduction

The investigation of direct electron transfer between electrodes and redox enzymes is important for understanding the kinetics and thermodynamics of biological redox processes [1–5]. The direct electrochemical behavior at the electrode surface provides a foundation for the fabrication of the mediator-free biosensors which simplify the preparation processes of sensing devices and avoid the toxicity of the mediator in comparison with the second-generation biosensors [6–8]. Horseradish peroxidase (HRP) is one of the most commonly studied enzymes in fabrication of electrochemical biosensors for hydrogen peroxide determinations, and generally used as a model peroxidase to investigate the mechanism of direct electron transfer [9,10]. The electroactive centers of enzymes usually embed deeply within the enzyme structure which prohibits electron transfer rates in most orientations around the redox center because of low electronic conductivity of the surrounding amino acid chains [11,12]. The redox centers of HRP-Fe(III)/Fe(II) are electrically insulated by a protein shell like those of most enzymes [13]. The direct electron transfer between HRP and the electrode is comparatively slow and difficult to detect. Therefore, it is attractive to find new materials to obtain their direct electron transfer between redox enzyme and supporting matrix and to retain their bioactivities. These various materials include nanomaterials [3,4], DNA films [14], polymer films [15,16], and mesoporous silicates [17].

Carbon nanotubes (CNTs) possess many unique properties such as high electrical conductivity, high chemical stability, and the ability to promote certain types of electron-transfer reactions in electrochemical reactions [18,19]. CNTs have been used for studies into the direct electron transfer of proteins or enzymes because their unique structure and electronic properties allow excellent communication between CNTs and redox active centers of enzymes. The CNT modified electrodes have been used to study the direct electron transfer of hemoglobin [20,21], myoglobin [22,23], and glucose oxidase [24,25]. However, a major problem in using CNTs for biological systems is their poor dispersability in all types of solvents because of the large intertube van der Waals interactions. In recent studies, a novel inorganic and noncovalent method to dispersion of CNTs in aqueous solutions through alumina-coated silica (ACS) nanoparticle halos for electroanalytical applications has been studied [26]. Moreover, the MWCNT/ACS has a tendency to self-assemble onto the surface of glassy carbon electrode [27].

In this work, the MWCNT/ACS nanocomposite was chosen as the host matrix for the immobilization of HRP which acts as a model redox enzyme. The HRP was immobilized onto the sur-

---

* Corresponding author. Tel.: +886 4 22857257; fax: +886 4 22854734.
E-mail address: yctsai@dragon.nchu.edu.tw (Y.-C. Tsai).

0925-4005/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.snb.2009.04.033

face of MWCNT/ACS nanocomposite to form a HRP/MWCNT/ACS nanobiocomposite modified glassy carbon electrode. The Nafion was coated on the HRP/MWCNT/ACS in order to prevent the leakage of HRP. Nafion has been extensively used to fabricate modified electrodes for electroanalysis because of the unique ion exchange, discriminative, and biocompatible properties [28]. The resulting Nafion/HRP/MWCNT/ACS nanocomposite film modified glassy carbon electrodes can provide a favorable microenvironment for HRP to perform direct electron transfer and retain its bioactivity. Electrochemical characterization of the Nafion/HRP/MWCNT/ACS nanocomposite modified electrode and its subsequent application in the development of electrocatalytic hydrogen peroxide biosensor are described. The possible interference behavior is also presented.

2. Experimental

2.1. Reagents

HRP (MW 44 kDa) and Nafion (5 wt% in lower aliphatic alcohol) were purchased from Sigma. A 30% H_2O_2 solution was purchased form Showa. The MWCNT (TECO Nanotech Co. Ltd., Taiwan) used in this study was synthesized by an electric arc discharge method and of ~99% purity. The MWCNTs are cylindrical with an inner diameter in the range 2–5 nm, an outer diameter in the range 20–40 nm, and a length of up to several micrometers. No impurity element was detected in MWCNTs by energy dispersive X-ray spectroscopy. Positively charged ACS spheres (Ludox CL) were the product from DuPont. The pH value of the ACS solution was adjusted with 0.1 M HCl. All solutions were prepared with deionized water of resistivity of not less than 18 MΩ cm which was taken from a Milli-Q water purification system (Milli-Q, USA).

2.2. Apparatus

All electrochemical experiments were performed with an Autolab PGSTAT30 Electrochemical Analyzer (Eco Chemie, Netherlands). A conventional three-electrode system was carried out with a 3-mm diameter glassy carbon electrode as the working electrode, an Ag/AgCl (3 M KCl) as the reference electrode, and a platinum wire as the counter electrode (all Metrohm., Switzerland). Scanning electron microscopy (SEM) images were carried out using a JSM-6700F (JEOL, Japan).

2.3. Preparation of Nafion/HRP/MWCNT/ACS nanobiocomposite modified glassy carbon electrodes

The preparation of MWCNT/ACS modified glassy carbon electrode has been described in our previous studies [26,27]. Before the surface modification, the 3-mm bare glassy carbon electrode was polished with 0.3 and 0.05 μm alumina slurries and washed with deionized water several times. MWCNTs (20 mg) were dispersed in a 1 mL of 1 wt% ACS aqueous solution (pH 2) with the aid of ultrasonic agitation for 2 h. The self-assembly of MWCNT/ACS onto the surface of glassy carbon electrode was conducted by dipping glassy carbon electrode into the prepared MWCNT/ACS solution for 1 h. The MWCNT/ACS modified glassy carbon electrode was rinsed with deionized water several times and the solvent was allowed to evaporate at room temperature in the air. The HRP/MWCNT/ACS nanobiocomposite modified glassy carbon electrode was prepared by casting a 6 μL of HRP (40 mg mL^{-1}) onto the surface of glassy carbon electrode and drying under room temperature in the air. Next, a 6 μL of 0.5 wt% Nafion was coated onto the surface of HRP/MWCNT/ACS. The prepared Nafion/HRP/MWCNT/ACS modified electrode was dried at room temperature and used for electrochemical investigations or stored at 4 °C when not in use.

3. Results and discussion

3.1. HRP/MWCNT/ACS nanobiocomposite characterization by SEM

The surface morphology of the HRP/MWCNT/ACS nanobiocomposite was characterized by SEM in order to compare the MWCNT/ACS and HRP/MWCNT/ACS nanocomposites. Fig. 1 shows the SEM images of the MWCNT/ACS before and after cast deposition of HRP. The surface of the prepared MWCNT/ACS (Fig. 1a) nanocomposite was rough and consisted of a great number of ACS nanoparticles. The individual MWCNT was well-dispersed within MWCNT/ACS nanocomposite. MWCNTs will act as high conductivity nanowires connecting nanocomposite domains throughout the MWCNT/ACS nanocomposite. After casting of HRP onto the surface of MWCNT/ACS, the surface morphology resulted in a smooth surface as shown in Fig. 1b. This is attributed to the fact that the HRP is absorbed on the surface of MWCNT/ACS. This result presents that the HRP/MWCNT/ACS was uniformly coated on the glassy carbon electrode.

3.2. Direct electrochemistry of HRP

To investigate the direct electron transfer of HRP at MWCNT/ACS modified electrode, cyclic voltammetry was performed. Fig. 2 shows the cyclic voltammograms of Nafion/HRP, Nafion/MWCNT/ACS, and Nafion/HRP/MWCNT/ACS modified glassy carbon electrodes in 0.1 M N_2-saturated phosphate buffer solution (pH 7) at a scan rate of 200 mV s^{-1}. No peaks were observed at the Nafion/HRP modified glassy carbon electrode (Fig. 2a), which showed that

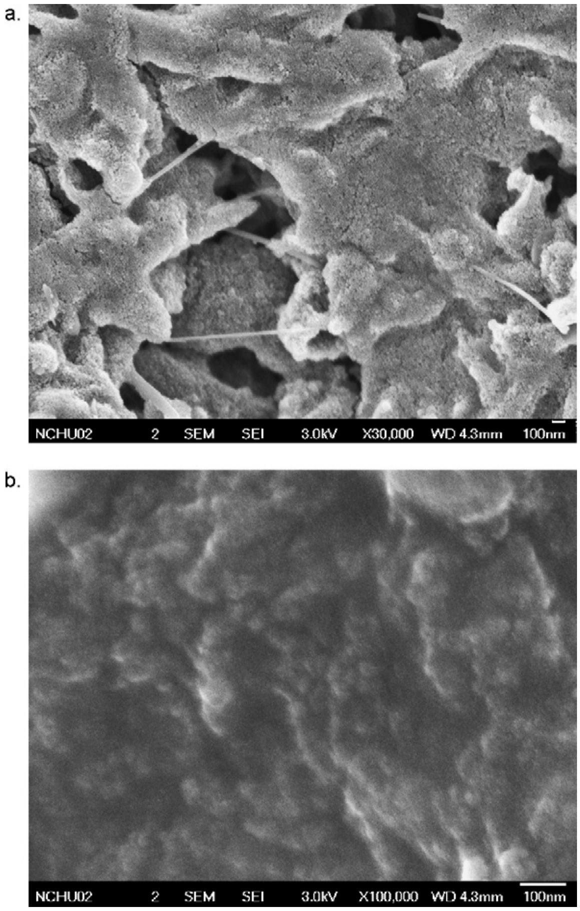

Fig. 1. SEM images of MWCNT/ACS nanocomposite (a) before and (b) after immobilization of HRP.