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ScienceDirect
Biosensors and Bioelectronics 22 (2007) 1268–1274
BIOSENSORS & BIOELECTRONICS
www.elsevier.com/locate/bios

Amperometric third-generation hydrogen peroxide biosensor based on the immobilization of hemoglobin on multiwall carbon nanotubes and gold colloidal nanoparticles

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Received 15 February 2006; received in revised form 7 May 2006; accepted 19 May 2006
Available online 3 July 2006

Abstract

A convenient and effective strategy for preparation nanohybrid film of multi-wall carbon nanotubes (MWNT) and gold colloidal nanoparticles (GNPs) by using proteins as linker is proposed. In such a strategy, hemoglobin (Hb) was selected as model protein to fabricate third-generation H_2O_2 biosensor based on MWNT and GNPs. Acid-pretreated, negatively charged MWNT was first modified on the surface of glassy carbon (GC) electrode, then, positively charged Hb was adsorbed onto MWNT films by electrostatic interaction. The $\{Hb/GNPs\}_n$ multilayer films were finally assembled onto Hb/MWNT film through layer-by-layer assembly technique. The assembly of Hb and GNPs was characterized with cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and transmission electron microscopy (TEM). The direct electron transfer of Hb is observed on Hb/GNPs/Hb/MWNT/GC electrode, which exhibits excellent electrocatalytic activity for the reduction of H_2O_2 to construct a third-generation mediator-free H_2O_2 biosensor. As compared to those H_2O_2 biosensors only based on carbon nanotubes, the proposed biosensor modified with MWNT and GNPs displays a broader linear range and a lower detection limit for H_2O_2 determination. The linear range is from 2.1×10^{-7} to 3.0×10^{-3} M with a detection limit of 8.0×10^{-8} M at 3σ. The Michaelies–Menten constant K_M^{app} value is estimated to be 0.26 mM. Moreover, this biosensor displays rapid response to H_2O_2 and possesses good stability and reproducibility.

Keywords: Carbon nanotubes; Gold colloidal nanoparticles; Hemoglobin; Hydrogen peroxide biosensor

1. Introduction

The rapid and accurate determination of hydrogen peroxide (H_2O_2) is of great importance because it is not only the product of the reactions catalyzed by many highly selective oxidases but also an essential compound in food, pharmaceutical and environmental analyses (Wang et al., 1993; Kulys et al., 1993). Among these techniques employed for hydrogen peroxide analysis, such as titrimetry, photometry, chemiluminescence, high performance liquid chromatography and electrochemistry, amperometric enzyme-based biosensors have received considerable interest, because this class of technique is characterized by sensitivity, convenience and high selectivity. Amperometric enzyme-based H_2O_2 biosensors could be divided into two categories, namely mediated biosensors and mediated-free biosensors. Although the mediated H_2O_2 biosensors could hold the detection limits as low as 10^{-7} to 10^{-8} M by use of electron transfer mediators such as ferrocene derivatives (Tatsuma et al., 1989), hexacyanoferrates (Schubert et al., 1991), tetrathiafulvalene (Wendzinski et al., 1997), or phenazine methosulphate (Qian et al., 1995), some mediator molecules will pollute sample or the electrode system or diffuse out the enzyme layer (Wang and Dong, 2000). Mediated-free biosensors, third-generation biosensors, which are based on the direct electron transfer between redox proteins and electrode, are gaining an increasing attention because they could overcome the disadvantages mentioned above of mediated biosensors, and make the design of biosensors simple without requirements for chemical mediators.

In order to achieve the direct electron transfer between redox proteins and electrode to obtain third-generation biosensor, extensive work has been done with redox protein films over the

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doi:10.1016/j.bios.2006.05.022

past 20 years. Successful examples for the direct electron transfer of proteins on films have included surfactants (Rusling and Nassar, 1993; Nassar et al., 1995; Zhang et al., 1997), polymers (Kong et al., 2003; Nassar and Rusling, 1996; Chen et al., 2000; Fan et al., 2001), and many inorganic materials (Li et al., 2001; Zhao et al., 2005a; Yao et al., 2006; Liu et al., 2004; Zhang et al., 2005). In addition, carbon nanotubes (CNTs), as a new class of nanomaterial, discovered in 1991 by Iijima (1991), have also been employed in biosensors. Such an application is attributed to their unique electrical properties, which make redox active center of proteins be close to the surface of proteins and direct electron transfer between proteins and electrode be achieved, accordingly, a new avenue for fabricating the third-generation biosensors is developed. Actually, several CNTs-based unmediated H_2O_2 biosensors have been reported (Zhao et al., 2002, 2004, 2005b,c).

Just recently, the research interest has extended to modify CNTs with other nanomaterials so as to optimize the use of them in various applications. Several semiconductor nanoparticles have been bound to the surfaces of CNTs, such as SiO_2 (Fu et al., 2002), TiO_2 (Banerjee and Wong, 2002), CdS (Shi et al., 2004), CdSe (Ravindran et al., 2003) and CdTe (Banerjee and Wong, 2003). Several metal nanoparticles such as Pd (Lim et al., 2005), Ag (Guo and Li, 2005), Pt (Yu et al., 1998) and Au (Liu et al., 2003) have also successfully been introduced onto the CNTs. Meanwhile, some investigations have been conducted for the fabrication of biosensors based on the nanocomposites consisted of CNTs and other nanomaterials. Yang et al. (2006b) and Tang et al. (2004) reported a glucose biosensor based on CNTs and Pt nanoparticles, respectively. Lim et al. (2005) reported a Pd-CNT-based glucose biosensor. Zhu et al. (2005) reported a DNA biosensor based on platinum nanoparticles combined CNTs. Yang et al. (2006a) reported a cholesterol biosensor based on layer-by-layer self-assembled multilayer films of CNTs and platinum nanoparticles with polyelectrolyte. Liu et al. (2005) reported a H_2O_2 biosensor based on microperoxidase immobilized on nanohybrid film of MWNT and gold nanoparticles. Such methods as electrodeposition of metal nanoparticle onto CNTs (Lim et al., 2005; Tang et al., 2004), dispersing two kind of nanomaterial in the same solution such as Nafion (Zhu et al., 2005), dispersing CNTs in the metal nanoparticle-doped chitosan (Yang et al., 2006a), and direct adsorption of gold nanoparticles onto the CNTs (Liu et al., 2005), were adopted to obtain the nanocomposites in above biosensors. Additionally, Jitianu et al. (2004) reported the preparation of nanocomposites of MWNT and TiO_2 by sol–gel method. Liu et al. (2003) reported the self-assembly of gold nanoparticles to CNTs using thiol-terminated pyrene as interlinker.

In this paper, a convenient and effective strategy by using proteins as linker to combine MWNT and GNPs is proposed. Prior to prepare nanohybrid, acid-pretreated, negatively charged MWNT were first dispersed into the solution of sodium dodecyl sulfate (SDS) to give stable homogeneous black suspension based on the hydrophobic interaction between the hydrophobic chain of SDS and the sidewall of the MWNTs (Jiang et al., 2003; Richard et al., 2003), and then the suspension of MWNT was cast on a cleaned GC electrode surface to form MWNT films. Subsequently, hemoglobin (Hb) was assembled onto the MWNT films by electrostatic interaction between negatively charged MWNT and positively charged Hb. Then, by electrostatic adsorption, negatively charged GNPs was adsorbed onto positively charged protein films to achieve the combination of MWNT with GNPs. TEM was used to monitor the assembly of GNPs and electrochemical impedance spectroscopy (EIS) was employed to investigate the modification process. Here, we choose Hb as linker not only because of its commercial availability and well-documented structure but also because of its enzymatic activity to reduce hydrogen peroxide. This proposed strategy for preparation nanohybrid of MWNT and GNPs is simple and effective. More importantly, by layer-by-layer assembly based on the electrostatic interaction between GNPs and oppositely charged proteins, $\{proteins/GNPs\}_n$ multilayer can be formed on the MWNT film, resulting in higher protein loading in the nanohybrid film. The resulting nanohybrid film provides a favorable microenvironment for Hb to perform direct electron transfer at glassy carbon electrode. We choose this nanohybrid film as a model films to fabricate a third-generation H_2O_2 biosensor based on Hb/GNPs/Hb/MWNT film modified electrode. The direct electrochemistry and analytical performance of this biosensor were investigated.

2. Experimental

2.1. Materials

The multi-wall carbon nanotubes (MWNT, >95% purity) synthesized by CVD method, were purchased from Chengdu Organic Chemicals Co. Ltd., of the Chinese Academy of Science. Prior to use, MWNT were treated with concentrated nitric acid in order to introduce carboxylic acid groups according to report (Guo et al., 2004). Bovine (horse heart) hemoglobin, gold chloride tetrahydrate and sodium citrate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). They were used directly without further purification. A stock solution of 10 mg/mL Hb was freshly prepared with a 0.1 M phosphate buffer solutions (pH 6.0). Sodium dodecyl sulfate (SDS) and hydrogen peroxide (30%, w/v solution) were purchased from Chemical Reagent Company, Chongqing, China. The concentration of the more diluted hydrogen peroxide solutions prepared from 30% hydrogen peroxide was determined by titration with potassium permanganate. Phosphate buffer solutions (PBS) with various pH were prepared with 0.1 M KH_2PO_4 and 0.1 M Na_2HPO_4. The supporting electrolyte was 0.1 M KCl. All other chemicals employed were of analytical grade and used as received, doubly distilled water was used throughout the experiments. Gold nanoparticles with mean size of 16 nm were produced by reducing gold chloride tetrahydrate with citric acid at 100 °C for half an hour (Enüstün and Turkevich, 1963).

2.2. Preparation of hydrogen peroxide biosensor

The glassy carbon (GC) electrode ($\Phi = 4$ mm) was polished with 1.0, 0.3 and 0.05 μm alumina slurry, respectively, and then ultrasonically cleaned in ethanol and water. In 10 mL