Sensors and Actuators B 152 (2011) 88–93

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Review

Electrochemical studies of bovine serum albumin immobilization onto the poly-*o*-phenylenediamine and carbon-coated nickel composite film and its interaction with papaverine

Li-Jun Feng, Xiu-Hua Zhang*, Dong-Ming Zhao, Sheng-Fu Wang

Ministry-of-Education Key Laboratory for the Synthesis and Application of Organic Functional Molecules & College of Chemistry and Chemical Engineering, Hubei University, Wuhan, PR China

ARTICLE INFO

Article history:
Received 24 March 2010
Received in revised form 17 June 2010
Accepted 13 September 2010
Available online 19 September 2010

Keywords:
Papaverine
Poly-*o*-phenylenediamine
Carbon coated nickel
BSA
Biosensor

ABSTRACT

A biosensor based on bovine serum albumin (BSA) and poly-*o*-phenylenediamine (PoPD)/carbon-coated nickel (C-Ni) nanobiocomposite film modified electrode has been developed to study the interaction of BSA with papaverine (PAP). The well-dispersed C-Ni nanoparticles were dripped onto the glassy carbon electrode (GCE) surface firstly, and PoPD films were subsequently electropolymerized by cyclic voltammetry (CV) to prepare PoPD/C-Ni/GCE. Finally, the BSA was easily immobilized on the PoPD films via electrostatic adsorption. The morphology and the electrochemical properties of the fabricated composite electrodes were examined by scanning electron microscope (SEM) and electrochemical impedance spectroscopy (EIS), respectively. The interaction of PAP with BSA was monitored by differential pulse voltammetry (DPV), using PoPD as the electrochemical indicator. The binding constant (K), obtained by DPV, was 1.7×10^4 L/mol, which was consistent with the fluorescence analysis. This constructed biosensor also exhibited a fine linear correlation with PAP concentration range of 2.5×10^{-9}–4.5×10^{-5} mol/L and a detection limit of 8.3×10^{-10} mol/L was achieved by DPV.

Contents

1. Introduction

Since the first synthesis of carbon-coated metal nanoparticles in 1993 [1,2], intensive attention has been paid on its potential application fields including magnetic recording, magnetic fluids [3], immobilization of proteins [4], detection of DNA hybridization [5], drug delivery [6] and so on. Carbon-coated nickel (C-Ni),

* Corresponding author. Tel.: +86 27 88662747; fax: +86 27 88663043.
E-mail address: zhanganal@yahoo.com.cn (X.-H. Zhang).

0925-4005/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.snb.2010.09.031

which is a novel material relating with the fullerene, could potentially absorb a micromolecular pharmaceutical and be used as a magnetism-targeted drug carrier in cancer treatments. This kind of material, which is protected by a layer of carbon, is quite stable and can overcome the agglomeration of nanoparticles. Our previous research indicated that C-Ni nanoparticles have the ability to promote electron-transfer reactions in electrochemical process [7,8]. In addition, nanostructures, including nanoparticles and nanotubes, have dimensions similar to those of biomolecules such as proteins and DNA. Thus, the combination of nanostructures with biomolecules would yield functional nanostructured biointerfaces with synergistic properties and functions [9]. The recent surge of research interest in the bioelectrochemical field is focused on the advanced design and preparation of such potential nanostructured biosensors [10].

The key to the fabrication of biosensors is the immobilization of biomolecules on different kinds of transducer surfaces, which is the significant factor for improving the stability, reproducibility and sensitivity of the biosensor [11,12]. With the abundant functional groups, the polymer film modified electrode has stable performance and the ability to link biomolecules [13]. Jiao and co-works prepared a DNA electrochemical sensor with poly-L-lysine/single-walled carbon nanotubes films for the detection of the transgenic plants gene fragment [14]. Also, an amperometric biosensor based on multiwalled carbon nanotube-poly(pyrrole)-horseradish peroxidase nanobiocomposite film modified gold electrode was successfully employed for determination of phenol derivatives [15].

Papaverine (PAP), together with morphine, narcotine, codeine, thebaine and narceine, are the six opium alkaloids which occur naturally in the largest amounts. PAP was first isolated in opium in 1848 by Merck [16]. It presents in the extent of 0.5–1% in most varieties of opium. In 1909, Pictet and Gams accomplished the synthesis of papaverine and confirmed its molecular structure [16]. Many methods have been reported for the determination of opium alkaloids in natural plants. It mainly includes Gas liquid chromatography (GLC) [17], High performance liquid chromatography (HPLC) [18–20], Gas chromatography–mass spectrometry (GC–MS) [21], Dragana and Dušanka [22] using reversed phase high performance liquid chromatography (RP-LC) for determination of PAP, the linear range was from 2.7×10^{-4} to 7.9×10^{-4} mol/L. J. Yan et al. [23] established a method for the determination of PAP based on indirect competitive enzyme-linked immunosorbent assay (ELISA), the linear range was from 2.6×10^{-7} to 8.0×10^{-5} mol/L. These methods have high sensitivity and specificity. However, all these methods which require a compressing system, temperature controlling systems or separation systems are obviously complex. As compared with those methods mentioned above, electrochemical methods are very useful and popular for trace analysis since voltammetric techniques are compact, efficient, and sensitive. Various voltammetry solutions have been found to have a low detection limit, depending on the working electrode systems. To date, only a few electrochemical methods have been described in the literature for the determination of PAP. Zeng and Zhang [24] investigated the characteristics of PAP using mercury electrode. Although this method could reach a low detection limit of 1.0×10^{-9} mol/L, the use of mercury may do harm to the operator and pollute the environment. Ziyatdinova et al. [25] adopted the graphite electrode to selectively detect PAP, with a detection limit of 3.9×10^{-6} mol/L, which was not satisfactory for the microscale analysis purposes.

In this paper, we prepared a novel BSA/poly-*o*-phenylenediamine/carbon-coated nickel nanoparticles modified glassy carbon electrode (BSA/PoPD/C-Ni/GCE). BSA was easily bound with the positively charged amino groups of PoPD films due to the strong electrostatic affinity. The immobilization of BSA was characterized by scanning electron microscope (SEM) and electrochemical impedance spectroscopy (EIS). In addition to electrostatic adsorption of BSA, PoPD can also act as electrochemical indicator to monitor the interaction of BSA with PAP at the electrode surface. This biosensor has also been applied to detect PAP within a wide concentration range of 2.5×10^{-9}–4.5×10^{-5} mol/L by differential pulse voltammetry (DPV). By this method a low detection limit of 8.3×10^{-10} mol/L was obtained.

2. Experimental

2.1. Chemicals and materials

C-Ni nanoparticles (obtained from Professor Xungao Zhang, Wuhan University, China) were rinsed ultrasonically in 3 mol/L HCl for 5 h. Then the purified C-Ni nanoparticles were washed to neutral by water. The nickel metal content of the carbon-coated nanoparticles was 64.5% by thermogravimetric analysis [26]. Phosphate buffer was comprised with Na_2HPO_4 and NaH_2PO_4. The pH value was adjusted with NaOH and H_3PO_4. *o*-Phenylenediamine (oPD) ~99% (Aldrich Chemical Co.) was sublimated under vacuum at 80 °C before use. Epinephrine (from Sigma), and dopamine (from Fluka) were used as received. Bovine serum albumin (BSA, tianyuan) was commercially purchased and accepted for use without further purification. The standard stock solutions of 0.68 mg/mL BSA were prepared by dissolving BSA in water. PAP hydrochloride injection was supplied by Jiangsu Wuxi. All other chemicals were of analytical-regent grade. All solutions were prepared with double-distilled water.

Electrochemical measurements were performed by a model CHI660C electrochemical workstation (CH Instruments, Chenhua Co., Shanghai, China) controlled by a personal computer. A three-electrode system was used in the measurements, with a bare GCE (2 mm in diameter) or modified GCE as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum wire as the auxiliary electrode. All the following potentials reported in this work are against the SCE. The electrochemical impedance spectroscopy (EIS) measurements were performed in the presence 5 mmol/L $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 mol/L KCl and plotted in the form of complex plane diagrams (Nyquist plots). They were recorded with a frequency range of 0.01 Hz to 100 kHz. The amplitude of the applied sine wave potential is 5 mV, with a formal potential 0.2 V. The observations of the morphology of samples were performed using a JSM-5510 LV (JEOL) scanning electron microscopy (SEM).

2.2. Preparation of BSA/PoPD/C-Ni/GCE

The bare GCE was carefully polished with 0.05 μm Gamma alumina powder (γ-Al_2O_3) on chamois leather, and then washed ultrasonically in water and ethanol, respectively. For the preparation of C-Ni/GCE, 10 μL of 1.0 mg/mL C-Ni nanoparticles suspension (1.0 mg C-Ni nanoparticles in 1.0 mL purified water containing 5.0 mmol/L SDS) was dropped onto the surface of a clean GCE, followed by evaporating the solvent under an infrared lamp. The PoPD/C-Ni/GCE was formed by dipping the above C-Ni/GCE into 5.0×10^{-4} mol/L of oPD solution prepared using 0.1 mol/L H_2SO_4 solution and then scanning in the potential range from −0.5 to +1.0 V at 100 mV/s for 6 cycles. After being rinsed with PBS solution (pH 5.0), the above PoPD/C-Ni/GCE coated with different concentrations of BSA (0.14, 0.34, 0.48, 0.54, 0.68, 1.4, 3.4, 6.8 mg/mL of 10 μL of stock solution) solution for 30 min to immobilize the BSA on the modified surface through electrostatic force, followed by washing the electrode with 0.2% SDS solution and then rinsing it with purified water to remove the unimmobilized BSA. Thus, the resulting electrode (hereafter denoted as BSA/PoPD/C-Ni/GCE) was used for the electrochemical measurements later.