Sensors and Actuators B 134 (2008) 625–631

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Highly sensitive, reagentless amperometric immunosensor based on a novel redox-active organic–inorganic composite film

Zhongyuan Liu, Ruo Yuan*, Yaqin Chai, Ying Zhuo, Chenglin Hong, Xia Yang

Key Laboratory on Luminescence and Real-Time Analysis (Southwest University), Ministry of Education, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China

ARTICLE INFO

Article history:
Received 23 November 2007
Received in revised form 28 April 2008
Accepted 4 June 2008
Available online 20 June 2008

Keywords:
Amperometric immunosensor
Gold nanoparticles
Redox-active organic–inorganic composite film
Prussian blue
Carcinoembryonic antigen (CEA)

ABSTRACT

A new highly sensitive amperometric immunosensor for the determination of carcinoembryonic antigen (CEA) had been constructed by employing a novel organic–inorganic composite film coupled with gold nanoparticles. At first, prussian blue (PB) was deposited on a glassy carbon electrode (GCE) and then a new porous organic material synthesized with 3,4,9,10-perylenetetracarboxylicdianhydride (PTCDA) and ethanediamine was coated on the surface of PB film to obtain a stable organic–inorganic composite film, which contained electrochemical redox activity and abundant amino groups on electrode interface. Furthermore, this porous organic material (PTC-NH_2) could prevent the leakage of the PB efficiently which led to the significant enhancement of the stability and sensitivity of the immunosensor, as well as provided abundant amino-groups to assemble gold nanoparticles (nano-Au) for immobilization of carcinoembryonic antibody (anti-CEA). The morphologies of PB and the organic–inorganic composite films (PTC-NH_2/PB) were studied by means of scanning electron microscope (SEM). In addition, the preparation procedure of the immunosensor was further investigated by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Under optimal conditions, the resulting immunosensor displayed a high sensitivity for the detection of CEA, and responded to the CEA concentration in two ranges from 0.05 to 2.0 ng/ml (R = 0.996) and from 2.0 to 40.0 ng/ml (R = 0.995) with a detection limit of 0.018 ng/ml. Moreover, the immunosensor exhibited high selectivity, long-term stability and good reproducibility.

1. Introduction

Carcinoembryonic antigen (CEA) is a kind of cell surface glycoprotein [1] with a molecular weight of about 200 kDa. It is an important tumor marker [2] which is expressed in many malignancies, such as ovarian carcinoma, lung cancer, colon cancer, breast cancer and others [3–5]. Increasing levels of CEA in the adult human serum is also related to the state of tumor. Therefore, the determination of the CEA level is of great importance and necessary. There are a number of methods to determine CEA, for example, radioimmunoassay (RIA), enzymelinked immunosorbentassy (ELISA) and immunohistochemical test (IHC) [6–8]. However, these methods exhibit the drawbacks of radiation hazards, tedious assay time, qualified personnel and sophisticated instrumentation. Thus, a simple, sensitive and convenient method for the determination of CEA in human serum is desirable.

Electrochemical immunosensors, which are based on the specificity of antigen–antibody interactions with electrochemical transduction for analytical purposes, have attracted wide interest due to their advantages such as simple pretreatment procedure, fast analytical time, precise and sensitive measurements, and inexpensive and miniaturizable instrumentation [9–11]. Thus, many kinds of electrochemical immunosensors have been developed, including amperometric, potentiometric, capacitive and impedance transducers, which respectively determine the level of analyte by detecting the changes of current [12], potential [13], conductance [14] and impedance [15] caused by the immunoreaction. Among them, the amperometric immunosensor is especially promising for its relatively low detection limit and high sensitivity [16]. Based on the ELISA principle with further amplifying process, many groups reported the amperometric immunosensors which had obtained an ultra-low detection limit [17,18]. However, the preparation and detection steps of these immunosensors were perplexing, inconvenient and time consuming. As a result, a series studies of the reagentless and label-free amperometric immunosensors, especially the immunosensors immobilized the electrochemical redox-active compound on the electrode surface, have caught the eyes due to their advantages such as to simplify the assay system, accelerate the electrode response as well as reduce the analytical time [11]. Thus, searching for a suitable

* Corresponding author. Tel.: +86 23 68252277; fax: +86 23 68252277.
E-mail address: yuanruo@swu.edu.cn (R. Yuan).

0925-4005/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.snb.2008.06.013

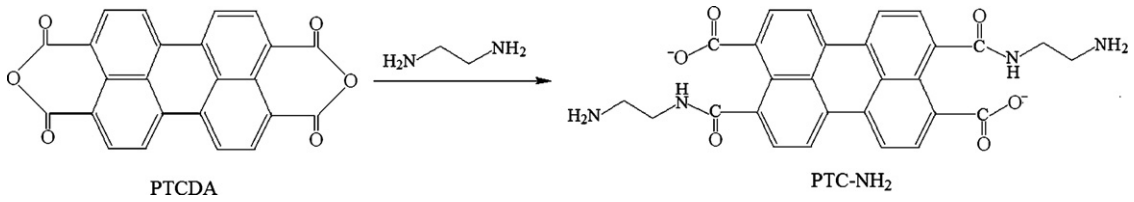

Fig. 1. The schematic diagram of the procedure of the synthesis of PTC-NH_2.

electrochemical redox-active compound and a good method to immobilize biorecognition substance on electrode surface are two key points of the research work. Taking into these considerations, a novel, simple and highly sensitive electrochemical immunosensor for the detection of CEA, as a model protein, are developed by means of the immobilization of biomolecules (anti-CEA) onto the nano-Au/PTC-NH_2/PB biocompatibility composite film in this contribution.

Among the numerous redox compounds, prussian blue (PB) combines several well-known properties such as redox characteristic, high chemical stability, facile preparation, low cost, and magnetic properties [19]. As a result, it is widely used to manufacture electrochemical sensor and biosensor. There are a number of methods to immobilize PB on the electrode surface including mechanically attachment, adsorption, entrapping or encapsulating, especially electrodeposition [20–23]. However, the main drawback of the PB layer is that it is not stable enough and easy to leak from the electrode surface [24,25]. Thus, great efforts have been made to avoid leaching of PB from the electrode surface in order to develop new sensors with high sensitivity, selectivity, and stability. In our present work, we have synthesized a new organic compound which is abbreviated to PTC-NH_2 (molecular structure shown in Fig. 1) with 3,4,9,10-perylenetetracarboxylicdianhydride (PTCDA, molecular structure shown in Fig. 1) and ethanediamine. Before we turn to the description of the advantages of PTC-NH_2, we briefly resume the most important structure and properties of PTCDA which is the archetype for PTC-NH_2 known so far [26]. The planar perylene derivative PTCDA has been widely used as model compound in studies of growth and electronic properties of semiconducting thin films, due to its well-known certified properties such as stable, easily to form porous film and transfer electrons [27,28]. It is also important as a starting material in the synthesis of various kinds of organic compounds [29], and has shown promising electronic properties that can be used in chemical sensing and other device applications [30,31]. With the isologous framework structure of PTCDA, PTC-NH_2 has not only the homologous advantages of PTCDA including stable, easily to form porous film and transfer electrons, as well as offers abundant amino groups. When the PTC-NH_2 is coated on the surface of PB film, the holes of the PTC-NH_2 can encrust with the PB islands to form a stable PTC-NH_2/PB organic–inorganic composite film with redox activity and abundant amino groups on its surface, which will prevent the leakage of the PB to enhance the stability and sensitivity of the immunosensor. Moreover, this PTC-NH_2/PB organic–inorganic composite film can absorb the nano-Au particles through abundant amino groups of the PTC-NH_2. It is well known that, the nano-Au particles [32,33] with large specific surface area and good biocompatibility can strongly interact with biomaterials and has been utilized as a intermediator to immobilize antibody to efficiently retain its activity and to enhance current response in the construction of a sensitive amperometric immunosensor. In this work, CEA is selected as a model system to be immobilized on the nano-Au interface to complete this immunosensor.

The prepared method is simplified, economical and efficient, and the resulting immunosensor exhibits high sensitivity and selectivity, long-term stability and good reproducibility.

2. Experimental

2.1. Reagent and materials

CEA and anti-CEA were purchased from Biocell Company (Zhengzhou, China). Bovine serum albumin (BSA, 96–99%), gold chloride ($HAuCl_4$) and sodium citrate were obtained from Sigma Chemical Co. (St. Louis, MO, USA). $K_3Fe(CN)_6$, $FeCl_3$ were purchased from Chemical Reagent Co., Sichuan, China. 3,4,9,10-Perylenetetracarboxylicdianhydride (PTCDA) were purchased from Lian Gang Dyestuff Chem. Co. (Liaoning, China). All chemicals and solvents used were of analytical grade. Double distilled water was used throughout all experiments. Phosphate-buffered solutions (PBS, pH 6.0) were prepared by mixing the solutions of KH_2PO_4, Na_2HPO_4 and KCl. Gold nanoparticles (nano-Au) were produced by reducing gold chloride tetrahydrate with citric acid at 100 °C for half an hour. The CEA was stored in the frozen state, and its standard solutions were prepared daily with PBS as in use.

2.2. Apparatus

Cyclic voltammetric (CV) measurements were carried out with a CHI 600B electrochemistry workstation (Shanghai CH Instruments, China). A three-compartment electrochemical cell contained a platinum wire auxiliary electrode, a saturated calomel reference electrode (SCE) and the modified glassy carbon electrode (Ø4 mm) as working electrode. The pH measurements were made with a pH meter (MP 230, Mettler-Toledo Switzerland) and a digital ion analyzer (Model PHS-3C, Dazhong Instruments, Shanghai, China). The morphologies of the PB film and PTC-NH_2/PB film were studied by means of scanning electron microscope (SEM) (1000B, AMRAY, American). The size of nano-Au was characterized by transmission electron microscope (TEM) (TECNAI 10, PHILIPS, Holland). The AC impedance of the immunoelectrode membrane was measured with a Model IM6e (ZAHNER Elektrick, Germany).

2.3. Preparation of PTC-NH_2

The PTC-NH_2 had been prepared as follows: 1 g of PTCDA was dissolved in 5 ml acetone firstly. After dissolved completely, 10 ml ethanediamine was added and the solution was stirred for 40 min at room temperature. The mixture was then filtered, rinsed with deionized water and ethanol, dried at room temperature for 2 days to obtain the purified PTC-NH_2. The schematic diagram of the procedure of the synthesis was shown in Fig. 1.

2.4. Preparation of the immunosensor

Prior to surface modification, the glassy carbon electrode (Ø4 mm) was polished to a mirror-like surface repeatedly with 0.3