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Label-free immunosensor based on Pd nanoplates for amperometric immunoassay of alpha-fetoprotein

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ABSTRACT

In this paper, Pd nanoplates were used as a kind of electrode materials for fabrication of an electrochemical immunosensor, which was applied for detection of cancer biomarker alpha-fetoprotein (AFP). Thanks to the unique structure and properties of Pd nanoplates, the antibody of AFP (Ab) was effectively immobilized onto the surface of the Pd nanoplates modified glassy carbon electrode (GCE). Moreover, the good electrochemical properties of Pd nanoplates greatly improved the electronic transmission rate and enhanced the electrochemical signal, which led to an increase of the detection sensitivity. Based on the specific antibody–antigen interaction, a label-free immunosensor based on Pd nanoplates was developed for sensing of AFP. The current method allows us to detect AFP over a wide concentration range from 0.01 to 75.0 ng/mL with a detection limit of 4 pg/mL. The proposed immunosensor has been used to determine AFP in human serum with satisfactory results.

1. Introduction

Alpha-fetoprotein (AFP) is an oncofetal glycoprotein with a molecular weight of approximately 70,000 Da. As is known to all, AFP is an important tumor marker and extensively employed for the early diagnosis of the patients with liver cancer (Su et al., 2011; Liu et al., 2011). The concentration of AFP is below 25 ng/mL in healthy human serum but increases greatly in patients with liver cancer (Lin et al., 2009; Jiang et al., 2010). Thus, the detection of AFP level in human serum plays an important role in the diagnosis and management of original liver carcinoma. Various methods, such as chemiluminescence (Fu et al., 2006), enzyme-linked immunosorbent assay (ELISA) (Darwish et al., 2013) and radioimmunoassay (Brummund et al., 1980), have been used for the detection of AFP level in human serum. However, these conventional immunoassays have some drawbacks such as being time-consuming, needing expensive instruments and requiring skillful operators.

Due to significant benefits such as high sensitivity, low detection limit, low cost, fast response and ease of handling and miniaturization, electrochemical immunosensors have aroused extensive interests in the past few years and have been applied to environmental pollutant detection, food industry, biotechnology, clinical diagnosis and pharmaceutical chemistry. Label-free electrochemical immunosensors have some other advantages, for instance, they could be directly used to monitor the binding process of antibody–antigen reaction and avoid disturbances from conjugated markers or handling with hazardous materials.

For fabrication of a label-free electrochemical immunosensor, the most crucial step is to efficiently and effectively immobilize the biomolecules. It is very important to adopt an optimal method to increase the immunosensor interface for immobilizing more antibodies and keeping their activity for subsequent assays. Covalent bonding is the most widely used method for immobilization of biomolecules, while glutaraldehyde and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide (EDC/NHS) is the commonly used crosslinking agent and has been widely used in the fabrication of immunosensors (Shi and Ma, 2011; Wang et al., 2013; Lin et al., 2011, 2013; Liu et al., 2013). However, there exists some problems, such as time consuming, not easy to clean up, moreover, excess glutaraldehyde will make biomolecules lose activity.

Electrode material is very important for electrochemical immunosensor, especially for label-free electrochemical immunosensor, since it is responsible for two major tasks: increasing specific surface area of electrode to immobilize more antibodies and improving conductivity so as not to hinder the electron transfer. Graphene, carbon nanotube and their derived materials have been widely applied to the electrochemical biosensor due to large specific surface area and good electrical conductivity, which attract widespread interests (Li et al., 2013a, 2013b; Zhao et al., 2011;

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Fam et al., 2011). Recently, a novel material, nanoporous gold film, was found by our research team and has been used to fabricate an immunosensor for label-free detection of cancer biomarker, which has achieved satisfactory results (Wei et al., 2011). Nanoporous gold film can be applied directly to the immobilization of antibodies without the participation of crosslinking agent, which improves efficiency and shorten the reaction time.

Here we report a highly sensitive immunosensor for amperometric immunoassay of alpha-fetoprotein using a novel nanomaterial, Pd nanoplates, as electrode material. Pd nanoplates with unique structure and function can be used as a good electrode material for the immobilization of biomolecules and the conduction of electrochemical signals. According to the literature (Mandal et al., 2004), the $-NH_2$ groups bind very strongly to Pd nanoplates, suggesting that Pd nanoplates can directly bound antibodies without the participation of crosslinking agent. Moreover, lamellar structure of Pd nanoplates enables the strong adsorption on the surface of electrode, which also do not need some film forming agent or adhesive such as nafion and chitosan. These characteristics greatly simplify the fabrication process of the electrochemical immunosensor, shorten the time, reduce the interference, and promote the stability. This method can find potential application in clinical analysis of AFP and other cancer biomarker.

2. Material and methods

2.1. Reagents and apparatus

The AFP antibody (Ab) and AFP were purchased from Beijing Kwinbon Biotechnology Co., Ltd. (Beijing, China). Bovine serum albumin (BSA, 96–99%) was purchased from Sigma (USA) and used as received. Human serum samples were purchased from a local hospital. All other chemicals were of analytical reagents grade and used without further purification. Five millimolar $K_3[Fe(CN)_6]$ was used as electrolyte for all electrochemistry measurement. Ultrapure water was used throughout the experiments.

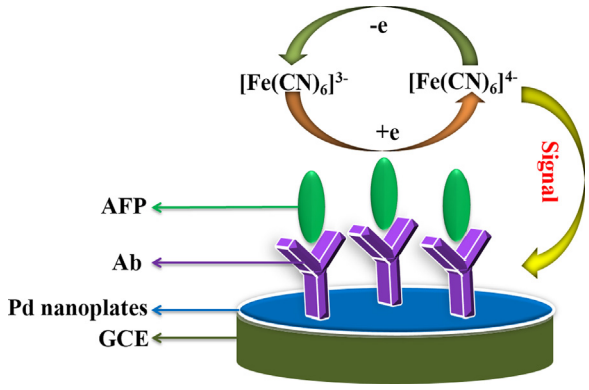
Electrochemical measurements were performed on a CHI 760D electrochemical workstation (Shanghai CH Instruments Co., China). Transmission electron microscope (TEM) images were obtained from a JEM-2100 microscope (Japan).

2.2. Preparation of Pd nanoplates, Pd nanoparticles and nanoporous gold film

Pd nanoplates was synthesized according to literature with some modification (Huang et al., 2010). Pd nanoplates were synthesized as follow, briefly, Pd(II) acetylacetonate ($Pd(acac)_2$, 50.0 mg), poly(vinylpyrrolidone) (PVP, MW = 30,000, 160.0 mg) and CTAB (185 mg) were mixed together with N,N-dimethylpropionamide (10 mL) and water (2 mL). The resulting homogeneous yellow solution was transferred to three-necked flask. The three-necked flask was then heated at 100 °C for 3.0 h in CO atmosphere. Afterwards, it was cooled to room temperature. The dark blue products were precipitated by acetone separated via centrifugation and further purified by an ethanol–acetone mixture. The resulting solid fresh product was obtained by dried under high vacuum. Pd nanoparticles were obtained by reduction of H_2PdCl_4 by sodium borohydride. Nanoporous gold film was prepared according to our previous literature (Wei et al., 2011).

2.3. Preparation of the immunosensor

The schematic diagram of the stepwise self-assemble procedure of the immunosensor was shown in Scheme 1. GCE was polished carefully with Al_2O_3 powder of 1.0, 0.3 and 0.05 μm



Scheme 1. Schematic representation of the electrochemical immunosensor for the detection of AFP.

respectively, to a mirror-like surface, then cleaned through sonication in anhydrous ethanol for half a minute and dried in air. To prepare the Pd nanoplates modified electrode (Pd/GCE), 5 μL of the Pd nanoplates (1 mg/mL) was dropped onto the electrode surface and dried. The electrode was then thoroughly rinsed with ultrapure water and dried in air. Then 5 μL of Ab solution (10 μg/mL) was added onto electrode surface and incubated for 1 h to yield an Ab/Pd/GCE. Then the resulting electrode was incubated in 1 wt% BSA solution for 1 h to eliminate nonspecific binding between the antigen and the electrode surface. Then it was rinsed with ultrapure water to wash away the excess BSA. Subsequently, the proposed electrode was incubated in AFP solution for 1 h with varying concentrations at room temperature to complete the immunoreaction. As a comparison, Pd nanoparticles and nanoporous gold films were used as electrode materials to fabricate analogous immunosensors.

2.4. Experimental measurements

A conventional three-electrode system was used for all electrochemical measurements: glassy carbon electrode (GCE), 4 mm in diameter as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a platinum electrode as the counter electrode. The cyclic voltammetry experiments (CVs) were recorded in 5 mM $K_3[Fe(CN)_6]$ at 100 mV/s. The square wave voltammetry experiments (SWVs) were performed in 5 mM $K_3[Fe(CN)_6]$ and the potential swept from −0.1 to 0.5 V. The electrochemical impedance spectroscopy (EIS) was scanned in 2.5 mM $[Fe(CN)_6]^{4-/3-}$ and 0.1 M KCl solution. All measurements were performed at room temperature.

3. Results and discussion

3.1. Characterization of Pd nanoplates

Fig. 1 is the TEM image of Pd nanoplates. As shown in Fig. 1, the synthetic nanomaterial shows a lamellar structure with a diameter of about 15 nm. The lamellar structure of Pd nanoplates enables strong adsorption on the surface of electrode, especially can more adequately contact with the electrode surface than other morphology of the nanoparticles, which will greatly improve the ability of electron transfer between electrolyte and surface of the electrode.

3.2. Electrochemical characterization of the immunosensor

In order to characterize the fabrication process of the immunosensor, SWVs and EIS at each immobilization step were recorded and shown in Fig. 2A and B. First, a film of Pd nanoplates