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Electrodeposition of gold–platinum alloy nanoparticles on carbon nanotubes as electrochemical sensing interface for sensitive detection of tumor marker

Ya Li, Ruo Yuan*, Yaqin Chai, Zhongju Song

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

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ABSTRACT

A novel electrochemical sensing interface, electrodeposition of gold–platinum alloy nanoparticles (Au–PtNPs) on carbon nanotubes, was proposed and used to fabricate a label-free amperometric immunosensor. On the one hand, the multiwalled carbon nanotubes (MWCNTs) could increase active area of the electrode and enhance the electron transfer ability between the electrode and redox probe; on the other hand, the Au–PtNPs not only could be used to assemble biomolecules with bioactivity kept well, but also could further facilitate the shuttle of electrons. In the meanwhile, horseradish peroxidase (HRP) instead of bovine serum albumin (BSA) was employed to block the possible remaining active sites and avoid the nonspecific adsorption. With the synergetic catalysis effect of Au–PtNPs and HRP towards the reduction of hydrogen peroxide (H_2O_2), the signal could be amplified and the sensitivity could be enhanced. Using alpha-fetoprotein (AFP) as model analyte, the fabricated immunosensor exhibited two wide linear ranges in the concentration ranges of 0.5–20 ng mL^{-1} and 20–200 ng mL^{-1} with a detection limit of 0.17 ng mL^{-1} at a signal-to-noise of 3. Moreover, the immunosensor exhibited good selectivity, stability and reproducibility. The developed protocol could be easily extended to other protein detection and provided a promising potential in clinical diagnosis application.

1. Introduction

The elevated concentration of tumor marker in serum may be an early indication of certain cancer. Therefore, it is necessary and important to develop a method with high sensitivity and selectivity for the determination of tumor marker levels in serum. Electrochemical immunosensor, based on highly specific recognition of antibody and antigen, is a very promising technique for the assay of tumor marker and has been applied widely in many fields such as environment analysis [1], food industry [2,3], and clinical applications [4], which was due to their potential utility as specific, simple, low cost, small size and short response time [5]. Various types of electrochemical immunosensors such as amperometric [6,7], potentiometric [8,9], capacitive [10,11] and impedance immunosensors [12,13] have been reported. Among these immunosensors, the amperometric immunosensor is especially promising for its relatively higher sensitivity, low detection limit and wider linear range [14,15].

In the fabrication process of immunosensor, immobilization of biomolecules with bioactivity kept well on the sensing electrode surface has been considered to be one of the most important points, and accordingly there are many literatures about immunosensor based on different immobilization matrix and sensing interface [16]. With the rapid development of nanotechnology over the past decade, various nanomaterials have been synthesized and used for the construction of biosensors, especially metallic nanoparticles. Recently, bimetallic alloys have been of considerable interest in the field of catalysis and sensors because of the interaction between two components in bimetallic alloys. They often present many favorable properties in comparison with the corresponding monometallic counterparts, which include high catalytic activity, catalytic selectivity and better resistance to deactivation. Gold–platinum alloy nanoparticles (Au–PtNPs) are very attractive among various bimetallic alloys. Besides large surface-to-volume ratio, good biocompatibility and satisfied conductive capability [17,18], Au–PtNPs possessed excellent catalytic activities towards H_2O_2, methanol and so on, due to the high synergistic action between gold and platinum [19]. So it is significant to develop Au–PtNPs based electrochemical sensors with appropriate characteristics such as high sensitivity, fast response time, wide linear range, better selectivity and reproducibility, which was attribute to the advantages of bimetallic nanoparticles. Based on the above reason, Luo et al. have reported Au–PtNPs for electrocatalytic methanol oxidation reaction and the result was satisfying [20].

On the other hand, carbon nanotubes (CNTs), which could possess unique advantages including enhanced electronic properties, a large edge plane/basal plane ratio, and rapid electrode kinetics, have also been incorporated into electrochemical sensors.

* Corresponding author. Tel.: +86 23 68252277; fax: +86 23 68253172.
E-mail address: yuanruo@swu.edu.cn (R. Yuan).

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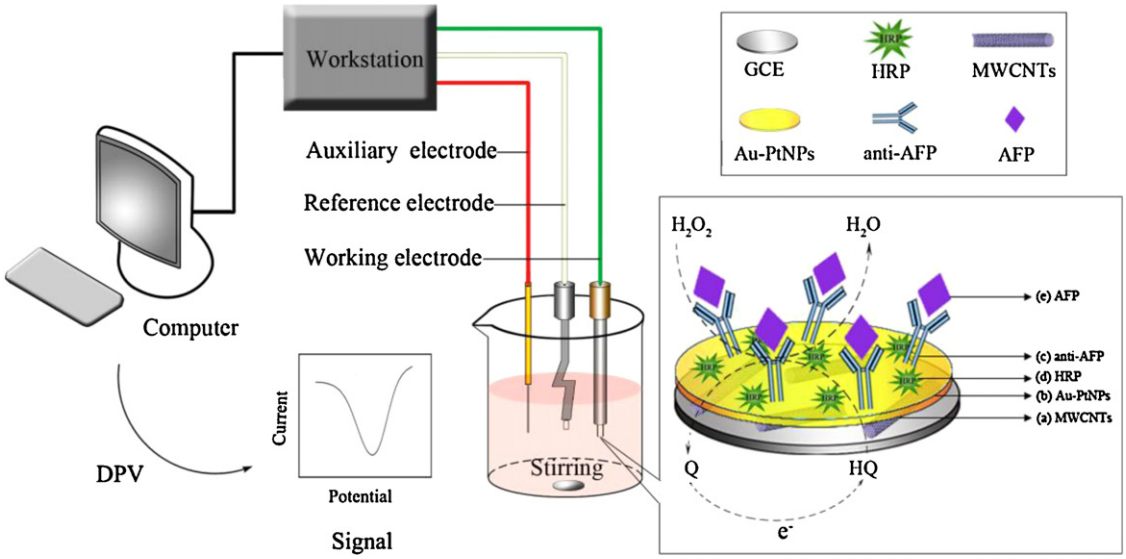

Fig. 1. Schematic illustration of the stepwise immunosensor fabrication process: (a) adsorption of MWCNTs film, (b) electrodeposition of gold–platinum alloy nanoparticles, (c) anti-AFP loading, (d) HRP blocking, (e) AFP loading.

CNT-based sensors generally have higher sensitivities, lower limits of detection, and faster electron transfer kinetics than traditional carbon electrodes [21].

Based on the above consideration, we utilized the unique properties of MWCNTs and Au–PtNPs to fabricate an amperometric immunosensor. Initially, Au–PtNPs were electrodeposited on the multiwalled carbon nanotubes (MWCNTs) modified glassy carbon electrode by constant potential stripping technique, and then anti-AFP was adsorbed onto the sensing interface of the Au–PtNPs/MWCNTs/GCE. Subsequently, horseradish peroxidase (HRP) instead of bovine serum albumin (BSA) was employed to block possible remaining active sites of the Au–PtNPs to avoid the non-specific adsorption and was further used to amplify response signal. The electron transfer between HRP and electrode surface is the limiting factor in the operation of amperometric signal detection. The hydroquinone (HQ) was used as an electron mediator to shuttle electrons between HRP and the electrode surface. Hydrogen peroxide (H_2O_2) was added into the working buffer as substrate, and the response signal could be amplified effectively by the synergistic catalysis action of Au–PtNPs and HRP towards the reduction of H_2O_2. Using alpha-fetoprotein (AFP) as model analyte, the proposed biosensor exhibited a wide linear response range for model analyte, good selectivity and sensitivity, which demonstrated the proposed immunosensor possessed potential applications in clinical screening of cancer biomarkers.

2. Experimental

2.1. Reagents and material

Anti-AFP and AFP were purchased from Biocell Company (Zhengzhou, China), stored in the frozen state before used. The multi-walled carbon nanotubes (MWCNTs, >95% purity, synthesized by CVD method) were purchased from Chengdu Organic Chemicals Co. Ltd. of the Chinese Academy of Science. Chloauric acid, chloroplatinic acid, bovine serum albumin (BSA, 96–99%), *N*, *N*-dimethylformamide (DMF) and horseradish peroxidase (HRP) were bought from Sigma Chemical Co. (St. Louis, MO, USA). Hydrogen peroxide (H_2O_2, 30%, w/v solution) and hydroquinol (HQ) were obtained from Chemical Reagent Co. (Chongqing, China). All of the chemicals used were of analytical grade and were used as received without further purification. Phosphate-buffered solution (PBS, 0.1 M) with various pH values was prepared with stock standard solutions Na_2HPO_4 and NaH_2PO_4. The supporting electrolyte was 0.1 M KCl. Serum specimens provided by Southwest Hospital of Third Military Medical University (Chongqing, China) were stored at 4 °C in a freezer. Double-distilled water was used throughout this study.

2.2. Apparatus

Electrochemical measurements were carried out by CHI 660D electrochemistry workstation (Shanghai CH Instruments Co., China). The scanning electron micrograph was taken with scanning electron microscope (SEM, S-4800, Hitachi). A conventional, three-electrode cell consisting of the modified glassy carbon electrode (GCE) as the working electrode, a platinum wire as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode was used.

2.3. Fabrication of the amperometric immunosensors

The bare GCE were respectively polished with 0.3 and 0.05 μm alumina slurry to obtain mirror-like surface and ultrasonically cleaned with ethanol and double distilled water for 5 min to remove the physically adsorbed substance. Then the electrodes were allowed to dry at room temperature. Subsequently, 10 μL of black suspension of MWCNTs was dispersed in *N*, *N*-dimethylformamide (DMF) was cast on the pretreated bare GC electrode surface and dried in air. After that, the MWCNTs modified GCE was immersed in 2 mL deposition solution (0.2 M Na_2SO_4 aqueous solution containing 1 mM $HAuCl_4$ and 1 mM H_2PtCl_6) [22] and applied a constant potential for 200 s at −0.2 V to obtain Au–PtNPs/MWCNTs modified electrode. Then, it was immersed in anti-AFP solution at 4 °C for 12 h. Finally, the obtained electrode was incubated in HRP solution for 4 h at 4 °C to eliminate non-specific binding effect and block possible remaining active sites. The schematic diagram of the immunosensor was shown in Fig. 1.

2.4. Synthesis of Au–PtNPs

Au–PtNPs were synthesized according to the reference [22]. 1 mL 1 mM $HAuCl_4$ and 1 mL 1 mM H_2PtCl_6 were mixed with 0.2 M Na_2SO_4 aqueous solution. The resulting solution was Au–PtNPs