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An ultrasensitive electrochemical immunosensor for carcinoembryonic antigen detection based on staphylococcal protein A—Au nanoparticle modified gold electrode

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ABSTRACT

The fabrication of staphylococcal protein A (SPA) film on Au nanoparticle (AuNPs)-modified electrode was done for the construction of electrochemical immunosensor. In this work, a highly sensitive electrochemical immunosensor for the detection of a tumor biomarker, carcinoembryonic antigen (CEA) was developed by absorption of anti-CEA antibodies on the SPA-AuNPs modified gold electrode. Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) techniques were employed in the determination of electrode responses and applicability. Under the optimized condition, the immunosensor displays a good linear response to CEA with a range from 1 pg/ml to 100 ng/ml and a detection limit of 0.1 pg/ml. Furthermore, samples of 1–50 ng/ml CEA in rat serum was detected using the immunosensor and the immunosensor showed acceptable recoveries when the CEA value is within the scope of clinical detection. It can be concluded that the immunosensor provides a promising ultrasensitive immunology strategy for clinical diagnosis.

1. Introduction

Electrochemical biosensors, a promising method for label-free, fast and sensitive analysis of biomolecules, is composed of a biological layer which can interact with the substance to be examined and a transducer system which can recognize the interaction and transform the biological signal into electrical signal [1]. The biological layer could be aptamer [2–4], probe DNA [5–8], enzymes [9–12], antibody, cells [13,14] and etc. Antibody-based electrochemical immunosensor, a device to detect biomolecules through the interaction between an antibody and an antigen, has shown great potential in bioanalysis [15–18].

The immobilization of antibody is a vital step in the fabrication of immunosensor since antibody acting as the recognition elements provides the sites of antibody-antigen reaction. A well-defined oriented antibody surface would largely increase antigen binding capacity and enhance the performance of the detection system. So the choice of the antibody immobilization method becomes one of the most important points in the design of an immunosensor. Several methods including physical [15,16,19,20] and chemical adsorption have been proposed for the preparation of oriented antibody molecular layers on the surface of the transducer. Self-assembly (SAM) technique is a frequently-used chemical adsorption method for immobilization of antibody [17,21–23]. Normally, a self-assembly monolayer was firstly on the solid matrix surface through chemical bonds such as Au-S, Si-OH and etc. Then the antibody is covalently attached to the monolayer through cross-linkers. Conducting polymers such as polypyrroles [24–26] is also used extensively in the design of electrochemical immunosensor for its good affinity to biomolecules. However, these immobilization methods have some limitations because of the unpredictable orientation of antibody on the self-assembly monolayer or polymers.

Staphylococcal protein A (SPA), a cell wall component of staphylococcus aureus, is commonly used in bioanalysis and clinical diagnosis [27]. On one hand, SPA can form a uniformly dense monolayer on the surface of gold electrode through the strong affinity to gold atom. On the other hand, SPA has four active sites to bind with the non-antigenic Fc portion of immunoglobulin G (IgG), leaving the antigen binding region Fab portion arranged in the lateral surface of the electrode to bind their target antigens [28,29]. Thus, it is expected that the antibody could be well-oriented on the surface of SPA coating and interact well with the specific antigen.

Gold nanoparticles (AuNPs) is one of the most widely-used nanomaterials in the fabrication of electrochemical immunosensor for its excellent physicochemical properties including good

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conductivity and biocompatibility [5,19,20]. Also, the AuNPs could effectively increase the electroactive area of the electrode when it was attached on the electrode. Thus it provides more active sites for the binding of SPA and electron-transfer and is better at maintaining the bioactivity of the SPA.

In our work, a label-free electrochemical immunosensor was developed for the direct assay of CEA. Firstly, an organic monolayer was formed by the self-assembly of 1,6-hexanedithiol (HDTs) through the strong specific interaction between the sulfur atom and the gold surface. At the same time, the thiol groups on the other end of the HDTs were exposed for further chemical interaction. Then the AuNPs were attached to the HDTs-modified electrode via the Au-S bond. After that, SPA was absorbed on the AuNPs surface through its strong affinity to Au atom. Subsequently, the anti-CEA antibody binds to SPA modified electrode via Fc regions. Before the immunosensor was employed for the determination of CEA, BSA was used to block the non-specific sites. The schematic description of immunosensor preparation procedures is shown in Fig. 1A. The proposed immunosensor and the analysis of CEA using the above immunosensor are investigated by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) using $[Fe(CN)_6]^{4-/3-}$ as the redox probe. The detection principle of antibody-based electrochemical immunosensor is shown in Fig. 1B. When the antigen is specifically attached on the antibody, the electron-transfer of $[Fe(CN)_6]^{4-/3-}$ is hindered by the combination, which can be reflected on the CV and EIS curves. The scanning probing microscope (SPM) and transmission electron microscopy (TEM) are used as supplementary methods to characterize the prepared AuNPs and the AuNPs modified electrode.

2. Experimental

2.1. Reagents and chemicals

All chemicals were of analytical grade. Human carcinoembryonic antigen (CEA), mouse monoclonal antibodies to human CEA and human chorionic gonadotropin (HCG) were obtained from Linc-Bio Science Co., Ltd. (Shanghai, China). Hydrogen tetrachloroaurate(III) hydrate ($HAuCl_4{\cdot}3H_2O$), albumin from bovine serum (BSA), potassium ferrocyanide ($K_4Fe(CN)_6$, 99%), potassium ferricyanide ($K_3Fe(CN)_6$, 99%), absolute ethyl alcohol (99.7%), sodium citrate (99%), hydrochloric acid (99%), nitric acid (99%), potassium chloride (99.5%), sulfuric acid(98%), hydrogen peroxide(30%) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Recombinant Staphylococcal Protein A was achieved from NeuroPeptide Biological Science and Technology Incorporation (Hangzhou, China). 1,6-hexanedithiol was purchased from Aladdin (Beijing, China). Phosphate buffered saline (PBS, 0.01 M, pH 7.2) was obtained from Solarbio Science & Technology Co., Ltd. (Beijing, China).

All solutions employed in the experiment were prepared using deionized Milli-Q water.

2.2. Preparation of AuNPs

The AuNPs used in this work were prepared by the sodium citrate reduction method. All glassware used in the preparation procedures was soaked in fresh aqua regia (HCl: HNO_3, 3:1) overnight, washed thoroughly with ultrapure water, and dried prior to use. 5 ml of 1% sodium citrate solution was added into 100 ml 0.01% boiling $HAuCl_4{\cdot}3H_2O$ solution with vigorous stirring. The color firstly changed from pale to light purple, then to burgundy. Boiling was continued for 15 min. All the AuNPs were stored at 4 °C for future use. Before using, the prepared AuNPs solution was concentrated by centrifuging to obtain the gold colloid with higher concentration.

2.3. Construction of the CEA immunosensor

The gold electrodes were first polished on 1200 mesh metallographic sandpaper, then polished to mirror surface with alumina slurries of 0.05 μm, rinsed thoroughly with ultrapure water and dried with nitrogen. Subsequently, the electrode were treated with fresh piranha's solution (H_2O_2:H_2SO_4, 3:7) for 5 min and rinsed thoroughly with ultrapure water, further sonicated in ethanol and ultrapure water for 5 min respectively. Lastly, the Au electrodes were cleaned by electrochemical scanning with the potential between 0 and 1.6 V vs Ag/AgCl at 0.05 mV/s in 0.1 M sulfuric acid until stable and typical CV curves of a clean Au electrode were obtained.

The cleaned gold electrode was immersed into the HDTs solution (0.5% in pure ethanol) for 8 h to obtain a self-assembled monolayer of HDTs. Then the electrode was thoroughly rinsed with pure ethanol and ultrapure water to remove physically adsorbed HDTs. Afterwards, the electrode was dipped in the colloidal AuNPs for 12 h in 4 °C refrigerator to obtain the AuNPs modified gold electrode. Then the AuNPs-HDTs-Au electrode was immersed into a solution of SPA (0.6 mg/ml in PBS) for 90 min at 37 °C to absorb SPA on electrode. After that, the SPA-modified electrode was dipped in 80 μg/ml anti-CEA antibody solution in PBS (0.01 M, pH 7.2) for 90 min at 37°C. Finally, the modified immunosensor was immersed into a solution of 1% BSA in PBS (0.01 M, pH 7.2) for 1 h at 37 °C. The immunosensor was stored at 4 °C for use. The CEA immunosensors were incubated in CEA solution in PBS with a concentration varying from 1 pg/ml to 100 ng/ml for 1 h at 37 °C. After each procedure, the electrode were rinsed thoroughly with PBS (0.01 M, pH 7.2) to remove unbound molecules.

2.4. Apparatus

All electrochemical measurements were performed with a PC controlled CHI-832 electrochemical analyzer (Chenhua, Shanghai, China). Cyclic voltammetry (CV), and electrochemical impedance (EIS) measurements were performed using a standard three-electrode electrochemical cell which composed of a modified gold electrode (1.6 mm diameter disk-shaped) as the working electrode, saturated Ag/AgCl (KCl) as the reference electrode, and a Pt wire as the counter electrode. EIS was measured from 100 kHz to 0.1 Hz at a bias potential of 0.2 V and ac amplitude of 5 mV. The impedance data were shown in the Nyquist plot, and the fitting software Zview was used to analyze the impedance spectra using the Randles model with some slight modification. In CVs, potential was cycled from −0.1 V to 0.5 V, with scan rate of 50 mV/s. All measurements were performed in 0.1 M KCl solution containing 10 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ as a redox probe.

The size of AuNPs was estimated from transmission electron microscopy (TEM, JEOL-JEM1230). The absorbance spectrum of colloidal AuNPs was recorded by microplate reader (Molecular Devices, USA). The morphological analysis of the gold electrode was performed with scanning probe microscope (SPM, MultiMode, Veeco Inc., USA) with tapping mode at room temperature and humidity of 60%. Images were acquired at a scan rate of 2 Hz with an antimony (n) doped silicon tip.

3. Results and discussion

3.1. Characterization of AuNPs

The TEM image of AuNPs is shown in Fig. 2A. As seen in the figure, the AuNPs are homogeneous in size, mono-dispersed and spherical