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A sensitive amperometric immunosensor for carcinoembryonic antigen detection with porous nanogold film and nano-Au/chitosan composite as immobilization matrix

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Abstract

A sensitive amperometric immunosensor for carcinoembryonic antigen (CEA) was prepared. Firstly, a porous nano-structure gold (NG) film was formed on glassy carbon electrode (GCE) by electrochemical reduction of $HAuCl_4$ solution, then nano-Au/Chit composite was immobilized onto the electrode because of its excellent membrane-forming ability, and finally the anti-CEA was adsorbed onto the surface of the bilayer gold nanoparticles to construct an anti-CEA/nano-Au/Chit/NG/GCE immunosensor. The characteristics of the modified electrode at different stages of modification were studied by cyclic voltammetry (CV). The gold colloid, chitosan and nano-Au/Chit were characterized by transmission electron microscopy and UV–vis spectroscopy. In addition, the performances of the immunosensor were studied in detail. The resulting immunosensor offers a high-sensitivity (1310 nA/ng/ml) for the detection of CEA and has good correlation for detection of CEA in the range of 0.2 to 120.0 ng/ml with a detection limit of 0.06 ng/ml estimated at a signal-to-noise ratio of 3. The proposed method can detect the CEA through one-step immunoassay and would be valuable for clinical immunoassay.

Keywords: Amperometric immunosensor; Carcinoembryonic antigen; Gold nanoparticle; Nano-Au/chitosan composite

1. Introduction

Carcinoembryonic antigen (CEA) is an important tumor marker responsible for clinical diagnosis of over 95% of all colon tumors, 50% of breast tumors, as well as tumors of the lung cancer, ovarian carcinoma, cystadenocarcinoma and others [1–9]. Furthermore, the CEA level in serum is also related to the state of tumor. Thus, the determination of CEA level is very helpful to clinical tumor diagnoses. Compared with conventional immunoassay methods for CEA quantitative determination, such as radioimmunoassay [10], chemiluminescence immunoassay [11], enzyme-linked immunosorbent assay (ELISA) [12,13], the electrochemical immunosensor has been one of attractive analytical tools due to potential utility such as specific, simple, direct detection techniques and reductions in size, cost and time of analysis compared with conventional immunoassay techniques [14–19]. As for the construction of an electrochemical immunosensor, the crucial step is the immobilization of immunoreagent onto the electrode surface. Thus, searching for an effective and simple immobilization method is of considerable interest in our test.

Recently, nanometer-sized gold (NG) particles have been used widely to immobilize protein because of unique physical and chemical features [20]. NG particles with small size may allow more freedom in orientation for the protein molecules anchored to maximize the utilization of their bioactive sites [20,21]. Tedious procedures were required, unfortunately, to assemble gold nanoparticles onto the electrode surface. Comparatively, electrochemical reduction of $HAuCl_4$ solution is a simple and promising method to form a nano-structure film on electrode. The electrochemically deposited NG particles provide a stable and porous surface for antibody immobilization. Moreover, high specific area of the porous gold can amplify the final sensitivity of the original flat surface device [22–24]. By this means, one can deposit NG particles electrochemically on the surface of electrode directly in a short time, and the sizes of

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the nanoparticles can be controlled by different conditions of electrochemical deposition with the advantageous properties being kept.

Moreover, organic–inorganic composite (or hybrid) materials have emerged in recent years. It combines the physicochemical attributes of components and improves their features. Gold nanoparticles (nano-Au) can firmly adsorb antibody because of its large specific surface area, good biocompatibility and high surface free energy [25,26]. Biopolymer chitosan is a polysaccharide derived by deacetylation of chitin. It has primary amino groups that have pKa values of about 6.3 [27]. It possesses many advantages such as excellent membrane-forming ability, high permeability towards water, good adhesion, biocompatibility, nontoxicity, high mechanical strength and susceptibility to chemical modification due to the presence of abundant primary amino groups [28]. It is well known that some functional groups such as cyano (–CN), mercapto (–SH), and amino (–NH_2) groups have a high affinity for Au. Therefore, nano-Au/Chit composite was prepared via covalent bonds between gold nanoparticles (nano-Au) and the –NH_2 groups of the chitosan. This material combined the advantages of inorganic nanoparticles and organic polymer.

The sensitivity of the immunosensor is dictated by the amount of receptor molecules immobilized on the sensor surface. An enlargement of the sensor area would allow for an increase of the binding capacity, hence a larger amount of immobilized receptor molecules. Thus, in this work, in order to improve the sensitivity of the immunosensor, the glassy carbon electrode (GCE) was modified with porous NG film and nano-Au/Chit composite to form gold nanoparticles bilayer to increase the amount of the antibody loaded.

2. Experimental section

2.1. Reagent and materials

CEA and anti-CEA were purchased from Biocell Company (Zhengzhou, China). Chitosan (Chit, MW — 1×10^6, 75–85% deacetylation), bovine serum albumin (BSA, 96–99%), chloroauric acid ($HAuCl_4$) and sodium citrate were purchased from Sigma (St. Louis, MO, USA). All chemicals and solvents used were of analytical grade. Double distilled water was used throughout all experiments. 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 measurements were carried out with a CHI 600 B electrochemistry workstation (Shanghai CH Instruments Co., China). A three-compartment electrochemical cell contained a platinum wire auxiliary electrode, a saturated calomel reference electrode (SCE) and the modified electrode as working electrode. The size of porous NG film was estimated from scanning electron microscopy (SEM) (1000B, AMRAY, American). The sizes of nano-Au, nano-Au/Chit and chitosan were characterized by transmission electron microscopy (TEM) (TECNAI 10, PHILIPS, Holand).

2.3. Preparation of nano-Au colloid

The nano-Au colloid were prepared according to the literature [29] by adding 2 ml of 1% (w/w) sodium citrate solution into 50 ml of 0.01% (w/w) $HAuCl_4$ boiling solution. The nano-Au colloid was stored in a refrigerator with a dark-colored glass bottle before use.

2.4. Preparation of nano-Au/Chit composite material

Appropriate amount of nano-Au colloid was mixed with 0.1% of chitosan (0.05 mM acetic acid). The mixture was stirred for 2 h, and then it was placed at 4 °C overnight. Finally, the nano-Au/Chit composite was formed. The composite was characterized by TEM.

2.5. Fabrication of the immunosensor

A GCE (diam = 4 mm) was first polished on micro cloth pad to a mirror-like finish with 1.0, 0.3 and 0.05 μm alumina slurries to remove adsorbed organic matter. After removal of the trace alumina from the electrode surface, the electrode was cleaned by water and ethanol in an ultrasonic bath and then allowed to dry at room temperature.

Modification with porous NG particles was performed by immersing the clean electrode into the 100 mg l^{-1} $HAuCl_4$ solution and by applying a constant potential of −0.2 V for 60 s. The modified electrode (NG/GCE) was washed with water. A volume of 25 μl nano-Au/Chit composite solution was pipetted onto the surface of the NG/GCE. The casting solution was allowed to dry at 4 °C overnight. Then the modified electrode (nano-Au/Chit/NG/GCE) was washed with water and then immersed in the anti-CEA solution at 4 °C for 12 h. At last the resulting electrode was incubated in BSA solution (w/w, 0.25%) about 1 h at 35 °C in order to block possible remaining active sites and avoid the non-specific adsorption. The finished immunosensor was stored at 4 °C when not in use. The procedures used for construction of the immunosensor are shown in Scheme 1.

2.6. Experimental measurements

Electrochemical measurements were done in an unstirred electrochemical cell at 35 °C and the potential swept from −0.2 to 0.6 V (versus SCE) with the scan rate of 50 mV/s. The immunoreaction was performed by incubating the immunosensor in 0.01 M PBS containing various concentrations of CEA (pH 7.0) at 35 °C for 20 min. The detection of CEA level was performed by detecting the changes of oxidation current response (ΔI) before and after the immunoreaction in 5.0 mM $[Fe(CN)_6]^{4-/3-}$ solution (pH 7.0) containing 0.1 M KCl.

3. Results

3.1. UV–vis spectroscopy

To investigate the interaction of nano-Au colloid with chitosan, UV–vis absorption spectrometry was carried out.