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Clinical Biochemistry 39 (2006) 309–314
CLINICAL BIOCHEMISTRY

Electrochemical detection of hepatitis B surface antigen using colloidal gold nanoparticles modified by a sol–gel network interface

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Received 1 October 2004; accepted 5 December 2005
Available online 28 February 2006

Abstract

Background: A novel potentiometric immunosensor for the detection of hepatitis B surface antigen has been developed by self-assembling gold nanoparticles to a thiol-containing sol–gel network.

Methods: A cleaned gold electrode was first immersed in a hydrolyzed (3-mercaptopropyl) trimethoxysilane sol–gel solution to assemble a three-dimensional silica gel, and then gold nanoparticles were absorbed onto the thiol groups of the sol–gel network. Finally, hepatitis B surface antibody was assembled onto the surface of the gold nanoparticles. The self-assembling procedure was characterized by cyclic voltammetry and electrochemical impedance spectroscopy. Detection is based on the change in potentiometric response before and after the antigen–antibody reaction.

Results: Tests relating to the detection of hepatitis B surface antigen demonstrate that the potentiometric immunosensor exhibited a rapid potentiometric response (<4 min), with high sensitivity, good reproducibility, and long-term stability. The linear range was from 4 to 960 ng·mL^{-1} with a detection limit of 1.9 ng·mL^{-1} (S/N = 3) and the lifetime was 1 month.

Conclusion: Analytical results of several specimens using the developed technique showed satisfactory agreement with those from an ELISA method. This method shows promise for detecting HBsAg in clinical specimens.

Keywords: Potentiometric immunosensor; Hepatitis B; Gold nanoparticles; Sol-gel MPS; Three-dimentional

Introduction

Immunosensors are of great interest because of their potential utility as specific, simple, rapid, label-free, direct detection techniques with small sample requirements compared with conventional immunoassay techniques [1]. Recently, there have been many reports on the use of immunosensors for a wide range of applications in clinical diagnosis [1], and biochemical and environmental analyses [1] due to the high specificity of recognition between antigen and antibody. Of these immunosensors, however, the binding reaction of antigen at the antibody-immobilized surface was often insufficient to produce a large signal change [2]. Therefore, most reports relied on the use of enzyme labels [3] (particularly alkaline phosphatase) that generate electrochemically detectable species and offered a biocatalytic signal amplification. On the other hand, various nonenzyme redox labels, including organic and inorganic tags such as polyvinylchlorene [4], polystyrene [5], colloidal gold [6–8], silver sols [9], Al_2O_3 [10], and SiO_2 [11] have been used in electrochemical immunoassays. However, in these nonenzyme redox label methods, the physicochemical signals associated with binding of an analyte to an antibody or receptor molecule were weaker than those of enzymatic reactions. Thus, exploring a simple and sensitive detection method with real-time output and low cost is of considerable interest.

Here, a new amplification strategy is introduced for improving the sensitivity of potentiometric immunosensor using colloidal gold nanoparticles and a three-dimensional sol–gel network as matrices on a gold electrode. The detection

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doi:10.1016/j.clinbiochem.2005.12.003

is based on the change in the potentiometric response before and after the antigen–antibody reaction.

Materials and methods

Chemicals

Hepatitis B surface antibody (HBsAb) and hepatitis B surface antigen (HBsAg) (1.28 μg·mL^{-1}) were purchased from Kehua Bioeng Co. (Shanghai, China). Bovine serum albumin (BSA, 96–99%), (3-mercaptopropyl) trimethoxysilane (MPS), gold chloride ($HAuCl_4$), and sodium citrate were obtained from Sigma (USA). A 30% hydrogen peroxide solution was purchased from Beijing Chemical Reagent Company (Beijing, China). All chemicals and solvents used were of analytical grade and were used as received. Double distilled water was used throughout this study. The colloidal gold nanoparticles were stored in a brown bottle at 4°C. The standard HBsAg stock solutions were prepared with a phosphate buffer solution (PBS, pH 7.4) and stored at 4°C. The HBsAb was stored frozen, and standard solutions were prepared daily with a phosphate buffer solution (pH 7.4), which was prepared with NaCl 8.0 g, Na_2HPO_4 1.15 g, KH_2PO_4 0.2 g, and KCl 0.2 g dissolved in 1000 mL water.

Preparation of the HBsAg immunosensor

The 16-nm gold nanoparticles were prepared according to the literature [12] by adding 2 mL of 1% (w/w) sodium citrate solution into 50 mL of 0.01% (w/w) $HAuCl_4$ boiling solution. The maximum adsorption of the synthesized colloidal Au in UV–vis spectra was at 520 nm and the solution was stored in a refrigerator with a dark-colored glass bottle before use. When 1.5/1.0 mL of 1% (w/w) sodium citrate solution was added into 50 mL of 0.01% (w/w) $HAuCl_4$ boiling solution, 30-nm and 51-nm gold nanoparticles were obtained, respectively. The particle sizes were confirmed by transmission electron microscopy (TEM, H600, Hitachi Instrument Co., Japan).

The MPS sol–gel was modified from the literature [13]. Silica sol was prepared by mixing MPS with 10% (v/v) of methanol, water at a 1:4 ratio, and 3.3% (v/v) of 0.1 mol·L^{-1} hydrochloric acid. The mixture was sonicated for 50 min until a clear and homogeneous solution resulted and was stored at room temperature for 2–3 h. Then, the homogeneous and pellucid solution was used to self-assemble the gold electrode. The formed MPS sol–gel-modified gold electrodes were immersed into the colloidal gold nanoparticle solution for 8 h at 4°C and then incubated in 1.28 μg·mL^{-1} hepatitis B surface antibody (in PBS, pH 7.4) for 12 h at 4°C to attach hepatitis B surface antibody molecules. All resulting electrodes were washed with water and stored at 4°C.

Performance test of the immunosensor

The electrochemical behavior of the immunosensors was tested by cyclic voltammetric and AC impedance technique. Cyclic voltammetric measurements were carried out with a CHI 660A electrochemistry work station (Shanghai CH Instruments Co., China). The AC impedance of the immunosensor was measured with a Model IM6e (ZAHNER Elektrick Co., Germany). All experiments were carried out using a conventional three-electrode system with the modified electrode as the working electrode, a platinum wire as the auxiliary electrode, and a saturated calomel reference electrode (SCE).

Potentiometric assay

Detection was based on the change in the potentiometric response before and after antigen–antibody reaction. The potentiometric responses were derived from a potentiometer. When the immunosensor was immersed in pH 7.4 phosphate buffer solution, the steady-state potentiometric value (E_1 vs. SCE) was recorded, then an appropriate volume of the standard positive or negative serum was added into the PBS solution, and the steady-state potentiometric value (E_2 vs. SCE) was obtained. The potentiometric response of the immunosensors towards hepatitis B surface antigen was evaluated with the following equation: $\Delta E = E_2 - E_1$.

Statistical analysis

Data were expressed as mean ± standard error of mean. The selectivity, repeatability, and the difference between the detection result of the proposed immunosensor and that of ELISA were analyzed with a χ^2 test. $P < 0.05$ was considered to be statistically significant.

Results

Figs. 1 and 2 show the electrochemical impedance spectra and cyclic voltammograms of the various modified electrodes

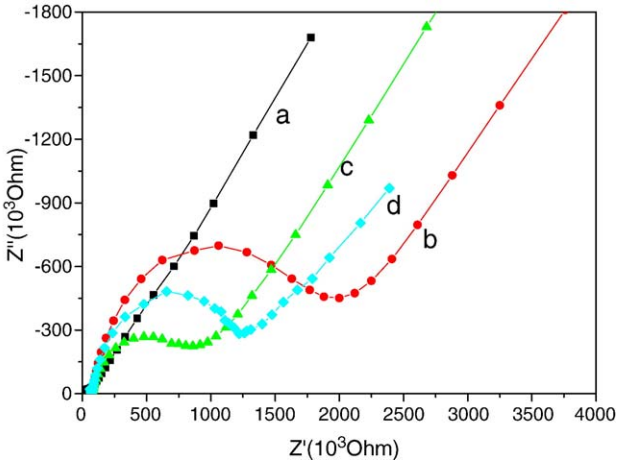

Fig. 1. Complex impedance plots (Z′ vs. Z″ at 220 mV vs. SCE) in 10 mM PBS (pH 7.4) + 0.1 M KCl + 2.5 mM $Fe(CN)_6^{4-/3-}$ solution at (a) bare gold electrode; (b) MPS-modified gold electrode; (c) Au-MPS-modified gold electrode; (d) HBsAb-Au-MPS-modified gold electrode. The frequency range is between 0.1 and 65 535 Hz with signal amplitude of 10 mV.