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Organic–inorganic matrix for electrochemical immunoassay: Detection of human IgG based on ZnO/chitosan composite

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Abstract

A new strategy to construct amperometric immunosensor for human IgG assay based on ZnO/chitosan composite as sensing platform has been described. This material, which combined the advantages of inorganic species, ZnO and organic polymer, chitosan, can maintain biological activity well. A sequential sandwich immunoassay format was performed on the ZnO/chitosan composite supported by glass carbon electrode (GCE) using goat-anti-human IgG antibody (IgG Ab) and human IgG as a model system. Amperometry was used to determine the amount of horse-radish peroxidase (HRP) fixed on the sensor surface, which was related to the content of the desired human IgG. Assay conditions that were optimized included the amount of labeled antibody, the incubation time and temperature, the pH of the substrate solution, etc. Using hydroquinone as a mediator, amperometric detection at −150 mV (versus SCE) resulted in a detection range 2.5–500 ng mL^{-1}, with a detection limit of 1.2 ng mL^{-1}. The simple manipulations of the construction of ZnO/chitosan composite, as well as low-cost and broad linear range, are the main features of the proposed immunosensing method.

Keywords: Electrochemical immunoassay; Organic–inorganic matrix; Human IgG; ZnO/chitosan composite

1. Introduction

In recent years, various kinds of immunosensors for clinical and environmental purposes [1–3] have been developed. Different immunoassay formats, such as electrophoretic immunoassay, enzyme-linked immunosorbent assay (ELISA), radio immunoassay and dot-immunobinding, have been adopted to meet the increasingly analytical needs. The electrochemical immunosensors, which combine simple, portable, low-cost electrochemical measurement systems with specific and sensitive immunoassay procedures, have gained considerable attention [4–6]. On the basis of the specific reaction of the antibody with the antigen, immunosensors provide a tool for the determination of immunoreagents. Here, the immunologic material is immobilized on a transducer; the analyte is measured through labeled species conjugated with one of the immunoreagents. Enzymes such as cholinesterase [7], horse-radish peroxidase (HRP) [8,9] and alkaline phosphatase [10,11] are used extensively as labels to improve the sensitivity of immunoassay by electrochemical amplification of the signals.

In the design and fabrication of electrochemical immunosensors, the development of a simple and effective strategy for immobilizing bioreagents on or into the electrode, is a crucial step. Entrapment [6], absorption [10] and covalent binding [12] are conventional immobilization methods. However, some of these immobilization methods are relatively complex, requiring expensive reagents or environmentally unattractive solvents, and result in relatively poor stability. Thus, new immobilized schemes and advanced materials that can improve the analytical capacities of sensor devices are highly desired. The inorganic ceramics exhibit relatively high mechanical strength, enhanced thermal stability and negligible swelling in both aqueous and organic solutions compared to most organic polymers [13]. Unfortunately, owing to some drawbacks, especially their brittleness, the practical application of ceramic materials is often limited. Efforts have been made to seek for new materials, which could overcome the cracking caused by sol–gel for biomolecular immobilization in biosensor construction.

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Organic–inorganic composite materials have emerged in recent years. They combine the physicochemical attributes of components and improve their features. Organic components benefit from the formation of defect-free inorganic membranes and make them less brittle. Organic membranes can have their chemical and thermal stability improved by an inorganic phase [14]. These organic–inorganic composite membranes have been used to immobilize enzymes to efficiently retain their activity [13,15,16]. To our knowledge, their utility as a sensing platform in electrochemical immunosensor design has not been yet explored. Therefore, it seems that the use of the organic–inorganic composite material, as a sensing platform in electrochemical immunosensor, is quite promising.

In this paper, we present for the first time a novel immunosensor based on nanoporous ZnO/chitosan inorganic–organic composite film as an immobilization matrix. This material combined the advantages of inorganic species, ZnO, and organic polymer, chitosan. Chitosan was chosen as the material to form the membrane due to its excellent film-forming and adhesion abilities, together with its nontoxicity and biocompatibility [17,18]. Moreover, a chitosn contains amino groups, thus providing a hydrophilic environment, which is compatible with the biomolecules. The immobilization of the antibody is based on the absorption of the nanoporous ZnO. The nanoporous structure of ZnO greatly enhances the active surface available for antibody binding over the geometrical area. To investigate the feasibility of this methodology, goat-anti-human IgG and human IgG were chosen as model systems, while horse-radish peroxidase served as the enzyme label. To quantify the amount of IgG-HRP on the surface of GCE, which is inversely proportional to the amount of the analyte, hydroquinone and H_2O_2 were used as substrates. This matrix can also be used to immobilize other biomolecules.

2. Experimental

2.1. Reagents and solutions

Peroxidase anti-human IgG (HRP-IgG Ab, 1.0 mg mL^{-1}) was purchased from Dingguo Biochemical Reagents (Beijing, China). Hydrogen peroxide (30% v/v aqueous solution) and hydroquinone were obtained from Shanghai Chemical Reagents (Shanghai, China). Goat-anti-human IgG antibody (IgG Ab, affinity purification), normal human reference serum (NHRS, containing 10.9 mg/mL immunoglobulin G (IgG)), bovine serum albumin (BSA) and trishydroxymethyl aminomethane (tris) were supplied by Shanghai Biochemical Reagents (Shanghai, China). Chitosan (CHIT, MW−1×10^{-6}, 75–85% deacetylation) was supplied by Sigma (St. Louis, MO, USA). Nanoporous ZnO was produced by Nano Material Application Engineering Technology Center (Zhejiang, China).

An incubating buffer, 0.1 mol L^{-1} tris–HCl and 1.0 mmol L^{-1} EDTA (pH 7.5), was used. IgG and IgG Ab solutions of the desired concentration were prepared by diluting stock IgG Ab and IgG solutions in the same tris–HCl buffer. A solution of 2% BSA in tris–HCl (pH 7.5) buffer was used as a blocking buffer. The washing solution was 0.1 mol L^{-1} tris–HCl/0.1 mol L^{-1} KCl buffer. The supporting electrolyte of the enzymatic substrate was 0.067 mol L^{-1} phosphate buffer saline (PBS) containing 0.1 mol L^{-1} KCl (pH 7.00). All solutions were prepared with double distilled water.

2.2. Apparatus

Cyclic voltammetric and amperometric analyses were carried out using a CHI 760b Electrochemical Analyzer (Chen Hua Instrument Inc, Shanghai, China). Scanning electron microscope (SEM) image of ZnO/CHIT matrix was taken with a KYKY 2800, using an accelerating voltage of 20 KV (Hitachi, Tokyo, Japan). The three-electrode system consisted of a glass carbon electrode (GCE) (of 4 mm in diameter) as the working electrode, the saturated calomel electrode (SCE) as the reference electrode and a Pt foil as the counter electrode. Cyclic voltammetric experiments were performed in unstirred solutions. Amperometric measurements were carried out in stirred substrate solutions with a steady-state background current that was first obtained before standard H_2O_2 solution was added into the buffer solution. All potentials were measured and reported versus the SCE. A magnetic stirrer and bar provided the convective transport.

2.3. Preparation of ZnO/CHIT solution

An appropriate amount of nanoporous ZnO was dispersed in 0.5% chitosan (0.05 M acetic acid), and the mass ratio of ZnO to chitosan was 1 to 100. The mixture was sonicated for 15 min after stirring for 1 h. Then, a high dispersed colloidal solution was formed.

2.4. Preparation of the immunosensor

A casting solution was obtained by mixing 20 μL of ZnO/chitosan composite solution with 20 μL of IgG Ab solution (56 μg mL^{-1}). An aliquot (10 μL) of this resulting casting solution was pipetted onto the surface of the GCE, being polished before each experiment with 0.05 μm of α-alumina powder, rinsed thoroughly with absolute alcohol and distilled water in ultrasonic bath and dried in the air. The casting solution was allowed to dry at 4 °C overnight. Then, the electrode, loaded with IgG Ab, was introduced into a 2% BSA blocking buffer for 15 min at 25 °C. Rinsed with washing solution, the electrode was immersed in the IgG Ag solution (74 μg mL^{-1}) and incubated for 35 min at 25 °C. Rinsed with washing solution again, the electrode was immersed in the HRP-IgG Ab solution and incubated for 35 min at 25 °C. The immunosensor was, then, rinsed thoroughly with washing buffer and stored in the incubating buffer solution prior to the amperometric measurement.

2.5. Measurement

The amperometric measurements were performed in an electrochemical cell, holding 10 mL of the supporting electrolyte and containing 1.0 mmol mL^{-1} hydroquinone. A three-electrode system was used at the applied potential of −150 mV versus