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Flow-injection electrochemical immunosensor for the detection of human IgG based on glucose oxidase-derivated biomimetic interface

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ABSTRACT

A newly flow-through electrochemical immunosensor for monitoring IgG in human serum has been developed by using core-shell SiO_2/Au nanocomposites and poly(amidoamine) G4 dendrimer as matrices. The ferrocenecarboaldehyde-labeled *anti*-IgG biomolecules were initially chemisorbed onto the nanoparticle surface, and then glucose oxidase (GOx), as a blocking reagent instead of bovine serum albumin (BSA), was backfilled onto the modified surface. The formation of the antibody–antigen complex by a simple one-step immunoreaction between the immobilized *anti*-IgG and IgG in sample solution introduced a barrier of direct electrical communication between the immobilized GOx and the base surface, and decreased the immobilized GOx toward the catalytic oxidation of glucose. The performance and factors influencing the performance of the immunosensor were evaluated. Under optimal conditions, the linear range of the developed immunosensor by using GOx as enhancer was from 5.0×10^{-6} to 9.6×10^{-4} mol/L with a detection limit of 8.0×10^{-7} mol/L IgG (at 3δ), while the detection limit by using BSA was 1.5×10^{-5} mol/L IgG (at 3δ) with the linear range from 3.5×10^{-5} to 1.2×10^{-3} mol/L. The selectivity, reproducibility and stability of the proposed immunosensor were acceptable. The IgG contents in 37 human serum samples obtained by the proposed method are identical with the data of clinical laboratory.

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1. Introduction

Immunoassay based on the antigen–antibody specific reactions has been considered a major analytical tool in clinical diagnoses, environment, and biochemical studies (McGlinchey et al., 2008; Henarea et al., 2008; Cui et al., 2007; Tang et al., 2007). Various immunoassay protocols, such as surface plasmon resonance (SPR), quartz crystal microbalance (QCM), chemiluminescence and electrochemical method, have been extensively developed for the detection of biomarkers (Ehrhart et al., 2008; Kim et al., 2008; Wasowicz et al., 2008; Yang et al., 2008). Among these methods, electrochemistry with high sensitivity, low cost, low power requirements and high compatibility has been a preferable approach for clinical and environmental immunoassays (Pohanka and Skladal, 2008; Marquette and Blum, 2006; Jie et al., 2007; Campas and Marty, 2007a; Tang et al., 2005). Up to date, many promising strategies such as enzymes, nanoparticles, liposome, and DNA-based polymerase chain reaction (PCR) have been employed for the signal amplification, however, it is a challenge to find new approaches that could improve the simplicity and sensitivity of the electrochemical immunoassays (Jung et al., 2008; Adler et al., 2008; Jiang et al., 2008; Campas et al., 2008a,b).

Protein-mediated assembly of nanoparticles is a potent tool for the creation of new materials (Russier-Antoine et al., 2008; Sarikaya et al., 2003; Yan et al., 2008). These materials combine tunable nanoparticle features (size, surface functionality, and core properties) with the unique physical and chemical properties of proteins (Ringler and Schulz, 2003; Yang et al., 2004; Kurppa et al., 2007). Various nanomaterials, such as magnetic gold nanospheres (Tang et al., 2008), gold nanocatalysts (Selvaraju et al., 2008), core-shell Ag/Au nanoparticles (Tang et al., 2006a), and core-shell Fe_3O_4/Ag nanoparticles (Tang et al., 2006b), have been used for the fabrication of electrochemical immunosensors. Recently, Zhu introduced a novel strategy for the signal amplification of the electrochemical immunoassay by using gold nanoparticle-colloidal carbon nanosphere hybrid material (Cui et al., 2008). The hybrid nanomaterials were used for the detection of protein with high sensitivity and low detection limit, and exhibited unique chemical and physical properties to enable new and advanced functions in comparison with bulk materials. Chang also described an electrochemical immunosensor by using a multi-functionalized gold nanoparticles for signal amplification, which displayed a wide linear range and a lower detection limit of 1.0 pg/mL protein A (Lin et al., 2008).

In addition to the nanomaterials, enzymes with bioactivity were used for the amplification of electrochemical signals (Campas and

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Marty, 2007b). Campas described an enzymatic recycling-based amperometric immunosensor for the ultrasensitive detection of okadaic acid (Campas et al., 2008a,b). The highlight of this method is the improvement of the labeled method of the secondary antibodies for the conventional ELISA, which used double-enzyme labels in combination with the relationship between substrate and product. Wilson used enzyme-labeled electrochemical immunoassays for the simultaneous detection of multiple tumor markers (Wilson, 2005; Wilson and Nie, 2006). The detection limit and sensitivity were greatly improved due to the bioelectrocatalytic reaction of the labeled enzyme. Rusling introduced another sandwich-type immunoassay method for the detection of PSA with a detection limit of 5 pg/mL by modifying HRP-bound antibodies with carbon nanotubes as secondary antibodies (Yu et al., 2006). Ju designed a disposable electrochemical immunosensor array for the simultaneous determination of four biomarkers by using the HRP-labeled antibodies (Wu et al., 2007).

In this contribution, we combined the advantage of the hybrid nanomaterials with the amplified property of the bioactive enzyme, and synthesized the core-shell SiO_2/Au composite nanoparticles, which were used for the fabrication of biomimetic interface on the poly(amidoamine) dendrimer-modified electrode. The amplification strategy is on the basis of backfilling the immobilization of GOx onto the antibody-functionalized core-shell SiO_2/Au nanoparticles surface toward the catalytic oxidation of glucose. The unique point of the method is that the signal amplification does not require the routine and cumbersome process such as enzyme-labeled secondary antibody.

2. Experimental method

2.1. Materials

Goat *anti*-human IgG monoclonal antibody (*anti*-IgG) and human IgG (IgG) were obtained from Sigma (USA). Poly(amidoamine) fourth-generation dendrimer, ferrocenecarboaldehyde (Fc-CHO), 3-3′-Dithio-bis(propionic acid *N*-hydroxysuccinimide ester) (DTSP), bovine serum albumin (BSA), dimetyl sulfoxide (DMSO) and glucose oxidase (GOx) were purchased from Sigma–Aldrich and used as received. All other reagents, unless specified, were of analytical grade and used without further purification. Deionized and distilled water was used throughout the study. Phosphate buffered solutions (PBS) of various pHs were prepared using 0.01 M Na_2HPO_4 and 0.01 M KH_2PO_4.

2.2. Preparation of Fc-CHO-labeled anti-IgG (Fc-anti-IgG)

50 μL of *anti*-IgG (1.28×10^{-3} mol/L) was initially dissolved in 1 mL of 0.01 M PBS and the pH was adjusted to 9.5 by using 10% (w/w) K_2CO_3, and then 50 μL of Fc-CHO dimethylformamide solution (200 mg/mL) was added into the *anti*-IgG PBS. After incubation for 30 min, 0.5 mg of sodium borohydride was injected for the reduction of Fc-CHO. Afterward, the mixture solution was adjusted to pH 7.0 by using 1 M NaH_2PO_4. Removal of unlabeled Fc-CHO was carried out by ultrafiltration for 12–15 times until the peak corresponding to ferrocene in the elution disappeared.

2.3. Synthesis of nanogold, nanosilica, and core-shell SiO_2/Au nanoparticles

16-nm nanogold particles were synthesized by reducing gold chloride tetrahydrate with citric acid at 100 °C for half an hour (Frens, 1973). 16-nm silica nanoparticles were prepared as described in the literature (Tang et al., 1992). The synthesis of the core-shell SiO_2/Au nanoparticles was as follows: 1.9 mg of SiO_2 nanoparticles was initially transferred into 100 mL of 0.3 mM sodium citrate aqueous solution, and the mixture solution was then heated to the boil with vigorous stirring. Following that, 1.0 mL of $HAuCl_4$ solution (1%, w/w) was injected as soon as possible. The solution color was changed from pale-white to brown and dark brown, and finally to the deep red. The absorption peak of the synthesized core-shell SiO_2/Au colloids in the UV–vis spectra was at 518 nm, which was stored in a dark-colored glass bottle at 4 °C when not in use.

2.4. Preparation of the electrochemical immunosensor

A cleaned gold electrode (4 mm in diameter) was initially dipped into a 5 mM DTSP–DMSO solution for 2 h at room temperature to form an amine-reactive self-assembly monolayer. When washing with DMSO and ultra-pure ethanol, the modified electrode was immersed into the poly(amidoamine) G4 dendrimer ethanol solution (0.5%, w/w) for 4 h at 4 °C. After that, the dendrimer-modified electrode was initially dipped into the core-shell SiO_2/Au colloids (1.92%, w/w) for 6 h at 4 °C, and then immersed in the prepared Fc-*anti*-IgG solution above for 6 h at 4 °C. (*For comparison, pure nanogold particles and pure nanosilica particles were also used.*) The formed film was carefully washed with water after each step. Subsequently, the Fc-*anti*-IgG/nanoparticles/dendrimer G4/DTSP-modified gold electrode was incubated into the GOx solution (0.25%, w/w) for 60 min at 37 °C. The backfilling immobilization of GOx could not only eliminate non-specific binding effect and block the remaining active groups, but also favor the amplification of electrochemical signal in the presence of glucose. The finished immunosensors were stored at 4 °C when not in use. The fabricated procedure of the electrochemical immunosensor is schematically illustrated in Scheme 1A.

2.5. Fabrication of the flow immunoassay (FIA) system

Scheme 1B represents the developed FIA system, which consists of a six-way control valve, two 1 mL syringe pumps and a flow cell. The carrier buffer (10 mM PBS, pH 7.0), the sample (IgG) and the regenerating solution (8 M urea) were automatically introduced into the detection cell at 100 μL/min. The electrochemical measurements were carried out with an AutoLab system (The Netherlands) by using a conventional three-electrode system containing a platinum wire auxiliary electrode, a saturated calomel reference electrode (SCE) and a modified gold electrode as working electrode. The flow immunoassay was carried out at room temperature (25 ± 1.0 °C) as the following steps: (i) the IgG standard sample with various concentrations was delivered into the detection cell, and incubated with the immobilized *anti*-IgG antibodies for 10 min at the stopping flow mode; (ii) after washing with pH 7.0 PBS, 10 mM of glucose-PBS (pH 7.0) was injected into the detection cell, at the same time, the cyclic voltammogram was recorded in the stopping flow mode; (iii) after each assay run, the regeneration of the contaminated immunosensor was performed by washing for 5 min with 8 M urea.

2.6. Scanning electron microscopy (SEM) and quartz crystal microbalance (QCM) measurement

SEM measurements were performed using a digital JSM-T220 scanning electron microscope (Hitchi Co., Japan) at an acceleration voltage of 15 kV and a working distance of 4–5 mm. The images were acquired in the tapping mode. Gold substrates were prepared by thermal evaporation of 5 nm Cr followed by 200 nm gold onto silicon wafers that were precleaned by heating in piranha solution at 90 °C for 1 h. Prior to assembly, the gold substrates were treated with concentrated HNO_3 for 10 min, washed exhaustively with water and ethanol, and dried. 10-MHz QCM device (PICBAL-

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