Analytical Biochemistry 421 (2012) 649–656

Contents lists available at SciVerse ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

A high-sensitivity electrochemical immunosensor based on mobile crystalline material-41–polyvinyl alcohol nanocomposite and colloidal gold nanoparticles

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ARTICLE INFO

Article history:
Received 21 September 2011
Received in revised form 28 November 2011
Accepted 9 December 2011
Available online 14 December 2011

Keywords:
Competitive immunosensor
Polyvinyl alcohol (PVA)
Mobile crystalline material-41 (MCM-41)
Gold nanoparticles (AuNPs)
Human serum albumin (HSA)

ABSTRACT

A novel competitive immunosensor was developed as a model system using anti-human serum albumin (HSA)-conjugated gold nanoparticles (AuNPs) as an electrochemical label and mobile crystalline material-41 (MCM-41)–polyvinyl alcohol (PVA) mesoporous nanocomposite as an immobilization platform. However, no attempt has yet been made to use the MCM-41 as the supporting electrolyte for the electrosynthesis of nonconducting polymer nanocomposite. This hybrid membrane was evaluated extensively by using field emission scanning electron microscopy (FESEM), cyclic voltammetry (CV), and differential pulse voltammetry (DPV) to determine its physicochemical and electrochemical properties in immunosensor application. FESEM revealed an appropriate and stable attachment between HSA and MCM-41 and also a dense layer deposition of MCM-41–HSA–PVA film onto the electrode surfaces. DPV was developed for quantitative determination of antigen in biological samples. A decrease in DPV responses was observed with increasing concentrations of HSA in standard and real samples. In optimal conditions, this immunosensor based on MCM-41–PVA nanocomposite film could detect HSA in a high linear range (0.5–200 μg ml^{-1}) with a low detection limit of 1 ng ml^{-1}. The proposed method showed acceptable reproducibility, stability, and reliability and could also be applied to detect the other antigens.

Accurate and sensitive detection of biomarkers is important for both preclinical research fields, such as biomedicine and biochemistry, and clinical diagnosis. From many developed methods for measuring the biomarkers, immunoassays are among the dominant biological techniques that have been used to detect or quantify a specific substance, the analyte, in a blood or body fluid sample. These assays have found widespread applications in disease diagnosis, food safety, and environmental analysis [1,2]. However, finding the new immunoassay system with lower cost and higher sensitivity and selectivity has attracted considerable interest.

Immunosensors are the miniaturized analytical devices that combine high specificity of immunological reaction with sensitivity of detection techniques. Currently, many electrochemical, optical, thermal, and microgravimetric immunosensors have been developed for biomarker detection [3]. Among them, electrochemical immunosensors have received increasing attention due to their lower cost, high sensitivity, simple instrumentation, and easy signal amplification [4–7]. However, it is still a challenge to find the new approaches that could improve the simplicity, selectivity, stability, and sensitivity of clinical immunoassays [8].

Nanomaterials have been implemented in bioanalysis in general, and in biosensors in particular, because of their capacity in improving the stability and sensitivity of biosensors. These advantages, along with capability of nanomaterials for improvement of cost efficiency of biosensors, have made them one of the most attractive materials employed in biosensors [9–11].

In this study, we have developed an electrochemical immunosensor, using nanogold microsphere-labeled antibodies as electrochemical signal probes and screen-printed carbon electrode (SPCE)[1] modified with mesoporous silica nanoparticles (MSNs) and polyvinyl alcohol (PVA) as the electrochemical transducer, for rapid and sensitive measurement of analytes in biological samples.

During recent years, the screen-printed electrodes have been used extensively in development of biosensor systems and especially for immunosensing technologies. These devices have the advantages of disposability, an important characteristic when working with real samples, and the small sensor dimension that allows their combination with a portable measuring system [12].

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[1] *Abbreviations used:* SPCE, screen-printed carbon electrode; MSN, mesoporous silica nanoparticle; PVA, polyvinyl alcohol; MCM-41, mobile crystalline material-41; UV, ultraviolet; AuNP, gold nanoparticle; HSA, human serum albumin; mAb, monoclonal antibody; DPV, differential pulse voltammetry; FCS, fetal calf serum; BSA, bovine serum albumin; $HAuCl_4$, chloroauric acid; $Na_3C_6H_5O_7$, trisodium citrate; PBS, phosphate buffer solution; FESEM, field emission scanning electron microscopy; CV, cyclic voltammetry; ELISA, enzyme-linked immunosorbent assay; TEM, transmission electron microscopy; RSD, relative standard deviation; IT, immunoturbidimetry; CLIA, chemiluminometric immunoassay.

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doi:10.1016/j.ab.2011.12.022

Mesoporous materials with a pore size of 2–50 nm are a series of inorganic nanomaterials with specific characteristics such as large surface area; high mechanical, thermal, and chemical stability; good adsorption quality; and low toxicity. They can also act as an appropriate platform to transfer the electron from the environment into the electrode [13,14]. MSNs are more effective in protein immobilization than conventional materials [15,16]. Among mesoporous materials, mobile crystalline material-41 (MCM-41), a hexagonal mesoporous silica with a high surface area and loading capacity, ordered uniform pore structure, good biocompatibility, and fine environment for electron transfer, has been considered by many investigators as an excellent candidate for protein immobilization in electrochemical biosensor [14,17,18]. In particular, the combination of nanocomposite MCM-41 with PVA (MCM-41–PVA) as a sensing platform for development of electrochemical biosensors has attracted considerable interest [13,14,18]. PVA is a biocompatible nontoxic synthetic polymer that has exclusive features, including flexible molecular chains, chemical stability, and ductile nature [19,20]. Indeed the excellent film-forming property and electrode adhesion capacity of PVA makes it an appropriate matrix for development of biosensor [21]. Photo polymerization of PVA by ultraviolet (UV) radiation produces a suitable three-dimensional structure for immobilization of biological molecules. Presentation of biological molecules in this step to PVA would result in entrapment of molecules [19,21]. Considering this property, we used PVA as a polymeric binder backbone to efficiently immobilize the functional antigen molecules on the electrode surface.

Gold nanoparticles (AuNPs) with special characteristics of high-specificity surface area and good biocompatibility and conductivity have been employed as a label in biosensor to amplify the transduced signals of antibody–antigen interactions [9,22]. Electrooxidation of AuNPs in HCl with production of electroactive $AuCl_4$ has been recently proposed [23–28] as a manner in which to increase the sensitivity of electrochemical immunosensors.

Incorporation of MCM-41 with PVA provides hybrid materials with synergetic properties that result in improvement of electrocatalytic properties of modified electrodes. However, to the best of our knowledge, there is no evidence of applying the MCM-41–PVA combination or MCM-41 alone as an immobilizing agent in electrochemical immunosensor system development.

In this study, we used human serum albumin (HSA) as a model protein and biological marker to be measured by our biosensor. MCM-41 was employed for immobilization of HSA, and PVA was used as a sensor platform for better immobilization of MCM-41–HSA on the SPCE surface. By conjugation of a homemade mouse monoclonal antibody (mAb) against HSA to AuNPs (Ab–AuNPs), a competitive immunoreaction was produced. The detection process was based on the reaction of analyte with the Ab–AuNP complex. Preoxidization of this complex in 0.1 M HCl at1.3 V for 80 s followed by reduction of $AuCl_4^-$ to Au^o in differential pulse voltammetry (DPV) mode would produce the electrochemical signals. In fact, by using the oxidoreduction properties of the AuNPs in acidic medium, the quantification of nanoparticles, and in turn identification of the analyte in the serum sample, would be plausible [23–29].

A schematic representation of competitive electrochemical detection of HSA is provided in Fig. 1.

Materials and methods

Materials

RPMI 1640, fetal calf serum (FCS), streptomycin, penicillin, bovine serum albumin (BSA), HSA, hemoglobin, protein G column, chloroauric acid ($HAuCl_4$), trisodium citrate ($Na_3C_6H_5O_7$), sodium azide, skim milk, and vinyl alcohol all were purchased from Sigma–Aldrich (St. Louis, MO, USA). The analytical-grade HCl and other chemicals were purchased from Merck (Spain) and used as received. The phosphate buffer solution (PBS, pH 7.5) consisted of 0.01 M phosphate-buffered saline, 0.137 M NaCl, and 0.003 M KCl. Mesoporous MCM-41 was prepared in the electrochemistry laboratory of Tehran University based on a previously established synthetic procedure [30,31]. All solutions were prepared in distilled water.

Apparatus

All electrochemical experiments were carried out using a DropSens potentiostat equipped with connector for disposable SPCE, including round-shaped graphite working electrode (3 mm diameter), Ag/AgCl reference electrode, and graphite auxiliary electrode (DropSens, Spain). Electrochemical studies were performed using a single Teflon-based electrochemical cell with a 5 mm diameter (50 μl volume) in the sensing area, as was reported in our previous work [23]. Centrifugation of samples was carried out by using the centrifuge instrument (Avanti J-25I, Beckman, UK). The morphology of the modified electrode was characterized using Hitachi model s-4160 field emission scanning electron microscopy (FESEM) at 15 kV (Hitachi, Tokyo, Japan).

Production and purification of albumin monoclonal antibody

Albumin mAb-producing hybridoma cell lines were generated from the fusion of mouse spleen lymphocyte and Sp2/0 myeloma cells based on the method reported by Omidfar and coworkers [32]. Briefly, the hybridoma cells were cultured in a 50-ml flask containing 3% FCS in RPMI 1640. Then, antibodies were purified from the culture supernatants using affinity chromatography on HiTrap protein G columns. Finally, the eluted antibodies were dialyzed against PBS and concentrated in Ulterafree-15 centrifugal filter units (Millipore, Billerica, MA, USA) [32].

Preparation of AuNPs and modification with mAb

In this study, the 20 nm AuNPs was selected as label due to the best electrochemical response for AuNP suspension obtained by this size [29]. The 20 nm AuNPs was synthesized by reducing $HAuCl_4$ using trisodium citrate according to our previous work [33]. In brief, 100 ml of tetrachloroauric acid (0.01% [w/v] $HAuCl_4$) was brought to a boil, and then a solution of 1% trisodium citrate was added with constant stirring. When the color of the solution changed from yellow to wine red after approximately 8 min, the solution was cooled down to room temperature. The pH was adjusted to 8.5 with concentrated sodium hydroxide, and then 0.01% (w/v) sodium azide was added. The obtained AuNP solution was stored at 4 °C in a dark glass bottle.

Bioconjugation was carried out by adding 300 μg of HSA mAb (30 μg ml^{-1}) in 0.01 M PBS (pH 7.5) to 10 ml of pH-adjusted colloid Au solution at room temperature, followed by gentle continuous overnight stirring at 4 °C. Then, the 10% BSA solution was added to block the residual surface of the colloidal AuNPs. The mixture was stirred for 5 min at room temperature and then centrifuged at 15,000*g* (45 min, 4 °C). The pellet was collected and suspended in 10 ml of PBS containing 1% (w/v) BSA and 0.05% sodium azide. Finally, the conjugate was centrifuged several times, and the pellet was resuspended in 10 ml of PBS and stored at 4 °C [33].

Preparation of MCM-41–HSA conjugate

MCM-41 (2 mg) was dispersed in 1 ml of PBS (pH 7.2, 0.01 M) to obtain a suspension of MCM-41. To immobilize HSA on the mesoporous materials, 2 μl of solution containing 60 μg of HSA in PBS was added to the 150-μl MCM-41 suspension and allowed to stir