Biosensors and Bioelectronics 109 (2018) 171–176

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Improved sandwich-format electrochemical immunosensor based on "smart" SiO_2@polydopamine nanocarrier

Dongsheng Zhang[1], Weixiang Li[1], Zhanfang Ma*

Department of Chemistry, Capital Normal University, Beijing 100048, China

ARTICLE INFO

Keywords:
Methylene blue
Mesoporous silica nanoparticles
Polyaniline hydrogel
Electrochemical immunoassay
Prostate specific antigen
Tumor marker

ABSTRACT

An improved sandwich-type electrochemical immunosensor based on the novel signal amplification strategy was developed. Methylene blue (MB) loaded mesoporous silica nanoparticles (MSN) with polydopamine coating (PDA) were employed as "smart" labels, while phytic acid doped polyaniline hydrogel (PANI) with high adsorption capacity was acted as substrate. In this strategy, amount of redox species (MB) encapsulated in MSN by PDA will be released under acidic condition and then absorbed stably by PANI. Meanwhile, the label tended to drop down due to the destruction of polydopamine coating and the disassociation of antibody-antigen composites. The advantages of as-prepared immunosensor are as follows: (1) Higher electron transfer efficiency was obtained because of the decrease of relative position between MB and the electrode; (2) Lower impedance was achieved due to the loss of the labels and the dissociation of antigen-antibody insulating layer; (3) Abundant MB molecules were loaded on MSN/PDA nanocarrier with large pore volume for signal amplification. Under optimum conditions, the proposed immunosensor exhibited a low detection limit of 1.25 fg mL^{-1} and a wide linear range from 10 fg mL^{-1} to 100 ng mL^{-1} for prostate specific antigen detection. Importantly, present method showed good stability, selectivity, and reproducibility, which possessed wide potential applications for the detection of other biomarkers.

1. Introduction

Taking advantage of high bio-specific recognition of antibody-antigen, the sandwich-type structure has been widely used in various assay techniques in immunoassays, i.e. electrochemical immunoassay (Zhang et al., 2015; Zhao et al., 2018), chemiluminescence immunoassay (Zeng et al., 2016; Zhang et al., 2018a, 2018b), enzyme-linked immunosorbent assay (Preechakasedkit et al., 2018). Sandwich-type electrochemical immunosensor based on high sensitivity electroanalytical methods and inherent specificity of immunological reaction possesses significant advantages such as good stability, fast response, simple instrument and low cost (Tang et al., 2008; Yang et al., 2018). They have been extensively developed for the detection of tumor markers (Jia et al., 2014; Liu and Ma, 2014; Tang and Ma, 2017). However, there are still challenges of improving sensitivity and detection limit of sandwich-type amperometric immunosensor to meet the demand of modern clinical diagnosis and biomedical research applications.

For sandwich-type amperometric immunosensor, the primary antibodies (Ab_1) are usually immobilized on the electrode to selectively capture the analyte protein from the sample, while the sandwich immunocomplex is formed after a sandwich immunoreaction between the immobilized Ab_1 and signal antibodies. Quantification is generally achieved by monitoring the detectable signal derives from the label which is proportional to the amount of targets presented in sample (Jiang et al., 2018; Wu et al., 2015; Yáñez-Sedeño et al., 2016; Zhang et al., 2013). Therefore, various appropriate strategies for improving the performance were carried out with following two considerations: (1) Increasing the amount or activity of immobilized antibodies to facilitate the possibility of antigen-antibody reaction (Wang et al., 2016; Zhang et al., 2018a, 2018b); (2) Amplifying the detectable signal by multi-functional label (usually enzyme-labeled antibodies or nanoparticle-labeled antibodies) (Chikkaveeraiah et al., 2012; Liu and Lin, 2007; Pei et al., 2013; Shan and Ma, 2017; Soleymani et al., 2011). Although these strategies have improved the performances of sandwich amperometric immunoassay to some extent, some innate drawbacks of sandwich amperometric immunoassay still exist, which can limit the performance seriously. First, the specific recognition between antibody and antigen generates an insulating layer on the modified electrode, which can hinder the electron transfer (Arkan et al., 2015; Katz et al.,

* Corresponding author.
E-mail address: mazhanfang@cnu.edu.cn (Z. Ma).
[1] These authors equally contributed to this work.

https://doi.org/10.1016/j.bios.2018.03.027
Received 26 December 2017; Received in revised form 20 February 2018; Accepted 12 March 2018
Available online 13 March 2018
0956-5663/

2001). Second, the label itself possesses high impedance, which can seriously decrease the current response (Fan et al., 2014; Wang et al., 2015). Predictably, effective improvement of the performances of sandwich-type amperometric immunosensor can be reached when these drawbacks are overcome.

In view of the above problems, an improved sandwich-type ampermetric immunosensor was designed by employing a pH-response nano-carrier loaded with redox species as smart label and an electrical-deposition hydrogel film as substrate for capturing redox species. Different from the conventional immunosensor, the formed the sandwich immune-complex was just acted as a linker, which was sacrificed prior to detection. In brief, both of the labels and antibody-antigen complex were destroyed under acid condition and then washed away, while the redox species released from the smart labels were absorbed by hydrogel modified electrode for signal generation. In this case, the issues existed in sandwich-type ampermetric immunosensor were overcome and brought the as-prepared immunosensor with the following advantages: (1) amounts of redox species loaded on the label enhanced the current response; (2) the immunosensing interface resistance was decreased due to the loss of the labels and the disassociation of antigen-antibody insulating layer; (3) the electron transfer efficiency between redox species and the electrode was increased for the decrease of relative position between them (Li et al., 2016, 2017).

In order to demonstrate the feasibility of this strategy, an immunosensor was developed for the detection of prostate specific antigen (PSA). In the immunosensor, mesoporous silica nanoparticles (MSN) with methylene blue (MB) sealed with polydopamine coating (MB-MSN/PDA) were used as labels and electro-polymerized polyaniline hydrogel (PANI) was employed as substrate. One should be emphasized that the label (MB-MSN/PDA) can release MB molecules and then automatically fall off from the electrode with the dissociation of PDA coating under acidic condition since the PDA acted as gate keeper to seal MSN and a linker to immobilize antibody. Meanwhile, the released MB molecules can be captured by PANI hydrogel modified on the electrode. These features make sure it meets the requirements of this strategy to overcome the innate drawbacks of sandwich amperometric immunoassay. An ultra-low limit of detection (LOD) at femtogram levels (fg mL^{-1}) was obtained, indicating that this strategy can extremely improve the sensitivity of sandwich ampermetric immunosensor and possesses large potential for the quantitative determination of biomarkers in clinical diagnosis.

2. Materials and methods

2.1. Materials and reagents

Prostate specific antigen (PSA), PSA antibody (Ab_1, Ab_2) and carcinoembryonic antigen (CEA),α-fetoprotein (AFP), cancer antigen 125 (CA125), cancer antigen 125 (CA153), were purchased from Shanghai Linc-Bio Science Co. Human immunoglobulin G (IgG) was obtained from Chengwen Biological Company (Beijing, China). Bovine serum albumin (BSA) was purchased from BJXJKSW (Beijing, China). Human serum albumin (HSA), tetraethyl orthosilicate (TEOS), cetyltrimethyl ammonium bromideand (CTAB), and Pluronic® F-127 were purchased from Sigma. Human serum samples were obtained from Beijing GENIA Biotechnology Co. Ltd. (Beijing, China). Hydrogen tetrachloroaurate hydrate ($HAuCl_4{\cdot}XH_2O$, 99.9%) were purchased from Alfa Aesar. Glucose (Glc) and dopamine (DA) were purchased from Beijing Chemical Reagents Company (Beijing, China). Methylene blue (MB) was purchased from AcrosOrganics. Ultrapure water (18.2 MΩ cm) was used throughout the experiments.

2.2. Apparatus

All electrochemical measurements were performed on a three-electrode system with CHI832 electrochemical workstation (Chenhua Instruments Co., Shanghai, China). The three-electrode system was used for the proposed electrochemical measurements with a glass carbon electrode as working electrode (GCE, 4 mm in diameter), an Ag/AgCl electrode as reference electrode and a platinum wire as counter electrode. X-ray photoelectron spectroscopy (XPS) was obtained on an Escalab 250 × -ray Photoelectron Spectroscope (Thermofisher, American). Scanning electron microscope (SEM) images and energy disperse spectroscopy (EDS) were carried out with a Hitachi SU8010 SEM. The transmission electron microscopy (TEM) images were obtained with a Hitachi (H7650, 80 kV) TEM. UV–vis measurements were carried out on a UV-2550 UV–Vis spectrophotometer (Shimadzu, Japan). The water used was purified through an Olst ultrapure K8 apparatus (Olst, Ltd., China).

2.3. Synthesis of the antibody (Ab_2)-functionalized MSN-MB/PDA labels

MSN was synthesized with the reported method (Guillet-Nicolas et al., 2013). Briefly, CTAB (0.50 g) and Pluronics® F-127 (2.05 g) were dissolved in the mixture of 43.1 mL ethanol, 96 mL H_2O and 11.2 mL $NH_3{\cdot}H_2O$ (28 wt%). TEOS (1.93 mL) was added into the mixture with rapid stirring. After stirring for 1.5 min, the obtained mixture solution was kept under static condition at room temperature for 24 h. The product was collected by centrifugation, washed with distilled water and ethanol and then dried under vacuum at 60 °C.

MSN (5.0 mg mL^{-1}) and equal volume MB solutions with different concentration were mixed together by 30 min ultrasonication and then stirred for 4 h to make MB molecules loaded into the pores of MSN. 5.0 mg mL^{-1} dopamine solution (Tris-HCl buffer, pH 8.5) was added into the mixture and stirred overnight to encapsulate MB molecules by forming a polydopamine film on the surface of MSN. The MB-loaded nanoparticles were purified by being dialyzed against water for 3 days in order to remove the non-reacted dopamine reactants and free MB molecules. After centrifugation, MB-loaded nanoparticles were re-dispersed in phosphate buffer (PB, pH 8.0) for further use.

100 μL of the dispersion of the MB-loaded nanoparticles and 200 μL of PSA antibody (Ab_2) were mixed together and stirring for 12 h. After that, 200 μL BSA blocking buffer (2.0 wt%) was added into the mixture and stirring for another 1 h. The Ab_2 functional labels (MB-MSN/PDA) were obtained after centrifugation and washing by PB (pH 7.4), then dispersed in the phosphate buffer saline (PBS, pH 7.4).

2.4. Fabrication of the immunosensor

Firstly, GCE was polished carefully with polishing powder (Al_2O_3) and then washed thoroughly to get a mirror-like surface. The phytic acid doped polyaniline hydrogel composite film was successfully deposited on the GCE via in-situ electrochemical co-deposition with a constant potential at 0.80 V for 400 s in an aqueous solution containing aniline (PA), phytic acid and KCl solution. After being washed several times, AuNPs were deposited on the PANI film by applying potential cycling from − 1.0–0.20 V at a scan rate of 50 mV s^{-1} for 10 cycles in 0.50 mM $HAuCl_4$ solution (containing 0.10 mM KCl). After washing, Ab_1 (80 μL, 200 μg mL^{-1}) dispersion was added on to the modified electrode (Au/PANI/GCE) and incubated in the refrigerator at 4 °C overnight. BSA blocking buffer (2.0 wt%, 20 μL) was added onto the Ab_1- Au/PANI/GCE and incubated at room temperature for 1.0 h to eliminate nonspecific binding sites of the modified electrode. After washing, the electrode was ready for measurement.

2.5. Electrochemical detection of PSA

The immunosensor (Ab_1-BSA/Au/PANI/GCE) was first incubated with various concentrations of PSA antibody standard solution or different serum samples for 50 min at 37 °C. Then, PSA-Ab_1-BSA/Au/PANI/GCE was washed carefully to remove unbounded PSA antigen. Subsequently, 40 μL of the as-prepared labels were added onto the