Journal of Electroanalytical Chemistry 809 (2018) 14–21

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

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

Highly sensitive immunosensor for Hepatitis B surface antigen detection based on a novel signal amplification system of gold nanorods and mesoporous Au@Pd@Pt core-shell nanospheres

Mingdang Li, Ping Wang*, Fubin Pei, Haoxuan Yu, Peng Chen, Yunhui Dong, Yueyun Li, Qing Liu, Degang Li

School of Chemical Engineering, Shandong University of Technology, 255049 Zibo, PR China

ARTICLE INFO

Keywords:
Sandwich-type immunosensor
Hepatitis B surface antigen
Gold nanorods
Mesoporous structure
Core-shell nanospheres

ABSTRACT

In this work, a highly sensitive sandwich-type electrochemical immunosensor was fabricated by using the gold nanorods (Au NRs) as the substrate material and the mesoporous Au@Pd@Pt core-shell nanospheres (m-Au@Pd@Pt) as the label of the detection antibodies (Ab_2, Ab_2 label) for detecting Hepatitis B surface antigen (HBs Ag). The Au NRs with excellent conductivity and biocompatibility can accelerate the electron transfer on the electrode interface and enhance the load capacity of capture antibodies (Ab_1). The m-Au@Pd@Pt with mesoporous structure can not only provide high specific surface area and abundant catalytically active sites, but also offer great pore accessibility of guest species from outside. The detection signal of the immunosensor was effectively improved by the novel Ab_2 label. With the good cooperation between Au NRs and Ab_2 label, a linear relationship between current signal and the logarithm of the HBs Ag concentration was obtained in the wide range of 20 fg/mL to 200 ng/mL and the detection limit of HBs Ag was 6.7 fg/mL (signal-to-noise ratio of 3). Besides, the designed immunosensor shows good reproducibility, long-term stability and high specificity. Crucially, the sensitive and selective response to HBs Ag in real human serum samples confirms that the designed immunosensor will be promising in the clinical diagnosis of biomarkers.

1. Introduction

Hepatitis B virus (HBV) infection remains a serious global health issue. Regrettably, HBV carriers are faced with the risk of complications from this persistent infection, such as chronic hepatitis, cirrhosis, even the hepatocellular carcinoma [1]. However, the incubation period of HBV is approximately half a year, early diagnosis of HBV is of extreme significance for the reduction of morbidity [2]. Hepatitis B surface antigen (HBs Ag) as a crucial HBV marker can be detected in the blood during the incubation period. It is reliable that the diagnosis of HBV infection is based on the level of HBs Ag in the blood [3]. As a consequence, the accurate and quantitative detection of HBs Ag by highly sensitive and fast method is significant for early diagnosis of HBV infection. In recent years, various analytical techniques have been developed for detecting HBs Ag, including enzyme-linked immunosorbent assay [4], real-time polymerase chain reaction [5], and electrochemical immunosensor [6]. In the assay above, electrochemical immunosensors based on the highly specific interaction between antigen and antibody are widely used to detect tumor or virion markers, and have attracted increasing interest because of the meaningful advantages involving fast response, low detection limit, high sensitivity, ease of operation and low manufacturing cost. Crucially, the application of high-performance nanomaterials has provided the basis for ultrasensitive immunoassay. The high performance of the sandwich-type electrochemical immunosensor should attribute to the good cooperation between a stable substrate material and an efficient signal amplification strategy. Thus, there is still a challenge to develop nanomaterials to improve the sensitivity, reproducibility and selectivity of the immunosensor.

A sandwich-type immunosensor herein was designed by using the gold nanorods (Au NRs) as the substrate material and the mesoporous Au@Pd@Pt core-shell nanospheres (m-Au@Pd@Pt) as signal amplifier for detecting the trace amounts of HBs Ag. Currently, gold nanostructures such as gold nanoparticles (Au NPs) [7], gold nanoflowers (Au NFs) [8] and Au NRs [9] have been applied extensively in various fields, especially used in electrochemical immunoassay. Among the various gold nanostructures, Au NRs becomes an ideal nanomaterial gradually in the fabrication of immunosensors on account of the stable biocompatibility, the excellent ability of electron transfer and the high

* Corresponding author.
E-mail address: wangping876@163.com (P. Wang).

https://doi.org/10.1016/j.jelechem.2017.12.044
Received 28 September 2017; Received in revised form 15 December 2017; Accepted 17 December 2017
Available online 18 December 2017
1572-6657/

surface area particularly [10,11]. Definitely, such an excellent substrate material lays a solid foundation for fabricating the designed immunosensor, because the Au NRs can not only effectually accelerate the electron transfer, but also provide a suitable microenvironment for immobilizing capture antibodies (Ab_1) [12–14].

In recent years, metallic core-shell nanoparticles with unique nanoarchitectures have attracted widespread attention and shown excellent conductivity and catalytic activity which are unreachable by their monometallic nanostructures [15]. To improve the sensitivity of the immunosensor, m-Au@Pd@Pt herein was prepared by soft template method to amplify current responses in differential pulse voltammetry (DPV) measurements towards the reaction between hydrogen peroxide (H_2O_2) and *o*-phenylenediamine (*o*-PD). The mesoporous structure of m-Au@Pd@Pt was formed by dendrite growth of Pt on the surface of the Au@Pd cores. The nanodendritic Pt shell with rich edges and corner atoms provides high specific surface areas and abundant catalytically active sites, which are highly significant for improving the catalytic performance of Pt. In addition, the mesopore of m-Au@Pd@Pt provides opportunities for the guest species to access the inner cores, which can further enhance the catalytic performance [16]. Thus, the m-Au@Pd@Pt was considered as the superior signal amplifier for the designed immunosensor. Furthermore, the detection antibodies (Ab_2) can immobilize on the m-Au@Pd@Pt to form Ab_2 label. The m-Au@Pd@Pt with good biocompatibility and high surface areas can stably bind Ab_2 via the interaction of Pt-NH_2 [17,18]. As a result, the m-Au@Pd@Pt can be assembled to the immunosensor successfully by the specific interaction between HBs Ag and Ab_2, and exert positive influence on the amplification of current responses under DPV measurements. On the basis of the stable substrate and high performance Ab_2 label, the designed immunosensor shows a sensitive detection of HBs Ag. The designed immunosensor shows an excellent performance in the detection of real human serum samples, which ascribes to its satisfactory reproducibility, stability and selectivity. Credibly, the designed immunosensor shows a bright prospect in the early clinical diagnosis of various diseases.

2. Materials and methods

2.1. Reagents and apparatus

HBs Ag and HBs antibody were purchased from Linc-Science Co., Ltd. (Shanghai, China). Human HBs Ag ELISA Kit was purchased from Hushang Biochemical Reagents Co., Ltd. (Shanghai, China). Bovine serum albumin (BSA, 96–99%) was purchased from Sigma reagent Co., Ltd. (St. Louis, MO, United States). Chloroauric acid ($HAuCl_4{\cdot}4H_2O$) was obtained from Sigma-Aldrich Co., Ltd. (Beijing, China). Cetyl trimethyl ammonium bromide (CTAB), Pluronic F127 and chloroplatinic acid ($H_2PtCl_6{\cdot}6H_2O$) were purchased from Macklin Biochemical Co., Ltd. (Shanghai, China). Sodium borohydride ($NaBH_4$), ascorbic acid (AA), *o*-PD and sodium tetrachloropalladate (Na_2PdCl_4) were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). Potassium ferricyanide ($K_3[Fe(CN)_6]$) and potassium ferrocyanide ($K_4[Fe(CN)_6]$) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Phosphate buffered saline (PBS) used as the electrolyte in the process of electrochemical measurements, was prepared by compounding the solution of disodium hydrogen phosphate (Na_2HPO_4) and potassium phosphate monobasic (KH_2PO_4). The level of other chemicals and solvents was of analytical grade, and they were used without further purification. Ultrapure water (18.25 MΩ cm, 24 °C) was used in all the experimental processes.

All the electrochemical measurements were performed on CHI760E electrochemical workstation (Shanghai Chenhua Instruments Co., China). A conventional three-electrode system was used for all the electrochemical measurements: a modified glassy carbon electrode (GCE, 4 mm in diameter) used as the working electrode, a platinum wire electrode used as the counter electrode and a saturated calomel electrode (SCE) used as the reference electrode. Scanning electron microscope (SEM) images were obtained using Quanta FEG250 field emission environmental SEM with energy-dispersive spectrometer (EDS) analysis (Field Electron and Ion Co., United States). Transmission electron microscope (TEM) images were obtained from a Tecnai G^2 F20 transmission electron microscope (Field Electron and Ion Co., United States). Nitrogen (N_2) adsorption-desorption data was obtained by using an ASAP 2020 apparatus (Micromeritics Instrument Co., United States). UV–vis spectra were obtained by UV–vis spectrophotometer (UV 2450, Shimadzu, Japan).

2.2. Preparation of Au NRs

Single-crystalline cylindrical Au NRs were prepared by the seed-mediated growth method [19]. 2.5 mL of CTAB solution (0.2 M) and 1.5 mL of $HAuCl_4$ (1.0 mM) were added into a 50 mL flask. The mixture was subject to vigorous magnetic stir for 30 min at 35 °C. Then, 0.6 mL of freshly prepared ice-cold $NaBH_4$ (0.01 M) was quickly added to the solution and the stir was stopped. The resulting brownish yellow solution was kept at 35 °C for 3 h to be used as the seed solution. For seed-mediated growth, 50 mL of CTAB (0.2 M), 5 mL of $HAuCl_4$ (15.0 mM), 2.5 mL of $AgNO_3$ (4.0 mM) and 45 mL of deionized water were added into 250 mL flask. And then, 1.25 mL of AA (0.08 M) was slowly added to the mixture. After the solution stabilized, 300 μL of Au seed solution was added into the solution drop by drop and allowed to grow for 24 h. Finally, the color of the solution turned dark-red. After washing with ultrapure water and drying at 35 °C, the Au NRs were ready for use.

2.3. Preparation of m-Au@Pd@Pt

The m-Au@Pd@Pt was synthesized with reference to a published method [20]. Briefly, 2.5 mL of $HAuCl_4$ (20.0 mM), 2.5 mL of Na_2PdCl_4 (20.0 mM), 4.0 mL of $H_2PtCl_6{\cdot}6H_2O$ (20.0 mM) and 0.1 g Pluronic F127 were placed in a 50 mL flask, then 1.5 mL of 0.4 M AA was quickly added under stirring. The mixture solution was stirred for 1 h at 80 °C, and the color of the reaction solution was changed from brownish-yellow to opaque black. The black precipitate was obtained by consecutive washing/centrifugation cycles five times with ultrapure water, and then dried up at room temperature.

2.4. Preparation of Ab_2 label

The m-Au@Pd@Pt dispersion (3.0 mg/mL, 1.0 mL) was mixed with the solution of Ab_2 (20.0 μg/mL, 1.0 mL) and oscillated at 4 °C for 12 h. Subsequently, the dispersion was centrifuged and rinsed with PBS (pH = 7.0) to remove the unbound Ab_2. The sediment was re-dispersed in 2.0 mL of PBS (pH = 7.0) and stored at 4 °C.

2.5. Fabrication for working electrode of the immunosensor

Fig. 1 has explained the fabrication process for working electrode of the immunosensor. First, the GCE was polished by alumina polishing powders with particle size of 1.0, 0.3, and 0.05 μm in sequence, followed by sonication in ethanol and rinsed by ultrapure water. Then, the clean surface of GCE was covered with Au NRs suspension (2.0 mg/mL, 6.0 μL) and dried at room temperature. After that, the integrate Au NRs/GCE was incubated with Ab_1 (10.0 μg/mL, 6.0 μL) and dried at 4 °C. After rinsing with PBS (pH = 7.0), BSA solution (1.0 wt%, 3.0 μL) was coated onto the surface of electrode against non-specific adsorption. After 1 h incubation, the surface of electrode was rinsed with PBS (pH = 7.0) and covered by 6.0 μL of HBs Ag solution with a series of concentrations at 4 °C for another 1 h to ensure the specific binding between HBs Ag and Ab_1. Finally, Ab_2 label (1.5 mg/mL, 6.0 μL) was dropped onto the surface of electrode evenly, and then stored at 4 °C for 35 min. The prepared electrodes were rinsed with PBS (pH = 7.0) to remove unbound Ab_2 label and stored at 4 °C for further measurement.