Talanta 156-157 (2016) 11–17

Contents lists available at ScienceDirect

Talanta

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

Ultrasensitive electrochemical immunosensor for quantitative detection of tumor specific growth factor by using Ag@CeO_2 nanocomposite as labels

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ARTICLE INFO

Article history:
Received 30 December 2015
Received in revised form
14 April 2016
Accepted 24 April 2016
Available online 26 April 2016

Keywords:
Electrochemical immunosensor
Sandwich-type
Tumor specific growth factor
Ag@CeO_2 nanocomposite

ABSTRACT

In this paper, an ultrasensitive electrochemical immunosensor was developed for the detection of tumor specific growth factor (TSGF). Reduced graphene oxide-tetraethylene pentamine (rGO-TEPA) was used to modify the surface of glassy carbon electrode (GCE). Meanwhile, Ag@CeO_2 nanocomposite was synthesized and applied as secondary-antibody (Ab_2) labels for the fabrication of the immunosensor. The amperometric response of the immunosensor for the reduction of H_2O_2 was recorded. Simultaneously, electrochemical impedance spectroscopy (EIS) and Cyclic voltammetry (CV) were used to characterize the fabrication process of the immunosensor. The anti-TSGF primary antibody (Ab_1) was immobilized onto the rGO-TEPA modified GCE via cross-linking with glutaraldehyde (GA). And then the TSGF antigen and Ab_2-Ag@CeO_2 were modified onto the electrode surface in sequence. Under the optimal conditions, the immunosensor exhibited a wide linear range (0.500–100 pg/mL), a low detection limit (0.2 pg/mL), good reproducibility, acceptable selectivity and excellent stability. The proposed sensing strategy may provide a potential application in the detection of other cancer biomarkers.

1. Introduction

Tumor specific growth factor (TSGF) is a gene which can facilitate the proliferation of tumor and peripheral capillaries and can be released into the peripheral blood. It was first discovered by scientists of Canada in 1989 [1]. And it plays an important role in malignant tumor blood vessel growth [2,3]. The content of it increases significantly in the blood of the early patients with malignant tumor. Hence, it has clinical significance and application value in the preliminary screening, early auxiliary diagnosis, therapeutic efficiency evaluation of malignant tumor and the predictor of tumor recurrence. No similar correlation has been found between TSGF and hyperplasia of non-tumor blood vessels. Many studies have indicated that TSGF possesses high sensitivity for the detection of malignant tumors [4–7]. Therefore, TSGF can be used as a kind of tumor marker for clinical application.

In recent years, electrochemical immunoassay has received more and more attention due to their simple pretreatment procedure, short analytical time, high sensitivity, precise current measurement and miniaturized instrument [8–17]. Simultaneously, hardly has anyone used the electrochemical immunoassay method for the detection of TSGF. Hence, a simple and ultrasensitive electrochemical immunosensor was designed for sensitive and selective determination of TSGF.

For the electrochemical immunosensors, the materials applied to modify electrode surface and labeled antibodies are very crucial [18–21]. Reduced graphene oxide (rGO), a one-atom-thick planar sheet of sp^2-bonded carbon atoms densely packed in a honeycomb crystal lattice, has gained extensive attention in fabricating immunosensors due to its excellent electronic properties and large surface area [22–24]. However, the irreversible agglomeration of rGO affects the stability of the electrochemical immunosensors severely. Hence great efforts have been made for improving the stability of rGO [25,26]. Reduced graphene oxide-tetraethylene pentamine (rGO-TEPA) is a novel material combining rGO and TEPA by covalent bonding [10]. A great amount of amino groups from rGO-TEPA can form covalent bonds with more antibodies. So it is an ideal material acting as the substrate material for the modification of electrode. Meanwhile, cerium oxide (CeO_2) nanoparticle as a kind of rare earth materials has a wide range of applications in catalysis, biosensing and optoelectronics. It has been reported that CeO_2 has been used in the immobilization of biological enzyme or protein on the surface of the electrode [27,28]. It

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http://dx.doi.org/10.1016/j.talanta.2016.04.050
0039-9140/

has no toxic effect on biological molecules, and it can improve the electrons transfer effect between the electrode and materials modified on the electrode surface [29–31]. At the same time, the noble metal silver has showed excellent electroconductibility in the preparation of immunosensors [20,32–38]. Therefore, silver-cerium oxide ($Ag@CeO_2$) nanocomposite is a favorable material for the preparation of electrochemical immunosensors.

In this study, an ultrasensitive electrochemical immunosensor was developed for the detection of TSGF. $Ag@CeO_2$ nanocomposite was synthesized and used to prepare the Ab_2-$Ag@CeO_2$ conjugation. A sandwich-type strategy was used to fabricate the immunosensor, with the anti-TSGF primary antibody (Ab_1) immobilized onto the rGO-TEPA modified electrode. Afterwards, the TSGF and Ab_2-$Ag@CeO_2$ were sequentially modified onto the electrode. Finally, the properties of the immunosensor were measured by recording the reduction of H_2O_2.

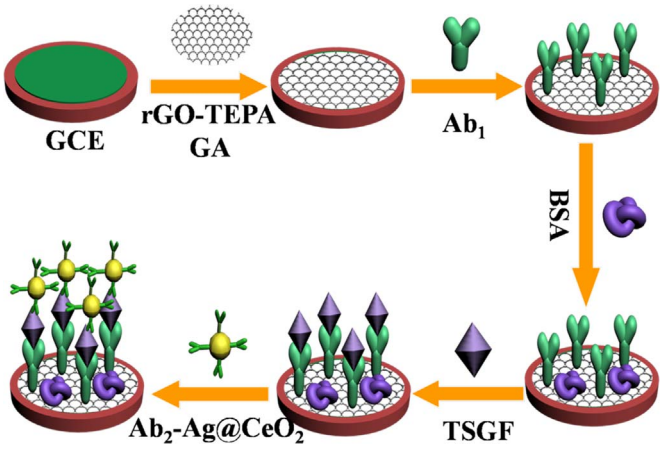

Fig. 1. Schematic representation of the preparation of immunosensor. The reticular structures represent rGO-TEPA. Ab_1 represents the anti-TSGF antibody. The rGO-TEPA, GA, Ab_1, BSA, TSGF and Ab_2-$Ag@CeO_2$ are modified onto the GCE in sequence.

2. Materials and methods

2.1. Apparatus and reagents

TSGF and TSGF antibody (Ab_1 and Ab_2) were purchased from Shanghai chenglin biological technology Co., Ltd. (Shanghai, China). Reduced graphene oxide-tetraethylene pentamine (rGO-TEPA) was obtained from NanoInnova Technologies Co., Ltd. (Madrid, Spain). $AgNO_3$, $NH_3 \cdot H_2O$, $K_3Fe(CN)_6$ and glutaraldehyde (GA) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Bovine serum albumin (BSA, 96–99%) was purchased from Sigma-Aldrich (USA). The actual human serum samples were applied for the real sample analysis by using standard addition methods. All other chemicals were of analytical reagent grade. Phosphate buffered saline (PBS, 0.1 mol/L) was used as electrolyte for all electrochemical measurements. Ultrapure water was used throughout the experiments.

All electrochemical measurements were performed on a CHI 760D electrochemical workstation (Chenhua Instrument Shanghai Co., Ltd., China). Transmission electron microscope (TEM) images were obtained from a Hitachi H-800 microscope (Japan). Scanning electron microscope (SEM) images were obtained by using a field emission SEM (Hitachi S-4800). Energy dispersive X-ray spectroscopy (EDX) was recorded by JEOL JSM-6700F microscope (Japan). Fourier transform infrared (FTIR) spectrum was recorded with KBr pellet in the range of 4000–400 cm^{-1} on Spectrum One FTIR Spectrometer (PerkinElmer). X-ray diffraction (XRD) pattern was recorded by a D8 FOCUS X-ray diffraction spectrometer (Bruker, German) with a Cu Kα target at a scan rate of 0.03° 2θ s^{-1} from 5° to 80°.

2.2. Preparation of $Ag@CeO_2$ nanocomposite

The synthetic procedure of $Ag@CeO_2$ nanocomposite was as follows: Firstly, 0.2 g of polyvinylpyrrolidone (PVP) was dissolved in 20 mL of methanol. Then 1.0 g of CeO_2 was added, as well as 0.157 g of $AgNO_3$ and 0.5 mL of $NH_3 \cdot H_2O$. The solution was stirred until the color changed. Secondly, the obtained solution was enclosed in stainless steel reactor. Afterwards, the reactor was put into drying oven at the temperature of 160 °C and 200 °C for 24 h in sequence. After cooled to room temperature, the product was collected and centrifugally washed three times with ethanol. Finally, the obtained product was dried at 80 °C and then grinded. The powder was calcinated at 500 °C for 5 h and the $Ag@CeO_2$ nanocomposite was obtained successfully.

2.3. Preparation of Ab_2-$Ag@CeO_2$ conjugation

The Ab_2-$Ag@CeO_2$ conjugation was prepared as follows: 70 mg of $Ag@CeO_2$ nanocomposite was dispersed in 1 mL of PBS at pH 7.40. Then 1 mL of 100 μg/mL anti-TSGF secondary antibody and 0.5 mL of 1% BSA were added under stirring for 12 h at 4 °C. The resulting Ab_2-$Ag@CeO_2$ conjugation was obtained by centrifugation and washed with PBS (pH 7.40). Finally, the obtained Ab_2-$Ag@CeO_2$ conjugation was dispersed in 1 mL of PBS (pH 6.92) and stored at 4 °C until use.

2.4. Fabrication of the immunosensor

The fabrication process of the immunosensor is shown in Fig. 1. The glassy carbon electrode (GCE) was polished repeatedly using alumina powder (particle sizes of 1.0, 0.3, and 0.05 μm in turn) and then thoroughly cleaned with ultrapure water. Firstly, 6 μL of 3 mg/mL rGO-TEPA solution was dropped onto the electrode surface. After drying, 3 μL of 2.5% GA solution was added onto the electrode surface. As the electrode surface dried, 6 μL of Ab_1 (10 μg/mL) was dropped onto it. After the step of drying and washing, 3 μL of 1% BSA solution was used to eliminate nonspecific binding sites between the antigen and the electrode surface. Subsequently, TSGF solutions with different concentrations were dropped onto the electrode. After incubation for 1 h at 4 °C, the electrode was washed thoroughly to remove the unbounded TSGF. Finally, the prepared Ab_2-$Ag@CeO_2$ conjugation solution was dropped onto the electrode surface. After the electrode turned dry, it was washed and then ready for measurements.

2.5. Characterization of the immunosensor

A conventional three-electrode system was used for all the electrochemical measurements: GCE, 4 mm in diameter, as the working electrode, a saturated calomel electrode as the reference electrode, and a platinum wire electrode as the counter electrode (All electrodes were purchased from Wuhan Gaossunion Technology Co., Ltd). Cyclic voltammetry (CV) was performed at 100 mV/s. The detection voltage of the amperometric measurement was selected as −0.4 V. After the background current was stabilized, 5.0 mmol/L H_2O_2 was injected into the buffer solution and the current change was recorded.