Biosensors and Bioelectronics 53 (2014) 160–166

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

A label-free immunosensor based on graphene nanocomposites for simultaneous multiplexed electrochemical determination of tumor markers

Xinle Jia [1], Zhimin Liu [1], Na Liu, Zhanfang Ma *

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

ARTICLE INFO

Article history:
Received 10 August 2013
Received in revised form
22 September 2013
Accepted 23 September 2013
Available online 30 September 2013

Keywords:
Graphene nanocomposites
Label-free immunosensor
Simultaneous multiplexed electrochemical detection
Tumor markers

ABSTRACT

Here we prepared a label-free electrochemical immunosensor employing indium tin oxide (ITO) sheets as working electrodes and graphene nanocomposites as supporting matrix for simultaneous determination of carcinoembryonic antigen (CEA) and α-fetoprotein (AFP). Reduced graphene oxide/thionine/gold nanoparticles nanocomposites were synthesized and coated on ITO for the immobilization of anti-CEA while reduced graphene oxide/Prussian Blue/gold nanoparticles were used to immobilize anti-AFP. The immunosensor determination was based on the fact that due to the formation of antibody–antigen immunocomplex, the decreased response currents of thionine and Prussian Blue were directly proportional to the concentrations of corresponding antigens. Experimental results revealed that the multiplexed immunoassay enabled the simultaneous determination of CEA and AFP with linear working ranges of 0.01–300 ng mL^{-1}. The limit of detections for CEA is 0.650 pg mL^{-1} and for AFP is 0.885 pg mL^{-1}. In addition, the methodology was evaluated for the analysis of clinical serum samples and received a good correlation with the enzyme linked immunosorbent assay.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

In clinical analysis, the determination of a single tumor marker often limits diagnostic value because most cancers have more than one marker associated with their incidence (Ferrari, 2005; Kulasingam and Diamandis, 2008). To address this, simultaneous determination of two or more tumor makers have stimulated intense research recently (Liu and Ma, 2013b; Chen et al., 2013, 2012; Yang et al., 2009; Wilson and Nie, 2006). The multiplexed immunoassay which quantitatively detects a panel of biomarkers can effectively improve the diagnostic specificity, shorten analytical time, enhance detection throughput and detection cost.

Electrochemical immunoassay could be selected as an ideal strategy among various measurement techniques for tumor maker determination because of its portability, low cost and high sensitivity (Chikkaveeraiah et al., 2012; Li et al., 2012a). To date, two primary approaches: multiple labels (Bai et al., 2012; Li et al., 2012b) and spatial resolution (Lai et al., 2011; Wu et al., 2008) have been developed for electrochemical multiplexed immunoassay. In the former mode, multiple-labeled antibodies are usually selectively bound onto the electrode surface through "sandwich" immunoreaction and the recognition event thus yield a distinguishable voltammetric peak, whose position and size reflect the identity and concentration of the corresponding antigens. It had obtained good sensitivity and limit of detection. For example, Qian et al. (2011) used quantum dots coated silica nanoparticles as labels for simultaneous detection of dual proteins. Song et al. (2010) reported a sensitive electrochemical immunoassay using horseradish peroxidase functionalized Pt hollow nanospheres and multiple redox probes as trace labels. However, the introduction of the operation for labeling secondary antibodies will make the operation process more complex, time consuming. Moreover, the cross-talk between the different antigens is inevitable and the repeatability of immunoassay will also be affected. At the same, the labeled antibodies usually have lower biological activity and narrowed the working ranges and decreased detection limits. Thus, its application in diagnosis gets limited. The detection based on spatial resolution overcomes the problems to some extent, each electrode surface has a different antibody attached, rendering the senor to simultaneously respond to multiple targets in a complex sample. The cross-talk could be effectively avoided. In addition, non-enzymatic reaction can enhance the signal reproducibility as well as make it cheaper. In this case, we carried out a simultaneous immunoassay based on spatial resolution without enzymatic reaction.

Another important issue for developing a successful multiplexed immunosensor is to search ideal materials for immobilizing distinguishable redox probes as trace labels. Owing to the favorable electronic properties and impressive surface area, graphene was proved to be a promising material in designing and preparing

* Corresponding author. Tel.: +861068902491.
E-mail address: mazhanfang@cnu.edu.cn (Z. Ma).
[1] The authors contribute equally to this work.

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.09.050

electroactive nanocomposites (Chen et al., 2010; Liu et al., 2013a). Up to now, one of the most common methods for loading nanomaterials onto graphene is to covalently functionalize graphene via coupling reaction (such as amidation, esterification). However, in this method the strict requirement for special coupling reagents (Feng et al., 2011; Yuan et al., 2011), harsh reaction conditions (such as high temperature, microwave, non-aqueous solvent) (Jin et al., 2012; Avinash et al., 2010) limits its application. To resolve this issue, here we show a polyethyleneimine (PEI) functionalized reduced graphene oxide (rGO) for directly loading electroactive species thionine (Thi), Prussian Blue (PB) and gold nanoparticles (AuNPs). Thi molecules were noncovalently attached to rGO surface via π–π stacking while PB nanoparticles were grown on rGO surface by an in situ reduction of ferric chloride and potassium ferricyanide solution. AuNPs were immobilized onto rGO/Thi and rGO/PB nanocomposites via the interaction between the amine groups of rGO and the AuNPs. Compared with the routine method, our work shows two advantages: (1) the nanocomposites are synthesized with simply synthetic process and mild conditions; (2) the size and amount of AuNPs could be flexibly controlled which are critical for optimizing the performance of electrodes.

In this work, the prepared rGO/Thi/AuNPs and rGO/PB/AuNPs nanocomposites were used as substrate simply to construct electrochemical immunosensing for simultaneous detection of multiple tumor marker CEA and AFP. In summary, the graphene plays two main roles in the proposed immunosensor: (1) Owing to the impressive surface area, a large number of redox molecules (Thi and PB) and AuNPs were immobilized onto the ITO surface, which is very important for absorbing antibodies and signal generation; (2) as for the favorable electronic properties, the graphene could accelerate electron transfer, which makes the signal amplification achieved easily. Such a general method offers several advantages over some conventional techniques such as wide linear working ranges, low limit detection, high simplification and potential for high-throughput parallel analysis.

2. Experimental section

2.1. Reagents and materials

Indium tin oxide (ITO) sheets (resistance $<7\ \Omega\ sq^{-1}$) were purchased from Zhuhai Kaivo Electronic Components Company (Shenzhen, China). Mouse monoclonal anti-AFP and anti-CEA, AFP and CEA were purchased from Linc-Bio Company (Shanghai, China). Graphene oxide was obtained from JCNANO (Nanjing, China). Chloroauric acid ($HAuCl_4{\bullet}4H_2O$), ascorbic acid (AA) and uric acid (UA), D-(+)-glucose (Glu), thionine acetate (Thi) were obtained from Alfa Aesar. Ferric chloride ($FeCl_3{\bullet}6H_2O$), Chitosan (CHIT), potassium ferricyanide ($K_3Fe(CN)_6$) and bovine serum albumin (BSA) were purchased from Beijing Chemical Reagents Company (Beijing, China). Polyethyleneimine (PEI, molecular weight: 25,000) was obtained from Sigma-Aldrich. Clinical serum samples were available from Capital Normal University School Hospital. All the reagents were analytical grade and used as received.

2.2. Apparatus

In all the procedures, the water used was purified through an Olst ultrapure K8 apparatus (Olst, Ltd., resistivity $>18\ M\Omega$). Images of the nanomaterials were taken via a Hitachi (H7650, 80 kV) transmission electron microscope (TEM). X-ray photoelectron spectroscopy (XPS) analysis was carried on an Escalab 250 X-ray Photoelectron Spectroscope (Thermofisher, American) using an Al (mono) $K\alpha$ radiation. All electrochemical experiments were measured on a CHI1140 electrochemical workstation (Shanghai, China). A three-electrode electrochemical cell was composed of a modified ITO as the working electrode, a platinum wire as the auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode. Field emission scanning electron microscopy (FE-SEM, Hitachi S4800) was used to confirm the modified nanomaterials on ITO.

2.3. Preparation of rGO

rGO were prepared with a slight modification according to a reference (Cao et al., 2010). To prepare rGO, 10 mL stable dispersion of graphene oxide ($1\ mg\ mL^{-1}$) was mixed with 1 mL PEI (3%) and then heated at 135 °C under reflux for 3 h. After several washings and centrifugations, the final products were re-dispersed in 10 mL ultrapure water for further use.

2.4. Preparation of AuNPs

The AuNPs with sizes of 5 nm, 10 nm, 15 nm, 20 nm and 25 nm used in this work were prepared according to our previous work (Wang et al., 2012). Samples of 5 nm and 10 nm AuNPs were obtained at room temperature through adding 1 mL sodium citrate solution (1%) to $HAuCl_4$ aqueous solution (0.01%). After stirring for 1 min, 1.6 mL 0.0075% $NaBH_4$ solution for 5 nm AuNPs or 1 mL for 10 nm AuNPs were added under continuously string until its color turned red. For the preparation of 15 nm, 20 nm and 25 nm AuNPs, 5 mL, 4.6 mL or 5 mL sodium citrate solution (1%), respectively, were added into 100 mL boiling aqueous $HAuCl_4$ solution with vigorous stirring. The color changed from pale to burgundy. After boiling continued for 15 min, the sample was stirred until it had cooled to room temperature.

2.5. Preparation of rGO/Thi/AuNPs nanocomposites

For preparation of rGO/Thi/AuNPs, we firstly synthesized rGO/Thi nanocomposite. Briefly, after ultrasonication for 40 min at room temperature, 2 mL rGO dispersion ($1\ mg\ mL^{-1}$) was mixed with 2 mL Thi solution ($2\ mg\ mL^{-1}$) and stirred vigorously for 24 h. Finally, rGO/Thi nanocomposites were obtained by removing the non-integrated Thi away through centrifugation and washing with ultrapure water. Subsequently, the rGO/Thi/AuNPs nanocomposites were prepared as follows: the as-prepared rGO/Thi nanocomposites were dispersed in 2 mL water. Then 10 mL AuNPs solution was added into this dispersion and reacted for overnight under stirring. After several washing and centrifugation, the collected samples were re-dispersed in 2 mL 0.1% CHIT solution and stored at 4 °C before use.

2.6. Preparation of rGO/PB/AuNPs nanocomposites

rGO/PB nanocomposites were synthesized through an in stiu reduction process. Typically, 2 mL rGO dispersion ($1\ mg\ mL^{-1}$) was added into 2 mL (pH 1.5, adjust with HCl) aqueous solution containing 15 mmol L^{-1} $K_3Fe(CN)_6$ and 15 mmol L^{-1} $FeCl_3{\bullet}6H_2O$. After stirring for 2 h, the rGO/PB nanocomposites were formed with the color changing from yellow brown to dark cyan. The as-prepared nanocomposites were collected by several centrifugation and washing with ultrapure water, and re-dispersed in 2 mL water for further use. AuNPs were attached to rGO/PB nanocomposite through several steps as follows: Briefly, the as-prepared rGO/PB nanocomposites (2 mL) were mixed with 10 mL AuNPs solution. The mixture was reacted overnight with continuously string at room temperature. Following several centrifugation and washing, the resulting rGO/PB/AuNPs nanocomposites were re-dispersed in 2 mL 0.1% CHIT solution and stored at 4 °C in refrigerator for use.

Download English Version:

https://daneshyari.com/en/article/866686

Download Persian Version:

https://daneshyari.com/article/866686

Daneshyari.com