Analytica Chimica Acta 986 (2017) 138–144

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Ultrasensitive electrochemical immunosensor for alpha fetoprotein detection based on platinum nanoparticles anchored on cobalt oxide/graphene nanosheets for signal amplification

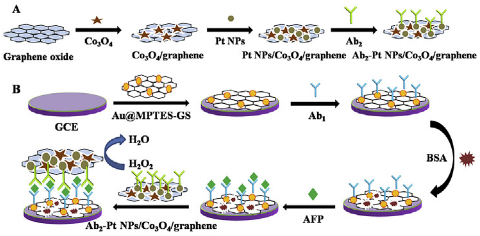
Li Liu, Lihui Tian, Guanhui Zhao, Yuzhen Huang, Qin Wei, Wei Cao*

Key Laboratory of Chemical Sensing & Analysis in Universities of Shandong, School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, PR China

HIGHLIGHTS

- An incorporated signal amplification strategy was proposed.
- Co_3O_4 nanoparticles exhibited multifunctional character.
- Au@MPTES-GS possesses high surface area and excellent conductivity.
- The proposed immunosensor exhibited a low detection limit of 0.029 pg mL^{-1} for AFP.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 18 March 2017
Received in revised form
7 July 2017
Accepted 11 July 2017
Available online 14 July 2017

Keywords:
Sandwich-type electrochemical immunosensor
Catalytic activity
Signal amplification mechanism
Tumor markers

ABSTRACT

An ultrasensitive sandwich-type electrochemical immunosensor was developed for quantitative monitoring of Alpha fetoprotein (AFP). To achieve this objective, an incorporated signal amplification strategy of platinum nanoparticles anchored on cobalt oxide/graphene nanosheets (Pt NPs/Co_3O_4/graphene) was proposed by acting as the label of secondary antibodies. The prepared label not only empowered by advantages of each component but exhibited better electrochemical performance than single Pt NPs, Co_3O_4 and graphene, which has shown large specific surface area and good catalytic activity towards the reduction of H_2O_2. Meanwhile, the nanocomposite of gold nanoparticles adhered on 3-mercaptopropyltriethoxysilane functionalized graphene sheets (Au@MPTES-GS) was used as matrix to accelerate electron transfer and immobilize primary antibodies in this system. The signal amplification mechanism of the matrix and the label were explored successfully. Under optimal conditions, the electrochemical immunosensor exhibited a wide linear range from 0.1 pg mL^{-1} to 60 ng mL^{-1} with a low detection limit of 0.029 pg $mL^{-1}$for AFP. The proposed immunosensor may have promising application in the clinical diagnosis of AFP and other tumor markers.

1. Introduction

Alpha fetoprotein (AFP) is a sort of glycoprotein with the molecular weight of 69000, which generated by the yolk sac and existed in liver of embryonic development [1–3]. Along with the baby's birth, the level of AFP concentration reduces gradually. Generally speaking, the average concentration of AFP in healthy human serum is less than 25 ng mL^{-1} [4–7]. The increasing of AFP concentration in human serum may indicate some pathological conditions, such as carcinogenesis of germinal tumors, hepatocellular carcinoma and extrahepatic adenocarcinoma [8]. Therefore, monitoring human serum AFP level plays a significant role in early

---

* Corresponding author.
E-mail addresses: jncw88@163.com, jn_chm302@163.com (W. Cao).

http://dx.doi.org/10.1016/j.aca.2017.07.025

diagnosis of cancer [9].

Correspondingly, numerous methods for determining the concentration of AFP have been reported, such as enzyme-linked immunosorbent assay [10], chemiluminescence [11], fluoroimmunoassay [12] and immunoradiometric assay [13]. These methods suffer the drawbacks of time-consuming, high cost and the narrow dynamic range [14,15]. As a promising detection strategy, electrochemical immunosensor have gained extensive attention due to the advantages of high sensitivity, fast response, low cost and easy miniaturization [16–19]. It has been extensively applied in many fields like foodadditives, environmental pollution and biomarkers [20–22]. Among all kinds of electrochemical immunosensors, the sandwich-type structure is most widely used. For sandwich-type immunosensors, the label materials of secondary antibody (Ab_2) were a crucial factor for improving the sensitivity and lower the limit of the detection [23–25].

Transition metal oxide, cobalt oxide (Co_3O_4) has been widely utilized in biosensors [26–28], gas sensors [29,30], supercapacitors [31,32] and so on [33–35]. Mu has reported that Co_3O_4 exhibited intrinsic peroxidase-like activity and catalase-like activity. And Co_3O_4 nanoparticles were successfully used as mimetics peroxidase for H_2O_2 and glucose determination [36]. Nanostructured Co_3O_4 has shown superior performance in its traditional stage and also exhibited some unique properties in terms of its specific morphology. However, pure Co_3O_4 nanoparticles readily generate aggregates during growth [37], which can cause the decrease of catalyst surface area and directly influence the catalytic performance of Co_3O_4 nanoparticles. Graphene nanosheets have been widely used in the fabrication of electrochemical immunosensors due to the advantages of large specific surface area, good electron transfer properties, strong mechanical stability and high thermal stability [38,39]. Nevertheless, graphene nanosheets tend to form irreversible agglomerates due to strong π-π stacking and Vander Waals interactions [40], which would seriously influence the stability of electrochemical immunosensors. These unfavorable phenomena inspired us to attempt to minimize the agglomeration and improve the analytical performance of the proposed immunosensor.

To address the problems above, we have prepared Co_3O_4/graphene nanocomposite by a facile one-spot solvothermal method. Wang has reported that there are many surface groups on the graphene nanosheets, the possible linkage between these groups of graphene nanosheets and Co_3O_4 nanoparticles might be helpful to stabilize the particles [41]. Co_3O_4 nanoparticles which loaded on the graphene nanosheets can act as spacers to prevent the stacking of graphene and the incorporation of Co_3O_4 nanoparticles and graphene nanosheets can form a porous network [37]. The interaction between Co_3O_4 nanoparticles and graphene nanosheets enhances the electrochemical performance of the composite. In addition, the special structure enlarges its surface area, which is beneficial to the electron transfer and improvement of catalytic activity. Therefore, Co_3O_4/graphene nanocomposite can be a promising template for the loading of platinum nanoparticles (Pt NPs). Pt NPs, as a kind of noble nanoparticles, were used as signal amplifier due to the excellent electrical conductivity and high catalytic activity [42]. Moreover, the application of Pt NPs can improve the immobilizing amount of Ab_2 and the sensitivity of the proposed electrochemical immunosensor via the interaction of Pt-NH_2.

The immobilization of primary antibodies (Ab_1) is also an important factor for the signal amplification and ultrasensitive detection [43]. Gold nanoparticles adhered on 3-mercaptopropyltriethoxysilane functionalized graphene nanosheets (Au@MPTES-GS) were used as the matrix for the immobilization of Ab_1. Its large specific surface area, good biocompatibility and outstanding electron transfer ability are beneficial to the signal amplification of the electrochemical immunosensor [44]. Sensitivity has been greatly improved by using Au@MPTES-GS as the signal platform in the proposed electrochemical immunosensor.

2. Experimental section

2.1. Apparatus and reagents

All electrochemical measurements were performed on a CHI760D electrochemical workstation (Chenhua Instrument Shanghai Co., Ltd., Shanghai, China). Scanning electron microscope (SEM) images were obtained from JSM-6700F microscope (JEOL, Japan). FT-IR spectra were collected using a FTIR-410 infrared spectrometer (JASCO, Japan). Energy dispersive X-ray spectroscopy (EDS) was recorded by JSM-6700F microscope (JEOL, Japan). A UPT-I-10 20 T ultrapure water machine (Ultrapure Technology Chengdu Co., Ltd., Chengdu, China) was used to provide ultrapure water. A conventional three-electrode system was used for all electrochemical measurements: a glassy carbon electrode (GCE) (4 mm in diameter) was used as the working electrode, a saturated calomel electrode (SCE) was used as the reference electrode, and a platinum wire electrode was used as the counter electrode.

Bovine serum albumin (BSA) was purchased from Sigma-Aldrich Chemical Co., Ltd., Beijing, China. Ammonia (25%) and cobalt nitrate hexahydrate were obtained from Sinopharm Chemical Reagent Co., Ltd., Beijing, China. 3-mercaptopropyl triethoxysilane (MPTES) was purchased from Aladdin Chemical Shanghai Co., Ltd., Shanghai, China. Phosphate buffered saline (PBS, 1/15 mol L^{-1} KH_2PO_4 and 1/15 mol L^{-1} Na_2HPO_4) was used as electrolyte for all electrochemical measurements. All other chemicals were of analytical reagents grade and used without further purification. Ultrapure distilled and deionized water (18.25 MΩ) was used in all experiments.

2.2. Preparation of the Au@MPTES-GS

Graphene oxide (GO) was synthesized by the further improved Hummers method [45]. An ethanol dispersion of GO (1 wt%, 10 mL) mixed with 0.3 mL of 95% 3-mercaptopropyl triethoxysilane (MPTES) and heated to 70 °C under stirring for 2 h. Then, 0.1 mL of 80% hydrazine hydrate was added dropwise under 95 °C for another 1.5 h. The resulting black product was washed with ultrapure water and absolute alcohol for several times and finally dried at 35 °C under vacuum for 24 h.

Au nanoparticles (Au NPs) were synthesized by the classical Frens method [46]. In short, 100 mL of 0.01% $HAuCl_4$ was heated to boiling. Then, 2.5 mL of 1% trisodium citrate was added dropwise. The mixed solution turned a brilliant ruby-red in about 15 min, indicating the formation of small size spherical nanoparticles, and then it was cooled to room temperature.

10 mL of ultrapure water containing 20 mg of MPTES-GS was under ultrasound for 1 h to disperse homogeneously, then 30 mL of the resultant Au NPs solution was added, and the mixture was stirred for 12 h. The product was washed with ultrapure water for three times. The powder of Au@MPTES-GS was obtained after dried in vacuum at 35 °C for 24 h.

2.3. Preparation of Pt NPs/Co_3O_4/graphene

In a facile one-spot solvothermal method, the preparation process of Co_3O_4/graphene was showed as follows:

0.29 g of $Co(NO_3)_2 \cdot 6H_2O$ was dissolved in 20 mL ethanol, 20 mg of GO was dispersed in 20 mL ethanol under ultrasonic dispersion. Then, the synthesized two solutions were mixed by stirred vigorously. Simultaneously, 4 mL of ammonia solution ($NH_3 \cdot H_2O$, 25%) was added dropwise. After completion, the as-prepared mixture