Talanta 107 (2013) 176–182

Contents lists available at SciVerse ScienceDirect

Talanta

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

Graphene oxide as nanocarrier for sensitive electrochemical immunoassay of clenbuterol based on labeling amplification strategy

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ARTICLE INFO

Article history:
Received 2 August 2012
Received in revised form
1 January 2013
Accepted 4 January 2013
Available online 16 January 2013

Keywords:
Electrochemical immunosensor
Clenbuterol
Graphene oxide
Gold nanoparticles
Glucose oxide
Prussian blue

ABSTRACT

A novel electrochemical immunosensor for sensitive detection of clenbuterol (CLB) is fabricated using glucose oxidase (GOD)-functionalized grahene oxide (GO) nanocomposites to label CLB. The immunosensor was constructed by layer-by-layer assembly colloidal prussian blue (PB), multiwalled carbon nanotubes (MWCNTs) and CLB antibodies (Abs) on a glassy carbon electrode (GCE). In this competitive immunoassay system, PB acts as the redox mediator to reduce H_2O_2 originated from the catalyst cycle of GOD. The high ratio of GOD to GO effectively amplified the signal for this competitive-type immunoassay. Under optimized conditions, the immunosensor shows a wide linear range from 0.5 to 1000 ng/mL with a low detection limit of 0.25 ng/mL. The dual signal amplification of GOD-functionalized GO nanocomposites as a label is promising to be applied to design other sensitive immunosenseors.

1. Introduction

Clenbuterol (CLB), as a β-agonist, can reduce stress symptoms and asthma, leading to its widespread use in the treatment of human depression and pulmonary diseases [1]. However, due to its unique function of promoting fat consumption [2,3], some illegal producers often add CLB at high doses to the feed of livestocks to improve the production of lean meat. Large amounts of CLB residues in the produced meat associate with serious side effects and food poisoning [4]. For this reason, several countries have forbidden the use of CLB as growth promoter. In the past few years, the illegal misuse of CLB has caused several serious food poisoning tragedies in China, Italy and France [5,6]. It is followed that establishing a simple, rapid and sensitive method for the determination of CLB is extremely important to ensure food safety. At present, various methods have been reported for CLB detection including high-performance liquid chromatography [7,8], gas chromatography—mass spectrometry [9,10], capillary electrophoresis [11], enzyme-linked immunosorbent assay [12,13] and immunosensor based on surface plasmon resonance [14].

Although these methods for the determination of CLB are very promising due to high sensitivity and selectivity, most of them suffer from several disadvantages such as expensive instruments, complicated operating processes. Electrochemical immunosensors have attracted considerable attentions since they not only enjoy the high specificity of immunoassay, but also feature the sensitivity and low expenses of electrochemical systems. So far, only few electrochemical immunosensors [15–17] are constructed for the determination of CLB. It may be attributed to that lots of electrochemical immunosensors are based on the embarrassment of electron transfer of the electrochemical mediator in solutions caused by macrobiomolecules, but CLB, as a small molecular, cannot have significant embarrassment effect on the electron transfer. Therefore, it is a challenge to improve the sensitivity and stability for the determination of CLB with a lower detection limit using electrochemical immunosensors.

In order to enhance the sensitivity, many nanomaterials have been applied to construct electrochemical immunosensors. The functions of these nanomaterials can be divided into two sorts: (1) metal and semiconductor nanoparticles are directly used as electroactive labels to amplify the electrochemical responses [18,19], (2) nanomaterials, with large surface areas, are used as carriers to load electroactive species or enzyme for the amplification of the detectable signal [20,21]. Carbon nanotubes [22,23], carbon nanospheres [24], silica nanoparticles [25] and carboxylated magnetic beads [26] have been used as carriers of electroactive species or enzymes for signal amplification. Graphene oxide (GO), a novel one-atom thick and two-dimensional graphitic carbon system with abundant oxygen functional groups, has attracted increasing attentions in recent years due to unique physical and chemical properties. GO has potential applications in

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http://dx.doi.org/10.1016/j.talanta.2013.01.002

sensors, nanoelectronic devices and nanomaterials [27–30]. According to previous reports [30–33], the loading ratio of GO could reach 200%, much higher than that of other nanocarriers. Based on the high loading ratio, good biocompatibility, and physiological stability, GO can be used as efficient nanocarriers in electrochemical immunoassay system. Recently, Du's group [34] reported an electrochemical immunosensor based on multi-enzyme amplification strategy for ultrasensitive detection of phosphorylated p53 at Ser392 using GO as a nanocarrier. The immunosensor achieves higher sensitivity and lower detection limit than that of the traditional sandwich electrochemical immunosensors.

Gold nanoparticles (AuNPs) are well known as conductive and biocompatible labels for electrochemical signal amplification. So AuNPs decorated GO nanocomposite (AuNPs/GO) is very promising for electrochemical biosensors for it has large surface area to volume ratio, but better electrical conductivity than GO. Besides, several researches have revealed that AuNPs can effectively absorb biomolecules including enzymes [35], antibodies [22] as well as maintaining their bioactivity. Recently, Izquierdo's group [36] found CLB can be absorbed on the surface of AuNPs by electrostatic interaction or coordination bond at different pHs. Our work is motivated by utilizing AuNPs/GO as nanocarrier to co-immobilize glucose oxidase (GOD) and CLB simultaneously, yielding GOD/GO/AuNPs-CLB (Fig. 1A). AuNPs/GO was prepared by electrostatic interaction using positive (poly-diallyldimethylammonium chloride) (PDDA) as a linker. The electrochemical immunosensors was constructed by layer-by-layer assembly colloidal prussian blue (PB), multiwalled carbon nanotubes (MWCNTs), and CLB antibodies (Abs) on a glassy carbon electrode (GCE) (Fig. 1B). Herein, the PB acts as electron transfer mediator to catalyze reduction of H_2O_2 produced in a GOD directed enzyme reaction [22]. The MWCNTs with abundant carboxyl groups can provide effective scaffolds for the attachment of Abs. Through competitive immuno-recognition, GOD was immobilized onto GCE via the immuno-reaction of GOD/GO/AuNPs-CLB with Abs on the electrode. Electrochemical detection of enzymatic products is performed in the presence of glucose as shown in Fig. 1C. The enzymatic cycle with PB dually amplifies the electrochemical signal. Therefore, a highly sensitive and selective electrochemical immunosensor is developed for CLB with a low detection limit. Moreover, the stability, reproducibility and accuracy of the proposed method are all very satisfactory and suitable for the determination of CLB in real feed samples.

2. Experimental section

2.1. Reagents

CLB, bovine serum albumin (BSA), and chitosan (CS, ≥85% deacetylation), GOD (10,000 units/g, from Aspergillus niger), PDDA (20% w/w in water, MW: 200,000–350,000), 1-(3-(Dimethylamino)-propyl)-3-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysulfo-succinimide (NHS) were all obtained from Sigma-Aldrich Chemical Co. (Shanghai, China) and were used as received. Mouse monoclonal CLB Abs (85%, 8.4 mg/mL, ELISA IC50 0.6 ppb) was purchased from Ucando Biotechnology Co. (Guangzhou, China). MWCNTs (diameter 60–100 nm) were obtained from nanotechnology Co. (Shenzhen, China). Graphite powder (99.95%, 325 mesh) was obtained from Alfa Aesar. All other chemicals were of analytical grade. Phosphate buffered saline (PBS, 0.05 M) with different pH was prepared by mixing the stock solutions of KH_2PO_4 and Na_2HPO_4. The washing buffer was PBS (0.05 M, pH 7.0) containing 0.05% (w/v) Tween 20 (PBST). Blocking solution was 2% (w/v) BSA containing 0.05% Tween 20. All water used was double-deionized water (Milli-Q, Millipore Corporation, Bedford, MA).

2.2. Apparatus

Differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) were carried out on CHI 660A electrochemical workstation (CHI, USA) with a conventional three-electrode system, a modified GCE as the working electrode, an Ag/AgCl electrode as the reference electrode and a platinum electrode as the counter electrode. Transmission electron microscopy (TEM) was carried out on a JEOL JEM-2010 (JEOL, Japan). Surface morphological images were taken by a HITACHI S-4800

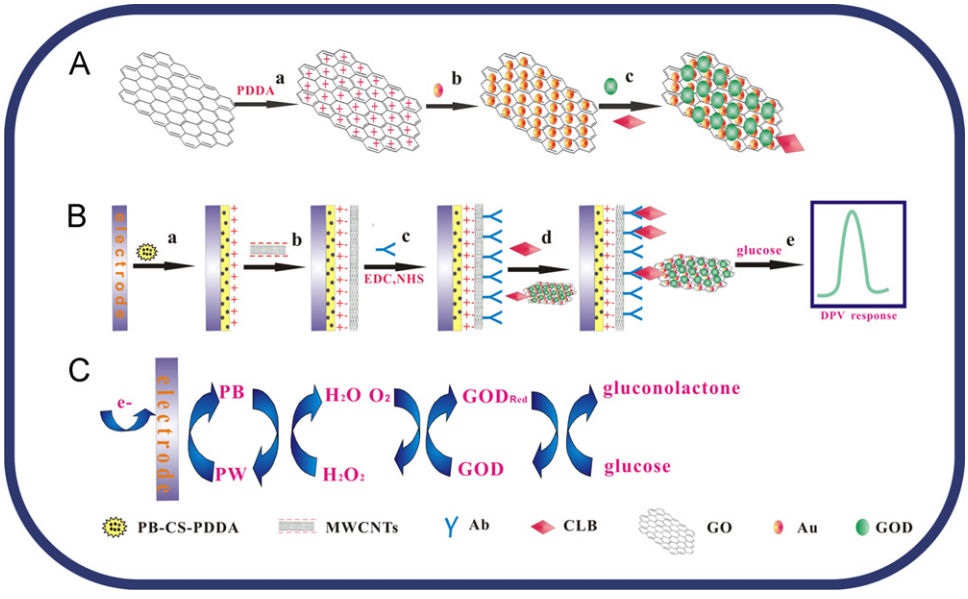

Fig. 1. Schematic representation of (A) preparation procedure of GOD/AuNPs/GO-CLB. (a) formation of PDDA-GO, (b) assembly of AuNPs on PDDA-GO and (c) co-immobilization of GOD and CLB on AuNPs/GO. (B) Construction of immunosensor and competitive electrochemical immunoassay. (a) Formation of a PB-CS-PDDA membrane on GCE, (b) modification with MWCNTs, (c) immobilization of Abs on the modified GCE, (d) competitive immunoreation of CLB in GOD/AuNPs/GO-CLB and in analyte with Abs on the modified GCE and (e) electrochemical measurements of the incubated immunosensor in 10 mM glucose solution. (C) Electrochemical response mechanism of the immunosensor.