



Graphene/polyaniline/gold nanoparticles nanocomposite for the direct electron transfer of glucose oxidase and glucose biosensing

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ABSTRACT

A novel glucose biosensor was developed based on the direct electrochemistry of glucose oxidase (GOD) adsorbed in graphene/polyaniline/gold nanoparticles (AuNPs) nanocomposite modified glassy carbon electrode (GCE). Compared with graphene, polyaniline (PANI) or graphene/PANI, the graphene/PANI/AuNPs nanocomposite was more biocompatible and it offered a favorable microenvironment for facilitating the direct electron transfer between GOD and electrode. The adsorbed GOD displayed a pair of well-defined quasi-reversible redox peaks with a formal potential of -0.477 V (vs. SCE) and an apparent electron transfer rate constant (k_s) of 4.8 s⁻¹ in 0.1 M pH 7.0 PBS solution. The apparent Michaelis–Menten constant of the adsorbed GOD was 0.60 mM, implying a fabulous catalytic activity and a remarkable affinity of the adsorbed GOD for glucose. The amperometric response of GOD-graphene/PANI/AuNPs modified electrode was linearly proportional to the concentration of glucose in the range of 4.0 μ M to 1.12 mM with a low detection limit of 0.6 μ M at signal-to-noise of 3 . The combination of the direct electron transfer character of GOD and the promising feature of graphene/PANI/AuNPs nanocomposite favors the selective and sensitive determination of glucose with improved analytical capabilities.

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1. Introduction

Biosensors based on the direct electrochemistry of redox enzymes/proteins have been investigated extensively over the past years [1,2]. These biosensors could avoid expensive or harmful mediators and their selectivity and sensitivity are high. An in-depth study of the direct electrochemistry of redox enzymes/proteins could also help to gain further insight into the mechanisms of electron transfer in biological systems [3,4]. Diabetes is a worldwide public health problem. Reagentless glucose biosensors based on the direct electrochemistry of glucose oxidase (GOD) play a leading role in the monitoring of serum or urine glucose levels [5]. However, the two bound redox-active flavin adenine dinucleotide (FAD) cofactors of GOD are deeply buried within the insulated prosthetic shells, rendering them inaccessible for direct electron transfer (DET) with bare electrodes [6]. Intensive efforts have been devoted to the development of retention of the biological activity and promote DET behaviors of GOD via selected matrix [7–10].

The development of nanotechnology offers new horizons for the application of nanomaterials in bioelectrochemical analysis [11–13]. Graphene, a single-atom thick, two-dimensional sheet of sp² bonded carbon, have been given considerable attention in recent years [14,15]. Owing to its extraordinary electrical, mechanical and chemical properties, fast electron transfer kinetics and excellent electrocatalytic characteristics, graphene has the most prospective application in synthesizing nanocomposites and constructing biosensors [12,16–19].

Recent research interest has focused on the preparation of nanomaterials/nanocomposites involving the combination of graphene and conducting polymers [20,21]. Incorporation of graphene in the polymer matrix with hydrophilic groups or molecules can improve water solubility, mechanical and thermal properties and enhance electrical conductivity of graphene. Among the various conducting polymers, polyaniline (PANI) is a perfect matrix for the immobilization of enzyme because of its biocompatibility, good environmental stability and controllable electrochemical properties [22]. PANI also provides an excellent matrix for dispersing noble metals, which expands its usage, and has been used in the fields of catalysts and sensors [23]. The graphene/PANI/metal nanoparticles composite could combine the electrical characteristics of graphene and metals, the mechanical and processing properties of polymers.

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Many of these composites show enhanced electrocatalytic activity and catalytic applications, as compared to those of pure PANI [24], graphene or AuNPs. The strong electronic interactions between graphene, metal nanoparticles and PANI matrix could improve the electron transfer rate of proteins. This is particularly important for studying the DET of proteins [25].

In this work, graphene was successfully modified and wrapped using PANI to provide hydrophilic functional $-NH_2$ groups. The functional $-NH_2$ groups paved the way for the following AuNPs loading. The graphene/PANI/AuNPs nanocomposite with AuNPs homogeneously decorated on PANI wrapped graphene was developed. Combine with the electrical/mechanical properties of graphene, the optical and catalytic abilities of both PANI and AuNPs, the resultant nanocomposite is more biocompatible and it offers a favorable microenvironment for facilitating DET between GOD and electrodes. Furthermore, the adsorbed GOD retains its native structure and bioactivity activities to glucose, indicating a new reagentless amperometric glucose biosensor can be constructed based on this new nanocomposite.

2. Experimental

2.1. Reagent and materials

Graphite, hydrazine solution (50 wt %), ammonia solution (28 wt %), aniline, $H AuCl_4 \cdot 3H_2O$, sodium borohydride ($NaBH_4$), ammonium peroxydisulfate (APS), chitosan and D-(+)-glucose were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Glucose oxidase (GOD) (EC 1.1.3.4, 109 U/mg, Type VII from *Aspergillus niger*) was obtained from Amresco Chemical Co., Ltd. and used as received. Phosphate buffer solutions (PBS) were prepared by mixing the stock solutions of 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 , and then adjusting the pH with H_3PO_4 or NaOH. The glucose stock solution was prepared by 0.1 M pH 7.0 PBS and was allowed to mutarotate at room temperature overnight before use. All other chemicals were of analytical grade and were used as received without any purification process. Deionized double-distilled water (18.6 M Ω) (Millipore Co. Ltd.) was used throughout the experiment.

2.2. Apparatus

The morphologies of graphene, PANI, graphene/PANI and graphene/PANI/AuNPs were investigated by using a Hitachi S-4800 Field Emission Scanning Electron Microscope (Hitachi Co., Japan). The cyclic voltammetric and amperometric measurements were performed on a CHI 840 electrochemical analyzer (Shanghai Chenhua, China), using a conventional three-electrode system with a platinum foil as the auxiliary electrode and a saturated calomel electrode (SCE) as the reference electrode. Working electrodes were bare or modified glassy carbon electrodes (GCE) (3 mm in diameter, Shanghai Chenhua, China). Before each experiment, the GCE was polished with 0.3 and 0.05 μm alumina slurries, rinsed with doubly distilled water, and then purified sequentially by HNO_3 (1:1, v/v), ethanol (1:1, v/v) and distilled water.

2.3. Preparation of graphene/PANI/AuNPs nanocomposite

The graphene was prepared according to the method proposed by our previous work [26]. Graphene/PANI composite was synthesized by using the following procedures. The mass ratio of aniline to graphene was 100:1. Typically, 100.0 mg aniline and 1.0 mg graphene were added into 20 mL of 2.0 mol/L HCl solution under magnetic stirring at room temperature for 0.5 h. Then 0.46 g APS in 10 mL aqueous solution was slowly added and the resulting solution continued to be stirred. During the reaction process, the color of the solution was changed from colorless to dark green. After

five hours, the obtained graphene/PANI nanocomposite was centrifuged at high speed, washed several times with double-distilled water, and dried at 60 $^{\circ}C$.

The self-assembly method was used to obtain the three-component graphene/PANI/AuNPs nanocomposite. AuNPs were prepared using the classical citrate reduction method [19]. 0.01 g portion of the graphene/PANI nanocomposite was added into 25 mL of the obtained Au colloid under stirring. The reaction was allowed to proceed for 12 h, and then the resultant product was centrifuged, washed several times with double-distilled water, and dried at 60 $^{\circ}C$.

2.4. Fabrication of GOD-graphene/PANI/AuNPs/GCE

In this work, chitosan was used to help the stable dispersion of graphene/PANI/AuNPs in aqueous solution because of its good biocompatibility and excellent film forming ability [27]. Chitosan solution (0.5%) (w/v) was prepared by dissolving 0.5 g chitosan powder in 100 mL 1.0% acetic acid solution. 4 mg of graphene/PANI/AuNPs was added into 2 mL of 0.5% chitosan solution to form a homogeneous dispersion with ultrasonication. 50 μL of 4 mg/mL of GOD solution was mixed 50 μL of 2 mg/mL of graphene/PANI/AuNPs suspension, and then the mixture was shaken strongly for about 30 min at room temperature for the adsorption of GOD. Electrode modification was achieved by depositing 5 μL of the above obtained mixture on the center of the pretreated GCE, which was left to dry at 4 $^{\circ}C$. When not in use, the modified electrode was stored at 4 $^{\circ}C$.

3. Results and discussion

3.1. Characterization of graphene/PANI/AuNPs and GOD-graphene/PANI/AuNPs

Scanning electron microscope was used to characterize the morphology of the as-prepared graphene, PANI, graphene/PANI and graphene/PANI/AuNPs. The scanning electron microscopy (SEM) images show typically curved, layer like structure morphology for graphene (Fig. 1A) and fibrous structures for PANI (Fig. 1B). Fig. 1C shows the SEM image of graphene/PANI nanocomposites. Significant changes in morphology are seen in the nanocomposites. Instead of the fibrous PANI, PANI is homogeneously surrounded around graphene (Fig. 1C). In situ polymerization of aniline in the presence of graphene in HCl aqueous solution allows us to prepare homogeneous composites in which PANI and graphene are believed to intercalate with each other instead of individually being in an agglomerated form [28]. Fig. 1D shows SEM image of the graphene/PANI/AuNPs nanocomposite. It is evident that AuNPs are distributed well in the graphene/PANI matrix. The method employed in the present study is satisfactory in preparing graphene/PANI/AuNPs nanocomposite.

FT-IR spectra were recorded to characterize the structure of graphene/PANI/AuNPs nanocomposites and illuminate the existing state of the adsorbed GOD. Fig. 2 shows the FT-IR spectra of PANI (a), graphene/PANI/AuNPs (b), native GOD (c) and the adsorbed GOD samples (d). Fig. 2a is the spectrum of the pure PANI. In this spectrum, prominent absorption peaks at 1576 and 1490 cm^{-1} are attributed to C=C stretching deformation of quinoid and the C=N stretching of secondary aromatic amine [29]. All character bands of PANI chains are observed in the graphene/PANI/AuNPs nanocomposite (Fig. 2b), showing the nanocomposite contains PANI. The peak at ca. 1140 cm^{-1} is attributed to C-N stretching of the secondary aromatic amine. It is the character of the conductivity of PANI [30]. Fig. 2b shows that the relative intensity of the peak at ca. 1140 cm^{-1} in graphene/PANI/AuNPs increases, suggesting the increase of the the conductivity of PANI in the nanocomposites increases. Thus, graphene/PANI/AuNPs nanocomposites can

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