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Electrochemistry of cholesterol biosensor based on a novel Pt–Pd bimetallic nanoparticle decorated graphene catalyst

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ABSTRACT

A new electrochemical biosensor with enhanced sensitivity was developed for detection of cholesterol by using platinum–palladium–chitosan–graphene hybrid nanocomposites (PtPd–CS–GS) functionalized glassy carbon electrode (GCE). An electrodeposition method was applied to form PtPd nanoparticles-doped chitosan–graphene hybrid nanocomposites (PtPd–CS–GS), which were characterized by scanning electron microscopy (SEM) and electrochemical methods. The presence of the PtPd–CS–GS nanocomposites not only accelerated direct electron transfer from the redox enzyme to the electrode surface, but also enhanced the immobilized amount of cholesterol oxidase (ChOx). Under optimal conditions, the fabricated biosensor exhibited wide linear ranges of responses to cholesterol in the concentration ranges of 2.2×10^{-6} to 5.2×10^{-4} M, the limit of detection was 0.75 μM (S/N=3). The response time was less than 7 s and the Michaelis–Menten constant (K_m^{app}) was found as 0.11 mM. In addition, the biosensor also exhibited excellent reproducibility and stability. Along with these attractive features, the biosensor also displayed very high specificity to cholesterol with complete elimination of interference from UA, AA, and glucose.

1. Introduction

Direct electrochemistry of redox enzymes not only provides a model for the study of electron transport of enzymes in biological systems, which is important to understand the material metabolism and energy transformation in life processes, but also achieves direct electron exchange between redox enzymes and electrodes, establishes a foundation for the fabrication of third generation of electrochemical biosensors [1–3]. In a direct electrochemistry-based biosensor, enzymes are integrated with electrodes, the direct electron transfer (DET) of enzymes on an electrode is generally difficult since the enzyme active sites are deeply buried in the protein matrix. To achieve DET, efforts have been devoted to shortening the electron transfer distance and designing biocompatible matrix to retain the native structure of the redox enzymes [4,5]. Nanomaterials are suitable for acting as "electronic wires" to shorten the electron transfer distance, enhance the electron transfer between redox centers of the enzyme and the electrode surface and simultaneously retain the biological activity of the redox enzymes [6]. Graphene-based hybrid nanomaterials are good examples, which could provide larger electrochemically active surface areas for the adsorption of enzymes and effectively accelerate the electron transfer between electrode and detection molecules [7]. Zeng and his colleague successfully synthesized palladium nanoparticle/chitosan-grafted graphene nanocomposites for construction of a glucose biosensor [8]. The hybrid nanocomposites showed a significant increase of electronic conductivity. Dong group successfully synthesized graphene/platinum hybrid nanostructures by alternatively assembling the ionic liquid-modified grapheme nanosheets and platinum nanoparticles [9]. The obtained hybrid nanomaterials exhibited good electrochemical properties. Our group demonstrated a facile strategy to incorporate high-quality hollow CoPt bimetal alloy nanoparticles onto reduced graphene oxide sheet [10]. The formed conjugates provided large surface area for loading plentiful redox probe thionine and showed a significant increase of electronic conductivity. These hybrid nanomaterials displayed superior electrochemical performance in comparison with that of alone nanomaterial. In this work, we tried to fabricate platinum (Pt) and palladium (Pd) nanoparticles-doped graphene hybrid nanomaterials for the preparation of the electrochemical cholesterol biosensor. Due to the unique catalytic activity and chemical selectivity of the bimetallic nanoparticles [11,12], the PtPd nanoparticles-doped grapheme hybrid nanomaterials were found to play the dual roles of catalyzing cholesterol redox reactions and also assisting direct electron transfer from the redox enzyme to the electrode surface.

Chitosan (CS) is a biocompatible polymer. It has been widely used as an immobilization matrix for biofabrication due to its high permeability towards water, excellent membrane forming

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ability, good adhesion and biocompatibility [13]. Many literatures also reported that CS can accumulate metal ions through various mechanisms, such as chelation, electrostatic attraction, and ion exchange, depending on the nature of the metal ion and pH of the solution [14,15]. So in this study, chitosan was joined into the dispersed graphene nanosheets (GS) to form homogeneous GS–CS solution. Herein, chitosan not only acts as an effective solubilizing agent for dispersing graphene nanosheets (GS–CS), which can have a good film-forming, but also as a polymer for accumulating Pt Pd ions to obtain a nanostructural graphene–chitosan–Pt–Pd composite membrane with the further enhancement of the porosity, surface area and electronic transfer rate.

In view of the advantageous features of Pt–Pd nanoparticles-doped graphene hybrid nanomaterials and GS–CS composite membrane, a sensitive electrochemical biosensor was constructed using cholesterol as the model analyte. The aim of this study is to design an enzyme sensing biosensor to study the direct electrochemistry of cholesterol oxidase (ChOx) and biosensing for cholesterol. Based on the PtPd nanoparticles-doped chitosan–graphene hybrid nanocomposites (PtPd–CS–GS), direct electron transfer from the redox enzyme to the electrode surface could be achieved by efficient catalysis of the ChOx towards the electrochemical reduction of cholesterol, resulting in the low detection limit of cholesterol. The details of the attractive response performances of the proposed biosensor and potential merits for protein detection are substantiated as follows.

2. Experimental

2.1. Reagents and materials

Graphene sheets (GS) were obtained in Pioneer Nanotechnology Co. (Nanjing, China). Cholesterol oxidase (ChOx, EC 1.1.3.6, $\geq$ 50 units/mg, from Brevibacterium sp.), Cholesterol ($C_{27}H_{46}O$, Mr: 386.67, $\geq$ 99% purity, from lanolin), Chitosan (CS), Triton X-100 ($C_{34}H_{62}O_{11}$, MW: 646.85), poly (diallyldimethylammonium chloride) (PDDA, 20% aqueous solution), $PdCl_2$, $HAuCl_4$, H_2PtCl_6 were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Phosphate buffered solutions (PBS) (pH 7.0) were prepared using 0.1 M Na_2HPO_4, 0.1 M KH_2PO_4, 0.1 M KCl and kept at 4 °C before use. The stock solution was prepared by dissolving cholesterol in the mixture of 2-propanol and Triton X-100, and then diluted it only with Triton X-100 solution for preparing standard solutions. Distilled water was used throughout this study. All other chemicals were of analytical grade and used as received without further purification.

2.2. Apparatus

The cyclic voltammetric (CV) experiments were carried out on a CHI 600D electrochemistry workstation (Shanghai CH Instruments, China), connected to a personal computer. All experiments were performed with a conventional three-electrode system. The modified glassy carbon electrode (GCE) as working electrode, a platinum wire as counter electrode and a saturated calomel electrode (SCE) or Ag/AgCl (sat. KCl) as reference electrode. The assembling interface was tracked by scanning electron microscopy (SEM, S-4800, Hitachi, Japan) and cyclic voltammetric. The pH measurements were made with a pH meter (MP 230, Mettler-Toledo Switzerland).

2.3. Fabrication of the proposed biosensor

A glassy carbon electrode (GCE) with 4 mm diameter was firstly polished with 0.3 and 0.05 μm alumina to obtain mirror-like surface, respectively. To remove the physically adsorbed substance, it was rinsed with deionized water and ethanol in ultrasonic bath and dried at room temperature.

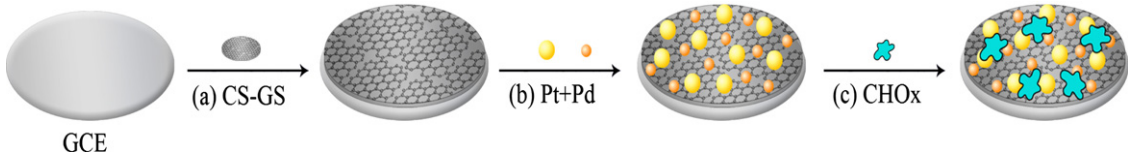
Firstly, 1 mg GS and 2 mg CS were dissolved in 1 mL 1% PDDA solution by ultrasonic dispersion, 10 μL of the resulting suspension was coated onto a pretreated GCE surface and dried in the air. Secondly, the electrochemical deposition of Pt–Pd was performed on CS–GS modified GCE to form PtPd–CS–GS nanocomposites in aqueous solution containing 0.5 mM H_2PtCl_6 and 0.5 mM $PdCl_2$. The deposition time was 200 s and the potential was −0.2 V. Subsequently, 6 μL ChOx (1 mg/mL in 0.1 M PBS, pH 7.0) solutions were dropped on the surface of the electrode to construct a cholesterol biosensor (noted as ChOx/PtPd–CS–GS/GCE). Ultimately, the obtained biosensor was stored at 4 °C when not in use. Scheme 1 showed the schematic diagram of the fabrication of the cholesterol biosensor.

3. Results and discussion

3.1. Characterizations

In order to confirm the microstructure and morphology of as-prepared nanomaterials, scanning electron microscopy (SEM) was used to investigate (Fig. 1). The SEM of graphene sheets (GS) dispersed by CS (Fig. 1A) clearly displays a wrinkled paper-like structure with slightly scrolled edges shapes, which is the standard morphology of Graphene sheets. Then the Pt–Pd nanoparticles appear as bright dots, which occupy almost all of the surface of CS–GS with fairly even, ordered, and close-packed distribution in Fig. 1B, forming an interpenetrating network for favorable conduction pathways of electron transfer. The diameter of the formed randomly dot structures vary from tens to hundreds of nanometers. As ChOx was immobilized, a fairly beautiful film was formed like cotton ball (Fig. 1C), providing a good platform for biosensing.

The cyclic voltammetric behavior of the step by step surface modification of the GCE in 5 mM potassium ferricyanide solution (pH 7.0) containing 0.1 M KCl from −0.2 to 0.6 V at a scan rate of 50 mV/s is shown in Fig. 2. The CV wave of bare GCE showed a well defined redox wave in Fig. 2a, corresponding to the reversible redox reaction of ferricyanide ions. The curve b in Fig. 2 was the CV of CS–GS modified GCE. Compared with the bare GCE, the redox wave was obtained at more negative potentials because the positive surface of PDDA modified electrode would attract $Fe(CN)_6^{4-/3-}$ redox couple. When the Pt–Pd nanoparticles were deposited in CS–GS membrane (curve c in Fig. 2), a large peak current was obtained owing to Pt–Pd nanoparticles may act as a bridge of electron transfer and promote the electron transfer. After ChOx was successively loaded onto the electrode surface,



Scheme 1. The schematic diagram of the fabrication of the cholesterol biosensor.