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Direct electrochemistry of hemoglobin based on nano-composite film of gold nanopaticles and poly (diallyldimethylammonium chloride) functionalized graphene

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ABSTRACT

The poly (diallyldimethylammonium chloride) (PDDA) functionalized graphene nanosheets (PDDA-G) were prepared and gold nano-particles (AuNPs) were assembled on the surface of PDDA-G film through electrostatic interaction to construct the biocompatible interface. The resulting AuNPs/PDDA-G nanocomposite film exhibited enhanced capability for hemoglobin (Hb) immobilization and realized its direct electrochemistry. The surface concentration (Γ^*) of electroactive Hb on the surface of GCE was calculated to be 3.85×10^{-9} mol/cm^2. Moreover, the AuNPs/PDDA-G based sensor showed prominent electrocatalytic activity for the detection of H_2O_2 with a wide linear range from 6 μM to 1010 μM and a low detection limit of 0.39 μM at 3σ. Meanwhile, the apparent Michaelis–Menten constant (K_m) was 0.51 mM. This biosensor exhibited high sensitivity and fast responses, which may provide a novel way for fabrication of other graphene based sensors and broaden the application of graphene in biosensing.

1. Introduction

As an extraordinary protein, hemoglobin is not only an oxygen carrier but also a pro-oxidant taking part in complex redox process in blood. It consists of two α and two β subunits with a molecular weight of nearly 67,000, each of them has one peptide chain and one protoheme [1–3]. Due to its structural similarity to the peroxidase, it can be employed to catalyze reduction of hydrogen peroxide through electrochemical catalysis [4–6]. Furthermore, protein-based biosensor is a significant research field with wide potential application of analytical chemistry, such as catalytic bioreactors, food industry, environmental monitoring and clinical diagnosis [7]. Meanwhile, direct electrochemistry of proteins or enzymes can provide a suitable model for studying mechanism of electron's transferring in real biological systems.

Graphene, a flat monolayer of sp^2 hybrid carbon atoms in a two-dimensional (2D) lattice which is the basic building block of carbon materials, has attracted tremendous attention because of its fascinating properties such as superior mechanical, large specific surface area and high conductivity [8]. However, the exclusive two-dimensional structure of graphene often tends to form conglomeration or even re-graphitized to graphite because of the van der Waals interaction and strong π–π piling which limit its further biological applications [9]. Therefore, graphene-modified materials, which are functionalized through noncovalent or covalent by polyelectrolytes or surfactants, becomes an other hot topic in material science for their enhanced electronic, solubility, and the electrochemical properties [10]. Poly (diallydimethylammonium chloride) (PDDA), is not only an electronic conducting polymer but also a strong ionic polymer, has excellent binding capability with graphene to form positive charge PDDA functionalized graphene (PDDA-G) and can maintain the electronic structure of graphene. Meanwhile, the dispersity of PDDA-G is greatly enhanced and can be used to construct the biosensor. On the other hand, gold nano-particles (AuNPs) are one of most stable metal nanoparticles. Due to its novel optical, electrical, catalytic properties and favorable biocompatibility, AuNPs have been widely applied in analytical chemistry [11]. It can hybrid with positive PDDA-G to form AuNPs/PDDA-G nanocomposite through electrostatic attraction. The obtained nanocomposite can be an ideal substrate material to provide a biocompatible microenvironment for the immobilization of biomolecules as well as retaining its native bioactivity due to the good affinity and lower biotoxicity of AuNPs for biomolecules.

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Meanwhile, due to the outstanding electric conductivity and the synergetic effect of PDDA polymer matrix and AuNPs [12,13], it can also accelerate the electron transfer on the electrode surface to amplify the electrochemical signal.

In the present work, AuNPs are assembled on the surface of PDDA-G film through electrostatic interaction to construct the biocompatible interface. Then, hemoglobin was immobilized on the surface of AuNPs/PDDA-G film modified electrode due to the good bioaffinity of AuNPs and realizes its direct electrochemistry of hemoglobin. The constructed biosensor displayed fast electron transfer and prominent catalytic ability to hydrogen peroxide with wide linear range, low detection limit and large surface concentration. Therefore, this work opens a new way to broaden the applications of graphene in electrochemical biosensors.

2. Experiment

2.1. Reagents and apparatus

Graphite was purchased from Alfa Aesar. Hemoglobin and PDDA (MW = 200,000–350,000) were obtained from Sigma. Hydrazine hydrate and Phosphates were purchased from TianJin guangfu Chemical Co. All of the other chemical reagents were of analysis grade and used without any further purification. Ultrapure water was used throughout the experiments.

Study of Electrochemical and electrocatalytic behaviors was fulfilled in CHI660 workstations (Shanghai Chenhua, China), a three-electrode system which comprised a platinum wire as counter electrode and a saturated calomel as reference and the modified glass carbon (GCE) as working electrode. Characteristics were achieved via field-emission scanning electron microscopy (FESEM, HITACHI S-4800), Fourier-transform infrared (FT-IR NEXUS 670) and X-ray powder diffraction (Panalytical X' Pert PRO).

2.2. Synthesis of PDDA-G and AuNPs

PDDA-G was prepared according to our previous work [9]. AuNPs were prepared with method reported by other article [14]. All vessels were immersed in aqua regia completely for more than 12 h and washed 3 times at least with ultrapure water before use. Prior to use, 2 ml 1.0% $HAuCl_4$ and 5 ml 1% trisodium citrate were mixed well with 200 ml ultrapure water in a 400 ml beaker. By vigorous stirring, the color changed from blue to wine red. And the solution was heated continually for an additional 10 min and stirred for another 2 min after diffusing the heater to produce about 24 nm diameter colloidal Au particles. The product was stored in refrigerator at 4 °C.

2.3. Construction of biosensor

The procedures used for preparation of the biosensor were shown in Scheme 1. Firstly, 5 μl of the PDDA-G was dropped on the cleaned GEC and dried in the air. Then the modified electrode was immersed into the AuNPs solution for 1 h to capture AuNPs. After that, the modified electrode was rinsed with ultrapure water and dried in the refrigerator at 4 °C. Finally, 5 μl of 5 mg/ml hemoglobin solution was dropped onto the modified electrode and stored at 4 °C for 12 h and then washed thoroughly with PBS to remove unbounded Hb. All the other compared modified electrodes were prepared with the similar process.

3. Result and discussion

3.1. Characterizations

As could be seen from Fig. 1A, the UV–vis spectra of AuNPs showed a characteristic surface plasma resonance absorption band at about 520 nm which indicated the diameter of AuNPs was about 20 nm [12]. The symmetric and narrow peak suggested a smaller average particle size and more mono-disperse size range. The surface morphologies of PPDA-G and AuNPs/PDDA-G composite were observed by SEM in Fig. 1B. As shown in inset of Fig. 1B, PDDA-G clearly showed the translucently silk-like shapes of graphene sheets. However, in AuNPs/PDDA-G composite, monolayer AuNPs homogeneously absorbed on the surface of PDDA-G and no aggregation was observed. Due to the high electric conductivity of graphene and good biocompatibility of AuNPs, the AuNPs/PDDA-G composite could accelerate the electron transfer on the electrode surface and provided a biocompatible microenvironment for biomolecules loading as well as retaining native bioactivity. Therefore, it would be an ideal substrate material for electrode modification to immobilize biomolecules.

Moreover, as could be seen from the XRD pattern in Fig. 1C, graphite (Fig. 1C) showed a strong characteristic peak centered at 26.4° (curve a). After oxidation, the characteristic peak of graphite was absent and the GO (curve b) exhibited a new characteristic peak at 9.9° which indicated that the graphite had been oxidized completely. After the reduction with hydrazine, graphene (curve c) showed a weakly broad peak centered at 23.6° and PDDA-G (curve d) showed two peaks centered at 22.9° and 16.9°, respectively, which indicated the ideal reduction of GO. The image of water dispersion of graphene without (left) and with PDDA (right) was exhibited in the inset of Fig. 1C. Because of the adsorption of PDDA, the PDDA-G has positive charge and prevented itself from aggregating. As a result, it can maintain in excellent dispersity within two months. In contrast, the water dispersion of graphene without PDDA aggregated more easily.

Furthermore, the FT-IR spectroscopy was used to study the functionalization process of graphene as illustrated in Fig. 1D. As could be seen, graphite (curve a) showed an absorption band about 1636 cm^{-1} which is the skeletal vibration of C=C. The characteristic peak of –OH (3429 cm^{-1}), C=O (1742.9 cm^{-1}), C–OH (1398 cm^{-1}) and C–O (1051 cm^{-1}) in GO (curve b) indicated that the graphene oxide had been synthesized successfully. The disappearance of oxo-group in graphene (curve c) and PDDA-G (curve d) showed the complete reduction of GO. Moreover, the peaks at 2925 cm^{-1} ($-CH_n-$), 1455 cm^{-1}($-CH_2-$), and 1111 cm^{-1} (C–N) in PDDA-G corresponded to the characteristic bands of PDDA which indicated the functionalization of graphene with PDDA.

3.2. Direct electrochemistry of Hb/AuNPs/PDDA-G film modified electrode

The direct electrochemistry of hemoglobin microbelts was studied by CV. Fig. 2 illustrates the cyclic voltammograms of different modified electrodes in 0.1 M pH 7.4 N_2-saturated PBS buffer solution at the rate of 100 mV/s. As shown in Fig. 2A, no obvious voltammetric peaks were observed at the bare GCE (curve a), PDDA-G modified electrode (curve b), and AuNPs/PDDA-G film-modified electrode (curve c). In contrast, the Hb/AuNPs/PDDA-G film-modified electrode (curve d) showed a couple of well-defined and quasi-reversible redox peaks at −0.413 V and −0.330 V with a peak-to-peak separation of about 83 mV, indicating the characteristic of heme Fe (III)/Fe (II) redox couples of Hb [1,12]. Fig. 2B displays the direct electrochemistry of Hb on different modified electrode. As can be seen from Fig. 2B, both ΔE and the anodic or cathodic peak current of Hb/AuNPs/PDDA-G film-modified electrode (curve