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Mimetic biomembrane–AuNPs–graphene hybrid as matrix for enzyme immobilization and bioelectrocatalysis study

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ABSTRACT

A hybrid composite constructed of phospholipids bilayer membrane, gold nanoparticles and graphene was prepared and used as matrices for microperoxidase-11 (MP11) immobilization. The direct electrochemistry and corresponding bioelectrocatalysis of the enzyme electrode was further investigated. Phospholipid bilayer membrane protected gold nanoparticles (AuNPs) were assembled on polyelectrolyte functionalized graphene sheets through electrostatic attraction to form a hybrid bionanocomposite. Owing to the biocompatible microenvironment provided by the mimetic biomembrane, microperoxidase-11 entrapped in this matrix well retained its native structure and exhibited high bioactivity. Moreover, the AuNPs–graphene assemblies could efficiently promote the direct electron transfer between the immobilized MP11 and the substrate electrode. The as-prepared enzyme electrode presented good direct electrochemistry and electrocatalytic responses to the reduction of hydrogen peroxide (H_2O_2). The resulting H_2O_2 biosensor showed a wide linear range (2.0×10^{-5}–2.8×10^{-4} M), a low detection limit (2.6×10^{-6} M), good reproducibility and stability. Furthermore, this sensor was used for real-time detection of H_2O_2 dynamically released from the tumor cells MCF-7 in response to a pro-inflammatory stimulant.

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1. Introduction

The direct electrochemistry of enzyme has remained a focus of study in recent years [1–3]. It can not only be applied to investigate electron transfer mechanisms in biological systems [4,5], but also offers great potential in many fields such as the construction of ultrasensitive third-generation biosensors, enzyme-based biofuel cells and bioelectrocatalysis [6,7]. However, it is difficult to realize direct electron transfer between enzymes and substrate electrodes due to their easy denaturation and deeply embedded redox active centers [8]. Therefore, one of the main challenges in this area is to explore appropriate host matrices that can both provide a biocompatible microenvironment for enzymes and enhance direct electron transfer between the enzymes and substrate electrodes [9,10]. Among various materials, biomaterials (e.g., phospholipids [11] and chitosan [12]) and two-dimensional layered inorganic nanomaterial (graphene [13], layered titanate [14], and layered hydroxide nanosheets [2]) are very attractive as support matrices for enzymes immobilization due to their intrinsic properties and unique structure.

Recently, graphene-based bionanocomposites have attracted great attention because of their unique and novel properties as structural or functional biomaterials [15,16]. With appropriate designs, graphene-based bionanocomposites can be furnished with tailor-made biological properties and improved biocompatibility derived from the modified biomaterials, effectively extending the application of graphene in biological science [17]. Artificial phospholipid membrane has been used as a mimetic biomembrane model in studying some membrane properties and biological functions [18–20]. It has been reported that lipid films can bind enzymes deeply, maintain their bioactivity, and offer a perfect biological microenvironment for electrochemical biosensors [11].

In this work, a biocompatible and conductive graphene-based bionanocomposite was prepared through facile electrostatic assembly of positively charged polyelectrolyte modified graphene

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and negatively charged phospholipid bilayer membrane protected AuNPs. The as-synthesized bionanocomposite was further used as a host matrix for microperoxidase-11 (MP11) immobilization. An enzyme electrode was fabricated by casting a suspension of MP11 mixed with this bionanocomposite onto a glassy carbon electrode (GCE). The resulting enzyme electrode presented a good direct electron transfer reaction and electrocatalytic response to the reduction of H_2O_2. The as-prepared biosensor was successfully employed for H_2O_2 detection and exhibited good performance. Furthermore, a rapid and real-time measurement was developed to detect the H_2O_2 released from living cells in response to a pro-inflammatory stimulant.

2. Materials and methods

2.1. Materials

Graphite was obtained from Alfa Aesar. Poly (diallyldimethylammonium chloride) (PDDA, MW < 10,000, 35 wt%) was purchased from Aldrich. Microperoxidase-11 (MP11), from horse heart cytochrome c, was obtained from Sigma (USA). 1,2-Dimyristoyl-sn-glycero-3-phosphatidil glycerol (DMPG) was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). $HAuCl_4 \cdot 4H_2O$ was obtained from Shanghai Chemical Factory (Shanghai, China). Unless otherwise stated, all other chemicals were of analytical grade and used as received without further purification. The ultrapure water (18.25 MΩ cm) used to prepare all aqueous solutions was purified with a Milli-Q Plus system (Millipore).

2.2. Fabrication of the sensing interface

The PDDA-modified-graphene nanosheets (PDDA-G) and the DMPG bilayer-membrane-protected AuNPs (DMPG-AuNPs) were synthesized respectively according to the previous work [21,22]. A 0.2 mL of PDDA-G (0.1 mg/mL) solution and 1 mL of DMPG-Au solution were mixed together overnight and centrifuged at 3000 rpm/min for 5 min, then the sediment (DMPG-AuNPs/PDDA-G) was obtained. The sediment (DMPG-AuNPs/PDDA-G) was resuspended with water at the final concentration of 0.5 mg/mL. The process of constructing DMPG-AuNPs/PDDA-G was shown in Scheme 1. Then, a glassy carbon electrode (GCE, 3 mm in diameter) was polished successively with 1.0 µm, 0.3 µm, and 0.05 µm alumina slurry, sonicated in ultrapure water for 1 min, and dried with high purified nitrogen steam. Aqueous solutions of DMPG-AuNPs/PDDA-G (0.2 mg/mL, 0.1 mL) and MP11 (0.2 mg/mL, 0.1 mL) were mixed together, and 5 µl of the mixture was dropped onto the freshly polished GCE surface and dried at 4 °C in order to obtain the MP11/DMPG-AuNPs/PDDA-G/GCE. As a control experiment, MP11/DMPG-AuNPs/GCE, MP11/PDDA-G/GCE, DMPG-AuNPs/PDDA-G/GCE and hemin/DMPG-AuNPs/PDDA-G/GCE were prepared according to the same procedures.

2.3. Electrochemical measurements

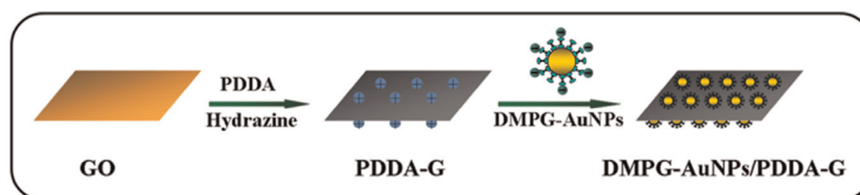
Electrochemical experiments were carried out with a CHI620a electrochemical analyzer (Shanghai Chenhua Equipment, China). A conventional three-electrode cell was employed in the PBS solution (0.1 M, pH 7.0) including a modified glassy carbon electrode as the working electrode, an Ag/AgCl electrode as the reference electrode, and a platinum wire as the counter electrode.

3. Results and discussion

3.1. Characterization of the as-prepared biosensor

As shown in Fig. 1, the TEM image of DMPG-AuNPs/PDDA-G demonstrates that DMPG-AuNPs are uniformly assembled on the PDDA-G surface. Because DMPG-AuNPs could be absorbed on both sides of PDDA-G, it is beneficial for maintaining a high surface area and providing a well biological microenvironment for enzymes immobilization. As MP11 is a heme-containing enzyme [23,24], UV–vis spectrometry was used to examine its secondary structure of MP11 because the information of heme enzymes conformational changes and denaturation can be obtained from the position of Soret absorption band of heme [25]. For the case of MP11 immobilized in the matrix of DMPG-AuNPs/PDDA-G, a nearly identical position of Soret absorption band is observed at the same wavelength with the dry MP11 film in Fig. 2, demonstrating that the MP11 retains a similar secondary structure and the matrix of DMPG-AuNPs/PDDA-G might provide a favorite biological microenvironment for the immobilization of MP11.

Cyclic voltammograms (CVs) of various modified GCE were obtained in N2-saturated phosphate-buffered saline (PBS: 0.1 M, pH 7.0), as shown in Fig. 3A. Compared with MP11/GCE, the current of MP11/DMPG-AuNPs/PDDA-G/GCE increased obviously. Moreover, MP11/DMPG-AuNPs/PDDA-G/GCE gives a pair of well-defined redox peaks; the anodic and cathodic peaks are located at −0.27 V and −0.35 V respectively, indicating that the composite played the role of "electrical wire" in the biosensor. However, no obvious electrochemical response is observed for either MP11/PDDA-G/GCE or DMPG-AuNPs/PDDA-G/GCE shown in Fig. 3. For the MP11 immobilized either in the DMPG-AuNPs or the PDDA-G matrix, a pair of peaks with low magnitudes were observed. And for the case of hemin immobilized in the matrix of DMPG-Au/PDDA-G, no obvious redox peak was observed. When MP11 was immobilized in the matrix of DMPG-AuNPs/PDDA-G hybrid, the peak current response became significantly enhanced. The peak-to-peak potential separation is about 80 mV, lower than that of MP11/chitosan/graphene [26], indicating an efficient direct electron transfer process between the active center of MP11 and the substrate electrode. This mainly attributes to a combination of the individual benefits of DMPG-AuNPs and PDDA-G, and a high electrochemical active area of the DMPG-AuNPs/PDDA-G nanocompisite material. The current response of MP11/DMPG-AuNPs/PDDA-G as a function of scan rate is shown in Fig. 4. Both the anodic and cathodic peaks were found to be increased linearly with the scan rate increasing from 50 mV/s to 500 mV/s. The linear dependence of peak current on scan rate is



Scheme 1. Schematic illustration of the self-assemble process of DMPG-AuNPs/PDDA-G.

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