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Graphene oxide directed in-situ synthesis of Prussian blue for non-enzymatic sensing of hydrogen peroxide released from macrophages

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ABSTRACT

A novel electrochemical non-enzymatic hydrogen peroxide (H_2O_2) sensor has been developed based on Prussian blue (PB) and electrochemically reduced graphene oxide (ERGO). The GO was covalently modified on glassy carbon electrode (GCE), and utilized as a directing platform for in-situ synthesis of electroactive PB. Then the GO was electrochemically treated to reduction form to improve the effective surface area and electroactivity of the sensing interface. The fabrication process was characterized by scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX) and atomic force microscopy (AFM). The results showed that the rich oxygen containing groups play a crucial role for the successful synthesis of PB, and the obtained PB layer on the covalently immobilized GO has good stability. Electrochemical sensing assay showed that the modified electrode had tremendous electrocatalytic property for the reduction of H_2O_2. The steady-state current response increased linearly with H_2O_2 concentrations from 5 μM to 1 mM with a fast response time (less than 3 s). The detection limit was estimated to be 0.8 μM. When the sensor was applied for determination of H_2O_2 released from living cells of macrophages, satisfactory results were achieved.

1. Introduction

Hydrogen peroxide (H_2O_2), as a common molecule in nature, shows widespread applications in pharmaceutical, clinic, environmental, mining, textile, paper, food manufacturing and chemical industries [1]. In biological field, it has been reported that the concentration level of H_2O_2 is an essential biological parameter in monitoring and maintaining the physiological balance of living cells, and the abnormal concentration level of H_2O_2 in body will lead to cell aberrance and apoptosis, causing different kinds of diseases, such as Parkinson's, Alzheimer's, cancer, diabetes, cardiovascular and neurodegenerative disorders [2,3]. Therefore, the development of highly sensitive, accurate, rapid and economical method for the determination of H_2O_2, especially released from living cells is of utmost importance in both biomedical and environmental studies [4–6]. Due to the intrinsic simplicity, rapidness, high sensitivity and selectivity, low cost, electrochemical method has been extensively employed in H_2O_2 sensor design and construction [7–9]. The enzyme-based electrochemical H_2O_2 sensors exhibit outstanding advantages of high sensitivity and high selectivity, but their utility is usually debased by the prohibitive costs of enzymes, complicated electrode fabrication process, poor stability, and harsh condition of test. Alternatively, non-enzymatic H_2O_2 sensors on the basis of nanomaterials are good candidates for robust detection of H_2O_2 due to their high stability, abundant active sites, excellent catalytic activity [10,11]. Up to now, numerous nanomaterials have been successfully used for the fabrication of non-enzymatic H_2O_2 sensors, such as noble metals, metal oxides, and carbonaceous materials [5,12–15]. But the high price and complicated synthesis procedures these materials restrains their practical application.

Prussian blue (PB) is a typical inorganic material, which shows extensive application in various fields such as capacitor material, electrochromic devices, catalysis, medicines, and so on [16]. PB has also been denoted as an “artificial peroxidase” because of its catalytic effect toward many biological important molecules such as cholesterol [17] and glucoses [18]. In addition, due to its excellent electroactivity, high selectivity and porous structure, PB or its composite has also been utilized as redox mediator in H_2O_2 biosensor construction [19,20].

The fabrication of PB-based biosensors can be commonly accomplished by direct electrosynthesis [21] or physical coating coupled with chemical pre-synthesis [22]. The electrodeposition of PB on the electrode surface is convenient and thickness controllable, but the too fast growth process of PB arising from its ultralow solubility product constant ($K_{sp} = 3.3 \times 10^{-41}$) makes the morphology and size of the electrosynthesised product hard to be controlled. PB synthesized through homogeneous chemical method also has above mentioned disadvantages, and meanwhile the modification process of the pre-

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synthesized PB on solid electrode surface is complicated. In addition to this, some film-forming reagent or binders are necessary, which will cause inferior electronic conductivity as well as the low sensitivity of the sensors.

In contrast, the in-situ self-assembly method based on successive adsorptions of Fe^{3+} and $Fe(CN)_6^{4-}$ on an appropriate matrix to synthesize PB not only can effectively control the growth rate as well as uniformity and morphology of PB, but also can greatly enhance its physicochemical properties such as mechanical strength and chemical stability [23]. Based on these merits, Manivannan et al. [24] used amine-functionalized silicate sol-gel reduced graphene oxide composite as the matrix for the in-situ synthesis of PB, leading to a three-dimension cage-like porous nanostructure. Electrochemical experiment showed that the unique electron-transfer mediating process of PB integrated with highly conductive reduced graphene oxide synthesized via the in-situ assembly method facilitated synergistic electrocatalytic activity for methanol oxidation. Ojani et al. [25] also synthesized PB through in-situ assembly method on a poly (o-phenylenediamine) modified electrode, which was utilized as an electrochemical sensor for H_2O_2 in acid media. However, in order to successfully obtain the in-situ synthesized PB, a non-conductive supporting matrix such as amine-functionalized silicate sol-gel [24] or poly (o-phenylenediamine) [25] was used, which not only increased fabrication cost and procedure, but also decreased the electrochemical activity of PB.

In order to overcome these disadvantages that happened in the conventional synthesis method of PB, the graphene oxide (GO) with rich functional groups was used as a directing platform for in-situ synthesis of PB in this work. The GO was first immobilized on a glassy carbon electrode (GCE) via a covalent bonding method, then the PB was grown on GO through sequential dipping in Fe^{3+} and $Fe(CN)_6^{4-}$ solutions. Characterization experiments suggested that the functional oxygen-containing groups were crucial for the GO-directed growth of PB. Then in order to improve the electroactivity of the synthesized PB, the modified electrode was electrochemically treated to transfer GO film to the reduced form (ERGO), which was more convenient and safe than the traditional chemical reduction methods [26,27]. So, the proposed method that combines in-situ growth of PB and direct electro-reduction of GO presented the advantages of higher stability, easier fabrication process and better analytical performance. Electrochemical analytical results showed that the prepared electrode could been utilized as a robust sensor for electrochemical analysis of H_2O_2 with wide linear range, fast response rate and excellent anti-interference ability. Based on these features, the electrode was applied as an electrochemical sensor for the effective monitoring of H_2O_2 released from living cells.

2. Experimental

2.1. Reagents and apparatus

Graphite and ascorbic acid (AA) of analytical grade were obtained from Guangdong Xilong Chemical Co., Ltd. (China). Dopamine (DA) was acquired from Aladdin Reagent Co., Ltd. (China). H_2O_2 and glucose (Glu) were provided by Hengmao Chemical Reagent Co., Ltd. (China). Lipopolysaccharide (LPS) and catalase were obtained from Sigma-Aldrich (China). N-Hydroxy succinimide (NHS), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), potassium ferrocyanide ($K_4Fe(CN)_6$) and ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) were purchased from Shanghai Jingchun Reagent Co., Ltd. (China). The macrophages were provided by BeNa Culture Collection Co., Ltd. (China). Uric acid (UA) was supplied by Sangon Bioengineering Co., Ltd. (China). Nafion was obtained from Shanghai Newenergy Technology Co., Ltd. (China). All the other chemicals were of analytical reagent grade and obtained commercially. Doubly distilled water was used throughout the experiments.

Morphology of the modified electrode was recorded on JEOL JSM-5600F Field-Emission Scanning Electron Microscopy (SEM, Japan). Atomic force microscopy (AFM) measurements were carried out on CSPM5500 scanning probe microscope (China). The pH values were tested on a model pHS-25 digital acidometer (China). Electrochemical measurements were measured by a CHI 650C electrochemical analyzer (China) with a conventional three-electrode system composed of Pt wire as auxiliary, Ag/AgCl electrode as the reference electrode, and a glassy carbon electrode (GCE, $\Phi = 3$ mm) modified with different materials as the working electrode. The SEM and AFM characterization on the morphology of the electrode were performed on a detachable working electrode purchased from Gaoss Union Tech. Co., Ltd. (China).

2.2. Covalent immobilization of GO on GCE

Prior modification, the bare GCE was polished to a mirror-like surface with 1.0, 0.3, and 0.05 μm α-Al_2O_3, followed by ultrasonic rinsing with water, ethanol-water mixture (V/V = 1:1), and water in turn. Then the electrode was dried under a high-purity N_2 stream. GO was synthesized from graphite powder according to Hummer's method [28]. The homogeneous GO suspension (1.0 mg mL^{-1}) was prepared by dispersing 5 mg GO in 5 mL water with ultrasonication for 5 h. The fabrication procedure of the sensor was illustrated in Scheme 1. In detail, the cleaned electrode was oxidized at +0.5 V for 60 s in 0.05 M PBS (pH 7.0) to generate oxygen-containing functional groups on the electrode surface [29]. Then the oxidized GCE was activated in 200 μL mixture solution containing 5.0 mM EDC and 8.0 mM NHS for 2 h, followed by rinsing with water. Afterward, 10 μL of prepared GO suspension was dropped onto the activated GCE surface and dried in air. After sufficiently washed with water to remove the loosely absorbed GO, the GO covalently modified GCE (GO/GCE) was prepared.

2.3. In-situ growth of PB on GO/GCE and the electro-reduction treatment

For the in-situ growth of PB on GO/GCE, the GO/GCE was subsequently immersed in Solution A (0.01 M $FeCl_3$, 0.1 M HCl and 0.1 M KCl) for 1 min, water for 30 s, Solution B (0.01 M $K_4[Fe(CN)_6]$, 0.1 M KCl) for 1 min, and water for 30 s at 37 °C. Such a dipping process was repeated for 10 times to make sure of adequate growth of PB nanoparticles on GO/GCE. The obtained electrode was denoted as PB-GO/GCE. Afterwards, the PB-GO/GCE was immersed into 0.05 M PBS (pH 7.0), and cyclically swept within a potential range of −1.6–0.6 V at 50 mV s^{-1} to transform GO to the reduced form (ERGO) [30]. The obtained electrode was denoted as PB-ERGO/GCE. Finally, in order to improve selectivity and stability of the prepared electrode, 5 μL 0.5% Nafion was cast on the PB-ERGO/GCE surface. After dryness and washed with water, the sensor (Nafion/PB-ERGO/GCE) was achieved.

In addition, in order to probe whether PB was grown specifically on GO modified electrode, an electrode of GO/GCE was electro-reduced first, and then used as the directing platform for growth of PB. The obtained control electrode was named as PB-pERGO/GCE. Also, in order to investigate sensing performance of each constitute in the sensing film, the electrodes of GO/GCE and PB-GO/GCE were fabricated through the same procedures.

3. Results and discussion

3.1. Morphologic and structure characterization

The appropriate matrix with functional groups is very attractive as support and stabilizer for the nanoparticles in both solution and solid phase [31]. In this work, the GO with rich functional groups of —COOH, —OH, and epoxy that covalently anchored on GCE was utilized as solid-phase support for the in-situ growth of PB particles. The growth process was characterized by SEM, and the results were displayed in Fig. 1. As seen, the bare GCE showed a flat and smooth surface (Fig. 1A). Upon modification of GCE with GO, some corrugated regions (Fig. 1B, see the arrow) that was consistent with the characteristic of GO was