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A novel electrochemical biosensor based on hemin functionalized graphene oxide sheets for simultaneous determination of ascorbic acid, dopamine and uric acid

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ABSTRACT

Graphene oxide (GO) has been demonstrated to be functionalized by hemin without any other reducing agents and the hemin functionalized graphene oxide (H-GO) hybrid nanomaterial was obtained under the condition of sonication, which could be ascribed to the π–π stacking and cation-π interactions between GO and hemin molecules. H-GO composite, characterized by scanning electron microscopy and UV–vis absorption spectroscopy, was used for preparation of the modified electrode considering its great electrocatalytic activity, large surface area, and abundant active sites. Due to the unique structure and excellent properties, H-GO modified glassy carbon electrode (GCE) showed high electrocatalytic activities for the oxidation of ascorbic acid, dopamine, and uric acid, achieving the simultaneous determination for those three different kinds of biomolecules with detection limits of 0.3, 0.17, and 0.17 μmol L^{-1} ($S/N = 3$), respectively. In addition, the proposed biosensor exhibited high selectivity and good stability, which could be applied for the electrochemical determination in practical samples with satisfying results.

1. Introduction

Functionalized graphene with distinct selectivity and high catalytic activity has received a great deal of interests in recent years. With large surface area and high chemical stability under ambient conditions, graphene consists of carbon atoms organized in a two dimensional (2D) honeycomb structure [1,2], exhibiting potential applications in various fields, such as energy storage, electronic structure, biosensor and so on [3,4]. As graphene oxide (GO) is decorated with oxygen-containing groups such as hydroxyl, carbonyl, carboxyl and epoxy group, the long-range conjugated structure of graphene had been destroyed, resulting in inferior electrical property [5]. Therefore, the reduction of GO became a hot topic nowadays and some routes that using hydrazine [6] and $NaBH_4$ [7] as reduced reagents for GO reduction were proposed, which were limited by the heavy toxicity and plenty of time. Accordingly, an approach that using the reagents which are environmentally friendly and non-toxic to simultaneous reduce and functionalize GO is desirable urgently [8]. Recently, covalent and non-covalent interaction could be used for graphene functionalization [9] and some reports have shown that GO can be reduced and functionalized by organic molecules via π–π stacking interaction between graphene basal plane and aromatic molecules such as hemin [10], ascorbic acid [11] or tetrathiafulvalene [12] under mild conditions.

Hemin, one of iron porphyrin derivatives, is well known as the active center of heme-proteins, having abilities to mimic the active site of various enzymes [13,14]. Nevertheless, direct application of hemin is of significant challenge because of its molecular aggregation in aqueous solution to form catalytic inactive dimers and oxidative self-destruction in the oxidizing media. Therefore, the development of novel materials such as hemin supports to achieve biomimetic catalysts with enzyme-like activity is highly desired. Recently, it was reported that hemin could be adsorbed on graphene surface by π–π interactions to form complex nanosheets, which were exhibiting great properties and various applications. Meanwhile, it is worthy noted that many small molecules including nitric oxide [13], nitrite [15], and hydrogen peroxide [16] could be reduced or oxidized based on the active groups of hemin [17] or reversible redox potential of $Fe^{2+/3+}$ [18].

The formation of hemin-reduced graphene (H-rGO) hybrid material with high catalytic ability and effective electronic transmission has been reported in recent years. The H-rGO was achieved through two steps included reduction of GO using hydrazine and then immobilizing hemin on the plane of rGO [14,19,20]. It is time

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consuming and most of the reducing agents are toxic. Interestingly, hemin has been used as reducing agents for reduction of GO under ultrasonication at room temperature [10]. The result exhibited that GO was not only functionalized but also reduced based on the facile and environmentally friendly method. To our best knowledge, some objectives including nitrite [21], hydrogen peroxide [22], toluene [20], and a few of biochemical molecules could be detected by hemin-functionalized reduced graphene oxide (H-rGO) hybrid due to the excellent catalysis of hemin and unique electronic properties of rGO.

Ascorbic acid (AA), which is present in daily diet as vitamin C, as an excellent agent, can take part in treatment of the common cold, mental illness, and infertility [23]. Dopamine (DA) is one of the excitation neurotransmitters in mammalian central nervous system that can transfer messages such as happiness and stimulation [24]. Additionally, abnormal dopamine concentration in brain can lead to Parkinson's disease [25]. Uric acid (UA) is another important biomolecule in human body, which is the primary product of purine metabolism [26]. Its abnormal concentration level results in several diseases, such as hyperuricaemia and gout [27]. AA, DA, and UA undergo the similar potential at bare electrodes, resulting in poor selectivity and reproducibility [28,29]. Since these three molecules coexist in human's diet so that the effective strategies for detecting AA, DA, and UA simultaneously are highly desirable.

In this report, the intriguing and facile approach allowing functionalization of GO using hemin without any other reducing agents has been done successfully. The structure of GO has been changed and the conductivity of GO has been improved after the functionalization with hemin. Due to the great electrochemical activity and high selectivity of H-GO, a novel electrochemical biosensor based on H-GO modified glassy carbon electrode (H-GO/GCE) was prepared for simultaneous determination of AA, DA, and UA, which was applied to the practical determination in real samples with satisfactory results.

2. Experimental

2.1. Reagents

Graphene powder was obtained from Shanghai Huayi Group Huayuan Chemical Co., Ltd., (Shanghai, China). Hemin was purchased from Sigma Chemical Co. (USA). Ascorbic acid, dopamine, and uric acid were purchased from Aladdin Chemical Reagent Company, (Shanghai, China). The Britton–Robinson (B–R) buffer solutions with various pH values were prepared by using the stock solution of 0.2 mmol L^{-1} NaOH, 0.04 mmol L^{-1} mixed acid of boric acid, phosphoric acid, and acetic acid. Double-distilled water was used in the whole experiments and the solutions were deaerated by bubbling high-purity nitrogen before the experiments were carried out. All the experiments were performed at room temperature.

2.2. Apparatus

All the electrochemical experiments were performed using a CHI660B electrochemical workstation (Chen Hua Instrument Co., Shanghai, China) with a conventional three-electrode cell including a modified glassy carbon electrode (3 mm in diameter) as the working electrode, a saturated Ag/AgCl reference electrode, and a platinum wire auxiliary electrode. The electrochemical measurements were recorded in a 10 mL electrochemical cell. A pHs-3B pH meter (Dazhong, Shanghai, China) was used for measuring pH. The UV–vis absorption spectra were carried out on a UV–vis 2450 spectrophotometer (Shimadzu, Japan). A KQ-250B ultrasonic bath (250 W, Kun Shan Ultrasonic Instruments Co., Ltd., China) was used for the preparation of H-GO.

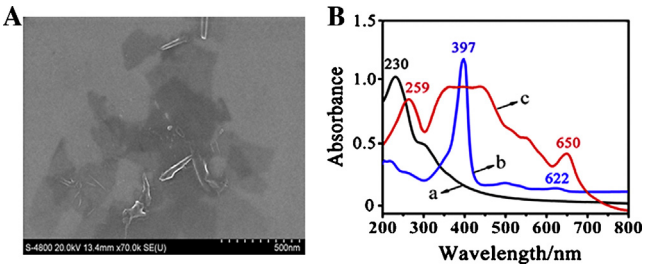

Fig. 1. (A) SEM image of H-GO; (B) UV–vis absorption spectra of GO (a), hemin (b), and H-GO (c).

2.3. Preparation of hemin functionalized graphene oxide

Graphene oxide was synthesized according to modified Hummers method [30]. A stable GO suspension (0.5 mg mL^{-1}) in double distilled water and 1 mg mL^{-1} hemin dissolved in ethanol were mixed with a volume ratio of 1:1 by sonicating for 5 h at 50 °C [10]. After sonication, the mixture was centrifuged at 14,000 rpm for 30 min to remove the supernatant. The precipitate was purged and collected by washing with double distilled water two times, then the pure composite was dried in an oven at 85 °C for 5 h, finally, the production was steadily dispersed in double distilled water with the concentration of 1 mg mL^{-1}.

2.4. Preparation of the modified electrode

The glassy carbon electrode (GCE) was polished with 0.05 μm alumina powder for about 10 min, and then rinsed by double distilled water and dried with high-purity nitrogen before modification. H-GO composite was dispersed with double distilled water and a homogeneous dark brown suspension (1 mg mL^{-1}) was obtained after sonication for 30 min. The H-GO/GCE was fabricated by dropping 5 μL of the above-mentioned suspension using a pipette on the pretreated GCE, and then stored in a vacuum desiccator to self-assemble for 5 h under room temperature (37 °C).

2.5. Electrochemical measurements

Electrochemical measurements to characterize the modified electrodes were performed in 5.0 mmol L^{-1} $[Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$ solution at room temperature (25 °C). The electrolytic solutions were purged with bubbling high-purity nitrogen for 10 min before the measurements. Current measurements were carried out by differential pulse voltammetry (DPV) in the potential range between −0.10 and 0.50 V. The following instrumental parameters were used to record DPV: Pulse amplitude of 50 mV, pulse width of 0.05 s, and scan rate of 5 mV s^{-1}.

3. Results and discussion

3.1. Structure characterization of graphene oxide, hemin, and hemin functionalized graphene oxide

The morphology and microstructure of the H-GO were investigated by means of scanning electron microscopy (SEM). The SEM image of the morphology of nanosheet H-GO on the sampling support is shown in Fig. 1A. The homogeneous nanostructure provided higher effective surface for the loading of biomolecules than that of the bare electrode. Besides, GO, hemin, and H-GO were all characterized by UV–vis absorption spectroscopy and the results are shown in Fig. 1B. A dispersion of brown colored GO in water displays a main absorption at 230 nm (curve a), with shoulder around 300 nm, which is attributed to the π–π transition of double bonds in aromatic skeleton and n–π transition of carbonyl functions,

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