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Direct electrochemistry and electrocatalysis of glucose oxidase on three-dimensional interpenetrating, porous graphene modified electrode

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ABSTRACT

Direct electrochemistry of glucose oxidase (GOD) on three-dimensional (3D) interpenetrating porous graphene electrodes has been reported, which have been fabricated by one-step electrochemical reduction of graphene oxide (GO) from its aqueous suspension. The electrochemically reduced GO (ERGO) modified electrodes exhibited excellent electron transfer properties for GOD and enhanced the enzyme activity and stability by the assistance of chitosan. The immobilized GOD shows a fast electron transfer with the rate constant (k_s) of 6.05 s^{-1}. It is worth mentioning that in the air-saturated phosphate buffer solution without any mediator, the resultant modified electrodes exhibited low detection limit of 1.7 μM with wide linear range of 0.02–3.2 mM and high sensitivity and high selectivity for measuring glucose. It would also be extended to various enzymes and bioactive molecules to develop the biosensor or other bio-electrochemical devices.

1. Introduction

Glucose oxidase (GOD) has attracted especial attention due to the extensive use in various fields including food, biofuel cells, textile industry, health and medical system [1–3]. To better understand the electron transfer mechanism of GOD redox in the biological systems and to develop biosensors, the direct electrochemistry of GOD has been developed widely [4–6]. On account of the active site (the flavin adenine dinucleotide (FAD)) of GOD being deeply embedded within a protective protein shell, the realizing of the direct electron transfer (DET) for GOD is extremely difficult, which limited the development of the third-generation glucose biosensor. A variety of materials including polymers [7], ionic liquid [8], metal oxides [9], metal nanoparticles [10,11], carbon nanotubes [12] and graphite nanosheet [13] have already been explored to modify the electrodes for improving and facilitating the DET between GOD and electrode. In recent years, graphene [14–16] embodied excellent property in graphene-based biosensors and enhanced DET between enzymes and electrodes, due to its large surface areas, high conductivity, good biocompatibility, thermal and chemical stability [14,17,18]. Graphene can be obtained from its precursor, graphene oxide (GO), reduced by the chemical [15] or electrochemical method [19]. Cai et al. [15] and Wei et al. [19] prepared graphene sheet with a typically crumpled and wrinkled structure by the above methods, respectively. However, due to the strong planar stacking during the electrode assembly [20], 2D graphene film would lead to a drastic loss of electroactive sites. 3D porous structure [21,22] of graphene is highly desirable for catalyst loading to facilitate the mass transfer and maximize the accessibility to the catalyst surfaces [20].

In this work, graphene with 3D interpenetrating porous networks was directly deposited on glass carbon electrode (GCE) by eletrochemical reduction of GO [23], which is fast, convenient and readily controllable compared with chemical reduction. The eletrochemically reduced graphene oxide (ERGO) has higher conductivity than that of its GO precursor and also have more accessible plane and edge sites [24] than that of 2D graphene film, which could be more likely to improve the communication between GOD and ERGO active electrode, and thus enhance DET between enzymes and electrodes. On the other hand, ERGO sheets still retain some oxygen-containing functional groups originating from their GO precursors, which make it easy for the formation of covalent linkage with the free amino groups of GOD. Chitosan (CS), a natural polymer with abundant amino groups, is a very suitable biocompatible matrix for the immobilization of enzyme with attractive properties including excellent film-forming ability, good adhesion, nontoxicity, and biocompatibility [25,26]. It is reasonable to expect

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the enhancement of direct electrochemistry of glucose oxidase via synergy effect of 3D porous graphene and CS matrix.

Herein, GOD was mixed with chitosan and immobilized on the ERGO modified glass carbon electrode (GCE). The electrochemical characteristics and high performance of the resultant electrode (CS-GOD-ERGO/GCE) with respect to linear range, the detection limit, reproducibility, selectively and stability is investigated in detail.

2. Experimental

2.1. Materials and apparatus

D-(+)-glucose and glucose oxidase (GOD) were obtained from Sigma–Aldrich. Chitosan (CS), graphite powder, L-cysteine (L-Cys), ascorbic acid (AA), urea, $LiClO_4$, potassium ferricyanide, disodium hydrogen phosphate and potassium dihydrogen phosphate were purchased from a chemical supplier (Sinopharm Chemical Reagents Co. Ltd., Beijing, China). 0.01 M phosphate buffer solution (PBS) was prepared freshly from Na_2HPO_4 and KH_2PO_4 reagents. The desired solution pH was adjusted by different amount of H_3PO_4 and NaOH solutions. All aqueous solutions were prepared with ultrapure water using a Milli-Q filter (Research UV, Hetai Instrument Co. Ltd., Shanghai, China). All chemicals were of analytical grade or better and used as received without further purification.

Alumina polishing powder (1.0 μm, 0.3 μm, 0.05 μm), glass carbon disk working electrode (1.5 mm in radius), platinum wire counter electrode (0.5 mm in radius) and saturated calomel electrode (SCE) reference electrode were purchased from CH Instrument (Chenhua Instrument Co., Ltd., Shanghai, China).

Electrochemical experiments were carried out using a potentiostat (CHI 920C, Chenhua Instrument Co. Ltd., Shanghai, China). All potentials were reported against the SCE reference electrode. Scanning electron microscopic (SEM) measurements were carried out on a scanning electron microscope (JEOL JSM-6700F) at 15 kV.

2.2. Preparation of graphene oxide (GO)

GO was prepared from natural graphite powder via acid-oxidation according to a modified Hummers method as mentioned in our previous paper [20].

2.3. Fabrication of the modified electrodes

The GCE was mechanically polished with 1.0, 0.3 and 0.05 μm alumina slurries in particle size successively. An ultrasonic cleaner was used to remove residual alumina loosely bound to the electrode surface. The polishing procedure was repeated until the working electrode was electrochemically clean for use. A 3 mg/mL GO dispersion containing 0.1 M $LiClO_4$ was used as the electrolyte. Electrochemical deposition of ERGO was carried out at room temperature under a constant potential of −1.2 V for 200 s using a CHI920C potentiostat under computer control. After electrodeposition, the graphene electrodes were washed with ultrapure water and then immersed in ultrapure water for 10 min to remove the residual GO absorbed on the electrodes. So, the modified glass carbon electrode by ERGO (ERGO/GCE) were obtained.

Secondly, the CS solution (1.0%) was prepared by dissolving 0.10000 g of chitosan in 10 mL acetic acid (0.05 M), filtered by a 0.45 μm syringe filter and stored in a refrigerator when not in use. 50 μL 18 mg/mL GOD solubilized in pH 7.3 PBS were mixed thoroughly with equal volume CS solution. Next, 6 μL of the above mixture was spread evenly onto the wet surface of ERGO/GCE with syringe. Finally, to get more uniform films, the modified electrode (CS-GOD-ERGO/GCE) was covered with a small bottle and allowed to stay for a while but not dry at 4 °C and then stored in the PBS atmosphere at 4 °C when not in use. To compare with CS-GOD-ERGO/GCE, GOD-ERGO/GCE and CS-GOD/GCE were also fabricated according to the same casting method.

3. Results and discussion

3.1. Morphology of ERGO/GCE

Fig. 1 shows the SEM images of the surface of ERGO modified electrode with different magnifications, which exhibit a well-defined and interconnected 3D porous network of graphene sheets, consistent with the 3D graphene synthesized by Shi [23]. From Fig. 1A, it can be seen that lots of porous and cavity-like structures formed by the graphene sheets with the pore sizes in the range of several micrometers to larger than 10 μm. Furthermore, those pores were interconnected with each other to form the 3D graphene network, which would provide the largely exposed surface area and the quantity of edges sites of graphenes with high activity, conducive to the enzyme adsorption and the direct electron transfer. The resulting 3D graphene network with high conductivity could also act as electronic mediator of enzyme, beneficial to the direct electrochemistry of enzyme. The close view of the 3D graphene structure (Fig. 1B) shows some smaller pores and cavities inside the bigger ones. The hierarchical structure of pores/cavities is also conducive to the enzyme adsorption and the direct electron transfer through the whole modified electrode. In addition, the much thin graphene sheets within the 3D graphene network may also decrease the electrons transfer resistance between the enzyme and graphene, helpful for direct electrochemistry of enzyme.

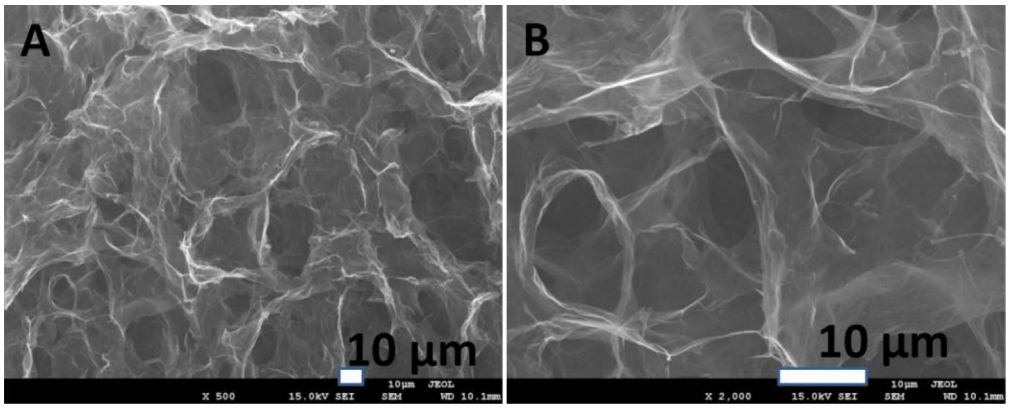

Fig. 1. SEM images the surface of ERGO modified electrode with different magnifications, respectively.