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Direct electron transfer of hemoglobin at 3D graphene–nitrogen doped carbon nanotubes network modified electrode and electrocatalysis toward nitromethane

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ABSTRACT

In this work, pulsed potentiostatic reduction method was presented to directly construct a three dimensional graphene-nitrogen doped carbon nanotubes (3DG-NCNTs) network on the surface of a glassy carbon electrode (GCE). Scanning electron microscopy (SEM), Raman spectra and electrochemical experiments revealed the characteristics of the 3DG-NCNTs network. Further, such 3DG-NCNTs network was employed to immobilize a model molecule, hemoglobin (Hb), for the construction of an electrochemical nitromethane (CH_3NO_2) biosensor. The electrochemical properties of the biosensor were studied in detail, including the direct electron transfer of Hb and the electrochemical determination of CH_3NO_2. Due to the 3DG-NCNTs network, electrochemical biosensor demonstrated fast electron transfer rate ($7.12\ s^{-1}$) and excellent catalytic activity toward CH_3NO_2. Under optimal conditions, the proposed biosensor exhibited a wide linear response in the range of 5.0×10^{-7}–$3.5 \times 10^{-4}\ mol\ L^{-1}$, with a low detection limit of $1.5 \times 10^{-7}\ mol\ L^{-1}$. In addition, the biosensor had high reproducibility, stability and selectivity, which provided the possibility for the monitoring of CH_3NO_2 in real samples.

1. Introduction

Direct electrochemistry of enzymes is of great importance to study of the intrinsic electrochemical properties of the enzyme, and is also the key to constructing the third-generation electrochemical biosensor [1]. But enzyme redox sites are often buried deep in the enzyme, and direct electron transfer (EDT) between the redox center and the electrode is difficult to achieve. So far, various materials have been employed as modifiers to promote and realize EDT, such as surfactant [2], conductive polymers [3], ionic liquids [4] and nanomaterials [5,6].

Graphene, as a two-dimensional (2D) carbon material, has attracted considerable attention recently owing to its excellent electrochemical stability, large specific surface area and high electrical conductivity [7,8]. Electrochemical biosensors based on graphene have been reported in a large number of documents [9]. However, graphene sheets tend to self-assemble and form 2D agglomerates due to the strong π-π interactions [10]. The strong planar stacking between graphene sheets result in the decrease of the effective surface area and catalytic sites, which limit the applications of graphene in the electrochemical biosensor. One way to overcome this issue is to design and construct three-dimensional (3D) graphene network with porous structures. This 3D graphene (3DG) network has an extraordinary surface area, allow target analyte to access the individual graphene sheets more easily and produce continuous ion transport channel due to their unique porous structure, which in turns decrease the mass transport resistance of the electrode (Scheme 1). Thus, 3DG network may be a more promising platform for electrochemical biosensors. In recent years, various methods have been proposed to construct 3DG including template method [11], chemical reduction method [12], chemical vapor deposition (CVD) method [13] and electrochemical reduction of graphene oxide (GO) sheets method [14]. Among these methods, electrochemical reduction method has several advantages over other methods, including being green, efficient, inexpensive, and rapid. More importantly, the method can form a stable film on the electrode surface without any further treatment, and the electrochemically prepared 3DG are more conducting than that prepared from other methods [15,16]. In addition, it is the most convenient and effective method for electrode surface modification since it can directly prepare graphene-based nanocomposites from relatively stable GO dispersion.

On the other hand, carbon nanotubes (CNTs) viewed as a rolled

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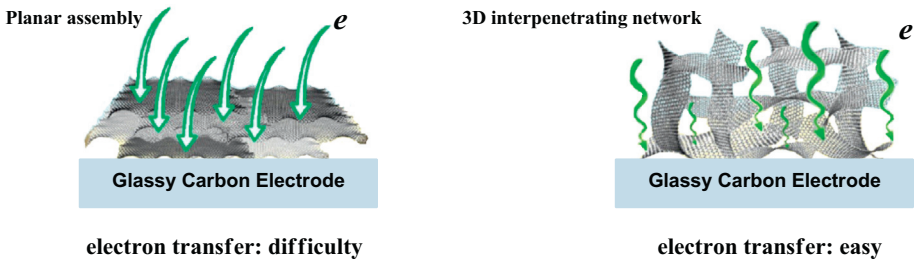

Scheme 1. Schematic illustration of electron transfer on 3D interpenetrating network and 2D planar assembly graphene surface.

graphene sheets also exhibit excellent mechanical, electrical and electrocatalytic properties [17]. Massive amount of electrochemical biosensor based on CNTs have been presented in the past decade [18]. But, a major challenge for developing such electrochemical biosensor of CNTs-based is the insolubility of CNTs in all solvents. To resolve the shortcoming, the most common route is to functionalize the surface of CNTs with specific bio/chemical molecules [19]. Now, some research groups confirmed that it is feasible to prepare a water dispersible and stable GO-CNTs hybrid material via non-covalent π–π stacking interactions [20]. And electrochemical biosensor platforms based on graphene-carbon nanotubes (G-CNTs) nanomaterials have been also reported [20]. However, G-CNTs nanomaterials usually obtained by two steps: drop-casting of GO-CNTs dispersion on the surface of glassy carbon electrode (GCE) and electrochemical reduction of above modified electrodes. So, the method to prepare G-CNTs nanomaterials usually showed 2D planar construction.

To the best of our knowledge, there are just few reports on the application of 3D graphene-carbon nanotubes (3DG-CNTs) for the electrochemical biosensor. Besides, recent studies demonstrated that nitrogen doped carbon nanotubes (NCNTs) can exhibit better conductivity, higher rate capability and wettability than that of CNTs [21,22]. Hence, in this work, electrochemical reduction method was presented to directly construct a three dimensional graphene-nitrogen doped carbon nanotubes (3DG-NCNTs) network on the surface of GCE. We creatively introduced the 3DG-NCNTs network to immobilizing a model molecule, hemoglobin (Hb), for the construction of an electrochemical nitromethane (CH_3NO_2) biosensor. The electrochemical properties of the biosensor were investigated in detail, including the direct electron transfer of Hb and the electrochemical determination of nitromethane (CH_3NO_2). Our results showed that the electrochemical biosensor exhibited excellent electrochemical responses with a couple of stable and nearly symmetric redox peaks of Hb presented, and showed excellent catalytic activity toward CH_3NO_2. It also indicated that the 3DG-NCNTs network are a promising matrix for application in electrochemical biosensors.

2. Experimental

2.1. Materials

High-purity flake graphite (325 meshes) and nitrogen doped carbon nanotubes (N content: 3.00 wt%; OD: 10–20 nm; L: 0.5–2 μm; purity > 95%) were purchased from Nanjing Xfnano Materials Tech. Co. Ltd. (Nanjing, China). All other reagents including chitosan (Cs), Hb, CH_3NO_2, $K_4[Fe(CN)_6]$, $NaNO_3$, $KMnO_4$, H_2O_2, HCl and H_2SO_4 were bought from Aladdin Chemistry Co. Ltd. (Shanghai, China). Phosphate buffer tablets were purchased from Aldrich (Shanghai, China). All the reagents were of a guaranteed grade and used as received. All solutions were prepared with double distilled water (DDW).

2.2. Preparation of GO and GO-NCNTs dispersion

Firstly, 1.0 g high-purity flake graphite and 0.5 g $NaNO_3$ were put into a 250 mL beaker, and 23.0 mL of concentrated H_2SO_4 was slowly added with stirring. Then 3.0 g $KMnO_4$ was added gradually using a spoon within 0.5 h. All processes are under the condition of ultrasonic and keep the temperature of the reaction mixture below 20 °C in the ice-bath. Subsequently, 46 mL of DDW was added slowly to the beaker, and the mixture was maintained between 32 °C and 38 °C for 1 h under the ultrasound condition. As the reaction progressed, the mixture gradually thickened and became pasty. At the end of 1 h, 140 mL DDW was slowly stirred into the paste. After a few minutes, the beaker was taken out from the ultrasonic instrument. The diluted suspension, now brown in color, was treated with H_2O_2 to reduce the residual MnO_4^- and MnO_2. Upon treatment with H_2O_2, the suspension turned bright yellow. The solid product was centrifuged at 4000 rpm to remove the unreacted graphite powder. It was then washed repeatedly with 5% HCl solution until SO_4^{2-} are removed and then washed with DDW repeatedly until it becomes free of Cl^-. Finally, the solid product was filtered and washed 3–4 times with acetone to make it moisture free, and then the residue obtained was dried in an air-oven at 50 °C overnight.

The exfoliated GO was obtained through ultrasonication for 3 h. Then, 3 mg NCNTs were added to 10 mL 3 mg mL^{-1} GO suspension, and then sonicated for 30 min to obtain GO-NCNTs homogeneous dispersion.

2.3. Construction of the biosensor

Prior to modification, the bare GCE (d = 3 mm) were polished (aqueous slurry of 0.05 μm α-alumina on a polishing cloth) and sonicated in DDW for 5 min, after being dried under N_2 blowing. The cleaned GCE was immersed in GO-NCNTs dispersion, and electrodeposition was carried out to obtain 3DG-NCNTs modified electrode (3DG-NCNTs/GCE) by pulsed potentiostatic method (PPM) under stirring. The parameters of electrodeposition were optimized and listed as follows: upper limit potential E_a, 0.1 V; lower limit potential E_a, −1.2 V; cathodic pulse duration t_c, 0.7 s; anodic pulse duration t_a, 0.3 s; experimental time t_{exp}, 100 s. Then 10 μL of Cs-Hb solution (10 mg Hb was dissolved 1 mL 1 mg mL^{-1} Cs solution) was dropped on the surface of the 3DG-NCNTs modified electrode, the biosensor denoted as Cs-Hb/3DG-NCNTs/GCE was constructed. Scheme 2 showed a schematic illustration of the construction of the biosensor. For a comparison, Cs-Hb/3DG/GCE was also prepared by a similar procedure.

2.4. Instruments and characterizations

Atomic force microscopy (AFM) analysis was performed by a Dimension FastScan AFM (Bruker Company, Germany). Scanning electron microscopic (SEM) images of the prepared 3DG-NCNTs networks were obtained using a field emission scanning electron microscopic (FESEM) of MERLIN (Zeiss Company, Germany). Scanning electron microscopy (SEM) images of prepared Cs/3DG-NCNTs and Cs-Hb/3DG-NCNTs were obtained with a Quonxe-250 scanning electron microscope (FEI Company, Czech). Raman spectra were conducted with the laser wavelength of 514 nm on an inVia Reflex Raman spectrometer (Renishaw Company, English). UV–vis absorption spectra were