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Reduced graphene oxide/PAMAM–silver nanoparticles nanocomposite modified electrode for direct electrochemistry of glucose oxidase and glucose sensing

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ABSTRACT

Reduced graphene oxide/PAMAM–silver nanoparticles nanocomposite (RGO–PAMAM–Ag) was synthesized by self-assembly of carboxyl-terminated PAMAM dendrimer (PAMAM-G3.5) on graphene oxide (GO) as growing template, and in-situ reduction of both $AgNO_3$ and GO under microwave irradiation. The RGO–PAMAM–Ag nanocomposite was used as a novel immobilization matrix for glucose oxidase (GOD) and exhibited excellent direct electron transfer properties for GOD with the rate constant (K_s) of 8.59 s^{-1}. The fabricated glucose biosensor based on GOD electrode modified with RGO–PAMAM–Ag nanocomposite displayed satisfactory analytical performance including high sensitivity (75.72 μA mM^{-1} cm^{-2}), low detection limit (4.5 μM), an acceptable linear range from 0.032 mM to 1.89 mM, and also preventing the interference of some interfering species usually coexisting with glucose in human blood at the work potential of −0.25 V. These results indicated that RGO–PAMAM–Ag nanocomposite is a promising candidate material for high-performance glucose biosensors.

1. Introduction

Electron transfer in biological systems is a very important phenomenon in the areas of biochemical and biophysical sciences. The direct electron transfer (DET) reactions between redox proteins and electrode surface have attracted considerable interest, because studies of DET are useful for elucidating the relationship between the structure and biological functions of redox proteins (Gooding et al., 2003; Nassar et al., 1996; Pulcu et al., 2007; Reed and Hawkridge, 1987; Rusling and Nassar, 1993; Wang et al., 2002), and especially meaningful for development of biosensor (Liu et al., 2005) and biofuel cell (Tanne et al., 2010; Tasca et al., 2008). Glucose oxidase (GOD), a flavin enzyme with molecular weight of 150–180 kDa, has been extensively used to monitor the blood glucose levels in diabetics for its catalytic ability to glucose. However, the realizing of DET between GOD and the electrode is extremely difficult due to the active site of GOD, flavin adenine dinucleotide (FAD), being deeply embedded within a protective protein shell (Liu et al., 2005). In order to promote the direct electron transfer between active site of GOD and electrode, many materials, including molecular wire (Liu et al., 2007a), polymers (Liu et al., 2007a; Wang and Chen, 2009; Wang et al., 2009; Zhao et al., 2009), metal or metal oxide nanoparticles (Gao and Zheng, 2009; Salimi et al., 2007; Zhao et al., 2006), carbon nanotube (Tominaga et al., 2008; Vaze et al., 2009; Zhao et al., 2010), have been used to modify the electrode to immobilize GOD for improving the DET of GOD on the surface of electrode.

Graphene, a monolayer of sp^2 hybridized carbon atoms packed into a dense honeycomb crystal structure (Allen et al., 2009), has received increasing attention during recent years because of its unique physicochemical properties including high surface area (Li et al., 2008), excellent electric conductivity (Geim and Novoselov, 2007), strong mechanical strength, biocompatibility, ease of functionalization and mass production (Shao et al., 2009). It has shown great promise in applications as electrode materials for immobilizing GOD and improving DET of GOD on electrode (Kang et al., 2009; Liu et al., 2010; Shan et al., 2009; Wang et al., 2009c). Moreover, graphene-based hybrids have been proved to generate synergy effect and thus enhance their performance in applications of biosensor (Shan et al., 2010; Wu et al., 2009). For example, coupling graphene with CdS quantum dots resulted in facilitating direct electron transfer and retaining the good bioactivity of GOD via synergy effect (Wang et al., 2011). Silver nanoparticles (Ag NPs) have attracted our attention due to their quantum

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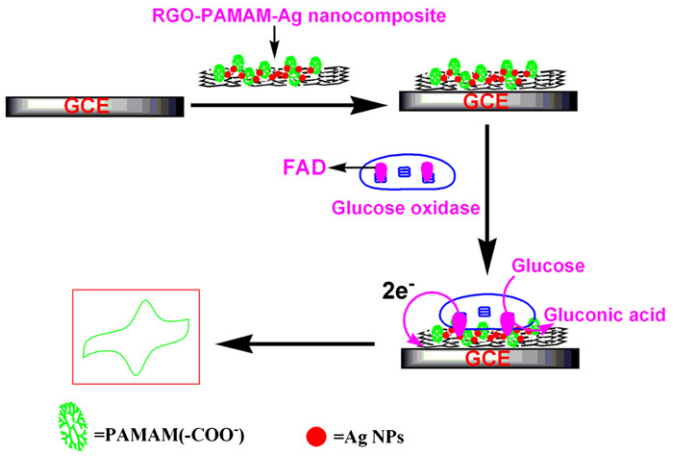

Fig. 1. Schematic diagram of RGO–PAMAM–Ag nanocomposite modified glassy carbon electrode to promote DET of GOD for detecting glucose.

characteristics and large specific surface area of small granule diameter as well as their ability to quickly transfer photoinduced electrons at the surfaces of colloidal particles (Liu et al., 2003). It has been reported that Ag NPs can greatly enhance the DET between some redox proteins and electrode (Gan et al., 2004; Liu and Hu, 2009; Ren et al., 2005).

Herein, RGO–PAMAM–Ag nanocomposite was synthesized by microwave-irradiation method and used to modify glassy carbon electrode (GCE). GOD was immobilized on the modified GCE and the DET between GOD and the modified GCE was studied, as can be illustrated in Fig. 1. The electrochemical catalytic activity of the fabricated electrode in response to glucose was also investigated. Due to the synergy effect of RGO and PAMAM–Ag NPs, the fabricated electrode showed excellent direct electrochemical behavior and the DET between the GOD and the modified electrode was easily achieved, indicating that RGO–PAMAM–Ag nanocomposite could be a good candidate material for immobilizing biomolecules and fabricating the third-generation biosensor.

2. Experimental section

2.1. Reagents and apparatus

Graphite, PAMAM dendrimer (ethylenediamine core, generation 3.5 solution in methanol), Chitosan (CS, medium molecular weight with viscosity 200–800 cP of 1% solution in 1% acetic acid, 75–85% deacetylated) and glucose oxidase (GOD, from *Aspergillus niger* Type X-S, lyophilized powder, 100,000–250,000 units/g solid) were purchased from Sigma-Aldrich. Acetone, potassium permanganate ($KMnO_4$, 99%), concentrated sulfuric acid (H_2SO_4, 98%), silver nitrate ($AgNO_3$, 99.9%) and hydrazine hydrate (50%) were purchased from Aladdin Reagent Co., Ltd. All reagents were of analytical grade and were used as received. Aqueous solutions were prepared with deionized water from a Millipore system.

The UV–vis absorption spectra were carried out with a UV–VIS–NIR spectrophotometer (Shimadzu **UV-3150**). Morphology and element analyses of RGO–PAMAM–Ag nanocomposite were carried out on high solution transmission electron microscopy (HRTEM) (JEOL, **JEM**-2010F **UHR**) equipped with an energy dispersive X-ray spectrometer (EDS), operating at 200 kV. The sample was prepared by dropping RGO–PAMAM–Ag nanocomposite on micro-grids supported on a 200 mesh copper grid and allowing deionized water to dry for observation. The electrochemical experiments were performed with a CHI660d electrochemical workstation (CHI, Austin, TX). All measurements were conducted at room temperature (25 °C) with a conventional three-electrode cell, which included a Ag/AgCl (saturated KCl solution) as reference electrode, a platinum wire as counter electrode, and a bare or modified GCE (3 mm in diameter) as working electrode. The cyclic voltammograms (CVs) experiments were carried out at a scan rate of 100 mV s^{-1} in a quiescent solution, which had been purged with high-purity nitrogen for at least 20 min prior to experiments. The chronoamperometric experiments were carried out in O_2-saturated phosphate buffered saline (PBS) with successive adding glucose solution at the work potential of −0.25 V.

2.2. Preparation of GO

Natural graphite was used for the preparation of GO. Graphite oxide was prepared from natural graphite powder by Hummers and Offeman's method with some modifications (Hummers and Offeman, 1958). In brief, 1 g of graphite powder and 30 mL of sulfuric acid were added into a reaction vessel in a dry ice bath, and stirred gently for 6 h. Then 3 g of potassium permanganate was added slowly with violent stirring. The reaction was allowed to proceed at below 20 °C for 30 min and at 35 °C for 30 min. Then 30 mL of deionized water was added into the reaction vessel slowly, and the reaction was kept at ~95 °C for 35 min. At last 140 mL of deionized water and 10 mL of 30% hydrogen peroxide were added into the reaction vessel for finishing the reaction. The resulting graphite oxide was filtered and washed with 5% hydrochloric acid and deionized water to remove the free SO_4^{2-}. The graphite oxide was suspended in the deionized water, and exfoliated through ultrasonication for 3 h. The colloidal solution was centrifuged at the speed of 5000 rpm for 10 min to remove the unexfoliated graphite oxide. The yellow-brown upper solution was used to prepare RGO–PAMAM–Ag nanocomposite.

2.3. Preparation of RGO–PAMAM–Ag nanocomposite

1 mL of GO (1 mg mL^{-1}) and 100 μL of PAMAM-G3.5 dendrimer methanol solution was added into a reaction vessel and diluted with 19.0 mL of deionized water. The mixture was stayed for 1 h in order to fully make PAMAM-G3.5 dendrimer self-assembled on GO. Then 260 μL of 0.2 M $AgNO_3$ aqueous solution was added and the mixtures were kept in the microwave reactor for 60 min under violent stirring at 80 °C. The resultant solution was firstly filtered by microporous membrane (pore size 0.22 μm) and rinsed with deionized water. The solid on the microporous membrane was dispersed in 20 mL deionized water, and the pH value of the colloidal solution was adjusted to be 10.0 with 1.0 M NaOH aqueous solution. Then 1.4 μL of hydrazine hydrate (50%) was added to further react for 2 h at 90 °C. The product was obtained through filtration by microporous membrane (pore size 0.22 μm) and rinsed with deionized water again. The RGO–PAMAM–Ag nanocomposite was redispersed in water (pH 10.0) and was used to modify the GCE.

2.4. Fabrication of modified electrode

GCE (3 mm diameter) was polished with 1.0, 0.3, and 0.05 μm α-alumina powders, respectively. After successive ultrasonication in ethanol and deionized water, the electrode was rinsed with deionized water and allowed to dry at room temperature. 6 μL of RGO–PAMAM–Ag suspension (=0.25 mg mL^{-1}) was dropped on the surface of GCE and dried in air. Then the modified electrode (GCE/RGO–PAMAM–Ag) was put into phosphate buffered saline (PBS) (pH 5.29) for 1 h to reduce the negative charges on the surface of RGO–PAMAM–Ag, and immersed into 10 mg mL^{-1} GOD solution in 0.1 M PBS (pH 7.0) for 24 h to load GOD. Finally, 10 μL