Technical Note

Direct Electrochemistry of Glucose Oxidase and Biosensing for Glucose Based on Graphene

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Direct Electrochemistry of Glucose Oxidase and Biosensing for Glucose Based on Graphene

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We first reported that polyvinylpyrrolidone-protected graphene was dispersed well in water and had good electrochemical reduction toward O_2 and H_2O_2. With glucose oxidase (GOD) as an enzyme model, we constructed a novel polyvinylpyrrolidone-protected graphene/polyethylenimine-functionalized ionic liquid/GOD electrochemical biosensor, which achieved the direct electron transfer of GOD, maintained its bioactivity and showed potential application for the fabrication of novel glucose biosensors with linear glucose response up to 14 mM.

Graphene, a single layer of carbon atoms in a closely packed honeycomb two-dimensional lattice, has attracted considerable attention from both the experimental and theoretical scientific communities in recent years.[1,2] The unique nanostructure and property of graphene provide potential applications in synthesizing nanocomposites[3−6] and fabricating various microelectrical devices, such as battery,[7] field-effect transistors,[8] ultrasensitive sensors,[9] and electromechanical resonators.[10] Graphene sheets, which have a high specific surface area, unless well separated from each other, tend to form irreversible agglomerates or even restack to form graphite through strong $\pi-\pi$ stacking and van der Waals interaction.[2] The prevention of aggregation is of particular importance for graphene sheets because most of their unique properties are only associated with individual sheets. The challenge of dispersing graphene has been addressed through their covalent modification[11,12] or noncovalent functionalization.[3,4,13]

The electrochemical biosensors utilizing nanomaterials have recently attracted considerable attention in the area of sensing.[14−16] Many kinds of nanomaterials, such as carbon nanotubes,[17−19] gold nanoparticles,[20−22] metal oxides,[23,24] and semiconductors,[25] have been used in biosensors in the medicine and food quality control field. Especially, because of the usefulness in diagnostic analysis of diabetes, glucose biosensors based on nanomaterials, such as carbon nanotubes and metal nanoparticles, have been extensively studied in recent years.[26−34] Although graphene has been exploited in many applications, it has not been reported that graphene can be used in electrochemical biosensors as far as we know. As an example, here we first communicated an application

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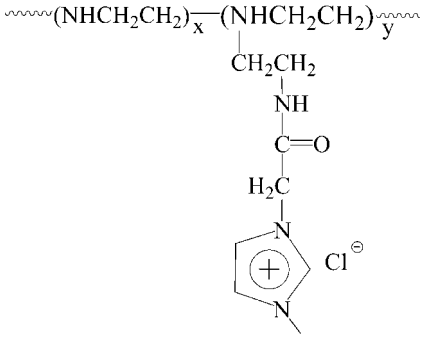
Scheme 1. Structure of Polyethylenimine-Functionalized Ionic Liquid



of graphene nanomaterial in the fabrication of an electrochemical glucose biosensor.

A polyethylenimine-functionalized ionic liquid (PFIL, as illustrated in Scheme 1), which has been reported by covalent attachment of a carboxyl terminated ionic liquid on polyethylenimine,[35] has good film stability and high ionic conductivity for enhanced electrochemical response.[36,37] Also it has wide solubility which is quite helpful for the dispersion of graphene. In addition, high biocompatibility and exchangability of the counter-anions in PFIL, e.g., with negatively charged glucose oxidase (GOD), are much favorable for further immobilization of biomolecules.[38] The PVP-modified graphene was also dispersed well in such a PFIL aqueous solution due to the high solubility of PFIL. So here, the PFIL is used to construct a graphene-GOD-FPIL biosensor.

EXPERIMENTAL SECTION

Materials. Graphite, hydrazine solution (50 wt %) and ammonia solution (28 wt %) was purchased from Sinopharm Chemical Reagent Co., Ltd. PFIL was prepared as described previously.[35] Polyvinylpyrrolidone (M_w = 360 000, PVP) was obtained from Aldrich. Glucose oxidase (EC 1.1.3.4, type X-S, lyophilized powder, 100−250 units/mg, from *Aspergillus niger*) and D-(+)-glucose (≥99.5%) were obtained from Sigma. Glucose stock solutions were stored overnight at room temperature before use. Hydrogen peroxide solution (30 wt % aqueous) was purchased from Beijing Chemical Reagent Co. Unless otherwise stated, reagents were of analytical grade and used as received. Aqueous solutions were prepared with double-distilled water from a Millipore system (>18 MΩ cm).

Instruments. Fourier transform infrared spectroscopy (FT-IR) was recorded on a Bruker Vertex 70 spectrometer (2 cm^{-1}). Transmission electron microscopy (TEM) micrographs were obtained using a JEOL 2000 transmission electron microscopy operating at 200 kV. X-ray photoelectron spectroscopy (XPS) analysis was carried out on an ESCALAB MK II X-ray photoelectron spectrometer. Energy dispersive X-ray spectroscopy (EDX) was measured on the 2000XMS instrument (EDAX, Inc.). Cyclic voltammetry measurements were performed using a conventional three-electrode cell with a platinum wire as the auxiliary electrode and an Ag/AgCl (saturated KCl) as reference in a CHI 660 Electrochemical Workstation (CHI). Working electrodes were bare or modified glassy carbon (GC) electrodes (d = 3 mm). Before use, GC electrodes were carefully polished to a mirror finish with 1.0, 0.3, and 0.05 μm alumina slurries, successively.

Synthesis of PVP-Protected Graphene and Pure Graphene. Graphite oxide was synthesized from graphite by a modified Hummers method.[39,40] PVP-protected graphene was prepared by a modified method according to the literature.[2] As-purified graphite oxide suspensions were then dispersed in water to create a 0.05 wt % dispersion by ultrasonication for 30 min. The obtained brown dispersion was then subjected to 30 min of centrifugation at 3 000 rpm to remove any unexfoliated graphite oxide (usually present in a very small amount). The homogeneous graphite oxide dispersion (5.0 mL) was mixed with 5.0 mL of 4 mg/mL PVP aqueous solution, and the mixed solution was then stirred at 50 °C for 12 h. After cooled to room temperature, to the resulting dispersion was added 3.5 μL of hydrazine solution and 40.0 μL of ammonia solution. After being vigorously shaken or stirred for a few minutes, the vial was put in a oil bath (95 °C) for 1 h. The stable black dispersion was obtained. The dispersion was filtered with a nylon membrane (0.22 μm) to obtain PVP-protected graphene that can be redispersed readily in water by ultrasonication. Additionally, the preparation of pure graphene was similar with PVP-protected graphene, just no PVP was added.

Preparation of PVP-Protected Graphene-GOD-PFIL Modified GC Electrodes. A total of 1 mg of PVP-protected graphene was added to 1 mL of a 1.5 mg/mL PFIL aqueous solution to form a homogeneous dispersion with mild ultrasonication. A volume of 2 μL of the resulting graphene-PFIL solution was dropped onto a GC electrode and allowed to dry in ambient air for 24 h. Then the graphene-PFIL modified GC electrode was soaked in 2 mg/mL GOD solution (0.05 M phosphate-buffered saline, pH 7.4) for 24 h at 4 °C to obtain the graphene-GOD-PFIL modified GC electrode. At this pH, GOD (pI ∼ 4.5) bears a net negative charge, allowing counter-anions in the PFIL film to exchange with GOD (−). The PFIL-modified and graphite-PFIL-modified GC electrodes used in the control experiment were prepared with 2 μL of 1.5 mg/mL PFIL aqueous solution and 2 μL of 1.5 mg/mL PFIL solution containing 1 mg/mL graphite by the same procedure, respectively.

RESULTS AND DISCUSSION

Characterization of Graphene and PVP-Protected Graphene. The graphene oxides, graphene and PVP-protected graphene were first characterized by FT-IR (Figure S1 in the Supporting Information). The spectrum of the graphite oxides showed the presence of O−H (ν_{O-H} at 3386 cm^{-1}), C=O ($\nu_{C=O}$ at 1733 cm^{-1} in carbonyl groups), C=C ($\nu_{C=C}$ at 1622 cm^{-1}), and C−O (ν_{C-O} at 1129 cm^{-1}). After the reduction with hydrazine, the FT-IR spectra of graphene were essentially featureless except the C=C conjugation (1545 cm^{-1}) and C−C bands (1190 cm^{-1}).

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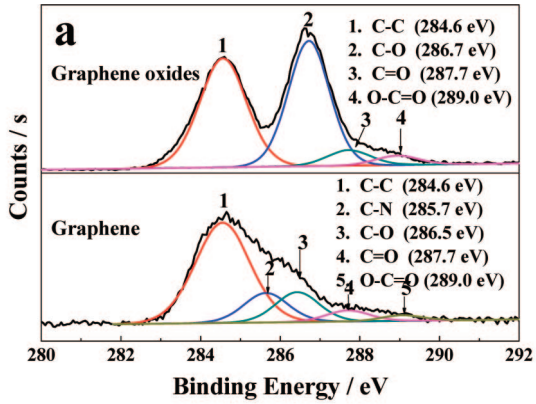

Figure 1. (a) The C1s XPS spectra of graphene oxides (up) and pure graphene (down). (b) TEM image of PVP-protected graphene.

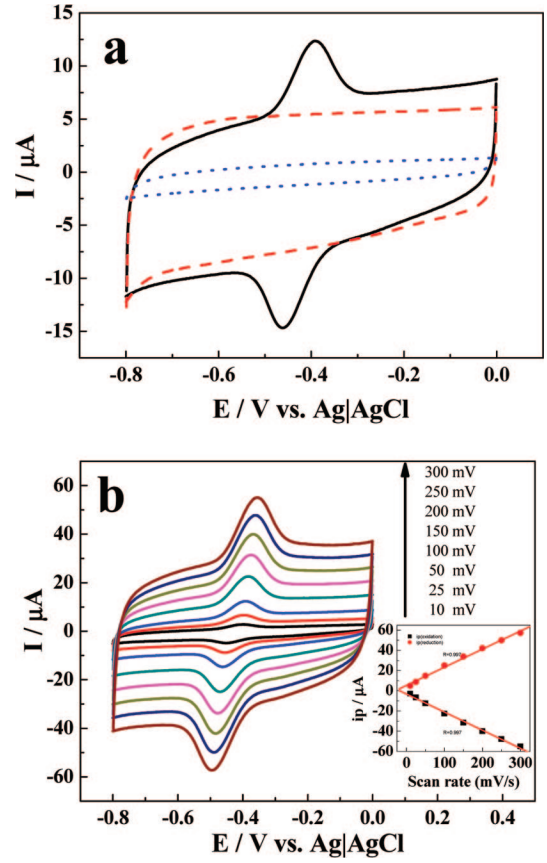

Figure 2. (a) Cyclic voltammograms of graphene-PFIL (dashed), graphite-GOD-PFIL (dotted), and graphene-GOD-PFIL (solid) modified electrodes in 0.05 M PBS solution (pH = 7.4) saturated with N_2 at a scan rate of 0.05 V s^{1-}. (b) Cyclic voltammograms at various scan rates from 0.01, 0.025, 0.05, 0.10, 0.15, 0.20, and 0.25 to 0.3 V s^{1-}, respectively. Inset: plot of peak current (ip) vs scan rate.

The FT-IR spectrum of PVP-protected graphene exhibited PVP absorption features, indicating the presence of the PVP component. The graphene oxide and graphene were also characterized by XPS in Figure 1a. Although the C1s XPS spectrum of graphene show the same oxygen functionalities with graphene oxide, the absorbance peaks of graphene at 286.5 (C—O), 287.7 (C═O), and 289.0 eV (O—C═O) were sharply decreased. In addition, there was an additional component at 285.7 eV corresponding to the carbon in the C—N bonds.[41] Energy-dispersive X-ray spectroscopy (EDX) also confirmed the decrease of oxygen content and the presence of a little nitrogen in the graphene (Figure S2 in the Supporting Information). These results indicated that graphene oxides were reduced into graphene with a spot of oxygen functionalities. The graphene oxide and graphene showed high purity except a very small amount of sulfur confirmed by EDX (Figure S2 in the Supporting Information). The morphology of the graphene was observed by TEM. Figure 1b shows the TEM image of graphene nanosheets, illustrating the flakelike shapes of graphene. Additionally, PVP-protected graphene can be redispersed readily in water via gentle ultrasonication, forming black suspensions (Figure S3 in the Supporting Information). At concentrations lower than 0.1 mg/mL, the dispersions are very stable, even for several months storage. More concentrated dispersions would generate a little precipitate after several days. However, they never completely settle down, even upon months of standing.

Direct Electrochemisty of Glucose Oxidase. Figure 2a shows cyclic voltammograms (CV) of graphene-PFIL, graphite-GOD-PFIL, and graphene-GOD-PFIL modified glassy carbon (GC) electrodes in N_2-saturated PBS solution. A pair of well-defined redox peaks at the graphene-GOD-PFIL modified electrode was obtained (Figure 2a, solid). The formal potential ($E^{\circ\prime}$) calculated by averaging the cathodic and anodic peak potentials was estimated as ~−0.43 V (vs Ag/AgCl in saturated KCl) with ~69 mV peak-to-peak separation and ~1 ratio of cathodic to anodic current intensity. In comparison with the CV curve of the graphene-PFIL modified GC electrode (Figure 2a, dashed), it can be concluded that the redox waves should be ascribed only to GOD, which is characteristic of reversible electron transfer process of redox active center (flavin adenine dinucleotide, FAD) in the GOD.[42−44] Thus, a direct electron transfer of GOD in graphene-PFIL film has been achieved successfully. The cyclic voltammograms of graphene-GOD-PFIL modified GC electrode

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at various scan rates were investigated too (Figure 2b). The peak-to-peak separation and the linear relationship between peak current and scan rate (up to 0.3 Vs^{1-}) indicated that the redox process of GOD in this composite film was a reversible and surface-confined process.

Additionally, we did also run the direct electrochemistry of GOD in the graphite-PFIL modified electrode as a control experiment (Figure 2a, dotted). It was obvious that the direct electrochemistry of GOD was not achieved at such a graphite-PFIL modified electrode. It is well-known that the active redox center of GOD, flavin adenine dinucleotide (FAD), is deeply embedded in a protective protein shell, which makes the direct electron communication with electrodes extremely difficult. The graphene has the extraordinary electron transport property[45,46] and high special surface area,[2] so we speculate that graphene can promote the electron transfer of the film and facilitate the direct electron transfer process between the GOD and electrode substrate.

Electrocatalysis of O_2 and H_2O_2 at the Graphene-PFIL Modified GC Electrode. The resulting nanocomposite of graphene-PFIL exhibited good electrocatalysis toward the reduction of O_2 and H_2O_2. An obvious reduction wave of O_2 at the graphene-PFIL modified electrode could be observed at ~−0.3 V, and the reduction potential was much more positive than these at PFIL-modified and graphite-PFIL-modified electrodes (Figure 3a). The electrocatalysis of H_2O_2 at the graphene-PFIL modified electrode was also examined (Figure 3b). The more positive reduction potential (onset potential at ~0 V) and more obvious reduction current indicated that the graphene-based modified electrode had much better electrocatalysis toward H_2O_2 than PFIL-modified and graphite-modified electrodes. The good electrocatalysis toward O_2 and H_2O_2 in the presence of graphene might originate from the extraordinary electron transport property of graphene, which promotes the electron transfer of the film and thus facilitates the electrocalalysis toward O_2 and H_2O_2.

Detecting Glucose at the Graphene-GOD-PFIL Modified GC Electrode. On the basis of the high electrocatalytic activity of graphene-PFIL nanocomposite toward O_2 and H_2O_2, a glucose biosensor was developed further. Figure 4 shows cyclic voltammograms of the graphene-GOD-PFIL modified electrode in 0.05 M PBS solution with different concentrations of glucose (saturated with O_2). The reduction current originating from reduction of O_2 and H_2O_2 became smaller and smaller with increases of the concentration of glucose because of the consumption of O_2. Moreover, the calibration curve corresponding to amperometric response (as inset in Figure 4) is linear against the concentrations of glucose ranging from 2 to 14 mM (R = 0.994). The prepared biosensor had good reproducibility. The relative standard deviation (RSD) of the current response to 6 mM glucose at −0.5 V was 3.2% for 10 successive measurements. The stability of the biosensor was also investigated. The response current increased by 3.8% of its initial response after 3 days and by 4.9% after 1 week.

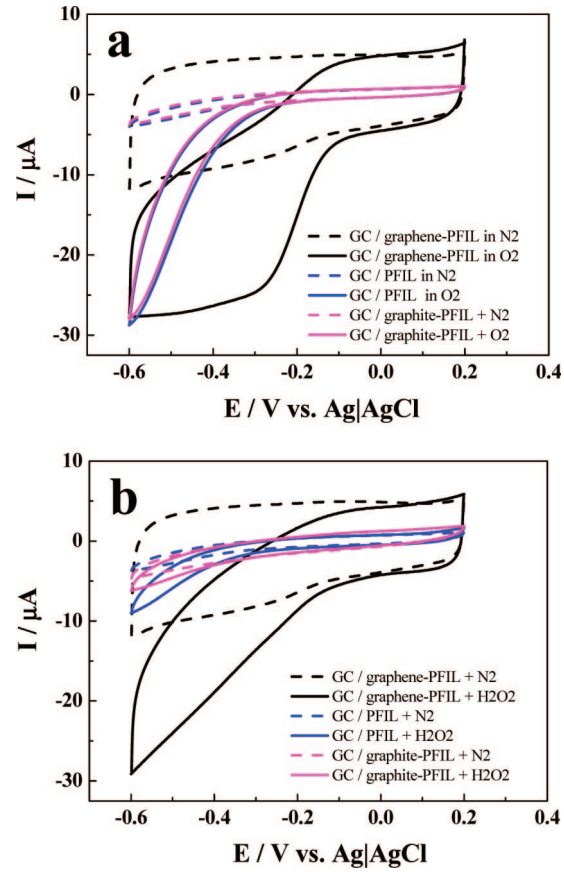

Figure 3. (a) Cyclic voltammograms at PFIL (blue), graphite-PFIL (magenta), and graphene-PFIL (black) modified electrodes in 0.05 M PBS solution saturated with O_2 (solid) and degassed with pure N_2 (dashed). (b) Cyclic voltammograms at PFIL (blue), graphite-PFIL (magenta), and graphene-PFIL (black) modified electrodes in 0.05 M PBS solution saturated with N_2 in the absence (dashed) and in the presence (solid) of 5 mM H_2O_2. Scan rate: 0.05 V s^{-1}.

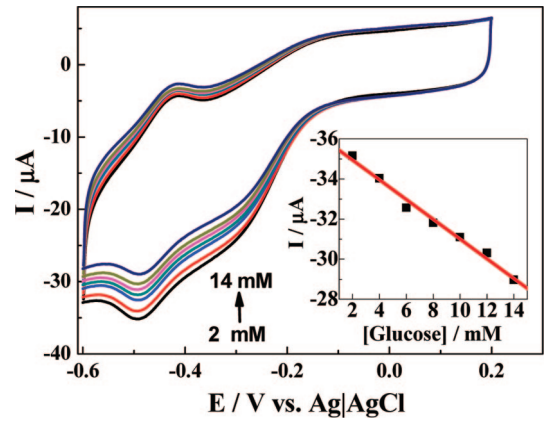

Figure 4. Cyclic voltammetric measurements at the graphene-GOD-PFIL modified GC electrode in various concentrations of glucose PBS solution saturated with O_2: 2, 4, 6, 8, 10, 12, and 14 mM from outer to inner. The inset is the calibration curve (R = 0.994) corresponding to amperometric responses at −0.49 V. Scan rate: 0.05 V s^{1-}.

Zhu et al.[47] fabricated a bienzymatic glucose biosensor based on coimmobilization of peroxidase and glucose oxidase on a carbon nanotubes electrode whose linear response range was from

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0.030 to 2.43 mM. Normally, the blood glucose level is maintained between about 4 and 6 mM. So the linear glucose response from 2 to 14 mM based on our novel graphene-PFIL modified electrode is enough and suitable for its practical application in determining blood sugar concentration. Wu et al.[48] constructed an amperometric glucose biosensor based on multilayer films via layer-by-layer self-assembly of multiwall carbon nanotubes, gold nanoparticles, and glucose oxidase, which exhibited linear response range toward glucose from 0.1 to 10 mM. Tsai et al.[49] constructed a biosensor based on a Nafion backbone, multiwalled carbon nanotube, and glucose oxidase by simple casting processes with a linear range from 0.025 to 2 mM, but the detection at positive potential (0.7 V) may be interfered with by some related biomolecules such as AA and DA by oxidation in the practical clinical analysis.[50] So the reduction detection by the graphene-based modified biosensor is more proper. Additionally, the graphene is better than carbon nanotube due to obtaining more easily.

CONCLUSION

In summary, graphene sheets protected by PVP, which could be stably dispersed in water, exhibited high electrocatalytic activity toward the reduction of O_2 and H_2O_2. Because of their good electronic properties and biocompatibility, graphene-based composites achieved the direct electron transfer of redox enzyme and maintained its bioactivity well. Finally, we have successfully fabricated the electrochemical biosensor based on such PVP-protected graphene/polyethylenimine-functionalized ionic liquid nanocomposites, which is a potential possibility for the further fabrication of biosensors.

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SUPPORTING INFORMATION AVAILABLE

FT-IR spectra of PVP, PVP-graphene, graphene and graphene oxides, energy dispersive X-ray (EDX) spectra of graphene oxides and graphene, and photograph of PVP-protected graphene and pure graphene. This material is available free of charge via the Internet at http://pubs.acs.org.

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