Direct electrochemistry of glucose oxidase assembled on graphene and application to glucose detection

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ABSTRACT

The direct electrochemistry of glucose oxidase (GOx) integrated with graphene was investigated. The voltammetric results indicated that GOx assembled on graphene retained its native structure and bioactivity, exhibited a surface-confined process, and underwent effective direct electron transfer (DET) reaction with an apparent rate constant (k_s) of 2.68 s^{-1}. This work also developed a novel approach for glucose detection based on the electrocatalytic reduction of oxygen at the GOx–graphene/GC electrode. The assembled GOx could electrocatalyze the reduction of dissolved oxygen. Upon the addition of glucose, the reduction current decreased, which could be used for glucose detection with a high sensitivity (ca. $110 \pm 3\,\mu A\,mM^{-1}\,cm^{-2}$), a wide linear range (0.1–10 mM), and a low detection limit ($10 \pm 2\,\mu M$). The developed approach can efficiently exclude the interference of commonly coexisting electroactive species due to the use of a low detection potential (-470 mV, versus SCE). Therefore, this study has not only successfully achieved DET reaction of GOx assembled on graphene, but also established a novel approach for glucose detection and provided a general route for fabricating graphene-based biosensing platform via assembling enzymes/proteins on graphene surface.

1. Introduction

Carbon materials have been widely used in both analytical and industrial electrochemistry because of their chemical inertness, their low residual current, their excellent conductivity, their wide potential window, and their electrocatalytic activity to a variety of redox reactions. Several forms of carbon materials such as graphite [1,2], highly ordered mesoporous carbon [3], carbon nanofiber [4], carbon nanotube [5–8], etc. have been studied to immobilize redox enzymes and used for developing enzyme-based electrochemical devices. Recently, a new form of large surface-to-volume ratio carbon material, graphene, which is a flat monolayer of carbon atoms tightly packed into a two-dimensional (2D) honeycomb lattice [9], has received a considerable attention. One of the factors makes graphene so attractive is its low energy dynamics of electrons with atomic thickness [10]. It is a semiconductor with zero band gap and high carrier mobility and concentration and shows nearly ballistic transport at room temperature [11,12]. These unusually electronic properties make graphene one of the most promising candidate materials for future nanoelectronic applications, including graphene-based field effect transistors [13], gas sensors [14], nanoelectromechanical switch [15], supercapacitors [16], lithium secondary batteries [17], and so forth. Of particular interest for us is to explore its application in the field of electrochemical research [18,19]. To fully exploit the electrochemical properties of graphene, it is important to understand the electrochemical characteristics of the graphene surface, including adsorption, the electron transfer (ET) kinetics of the redox system, and electrocatalysis. Several groups have demonstrated that graphene sheets show fast ET kinetics and excellent electrocatalytic characteristics compared with graphite and glassy carbon (GC) [18,20–23]. More recently, it has been found that graphene oxide can facilitate direct electron transfer (DET) of metalloproteins including cytochrome *c*, myoglobin, and horseradish peroxidase [24], and an ionic liquid, Nafion or chitosan modified graphene can support the DET of glucose oxidase (GOx) [25–27]. However, the reported procedures of immobilizing GOx were relatively complicated.

The unique properties of graphene may provide insight to fabricate novel biosensors for virtual applications. The high surface area is helpful in increasing the surface loading of the target enzyme molecules on the surface. The excellent conductivity and small band gap are favorable for conducting electrons from the biomolecule. Here, we describe the fabrication and characterization of a novel hybrid (GOx–graphene) in which glucose oxidase (GOx) is directly assembled onto the unmodified graphene surface. GOx is chosen as a model for its stability, its ability to be immobilized on the electrode surface, and its electrical communication with the electrode surface [28,29]. The results demonstrated that GOx immobilized on

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the surface of graphene could undergo the effective DET reaction and the hybrid exhibited good electrocatalytic activity toward the reduction of oxygen. Moreover, the concentration of glucose can be determined quantitatively based on the reduction of O_2 catalyzed by the GOx–graphene hybrid. In comparison with the previous report [27], although DET characteristics of GOx obtained in presented work are practically the same as that obtained by Kang et al. [27] at the GOx–graphene–chitosan/GC electrode, this work simplifies the electrode fabrication procedures significantly by directly dispersing graphene into water. However, Kang et al. dispersed graphene in chitosan solution, therefore complicating the electrode fabrication procedures. More importantly, the analytical performances of the GOx–graphene/GC electrode are much better that those of GOx–graphene–chitosan/GC electrode regarding the sensitivity ($110 \pm 3\,\mu A\,mM^{-1}\,cm^{-2}$ versus $37.93\,\mu A\,mM^{-1}\,cm^{-2}$) and the detection limit ($10 \pm 2\,\mu M$ versus $20\,\mu M$). Therefore, this study expands the scope of graphene applications to the field of bioelectroanalytical chemistry, which may open up a new challenge and approach to explore the electrochemical features of graphene or its hybrid materials for the potential utilizations. Our goal is not only to design a novel biosensing platform but also to present a new approach for preparation of a graphene-based hybrid, which has potential utility to bioelectroanalytical chemistry.

2. Experimental

2.1. Chemicals

GOx (EC 1.1.3.4, from *Aspergillus niger*, ∼200 U/mg, Sigma), β-D-glucose (Sigma), flavin adenine dinucleotide (disodium salt, FAD, 96%, Sigma), ascorbic acid (AA), uric acid (UA), 4-acetamidophenol (AP), hexaamineruthenium (III) chloride ($Ru(NH_3)_6Cl_3$, 99%, Strem Chemicals), and graphite powder (99.99995%, 325 mesh, Alfa Aesar) were used as received. All other chemicals were of analytical grade. Phosphate buffer solution (PBS, 0.1 M, pH 6.9) was made up from Na_2HPO_4 and NaH_2PO_4. Graphene was synthesized according to a published route involving the steps of graphite oxidation, exfoliation, and chemical reduction [30,31]. In brief, graphite oxide (GO) was prepared by a modified Hummers method, starting from graphite powder. Graphite was oxidized by concentrated H_2SO_4, $K_2S_2O_8$, and P_2O_5 to produce preoxidized graphite, which was then subjected to reoxidization by concentrated H_2SO_4 and $KMnO_4$. Exfoliation was carried out by sonicating GO dispersion (0.1 mg/mL) under ambient temperature for ca. 20 min. The resulting homogeneous yellow-brown dispersion was reduced by hydrazine. The reduction reaction was carried out by adding hydrazine (1.2 mL) into the dispersion of GO (60 mg of GO in 50 mL of water). After being sonicated for 1 h and kept stirring for 24 h at 50 °C, graphene sheets were obtained by filtering the product and drying in vacuum.

2.2. Fabrication of graphene/GC electrode and assembly of GOx

Glassy carbon electrode (GC, 3 mm in diameter, CH Instruments) was sequentially polished with metallographic abrasive paper (No .6) and slurries of 0.3 and 0.05 μm alumina to mirror finish. After rinsing with double-distilled water, it was sonicated with absolute ethanol and double-distilled water for about 1 min, respectively, for removing the trace alumina from the electrode surface.

Several experimental parameters were optimized to obtain the best voltammetric response. Typically, 2 mg of graphene was dispersed in 1 mL of water with the aid of ultrasonication forming homogeneous dark suspension (2 mg/mL). However, the homogeneous suspension cannot stand for a long time, the graphene will be precipitated within ca. 2 h. Therefore, the suspension need to be
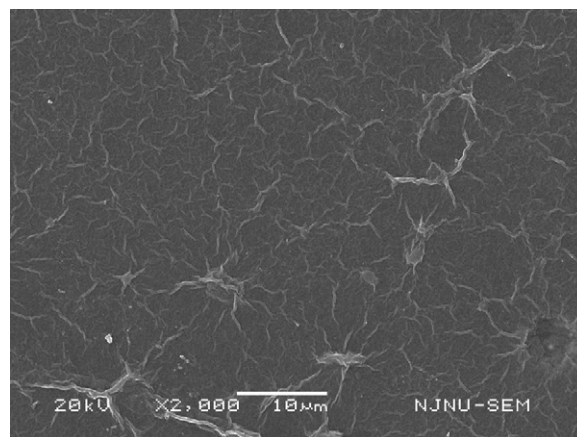

Fig. 1. Typical SEM image of graphene on electrode surface.

ultrasonicated again before next use. The graphene/GC electrode was fabricated by casting 5 μL of graphene suspension (2 mg/mL) onto the surface of the pretreated GC electrode with a microsyringe. The solvent was allowed to be evaporated at ambient temperature before use. Graphene can easily form a thin film on the GC electrode surface through π–π electronic interactions between graphene and GC. Fig. 1 is a SEM image of graphene on the GC electrode surface, revealing the typical crumpled and wrinkled graphene sheet structure.

GOx was assembled on graphene via physical adsorption by incubating the graphene/GC electrode in the enzyme solution (5 mg/mL in PBS) at 4 °C for 1 h. Afterward, the electrode (denoted as GOx–graphene/GC electrode) was thoroughly rinsed with water and stored at 4 °C when not in use. Using the similar procedures, the GOx/GC and FAD–graphene/GC electrodes were fabricated, and their electrocatalytic characteristics toward the reduction of O_2 were compared with that of the GOx–graphene/GC electrode. The concentration of FAD in PBS used for fabricating the FAD–graphene/GC electrode is ca. 5 μM.

2.3. Instruments and procedures

Atomic force microscopic (AFM) images were recorded with a Nanoscope IIIa scanning probe microscope (Digital Instruments, USA) using a tapping mode. The sample used for measurements was prepared by casting the suspension of graphene (0.1 mg/mL) or GOx–graphene (0.1 mg/mL) on the surface of a mica sheet. The solvent was allowed to be evaporated before measurements. Scanning electron microscopic (SEM) images were obtained using a LEO 1530 VP field-emitting scanning electron microscope (Germany). The sample of graphene used for recording SEM image was prepared by casting 5 μL of graphene suspension (at a level of 2 mg/mL in water) onto the surface of the GC plate (1 cm × 1 cm in size, 2 mm in thickness), which was also pretreated as GC electrode. Before SEM measurements, the solvent was allowed to be evaporated. Circular dichroic (CD) measurements were made on a JASCO Model J-810 dichrograph (Japan Spectroscopic Co. Ltd., Japan) in a 1 cm quartz cuvette. The final spectra were the mean of ten accumulated scans at a bandwidth of 2 nm, and were corrected for the unspecific dichroic absorbance of the medium by computer manipulation. The data were expressed in term of the molar ellipticity, $[\theta]$, in° $M^{-1}\,m^{-1}$. The FT-IR spectra were recorded on a Nexus 670 FT-IR spectrophotometer (Nicolet Instruments) using a KBr disk at a resolution of 4 cm^{-1}. To get the IR spectrum of GOx–graphene hybrid, graphene (2 mg) was dispersed into PBS (1 mL) with aid of ultrasonication forming 2 mg/mL graphene dispersion. Then, GOx (10 mg) was incorporated into the dispersion by stirring the mixture at 4 °C