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Water-dispersible triethylenetetramine-functionalized graphene: Preparation, characterization and application as an amperometric glucose sensor

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ABSTRACT

The triethylenetetramine-functionalized graphene (TFGn) was prepared using graphene oxide (GO) and triethylenetetramine as raw materials through a one-step reaction under alkaline condition. The triethylenetetramine not only acted as cross-linker to combine GO, but also as reductant of GO. The TFGn was characterized by its ultraviolet spectrum (UV), Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), X-ray diffraction (XRD), Raman spectroscopy and Scanning electron microscopy (SEM). The results showed that triethylenetetramine was successfully grafted onto the surface of the GO through covalent bonding between amine and epoxy groups. The resultant TFGn was uniformly dispersed in water over several weeks, suggesting that the introduction of amino groups greatly increased the hydrophilicity of TFGn. The triethylenetetramine-functionalized graphene was then applied to fabricate glucose biosensors with IO_4^- oxidized glucose oxidase (GOx) through layer-by-layer (LBL) self-assembly by the covalent bonding between the aldehyde groups of GOx and amino groups of TFGn. The gold electrodes modified with the $(GOx/TFGn)_n$ multilayer films were studied by cyclic voltammetry (CV) and showed outstanding electrocatalytical response to the oxidation of glucose when ferrocenemethanol was used as an artificial redox mediator. The response increased with an increasing number of GOx/TFGn bilayers, indicating that the analytical performance, such as the sensitivity of the glucose biosensor, could be adjusted by tuning the number of deposited GOx/TFGn bilayers. The linear response range of the biosensor constructed with six bilayers of GOx/TFGn to the concentration of glucose can extend to at least 8 mM with a sensitivity of 19.9 μA mM^{-1} cm^{-2}. In addition, the sensor exhibited good stability due to the covalent interactions between the GOx and TFGn.

1. Introduction

Recently, a new two dimensional nanomaterial, denoted as graphene, has attracted considerable attention, and its applications are being widely explored. Graphene exhibits excellent electrical conductivity, strong mechanical strength, high thermal conductivity and a high surface area [1–6]. The planar morphology and larger surface area together with its excellent electrical conductivity make graphene an ideal material for electrochemical sensors [7–9], and graphene may perform better than any other carbon-based electrodes as a sensor material [10]. Recently it was shown that the graphene-based electrodes can provide excellent capability in ultra sensitive electrochemical detection of single nucleotide polymorphisms of DNA [11] and early detection of leukemia using DPV [12–13].

The graphene prepared by chemical reduction has a strong tendency to stack and easily form irreversible agglomerates through van der Waals interactions. As a result, graphene is poorly dispersible in most solvents, leading to the limited applicability of chemically reduced graphene. For biomedical applications, it is extremely important that prepared graphene is water-dispersible. This property is achievable by the appropriate surface functionalization of graphene through covalent and noncovalent methods [14–17]. The covalently-functionalized graphene is more stable compared with the noncovalently-modified graphene, which is functionalized through π-π interactions and van der Waals interactions.

Glucose oxidase (GOx) has been widely used in the construction of glucose sensors by virtue of its high selectivity for glucose and high activity over a broad range of pH values. Among various enzyme immobilization methods for the construction of glucose biosensors, the layer-by-layer technique through covalent and noncovalent approaches has intrinsic advantages for the assembly of ultrathin films due to the simplicity of the procedure and thickness controllability in the nanometer range [18]. The reports about graphene-based LBL film glucose sensors are very few in the literature [19–20]. Liu's group reported that the pyrene-functionalized GOx was utilized to fabricate enzyme electrodes

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by alternating layer-by-layer self-assembly of graphene and pyrene-functionalized GOx via noncovalent π-π stacking interactions. Gu and co-workers prepared a composite material $\{IL\text{-}RGO/S\text{-}RGO\}_n$ through the layer-by-layer method using amine-terminated ionic liquid (IL-NH_2) and sulfonic acid (SO^{3-})-functionalized graphene along with immobilized glucose oxidase on $\{IL\text{-}RGO/S\text{-}RGO\}_n$ films to fabricate a glucose sensor through electrostatic interaction. The driving force of the assembly processes of glucose oxidase described above is noncovalent interactions, which are not stable or strong enough, most certainly leading to their poor limited application. Meanwhile, in the work of Gu's group a monolayer of glucose oxidase was immobilized on a composite material, which affected the sensitivity of the sensor. To overcome these drawbacks, direct LBL enzymic covalent attachment for the fabrication of ordered multilayer films is believed to be more advantageous since they are robust enough to withstand elevated temperatures, attack by a polar solvent, mechanical abrasion, etc.

In this work, we demonstrate an effective method for preparing water-soluble triethylenetetramine-functionalized graphene, which was prepared using GO and triethylenetetramine as raw materials through a one-step reaction. The advantage of this preparation process is not using the EDC/NHS [21] as activator, and the hydrazine hydrate [16,22] as reductant. This avoids the aggregation of the triethylenetetramine-functionalized graphene under the complicated steps. The triethylenetetramine not only acted as cross-linker to combine GO, but also as reductant of GO. We attempt to apply the TFGn with many amino groups to construct glucose biosensors via the layer-by-layer covalent self-assembly method without any cross-linker. The amino groups of TFGn can react with the aldehyde groups of GOx through the formation of a Schiff base. Multilayer film electrodes were fabricated by alternating the deposition between TFGn and GOx until the desired number of bilayers was achieved. The electrochemical behaviors of the $Au/CA/(GOx/TFGn)_n$ electrode and its electrocatalysis activity for glucose were investigated. The resulting electrode exhibited outstanding bioelectrocatalytic response to the oxidation of glucose and very effective electrochemical communication between the successive layers. Furthermore, the covalent attachment technology overcame the stability drawback of multilayer films based on the layer-by-layer electrostatic adsorption technique. The TFGn involved cannot only immobilize GOx without the loss of biological activity but also enhance the measurement signal because of its fast electron-transfer and large working surface area.

2. Experimental

2.1. Reagents

Glucose oxidase (GOx, EC 1.1.3.4, from *Aspergillus niger*, 100 U mg^{-1}) was purchased from Amresco. Natural graphite flakes and triethylenetetramine came from the Sinopharm Chemical Reagent Co., Ltd. The cystamine dihydrochloride (CA) and ferrocene methanol were obtained from Aldrich. The other chemicals were of analytical grade. All of the solutions were prepared using doubly distilled water. Phosphate buffer solutions (PBS, 0.1 M, pH = 6.82) were employed as a supporting electrolyte. The glucose stock solutions were prepared with 0.1 M PBS and were allowed to sit overnight before use.

2.2. Apparatus

UV–vis absorption spectra were recorded on an Agilent Cary 300 spectrophotometer (Agilent Cary, Australia). The spectra were plotted in the wavelength range from 200 nm to 400 nm. Fourier transform infrared spectroscopy characterization of GO and TFGn was performed using a Perkin Elmer 2000 spectrophotometer (Perkin Elmer Inc., USA). The spectra were recorded using KBr pellets in the IR range of 4000–400 cm^{-1}. The surface composition and functional groups present in the GO and TFGn were determined using an X-ray photoelectron spectroscopy (XPS) system (Thermo VG ESCALAB250, USA). The scanning electron microscope (SEM) used in this work was a Hitachi TM-3000 SEM (Hitachi Limited, Tokyo, Japan). A X-ray diffraction (XRD) system (Rigaku Co., Tokyo, Japan) was used for the X-ray analysis with Cu Kα radiation (λ = 1.54051 Å). Step scanning was used with 2θ intervals from 6° to 60° and a residence time of 1 s. The Raman spectra of the GO and TFGn were carried out using the DXR smart Raman spectrometer (Thermo Fisher, USA) under the 532 nm laser excitation.

The cyclic voltammetric and amperometric measurements were performed on a CHI 760E electrochemical workstation (Shanghai Chenhua, China). Experiments were performed in a standard three-electrode cell (10 mL) with a modified Au as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a platinum wire as the auxiliary electrode. The amperometric experiments of the $Au/CA/(GOx/TFGn)_n$ electrode with the sequential addition of a desired amount of glucose were carried out in a gently stirred air-saturated 0.1 M PBS buffer solution. The electrochemical impedance spectroscopy (EIS) measurements were performed in the presence of 5.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) mixture as a redox probe in the frequency range between 0.1 and 100,000 Hz. All experiments were performed at room temperature.

2.3. Preparation of GO and TFGn

The GO was prepared by a modified Hummers method using expanded graphite as the precursor [23]. The GO (20 mg) was dispersed in doubly distilled water (20 mL) and sonicated for 1 h to homogeneously disperse the GO sheets in solution. Two milliliters of 1% NaOH and 20 μL of triethylenetetramine were sequentially added dropwise to the above-mentioned solution, and the reaction mixture was refluxed and stirred at 60 °C for 24 h. Then, the mixture was centrifuged and washed with doubly distilled water three times. The preparation process is shown in Scheme 1A.

2.4. Synthesis of aldehyde glucose oxidase

The aldehyde glucose oxidase was prepared by the methodology already demonstrated by Zaborsky [24] with slight modifications. A 74.4 mg sample of GOx was dissolved in 10 mL of phosphate buffer solution (0.1 M, pH = 6.8) and was reacted with 120 mg of sodium metaperiodate at 0 °C in the dark for 1 h. Then, 31 μL of ethylene glycol was added to the above solution, and the reaction was continued at room temperature for 30 min. The product was purified and concentrated with ultrafiltration (molecular weight cutoff of 30,000).

2.5. Fabrication of the $Au/CA/(GOx/TFGn)_n$ electrode

The gold electrode was polished to a mirror-like surface with 1.0, 0.3, and 0.05 μm alumina powder sequentially and then washed with water and ethanol for a few minutes in an ultrasonic bath, respectively. The electrode was dried with nitrogen steam for the next decoration. The clean electrode was immersed into an aqueous cystamine solution (20 mg/mL) in darkness for 2 h. After thiol adsorption, multilayer films of GOx/TFGn were fabricated by alternatively dipping the modified electrode into the GOx solution and the above TFGn solution each for 1 h. After each dipping step, the electrode was carefully washed with doubly distilled water and then dried with nitrogen. This process was repeated until the desired GOx/TFGn bilayer number was achieved. The modified electrode was denoted as $Au/CA/(GOx/TFGn)_n$. The assembly process is shown in Scheme 1B. In comparative experiments, the multilayer film was fabricated following the same process using a GO solution instead of a TFGn solution. The resulting electrode was abbreviated to $Au/CA/(GOx/GO)_n$. The $Au/CA/(GOx/GO)_n$ electrode was stored at 4 °C when not in use.