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Reusable sensor based on high magnetization carboxyl-modified graphene oxide with intrinsic hydrogen peroxide catalytic activity for hydrogen peroxide and glucose detection

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ABSTRACT

We propose a new strategy to improve the enzyme stability, construction and sensitivity of a multifunctional sensor. An exfoliated graphene oxide sheet with carboxyl-long-chains (GO-CLC) was prepared in one step from primitive graphite via Friedel–Crafts acylation. Magnetic nanoparticles, glucose oxidase (GOD) and poly[aniline-co-N-(1-one-butyric acid) aniline] (SPAnH) were then incorporated to form an electrochemical film (SPAnH-HMGO-CLC-GOD) for the detection of hydrogen peroxide (H_2O_2) and glucose. The GO and Fe_3O_4 have intrinsic hydrogen peroxide catalytic activity and the activity will be enhanced by the combination of SPAnH coating and induces an amplification of electrochemical reduction current. This response can be used as a glucose sensor by tracing the released H_2O_2 after enzymatic reaction of bound GOD. Our sensor was linear within the range from 0.01 mM to 1 mM H_2O_2 and 0.1 mM to 1.4 mM glucose, with high sensitivities of 4340.6 $\mu A\,mM^{-1}\,cm^{-2}$ and 1074.6 $\mu A\,mM^{-1}\,cm^{-2}$, respectively. The relative standard deviations (RSD) were 5.4% for H_2O_2 detection and 5.8% for glucose detection. The true detecting range was 0.4–40 mM for H_2O_2 and 4–56 mM for glucose, which multiplied by 40-fold of dilution. This sensor based on the catalysis of organic SPAnH and the enzymatic activity of GOD can be used for both H_2O_2 and glucose sensing in potential clinical, environmental and industrial applications.

1. Introduction

Graphene oxide (GO), a one-atom thick planar carbon nanosheet, has generated intense interest among scientists and engineers due to its unprecedented physical and chemical properties (i.e. high surface area, high intrinsic mobility, high thermal conductivity, mechanical stiffness, excellent electrical conductivity, and good gas barrier properties) (Liu et al., 2009; Rao et al., 2009; Geim, Novoselov, 2007; Ni et al., 2007; Sykes and Charles, 2009; Li et al., 2008a; Stankovich et al., 2006a; Dikin et al., 2007; Li et al., 2008b), and significant progress has been made towards its use in nanoelectronics (Li et al., 2008a), nanocomposites (Liang et al., 2009), biosensors (Ping et al., 2011; Liu et al., 2011), and drug delivery (Liu et al., 2008; Sun et al., 2008). The undesirable tendency of chemically reduced GO to aggregate or re-stack irreversibly to form graphite (Shen et al., 2009) can be avoided by surface functionalization with epoxides, –OH, and –COOH groups to render it hydrophilic and well-dispersed in water (Szabo et al., 2005; Robinson et al., 2008), and to enhance its biological applications (Chang et al., 2008; Liu et al., 2008). Graphene oxide is an especially promising material for sensor applications because of its superior electron conductivity and single-atom thickness. Indeed, GO-modified electrodes show excellent electrocatalytic activity for H_2O_2, O_2, nicotinamide adenine dinucleotide (NADH), and other important electroactive species, and greatly improve performance in enzyme-based sensing (Li et al., 2009; Fang et al., 2010; Wang et al., 2009; Liu et al., 2010).

Serious illnesses can result from chemical imbalances in the human body, for example excess H_2O_2 could damage DNA or proteins and excessive levels of glucose in the blood may cause diabetes. Accurate determination of H_2O_2 and glucose is of practical importance for diverse applications, including clinical settings, and the fabrication of H_2O_2 and glucose sensors has

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therefore become increasingly important. Enzymes immobilized on nanomaterials retain their activity and show increased direct electron transfer between their active sites and the electrode, thus enabling selective and fast responses to specific substrates (Yu et al., 2003; Hua et al., 2012). Glucose oxidase (GOD) is commonly used as a glucose sensor by electrochemical detection of H_2O_2 that is produced along with gluconic acid through catalysis of electron transfer from glucose to oxygen. However, low stability and recovery and high operation cost have prevented the widespread practical application of GOD. Enzyme stability can be significantly enhanced by covalent immobilization, but activity is decreased due to the increased mass transfer limitation of binding the enzyme to the support, and it remains difficult to recover immobilized enzyme from the reaction mixture. To avoid the effects of enzyme inactivity, a number of sensors have been fabricated based on enzyme-free working electrodes such as using carboxylated polyaniline, Polybenzimidazole derivatives and Fe_3O_4 as catalytic material (Hua et al., 2011a; Hua et al., 2011b; Zhang et al., 2008). Besides, a potential solution has been the use of magnetic nanoparticles as enzyme carriers, also providing rapid separation in a magnetic field, lower cost and significant alleviation of the transfer barrier (Liao and Chen, 2001; Rossi et al., 2004; Horst et al., 2006; Zeng et al., 2006).

Here we develop high-magnetization carboxyl-long-chain (CLC)-modified GO (HMGO-CLC) with sufficient room to conjugate GOD (HMGO-CLC-GOD). Intrinsic H_2O_2 catalytic activity for glucose detection was achieved by coating the surface with poly[aniline-co-N-(1-one-butyric acid) aniline] (SPAnH) (Yang et al., 2012) to produce SPAnH-HMGO-CLC-GOD (Scheme 1A), which can detect H_2O_2 and glucose simultaneously. This system rapidly constructed a sensor in a magnetic field, stabilized GOD, and quickly separated it from the reaction mixture. In addition, SPAnH-HMGO-CLC-GOD attached firmly to the sensing area of a Au electrode in a magnetic field during glucose detection (Fig. 1A).

2. Experimental

2.1. Materials

Maleic anhydride (MA) was purchased from MP BiomedicalsTM. Aluminum chloride, 1-methyl-2-pyrrolidone (NMP) was purchased from ACROS. Aniline monomer, ammonium peroxydisulfate (APS, 98%), NaOH, $FeCl_2$, $FeCl_3$, and HCl (37 wt%) were purchased from Merck. Acetic acid was purchased from Scharlau. NMP and aniline monomer were distilled under reduced pressure prior to use. DI water was used throughout. FBS was purchased from Biological. PBS, GO (17,300 IU/g of lyophilized solid), 1-ethyl-3-(3-dimethylaminepropyl) carbodiimide hydrochloride (EDC · HCl), 2-(N-morpholino)ethanesulfonic acid hydrate (MES), ascorbic acid (AA), uric acid (UA) and immunoglobulin G (IgG), and N-hydroxysulfosuccinimide sodium salt (sulfo-NHS) were purchased from Sigma-Aldrich. Graphite was purchased from Alfa Aesar. The glucose assay kit (GLU-OD) was purchased from Medi Pro. The H_2O_2 assay kit was obtained from BioVision.

2.2. Preparation of HMGO-CSC and HMGO-CLC

The exfoliated graphene oxide sheet with carboxyl-long-chain (GO-CLC) was prepared from purified natural graphite using a chemical ring-opening reaction. Briefly, maleic anhydride (MA) (0.98 g) was refluxed with $AlCl_3$ (4.01 g) in dried NMP at approximately 90 °C under a dry nitrogen atmosphere for 3 h. Crude graphite (50 mg) was dispersed by sonication in dried NMP. The suspension was added slowly and dropwise to the mixture solution and then stirred at 160 °C for 72 h. After the reaction, the mixture was decomposed with DI water followed by 1 M HCl aqueous solution, and the solid component was purified by filtration through a 0.1 μm PVDF filter to remove the organic solvent. The filtered solid was resuspended in DI water and separated by centrifugation (10 min, 3000 rpm). The separate was re-purified by 0.1 μm PVDF filtration. Finally, the filtered solid was dried under vacuum for 48 h at 40 °C to obtain GO-CLC.

HMGO-CLC composites were synthesized by coprecipitation of $FeCl_3$ and $FeCl_2 \cdot 4H_2O$ in the presence of GO-CLC. Briefly, 200 mg of GO-CLC in 20 mL of DI water was ultrasonicated for 30 min. $FeCl_3$ (4.32 mmol) and $FeCl_2 \cdot 4H_2O$ (6.48 mmol) were dissolved in 380 mL DI water at room temperature, added to the GO-CLC and stirred for 5 min under N_2 gas. The solution was heated slowly to 50 °C and 30 mL of 0.576 N NaOH was added over a 70-min period, after which the temperature was increased to 80 °C for 20 min. The solution was then rapidly quenched in ice and 0.1 N HCl was added slowly until the pH was neutral. HMGO-CLC was separated from the solution by attraction to the wall of a separation funnel using a strong magnet, washed several times with DI water to remove unreacted material, and uniformly dispersed in DI water by sonication at 300 W for 1 h. Graphene oxide sheet with carboxyl-short-chain (GO-CSC) was synthesized from expandable graphitic flake using a method modified from Hummer (Liu et al., 2008). HMGO-CSC was prepared as mentioned above.

2.3. Enzyme immobilization process

Twenty-four milligrams of EDC and 27 mg of sulfo-NHS were dissolved in 2 mL of 0.5 M MES buffer (pH = 6.3) in the dark. A 0.2 mL aliquot of this solution was mixed with 0.2 mL of HMGO-CLC (10 mg/mL) at 25 °C and sonicated for 30 min in the dark to allow the formation of amide bonds between activated carboxyl groups. Activated HMGO-CLC was separated, washed with 0.8 mL 0.1 M MES buffer, resuspended in 0.2 mL of MES buffer, and then mixed with 0.1 mL of GOD at 25 °C by vortexing for 1 h followed by sonication for another 1 h. The primary amino groups of GOD interacted with the active ester, resulting in covalent conjugation of GOD on the surface of HMGO-CLC. The HMGO-CLC-GOD was then separated from the solution, washed with DI water to remove the MES buffer and unbound GOD, and dispersed in 0.2 mL DI water. The binding capacity was analyzed by optical density assay using a spectrometer (Lambda 800/900, Perkin Elmer) at 277 nm.

2.4. Kinetics of free and immobilized GOD

The kinetic parameters of free and immobilized GOD were studied by the initial rate method with the Michaelis constant (Ye et al., 2005). Different concentrations of GOD (0.1–1.0 mM) were reacted with O_2-saturated PBS that contained 10 mM glucose then using the H_2O_2 kit reagent at 25 °C to calculate the K_m and V_{max} values; V_{max} is the highest possible reaction velocity when GOD is saturated with substrate, K_m is the Michaelis constant, and k_{cat} is the catalytic activity, which is the substrate concentration that yields half the true maximum reaction velocity and represents the effective affinity between GOD and the substrate.

2.5. Fabrication of SPAnH-HMGO-CLC-GOD/Au electrode

Five milliliters of HMGO-CLC-GOD (10 mg mL^{-1}) were mixed with 1.5 mL of SPAnNa (5.5 mg mL^{-1}) and slowly doped by addition of 0.2 M HCl to induce formation and aggregation of SPAnH. SPAnH was coated on the surface of HMGO-CLC-GOD, then separated from the solution using a strong magnet, washed