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Direct electrochemistry of hemoglobin on graphene and titanium dioxide nanorods composite modified electrode and its electrocatalysis

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ABSTRACT

A biocompatible sensing platform based on graphene (GR) and titanium dioxide (TiO_2) nanorods for the immobilization of hemoglobin (Hb) was adopted in this paper. The GR–TiO_2–Hb composite-modified carbon ionic liquid electrode was constructed through a simple casting method with Nafion as the film forming material. UV–Vis and FT–IR spectra confirmed that Hb retained its native structure in the composite film. Direct electron transfer of Hb incorporated into the composite was realized with a pair of quasi-reversible redox waves appeared, indicating that the presence of GR–TiO_2 nanocomposite on the electrode surface could facilitate the electron transfer rate between the electroactive center of Hb and the substrate electrode. Hb modified electrode showed excellent electrocatalytic activity to the reduction of trichloroacetic acid in the concentration range from 0.6 to 21.0 mmol L^{-1}. These results indicated that GR–TiO_2 nanocomposite could be a friendly biocompatible interface for immobilizing biomolecules and keeping their native structure. The fabricated biosensor displayed the advantages such as high sensitivity, good reproducibility and long-term stability.

1. Introduction

Researches on direct electrochemistry of redox proteins have aroused increasing interests, which not only help to elucidate the intrinsic thermodynamic and kinetic properties of proteins, but also apply to fabricate bioelectronic devices (Armstrong et al., 1988; Leger et al., 2003). Also direct electron transfer between redox proteins and electrode can establish a model for the mechanism study on the electron exchange among enzymes in biological systems, and provide basis for fabricating electrochemical biosensors and catalytic bioreactors without mediators (Armstrong and Wilson, 2000). In general, direct electrochemistry of redox proteins on the conventional electrodes is difficult to be realized due to the deeply buried redox active center in the proteins or the unfavorable orientations of protein on the electrode surface. Therefore, great efforts have been developed to construct a matrix to enhance the electron transfer rate between the electrode and redox protein.

Recently graphene (GR) has become the hot research topics in different fields because of many intriguing attributes it displays (Geim and Novoselov, 2007; Guo and Dong, 2011). GR is a single layer of carbon atoms with a hexagonal arrangement in a two-dimensional lattice, which can be regarded as the basic building block for the related graphitic materials. GR exhibits high surface area and excellent electrical conductivity, and the increase of effective surface area helps to introduce a large number of active sites (Zhu et al., 2010). Other unique properties of GR include fast electron transportation, high thermal conductivity, excellent mechanical stiffness and good biocompatibility (Chen et al., 2008, 2010), which result in many applications including supercapacitors, batteries and electromechanical resonators. Electrochemistry and electroanalysis of GR had been reviewed by different research groups with its potentials applications described (Shao et al., 2010; Gan and Hu, 2011). Wang et al. (2009b) adopted the GR-modified electrode to selectively detect dopamine in a large excess of ascorbic acid. Li et al. (2009) fabricated a GR and Nafion based electrochemical platform for ultrasensitive detecting of cadmium ion. Wu et al. (2010) utilized chitosan-dispersed GR to immobilize cytochrome C for the electrocatalytic determination of nitric oxide. Xu et al. (2010) utilized chitosan-GR composite film for encapsulation of Hb to detect hydrogen peroxide. Deng et al. (2013) applied an electrochemically reduced graphene oxide modified glassy carbon electrode (GCE) for the detection of L-tryptophan and L-tyrosine.

As a semiconductive material, TiO_2 is widely used in cosmetics, solar cells, batteries, additives in toothpaste and white paint. Recently, there are considerable interests in using TiO_2 nanoparticles due to the properties such as high surface area, optical transparency

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and good biocompatibility. Various TiO_2 nanoparticles have been used to immobilize proteins or enzymes on electrode surface for either mechanistic study or fabricating electrochemical biosensors. Topoglidis et al. (2000, 2001) immobilized different proteins into nanoporous TiO_2 film electrodes as the electrochemical or optical biosensors. Li et al. (2001) applied nanocrystalline TiO_2 films on electrodes to entrap heme proteins and observed their direct electrochemistry. Bao et al. (2008) fabricated uniformly porous nanostructured TiO_2 materials for direct electrochemistry of glucose oxidase and glucose sensing. Sun et al. (2009b) investigated the direct electrochemistry of hemoglobin (Hb) on a chitosan and TiO_2 nanoparticle composite modified electrode and its electrocatalysis. Zhang et al. (2004) fabricated a hydrogen peroxide biosensor with horseradish peroxidase and TiO_2 nanoparticle modified electrode. So TiO_2 nanomaterials have been widely used in the fields of electroanalysis and electrochemical biosensors.

Nafion is a proton-conductive and biocompatible perfluorosulfonate linear polymer, which exhibits excellent film-forming ability and has been widely used for the immobilization of enzymes (Lu et al., 2007; Nadzhafova et al., 2004). The presence of Nafion can form a stable film to enhance the stability of protein immobilized on the electrode surface and prevent the leakage of protein from electrode into the solution. Carbon ionic liquid electrode (CILE), which is prepared by using ionic liquid (IL) as the binder and the modifier in carbon paste electrode, has been widely used in recent years. CILE has been proven to have the advantages such as easy preparation, high electrochemical stability, good ionic conductivity and wide electrochemical windows (Opallo and Lesniewski, 2011). Shangguan et al. (2008) applied CILE in the electrochemical detection of paracetamol. Zhang and Zheng (2007) investigated the electrochemical behavior of hydroquinone on CILE. Our group also applied CILE to the investigation on electrochemistry of protein and electroactive molecules (Sun et al., 2008, 2009a).

In this paper, a GR–TiO_2 nanocomposite was prepared and used for the investigation of the electrochemistry of Hb. GR based composites have aroused great attentions due to the synergistic properties originated from the individual components and their interaction (Singh et al., 2011). Zou et al. (2011) synthesized a GR–TiO_2 nanocomposite by refluxing mixing solutions of graphene oxide with peroxotitanium complexes and investigated their photoelectrical properties. Wang et al. (2009a) prepared a self-assembled TiO_2–GR hybrid nanostructure with enhanced Li-ion insertion/extraction properties. Zhang et al. (2010) applied GR–TiO_2 nanocomposite as the photocatalysis for the photodegradation of methylene blue with significant enhanced reaction rate. Sun et al. (2011) combined chitosan–IL–TiO_2–GR nanocomposite film-modified electrode for the investigation on electrochemistry of Hb. Jang et al. (2012) fabricated a glucose biosensor based on TiO_2–GR composite-modified GCE. The results indicated that GR–TiO_2 nanocomposite could exhibit specific photophysical and electrochemical properties, which had the potential applications for the electrochemical sensing. In this work, GR–TiO_2 nanocomposite was cast on the surface of CILE and further used for the immobilization of Hb. Direct electron transfer between Hb and CILE was further investigated by cyclic voltammetry. The composite film could retain the bioactivity of Hb and provide a favorable microenvironment for Hb to exchange the electrons. The Hb modified electrode exhibited good electrocatalysis to trichloroacetic acid. Therefore GR–TiO_2 composite film could be used as the biomaterial suitable for protein immobilization and biosensors preparation.

2. Experimental

2.1. Reagents

Bovine hemoglobin (Hb, MW. 64500, Sinopharm Chemical Reagent Ltd. Co., China), 1-butylpyridinium hexafluorophosphate ($BPPF_6$, Lanzhou Greenchem ILS. LICP. CAS., China), graphite powder (particle size 30 μm, Shanghai Colloid Chem. Co., China), Nafion (5%, Sigma, USA) and trichloroacetic acid (TCA, Tianjin Kemiou Chemical Ltd. Co., China) were used as received. Graphene (GR) was synthesized according to previous reports (Hummers and Offeman, 1958) and TiO_2 nanorods were synthesized according the reported method with minor modification (Wu et al., 2006). 0.2 mol L^{-1} phosphate buffer solutions (PBS) with various pH values were used as the supporting electrolyte. All the other chemicals used were of analytical reagent grade and doubly distilled water was used in the experiments.

2.2. Apparatus

All the electrochemical measurements were executed on a CHI 750B electrochemical workstation (Shanghai CH Instrument, China). A conventional three-electrode system was used with a Hb modified electrode as working electrode, a platinum wire as auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode. UV–Vis is absorption spectra and FT–IR spectra were recorded on a Cary 50 probe spectrophotometer (Varian Company, Australia) and Tensor 27 FT–IR spectrophotometer (Bruker Company, Germany). Scanning electron microscopy (SEM) was recorded on a JSM-6700 F scanning electron microscope (Japan Electron Company, Japan).

2.3. Procedure

CILE was fabricated according to a reported procedure (Sun et al., 2008). 3.0 g graphite powder and 1.0 g $BPPF_6$ were mixed thoroughly in an agate mortar. The homogeneous paste was packed into a cavity of a glass tube (Φ=4 mm) and a copper wire was inserted through the opposite end to establish an electrical contact. Prior to use the electrode was polished on a weighing paper to get a mirror-like surface.

The nanocomposite was prepared by mixing 60 μL of 2.0 mg mL^{-1} GR suspension solution, 100 μL of 2.0 mg mL^{-1} TiO_2 nanorods suspension solution and 120 μL of 30 mg mL^{-1} of Hb solution homogeneously. Then 8 μL of the mixture was cast on CILE surface and left to dry at room temperature. This resulted electrode was denoted as GR–TiO_2–Hb/CILE. Finally, 5 μL of 0.5% Nafion solution was spread evenly onto the surface of GR–TiO_2–Hb/CILE to get Nafion/GR–TiO_2–Hb/CILE. Other modified electrodes including Nafion/GR–Hb/CILE, Nafion/TiO_2–Hb/CILE, Nafion/Hb/CILE were prepared by the similar procedure and used for comparison.

3. Results and discussion

3.1. Scanning electron microscopic images

The surface morphologies of GR, TiO_2 nanorods and their hybrid films were examined by SEM. As shown in Fig. 1a, TiO_2 exhibited as nanorods with the average width of 150 nm. The SEM image of GR showed clearly large sheet-like shape with slightly scrolled edges, which was the typical result of GR nanosheets. Most GR nanosheets were lying flat with some fold together (Fig. 1b). Fig. 1c was that of GR–TiO_2 nanocomposite, which exhibited that TiO_2 nanorods were dispersed on GR surface and inserted into the layer structure of GR. The good connection between TiO_2 nanorods and GR provided a porous microstructure for the loading of proteins. As for the GR–TiO_2–Hb composite (Fig. 1d), it can be seen that Hb was embedded into GR–TiO_2 nanocomposite with the increase of surface roughness, indicating