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Myoglobin within graphene oxide sheets and Nafion composite films as highly sensitive biosensor

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ABSTRACT

A highly sensitive biosensor was fabricated by incorporating myoglobin (Mb) within graphene oxide (GO) sheets and Nafion composite films. The stable composite Mb–GO–Nafion films were characterized by electrochemistry, scanning electron microscopy, Fourier transform infrared spectroscopy and UV–vis spectroscopy. It was found that Mb in Mb–GO–Nafion films retained its secondary structure similar to its native states. Cyclic voltammetry of Mb–GO–Nafion films showed a pair of well defined, quasi-reversible peaks at about −0.312 V vs saturated calomel electrode (SCE) at pH 5.5, corresponding to direct electron transfer (DET) between Mb and the glassy carbon electrode. Electrochemical parameter of Mb in Mb–GO–Nafion film such as apparent heterogeneous electron transfer rate constant (k_s) and formal potential ($E^{o'}$) were obtained. The dependence of $E^{o'}$ on solution pH indicated that the DET reaction of Mb was coupled with proton transfer. Mb in the films displayed good electrocatalytic activities towards various substrates such as hydrogen peroxide, nitrite and oxygen, indicating that the composite films have potential applications in fabricating novel biosensors without using mediators.

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1. Introduction

Direct electron transfer (DET) of proteins has been receiving fast growing interests from the fields of biochemical and biophysical sciences [1,2]. Because of their larger molecular size and the inaccessibility of the protein redox centers to many substrates, it is generally difficult to achieve their DET [3,4]. Promoters and mediators are usually employed to prompt and/or facilitate DET. Recently, a new approach to realize direct electrochemistry of proteins is to incorporate the proteins into appropriate thin films modified on the electrode surface. Such thin films may provide a favorable microenvironment for the proteins and enhance DET between the protein and the electrode, thus offering opportunities for investigating the enzyme electrochemistry [5,6]. Direct electrochemistry of redox proteins can also provide a model for the mechanistic study of electron transfer between the enzymes in real biological systems and is of significance in fabricating the third-generation biosensors [7,8].

Myoglobin (Mb) is a heme protein which has a single polypeptide chain with an iron heme inside as the prosthetic group [9]. Electron transfer between Mb in solution and bare solid electrode is usually very slow and the electrochemical behavior is unstable and very sensitive to the sample purity and the nature of the electrode surface [10]. Efforts have been made to improve the electron transfer characteristics of Mb by using thin film modified on electrodes to enhance the DET of heme proteins [11–13].

Nanostructured thin films have proven to provide a favorable microenvironment for proteins or enzymes to conduct DET [14]. Several forms of carbon nanomaterial electrodes, such as carbon nanotubes and carbon nanofibers, have been used for electroanalytical applications [15–17]. Graphene and its derivatives have attracted much research interest as novel materials with an excellent electrical conductivity [18,19], mechanical strength and chemical stability [20–22]. Because of their electrocatalytic ability and ease of modification [23], graphene-modified electrodes have been fabricated to study electrochemical and catalytic behavior of some biomolecules [24–26]. Unlike rolled structured carbon nanotubes (CNTs), graphene is a two-dimensional plenary sheet with open structure, hence both sides of graphene could be utilized for supporting enzymes.

Graphene oxide (GO) sheets are chemically exfoliated graphite oxide sheets with carboxylic groups at the edges and phenol, hydroxyl and epoxy groups on the basal planes [27]. Recently, GO and GO composite materials have been used to fabricate pH sensor [28], photovoltaic device [29], supercapacitor [30] and biosensors [31]. GO films modified on the electrode surface have been used for the study of proteins [32].

Nafion membranes combine the extremely high hydrophobicity of the polytetrafluoroethylene backbone with the high hydrophilicity of the sulfonic acid side-groups alternatively arranged along the

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polymeric chain. The sulfonic acid groups that are known to aggregate into cluster, allowing fast transport of ions and serving as a polymer electrolyte. They are widely used as supporting materials for catalytic species and for modifying electrodes [33,34].

In this paper, GO sheets synthesized by Hummers method [35] were employed to fabricate thin film modified electrode. Mb was then incorporated within GO sheets and Nafion composite films. The composite films were characterized by Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), UV–vis spectroscopy and electrochemistry techniques. Electrochemical catalytic reductions of hydrogen peroxide (H_2O_2), sodium nitrite ($NaNO_2$) and oxygen (O_2) at the Mb–GO–Nafion films were also investigated.

2. Experimental

2.1. Chemicals

Horse heart myoglobin (Mb, M_W 17,800), Nafion (5 wt% aliphatic alcohols solution) and nature flake graphite were purchased from Sigma, and used as received without further purification. Potassium ferricyanide ($K_3[Fe(CN)_6]$) was obtained from Qingdao Chemical Engineering Plant. All other chemicals were of analytical grade.

The buffer was usually 0.05 M sodium acetate, 0.05 M boric acid, 0.05 M citric acid, or 0.05 M sodium dihydrogen phosphate, all containing 0.1 M potassium bromide. The pHs of buffers were regulated with HCl or NaOH solutions. Deionized (DI) water purified twice successively by ion exchange and distillation was used to prepare solutions. The working solutions of H_2O_2 and $NaNO_2$ were freshly prepared.

2.2. Preparation of GO sheets

GO was synthesized with the Hummers method [35]. Nature flake graphite was added to a flask with 50 mL of 98% H_2SO_4 in an ice bath, followed by slow addition of $KMnO_4$ and $NaNO_3$ under stirring. Then, DI water was added and the temperature was raised to 98 °C. H_2O_2 was subsequently added into the flask. After the resultant mixture cooled to room temperature, the mixture was filtrated and the filtered product was dried overnight at 60 °C. The product was suspended in water to yield a brown dispersion, which was subjected to dialysis to remove residual metal ions and acids. The purified dispersion was sonicated for 1.5 h at 300 W to exfoliate the GO, and unexfoliated GO was removed by centrifugation (4000 rpm, 5 min). At last, the GO sheets were obtained.

2.3. Preparation of Mb–GO–Nafion composite film

Glassy carbon (GC) electrode (geometric area 0.16 cm^2) was polished with metallographic SiC sand paper, followed by on a clean billiard cloths. After rinsing with DI water, the GC electrode was sonicated with DI water for about 30 s.

GO solution (1 mg/mL) was prepared by dissolving GO sheets in DI water with ultrasonication for about 1 h. To get the best cyclic voltammetry (CV) responses of Mb–GO–Nafion films, the concentration of Mb, the ratio of Mb/GO/Nafion, and the total volume of Mb–GO–Nafion dispensed on the GC electrode were optimized. Typically, 3 μL of 1 mg/mL GO suspension, 10 μL of 1 mg/mL Mb and 5 μL Nafion solution (0.05 wt%) were spread evenly onto a freshly abraded GC electrode respectively with a microsyringe. A small bottle was fit tightly over the electrode to serve as a closed evaporation chamber so that water was evaporated slowly. The Mb–GO–Nafion films were dried in air overnight. The GO–Nafion films were prepared in a similar way without the presence of Mb.

2.4. Apparatus and procedures

A CHI 760C electrochemical analyzer (CH Instruments) was used for cyclic voltammetry (CV), square-wave voltammetry (SWV) and alternating current impedance (AC impedance). In electrochemical measurements, a regular three-electrode cell was used with a saturated calomel electrode (SCE) as reference, a platinum wire as counter electrode, and a GC electrode coated with films as working electrode. All experiments were performed at ambient temperature (20 ± 2 °C). The Mb–GO–Nafion films electrode were stored in a refrigerator at 4 °C.

Voltammetry on Mb–GO–Nafion electrodes was carried on in buffers. Prior to electrochemical measurements, buffers were purged with highly pure nitrogen for at least 10 min, and nitrogen environment was then maintained over the solution in the cell to protect the solution from oxygen. In the experiments of oxygen, measured volume of air was injected via a syringe into the solution, which had been previously degassed with nitrogen.

UV–vis spectra were measured with a TU-1901 spectrophotometer. Scanning electron microscopy (SEM) measurements were conducted on a JSM-6390LV with an acceleration voltage of 20 kV. Fourier transform infrared (FT-IR) spectra were taken on a Nicolet 5700 spectrophotometer. Sample films for SEM, UV–vis measurements were prepared on the quartz slides. For example, the Mb–GO–Nafion films were prepared by deposition of the GO, Mb and Nafion suspension on the surface of the quartz slides.

3. Results and discussion

3.1. Characterization of GO sheets and Mb–GO–Nafion composite film

Fig. 1 shows the FT-IR spectra of samples. It is seen that the GO sheets carried the O—H (a strong absorption band at 3420 cm^{-1} due to O—H stretching vibrations), C=O (the C=O stretching vibrations from carbonyl and carboxylic groups at 1750 cm^{-1}), and C—O (C—OH stretching vibrations at 1200 cm^{-1} or O—C—O stretching vibrations at 1050 cm^{-1}) groups [36], indicating the attachment of oxo-groups on GO sheets after the chemical oxidation of flake graphite.

For graphite, only O—H stretching vibrations at 3450 cm^{-1}, and C=C stretching vibration at 1620 cm^{-1} were observed (Fig. 1a) [37].

For the Mb–GO–Nafion films (Fig. 1c), a strong absorption band appeared at about 3420 cm^{-1} which is attributed to the O—H stretching vibrations of GO. The absorption bands at 1240 cm^{-1} and 1160 cm^{-1} were much higher than that for GO films, most probably due to CF_2 asymmetric and symmetric stretching vibrations. The band at 1160 was due to the S—O symmetric stretching, and

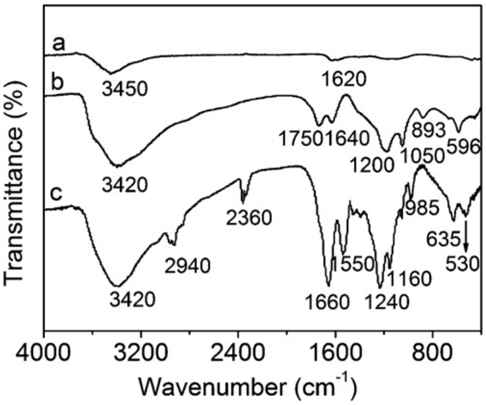

Fig. 1. FT-IR spectra of (a) graphite, (b) GO, and (c) Mb–GO–Nafion composite.

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