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Graphene dispersed cellulose microfibers composite for efficient immobilization of hemoglobin and selective biosensor for detection of hydrogen peroxide

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ABSTRACT

In the present work, we have investigated the electrochemical behavior and electrocatalysis of hemoglobin (Hb) immobilized on a glassy carbon electrode (GCE) modified with a graphene-cellulose microfiber (GR–CMF) composite. The GR–CMF composite was characterized by scanning electron microscopy, elemental analysis, and Raman and Fourier transform infrared spectroscopy. Well-defined electrochemical redox characteristics of Hb were observed for Hb immobilized on a GR–CMF composite modified GCE, with a formal potential of −0.306 V and a peak to peak separation of approximately 67 mV. Due to the high biocompatibility of the GR–CMF composite, the electrochemical behavior of the Hb heme redox couple (Fe^{II}/Fe^{III}) was enhanced for Hb immobilized on the GR–CMF composite when compared to Hb immobilized on pristine GR. The heterogeneous electron transfer constant (k_s) was calculated as 6.17 s^{-1}, and is higher than previously reported for Hb immobilized GR supports. The Hb immobilized GR–CMF composite modified electrode was used for the quantification of H_2O_2 under optimal conditions, and shows a wider linear amperometric response ranging from 0.05 to 926 μM. The limit of detection of the biosensor was 0.01 μM with the sensitivity of 0.49 μA μM^{-1} cm^{-2}. The biosensor also showed high selectivity in the presence of the range of interfering compounds and exhibits good operational stability and practicality in the detection of H_2O_2.

1. Introduction

Recent advances in the nanomaterials indicate a wide range of promising applications for their use in biosensor systems [1] as commonly applied to the detection of toxins and pathogens in food, clinical, and environmental analysis [2,3]. Given their high specificity, biosensors based on heme redox proteins are widely used for detection of small molecules such as hydrogen peroxide (H_2O_2) and nitrite (NO_2^-) in food and environmental samples [4]. Demonstrating greater stability than other commercially available redox heme proteins such as horseradish peroxidase, cytochrome C, and myoglobin, hemoglobin (Hb) is ideal for biosensor applications [5,6]. Hb-based biosensors are particularly suited to the selective detection of H_2O_2 due to their high electrocatalytic activity and narrow target specificity. The accurate detection of H_2O_2 in food, biological and pharmaceutical samples is fundamental to a wide range of industrial applications [7–10]. However, effective immobilization of Hb on the electrode surface is a limiting factor in the efficiency of Hb based biosensors. Accordingly, different micro and nanomaterials or approaches have been explored as a means to anchor the redox active center of Hb to the electrode matrix.

Over recent years, carbon nanomaterials [11–13], metal oxides [14], metal nanoparticles [15], ionic liquids [16] and conducting

Abbreviations: LRR, linear response range; LOD, limit of detection; MWCNT, multi-walled carbon nanotubes; GCE, glassy carbon electrode; NR, not reported; GR, graphene; CSc, hitosan; GTN, gelatin; CFEc, arbon fiber electrode; NPs, nanoparticles Hishistidine; GN, graphene.

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polymers [17] have been utilized as immobilization matrices for Hb. In particular, the 2D carbon nanomaterial graphene (GR) exhibits electronic conductivity and thermal stability superior to other carbon nanoforms [18,19], and make it an ideal support material in the fabrication of biosensors [19,20]. However, the direct immobilization of Hb on pristine GR surface is problematic due to the molecules hydrophobic nature, and the Hb redox active center is located deep within the proteins tertiary structure [21]. Accordingly, the immobilization of redox active proteins such as Hb has necessitated modification of GR with appropriate biocompatible materials. For instance, carbohydrate polymers and supramolecular adducts are widely used as a dispersing agent for GR and the resulting composite may enrich the biocompatibility of GR for immobilization of redox active proteins [22–26]. As a natural, renewable, abundant, and biodegradable carbohydrate polymer, cellulose has been utilized in a wide range of industrial and medical applications [27]. In particular, hydrophobic, water insoluble cellulose microfibers (CMF) represent a promising biomaterial for enzyme immobilization in biosensors due to their unique chemical properties and high biocompatibility [27]. In addition, CMF exhibits a high surface area, high porosity, and bond with the variety of conductive materials [27–30]. In the present work, we have exploited the aforementioned properties of CMF and, by dispersing GR in a CMF aqueous solution, prepared a GR CMF composite for the immobilization of Hb. In doing so, the inherent nature of the hydrophilic CMFs acts to effectively prevent aggregation of GR and form a stable GR–CMF composite for immobilization of Hb.

A review of the published literature indicates the great majority of GR and cellulose composites have been prepared by chemical reduction of graphene oxide and cellulose [28–30], but none have demonstrated the direct preparation of GR–CMF composite. However, we have recently demonstrated the direct preparation of GR–CMF composite as an immobilization matrix for laccase [31]. In the present work, we evaluate the electrochemical redox characteristics of Hb immobilized on a GR–CMF composite modified electrode and discuss this in relation to comparable modified electrodes for the immobilization of Hb. An H_2O_2 biosensor was fabricated based on the Hb immobilized GR–CMF composite modified electrode, and the detection parameters quantified using an amperometric method.

2. Experimental

2.1. Material and methods

The cellulose microfibers (medium) powder was purchased from Sigma Aldrich. Graphene 8 nm nanoflakes were obtained from UniRegion Bio-Tech, Taiwan. H_2O_2 (30 %) was received from Wako Pure Chemical Industries. Human blood serum samples were received from valley biomedical, Taiwan product & services Inc., and was approved by the ethics committee of Chang-Gung memorial hospital in contract no. IRB101-5042A3. Commercial contact lens cleaning solution was purchased from China Chemical and Pharmaceutical, Taipei, Taiwan. The whole milk was purchased from a local department store in Taipei, Taiwan. The supporting electrolyte was pH 7 phosphate buffer and was prepared with 0.05 M Na_2HPO_4 and NaH_2PO_4 in double distilled water. Adjustments to pH were made with 0.1 M H_2SO_4 and 0.1 M NaOH.

The surface morphologies of the as-prepared materials were characterized using an Hitachi S-4300SE/N High Resolution Schottky Analytical VP scanning electron microscope (SEM). Elemental analysis (EDS) and elemental mapping of the composite were performed using Hitachi S-4300SE/N High Resolution Schottky Analytical VP SEM attached BRUKER AXS elemental analyzer. Fourier transform infrared (FTIR) spectroscopy was acquired using a JASCO FTIR-6600 spectrometer. Raman spectra for the materials were taken using a Dong Woo 500i Raman spectrometer equipped with a charge-coupled detector. Cyclic voltammograms and amperograms (amperometric *i–t* curve) were taken using CHI1205B electrochemical workstation from CH Instruments. Hb immobilized on a GR–CMF modified glassy carbon electrode (GCE) was used as a working electrode, where the apparent electrode surface of the GCE was approximately 0.079 cm^2. Saturated Ag|AgCl and Pt wire were used as a reference and auxiliary electrodes, respectively. Amperometric *i–t* measurements were performed using a PRDE-3A (ALS Co., Ltd, Japan) rotating ring disc electrode (RDE), in which the geometric area of the electrode is 0.08 cm^2. The electrochemically active surface area (EASA) of the GR–CMF composite modified RDE was calculated as 0.27 cm^2, and was calculated using Randles–Sevcik equation by cyclic voltammetry response of 1 mM ferricyanide with 0.05 M KCl [32].

2.2. Fabrication of the biosensor

To fabricate the biosensor, first, the GR–CMF composite was prepared by dispersing GR (5 mg mL^{-1}) into the CMF solution using ultrasonication for approximately 30 min. The stable CMF solution was prepared by the addition of 10 mg mL^{-1} of CMF into the doubly distilled water and sonicated for 45 min at 10 °C. Then, about 6 μL of GR-μL of GR–CMF composite solution was dropped on precleaned GCE and allowed to dry in an air oven. Once dry, 6 μL of Hb solution (optimum) was dropped on the as-prepared GR–CMF composite modified electrode and dried at room temperature. Then, the resulting Hb immobilized GR–CMF (GR–CMF/Hb) composite modified electrode was used for further electrochemical studies. The schematic representation of the biosensor fabrication is shown in Scheme 1. The fresh Hb stock solution was prepared by dissolving 5 mg mL^{-1} of Hb at pH 7 and was stored at −4 °C when not in use. The Hb immobilized GR modified electrode was prepared by drop coating of 6 μL of Hb solution on GR modified electrode, while the GR dispersion was prepared by dispersing of 5 mg mL^{-1} of GR into the dimethylformamide (DMF) using ultrasonication for 30 min. The Hb immobilized CMF modified electrode was prepared by the same method. Optical images of GR–DMF, CMF and GR–CMF composite are shown in Fig. 1D. All electrochemical measurements were performed in oxygen-free atmosphere by purging high purity N_2 into pH 7 for at least 10 min, and the modified electrodes were stored under the dry condition when not in use.

3. Results and discussion

3.1. Characterizations

The surface morphological studies of the GR, CMF, and as-prepared GR–CMF composite were characterized by high-resolution SEM. Fig. 1 shows SEM images of pristine GR (A), CMF, (B) and GR–CMF composite (C). The SEM images shows the closely arranged layering of relatively small individual pristine GR nanosheets, and the typical dense fiber morphology of CMF. It is clear from Fig. 1C that the GR nanosheets were highly exfoliated by CMF compared with pristine GR. In addition, the optical image of GR–CMF composite (Fig. 1D) confirms the formation of GR–CMF composite and CMF is a suitable dispersing agent for GR. The GR–CMF composite was found to be highly stable even after storage for six days. We also performed EDS and elemental mapping of the GR–CMF composite and the results are shown in Fig. S1 and Fig. 2A and B. The EDS and elemental mapping of GR–CMF composite confirm the presence of carbon and oxygen in the composite, while oxygen is absent in the EDS and elemental mapping