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Direct electron transfer and electrocatalysis of hemoglobin adsorbed on mesoporous carbon through layer-by-layer assembly

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Abstract

Using chitosan as an effective linker between CMK-3 and glassy carbon electrode surface, $\{Hb/CMK\text{-}3\}_n$ multilayer film-modified electrodes were constructed through layer-by-layer assembly. The morphology of thus-formed $\{Hb/CMK\text{-}3\}_n$ film was characterized by scanning electron micrographs, and the interaction of hemoglobin (Hb) with CMK-3 was studied by UV–vis spectroscopy and electrochemical methods. Under optimal conditions, $\{Hb/CMK\text{-}3\}_6$ film showed a couple of stable and well-defined redox peaks at about −377 and −296 mV in pH 7.0 buffers. Furthermore, the $\{Hb/CMK\text{-}3\}_6$ film displayed excellent electrocatalysis to the reduction of both H_2O_2 and O_2. Based on thus-formed film and its direct electron transfer behavior, a novel biosensor was presented for the determination of H_2O_2 ranging from 1.2 to 57 μM with the detection limit of 0.6 μM at S/N = 3. CMK-3 provided a desirable matrix for protein immobilization and biosensor preparation.

Keywords: Mesoporous carbon; Layer-by-layer assembly; Hemoglobin; Direct electron transfer

1. Introduction

Recently, ordered mesoporous materials attracted increasing interest both in practical and fundamental field for its high specific surface areas and pore volumes, well-ordered pore structures and narrow mesopore-size distribution (Soler-Illia et al., 2002, 2003). These fascinating properties endowed them ideal candidates as hosts for biomolecules in bioadsorption and biocatalysis field (Hartmann, 2005). Moreover, changing the synthesis conditions could tailor the mesoporous host, which provide more freedom to encapsulate different size of proteins, enzymes, and other biomolecules (Vinu et al., 2003; Hartmann et al., 2005; Mureseanu et al., 2005; Lei et al., 2002). However, less work has been performed in connection with amperometric biosensors (Dai et al., 2004; Paddon and Marken, 2004; Xu et al., 2004), possible due to partly denaturation of these proteins and relatively lower conductivity of the above-mentioned matrices. Lately, mesoporous carbon materials and films become a hot subject, which are widely used in adsorption and separation field. Because mesoporous carbon has better electrical conductivity in comparison with other mesoporous materials. Therefore, the film based mesoporous carbon is a leading candidate to immobilize biomolecules with improved immobilization ability and enhanced electron transfer efficiency than simple ceramic or polymeric coatings (Vinu et al., 2003).

To fabricate mesoporous film, several methods have been developed. Successful examples include electrodeposition (Feng et al., 2005; Choi et al., 2002), evaporation-induced co-assembly (Choi et al., 2004), evaporation of metallic tin in an oxygen atmosphere (Wong et al., 2002), layer-by-layer (LBL) assembly (Liu et al., 2006; Xu et al., 2005; Zhao et al., 2005a), and so on. Particularly, LBL assembly is most prospective, which is a rich, versatile, low-cost, and easy-operation method. Moreover, the components, thickness and functionalization of thus-formed multilayer film can be finely tuned by incorporating different electroactive molecules and/or proteins for potential applications such as electronics, optics and biosensing (Hammond, 2004). Additionally, the fabricated procedures were performed in aqueous solution at mild conditions, which could further broaden the general utility and vitality of this system.

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In our experiment, chitosan just served as an effective linker between CMK-3 and glassy carbon electrode (GCE). As well known, chitosan is a natural biopolymer with a large amount of positive charges. On the other hand, LBL assembly process was usually pretreated via placing solid supports into positively/negatively charged polymer solution for several cycles to obtain a stable and positively/negatively charged surface for the following assembly. To simplify the assembly process and introduce more available solid supports to this biosensing field, we tried to just use simple electrodeposition of chitosan (Fernandes et al., 2003) to form positively charged surface on GCE. This strategy is simple, controllable, and universal. Namely, it can be performed on any conductive solid support whether this support possesses functional groups or not. Subsequently, CMK-3 and hemoglobin (Hb) were alternatively deposited on the outer layer of electrodeposited chitosan to construct $\{Hb/CMK\text{-}3\}_n$ films.

Here, the detailed preparation of $\{Hb/CMK\text{-}3\}_n$ film was presented. As expected, with film assembly, a layer of well-dispersed and continuous Hb film was obtained. More importantly, thus-formed film provided a desirable microenvironment to host Hb and retained its native structure. It also realized the direct electron transfer of Hb with underlying electrode. Additionally, the electrocatalytic ability of the immobilized Hb could be fine-tuned just through simply adjusting the number of assembly bilayers.

2. Experimental

2.1. Reagents

Chitosan (92.5% deacetylation from Nantong Shuanglin company) was dissolved in 0.05 M HCl and stirred for 3 h to form 0.2 wt.% solution (pH 3) at room temperature. After undissolved material was filtered, the pH was adjusted to about 5.0 using 1.0 M NaOH. Human hemoglobin (MW 66,000) was purchased from Sigma and used without further purification. Hydrogen peroxide (H_2O_2, 30%, w/w) was from Beijing Chemical Engineering Plant, its dilute solution was freshly prepared daily. Phosphate buffer solutions (PBS, 25 mM) with different pH values (3.0, 4.0, ..., and 9.0) were prepared by mixing the standard stock solutions of Na_2HPO_4 and KH_2PO_4 and adjusting the pH with 1.0 M H_3PO_4 or NaOH. Other reagents were of analytical reagent grade and used as received. All solutions were prepared with bi-distilled water.

2.2. Apparatus

UV–vis spectroscopy was performed with a UV-2201 spectrophotometer (Shimadzu, Kyoto, Japan). X-ray powder diffraction (XRD) experiments were performed with a Philips PW 1830 diffractometer, using a monochromatized X-ray beam with nickel-filtered Cu Kα radiation. N_2 adsorption–desorption isotherms were collected on a Micromeritics-Gemini adsorption analyzer at 77 K. The BET surface area was calculated from the linear part of the BET plot. The pore diameter is calculated by 4 × pore volume/surface area. The pore size distribution plots are obtained by using the Barret–Joyner–Halenda (BJH) model. Scanning electron micrographs (SEM) were obtained with a LEO-1530VP field-emission scanning electron microscope. Electrochemical experiments were performed with an Autolab PGSTAT 30 System (Ecochemie, Netherlands) in a three-electrode cell, where the modified GCE was used as a working electrode, a platinum wire as an auxiliary and a saturated calomel electrode as a reference one. Electrochemical impedance spectroscopy (EIS) experiments were performed in 0.1 M KNO_3 solution containing 5.0 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ (1:1), using an alternating current voltage of 5.0 mV. The impedance measurements were performed at an open circuit potential of 170 mV within the frequency range of 10^{-2} to 10^5 Hz. Voltammetric measurements were done in an unstirred electrochemical cell at room temperature. Solutions were deaerated for at least 20 min with a high-purity nitrogen stream and kept under a pressure of this gas during the experiments.

2.3. Synthesis of mesoporous carbon using SBA-15 as templates

Mesoporous carbon (CMK-3) was prepared by using SBA-15 as template and sucrose as the carbon source. SBA-15 was synthesized at 100 °C according to the method proposed by Hartmann and coworkers (Jun et al., 2000). The obtained SBA-15 (curve b) and CMK-3 (curve a) were characterized by XRD (see supporting information). This mesostructured silica (SBA-15) showed three well-defined peaks at 2θ values between 1° and 4° that can be indexed as (1 0 0), (1 1 0), and (2 0 0) Bragg reflections, typical of hexagonal (*p6mm*) SBA-15. Similar to the case of SBA-15, thus prepared CMK-3 possessed hexagonal (*p6mm*) meso-structure as well, in agreement with those reported by the above-mentioned authors.

Nitrogen adsorption measurements were done to further characterize the obtained CMK-3. Experimental data confirmed this material possessed high BET surface areas of 1060 m^2/g and large pore volumes of 1.1 cm^3/g (see supporting information). Obviously, a relatively small pore-size distribution was observed and the average pore diameter was about 3.2 nm.

Surface modification of CMK-3 with hydrophilic groups of –COOH groups was performed just through treated with 1 M H_2SO_4 for 3 h at 80 °C according to the literature (Zhu et al., 2005). This procedure could improve the hydrophilicity of CMK-3. The negatively charged surface is also favorable to immobilize Hb, which is positively charged in pH 7.0 PBS (Hb, IEP = 7.4).

2.4. Construction of $\{CMK\text{-}3/Hb\}_n$ multilayer film

GCE (d = 3 mm) was polished with 1.0, 0.3, and 0.05 μm alumina slurry, respectively, rinsed thoroughly with distilled water between each polishing step, then sonicated in 1:1 nitric acid solution (HNO_3:H_2O, v/v), acetone and water successively and allowed to dry at room temperature. The $\{Hb/CMK\text{-}3\}_n$