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Effects of pore structure of mesoporous silicas on the electrochemical properties of hemoglobin

Jinxia Li, Zhiying Xiong, Lihui Zhou *, Xia Han *, Honglai Liu

Key Laboratory for Advanced Materials and Department of Chemistry, East China University of Science and Technology, Shanghai 200237, China

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ABSTRACT

Effects of pore structure of mesoporous silicas (MPS) on the direct electron transfer of hemoglobin (Hb) were investigated. MCM-41 and SBA-15 were used to supply 1D channel and hexagonal mesoporous structure with the average pore diameters of 3.2 and 6.9 nm, respectively, while MCF was used to supply 3D and caged mesoporous network structure with the average pore diameter of 15.7 nm. Hb was immobilized by the physical adsorption method without significant denaturation and have the saturated binding amounts of 15.8, 240.0 and 325.7 mg g^{-1} for MCM-41, SBA-15 and MCF, respectively. The electrochemical properties of Hb/MPS/GC modified electrode were studied by cyclic voltammetry (CV). A couple of redox peaks were observed at Hb/SBA-15/GC and Hb/MCF/GC, while no obvious redox peak was found at Hb/MCM-41/GC modified electrode because Hb molecule could not enter into the channels of MCM-41. The CV signal of Hb/MCF/GC is about 2× as large as that of Hb/SBA-15/GC electrode when the same amount of Hb was immobilized in the pores of MCF and SBA-15, which suggests that the 3D porous structure may be the key factor for promoting the electron transfer of Hb.

1. Introduction

In recent years, the ordered mesoporous silicas (MPS) were found to be appealing materials for immobilizing enzymes and enzymatic catalysis, due to its good biocompatibility, low toxicity, large surface areas, and high stability to chemical and mechanical forces [1–3]. Indeed, these ordered mesoporous solids discovered in 1992 [4], having unique properties of regular and uniform channels varying from 1 nm to 30 nm (which match with the sizes of enzyme molecules). Many different enzymes have been encapsulated in the MPS since Diaz and Balkus [5] attempted to immobilize enzymes onto mesoporous MCM-41 for the first time in 1996. Previous studies show that either pore diameter or surface characteristics of MPS has a significant influence on the adsorption behavior of proteins [6–9]. The excellent thermal stability and high enzyme activities can be obtained by immobilizing enzymes in specially designed nanostructure of MPS, which may open possibilities for the application of enzyme-loaded MPS in the field of chemical synthesis, biosensor and delivery vehicle [10,11].

Direct electron transfer of enzymes with enhanced faradic responses is highly desired for development of the third generation biosensors with a high sensitivity and novel biofuel cells with a high performance [12]. However, it is difficult for enzymes to carry out a direct electrochemical reaction due to their deeply embedded electroactive centers [13]. Fortunately, MPS have been demonstrated to be able to promote direct electron transfer of proteins [14]. Many enzyme-loaded MPS systems have been explored to achieve the DET between enzyme and electrode surface [15]. For example, Balkus Jr. et al. [16] encapsulated cytochrome c into the mesoporous molecular sieves MCM-48 and SBA-15 and investigated its redox behavior. The results showed that cytochrome c retained its redox activity but the voltammetric signals were rather small, which maybe due to the low bonding amount or the blocking effect of mesoporous material. Dai and co-workers [17] reported the direct electrochemistry of heme protein immobilized on hexagonal mesoporous silica modified glassy carbon (GC) electrode. Yu and co-workers [18] reported that mesoporous Al_2O_3 had a significant promotion effect for the DET of hemoglobin (Hb). However, there is a lack of systematic studies clearly stating the influence of pore structure of MPS on the direct chemistry of redox enzyme.

In this work, we aimed at addressing the influence of pore structure of MPS (such as the pore size or the internal structure) on the electrochemistry properties of Hb. Three kinds of MPS materials with different pore sizes and structures, MCM-41, SBA-15 and MCF, were selected as carriers for immobilizing Hb. By a physical adsorption method the Hb molecule was immobilized on these carriers, the effects of the adsorption amounts of Hb and the pore structure on electrochemical properties of Hb/MPS/GC modified

* Corresponding authors. Address: Key Lab for Advanced Materials and Department of Chemistry, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China. Tel.: +86 21 64253235 (L. Zhou).
E-mail addresses: lhzhou@ecust.edu.cn (L. Zhou), xhan@ecust.edu.cn (X. Han).

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electrode were investigated in details. The differences in the amount of protein immobilization or the pore structure of the MPS were found to introduce different CV signal intensities, which one is the intrinsic factor for enhanced faradic responses was discussed.

2. Experiments

2.1. Reagents

Hb (MW 64,500, from bovine blood) was purchased from Sigma Co. Ltd. (USA). Pluronic P123 ($EO_{20}PO_{70}EO_{20}$, molecular weight 5800) was obtained from BASF (USA). Hydrochloric acid (HCl, 37%), 1,3,5-trimethylbenzene (TMB), tetraethylorthosilicate (TEOS) and hydrogen peroxide (H_2O_2, 30%) were obtained from Shanghai Chemical Reagent Co. Ltd. (China). Phosphate buffer solutions (PBS) containing 0.1 mol L^{-1} KCl (pH 7.0) were prepared by mixing stock standard solutions of Na_2HPO_4 (0.1 mol L^{-1}) and KH_2PO_4 (0.1 mol L^{-1}). All solutions were prepared with doubly distilled water.

2.2. Immobilization of Hb on MPS

Syntheses of MCM-41, SBA-15 and MCF were performed according to the literature [19–21]. The immobilization method of Hb is based on the procedure reported by Diaz and Balkus [5]. A standard Hb solution was prepared by dissolving Hb in PBS with pH 7.0. In each adsorption experiment, 0.1 g of MPS sample was added to 5 mL of Hb solutions with different concentrations. After stirred for 3 h at 4 °C, the mixtures were centrifuged with 10,000 rpm for 30 min. Then, the concentration of Hb in the supernatants was determined by means of UV spectra at 405.4 nm. The binding amounts of Hb onto MPS supports were calculated by subtracting the concentration of free Hb from the total concentration of Hb.

2.3. Electrode modification

Prior to preparation of modified electrode, GC electrode of 3.0 mm in diameter was mechanically polished to a mirror finish with 0.05 μm alumina slurry, then was ultrasonically washed with 1:1 HNO_3 (v/v), NaOH (1.0 mol L^{-1}), ethanol and redistilled water for 30 s, respectively. In a typical procedure, 10 μL of the Hb/MPS colloid solution (0.8 mg mL^{-1}) was dropped on the pretreated GC surface and dried for several hours at room temperature. The modified electrodes were noted as Hb/MPS/GC. The electrodes were stored at 4 °C in a refrigerator.

2.4. Apparatus and measurements

Electrochemical experiments were carried out on a CHI 430A electrochemical workstation (Shanghai Chenhua, China) with three-electrode system, a bare GC electrode or modified GC electrode as the working electrode, a platinum counter electrode as auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode. The cyclic voltammogram (CV) experiment was carried out in 5 mL of 0.1 mol L^{-1} PBS (pH 7.0) at room temperature. The solutions were bubbled with highly purified nitrogen for at least 20 min to remove oxygen and nitrogen atmosphere were maintained over the solutions during the experiments.

The low-angle X-ray powder diffraction (XRD) patterns were recorded using a Rigaku D/max 2550 VB/PC diffractometer with the Cu Kα radiation ($\lambda = 0.154056$ nm). The transmission electron microscope (TEM) images were observed on JEM-2010 TEM (JEOL, Japan). FTIR spectra were carried out on Nexus 670 Fourier transfer infrared spectrophotometer (Nicolet), KBr pellets. UV–vis spectra were recorded on Shimadzu UV-2450 spectrophotometer. Nitrogen adsorption isotherms were measured at 77 K using ASAP-2020 instrument (Micromeritics, USA).

3. Results and discussion

3.1. Structure of MCM-41, SBA-15 and MCF

The low-angle XRD patterns of MCM-41, SBA-15 and MCF are shown in Fig. 1. Both MCM-41 and SBA-15 show three obvious peaks at different 2θ degree, which indicates that they have the typical feature of hexagonal mesoporous structures [22]. The XRD pattern of MCF is not similar with others, which suggests that MCF has different mesoporous structure (Fig. 1, curve c). The pore size distributions of the as-prepared materials are shown in Fig. 2. As expected, MCM-41 and SBA-15 possess narrow pore size distributions. MCF was prepared with 1,3,5-trimethylbenzene as a swelling agent, which possess ultra-large pore structures. Table 1 shows the pore diameters and pore volumes as well as BET surface areas of the three carriers. The TEM micrographs show that MCM-41 and SBA-15 possess 2D hexagonal mesostructure while MCF has 3D caged mesoporous networks structure with large pore diameter (Fig. 3), which is consistent with the results of XRD and the pore size distributions.

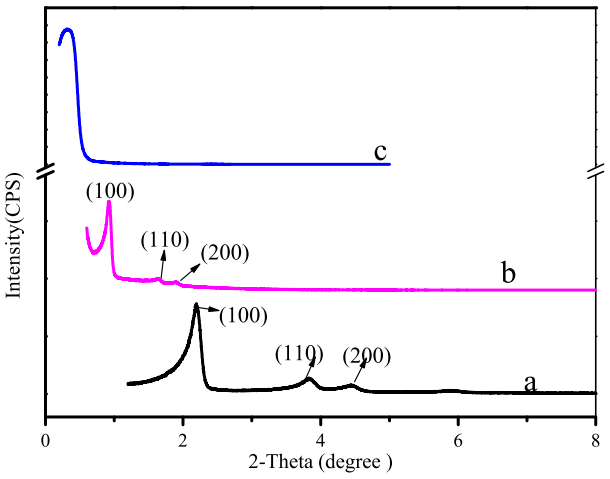

Fig. 1. Low-angle XRD patterns of (a) MCM-41, (b) SBA-15 and (c) MCF.

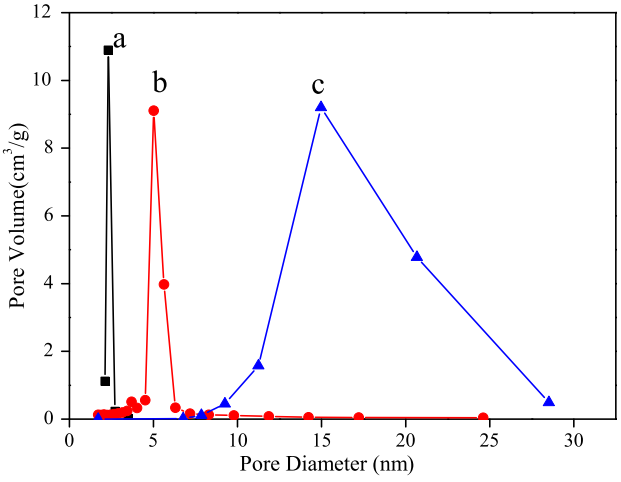

Fig. 2. Pore size distributions of (a) MCM-41, (b) SBA-15 and (c) MCF.