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Adsorption study of heme proteins on SBA-15 mesoporous silica with pore-filling models

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Abstract

The Langmuir-type adsorption of myoglobin occurred with monolayer coverage of the inner surface mesopore channels of SBA-15. Myoglobin occupation of the pores was calculated as ca. 50% based on N_2 adsorption/desorption isotherms. Pore-filling models revealed that myoglobin molecules are well packed in the pores. The maximum adsorption was observed near the isoelectric point of myoglobin, suggesting the important role of suppression of electric repulsion between the proteins and/or between the protein and the adsorbent. FT-IR spectroscopic studies confirmed that the myoglobin is stable even after the adsorption. The results obtained are comparable with those observed for another redox protein, cytochrome *c*.

Keywords: Mesoporous silica; Protein; Adsorption; Nanostructures

1. Introduction

Although recent developments in nanotechnology have realized many kinds of artificial molecular modules, they are still inferior to those present in naturally occurring systems [1]. For example, the efficiency of energy conversion in mitochondrial and photosynthetic systems exceeds significantly that obtained by artificial systems. Therefore, the fusion of current nanotechnology with existing biological systems is of substantial importance. Nano-bio electronics [2–6], which uses biomolecules as electrical components in nano-sized structures, will open a new age of science and technologies in electronics and related fields.

Amongst biomolecules, proteins are the most attractive materials for application in nano-bio electronics. They are made from simple sequences of amino acids but have incredibly well-designed three-dimensional structures, allowing material conversion and electron/energy transfer in highly specific and efficient ways. In particular, the redox activity of some kinds of proteins could be indispensable for the design of nano-bio electronic devices. In order to fabricate such devices, several methodologies to organize biomolecules in various molecular assemblies have been proposed including Langmuir–Blodgett (LB) [7,8] and layer-by-layer (LbL) [9–15] techniques.

Fixing biomolecules within well-defined and confined matrices has become a realistic aim following recent progress in mesoporous material processing [16–25]. These approaches permit new strategies in nano-bio electronics because molecules under restricted motion and conformation may show unexpected features in their electron transfer and conduction properties. However, the concept of "biomaterials in nanoporous media" is embryonic and there remain many unexplored frontiers from the viewpoints of practical applications and basic sciences.

Recently, we have been investigating immobilization of biomaterials such as proteins, vitamins, peptides, and amino acids on mesoporous materials [26–34]. In this paper, we describe preliminary results from immobilization of myo-

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globin, which is known as a typical redox protein [35–38], onto mesoporous silica, SBA-15 [39,40]. SBA-15 is known to have thicker walls and be more stable under hydrothermal conditions compared to other mesoporous silicates. Therefore, SBA-15 is suitable for use in aqueous media. In this paper, the loading behavior of macromolecules (myoglobin) in a pore with restricted dimensions is discussed and is based on several structural analyses such as X-ray diffraction (XRD) and N_2 adsorption/desorption techniques as well as IR analyses on structures of the adsorbed myoglobin. For the myoglobin adsorption, we also proposed pore-filling models, which have not been fully discussed in the previous reports on protein immobilization on mesoporous materials [41–45].

2. Experimental

2.1. Materials

Tetraethyl orthosilicate (TEOS) and triblock copolymer poly(ethylene glycol)-*block*-poly(propylene glycol)-*block*-poly(ethylene glycol) (Pluronic P123, molecular weight = 5800, $EO_{20}PO_{70}EO_{20}$) were obtained from Aldrich. Potassium phosphate, sodium carbonate, potassium chloride, and sodium hydroxide for buffer preparation were purchased from Wako Pure Chem. Horse heart myoglobin was obtained from Sigma and used without further purification.

2.2. Synthesis of mesoporous silica

SBA-15 was synthesized using the amphiphilic triblock copolymer P123 [29]. A typical synthesis was performed as follows: 4 g of P123 was dispersed in 30 g of water and 120 g of 2 M HCl solution and stirred for 5 h. Thereafter, 9 g of TEOS was added with stirring to the homogeneous solution. The resulting gel was aged at 40 °C for 24 h and finally heated to 100 °C for 48 h. After synthesis, the obtained solids were calcined in flowing air at 540 °C to decompose the triblock copolymer.

2.3. Characterization

The powder X-ray diffraction (XRD) patterns of mesoporous silica materials were collected on a Rigaku diffractometer using CuKα ($\lambda = 0.154$ nm) radiation. The diffractograms were recorded in the 2θ range of 0.8° to 10° with a 2θ step size of 0.01° and a step time of 10 s. Nitrogen adsorption and desorption isotherms were measured at −196 °C on a Quantachrome Autosorb 1 sorption analyzer. Prior to protein adsorption, all samples were outgassed at 250 °C for 3 h before the nitrogen adsorption measurements, while the protein-adsorbed samples were outgassed at 40 °C for 24 h. The specific surface area was calculated using the Brunauer–Emmett–Teller (BET) method [46]. The pore size distributions were obtained from the adsorption and desorption branch of the nitrogen isotherms by the Barrett–Joyner–Halenda (BJH) method [47]. FT-IR spectra of SBA-15 before and after the protein adsorption were recorded on a Nicolet Nexus 670 instrument.

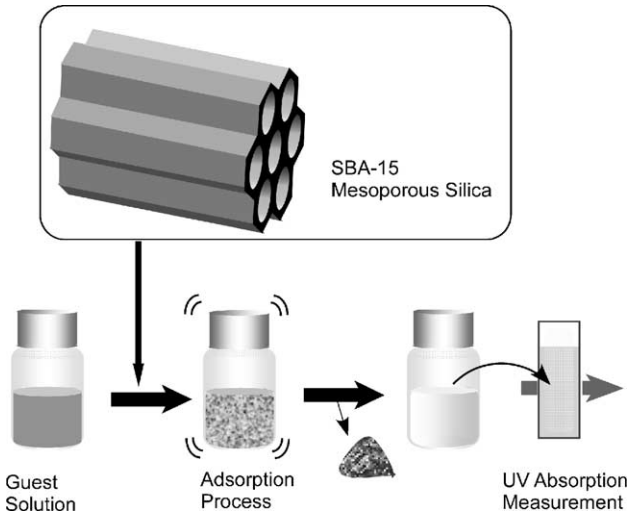

Fig. 1. Outline of adsorption study of myoglobin (guest) onto SBA-15 mesoporous silica.

2.4. Protein adsorption study

The protein adsorption study is outlined in Fig. 1. A series of standard myoglobin solutions with concentrations ranging from 14.7 to 235 μM was prepared by dissolving different amounts of myoglobin in 10 mM buffer solutions (potassium phosphate buffer for pH = 5.5, 6.5, 7.0, and 8.0; sodium bicarbonate buffer for pH = 10.5). In each adsorption experiment, 20 mg of the different mesoporous adsorbents were suspended in 4 g of the respective myoglobin solution. The resulting mixtures were shaken continuously in a shaking bath with a speed of 160 shakes min^{-1} at 20 °C until equilibrium was reached (typically 96 h). The amount of myoglobin adsorbed was measured by UV absorption at 409.5 nm.

3. Results and discussion

3.1. Basic behaviors of protein adsorption

The adsorption isotherm of myoglobin on SBA-15 at pH 7.0 is shown in Fig. 2. The isotherm exhibits a sharp initial rise and finally reaches a plateau, as denoted by Langmuir-type isotherm. By employing the Langmuir model, the monolayer adsorption capacity (n_m) was calculated using the Langmuir Eq. (1).

$$n_s = Kn_m c/(1 + Kc) \quad (1)$$

In this equation, K, c, and n_s represent the Langmuir (binding) constant, the myoglobin concentration at equi-