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Adsorption of protein on three dimensional large pore cage type mesoporous material

N. Gokulakrishnan[1], A.Vinu[2*], T.Mori[2] and K.Ariga[1]

[1]Supermolecules Group, [2]Nano-ionics Materials Group, NIMS, 1-1 Namiki, Tsukuba, Ibaraki 305 0044, Japan.

Email: vinu.ajayan@nims.go.jp,

Abstract:

Highly ordered three dimensional large pore cage type mesoporous silica (KIT-6) has been synthesized using P123 and *n*-butanol as a structure directing agent and a co-solvent respectively and characterized by XRD and N_2 adsorption. Adsorption of lysozyme on the above material has been studied as a model protein adsorption system and its adsorption isotherm has been fitted by Langmuir equation. The amount of lysozyme adsorbed over KIT-6 at the solution pH of 11, which is near the isoelectric point of lysozyme, is found to be 22.1μmol/. It has been found that the amount of lysozyme adsorbed over KIT-6 is much higher than that of MCM-48 which possesses three dimensional mesoporous structure with small mesopores.

Key words: KIT-6; lysozyme; mesoporous silica; protein; Langmuir isotherm

1. INTRODUCTION

The adsorption of proteins from solution on solid surfaces has received much attention due to its application in chemical and biological areas [1, 2]. The use of nano organized materials, such as nanoporous silica and carbons, nanotubes and silica capsules, foam type materials, as the supports leads to the development of nanosize reactors [3, 4] and sensors [5, 6]. Among these materials, mesoporous materials have been receiving much attention in the adsorption of large molecules such as proteins and vitamins due to their well-ordered pore structure with high specific surface area and pore volume [7-12]. The adsorption of biomolecules on the mesoporous materials with three dimensional porous structure with very high surface area and pore volume has received much attention because they provide easy access to all adsorption sites and support the easy diffusion of biomolecules in the three dimensional porous system. It has been found that MCM-48, which possesses a three dimensional structure, shows much higher adsorption capacity for proteins as compared to that of unidimensional mesoporous silica, MCM-41. This is due to the fact that access of pores for protein may be limited in one dimensional material, whereas for three dimensional materials, pores are accessible for protein in all sides because it contains two non-intersecting pore systems [13].

Lysozyme has long been considered a structurally stable. It is a small globular protein (molecular mass 14400 Da) with 18 cationic amino acid residues, has a single peptide chain of a total of 129 amino acids and prolate spheroid shape with two characteristic cross sections: a side of dimensions of roughly 3.0 × 4.5 nm^2 and an end of dimensions 3.0 × 3.0 nm^2 [14]. Lysozyme is a rigid and stable enzyme because of the four internal disulfide bonds help to maintain its tertiary structure.

Several authors have studied the immobilization of lysozyme on mesoporous materials. Kisler et al reported the adsorption of lysozyme on MCM-41 and surface coated MCM-41, in which they have observed that coated material shows a higher adsorption capacity compared to the parent MCM-41 [15, 16]. Qiao et al have studied the lysozyme adsorption on rod-like large-pore periodic mesoporous organosilica (PMO). They have reported that lysozyme is adsorbing more on the PMO compared to pure SBA-15 due to electrostatic interaction between lysozyme and PMO/SBA-15 [17]. Vinu et al have clearly described the adsorption of lysozyme on mesporous silica, aluminum substituted silica, and carbon molecular sieves, and studied the influence of solution pH, specific pore volume and pore diameter of the adsorbents [7, 8, 18] on the amount of protein adsorption. Recently Ryoo et al have reported that three dimensional large pore mesoporous silica with cubic Ia3d symmetry (KIT-6) can be synthesized by utilizing a triblock copolymer ($EO_{20}PO_{70}EO_{20}$)-butanol mixture for the structure direction in aqueous solution [19].

In this study, we report here on the adsorption of lysozyme over the three dimensional large pore mesoporous silica with cage type pore structure. The amount of lysozyme adsorbed over KIT-6 at the solution pH of 11, which is near the isoelectric point of lysozyme, is found to be 22.1μmol/.

2. EXPERIMENTAL SECTION

2.1 Materials

Hen egg white lysozyme (activity 25,000 units/mg of protein) was purchased from ICN biomedicals (Catalogue No. 100831) and used without further purification. Triblock copolymer Pluronic P123 (EO_{20} PO_{70} EO_{20}, MW=5800) and *n*-butanol were obtained from Aldrich and used as the template and the co-solvent respectively. TEOS (tetraethylorthosilicate), silica source was also obtained from Aldrich.

2.2 Synthesis of KIT-6

KIT-6 was synthesized using P123 as the structure directing agent and *n*-butanol as the co-solvent. In a typical synthesis of KIT-6 [19], 4 g of P123 was dissolved in 144 g of distilled water and 7.9 g of 35 wt% HCl solution and stirred at 35°C. Further, 4.0 g of *n*-butanol was added at once to the above mixture after complete dissolution. Then the mixture was stirred for 1 h at 35 °C. Then, 8.6 g of TEOS was added at once to the homogeneous clear solution and the stirring was continued for 24 h at 35 °C. Subsequently, the mixture was aged at 100 °C for 24 h in a closed polypropylene bottle under static conditions. The obtained while colored product was filtered without washing in water and dried at 100 °C for 24 h in an air oven. Then, the material was calcined at 550 °C in air.

2.3 Characterization

The X-ray powder diffraction pattern was collected on a Rigaku diffractometer with the use of CuKα (λ=0.154 nm) radiation. The diffractogram was recorded in the 2θ range of 0.8 to 10° with a 2θ step size of 0.01° and a step time of 1 s. Nitrogen adsorption and desorption isotherm was measured at -196°C on a Quantachrome Autosorb-1 sorption analyzer. The specific surface area was calculated with use of the Brunauer-Emmett-Teller (BET) method. The pore size distribution was obtained from the adsorption and desorption branch of the nitrogen isotherms using the Barrett-Joyner-Halenda method.

2.4 Lysozyme adsorption

A series of standard lysozyme solution with concentration ranging from 0.25 to 6 g/L was prepared by dissolving different amount of lysozyme in 25 mM sodium bicarbonate buffer with a pH of 11. In each experiment, 20 mg of the KIT-6 adsorbent was suspended in 4g of the respective lysozyme solution. The resulting mixture was continuously shaken in a shaking bath with a speed of 160 shakes per minute at 20 °C until equilibrium was reached (typically 96 h). The amount of lysozyme adsorbed was calculated by substracting the amount found in the supernatant liquid after adsorption from the amount of lysozyme present before addition of the adsorbent by UV absorption at 281.5 nm. Calibration experiments were done separately before each set of measurements with lysozyme solutions of different concentrations. Centrifugation prior to the analysis was used to avoid potential interference from suspended scattering particles in the UV-vis analysis.

3. RESULTS AND DISCUSSION

3.1 Characterization of the adsorbent

Figure 1 shows the powder X-ray diffraction pattern of KIT-6 molecular sieve. The sample exhibits a sharp peak at a lower angle and two higher order peaks which can be assigned to (211), (220) and (321) reflections of three dimensional mesoporous silica with *Ia3d* space group. The three dimensional structure of KIT-6 was also confirmed by high resolution transmission electron microscopic investigation. It should also be mentioned that the XRD pattern of the material is almost similar to that of KIT-6 reported in the literature. Nitrogen adsorption isotherm of KIT-6 is shown in the Figure 2. As can be seen, the material possesses type IV isotherm with a sharp capillary condensation step at p/p_o of ~ 0.65-0.8, characteristic condensation of nitrogen in uniform mesoporous materials.

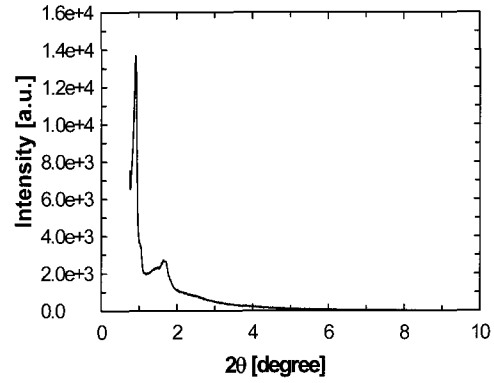

Fig. 1 X-ray diffraction pattern of KIT-6

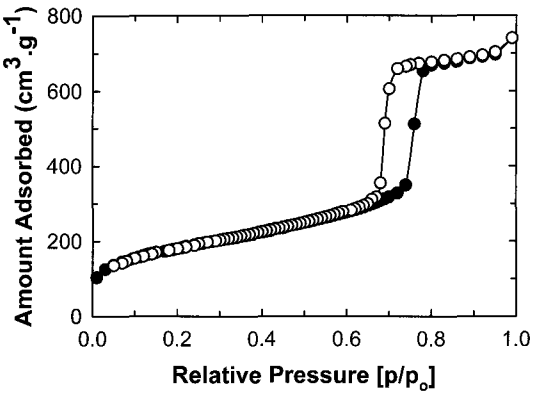

Fig. 2 Nitrogen adsorption/desorption isotherm of KIT-6

The wide H2 type hysteresis loop and the well defined steepness of the isotherm indicate the presence of large and uniform cage type structure similar to those reported in cage type mesoporous in the literature [19]. The surface area, pore volume and pore diameter of the material is found

to be 654 m^2/g, 1.145 cm^3/g and 7 nm respectively.

3.2 Adsorption of lysozyme

The adsorption isotherm of lysozyme on KIT-6 at pH 11 is shown in Figure 3. It has been observed that isotherm shows a sharp initial rise suggesting a high affinity between lysozyme and the adsorbent surface. Finally, the isotherm reaches a plateau (type L (Langmuir) isotherm). The solid line in the figure represents a fit of the experimental data by employing the Langmuir model. The monolayer adsorption capacity was calculated by using the Langmuir equation.

$$n_s = K\, n_m c/(1+Kc)$$

where K is the Langmuir constant, c is the lysozyme concentration, n_m is the monolayer adsorption capacity, and n_s is the amount of lysozyme adsorbed on the adsorbent.

Figure shows that the amount of lysozyme adsorption is increased with increasing the final solution concentration because the lysozyme molecule may adsorb on the solid support in various distinct orientations. At low bulk protein solution concentration, the prolate spheroid lysozyme molecule may be adsorbed with a side-on-type configuration perpendicular to the silica surface. On the other hand, when the bulk protein solution concentration is high, the protein molecules may be adsorbed with an end-on-type configuration that helps the molecules land close to each other with the long axis to reduce the increasing electrostatic repulsion protein molecules, resulting in a higher amount of lysozyme adsorption. Moreover, the high bulk concentration of lysozyme helps the close packing because of the decreased hydrophobic interactions between the protein and the mesoporous silica surface, mainly siloxane bridges, upon adsorption of some lysozyme molecule on the adsorbent surface.

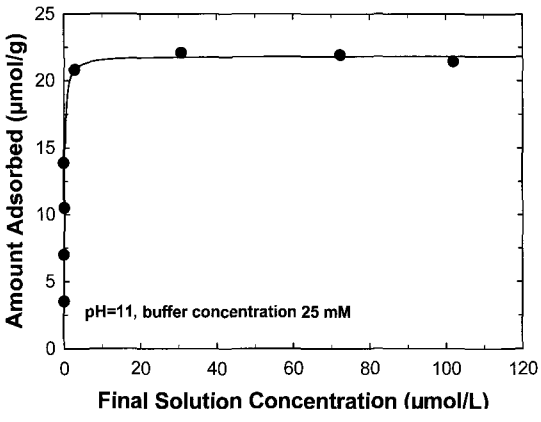

Fig. 3 Adsorption isotherm of lysozyme on KIT-6

The solution pH of 11 was used in the adsorption experiment because the isoelectric point of lysozyme is very close to that value where the net charge of the protein is low and coulombic repulsive force between the protein molecules is minimal. As a consequence, the protein molecules are tightly packed inside cage of KIT-6 adsorbent. The amount of protein adsorbed over KIT-6 is found to be 22.1μmol/g. It has been also found that the amount of lysozyme adsorbed over KIT-6 is much higher than that of MCM-48 which possesses three dimensional mesoporous structure with small mesopores. This could be mainly due to the fact that the pore size of KIT-6 is much larger than that of MCM-48 [16,18].

4. CONCLUSION

The three dimensional large pore cage type mesoporous silica (KIT-6) has been synthesized and characterized by XRD and N_2 adsorption. Adsorption of lysozyme has been done on KIT-6 as a model protein adsorption system at the solution pH of 11, where the net charge of the protein is low and coulombic repulsive force between the protein molecules is minimal.

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