Adsorption and Release Properties of Bovine Serum Albumin on SBA-15 Nanoparticles Functionalized with Aminosilanes

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INTRODUCTION

The mesoporous silica materials have played an important role in the fields of catalysis, separation, sensor design, nano-science and drug delivery system during the last decade [1,2]. In 1998, Stucky group synthesized the Santa Barbara Amorphous (SBA) materials by using neutral triblock template agents [3]. Among these SBA materials, SBA-15 attracted researcher's attention due to its prominent property. It has a narrow pore size distribution, high surface area and relatively high hydrothermal stability [4]. Furthermore, the pore size of SBA-15 can be tuned by using swelling agents or controlling the aging time and temperature. As a result, SBA-15 is utility material for separation large biomolecules such as proteins and enzymes [5]. Modified silica materials with amine or other organic moieties have been widely used as fixed phases in high performance liquid chromatography (HPLC), as adsorbents for the removal of organic compounds or metal ions from various sources and as catalysts. In some previous studies, multi-amine-functionalized mesoporous silicas have been prepared and used to adsorb heavy metal ions and carbon dioxide. There are two ways, including post-synthesis (grafting) and direct synthesis (co-condensation) methods to incorporate functional group into the mesoporous matrix [6].

In this study, we controlled the pore size of SBA-15 by using swelling agent at different conditions of aging such as temperature and time. Multi-amine-grafted mesoporous silicas SBA-15 were prepared by attaching 3-aminopropyltriethoxysilane, *N*-2(-aminoethyl)-3-aminopropyltrimethoxysilane and (3-trimethoxysilylpropyl) diethylenetriamine via post-synthesis method. The adsorption properties for Bovine Serum Albumin (BSA) such as equilibrium and kinetics were investigated. Moreover, the release study of initial samples and modified samples also were carried out under neutral pH condition.

EXPERIMENTAL

SBA-15 was synthesized by a procedure similar to that described previously {7}. An initial gel formation was done under acidic conditions using Pluronic P123 triblock copolymer as a structure-directing agent and tetraethyl orthosilicate (TEOS) as a silica source. In the specific procedure, dissolve completely 4 g of P123 in 104 g of deionized water and 20 mL of fuming HCl (37%) added under stirring condition for 2 h. TMB was used, in an appropriate ratio with the surfactant, as a swelling agent for increasing the pore size. Next, 8.56 g of TEOS was added drop wise and the mixture was vigorously stirred for 24 h at 35 °C. The synthesized gel was then transferred into Teflon-sealed autoclave at different temperatures and periods of time depending on targeted pore size. The solid products were filtered, washed with distilled water repeatedly and dried at room temperature overnight. The powdery SBA-15 was obtained by calcination for 6 h in ambient air from room temperature to 550 °C, with

a heating rate of 1 °C/min. Modified mesoporous materials with aminopropyl groups have alternatively been prepared by refluxing freshly activated mesoporous silica in toluene solution containing aminosilane. 5.0 g of calcined SBA-15, which was previously dried at 398 K for 6 hours in air, was refluxed in the toluene solution of aminosilane (1,7%, 250 mL) at 383 K for 24 hours under an Ar flow. The amine-functionalized SBA-15 was collected by filtration, washed with dry toluene, and dried at 333 K overnight. These materials are designated as APTES-SBA-15 (P1), AEAPS-SBA-15 (P2) and TA-SBA-15 (P3), where APTES, AEAPS and TA are aminopropyltriethoxysilane, *N*-2(-aminoethyl)-3-aminopropyltrimethoxysilane, and (3-trimethoxysilylpropyl)ethylenetriamine, respectively.

The initial and modified SBA-15 samples were characterized by XRD, nitrogen adsorption and desorption analysis, SEM, TEM and FT-IR. Protein adsorption was measured by the classical batch equilibration method. Batch adsorption experiments were carried out by contacting 50 mg of SBA-15 with 10 mL of different protein concentrations in buffer solution at 25°C. The adsorbent and solution were sealed and kept in a shaking incubator at 250 rpm for 24 h. The supernatant was diluted in a buffer and then filtered through a 0.2 μm HT Tuffryn low protein binding membrane filters. The concentration of BSA in the supernatant was analyzed on a UV spectrophotometer with wavelength of 280nm. For release experiments, 50 mg of treated SBA-15 was soaked in 10 mL of phosphate buffer solutions pH 7 under stirring with a speed of 250 rpm at 37°C for 3 days. The cumulative release amount was determined by a mass balance equation similar to the previous section.

RESULS AND DISCUSSION

The synthesized SBA-15 samples P, P1, P2 and P3 were characterized by XRD. The first diffraction peak is clearly observed in P, which could be indexed as (100) diffraction peak associated with *p6mm* hexagonal symmetry. For P1, P2 and P3, the intensities decrease drastically compared to that of P because of incorporating amine group onto the original sample P. After attaching the amine functional groups onto the silica surface, the XRD patterns changed. No peak is found in the XRD patterns of P1, P2 and P3. According to the previous studies, the XRD pattern of conventional was obtained from 1° to 7° of 2theta [3]. In this work, we want to get target sample with the large pore size by controlling aging conditions and using swelling agent. Hence, the peak of sample P was changed a little in position, but it still characterize the *p6mm* hexagonal symmetry. The pore size distribution and the nitrogen physisorption isotherms of original SBA-15 sample P gives a typical irreversible type IV isotherm with a H_1 hysteresis loop as defined by IUPAC. The nitrogen adsorption at low relative pressures (P/P_o<0.1) is accounted for by monolayer adsorption of nitrogen on the pore walls, and does not necessarily imply the presence of micropores. The sharp inflection in the P/P_o range from 0.6 to 0.8 of the isotherm is characteristic of capillary condensation within uniform mesopores, the position of which is clearly related to a diameter in the mesopore range. The pore volume and the surface area of the amine-grafted samples significantly decrease as compared with that of original SBA-15. The more amino groups or longer chain the silane coupling agents have, the smaller the pore diameter is and the lower surface area and pore volume are. The BET surface area and average pore volume of SBA-15 sample

are 658 m^2 g^{-1} and 1.08 cm^3 g^{-1}(not shown in this paper).

Because the pI of BSA is around 4.8, the protein adsorption experiment was carried out on samples at 293 K and pH value of 4.8. The iso electric point is the pH value in solution at which the sum of charges on the protein is zero. The protein BSA molecular is positively charged at a pH below the pI. On the contrary, it is negatively charged at a pH above the pI. The physical properties of BSA are listed in Table 1.

Table 1. Physical properties of Bovine Serum Albumin

UV analysis (nm)	317
Molecular weight	69,000
Isoelectric point	4.7 - 4.9
Molecular size (Å)	40 x 40 x 140

In order to investigate the properties of protein adsorption on SBA-15, the isotherm model Langmuir was applied. The isotherm parameters were determined by minimizing the mean percentage deviations between experimental and predicted amountadsorbed, based on a modified Levenberg-Marquardt method (IMSL routine DUNSLF). The object function, $E(\%)$, represents the average percent deviation between experimental and predicted results as follows:

$$E(\%) = \frac{100}{n}\sum_{k=1}^{n}\left[\frac{\left|q_{\exp,k} - q_{cal,k}\right|}{q_{\exp,k}}\right] \quad (1)$$

In equation (1), n is the number of experimental data, $q_{\exp,k}$ is the experimental adsorption capacity, and $q_{cal,k}$is the calculated adsorption capacity. The isotherm data were fitted with Langmuir equation very well. Figure 1 shows the adsorption isotherms of BSA at 293 K. The sample which has the highest adsorption capacity for BSA is the original sample P due to its largest pore size and internal surface area. After attaching of functional groups onto surface, the adsorption amounts of P1, P2 and P3 were decreased due to a significant decrease in pore size and surface area. The BSA adsorption capacity is decreased with increase in amine group amount attaching onto original SBA-15 surface. The P3 sample has the lowest adsorption capacity at the same condition considered with others. Compared with some previous reports, although the pore size of P is smaller than that of their samples, the adsorption capacity of P is very high because of the large surface area. The interactions between BSA and SBA-15 sample P are both chemical bond and physical bond due to the presence of hydroxyl groups on the surface of SBA-15 and the functional groups of proteins. After modification, the hydroxyl groups on surface of silica are partially lost because of high temperature hydrothermal and calcinations treatment. Moreover, the attachment of nonpolar and hydrophobic methyl groups in grafted chains made the surface of SBA-15 more hydrophobic and a decrease in pore size. Hence, the amount of proteins adsorbed on the amine-modified sample is reduced in comparison with to that before modification [5].

Figure 2 shows the release amount of BSA for all SBA-15 samples. Although the adsorption capacity of P was highest among all samples, the amount released by P1, P2 and P3 was higher than that of P in neutral solution. According to the results of Song et al, the release rate of BSA from amine-modified SBA-15 via post synthesis was very high and all the adsorbed protein could be released completely after 3 hours. Most of protein molecules adsorbed on the external surface of SBA-15 could be easily desorbed.

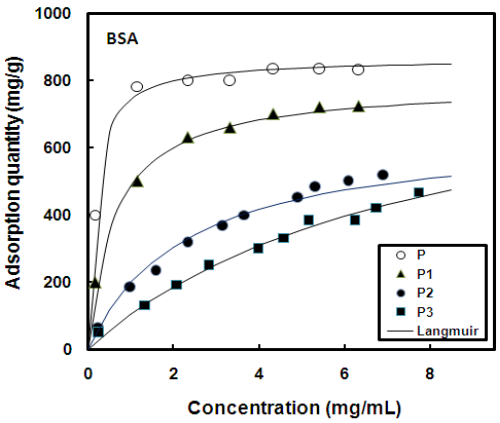

Fig. 1. Adsorption isotherms of BSA at 293 K

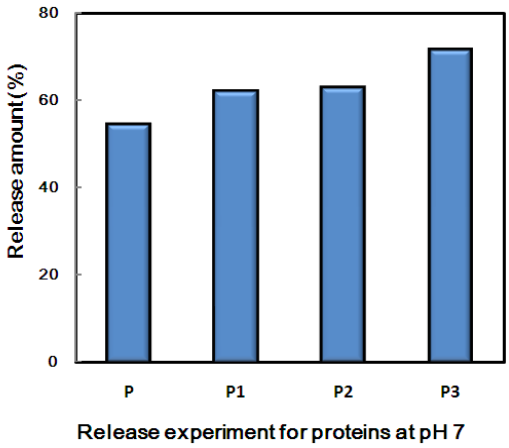

Fig. 2. Release experiment of BSA

CONCLUSION

In this study, we have made the SBA-15 sample with the large pore size by using swelling agent under certain conditions, i.e. at high aging temperature and prolonged aging time. In addition, the surface characteristics of SBA-15 can be modified by incorporating amine functional groups via post synthesis method. The adsorption equilibrium of BSA on conventional and amine-modified SBA-15 reaches the maximum value at the pI of protein because the lateral repulsion between adsorbed proteins is minimal. The original SBA-15 has the highest adsorption capacity for BSA due to its largest pore size and internal surface area. The isotherm data was fitted very well with Langmuir equation. It was also found that the adsorbed protein can be readily desorbed in the neutral solution on amine-modified samples. Thus, the modified samples can be applied for controlled drug delivery system.

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