Abstract The encapsulation and immobilization of biomolecules on solid materials, mesoporous SBA-15, have been studied. Highly ordered hexagonal mesoporous silicates (MPS) with different mean pore sizes have been synthesized using two kinds of triblock copolymers (P123 or L123) as a template. The mixture of tetraethyl orthosilicate (TEOS) and the triblock copolymer (poly(ethylene glycol)-poly(propylene glycol)-poly(ethylene glycol)) was stirred and hydrothermally treated at various temperatures to form the MPS structure. Higher temperatures during stirring and hydrothermal treatment led to the enlargement of pore size. UV-spectrometry was performed to determine the amount of bovine serum albumin (BSA) encapsulated in MPS. It was found that the adsorption processes of BSA on/in MPS could be well described by intraparticle diffusion model.

Keywords apatite; hydrogel; protein; release; cake; PBS

1 Introduction

Mesoporous silicates (MPS), which have ordered mesoscale pores, have been the subject of much interest in the applications of molecular sieve, catalyst, adsorbent and sensor due to their orientation and uniformity. There have been various developed methods to control the pore size in the sol-gel synthesis system using the nonionic surfactant as a template [4]. Pore diameters of 1.5–30 nm of the periodic mesoporous materials are close to the diameters of target molecules and the enclosure of the protein in a well-defined space may help to prevent denaturation. It is reported that the adsorption capacity of protein on the MPS materials is dependent of electrostatic interactions and protein and pore size [1]. But there remains the subject to be solved concerning the relations between the adsorption capacity and the powder characteristics. Uniformity of the pores of mesoporous materials may allow control of molecular adsorption based on size relation between mesopores and molecular dimensions of biomolecules. If the pore size of mesoporous materials is smaller than the molecular size, internal loading of biomolecules should be less. Generally the pore diameter at least three times the molecular diameter enables full access to the internal pores of mesoporous materials.

In the present work, MPS, which is highly ordered hexagonal mesoporous silica material with different mean pore sizes, specific surface areas and particle sizes, has been synthesized using two kinds of triblock copolymers as a template. The mixture of tetraethyl orthosilicate (TEOS) and triblock copolymer (poly(ethylene glycol)-poly(propylene glycol)-poly(ethylene glycol)) was stirred and hydrothermally treated at various temperatures to form the MPS structure. Then, adsorption of bovine serum albumin (BSA) onto as-synthesized MPS materials was analyzed using UV-spectrometry. Analysis for BSA adsorption to MPS was also investigated.

2 Materials and methods

2.1 Preparation of mesoporous materials

MPS was prepared using triblock copolymer, PEO$_{20}$PPO$_{70}$ PEO$_{20}$ (P123, Mw = 5,800) or PEO$_{5}$PPO$_{68}$PEO$_{5}$ (L123, Mw = 4,400), TEOS (Wako Pure Chemical Ind., Japan). In a typical synthesis, 2.0 g of P123 or L123 was dissolved in 60 g of 2M HCl (Wako Pure Chemical Ind., Japan) with continuous stirring at different temperatures varying from 35 ℃ to 50 ℃. Then, 1,3,5-trimethylbenzene (TMB; Wako Pure Chemical Ind., Japan) varying from 0.6 g to 1.2 g and 4.25 g of TEOS were added into the solution and further stirred for 1 h. The solution was hydrothermally treated at 100 ℃ for 24 h after stirring at each temperature for 24 h. The product was filtered, dried at 25 ℃, 50 ℃ or 80 ℃ for 24 h and calcined. The temperature was increased up to 550 ℃ with a heating rate at 1 ℃/min and kept at 550 ℃ for 6 h.

2.2 Characterization

Prepared MPS materials were characterized for d-spacing by powder X-ray diffraction (XRD; RINT2000, Rigaku Co., Japan) and for Brunauer-Emmett-Teller (BET) surface area and Berrett-Joyner-Halenda (BJH) pore size distribution by AUTOSORB-1 (Yuasa Ionics Inc., Japan). The specific surface areas of the samples were calculated from the
Table 1: Synthesis conditions and properties of MPS materials using P123.

| Sample | Stirring Temp. [°C] | Hydro-thermal Temp. [°C] | Drying Temp. [°C] | d(100) [nm] | a₀ [nm] | Pore size [nm] | BET surface area [m²/g] | Pore volume [cc/g] |
|--------|---------------------|-------------------------|------------------|-------------|---------|---------------|----------------------|-----------------|
| A-1    | 35                  | 100                     | 80               | 10.1        | 11.7    | 8.9           | 670                  | 1.02            |
| A-2    | 40                  | 100                     | 80               | 10.2        | 11.8    | 8.9           | 649                  | 1.12            |
| A-3    | 45                  | 100                     | 80               | 10.2        | 11.8    | 8.9           | 638                  | 0.97            |
| A-4    | 50                  | 100                     | 80               | 10.2        | 11.8    | 8.9           | 625                  | 0.93            |
| A-5    | 35                  | —                       | 80               | 8.77        | 10.1    | 5.3           | 458                  | 0.50            |
| A-6    | 35                  | —                       | 50               | 8.69        | 10.0    | 5.3           | 392                  | 0.42            |
| A-7    | 35                  | —                       | 25(RT)           | 8.77        | 10.1    | 6.2           | 486                  | 0.54            |

Table 2: Synthesis conditions and properties of MPS materials using L123.

| Sample | L121/TMB | Stirring Temp. [°C] | Hydro-thermal Temp. [°C] | Pore size [nm] | BET surface area [m²/g] | Pore volume [cc/g] |
|--------|----------|---------------------|-------------------------|---------------|----------------------|-----------------|
| B-1    | 2/0      | 35                  | 100                     | 9.4           | 530                  | 1.01            |
| B-2    | 2/0      | 40                  | 100                     | 10.4          | 538                  | 1.17            |
| B-3    | 2/0      | 45                  | 100                     | 14.5          | 489                  | 1.30            |
| B-4    | 2/0      | 50                  | 80                      | 8.7           | 616                  | 0.96            |
| B-5    | 2/0.5    | 35                  | 80                      | 12.6          | 568                  | 0.89            |
| B-6    | 2/1.2    | 35                  | 80                      | 15.9          | 461                  | 0.79            |

Figure 1: XRD pattern of MPS using L123.

2.3 Protein encapsulation
An amount of 50 mg of MPS and 50 ml of BSA (Wako Pure Chemical Ind., Japan) was put into phosphate buffer saline (Wako Pure Chemical Ind., Japan) in a tightly sealed bottle and cooled at 4 °C for 24 h statically. And then, the solution was filtered to remove SBA-15 and adsorbed BSA. An amount of BSA encapsulated onto SBA-15 was determined by absorbance of the solution at wavelength 279 nm using UV-spectrometry (JASCO V-500, JASCO, Japan).

2.4 Analysis for protein adsorption to MPS
The adsorption isotherm showed that the adsorption of BSA in/on MPS departed from the Langmuir and Freundlich equations. The pseudo-first order, pseudo-second order and intraparticle diffusion models were used to fit the experimental data [2].

3 Results and discussion
3.1 Materials
Tables 1 and 2 show the specific surface area, mean pore size and d-spacing of synthesized MPS using P123 or L123. The XRD pattern for MPS using L123 is shown in Figure 1. This shows a sharp peak with d(100) spacing of 10.1 nm and two weak peaks with d(110) and d(200) spacing. The d-spacing of each line shows that this material has lamella structure, although the MPS using P123 is 2d hexagonal structure. The BET surface areas for all materials are high and over 400 m²/g, and those of the materials synthesized by hydrothermal treatment (A-1–A-4) are higher than those of the materials without hydrothermal treatment (A-5–A-7). It shows that higher temperatures during stirring led to the decrease of BET surface area. As mentioned above, hydrothermal treatment increased pore volume. Therefore, the corresponding BET surface area is also increased.

3.2 Protein encapsulation
Previously we reported that the amount of adsorbed BSA was increased with an increase of the specific surface area.
of MPS materials but was not dependent on the pore size [2]. Katiyar et al. [3] have recently shown that an interpretation of the protein adsorption capacity must factor in the specific external surface area of mesoporous materials. In this study, the MPS materials having pores larger than the size of BSA (14 nm) resulted in a strong rise of adsorbed amount of BSA, and the adsorbed amount of BSA on/in MPS with pores smaller than 14 nm is in proportion to the pore size volume of MPS. The pore volume of mesopores over 1.5 times the molecular diameter show a close correlation with the quantity of adsorbed protein.

Figure 2 shows the plot between the measured and modeled time profiles for the adsorption of BSA. The standard deviations of the differences between the experimental data and the modeled data were estimated. The adsorption processes of BSA on/in MPS could be well described by intraparticle diffusion model. The first rapid increase is the external surface adsorption or instantaneous adsorption stage. The second portion is the gradual adsorption stage, where the intraparticle diffusion is rate-controlled. The third portion is the final equilibrium stage, where the “inside mesopores” diffusion starts to slow down [2]. The rate of mass transport processes of BSA on/in MPS can be interpreted using this model, but a further study should be done.

4 Summary

Mesoporous silicates (MPS) materials with different pore sizes have been synthesized using P123 or L123 and TEOS. A hydrothermal treatment process led to the enlargement of pore size and pore volume of materials. The MPS materials having pores larger than the size of BSA (14 nm) resulted in a strong rise of adsorbed amount of BSA and the adsorbed amount of BSA on/in MPS with pores smaller than 14 nm is in proportion to the pore size volume of MPS. The pore volume of mesopores over 1.5 times the molecular diameter shows a close correlation with the quantity of adsorbed protein. The adsorption processes of BSA on/in MPS could be well described by intraparticle diffusion model.

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References

[1] L. Ji, A. Katiyar, N. G. Pinto, M. Jaroniec, and P. G. Smirniotis, Al-MCM-41 sorbents for bovine serum albumin: relation between Al content and performance, Microporous Mesoporous Mater, 75 (2004), 221–229.
[2] Q. Sun and L. Yang, The adsorption of basic dyes from aqueous solution on modified peat-resin particle, Water Res, 37 (2003), 1535–1544.
[3] Y. Yokogawa, T. Toma, A. Nakamura, and I. Kishida, A study of mesoporous composite materials synthesis as a carrier of biomolecules, Arch Bioceram Res, 7 (2007), 235–238.
[4] D. Zhao, Q. Huo, J. Feng, B. F. Chmelka, and G. D. Stucky, Nonionic triblock and star diblock copolymer and oligomeric surfactant syntheses of highly ordered, hydrothermally stable, mesoporous silica structures, J Am Chem Soc, 120 (1998), 6024–6036.