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Fabrication of Mesoporous Carbon Materials as Adsorbents for Biomolecules

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Adsorption of lysozyme from buffered solution to mesoporous carbon materials with various pore diameters (CMK-1, CMK-3, CMK-3-130 and CMK-3-150) at different pHs (from pH = 6.5 to 12) has been studied. The maximum adsorption was observed near the isoelectric point of the lysozyme (pI ≈ 11), suggesting that suppression of electric repulsion between the enzymes plays an important factor in the adsorption process. The amount of adsorbed lysozyme also depends on the specific pore volume and pore diameter of the adsorbents. IR spectroscopic studies confirmed that the lysozyme used in this study is stable even after the adsorption on the mesoporous carbon. Adsorption behaviors of small biomolecules to the mesoporous carbons have been similarly investigated.

Key words: mesoporous carbon, lysozyme, Langmuir adsorption, isoelectric point, amino acid

1. INTRODUCTION

In structures controlled with nanometer-size precision, physical interaction and chemical reaction would have characteristics highly influenced by restricted molecular motion and orientation. Upon controlling such structures, chemical conversion and information processing with excellent efficiency and selectivity could be achieved. Immobilization of functional elements within nanostructured materials is the one of the most promising approaches along this concept. Biomelecules such as proteins and DNA show sophisticated functions and attractive candidates to be immobilized in the nanostructures. For examples, some kinds of enzymes have been immobilized onto nanometer-size ultrathin films such as Langmuir-Blodgett films[1,2] and layer-by-layer films[3,4] in order to fabricate sensors and microreactors.

Among various nanostructured materials, mesoporous silica with highly regular pore geometries has high potential in practical applications based on their huge surface area and high reliability of pore dimensions.[5] In addition, this kind of materials is easily removed from solutions by filtration or ultracentrifuge, which can be therefore regarded as environmentally friendly process. Several attempts to immobilize biomaterials such as peptides and proteins to mesoporous silica materials have been proposed.[6-9] Recently, Vinu et al. successfully immobilised of cytochrome c and lysozyme over various mesoporous silica molecular sieves.[8,9] Unfortunately, this approach might not be generally applicable to all the proteins, because strong electrostatic interactions between the surface silanol groups and proteins may seriously denature the structure of the proteins. In addition, the structural stability of the mesoporous silica adsorbent under aqueous condition is relatively poor due to the hydrolysis of their siloxane bridges. Therefore, mesoporous materials, which are much stabler than mesoporous silica, have to be applied for the immobilization of biomolecules.

As a new category of the materials, mesoporous carbon has been also paid much attention due to their applications in many areas, such as gas separation, adsorption of small gas molecules, catalysis, energy storage and capacitors.[10] These porous carbons are generally prepared through carbonization of carbon source using mesoporous silica as removable replica (see Figure 1A). They have also no charge on their surface and are highly tolerant in aqueous environment compared with silica materials. Therefore, the mesoporous carbon can be a promising candidate as a practical support for biomaterial immobilization. In spite of such potential capability of the mesoporous carbon materials, immobilization of biomaterials on this support is still primitive stage in research.

In this research, we have demonstrated superior ability of mesoporous carbon materials for immobilization of biomolecles such as a large protein (lysozyme) and a small amino acid (L-histidine).[11,12] The obtained results revealed that the adsorption behaviors of the biomaterials

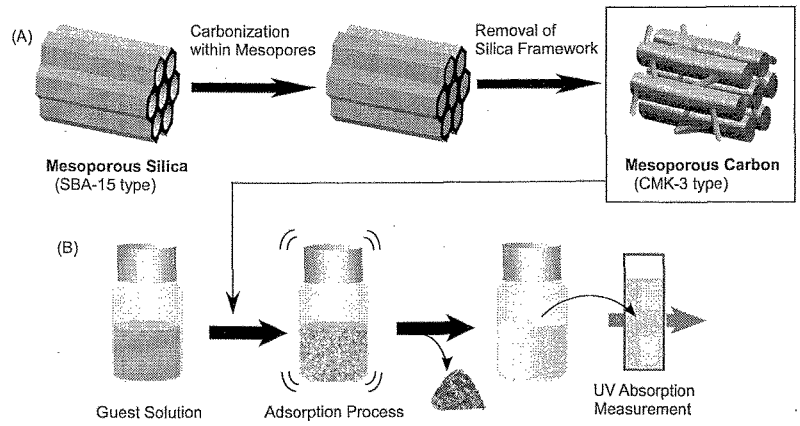

Figure 1 Schmatic illustration of outline of this research: (A) preparation of mesoporous carbon; (B) adsorption study.

onto mesoporous carbon materials depend on the solution pH as well as the specific pore volume and the pore diameter of the adsorbent.

2. EXPERIMENTAL

2.1 Materials

Potassium phosphate, sodium carbonate, potassium chloride, sodium bicarbonate, potassium dihydrogen phosphate, and sodium hydroxide used for buffer were purchased from Wako Pure Chem. Tri-block copolymer $EO_{20}PO_{70}EO_{20}$ (Pluronic P123, molecular weight 5800), where EO and PO represent ethylene oxide unit and propylene oxide unit respectively, tetraethyl orthosilicate, and sucrose were obtained from Aldrich. Chicken egg white lysozyme (E.C. 3.2.1.17) was obtained from Sigma and used without further purification. L-Histidine was purchased from Peptide Institute Incorporated. Activated carbon was supplied by Alfa Aesar.

2.2 Synthesis of mesoporous carbon[11]

Mesoporous silica SBA-15-X (X denotes the synthesis temperature of the silica material) were used as replica materials for synthesis of mesoporous carbons with different pore diameters (CMK-3-X). Typically, 1 g of replica (mesoporous silica material, SBA-15-X) was added to a solution obtained by dissolving 1.25 g of sucrose (carbon source) and 0.14 g of H_2SO_4 in 5 g of water, and keeping the mixture in an oven for 6 h at 100 °C. Temperature was subsequently raised to 160 °C for another 6 h. For completion of polymerization and carbonization of sucrose inside the silica mesopores, 0.8 g of sucrose, 0.09 g of H_2SO_4 and 5 g of water were further added to the pre-treated sample. The mixture was again subjected to the thermal treatment described above. The nanocomposites of silica and polymer were then pyrolyzed in a nitrogen flow at 877 °C and kept under these conditions for 6 h to carbonize the polymer. The mesoporous carbon obtained by dissolution of the silica framework in 5 wt % hydrofluoric acid was washed several times with ethanol and dried at 120 °C. CMK-1 was similarly synthesized by using MCM-48 as a replica.

2.3 Characterization

The powder X-ray diffraction (XRD) patterns of mesoporous carbon materials were collected on a Rigaku diffractometer using CuKα (λ = 0.154 nm) radiation. Nitrogen adsorption and desorption isotherms were measured at -196 °C on a Quantachrome Autosorb 1 sorption analyzer. The specific surface area was calculated using the Brunauer-Emmett-Teller (BET) method. The pore size distributions were obtained from the adsorption and desorption branch of the nitrogen isotherms by Barrett-Joyner-Halenda (BJH) method. FT-IR spectra were recorded on a Nicolet Nexus 670 instrument.

2.4 Adsorption study

Adsorption studies of biomolecules to mesoporous carbon materials are conceptually illustrated in Figure 1B. A series of standard lysozyme solutions was prepared by dissolving different amounts of lysozyme in 25 mM buffer solutions (pH = 6.5 potassium phosphate buffer, pH = 9.6, 10.5, and 12 sodium bicarbonate buffer). In each adsorption experiment, 20 mg of the different mesoporous adsorbents were suspended in 4 g of the respective lysozyme solution. The resulting mixture was continuously shaken in a shaking bath with a speed of 160 shakes/minute at 20 °C until equilibrium was reached (typically 96 h). The amount of lysozyme adsorbed was measured by UV absorption at 281.5 nm. Adsorption of L-histidine was similarly evaluated.

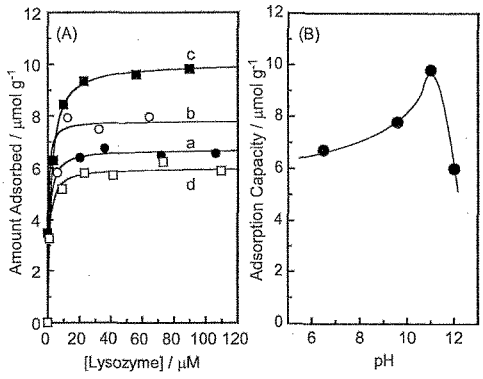

Figure 2 (A) Adsorption isotherms of lysozyme on CMK-3 at pH 6.5 (a), 9.6 (b), 11 (c), and 12 (d). (B) A pH profile of adsorption capacity of lysozyme to CMK-3.

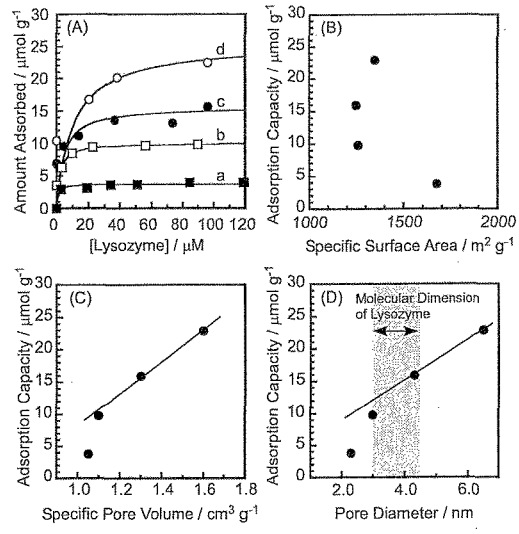

Figure 3 (A) Adsorption isotherms of lysozyme at pH 11 to various mesoporous carbon: (a) CMK-1; (b) CMK-3; (c) CMK-3-130; (d) CMK-3-150. Plots of adsorption capacity of lysozyme to mesoporous carbon materials as functions of surface area (B), pore volume (C), and pore diameter (D).

3. RESULTS AND DISCUSSION

3.1 Effect of electrostatic interaction

The powder XRD patterns of CMK-3 families (CMK-3, CMK-3-130, and CMK-3-150) are assigned to hexagonally ordered mesostructures, as evident from the presence of at least three XRD peaks indexed to (100), (110) and (200) reflections. CMK-1 exhibits three reflections which are indexed to (110), (211) and (220) reflections of the cubic space group $I4_132$.

The adsorption of lysozyme on CMK-3 at different solution pH were first measured (Figure 2A). The obtained isotherms exhibit a sharp initial rise and finally reach a plateau, as denoted type L (Langmuir) isotherm. By employing the Langmuir model, the monolayer adsorption capacity (n_m) was calculated by using the Langmuir equation.

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

In this equation, K, c, and n_s represents the Langmuir constant, the lysozyme concentration, and n_s is the amount of the lysozyme adsorbed on the adsorbent, respectively. The monolayer adsorption capacity significantly changes depending on the solution pH. The pH profile of the adsorption capacity is plotted in Figure 2B. The maximum adsorption of lysozyme amounts (9.8 μmol g^{-1}) was obtained at a pH 11, which is very close to the isoelectric point *p*I of the lysozyme ca. 10.8). The above-mentioned results suggest that suppression of the electrostatic repulsion between the lysozymes would promote the protein adsorption to rather hydrophobic mesoporous carbons from aqueous phase.

3.2 Size exclusion effect

Low-temperature (-196 °C) nitrogen adsorption isotherms allow calculation of specific surface area, specific pore volume and mesopore size distribution. CMK-3-130 and CMK-3-150 were estimated to possess pores of diameters about 4.3 and 6.5 nm with high BET surface areas of 1250 and 1350 $m^2\ g^{-1}$ and large pore volumes of 1.30 and 1.60 $cm^3\ g^{-1}$, respectively. These values are apparently larger than those assigned to CMK-3 (surface area, 1260 $m^2\ g^{-1}$; pore volume, 1.10 $cm^3\ g^{-1}$; pore diameter, 3.0 nm). CMK-1 exhibited type IV isotherm with high uptake at low relative pressure compared to CMK-3 material, indicating the presence of micropores. The specific surface area of CMK-1 (1675 $m^2\ g^{-1}$) is higher than the specific surface area of CMK-3-X materials, although pore volume (1.05 $cm^3\ g^{-1}$) and diameter (2.3 nm).

Figure 3A summarizes the adsorption isotherms of the lysozyme adsorbed on various mesoporous carbons at a solution pH of 11. The adsorption capacity does not show clear relevance with the surface area (Figure 3B). In contrast, positive correlation is obviously recognized between the adsorption capacity and the pore volume (Figure 3C). This relation sounds reasonable from the viewpoint of pore filling by lysozyme. Interestingly, unavoidable negative deviation from the expected line can be detected for the adsorption capacity to CMK-1. This result would be explained by the size exclusion effect at mesoporous media. As summarized in Figure 3D, a smaller pore diameter (2.3 nm for CMK-1) compared with dimensions of the lysozyme (crystallographic dimension of 4.5 x 3 x 3 nm in ellipsoidal[13]) resulted in very small adsorption capacity. Even comparable pore diameter (3.0 nm for CMK-3) is not advantageous for the lysozyme accommodation.

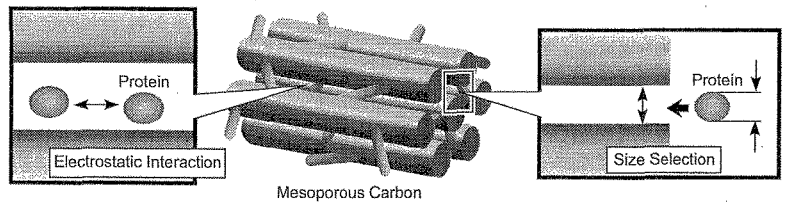

Figure 4 Factors that control adsorption behaviors of biomolecules onto mesoporous carbon materials.

Finally, FT-IR spectra, especially in amide I and amide II regions, were recorded for the lysozyme loaded mesoporous adsorbents CMK-3 to confirm the structural stability of the lysozyme on the mesoporous carbon. The amide band I due to the C=O stretching mode and the amide II band due to the N-H deformation were assigned to 1657 and 1522 cm^{-1}, respectively. The obtained values are very close to those observed for the lysozyme itself (1657 and 1527 cm^{-1}). Intensity ratio between these two bands did not virtually altered upon adsorption of the protein onto the mesoporous carbon. These results indicate the absence of serious denaturation accompanying changes in secondary structures through the adsorption process.

3.3 Adsorption of small biomolecules

In order to investigate adsorption behaviors of small biomolecules to mesoporous media, the adsorption of L-histidine onto CMK-3 mesoporous carbon were similarly investigated at different buffer solution pH ranging from 5 to 9.6. The adsorption capacity increased from pH 5.0 to 7.5 and decreases when the pH is increased to 9.6. The maximum adsorption capacity of L-hystidine was obtained as 1350 μmol g^{-1} at a pH of 7.5, which is close to the isoelectric point of L-hystidine. Near the the isoelectric point, the net charge of L-hystidine is zero and the coulombic repulsive force between the histodine molecules is minimal, and hydrophobic and/or π-π interactions would be enhanced between histidine molecules and/or between histidine and carbon framework.

Adsorption behaviors of L-histidine to activated carbon and mesoporous silica (SBA-15) were evaluated as control experiments. The adsorption capacity at pH 7.5 fell in order of CMK-3 > activated carbon >> SBA-15. Although SBA-15 has a higher mesopore volume and ultra large pore diameter as compared with other adsorbents used in this study, the adsorption capacity of L-histidine is quite low. Hydrophilic nature of the SBA-15 pore surface would be highly disadvantageous for the histidine accommodation. The obtained difference between CMK-3 and SBA-15 strikingly indicates indispensable role of hydrophobic interaction in the adsorption of the histidine to mesoporous medium. Interestingly, the adsorption capacity of the activated carbon is apparently inferior to that of CMK-3, although the activated carbon has the similar surface hydrophobicity and larger surface area. We may find here the importance of mesoporous geometry on filling small molecules. For efficient interactions between adsorbed molecules, wide space in the activated carbon is not probably favorable. In contrast, the guest molecules (histidines) in small mesoporous media can easily interact each other, resulting in more efficient adsorption.

4. CONCLUSION

In this research, mesoporous carbon is proved as a material suitable for adsorption of biomolecules such as large proteins and small amino acids. Figure 4 schematically summarizes factors that affects adsorption amounts. Adsorption capacities are easily tuneable through appropriate selection of solution condition such as pH, pore size, and so on. The obtained knowledge is highly useful for development of novel bioreactors and drug delivery systems.

5. Acknowledgement

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