Mesoporous Silicate Sequestration and Release of Proteins

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Received June 23, 1999

Porous silica materials, which have large surface areas and variable pore diameters, are attractive candidates to host large molecules, including proteins.[1−3,25] Extensive research has been performed on encapsulation of proteins in sol−gels for possible applications for biosensors.[4−6] However, a disadvantage in the use of sol−gels is their variability in pore size,[7] which cannot be tailored to isolate specific proteins. With the recent discovery of the well-ordered hexagonal (SBA-15, pore diameter = 50−150 Å)[8] and mesocellular siliceous foam (MCF, pore diameter = 170−420 Å)[9] mesoporous silica phases prepared with triblock copolymers, in which the phases and narrowly defined pore diameter are tuned by trimethylbenzene (TMB),[10] it is possible to design the selectivity of these hosts to specific protein characteristics, such as size and charge, by varying the pore diameter and by derivatizing the reactive silanol groups with functional organic silanes.[11−13] By varying the pore size from 60 to 160 Å and derivatizing the surface silanol groups with (3-aminopropyl)-triethoxysilane (APTS), 3-$NH_2(CH_2)_3Si(OCH_2CH_3)_3$, we have found it possible to specifically select proteins of different sizes with these mesoporous silicate materials. We report here the sequestration of anionic proteins[14] from aqueous solutions by SBA-15 and MCF, as well as the release of the sequestered proteins by increasing the ionic strength. This is the first utilization of mesoporous silicate materials to sequester and release proteins where both size exclusion and ion-exchange chromatography techniques are employed simultaneously.

Two mesoporous materials, SBA-15 and MCF, with varying pore size were silylated with APTS in toluene under Ar atmosphere, forming APTS-silylated SBA-15 (*d* spacing[15] (100) 87 Å, pore size 59 Å[16,17]) and APTS-silylated MCF (*d* spacing (100) 187 Å, pore size 160 Å). When APTS-silylated MCF (50 mg) reacted with conalbumin (5 mg, λ_{max} 280 nm, pI 6.0, MW 77 000)[18] in 0.005 M phosphate buffer (pH 7.0, 3 mL total volume), the absorbance of conalbumin in the solution decreased over time (typically <30 min; Figure 1 inset), indicating that the APTS-derivatized MCF had sequestered conalbumin from the solution. The conalbumin is tightly associated with the MCF material, since repeated washing with H_2O failed to release any conalbumin. However, when the ionic strength is raised (e.g., 0.5 M phosphate buffer, 3 mL total volume), the protein is fully released (Figure 1).[19] APTS-derivatized SBA-15 does not, however, sequester lysozyme (MW 14 000, pI 10), which would be positively charged at pH 7, demonstrating charge selection (data not shown).[14]

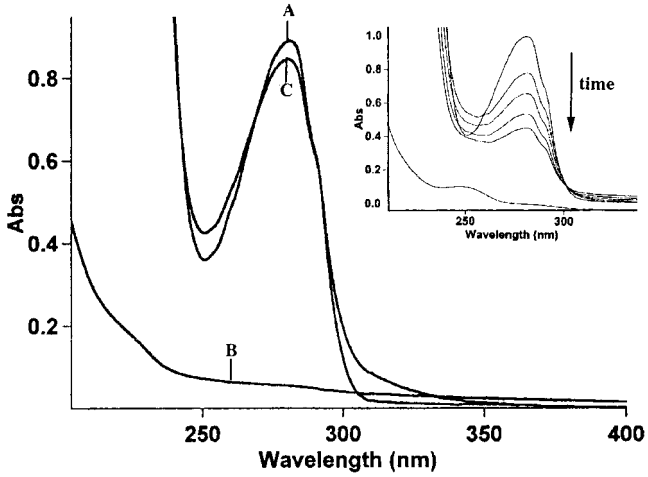

Figure 1. Uv−vis spectra demonstrating the adsorption and release of conalbumin (MW = 77 000, pI 6.0) with APTS-silylated MCF. (A) UV−vis spectrum of 7.8 μM conalbumin in 0.5 M phosphate buffer, pH 7.0; (B) UV−vis spectrum of the solution in part (A) after incubation with APTS-silylated MCF for 60 min; (C) UV−vis spectrum of the solution of conalbumin/APTS-silylated MCF from part (B) after incubation with 0.5 M phosphate, pH 7.0, for 60 min. The inset shows the time course of the adsorption process at ca. 5 min interval.

The size-exclusion properties of the small-pore and large-pore APTS-silylated SBA-15 and MCF were then investigated using three proteins of varying size, with similar isoelectric points: conalbumin (MW 77 000, pI 6.0),[18] chicken egg ovalbumin (MW 44 000, pI 4.9),[20] and soybean trypsin inhibitor protein (MW 14 000, pI 5.2).[21] APTS-silylated SBA-15 (50 mg) and APTS-silylated MCF (50 mg) were allowed to react with stock solutions of the three proteins in 5 mM phosphate buffer (pH 7.0) for 1 h.[22] The APTS-silylated materials were then removed by centrifugation and washed extensively with doubly deionized H_2O, before incubation in 0.5 M phosphate buffer (pH 7.0) for 1 h, to release the sequestered proteins back into solution. SDS PAGE

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(1) Moller, K.; Bein, T. *Chem. Mater*. **1998**, *10*, 2950.
(2) Diaz, J. F.; Balkus, K. J., Jr. *J. Mol. Catal. B: Enzymatic* **1996**, *2*, 115. Gimon-Kinsel, M. E.; Jimenez, V. L.; Washmon, L.; Balkus, K. J., Jr. *Stud. Surf. Sci. Catal.* **1998**, *117*, 373.
(3) Kumar, C. V.; McLendon, G. L. *Chem. Mater*. **1997**, *9*, 863.
(4) Dave, B. C.; Dunn, B.; Valentine, J. S.; Zink, J. I. *Anal. Chem.* **1994**, *66*, 1120A.
(5) Lan, E. H.; Dave, B. C.; Fukuto, J. M.; Dunn, B.; Zink, J. I.; Valentine, J. S. *J. Mater. Chem*. **1999**, *9*, 45.
(6) Chung, K. E.; Lan, E. H.; Davidson, M. S.; Dunn, B. S.; Valentine, J. S.; Zink, J. I. *Anal. Chem.* **1995**, *67*, 1505.
(7) Dave, B. C.; Dunn, B.; Valentine, J. S.; Zink, J. I. *Anal. Chem*. **1994**, *66*, 1120A.
(8) Zhao, D.; Feng, J.; Huo, Q.; Melosh, N.; Fredrickson, G. H.; Chmelka, B. F.; Stucky, G. D. *Science* **1998**, *279*, 548.
(9) Schmidt-Winkel, P.; Lukens, W. W.; Zhao, D.; Yang, P.; Chmelka, B. F.; Stucky, G. D. *J. Am. Chem. Soc.* **1999**, *121*, 254.
(10) Han, Y. J.; Schmidt-Winkel, P.; Yang, P.; Lettow, J. S.; Ying, J. Y.; Chmelka, B. F.; Butler, A.; Stucky, G. D. Manuscript in preparation.
(11) Le Grange, J. D.; Markham, J. L. *Langmuir* **1993**, *9*, 1749.
(12) Shephard, D. S.; Zhou, W.; Maschmeyer, T.; Matters, J. M.; Roper, C. L.; Parsons, S.; Johnson, B. F. G.; Duer, M. J. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2719.
(13) Golander, C.-G.; Kiss, E. *Colloids Surf. A* **1993**, *74*, 217.
(14) Cationic proteins (e.g., lysozyme, pI 10) were reacted as well with SBA-15 derivatized with the anionic organosilane $HOOC-CH_2CH_2-Si(OCH_2CH_3)_3$ which were readily adsorbed into the pores.
(15) Small-angle X-ray analysis was performed on a Scintag PADX diffractometer using Cu Kα radiation detected by a Si(Li) solid-state detector cooled by a Peltier cell.
(16) Pore sizes of the materials were determined by BdB-FHH pore size analysis from the data obtained by nitrogen sorption, carried out on a Micromeritics ASAP 2000 system at 77 K with samples outgassed at 180−200 °C under high vacuum for at least 4 h.
(17) Lukens, W. W.; Schmidt-Winkel, P.; Zhao, D. Y.; Feng, J. L.; Stucky, G. D. *Langmuir* **1999**, *15*, 5403.
(18) Cavatorta, P.; Crippa, P. R.; Tosi, A. M. *Experientia* **1978**, *34*, 849.
(19) Control experiments were performed with underivatized MCF. The underivatized MCF also sequestered conalbumin; however, when the ionic strength was increased (same conditions as the APTS-derivatized MCF), the protein was not released back into solution.
(20) Kidwai, S. A.; Ansari, A. A.; Salahuddin, A. *Biochem. J.* **1976**, *155*, 171.
(21) Kunitz, M. *J. Gen. Physiol*. **1947**, *30*, 311.

10.1021/ja992138r CCC: $18.00
Published on Web 10/13/1999

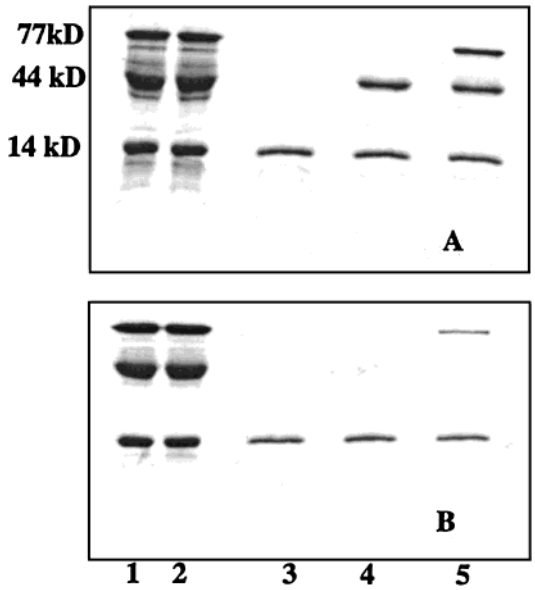

Figure 2. SDS PAGE (12%) of released proteins from (A) APTS-silylated MCF (160 Å) and (B) APTS-silylated SBA-15 (59 Å). Lanes 1 and 2 are a duplicate of the protein standard solutions. Lanes 3, 4, and 5 result from incubations with stock solution 1 (i.e., 3 mg of trypsin inhibitor protein), stock solution 2 (i.e., 3 mg each of trypsin inhibitor protein and ovalbumin), and stock solution 3 (i.e., 3 mg each of inhibitor protein, ovalbumin, and conalbumin), respectively, with MCF and SBA-15.[22] SDS PAGE was also performed on the supernatant of the solution recovered from SBA-15 and stock solution 3 reaction, which showed only the bands corresponding to the two larger proteins.

electrophoresis (Figure 2) of this solution revealed APTS-silylated SBA-15 (59 Å) sequestered and released the smallest protein, trypsin inhibitor (Figure 2B, column 3), but did not sequester ovalbumin (Figure 2B, column 4). A small amount of the largest protein, conalbumin, was sequestered and released by APTS-silylated SBA-15 (Figure 2B, column 5),[23] which may be expected due to the presence of the silylamines on the external surface. The large-pore MCF material adsorbed and released all three proteins (Figure 2A). Thus, in summary, we have demonstrated that SBA-15 and MCF function as combined size-exclusion and ion-exchange solid supports.[24]

Further work is in progress to develop a technique to coat the external surface with nonreactive functional groups (e.g., methylsilanes), before functionalizing the interior pore surfaces with APTS. In addition to matching selectively derivatized mesoporous silicate materials to the size and charge of a protein, as we have demonstrated here, we are pursuing application of these mesoporous materials in enzyme stabilization and enzyme catalysis. Preliminary results show that enzymes sequestered in the pores of these mesoporous materials are active and stable.[24]

Acknowledgment. This work is supported by grants from the Office of Naval Research (00014-98-083 to A.B.) and the National Science Foundation (CHE-9529374 to A.B. and DMR-96 34396 to G.D.S.). This work made use of the MRL Central Facilities supported by the National Science Foundation under Award No. DMR96-32716.

JA992138R

(22) Three protein stock solutions were prepared: Stock solution 1 contained 3 mg of trypsin inhibitor protein; stock solution 2 contained 3 mg of trypsin inhibitor protein and 3 mg of ovalbumin; stock solution 3 contained 3 mg of trypsin inhibitor protein, 3 mg of ovalbumin, and 3 mg of conalbumin, in 9 mL of 5 mM phosphate buffer (pH 7.0).

(23) The staining of the gel was performed with Coomassie Brilliant Blue R-250, which binds to quaternary NH_3^+ groups of the protein electrostatically. Due to the significant weight differences in the proteins used, same molar amount of the proteins would yield very different intensities in the bands observed. To demonstrate the size selectivity of these mesoporous materials, we have worked with same weight amounts of proteins, which allowed similar intensity bands to be observed in the gels.

(24) While trypsin inhibitor protein, conalbumin, or ovalbumin do not have catalytic activities, the enzyme FeHeme chloroperoxidase does function normally, both when sequestered by the silicate materials and after release from these materials; further work on the chloroperoxidase activity is in progress: Han, Y. J.; Watson, J. T.; Stucky, G. D.; Butler, A.

(25) Behrens, P.; Stucky, G. D. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 696.