Hierarchically Imprinted Stationary Phases: Mesoporous Polymer Beads Containing Surface-Confined Binding Sites for Adenine

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Robust molecular recognition elements can be produced by the copolymerization of commodity monomers, for example methacrylic acid, vinylpyridine, and acrylamide, with methacrylate-based cross-linking monomers in the presence of a template molecule.[1] This approach has been used to generate porous materials exhibiting pronounced recognition for a large variety of template structures. Conditions that are optimal for generating the templated binding sites at a molecular level often lead to undesirable properties at the nano- or microscopic level, that is, undesirable pore sizes, surface areas, and swelling properties.[2] Structural control at both length scales is of particular importance for larger template molecules, which can only access the surface of larger mesopores or macropores. Approaches to confine the binding sites to highly accessible domains of the polymer matrix are therefore being assessed.[3−7] One of these consists of the grafting of the polymers to[4] or from[8] porous supports with well-defined particle size and shape, pore systems, and pore size distributions. Another means of confining templated sites to accessible surfaces is through hierarchical templated synthesis.[5−7] Thus, immobilization of a template on the surface of a porous silica mold and polymerization in the mold followed by dissolution of the silica results in a "mirror image" pore system containing binding sites uniquely residing at the surface.[7] Here, we report the first application of hierarchical imprinting to produce methacrylate-based mesoporous beads useful for chromatographic applications.[9] These feature surface-confined binding sites for adenine or triaminopyrimidine, which are also capable of recognizing larger structures containing the template functionality.

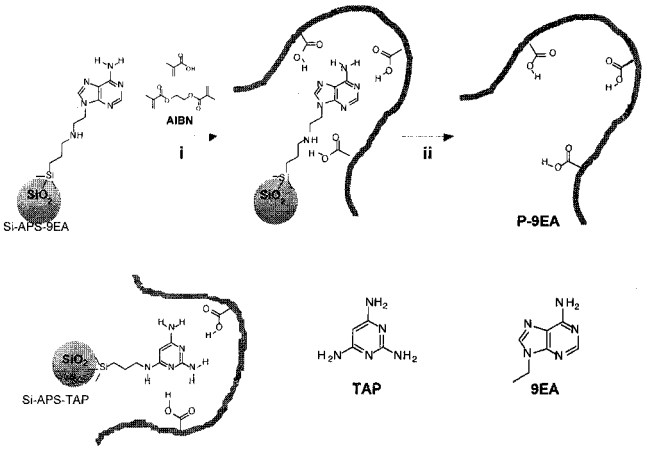

Figure 1. Approach used to prepare surface-confined templated sites for structures containing the adenine or triaminopyrimidine functionality.

The template precursors, 9-(2-bromoethyl)adenine and 6-chloro-2,4-diaminopyrimidine (CDAP), were immobilized by reaction with the amino groups of porous silica particles (11.5-nm average pore diameter) modified with aminopropyl(triethoxy)silane (APS) (Figure 1). Under the employed coupling conditions the coverage of surface template molecules corresponded to a 2% conversion of the reactive silanol groups (8 μmol/m^2) on the surface. This implies that the template molecules are separated by an average distance of ≈3 nm, which should be sufficient for complete separation of the templated sites. The pore system of the silica was completely filled with a mixture of ethyleneglycol dimethacrylate (83%), methacrylic acid (17%), and dissolved initiator (Figure 1(i)) by repeated vacuum-nitrogen purge cycles. Polymerization was then performed by heating the particles to 60 °C over a period of 24 h and the silica template subsequently dissolved by treatment of the composite particles in $(NH_4)HF_2$ solution over a 4-day period (Figure 1(ii)). The resulting mass loss (≈50%) and the elemental composition of the polymers indicated that this treatment successfully removed the majority of the silica template. The resulting polymer particles exhibit a structure and morphology similar to the "mirror image" of the original silica template. Thus, the SEM micrographs show spherical particles with an average diameter close to the original silica particles used as a template (Figure 2A,B).

The high surface area (293 m^2/g for P-TAP) and mesoporosity are also close to those of the precursor particles (350 m^2/g). Particularly striking is the narrow pore size distribution observed, around 8−9 nm, which stands in stark contrast to the broad distribution observed for the conventional bulk materials.[2] Further-

(1) (a) Sellergren, B., Ed. *Molecularly imprinted polymers. Man made mimics of antibodies and their applications in analytical chemistry*; Elsevier Publishers: Amsterdam, 2001; Vol. 23. (b) Haupt, K.; Mosbach, K. *Trends Biotechnol.* **1998**, *16*, 468−475. (c) Sellergren, B. *Angew. Chem., Int. Ed.* **2000**, *39*, 1031−1037.

(2) Sellergren, B.; Shea, K. J. *J. Chromatogr.* **1993**, *635*, 31.

(3) (a) Nakamura, S.; Takeuchi, H.; Nakamura, H.; Maeda, T. M. *Trans. Mater. Res. Soc. Jpn.* **1999**, *24*, 453−456. (b) Markowitz, M. A.; Kust, P. R.; Deng, G.; Schön, P. E.; Dordick, J. S.; Clark, D. S.; Gaber, B. P. *Langmuir* **2000**, *16*, 1759. (c) Piletsky, S. A.; Matuschewski, H.; Schedler, U.; Wilpert, A.; Piletska, E. V.; Thiele, T. A.; Ulbricht, M. *Macromolecules* **2000**, *33*, 3092−3098. (d) Perez, N.; Whitcombe, M. J.; Vulfson, E. N. *Macromolecules* **2001**, *34*, 830−836.

(4) (a) Wulff, G.; Oberkobusch, D.; Minarik, M. *React. Polym., Ion Exch., Sorbents* **1985**, *3*, 261−75. (b) Reinholdsson, P.; Hargitai, T.; Isaksson, R.; Törnell, B. *Angew. Makromol. Chem.* **1991**, *192*, 113. (c) Plunkett, S. D.; Arnold, F. H. *J. Chromatogr., A* **1995**, *708*, 19−29.

(5) Dai, S.; Burleigh, M. C.; Ju, Y. H.; Gao, H. J.; Lin, J. S.; Pennycook, S. J.; Barnes, C. E.; Xue, Z. L. *J. Am. Chem. Soc.* **2000**, *122*, 992−993.

(6) Johnson, S. A.; Ollivier, P. J.; Mallouk, T. E. *Science* **1999**, *283*, 963−965.

(7) Yilmaz, E.; Haupt, K.; Mosbach, K. *Angew. Chem., Int. Ed.* **2000**, *39*, 2115−2118.

(8) Sulitzky, C.; Rückert, B.; Hall, A. J.; Lanza, F.; Unger, K.; Sellergren, B. *Macromolecules*, in press.

(9) This approach was independently used by Yilmaz et al. for the preparation of divinylbenzene-based particles exhibiting selectivity for theophylline in batch equilibrium rebinding experiments (see ref 7).

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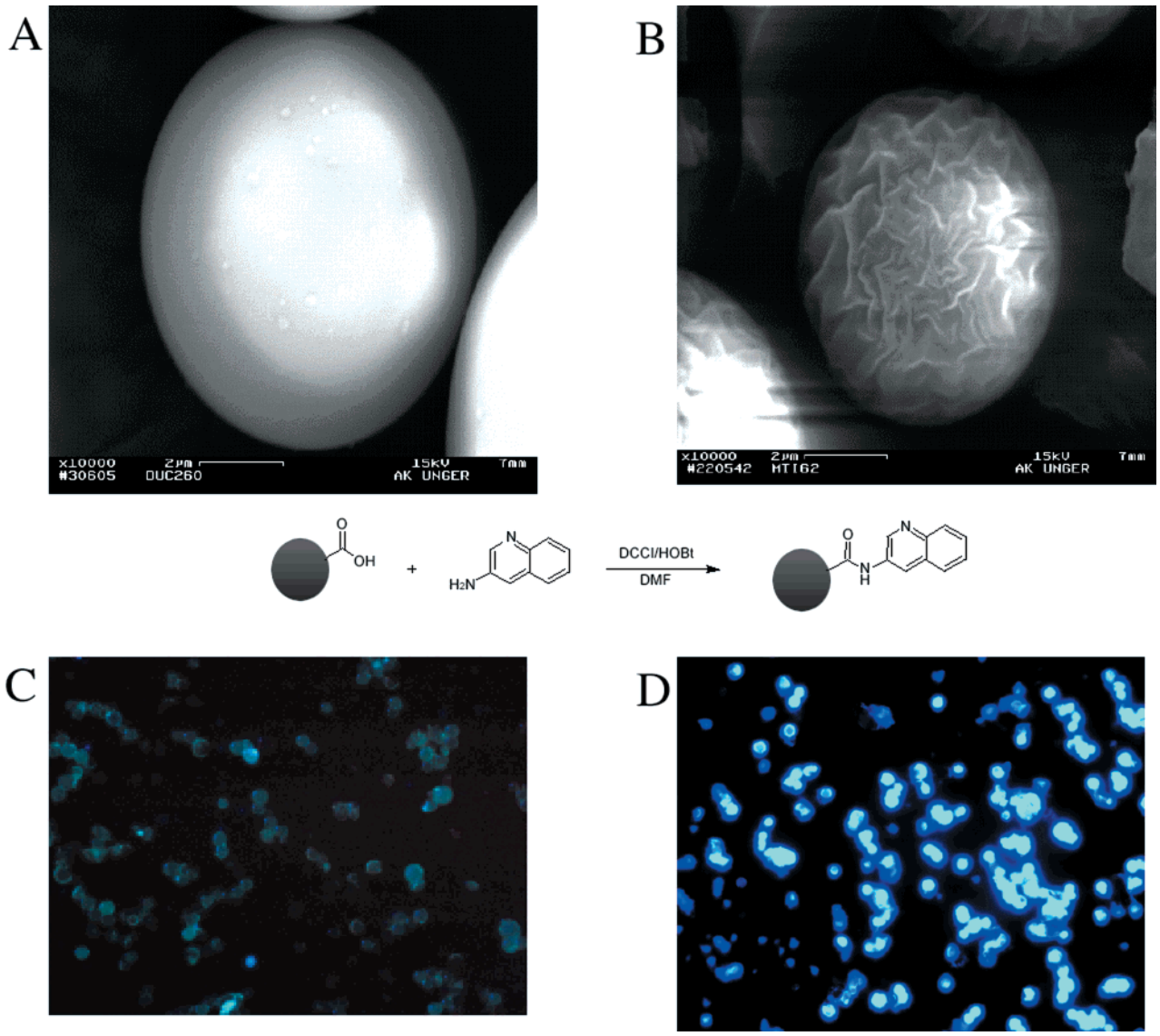

Figure 2. Scanning electron micrographs of the precursor silica template (A) and of P-TAP after dissolution of the silica template (B). The micrographs were obtained using a Zeiss DSM 962 (Zeiss, Oberkochen, Germany) at a magnification of 10 000×. (C) and (D) show fluorescence micrographs of P-9EA before (C) and after (D) dissolution of the silica template. The particles were post-labeled with 3-aminoquinoline as indicated and studied using a Fluorescence Microscope Leica DM R HC (Leica Microsystems Ltd., Bensheim, Germany).

more, the low bulk swelling factors (<1.15 (mL/mL) in acetonitrile) imply that the materials have a relatively homogeneous distribution of cross-links; this is also in contrast to the bulk-imprinted counterparts. To investigate the accessibility of the carboxylic acid functional groups on the surface, these were fluorescently labeled by reaction with 3-aminoquinoline. The labeled particles were then studied with respect to the distribution of the fluorescence intensity between and within the particles. Only weak intensity was observed after labeling the particles still containing the silica template (Figure 2C). This efficiently blocks access to the carboxylic acid groups. However, particles no longer containing the silica template exhibited a strong fluorescence, indicative of a high accessibility of the surface carboxylic acid groups (Figure 2D).

The particles were slurry-packed in columns and assessed in the liquid chromatographic mode for their ability to retain the templates and analogues, including the DNA bases and corresponding nucleotides. When an organic-based mobile phase system (acetonitrile/acetic acid: 99/1 (v/v)) was used, the imprinted polymers exhibited a clear selectivity for the template and analogues (Figure 3).

Thus, 9-ethyladenine (9EA) was retained ≈3.5 times more on P-9EA than on the non-imprinted polymer P−N. Likewise, triaminopyrimidine (TAP) was retained ≈3 times more on P-TAP than on P−N. Both of the imprinted polymers selectively retained purines and pyrimidines containing exocyclic amino groups common to the template structures, whereas the non-related bases uracil and thymine were weakly retained with lower selectivity. More direct evidence for the fidelity of the binding sites is given by the cross-retentitivity observed for 9EA and TAP. Thus, both templates are more retained on their complementary polymers than on the analogue-imprinted polymer. Free adenine, however, is more retained on P-TAP than on P-9EA. This can be explained considering the anticipated orientation of the carboxylic acid groups in the binding sites. The predominant interactions between the immobilized templates and carboxylic acids in aprotic

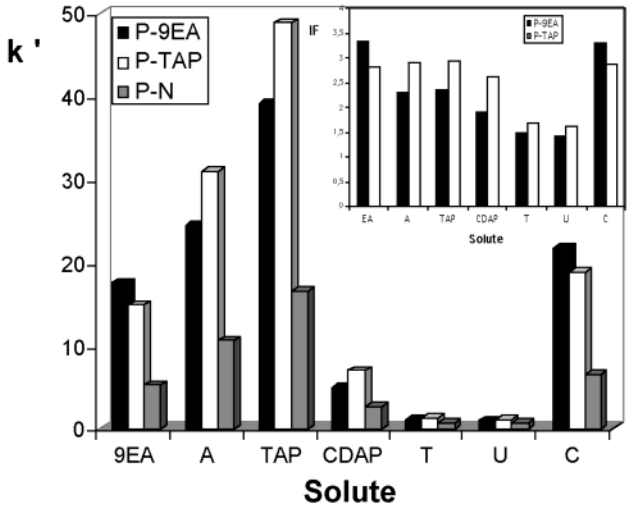

Figure 3. Retention of templates and structural analogues injected (10 μL of 1 mM stock solutions) on columns (50 × 5 mm) packed with P-9EA, P-TAP, or P−N using a mobile phase of acetonitrile/acetic acid 99/1 (v/v) and a flow rate of 1.0 mL/min. The retention was calculated as the capacity factor, $k' = (t - t_0)/t_0$ where t_0 is the elution time of the void marker acetone. The selectivity is reflected in the imprinting factor IF (inserted diagram. Example for P-9EA: IF = k'_{P-9EA}/k'_{P-N}). P−N was synthesized analogous to P-9EA and P-TAP but using APS-modified silica as the template.

media, based on previously characterized homogeneous systems,[10,11] have been indicated in Figure 1. From this it is seen that the carboxylic acid group interacting with the pyrimidine ring nitrogen *ortho* to the site of coupling of TAP may offer complementary hydrogen-bonding interactions to both N3 and H9 of adenine. The polymers also showed selectivity for larger template analogues.[11] Thus, when an aqueous mobile phase (phosphate buffer (10 mM, pH 3) containing 5% acetonitrile) was used, P-TAP retained the 2,4-diaminopteridine drug methotrexate with an IF of 1.5.

When the traditional monolith-imprinting procedure was used, the strong solution complexation between carboxylic acids and 9EA and the TAP analogue trimethoprim promote a high yield of strong, highly discriminating templated binding sites.[11−13] This system serves as an excellent reference system for comparison with the retention data observed here. Overall, the hierarchically imprinted polymers exhibit lower selectivity than the monolith counterpart. Whereas the monolithic molecularly imprinted polymers (MIPs) for 9EA exclusively retain adenine derivatives, the hierarchical MIPs exhibit a considerably broader selectivity and lower imprinting factors. This is a reasonable consequence of the steric hindrance caused by the coupling to the silica surface, which prevents complementary interactions to develop at this point. Moreover, the fluoride treatment may to some extent degrade the templated sites, although monolithic MIPs appear stable toward similar treatments.[14]

We are presently taking advantage of the accessible configuration of the hierarchically imprinted sites to achieve selective retention of larger molecules containing the template substructure.

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Supporting Information Available: Procedure for synthesis and evaluation of the polymers and characterization data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) Lancelot, G. *J. Am. Chem. Soc.* **1977**, *99*, 7037.
(11) Quaglia, M.; Chenon, K.; Hall, A. J.; De Lorenzi, E.; Sellergren, B. *J. Am. Chem. Soc.* **2001**, *123*, 2146−2154.
(12) Shea, K. J.; Spivak, D. A.; Sellergren, B. *J. Am. Chem. Soc.* **1993**, *115*, 3368−3369.
(13) In the case of the triaminopyrimidine analogue trimethoprim, this template forms a 1:2 complex with methacrylic acid in $CDCl_3$, leading to an apparent quantitative yield of templated binding sites.
(14) Sellergren, B.; Shea, K. J. *Tetrahedron Asymmetry* **1994**, *5*, 1403.