Catalytic Silica Particles via Template-Directed Molecular Imprinting

Michael A. Markowitz,*,† Paul R. Kust,† Gang Deng,† Paul E. Schoen,† Jonathan S. Dordick,‡ Douglas S. Clark,§ and Bruce P. Gaber†

Laboratory for Molecular Interfacial Interactions, Code 6930, Center for Bio/Molecular Science and Engineering, Naval Research Laboratory, Washington, D.C. 20375, Department of Chemical Engineering, Rensselaer Polytechnic Institute, Troy, New York 12180, and Chemical Engineering Department, University of California, Berkeley, California 94720

Received June 23, 1999. In Final Form: September 24, 1999

The surfaces of silica particles were molecularly imprinted with an α-chymotrypsin transition-state analogue (TSA) by utilizing the technique of template-directed synthesis of mineralized materials. The resulting catalytic particles hydrolyzed amides in an enantioselective manner. A mixture of a nonionic surfactant and the acylated chymotrypsin TSA, with the TSA acting as the headgroup at the surfactant−water interface, was used to form a microemulsion for silica particle formation. Incorporation of amine-, dihydroimidazole-, and carboxylate-terminated trialkoxysilanes into the particles during imprinting resulted in enhancement of the rates of amide hydrolysis. Acylated imprint molecules formed more effective imprints in the presence of the functionalized silanes than nonacylated imprint molecules. Particles surface-imprinted with the chymotrypsin TSA were selective for the trypsin substrate, and particles surface-imprinted with the L-isomer of the enzyme TSA were enantioselective for the D-isomer of the substrate.

Introduction

The synthesis and characterization of polymeric and metal oxide materials that selectively catalyze the hydrolysis or transformation of organic molecules has been of keen interest for some time.[1−5] One of the most active approaches to fabricating these catalytic materials has been to exploit the principles of molecular recognition to "imprint" the shape and functionality of the molecule to be hydrolyzed or transformed into polymers.[6−14] During a typical imprinting process, a mixture of functionalized and nonfunctionalized monomers surrounding the molecule to be imprinted are polymerized, thereby encasing the imprinted site within the polymer. Complementary binding groups, arising from the functionalized polymer groups incorporated during the imprinting, are utilized to enhance the preferential substrate binding and subsequent catalysis. To allow access by substrate molecules to the imprinted sites, either the polymer is ground up or an inert solvent incorporated into the polymer during formation is washed away, thereby exposing the sites. This process, however, results in the deformation of a large number of the binding sites, which necessarily adversely affects selectivity and activity since the shape-specificity and the complementary binding of the site are irreversibly altered. Recently, efforts have been made to deal with the issue of accessibility by imprinting on silica or polymer surfaces.[15,16] In general, the approach involves linking complementary hydrogen-bonding functionalized silanes to the imprint molecule and then creating the molecular recognition site by attaching this "scaffolding" to the silica or polymer surface. After the imprint molecule is washed away, a binding site with affinity for specific molecules remains on the surface.

We aim to develop a general surface-imprinting method for the formation of molecular imprints into metal oxide surfaces and into the pore surfaces of mineralized mesoporous materials. Such an imprinting method could result in robust, selective materials with very high catalytic activity per unit volume. The approach we have chosen is to synthesize a surfactant analogue of the molecule to be imprinted, with the imprint molecule portion serving as the surfactant headgroup (Figure 1). A mixture of this imprint surfactant and another surfactant would then be allowed to self-assemble into a microstructure such as a micelle, reverse micelle, vesicle, bicontinuous cubic phase, hexagonal phase, or tubule. Using well-established template-directed synthetic methods,[17,18] this microstructure would be mineralized. During mineralization, the imprint molecule "headgroup" is in

* To whom correspondence should be addressed.
† Naval Research Laboratory.
‡ Rensselaer Polytechnic Institute.
§ University of California.

(1) Davis, M. E.; Katz, A.; Ahmad, W. R. *Chem. Mater.* **1996**, *8*, 1820.
(2) Wulff, G. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1812.
(3) Hollinger, P.; Hoogenboom, H. R. *Trends Biochem.* **1995**, *13* (1), 7.
(4) Mosbach, K. *Trends Biochem. Sci.* **1994**, *19*, 914.
(5) Haupt, K.; Mosbach, K. *Trends Biotechnol.* **1998**, *16*, 468−475.
(6) Wulff, G.; Gross, T.; Schonfeld, R. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1962.
(7) Beach, J. V.; Shea, K. J. *J. Am. Chem. Soc.* **1994**, *116*, 379.
(8) Matsui, J.; Nicholls, I. A.; Karube, I.; Mosbach, K. *J. Org. Chem.* **1996**, *61*, 5414.
(9) Wulff, G.; Lauer, M.; Bohnke, H. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 188.
(10) Whitcombe, M. J.; Rodriguez Villar, P.; Vulfson, E. N. *J. Am. Chem. Soc.* **1995**, *117*, 7105.
(11) Matsuishi, T.; Shimada, T.; Morihara, K. *Chem. Lett.* **1992**, 1921.
(12) Shimada, T.; Kurazono, R.; Morihara, K. *Bull. Chem. Soc. Jpn.* **1993**, *66*, 836.
(13) Heilmann, J.; Maier, W. F. *Z. Naturforsch.* **1995**, *50b*, 460.
(14) Karmalkar, R. N.; Kulkarni, M. G.; Mashelkar, R. A. *Macromolecules* **1996**, *29*, 1366.
(15) Lele, B. S.; Kulkarni, M. G.; Mashelkar, R. A. *React. Funct. Polym.* **1999**, *39*, 37.
(16) Hwang, K.-O.; Yakura, Y.; Ohuchi, F. S.; Sasaki, T. *Mater. Sci. Eng., C* **1995**, *3*, 137.
(17) Vartuli, J. C.; Schmitt, K. D.; Kresge, C. T.; Roth, W. J.; Leonowicz, M. E.; McCullen, S. B.; Hellring, S. D.; Beck, J. S.; Schlenker, J. L.; Olson, D. H.; Sheppard, E. W. *Chem. Mater.* **1994**, *6*, 2317.

10.1021/la990809t CCC: $19.00
Published on Web 12/07/1999

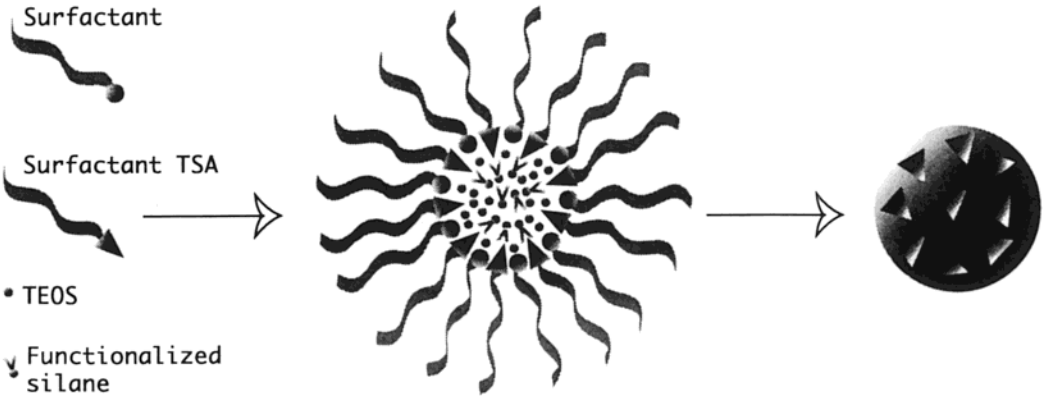


Figure 1. Template-directed molecular imprinting of silica particles. A water-in-oil microemulsion is formed by mixing a nonionic surfactant and the surfactant TSA with cyclohexane, ammoniated ethanol, and water. TEOS and functionalized silanes are added to begin particle formation and surface-imprinting. After particle formation is complete, the surfactants are washed away to complete the preparation of surface-imprinted particles.

contact with the surface of the metal oxide or polymer structure as it forms, creating a negative image or "imprint" of the shape of the imprint molecule in the new material's surface. Silanes containing hydrogen-bonding groups, which can bind to the imprint molecule and enhance substrate binding to the imprinted sites and/or furnish reactive sites for catalytic activity, may be present during the mineralization. After the surfactant is washed away, a robust material containing catalytic active sites in its surfaces remains.

We describe here a process for making catalytic silica particles using a combination of existing molecular imprinting and template-directed materials synthesis methods. Specifically, we investigated the feasibility of our surface-imprinting approach by adapting a microemulsion technique for forming silica particles to prepare catalysts for amide hydrolysis.[19,20] Both an α-chymotrypsin transition-state analogue and an inhibitor were acylated and used as the imprint molecules. The catalytic activity and selectivity of the surface-imprinted silica particles for chymotrypsin and trypsin substrates were determined.

Experimental Section

3-(Aminoethylaminomethyl)-phenyltrimethoxysilane (PEDA), carboxyethylsilanetriol (CTES), and *N*-(3-triethoxysilylpropyl)-4,5-dihydroimidazole (IPTS) were purchased from Gelest (Tullytown, PA); tetraethoxysilane (TEOS), Igepal CO-520 (polyoxyethylene(5) nonylphenyl ether, NP-5), 3-aminophenylboronic acid, 2-aminopyridine, dicyclohexylcarbodiimide (DCC), trifluoroacetic acid (TFA), ethanol, cyclohexane, acetonitrile, and dimethyl sulfoxide (DMSO) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide (Suc-AAPF−PNA), benzoyl-DL-arginine-*p*-nitroanilide (DL-BAPNA), benzoyl-D-arginine-*p*-nitroanilide (D-BAPNA), and benzoyl-L-arginine-*p*-nitroanilide (L-BAPNA) were purchased from Sigma Chemical Co. (St. Louis, MO). *N*-α-*t*-BOC-L-phenylalanine was purchased from Calbiochem-Novabiochem Corp. (San Diego, CA); methanol and glacial acetic acid were purchased from Fisher Scientific (Pittsburgh, PA). All chemicals were used as received. Saturated solutions of ammonia in ethanol were prepared by passing ammonia gas into denatured ethanol at 20 °C for 5−6 h. Electron microscopy was performed with a Zeiss EM-10 transmission electron microscope operated at 60 kV. UV/vis spectroscopy was performed with a Beckman DU-650 spectrophotometer.

Particle Synthesis. Silica particles that did not contain functionalized silanes were prepared by stirring a saturated solution of ammonia in ethanol with cyclohexane, NP-5, and water for 30 min at room temperature and then adding TEOS. Stirring continued overnight. Silica particles with functionalized silanes for surface modification were prepared by including measured amounts of PEDA, IPTS, and CTES before stirring and addition of TEOS. Imprinted particles, both with and without functionalized silanes, were prepared by mixing the NP-5 surfactant with a measured amount of the imprint molecule and dissolving in ethanol/cyclohexane before adding to the ammonia/ethanol mixture. The volume of the reaction mixture was reduced by vacuum evaporation, and the particles were separated from the remaining reaction mixture by centrifugation, washed three times with a wash solution consisting of four parts methanol, one part glacial acetic acid, and one part water, followed by washing three times with acetonitrile. The particles were then air-dried overnight. Unstained, unwashed particles on copper grids were observed by electron microscopy to determine particle size.

Assay for Hydrolytic Activity. Hydrolytic activity of the silica particles was determined by observing the hydrolysis of succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide (Suc-AAPF−PNA), a chymotrypsin substrate, and benzoyl-DL-arginine-*p*-nitroanilide (DL-BAPNA), a trypsin substrate. Substrate stock solutions were prepared by dissolving ∼50 mg BAPNA or suc-AAPF−PNA in DMSO; these stock solutions were then diluted with DMSO and aqueous 0.1 M Tris/HCl buffer (pH 7.4) to provide substrate solutions of various concentrations (final concentration of DMSO in each substrate solution was 15% v/v). A measured quantity of particles, 30−100 mg, were placed in a microcentrifuge tube; 1.3 mL of substrate solution was added, and the mixture was bath-sonicated to completely disperse the particles. The mixture was then placed in a water bath at 30 °C for 5−6 h, with additional agitation provided every 45−60 min. The mixture was then removed from the water bath and centrifuged at 14,000 rpm for 30 min. The reaction time was measured from initial sonication to the beginning of centrifugation. Catalytic activity was determined from the increase in free *p*-nitroanilide concentration, measured by UV/vis spectroscopy at 410 nm with an extinction coefficient of 8500 M^{-1} cm^{-1}. The measured absorbance was compared to that of the substrate solution at 410 nm and to the absorbance due to light scattering of the particle dispersion of particles in 0.1 M Tris buffer (prepared identically as above) at 410 nm. These contributions to the absorbance were subtracted from the total absorbance at 410 nm to obtain the absorbance due to free *p*-nitrophenol. Each data point was calculated based on the average of 3 to 7 trials.

Synthesis of *N*-α-Decyl-L-phenylalanine-2-aminopyridine (α-Chymotrypsin TSA). The acylated phenylalanine anilide imprint molecule was synthesized in three steps. *N*-α-*t*-BOC-L-phenylalanine was coupled with 2-aminopyridine using DCC to give *N*-α-*t*-BOC-L-phenylalanine-2-aminopyridine. The α-amine was then deprotected with TFA to give *N*-α-L-phenylalanine-2-aminopyridine. The free amine was then acylated with

(18) Huo, Q.; Margolese, D. I.; Ciesla, U.; Demuth, D. G.; Feng, P.; Gier, T. E.; Sieger, P.; Firouzi, A.; Chmelka, B. F.; Schuth, F.; Stucky, G. D. *Chem. Mater.* **1994**, *6*, 1176.
(19) Stober, W.; Fink, A.; Bohn, E. J. *J. Colloid Interface Sci.* **1968**, *26*, 62.
(20) Lindberg, R.; Sjoblom, J.; Sundholm, G. *Colloids Surf. A* **1995**, *99*, 79.

decanoic acid using DCC to give *N*-α-decyl-L-phenylalanine-2-aminopyridine.

***N*-α-*t*-BOC-L-phenylalanine-2-aminopyridine.** DCC (4.4 g, 21.1 mmol) was added to a solution of *N*-α-*t*-BOC-L-phenylalanine (10 g, 37.7 mmol) in 200 mL THF at 0 °C. The mixture was stirred for 30 min and then filtered. 2-Aminopyridine (4.5 g, 41.5 mmol) was added to the filtrate in a round-bottom flask, and the mixture was stirred overnight. The reaction mixture was filtered, and the solvent was evaporated under reduced pressure until approximately 20 mL of THF remained. Petroleum ether was added, and the product was precipitated from solution overnight. The semisolid precipitate collected by filtration was purified by silica gel column chromatography (95:4:1 $CHCl_3$:CH_3OH:H_2O, v/v/v) to give 8 g (60% yield) of the desired amide. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.37 (9H, s), 3.20 (2H, m), 4.55 (1H, s), 5.27 (1H, s), 7.06 (1H, d), 7.22 (5H, m), 7.75 (1H, d), 8.21 (1H, d), 8.27 (1H, d). ^{13}C NMR (100 MHz, CD_3OD): δ 28.6, 39.1, 58.0, 115.5, 121.2, 127.7, 129.4, 130.4, 138.4, 139.4, 139.5, 139.53, 139.6, 149.1, 152.6, 157.7, 173.1.

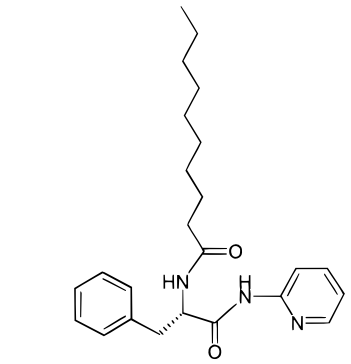
***N*-α-L-Phenylalanine-2-aminopyridine.** *N*-α-*t*-BOC-L-phenylalanine-2-aminopyridine (2 g, 5.8 mmol) was dissolved in 10 mL 1:1 TFA:CH_2Cl_2 (v/v) and stirred vigorously for 10 min. The TFA:CH_2Cl_2 solution was then evaporated under a stream of N_2. The residue was dissolved in 1 mL $CHCl_3$ and purified by silica gel column chromatography (80:18:2 $CHCl_3$:CH_3OH:H_2O, v/v/v) to give 1.4 g of *N*-α-L-phenylalanine-2-aminopyridine in quantitative yield. ^{1}H NMR (400 MHz, CD_3OD, δ): 3.11 (1H, m), 3.33 (1H, m), 4.28 (1H, t), 7.12 (1H, m), 7.30 (5H, m), 7.77 (1H, m), 8.05 (1H, m), 8.26 (1H, m). ^{13}C NMR (100 MHz, CD_3OD): δ 38.7, 56.3, 115.6, 121.7, 127.3, 128.4, 128.9, 130.2, 130.5, 135.4, 139.6, 149.4, 152.2, 168.5.

***N*-α-Decyl-L-phenylalanine-2-aminopyridine.** DCC (1.0 g, 4.85 mmol) was added to a solution of decanoic acid (1.50 g, 8.7 mmol) in 10 mL of chloroform under a nitrogen atmosphere. A white suspension was formed after stirring for 3 h at room temperature. The solid was removed by filtration. To the chloroform solution, 10 mL of a THF solution of *N*-α-L-phenylalanine-2-aminopyridine (1.0 g, 4.1 mmol) was added. After stirring at room temperature for 12 h, a white suspension was formed. The solvents were removed under reduced pressure, and the resulting solid was purified first by silica gel column chromatography with a mixture of chloroform/methanol (95:5(v/v)) as the eluent, then by crystallization in a mixture of hexanes and toluene to give *N*-α-decyl-L-phenylalanine-2-aminopyridine as a white crystalline solid (1.1 g, yield: 67%). ^{1}H NMR (400 MHz, C_6D_6, δ): 0.95 (3H, m), 1.24 (12H, m), 1.57 (2H, m), 1.95 (2H, m), 3.16 (2H, m), 4.48 (2H, d), 5.11 (1H, m), 6.56 (1H, m), 6.77 (1H, d), 7.23 (6H, m), 8.32 (1H, d). ^{13}C NMR (100 MHz, CD_3OD): δ 14.4, 23.7, 26.9, 30.2, 30.4, 30.5, 30.6, 33.1, 36.9, 38.9, 45.3, 56.3, 122.6, 123.7, 127.8, 129.5, 130.3, 138.5, 138.9, 149.6, 159.0, 174.0, 176.3.

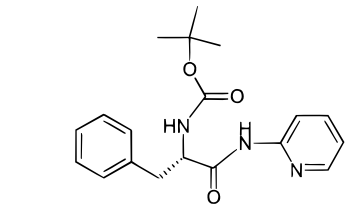
Synthesis of 3-Octylamidophenylboronic Acid (α-Chymotrypsin Inhibitor). Octanoyl chloride (4.6 g, 28.3 mmol) was added slowly to a solution of 3-aminophenylboronic acid (5.0 g, 26.9 mmol) in DMSO (50 mL) containing pyridine (2.3 mL, 28.3 mmol). After addition was complete, the reaction mixture was stirred overnight. The reaction mixture was diluted with 500 mL water and extracted with chloroform. The organic fractions were collected, dried over $MgSO_4$, and filtered; the solvent was then evaporated under reduced pressure to give the crude product as an oil. The oil was redissolved in a small amount of chloroform and passed through a cation exchange column (Bio-Rad AG50W−X8 resin, 20−50 mesh, hydrogen form) to remove any pyridine bound to the boronic acid. The crude product was then purified by silica gel column chromatography (98:2 $CHCl_3$:CH_3OH, v/v) to give 2.5 g, (35.6% yield) of the desired product. ^{1}H NMR (400 MHz, CD_3OD, δ): 0.87 (3H, t), 1.33 (10H, m), 1.66 (2H, m), 7.25−7.76 (4H, m). ^{13}C NMR (100 MHz, CD_3OD): δ 14.4, 23.7, 26.9, 30.1, 30.2, 31.6, 32.9, 37.9, 69.1, 130.3, 130.6, 133.6, 135.2, 177.7.

Results

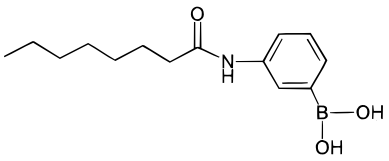
Effect of Imprint Type and Presence of Functional Surface Silanes on Hydrolytic Activity. The following imprint molecules were combined with the NP-5 (polyoxyethylene(5) nonylphenyl ether) surfactant used to form the microemulsion from which the imprinted silica particles were prepared: *N*-α-decyl-L-phenylalanine-(2-aminopyridine)amide; nonacylated, *N*-α-*t*-BOC-L-phenylalanine-(2-aminopyridine)amide; 3-octylamidophenylboronic acid (Figure 2). *N*-α-L-Phenylalanine-(2-aminopyridine)amide is an α-chymotrypsin transition-state analogue (TSA), and 3-aminophenylboronic acid is an α-chymotrypsin inhibitor. Silica particles were prepared in the presence and absence of a mixture of incorporated amine (PEDA), dihydroimidazole (IPTS), and carboxylate-terminated trimethoxysilanes (CTES), measured as a weight percentage of the amount of TEOS used in the particle synthesis. These silanes were chosen because of their similarity to the amino acid residues of serine proteases that are known to participate in the binding and hydrolysis of peptides and a variety of esters and amides in water and organic solvents.[21]



N-α-decyl-L-phenylalanine-(2-aminopyridine)amide



t-BOC-L-phenylalanine-(2-aminopyridine)amide



3-Octylamidophenylboronic acid

Figure 2. Structures of imprint molecules.

The imprint molecules were added as a mole percentage of the total surfactant. As controls, functionalized (amine, dihydroimidazole, and carboxylate) and nonfunctionalized particles were prepared in the absence of imprint molecules. Incorporation of these functionalized trimethoxysilanes during particle synthesis has been demonstrated to produce particles with surface amine, dihydroimidazole, and carboxylate groups capable of hydrogen bonding without affecting particle size.[22] Incorporation of the

(21) Fersht, A. *Enzyme Structure and Mechanism*; W. H. Freeman and Co.: San Francisco, 1985; pp 16−21.

(22) Markowitz, M. A.; Schoen, P. E.; Kust, P.; Gaber, B. P. *Colloids Surf. A* **1999**, *150*, 85.

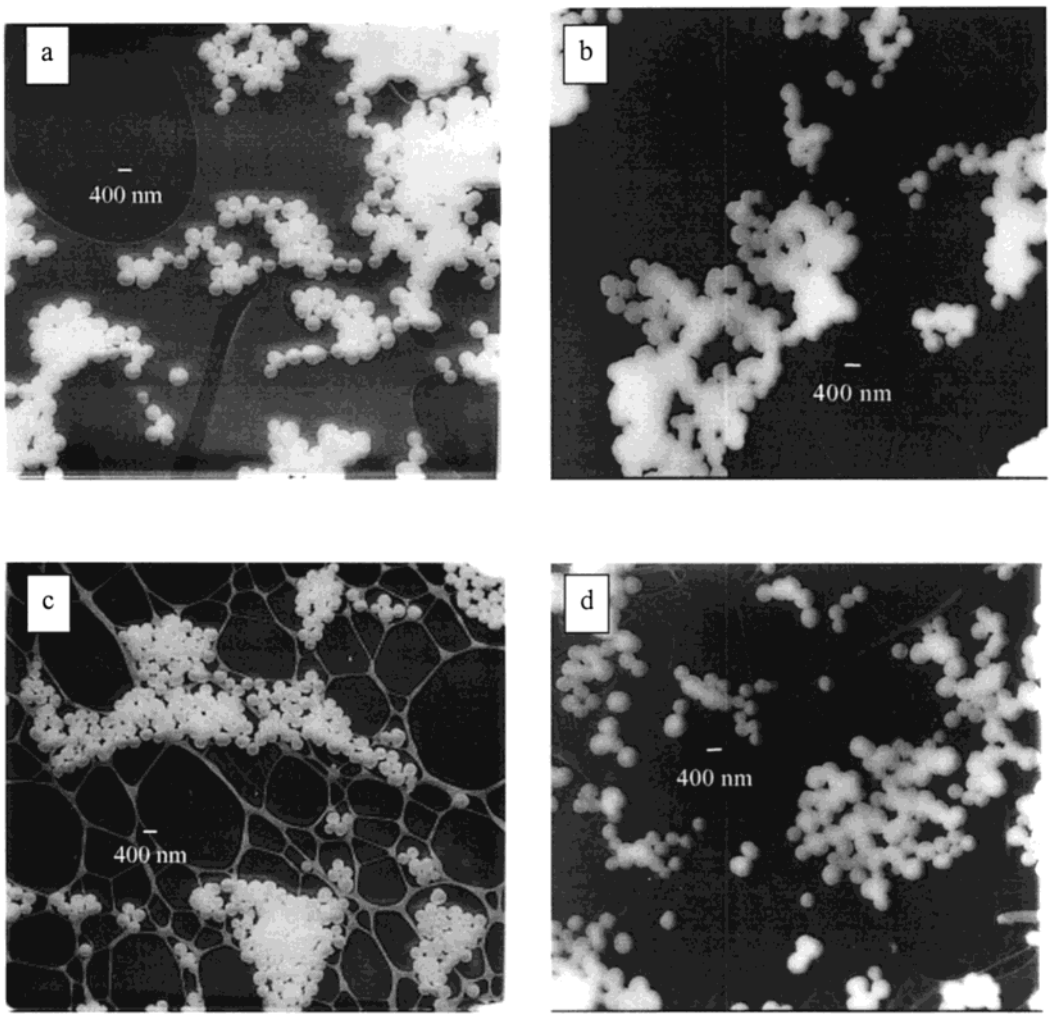


Figure 3. Transmission electron micrographs of (a) nonimprinted silica particles formed in the presence of PEDA, CTES, and IPTS functionalized silanes (5% wt/wt of total silica); (b) particles imprinted with *N*-α-decyl-L-phenylalanine-2-aminopyridine (10% wt/wt of total surfactant) in the presence of PEDA, CTES, and IPTS functionalized silanes (5% wt/wt of total silica); (c) particles imprinted with *N*-α-decyl-L-phenylalanine-2-aminopyridine (20% wt/wt of total surfactant) in the presence of PEDA, CTES, and IPTS functionalized silanes (5% wt/wt of total silica); and (d) particles imprinted with *N*-α-decyl-L-phenylalanine-2-aminopyridine (40% wt/wt of total surfactant) in the presence of PEDA, CTES, and IPTS functionalized silanes (5% wt/wt of total silica).

Table 1. Initial Rate Data for the Hydrolysis of DL-BAPNA Catalyzed by Silica Particles Surface-Imprinted with Different Imprint Molecules

imprint molecule	wt % of added functionalized silanes[a]	initial rate (μM/mg/min × 10^5)	σ (μM/mg/min × 10^5)[b]
none	0	0.47	0.128
none	5	0.83	0.302
3-octylamidophenylboronic acid	0	1.04	0.26
3-octylamidophenylboronic acid	5	1.81	0.324
BOC-L-Phe-2-aminopyridine	0	1.30	0.162
BOC-L-Phe-2-aminopyridine	5	1.41	0.156
N-decyl-L-Phe-2-aminopyridine	0	1.37	0.302
N-decyl-L-Phe-2-aminopyridine	5	3.98	0.489

[a] Mixture of PEDA, CTES, and IPTS. [b] Based on an average of 3−7 measurements.

surfactant imprint molecule into the microemulsion used to form the particles also did not have an effect on particle size (Figure 3). In each case, the diameters of the silica particles ranged from 400 to 600 nm.

All substrate solutions contained 15% (v/v) of DMSO. Table 1 summarizes the initial rate data for the hydrolysis of 0.2 mM DL-BAPNA solutions by particles prepared using the different imprinting molecules. The trypsin catalyzed rate of substrate BAPNA hydrolysis was calculated from a literature report as 6.7 μM mg^{-1} min^{-1} at 25 °C.[23] Amide hydrolysis was performed at 30 °C. Three to seven hydrolysis measurements were made for each type of particle. An analysis of variance at a 95% confidence level indicates that both the presence of the functional silanes on the particle surface and the presence of the imprint molecule during particle synthesis result in improvement in hydrolysis rate and that there is a significant correlation between these two factors.

A multiple comparison test of mean hydrolysis rates, using a Newman-Keuls range test,[24] was conducted to

(23) Erlanger, B. F.; Kokowsky, N.; Cohen, W. *Arch. Biochem. Biophys.* **1961**, *95*, 271.

(24) Hicks, C. R. *Fundamental Concepts in the Design of Experiments*, 3rd ed.; Holt, Rinehart, and Winston: New York, 1982; pp 51−52.

Table 2. Initial Rate Data for the Hydrolysis of DL-BAPNA Catalyzed by Silica Particles Surface-Imprinted with *N*-α-Decyl-L-phenylalanine-2-aminopyridine

amount of imprint molecule (mol % of total surfactant)	amount of functionalized silanes[a] (wt % of total silica)	substrate concentration (mM)	initial rate (μM/mg/min × 10^5)	σ (μM/mg/min × 10^5)[b]
0	5	0.2	0.83	0.30
10	5	0.2	2.65	0.42
10	5	0.4	3.41	0.33
10	10	0.4	3.92	0.89
10	15	0.4	4.17	0.38
20	5	0.2	3.98	0.49
20	5	0.4	5.55	0.15
20	10	0.4	4.12	0.37
40	5	0.2	7.11	0.97
40	5	0.4	9.13	0.32
40	15	0.4	6.28	0.62

[a] Mixture of PEDA, CTES, and IPTS. [b] Based on an average of 3−7 measurements.

elucidate the nature of the effects found significant during the analysis of variance. When there are no functional silanes present in the particles, there is a significant improvement in hydrolysis rate when an imprint molecule is used in the preparation of silica particles. However, there is no significant difference in effect provided by the different imprint molecules; each imprint molecule imparts the same rate improvement to the particle. The presence of 5% functional silanes (PEDA, IPTS, and CTES mixture) in the nonimprinted silica is sufficient to provide some rate improvement to the particles. Particles surface-imprinted with the acylated phenylalanine and aminophenylboronic acid imprint molecules demonstrate an increased enhancement of hydrolytic activity in the presence of functional surface silanes. In contrast, particles surface-imprinted with nonacylated phenylalanine in the presence of functionalized silanes do not reveal a similar rate enhancement.

Effect of Increasing Amount of Functional Silanes and Imprint Molecule on Hydrolytic Activity. Table 2 summarizes how the initial rate of hydrolysis of DL-BAPNA is affected by differences in the amount of functional surface silanes and the amount of imprint molecule used during the preparation of catalytic particles. Three to seven hydrolysis measurements were made for each particle type. An analysis of variance on the results (0.4 mM DL-BAPNA) indicates that there is no significant difference in hydrolysis rate resulting from increasing the amount of functional silanes present in the particle. Therefore, although the presence of a small amount of functional silanes in the catalytic particle provides some improvement in hydrolysis rate, there is no added benefit from increasing the amount of functional silanes beyond a certain level. In fact, in some cases, adding greater than 5% of functionalized silanes resulted in lower hydrolysis rates.

There is, however, a significant benefit from increasing the amount of imprint molecule used in the synthesis of the catalytic particles. Table 2 shows that hydrolysis rates increase with increased amounts of imprint molecule added to the NP-5 surfactant used in the microemulsion to prepare the particles. The observed rate enhancements are comparable to those noted in reviews of other catalytic enhancements through molecular imprinting,[1] including imprinting in polymers,[5,8,9] gels,[15] and silica,[6,7,11−14] where rate increases from 2 to 10 times over nonimprinted solids have been reported.

Evaluation of Kinetic Parameters for Catalytic Particles. The catalytic activity of imprinted silica particles was studied over a range of initial substrate concentrations, from about 0.1 mM to 1.0 mM. Figure 4 shows data for a series of silica particles synthesized with

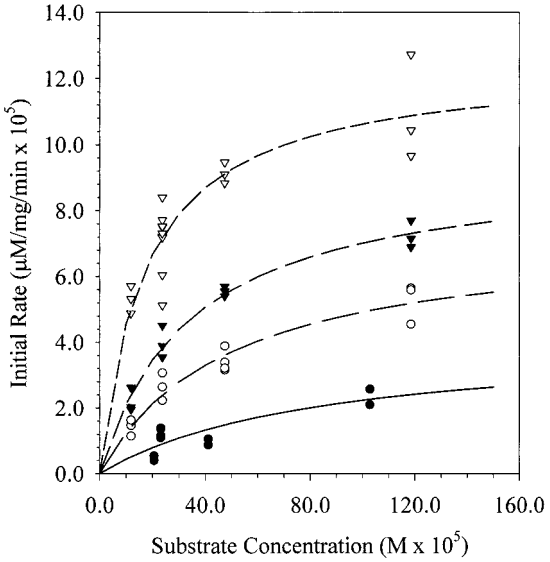


Figure 4. Kinetics of the hydrolysis of DL-BAPNA catalyzed by nonimprinted silica particles (●) and silica particles surface-imprinted with 10% (○), 20% (▼), and 40% (▽) *N*-α-decyl-L-phenylalanine-2-aminopyridine. All particles contain 5 wt % (total silica) of a mixture of PEDA, IPTS, and CTES.

5% incorporated functional silanes (PEDA, IPTS, CTES mixture) and 0, 10, 20, and 40% added acylated phenylalanine-2-aminopyridine anilide imprint molecule. Data for hydrolysis catalyzed by nonimprinted silica is included in the plot for comparison. Solid lines in the figure represent curves fitting the data to the following equation:

$$r_A = K_1 C_A/(K_2 + C_A)$$

using parameters for K_1 and K_2 derived from nonlinear regression. This equation was derived by applying the Langmuir−Hinshelwood method to describe the catalysis (Figure 4).[25] In this derivation, the catalytic active site is considered as a chemical entity capable of participating in the chemical reaction. We can then consider the catalyzed reaction of reactant A to product in terms of the combination of reactant with the catalytic site (adsorption to the surface) to form a complex and the subsequent reaction of the complex at the surface. For our particles, we assume that the reaction at the surface is irreversible. The kinetics can then be analyzed through the kinetic equations for each step in the reaction and a material balance on the catalytic sites. The equations are then

(25) Froment, G. F.; Bischoff, K. B. *Chemical Reactor Analysis and Design*, 2nd ed.; John Wiley and Sons: New York, 1989; p 76.

Table 3. Kinetic Constants for the Hydrolysis of DL-BAPNA Catalyzed by Nonimprinted Silica Particles and by Silica Particles Surface-Imprinted with *N*-α-Decyl-L-phenylalanine-2-aminopyridine[a,b]

amount of imprint molecule (mol % of total surfactant)[c]	amount of functionalized silanes (wt % of total silica)	K_1 (μM/mg/min × 10^5)	σ (μM/mg/min × 10^5)	K_2 (mM)	σ (mM)	K_1/K_2 (mg^{-1} min^{-1})
10	5	7.29	0.71	0.48	0.10	0.15
20	0	5.24	0.78	0.65	0.18	0.08
20	5	9.41	0.05	0.34	0.04	0.28
40	5	12.5	0.08	0.17	0.03	0.74

[a] 20 mol % (total surfactant) of imprint molecule used for imprinting. [b] Particles contain 5 wt % (total silica) of functionalized silanes (PEDA, IPTS, CTES mixture). [c] Each data point was determined from at least two trials.

Table 4. Selectivity of Catalysis by Silica Particles Surface-Imprinted with *N*-α-Decyl-L-phenylalanine-2-aminopyridine[a−c]

substrate	K_1 (μM/mg/min × 10^5)	σ (μM/mg/min × 10^5)	K_2 (mM)	σ (mM)	K_1/K_2 (mg^{-1} min^{-1})
suc-AAPF-PNA	2.70	0.69	0.52	0.24	0.05
DL-BAPNA	9.41	0.48	0.34	0.04	0.28
D-BAPNA	9.43	0.54	0.24	0.04	0.39
L-BAPNA	0.65	0.11	0.55	0.21	0.01

[a] 20 mol % (total surfactant) of imprint molecule used for imprinting. [b] Particles contain 5 wt % (total silica) of functionalized silanes (PEDA, IPTS, CTES mixture). [c] Each data point was determined from at least two trials.

derived using the steady-state approximation and the assumption of either an adsorption limiting step or a surface-reaction limiting step. The derivation according to adsorption limited kinetics is identical to the commonly used form of the Michaelis−Menten equation for enzyme catalysis.[26] Table 3 summarizes K_1 and K_2 parameters for a number of different batches of imprinted silica particles. Unfortunately, we have no ready means of calculating the "active site" concentration of the particles and, therefore, we cannot calculate k_{cat} or k_{cat}/K_2, the turnover number, for our catalytic particles. However, K_1/K_2 values can give some measure of the relative catalytic efficiency of particles for an identical amount of silica, as long as the amount of imprint molecule used to form the catalytic sites is identical for each set of particles. One observed trend is that the value of K_1/K_2 increases with increasing amount of imprint molecule used during particle synthesis as well as when functional silanes are present in the catalyst.

Selectivity of Molecularly Imprinted Catalysts to Several Substrates. Figure 5 presents data for the catalyzed hydrolysis of racemic trypsin substrate DL-BAPNA, the optically pure substrates D- and L-BAPNA, and the α-chymotrypsin substrate succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide. Table 4 presents the kinetic constants K_1, K_2, and K_1/K_2 for succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide and D- and DL-BAPNA calculated from these data. There are two interesting features of this data set. First, the catalyst particle seems to be selective for the trypsin substrate D-BAPNA over the chymotrypsin substrate, even though the imprint molecule more closely mimics the chymotrypsin substrate. Second, the particles are highly selective for the D-isomer of the trypsin substrate, even though the imprint molecule had the L-isomer configuration. This selectivity was observed for separately prepared batches of surface-imprinted particles.

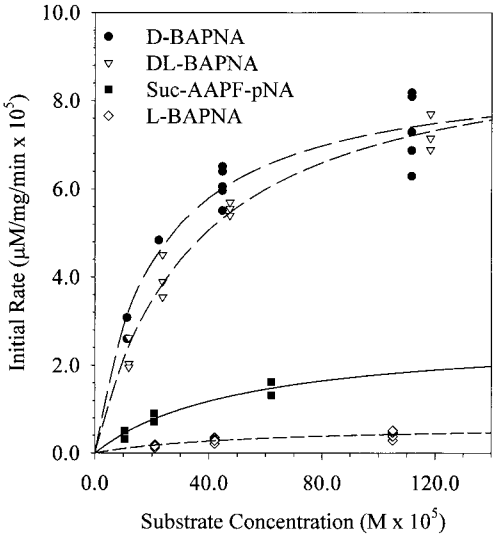


Figure 5. Kinetics of the hydrolysis of various substrates by silica particles surface-imprinted with *N*-α-decyl-L-phenylalanine-2-aminopyridine. All particles contain 5 wt % (total silica) of a mixture of PEDA, IPTS, and CTES.

Discussion

Template-directed molecular imprinting is designed to utilize the surfactant form of an enzyme transition-state analogue or inhibitor to stamp specific molecular recognition sites into the surfaces of functionalized silica colloids as they form. We examined the feasibility of template-directed molecular imprinting by using this approach to imprint silica particle surfaces with a transition-state analogue (TSA) and an inhibitor of chymotrypsin. Specifically, L-phenylalanine-2-aminopyridine (a chymotrypsin TSA) and 3-aminophenyl-boronic acid (a chymotrypsin inhibitor) were acylated to form amphiphilic imprint molecules (Figure 2), which were then mixed with the nonionic surfactant polyoxyethylene(5) nonylphenyl ether (NP-5). Microemulsions formed from these surfactant mixtures were utilized as templates to synthesize surface-imprinted silica particles. These results demonstrate that template-directed molecular imprinting is a viable method of creating robust, enantioselective catalytic silica particles. To our knowledge, this is the first example of forming catalytic silica particles by imprinting reactive sites exclusively into the surface of the particle. Our efforts represent an alternative to conventional molecular imprinting techniques.

Since the silica particles are formed using a microemulsion process, the imprint molecule, which acts as the headgroup of the surfactant *N*-α-decyl-L-phenylalanine-2-aminopyridine, should be positioned at the surfactant−water interface of the reverse micelles within which the silica particles are formed. As a consequence, catalytic

(26) Cornish-Bowden, A. *Fundamentals of Enzyme Kinetics*; Butterworth: London, 1979; p 20.

sites should only be formed on the surfaces of the silica particles. In any case, the imprinted silica particles are left intact after imprinting and, therefore, substrates would not be able to access any buried sites that may have formed. Surface-imprinting was strongly suggested by the effect of increasing the amount of acylated imprint molecule, *N*-α-decyl-L-phenylalanine-2-aminopyridine, on initial rate and K_1/K_2 (measure of catalytic efficiency per amount of surface-imprinted silica) values of DL-BAPNA amide hydrolysis (Tables 2 and 3). As the amount of *N*-α-decyl-L-phenylalanine-2-aminopyridine used to surface-imprint the silica particles increased, the catalytic efficiency of the particles increased. In addition, the observation of enantioselectivity (Table 4) implies that defined, surface-imprinted catalytic sites have been formed using template-directed molecular imprinting.

While both the imprint molecule and the functionalized amine and carboxylate silanes have a positive effect on the hydrolysis rates obtained, it is their combination that produces the best catalytic particles. The importance of the functionalized silanes can be seen from the data in Tables 1 and 2. The initial rates for particles imprinted in the absence of functionalized silanes indicate that imprint molecule shape has only limited impact on the efficacy of the molecular recognition site created. Incorporation of functionalized silanes into the particles reveals two important features of this imprinting method: (1) The lack of a rate enhancement for the nonacylated chymotrypsin TSA in the presence of silanes as compared to the rate enhancement observed for the acylated imprint molecules strongly suggests that acylation aids in effectively positioning the imprint molecule at the surfactant−water interface at which the particle forms; (2) the presence of complementary hydrogen-bonding silanes at the surfactant−water interface as the silica particle is forming is essential to forming reactive catalytic imprints. The results suggest that some or all of the surface amine and carboxylate groups, along with the surface hydroxyl groups of the silica particles, are capable of interacting with the substrate amide carbonyl group to enhance its reactivity. The observation that shape of the imprinted cavity alone does not produce effective imprinted catalytic sites within polymers has been previously reported.[6−8]

There are a number of different types of interactions that will influence what the optimal wt % of functionalized silanes should be to achieve the maximum catalyzed rate of substrate hydrolysis. Some of these interactions include interactions between imprint molecule and functionalized silanes, interactions between different functionalized silanes, and interactions between functionalized silanes and the silica particle surface. These interactions may be further affected by changes in the aqueous or organic media in which the catalyzed reaction occurs. In our work, results obtained in determining the effect of increasing the wt % of the functionalized silanes in the imprinted silica particles reveal that doing so results in a decrease in rate of amide hydrolysis (Table 2). On the basis of these reports, this is possibly due to hydrogen bonding between the functionalized silanes, leaving fewer available for interaction with the substrate. From previous reports, it is known that surface functional groups will form hydrogen bonds with the native surface hydroxyl groups of the silica particles as well as with each other, thereby decreasing their basicity and their ability to hydrogen bond to other molecules.[22,27]

The most interesting kinetic data deal with the specificity observed for amide hydrolysis that is catalyzed by silica particles with surfaces imprinted with *N*-α-decyl-L-phenylalanine-2-aminopyridine. The surface-imprinted particles have selectivity for the trypsin substrate over the chymotrypsin substrate even though imprinting was done with the chymotrypsin TSA (Table 4, Figure 5). The magnitude of the kinetic constants obtained for the hydrolysis of L-, D-, and DL-BAPNA catalyzed by the surface-imprinted silica particles is consistent with a D-enantioselective mode of hydrolysis. In fact, the hydrolysis of D-BAPNA catalyzed by the surface-imprinted particles is 10 times faster (K_1) and 39 times more efficient (K_1/K_2) than the hydrolysis of L-BAPNA catalyzed by the particles. On the basis of the observed enantioselectivity, at least three of the groups surrounding the chiral methine carbon of D-BAPNA must be bound to the catalytic site surface-imprinted silica particle.[28] This reversal of stereoselectivity has been observed to occur for substrate hydrolysis or transformation catalyzed by enzymes such as α-chymotrypsin,[29] lipase,[30] peptidases, and β-lactamases.[31] The enantioselectivity of the hydrolysis strongly suggests that molecular structure affected substrate packing within the catalytic site. Substrate substituent effects have been observed to be a major factor influencing enzyme enantioselectivity.[32−35] Since the substrate is structurally different than the imprinting molecule, the observed enantiopreference of the amide hydrolysis catalyzed by the surface-imprinted silica particles may arise because the D-BAPNA packs more readily into the imprinted catalytic site than the L-BAPNA.

Acknowledgment. The authors would like to thank Drs. Alok Singh, Banahalli Ratna, and David Turner of the Naval Research Laboratory for their helpful advice. Paul Kust is a NRC postdoctoral research fellow. This project was funded by the Department of Energy's Advanced Energy Projects Program and the Office of Naval Research.

LA990809T

(27) Horr, T. J.; Arora, P. S. *Colloids Surf. A* **1997**, *126*, 113.
(28) Bergman, M.; Fruton, J. S. *J. Biol. Chem.* **1937**, *117*, 193.
(29) Schwartz, H. M.; Wu, W. S.; Marr, P. W.; Jones, J. B. *J. Am. Chem. Soc.* **1978**, *100*, 5199.
(30) Xie, Z.-F. *Tetrahedron: Asymmetry* **1991**, *2*, 733.
(31) Damblon, C.; Zhao, G.-H.; Jamin, M.; Ledent, P.; Dubus, A.; Vanhove, M.; Raquet, X.; Christiaens, L.; Frere, J.-M. *Biochem. J.* **1995**, *309*, 431.
(32) Jones, J. B.; Desantis, G. *Acc. Chem. Res.* **1999**, *32*, 99.
(33) Weissfloch, A. N. E.; Kazlauskas, R. J. *J. Org. Chem.* **1995**, *60*, 6959.
(34) Chenevert, R.; Goupil, D.; Rose, Y. S.; Bedard, E. *Tetrahedron: Asymmetry* **1998**, *9*, 4285.
(35) Bayrich, T.; Theiss, R. *Pharmazie* **1998**, *53*, 853.