

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/228629393>

Amine-Catalyzed Biomimetic Hydrolysis and Condensation of Organosilicate

ARTICLE *in* CHEMISTRY OF MATERIALS · JUNE 2005

Impact Factor: 8.35 · DOI: 10.1021/cm051389y

CITATIONS

51

READS

44

2 AUTHORS, INCLUDING:



Nita Sahai

University of Akron

86 PUBLICATIONS 1,953 CITATIONS

SEE PROFILE

Amine-Catalyzed Biomimetic Hydrolysis and Condensation of Organosilicate

Katya M. Delak^{*,†} and Nita Sahai^{†,‡}

Department of Chemistry, 1101 University Avenue, University of Wisconsin, Madison, Wisconsin 53706,
and Department of Geology and Geophysics, 1215 West Dayton Street, University of Wisconsin,
Madison, Wisconsin 53706

Received September 20, 2004. Revised Manuscript Received March 23, 2005

Biogenic silica production occurs at mild conditions with greater control of pore size, shape, and micropatterning than is possible with typical industrial sol–gel methods, providing inspiration for potential alternative routes to silica synthesis. Researchers have implicated the amine moieties, histidine and polylysine, on proteins isolated from sponges and diatoms as catalysts for biogenic silica precipitation. Different mechanistic roles have been ascribed to the amines, but few systematic, quantitative studies isolating one effect from another have been conducted. In the present study, we use ²⁹Si NMR spectroscopy to systematically examine the different possible mechanistic roles of mono- and polyamines in catalyzing silica synthesis at mildly acidic pH (~5) from an organosilicate starting compound, trimethylethoxysilane (TMES). TMES has a single organosilicate bond, so there are no competing reactions and the reaction progress can be followed with little ambiguity. Hydrolysis and condensation (dimerization) of TMES lead to the products trimethylsilanol (TMSiOH) and hexamethyldisiloxane (HMD). The Refocused Insensitive Nuclei Enhanced by Polarization Transfer pulse sequence (RINEPT+) provides unambiguous, quantitative ²⁹Si NMR spectra from which the hydrolysis and condensation rates in the presence of each amine can be obtained. For both mono- and polyamines, the catalytic efficiency scales with the concentration of conjugate base form and inversely with pK_a. Thus, catalysis is most efficient with more acidic monoamines, such as pyridine and imidazole, as well as for the longer polyamines, where the most acidic protonation constant is lower than the experimental pH (~5). We postulate a nucleophile-catalyzed hydrolysis mechanism where the conjugate base of the amine attacks Si to form a pentacoordinate intermediate with TMES. Condensation is interpreted as an acid-catalyzed S_N2 mechanism. Our findings potentially explain the evolutionary selection of histidine-containing proteins for biogenic silica synthesis by sponges and address the chemical mechanisms at work for the precipitation of silica by polylysine-containing proteins in diatoms. Along with the physical mechanisms suggested by other research groups, the systematic results from the present study indicate that amines may be employed in more than one type of mechanistic strategy for catalyzing biogenic and biomimetic silica polymerization.

Introduction

Microorganisms such as sponges, radiolaria, and diatoms assimilate aqueous silicon from their surroundings to produce mineralized skeletal elements of exquisitely patterned, mesoporous amorphous silica that provide rigidity and strength to the cell.^{1–4} It is currently possible to synthesize mesoporous silica, such as MCM-41, but often some form of deliberate chemical templating, extreme pH, or high temperature is required.^{5–7} In contrast, biogenic mesoporous silica is produced at mildly acidic pH and room tempera-

tures.^{1,8–11} Biogenic processes have inspired materials scientists to elucidate the underlying chemical mechanisms as part of a greater effort to identify elegant routes to mesoporous materials synthesis. Such materials have potential applications as components of micromachined materials or as molecular sieves and filters for chemical separations processes.^{12, 13}

Work carried out to date indicates that biological macromolecules are effective in precipitating silica.^{14–17} Isolation

* Corresponding author. Telephone: (608) 265-5192. Fax: (608) 262-0693. E-mail: kdelak@geology.wisc.edu.

[†] Department of Chemistry.

[‡] Department of Geology and Geophysics.

- (1) Iler, R. K. *The Chemistry of Silica*; John Wiley & Sons: New York, 1979.
- (2) Poulsen, N.; Sumper, M.; Kroger, N. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 12075.
- (3) Kroger, N.; Deutzmann, R.; Sumper, M. *Science* **1999**, *286*, 1129.
- (4) Cha, J. N.; Shimizu, K.; Zhou, Y.; Christiansen, S. C.; Chmelka, B. F.; Stucky, G. D.; Morse, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 361.
- (5) Kresge, C. T.; Leonowicz, M. E.; Roth, W. J.; Vartuli, J. C.; Beck, J. S. *Nature* **1992**, *359*, 710.
- (6) Sayari, A.; Hamoudi, S. *Chem. Mater.* **2001**, *13*, 3151.

- (7) Barton, T. J.; Bull, L. M.; Klemperer, W. G.; Loy, D. A.; McEnaney, B.; Misono, M.; Monson, P. A.; Pez, G.; Scherer, G. W.; Vartuli, J. C.; Yaghi, O. M. *Chem. Mater.* **1999**, *11*, 2633.
- (8) Kroger, N.; Sumper, M. In *Biomimetalization: From Biology to Biotechnology and Medical Application*; Bauerlein, E., Ed.; Wiley-VCH: Weinheim, Germany, 2000; pp 151–170.
- (9) Martin-Jezequel, V.; Hildebrand, M.; Brzezinski, M. A. *J. Phycol.* **2000**, *36*, 821.
- (10) Wetherbee, R. *Science* **2002**, *298*, 547.
- (11) Vrieling, E. G.; Gieskes, W. W. C.; Beelen, T. P. M. *J. Phycol.* **1999**, *35*, 548.
- (12) Parkinson, J.; Gordon, R. *Tibtech* **1999**, *17*, 190.
- (13) Drum, R. W.; Gordon, R. *Trends Biotechnol.* **2003**, *21*, 325.
- (14) Kroger, N.; Deutzmann, R.; Sumper, M. *J. Biol. Chem.* **2001**, *276*, 26066.
- (15) Zhou, Y.; Shimizu, K.; Cha, J. N.; Stucky, G. D.; Morse, D. E. *Angew. Chem., Int. Ed.* **1999**, *38*, 780.

and purification of biomolecules is laborious, and many polymers used thus far must be custom-synthesized. Furthermore, these biomolecules are appropriated into the silica structure and cannot be retrieved for reuse.^{4,14,15} Thus, industrial silica production through the use of large macromolecules and/or biomolecules may not prove cost-effective, though avenues for combinatorial approaches have opened up with the recent completion of the diatom genome of *Thalassiosira pseudonona*.¹⁸ It is evident that simpler, less expensive, and more controlled bioinspired routes are required rather than attempting direct duplication of natural processes. As such, deciphering the molecular mechanisms, of enzyme-catalyzed silica precipitation is worthwhile.

The details of cell physiology and molecular biology that enable organisms to appropriate aqueous silicon from the environment are still murky, and the form in which silicon is stored intracellularly is not known. However, significant insight has been gained into some of the subsequent steps involving silica mineralization from stored silicon.^{8–11,19–22} Silicatein, a protein isolated from the sponge *Tethya aurantia* was found to have a large degree of homology with the digestive hydrolase, cathepsin.²³ When combined with an organosilicate starting material, silicatein catalyzes the formation of silica.^{4,15,23} Similarly, the silaffins, proteins isolated from the diatom *Cylindrotheca fusiformis*, catalyze precipitation of silica from aqueous solutions of orthosilicic acid.^{3,14,16} Significantly, silicatein and the silaffins share in common the presence of amine-based peptides that have been hypothesized to polymerize aqueous silicon monomers. In the case of silicatein, the active site consists of histidine and serine, where the histidine contains an imidazole moiety as its side chain. Elimination of the histidine through site-directed mutation results in a loss of the protein's ability to precipitate silica.¹⁵ Silaffins, in contrast, have not been demonstrated to have a single active site. Instead, they contain phosphorylated serines on the peptide backbone and polylysine-based side chains.¹⁷ Fragments of silaffins containing these polylysine-based groups have the capacity to catalyze silica precipitation, as do simpler long-chain polyamines isolated from diatom silica.^{16,24}

Drawing on the findings detailed above, numerous investigators have successfully shown that large polymer mol-

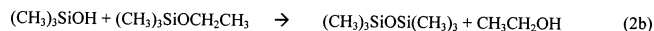
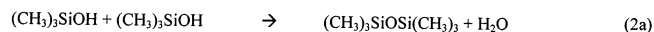
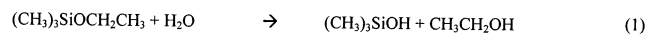
ecules with amine functionalities precipitate silica.^{24–28} Mizutani showed that, at pH 8.5, longer chain polyamines are significantly more effective at inducing silica precipitation from sodium silicate solutions than their monoamine counterparts.²⁹ Similarly, in studies carried out at neutral pH, Coradin showed that while the amino acids proline and lysine aid in the polycondensation of silicic acid, their polypeptides do so far more effectively.^{27,28}

The mechanism of amine catalysis is not fully understood, and polyamines may be involved in at least two separate stages of silica biomineralization. Recent in vitro work suggests that microscopic phase separations are induced by the presence of diatom silaffins that direct the formation and morphology of silica precipitates.^{30,31} This points to a physical mechanism for morphological control. In diatoms, silicon may be stored intracellularly as an organosilicate precursor,⁹ with the advantage that polymerization rates are slower than when silicic acid is used as a precursor, thus allowing a greater degree of morphological control. As discussed above, amines may also influence the first steps of hydrolysis and polycondensation that provide the initial nuclei for precipitation. Because the relative rates of hydrolysis and condensation also influence the size of nuclei and, consequently the final precipitate morphology,^{1,32,33} it is important to establish how amine-based compounds influence these rates at mildly acidic pH.

Silica hydrolysis and condensation kinetic studies are generally carried out at either extreme of the pH scale in an effort to substantiate the hypothesized S_N2 reactions that take place at these pH regimes.^{1,34–36} However, silica formation in diatoms takes place in the silica deposition vesicle at approximately pH 5, suggesting that a different mechanism may be at work. Iler and Corriu have suggested that, in the presence of amines, where nitrogen has a free lone pair of electrons, Si expands its coordination sphere to a pentacoordinate intermediate (a structure similar to that found in silatranes).^{1,37,38} Hydrolysis and condensation are enhanced in a mechanism that includes this pentacoordinate intermediate and a hexacoordinated transition state. Quantum mechanical molecular orbital calculations have shown that, in this pentacoordinate intermediate, the Si–O bond distances are elongated up to 1.7 Å, thus providing an opportunity for water to attack.²²

- (16) Kroger, N.; Deutzmann, R.; Bergsdorf, C.; Sumper, M. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 14133.
- (17) Kroger, N.; Lorenz, S.; Brunner, E.; Sumper, M. *Science* **2002**, *298*, 584.
- (18) Armbrust, E. V.; Berges, J. A.; Bowler, C.; Green, B. R.; Martinez, D.; Putnam, N. H.; Zhou, S. G.; Allen, A. E.; Apt, K. E.; Bechner, M.; Brzezinski, M. A.; Chaal, B. K.; Chiovitti, A.; Davis, A. K.; Demarest, M. S.; Detter, J. C.; Glavina, T.; Goodstein, D.; Hadi, M. Z.; Hellsten, U.; Hildebrand, M.; Jenkins, B. D.; Jurka, J.; Kapitonov, V. V.; Kroger, N.; Lau, W. W. Y.; Lane, T. W.; Larimer, F. W.; Lippmeier, J. C.; Lucas, S.; Medina, M.; Montsant, A.; Obornik, M.; Parker, M. S.; Palenik, B.; Pazour, G. J.; Richardson, P. M.; Rynearson, T. A.; Saito, M. A.; Schwartz, D. C.; Thamatrakoln, K.; Valentin, K.; Vardi, A.; Wilkerson, F. P.; Rokhsar, D. S. *Science* **2004**, *306*, 79.
- (19) Coradin, T.; Lopez, P. J. *ChemBioChem* **2003**, *4*, 251.
- (20) Kinrade, S. D.; Gillson, A. M. E.; Knight, C. T. G. *J. Chem. Soc., Dalton Trans.* **2002**, 307.
- (21) Sahai, N.; Tossell, J. A. *Geochim. Cosmochim. Acta* **2001**, *65*, 2043.
- (22) Sahai, N. *Geochim. Cosmochim. Acta* **2004**, *68*, 227.
- (23) Shimizu, K.; Cha, J.; Stucky, G. D.; Morse, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6234.
- (24) Menzel, H.; Horstmann, S.; Behrens, P.; Barnreuther, P.; Krueger, I.; Jahns, M. *Chem. Commun.* **2003**, 2994.

- (25) Vrieling, E. G.; Beelen, T. P. M.; van Santen, R. A.; Gieskes, W. W. C. *J. Phycol.* **2000**, *36*, 146.
- (26) Patwardhan, S. V.; Mukherjee, N.; Steinitz-Kannan, M.; Clarkson, S. J. *Chem. Commun.* **2003**, 1122.
- (27) Coradin, T.; Livage, J. *Colloids Surf. B* **2001**, *21*, 329.
- (28) Coradin, T.; Durupthy, O.; Livage, J. *Langmuir* **2002**, *18*, 2331.
- (29) Mizutani, T.; Nagase, H.; Fujiwara, N.; Ogoshi, H. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 2017.
- (30) Sumper, M. *Science* **2002**, *295*, 2430.
- (31) Brunner, E.; Lutz, K.; Sumper, M. *Phys. Chem. Chem. Phys.* **2004**, *6*, 854.
- (32) Brinker, C. J. *J. Non Cryst. Solids* **1988**, *100*, 31.
- (33) Osterholtz, F. D.; Pohl, E. R. *J. Adhes. Sci. Technol.* **1992**, *6*, 127.
- (34) Fyfe, C. A.; Aroca, P. P. *Chem. Mater.* **1995**, *7*, 1800.
- (35) Rankin, S. E.; Sefcik, J.; McCormick, A. V. *J. Phys. Chem. A* **1999**, *103*, 4233.
- (36) Rankin, S. E.; McCormick, A. V. *Magn. Reson. Chem.* **1999**, *37*, S27.
- (37) Corriu, R. J. P.; Belot, V.; Guerin, C.; Henner, B.; Leclercq, D.; Mutin, H.; Vioux, A.; Wang, Q. *Mater. Res. Symp. Proc.* **1990**, *180*, 3.
- (38) Corriu, R. J. P.; Guerin, C.; Henner, B. J. L.; Man, W. W. C. W. C. *Organometallics* **1988**, *7*, 237.

Scheme 1. Trimethylethoxysilane Hydrolysis, Followed by Either Water Condensation or Ethanolic Condensation Paths

Currently, there is little quantitative evidence to substantiate these postulated mechanisms. In this study, we use ^{29}Si NMR spectroscopy to measure rates of amine catalysis quantitatively at mildly acidic to circum-neutral pH conditions and to isolate the different possible mechanistic roles played by amines. We examine the effects of total chain length and carbon chain spacing in polyamines, as well as nucleophilicity, steric effects, and solvation of both mono- and polyamines on the hydrolysis and condensation rates of trimethylethoxysilane (TMES, $(\text{CH}_3)_3\text{SiOCH}_2\text{CH}_3$).

Trimethylethoxysilane does not directly reflect naturally occurring silicon species, since silicon found in nature is typically coordinated to oxygen and not carbon.¹ However, in contrast to previous studies where the starting silicon compound has been either silicic acid or sodium silicate,^{3,14,27–29} TMES exhibits an organosilicate functionality. This is significant because organosilicates are postulated to be a means by which diatoms store silica in vesicles at concentrations exceeding the saturation level of monosilicic acid. If this is the case, catalysis of the hydrolysis step becomes a critical factor in biogenic silica precipitation.

Furthermore, TMES has a single reactive bond with respect to hydrolysis and dimerization, where the reactions proceed according to Scheme 1. As a consequence, the concentrations of only three species need to be tracked during the reaction: TMES, trimethylsilanol (TMSiOH, $(\text{CH}_3)_3\text{SiOH}$), and hexamethyldisiloxane (HMD, $(\text{CH}_3)_3\text{SiO}(\text{CH}_3)_3$). The catalysts we chose included monoamines pyridine (PYP), imidazole (IMI), ethylamine (ET), and piperidine (PIP) and the polyamines 1,4-diaminobutane (DB), spermidine (SPD), spermine (SPM), triethylenetetramine (TET), and tetraethylenepentamine (TEP) (Figure 1).

Experimental Methods

TMES (Gelest, twice distilled) is not soluble in water, so a 4 M TMES solution was prepared in ethanol (Aaper) as the starting Si solution. Catalyst solutions containing amines (TEP: Aldrich, technical grade; TET: Aldrich, 98%; PYP: Acros ACS reagent grade; IMI, PIP: Aldrich 99+%; SPM, SPD, DB: Fluka, $\geq 99\%$) were first prepared as 18 mM aqueous solutions (H_2O , 18 M Ω), where the pH had been adjusted to pH ~ 4.5 through the addition of acid or base (HCl, NaOH: Fisher ACS reagent grade). A 1 mL aliquot of this aqueous catalyst solution was then combined with 445 μL of water and made up to a total volume of 10 mL with ethanol, yielding an ethanolic catalyst solution that was 8 M in H_2O , 1.83 mM in amine catalyst, and at a H^+ ion concentration that would be equivalent to pH ~ 5.5 in aqueous solution. For each experiment, the ethanolic catalyst solution was combined with an equal volume of the silicon starting solution (350 and 350 μL , respectively) in an NMR tube. Thus, the final concentrations of reagents in the reaction were 4 M H_2O , 2 M TMES, and 0.915 mM amine. Mixing was assured by complete inversion of the tube three times, and the sample was then inserted into the spectrometer.

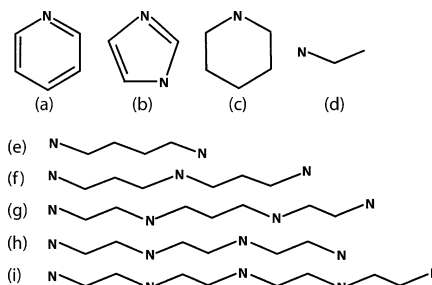


Figure 1. Amines used in the present study: (a) pyridine, PYP; (b) imidazole, IMI; (c) piperidine, PIP; (d) ethylamine, ET; (e) 1,4-diaminobutane, DB; (f) spermidine, SPD; (g) spermine, SPM; (h) triethylenetetramine, TET; and (i) tetraethylenepentamine, TEP.

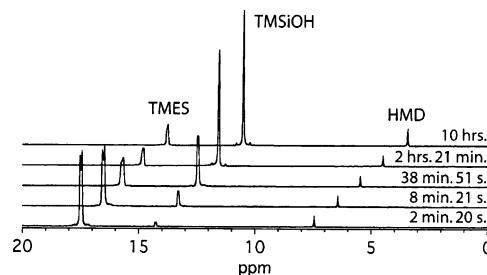


Figure 2. Time-elapased NMR spectra for the hydrolysis and condensation reactions. Spermidine is the catalyst. Peaks are referenced with respect to HMD (7.3 ppm).

Less than 1 min passed between the mixing of solutions and the initialization of the NMR experiment. In addition to the experiments run with amine catalysts, a control experiment was carried out where the sample contained no amine and was prepared by dilution of an aqueous solution of HCl (pH 4.5) with ethanol and water in the manner described above.

All experiments were run using the Refocused Insensitive Nuclei Enhanced by Polarization Transfer (RINEPT+) pulse sequence on a Varian UNITY spectrometer with a broad-band probe operating at 99.326 MHz on the ^{29}Si observe channel and ^1H decoupling operating at 499.625 MHz. A detailed explanation of the method is given elsewhere.³⁹ Temperature was checked with a methanol standard and found to be typically at 26.3 $^\circ\text{C}$.⁴⁰ Because ethanol was used as a solvent, the experiment was run without a lock. Spectra were referenced using HMD as an internal standard ($\delta = 7.3$ ppm)³⁵ and were obtained using two steady-state scans followed by eight transients. The preacquisition delay was arrayed such that the delay between the first two spectra was 30 s, and this delay was then increased from that of the previous delay by 30 s (i.e., preacquisition delay increment, 30, 60, 120, and 150 s, etc.).

Data were obtained from spectra through routine integration with normalization of peak integrals using VNMR software. Concentrations of the material present were then calculated on the basis of the initial amount of starting material using the normalized integral values from each spectrum.

Results

The reactions for TMES hydrolysis and condensation are detailed in Scheme 1. Sample NMR spectra are shown in Figure 2. To obtain a quantitative ranking of TMES hydrolysis rates in the presence of each amine, pseudo-rate constants (k_{hyd}) are obtained by fitting straight lines to the linear portion of the TMES data (Figure 3a). The dimeriza-

(39) Delak, K. M.; Farrar, T. C.; Sahai, N. *J. Non-Cryst. Sol.*, submitted for publication.

(40) Vangeet, A. L. *Anal. Chem.* **1970**, 42, 679.

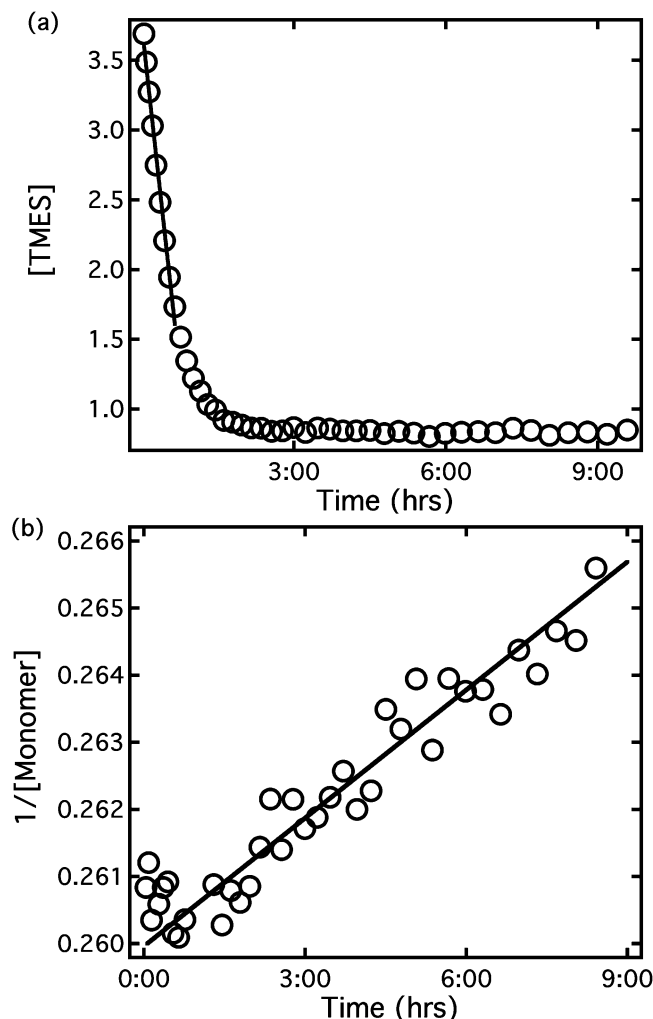


Figure 3. Representative graph showing hydrolysis kinetics as the decrease in TMES concentration over time (a) and condensation kinetics as the decrease in $1/[\text{monomer}]$ vs time, where $[\text{monomer}] = [(\text{CH}_3)_3\text{SiOCH}_2\text{CH}_3] + [(\text{CH}_3)_3\text{SiOH}]$ (b). In this case, the catalyst is spermidine. The pseudo-zero-order hydrolysis rate constant, k_{hyd} , is obtained from a straight-line fit to the linear portion of the data. The second-order condensation rate constant, k_{cond} , is obtained from a linear fit to the data.

tion reaction can occur through two pathways, in which either water or ethanol are produced. (eqs 2a,b in Scheme 1). In general, the water-producing pathway is favored,³⁵ but for the kinetic analyses of dimerization rates, the total monomer concentration must be taken into account.³⁶ A representative plot of the inverse concentration of total monomer $\{1/([\text{TMSiOH}] + [\text{TMES}])\}$ with spermidine as a catalyst, is shown in Figure 3b. The data are approximately linear, indicating a second-order character for the condensation mechanism. Rate constants from all the analyses are collected in Table 1. In general, hydrolysis rates are orders of magnitude larger than dimerization rates, in agreement with previous results obtained for strong acid/base catalysis.³⁶

The effects of polyamine chain length on hydrolysis and condensation rates are shown in Figure 4, where the logarithms of the rate constants k_{hyd} and k_{cond} are plotted against the number of amines in the polyamine. In general, catalytic efficiency scales with the polyamine chain length, thus reinforcing the findings of Mizutani.²⁹ This effect may be due to a cooperative, steric interaction between neighboring or alternate amine groups on longer chains, or due to

the optimal ("carbon spacer") distance between neighboring amine groups. Alternatively, the greater efficiency of longer polyamines may also be simply due to a stoichiometric effect relating to a larger total amine concentration. To separate out stoichiometric effects from polyamine chain length, we examined the effects of increased total monoamine and diamine concentration on both reaction rates. In the case of ethylamine, increased concentration has no effect. Results for decreased concentrations of tetraethylenepentamine are more variable, while a doubled concentration of diaminobutane yields results similar to the tetramine spermine. Thus, the data show that changes in the total amine concentration do not consistently affect catalysis.

As an alternative method of analysis, we use amine $\text{p}K_{\text{a}}$ as a measure of amine reactivity. From $\text{p}K_{\text{a}}$, we calculate the concentration of the conjugate base at the start of the reaction.^{41,42} We observe a direct relationship between the conjugate base concentration and the hydrolysis and condensation rate constants. Figure 5 shows plots of $\log(k_{\text{hyd}})$ and $\log(k_{\text{cond}})$ versus the negative log of the conjugate base concentration ($-\log(B)$), and correlation coefficients are 0.9554 and 0.9109 for hydrolysis and condensation, respectively.

Discussion

Our analysis of the influence of amines on TMES hydrolysis and condensation are complicated by the fact that amines are weak acids or bases and that they exist at different degrees of protonation depending on their $\text{p}K_{\text{a}}$ s. Furthermore, other important properties of amines, such as nucleophilicity, steric effects, and solvation behavior, are reflected in the $\text{p}K_{\text{a}}$ s of the amines. For these reasons, we have used $\text{p}K_{\text{a}}$ as a combined measure of amine reactivity in solution.

The $\text{p}K_{\text{a}}$ s used in our analyses reflect protonation constants in aqueous systems. The solvent system used here is a mixed ethanol–water system, in which the corresponding $\text{p}K_{\text{a}}$ values would not apply directly. However, the overall relative ranking for amine $\text{p}K_{\text{a}}$ s is typically retained for mixed ethanol–water systems,^{43–45} so the use of the aqueous $\text{p}K_{\text{a}}$ is an appropriate quantitative reflection of the characteristics listed above. For monoamines, there is only a single $\text{p}K_{\text{a}}$, and for polyamines, the number of $\text{p}K_{\text{a}}$ s is related to the number of amine groups in the molecule (Table 2).^{41,42,46} The $\text{p}K_{\text{a}}$ s are used to calculate the overall concentration of conjugate base present for a given amine. This compensates for any variability in pH encountered during sample preparation. Overall, we observe a linear log–log relationship between the concentration of conjugate base and the hydrolysis and condensation rates (Figure 5).

The effect of polyamine chain length on catalysis becomes more clear when examined in the context of the most acidic

- (41) De Robertis, A.; Foti, C.; Giuffrè, O.; Sammartano, S. *J. Chem. Eng. Data* **2001**, *46*, 1425.
- (42) De Stefano, C.; Foti, C.; Gianguzza, A.; Sammartano, S. *Anal. Chim. Acta* **2000**, *418*, 43.
- (43) Oszczapowicz, J.; Czuryłowska, M. *Talanta* **1984**, *31*, 559.
- (44) Kilic, E.; Gokce, G.; Canel, E. *Turk. J. Chem.* **2002**, *26*, 843.
- (45) Kilic, E.; Koseoglu, F.; Basgut, O. *Anal. Chim. Acta* **1994**, *294*, 215.
- (46) Parkhurst, D. *User's Guide to PHREEQC: A Computer Program for Speciation, Reaction-Path, Advective-Transport, and Inverse Geochemical Calculations*; U. S. Geological Survey: Lakewood, CO, 1995.

Table 1. Rate Constants from Kinetic Experiments with Monoamine and Polyamine Catalysts, and the pK_a s^a of the Amines Listed in Order of Increasing Hydrolysis and Condensation Rates

| name | catalyst | no. of functional groups | pK_a | k_{hyd} (10^{-4} mol L ⁻¹ s ⁻¹) | k_{cond} (10^{-8} L mol ⁻¹ s ⁻¹) | conjugate base concn ^b (10^{-6} mol L ⁻¹) |
|-----------------------|------------------------|--------------------------|--------|--|---|--|
| HCl | (control) | 0 | | 0.96 ± 0.04 | 1.6 ± 0.1 | 0.0063 (OH ⁻) |
| polyamines | | | | | | |
| DB | 1,4-diaminobutane | 2 | 9.1 | 0.77 ± 0.09 | 0.5 ± 2.8^c | 0.54 |
| SPD | spermidine | 3 | 7.82 | 12 ± 2 | 21 ± 5 | 4.01 |
| SPM | spermine | 4 | 7.18 | 27 ± 2 | 43 ± 7 | 35.8 |
| TET | triethylenetetramine | 4 | 2.38 | 203 ± 1^d | 1100 ± 100 | 1200 |
| TEP | tetraethylenepentamine | 5 | 1.88 | 418.0 ± 0.4^d | 1497 ± 5 | 1830 |
| monoamines | | | | | | |
| PIP | piperidine | 1 | 11.24 | 0.46 ± 0.02 | 4 ± 1 | 0.0031 |
| ET | ethylamine | 1 | 10.63 | 0.43 ± 0.03 | 2.9 ± 0.6 | 0.014 |
| IMI | imidazole | 1 | 6.95 | 42 ± 9 | 230 ± 30 | 68 |
| PYR | pyridine | 1 | 5.63 | 419 ± 4^d | 2000 ± 1000 | 550 |
| multiple amine concns | | | | | | |
| 2 × ET | ethylamine | 1 | 10.63 | 0.53 ± 0.07 | 3 ± 3 | 0.030 |
| 4 × ET | ethylamine | 1 | 10.63 | 0.38 ± 0.01 | 2.2 ± 0.6 | 0.072 |
| 2 × DB | 1,4-diaminobutane | 2 | 9.1 | 20 ± 2 | 55 ± 2 | 1.53 |
| 0.5 × TEP | tetraethylenepentamine | 5 | 1.88 | 430 ± 2 | 600 ± 300 | 1470 |
| 0.8 × TEP | tetraethylenepentamine | 5 | 1.88 | 376 ± 3 | 2309 ± 90 | 915 |

^a Most acidic pK_a for polyamines and imidazole. ^b Conjugate base concentrations calculated on the basis of published pK_a ^{41,42} values using PHREEQC.⁴⁶

^c The exceptionally large error of the sample is due to a significant amount of noise in the data, which made linear fits difficult. ^d Hydrolysis was mostly complete by the time the first data point was acquired, so the value was calculated using a starting concentration of 3.76 M (starting concentration based on the y-intercept from other analyses) and the first point from this data set.

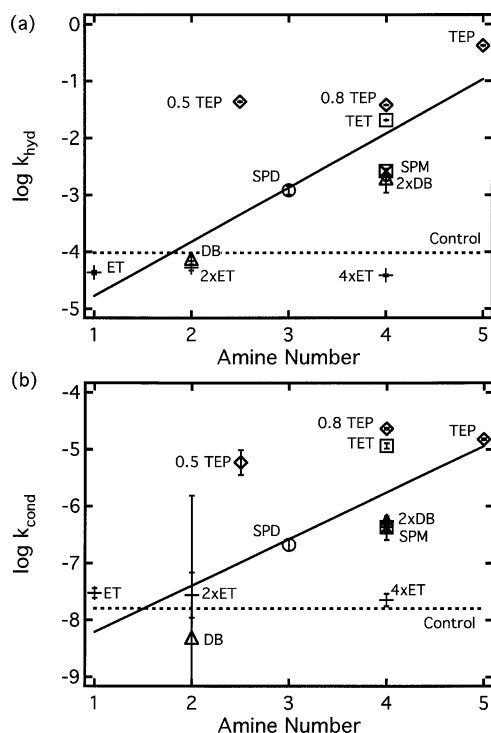


Figure 4. (a) Hydrolysis (k_{hyd}) and (b) condensation rate constants (k_{cond}) as a function amine number (n). The graph shows variable concentrations of ethylamine (crosses), diaminobutane (triangles), and tetraethylenepentamine (diamonds), in addition to the polyamines spermidine (open circle), spermine (crossed square), and triethylenetetramine (square). The straight line shows the log-normal relationship between the number of amines on the chain and the rates of hydrolysis and condensation ($r^2_{hyd} = 0.9466$; $r^2_{cond} = 0.8849$), but this relationship is insufficient to explain the nonstoichiometric effects of some amines on catalysis. Units for k_{hyd} and k_{cond} are (mol L⁻¹ s⁻¹) and (L mol⁻¹ s⁻¹), respectively.

polyamine pK_a and the overall conjugate base concentration of the polyamine. For the polyamines studied here, the most acidic pK_a decreases with both increasing polymer chain length and the length of the carbon spacer between amines.⁴⁷

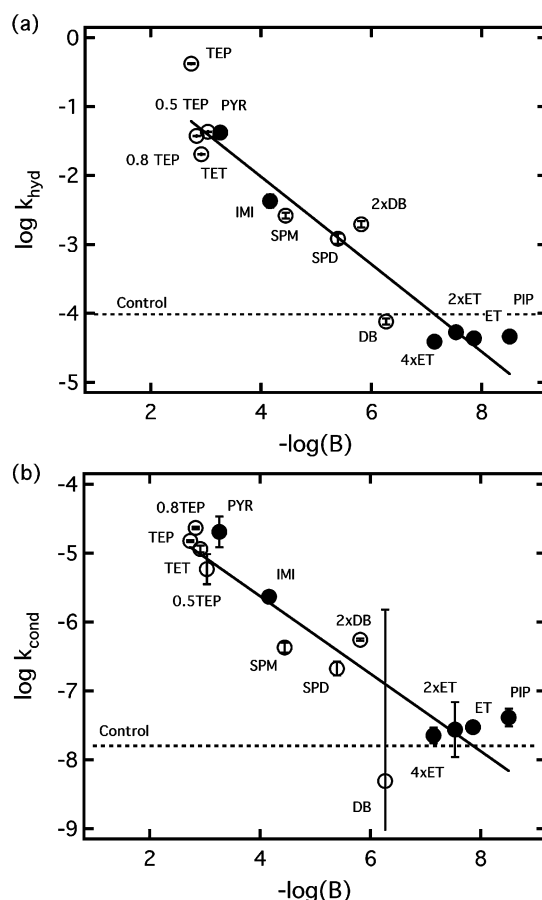


Figure 5. (a) Hydrolysis (k_{hyd}) and (b) condensation rate constants (k_{cond}) as a function of the negative logarithm of the conjugate base concentration ($-\log(B)$): monoamines (filled symbols), polyamines (open symbols), and HCl control (dashed line). The straight line shows the log-normal relationship between the pK_a and the reaction rate, with $r^2 = 0.9554$ for hydrolysis and $r^2 = 0.9109$ for condensation. Units for k_{hyd} and k_{cond} are (mol L⁻¹ s⁻¹) and (L mol⁻¹ s⁻¹), respectively.

As such, the five-amine chain of TEP is the most acidic ($pK_a = 1.88$), followed by TET ($pK_a = 2.38$),⁴² SPM ($pK_a = 7.18$),⁴² SPD ($pK_a = 7.82$),⁴² DB ($pK_a = 9.1$),⁴¹ and ET ($pK_a = 10.63$). So TEP, which is one repeat unit longer than TET,

(47) Koper, G. J. M.; van Duijvenbode, R. C.; Stam, D. D. P. W.; Steurle, U.; Borkovec, A. *Macromolecules* **2003**, *36*, 2500.

Table 2. Polyamine pK_a s^a

| polyamine | pK_{a1} | pK_{a2} | pK_{a3} | pK_{a4} | pK_{a5} |
|-----------|-----------|-----------|-----------|-----------|-----------|
| DB | 10.54 | 9.1 | | | |
| SPD | 10.8 | 9.58 | 7.82 | | |
| SPM | 10.7 | 9.7 | 8.31 | 7.18 | |
| TET | 9.67 | 8.87 | 6.11 | 2.38 | |
| TEP | 9.83 | 9.01 | 7.73 | 3.91 | 1.88 |

^a Derived from refs 41 and 42.

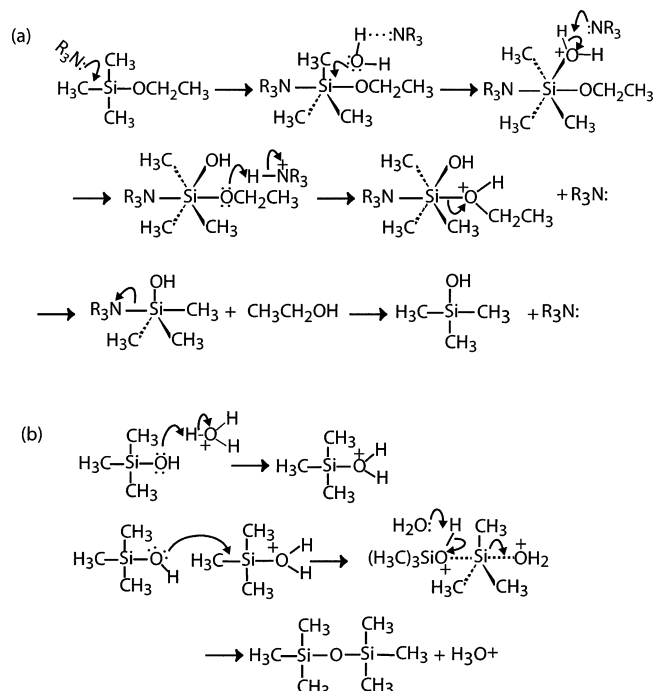
is slightly more acidic because of its longer chain length, and TET is more acidic than SPM, because of smaller carbon chain spacing between the amine groups (two for TET, two and three for SPM). The effect of polyamine chain length on catalysis is then simply a result of the pK_a and the corresponding conjugate base concentration, as seen in (Figure 5). The correlation between catalytic efficiency and conjugate base concentration explains why the effect of concentration on rates is not seen for ET and TEP. For instance, ET ($pK_a = 10.63$) is an ineffective catalyst because it exists exclusively in the protonated form at the experimental pH. The results for DB and $0.8 \times$ TEP are simply minor deviations from the trend rather than stoichiometric effects.

The effect of amine pK_a on catalysis extends to monoamines as well. Overall, we observe that more acidic monoamines, such as PYR and IMI, are more effective catalysts for organosilicate hydrolysis and condensation than basic monoamines (ET and PIP).

Recent studies of biogenic silica formation have attributed a catalytic role to silaffins, the polyamine-containing proteins found in diatoms. The proteins are postulated to catalyze silicic acid polymerization through predominantly physical mechanisms, whereby silica precipitates on the external surface of small protein or polyamine globules, whose aggregation is induced by the presence of phosphate.^{30,31} At near-neutral pH, the positively charged polyamines are attracted to negatively charged polysilicate oligomers that form when the concentration of silicic acid is greater than 2 mM. Regions of high polysilicate concentrations occur proximate to the interface between the protein and the aqueous surroundings, accelerating silica precipitation.^{27,28}

These physical mechanisms are inappropriate for describing our system. First, the use of a monofunctional organosilicate as a starting material precludes formation of negatively charged polysilicates, while providing a proxy for the reactivity of an organosilicate precursor. Second, the intermediate product, TMSiOH, has a pK_a of 12.7 and is neutrally charged in the pH regime studied here. Taken together, these two factors eliminate the consideration of electrostatically driven condensation while providing the appropriate environment necessary to examine the chemical mechanisms.

On the basis of the observed relationship in Figure 5 whereby the increased presence of the conjugate base enhances catalysis, we postulate a mechanism for hydrolysis (Scheme 2a), which incorporates aspects of peptide bond hydrolysis by chymotrypsin as well as the nucleophile-catalyzed mechanism of R_3SiX hydrolysis put forth by Corriu.^{37,38,48} In our scheme, the conjugate-base form of the

Scheme 2. Amine-Catalyzed Mechanism for Hydrolysis of TMES and S_N2 Acid-Catalyzed Condensation of (TM)SiOH

amine acts as a nucleophile toward the Si center in TMES, forming a pentacoordinate reactive intermediate with a direct Si-N bond (Scheme 2a).³⁷ This would result in a labilized, longer Si-O(C₂H₅) bond which is more susceptible to hydrolysis. In the next step, a second amine group H-bonds to a H₂O molecule, which encourages the H₂O to attack the pentacoordinated Si intermediate, forming a hexacoordinated Si transition state. The second amine group at this point abstracts the H⁺ from H₂O, forming R₃NH⁺. Finally, the R₃NH⁺ group interacts with the -OC₂H₅ leaving group, thus helping formation of C₂H₅OH and the R₃NH⁺ reverts to R₃N. In the analogous chymotrypsin-catalyzed hydrolysis of a peptide bond, the serine -OH group in chymotrypsin is the nucleophile that attacks the trigonal planar carbon center in the peptide to form a tetrahedral intermediate. Subsequently, histidine from chymotrypsin H-bonds to water, which promotes the water molecule to attack the tetrahedral acyl carbon intermediate.⁴⁸ Thus, the histidine in chymotrypsin is analogous to the second amine group in our mechanism. In principle, our proposed mechanism is very similar to the one suggested previously for the hydrolysis of tetraethylorthosilicate by silicatein, but differs in the details.

For the condensation step, consistent with the second-order rate law (e.g., Figure 3b), we propose an acid-catalyzed S_N2 mechanism, as is appropriate for the experimental pH (Scheme 2b). Typically, base-catalyzed condensation is the favored mechanism for silica polymerization between pH 2 and 7 once silica particles have nucleated.¹ This is because surface silanol groups are deprotonated and can act as nucleophiles when the pH is above the isoelectric point of silica (i.e. pH > 2). In our experiment, the pH conditions do not favor formation of TMSiO⁻, so we exclude base catalysis as an option. Furthermore, we do not expect that condensation is the direct result of amine catalysis primarily because the formation of a hexacoordinated transition state

(48) Stryer, L. *Biochemistry*; W. H. Freeman and Co.: New York, 1988.

involving two trimethylsilyl groups seems sterically prohibitive. Acid catalysis, where water acts as the proton-transfer agent (because of its much higher concentration than the amines), remains the only plausible mechanism for the condensation reaction. The parallel trend seen between the hydrolysis and condensation rates is then merely a reflection of the rates at which TMSiOH is produced.

The acid-catalyzed condensation mechanism operates in stark contrast to the postulated physical mechanism for polysilicate precipitation on positively charged amine surfaces.^{28,30} The complementary charge attraction that acts to foster silica precipitation cannot be taken advantage of here, because of the neutral charge on TMSiOH . If the mechanism of complementary charge attraction holds in natural systems, we would expect silica precipitation to be rapid once silicate clusters have formed.

The chemical mechanisms suggested above are only postulated, and we are carrying out calculations to validate these hypothesized scenarios. Our results are significant because they suggest that histidine, with its imidazole functionality, was the evolutionary choice for silicatein because the amine can exist in the conjugate base form under the mildly acidic conditions where silica deposition takes place.^{9,11} Furthermore, the efficient catalysis of silica formation by long-chain polyamines and silaffins has less to do with the absolute polyamine chain length and more to do with the increased presence of amine groups existing in the conjugate base form, a direct byproduct of the "smearing out" of the amine pK_a s that occurs with large polyelectrolytes.^{47,49} Our observation of chemically catalyzed organo-silicate hydrolysis and condensation does not discredit the possibility that longer chain polyamines such as those found in diatom proteins contribute to silica precipitation through a combination of electrostatic and cooperative phase-separa-

tion effects once polysilicate clusters have been formed. More likely, both mechanisms may be involved in different stages of silica precipitation, with the chemical mechanism dominating the early stages of hydrolysis of the putative Si(OR)_4 precursor and the physical mechanisms controlling the later stages (condensation) of Si(OH)_4 and aggregation of silica nanospheres.

Conclusions

Our experiments show that there is a direct correlation between the concentration of the amine conjugate base and its ability to catalyze hydrolysis and condensation of TMES. The ability of the mildly acidic amines to catalyze at the experimental pH suggests a nucleophile-driven reaction mechanism for hydrolysis that differs from conventional base-catalyzed $\text{S}_{\text{N}}2$ mechanisms.

We conclude that amines may act in biogenic and biomimetic silica polymerization in a number of redundant catalytic pathways. The quantitative ^{29}Si NMR approach taken here combined with the use of a starting compound with a single reactive bond has thus allowed us to separate out some of these pathways and to identify the properties of the amines which control the rates of some of these pathways, as a first step toward controlled amine catalysis of biomimetic silica synthesis.

Acknowledgment. We thank Prof. Robert West and Prof. Thomas Farrar, of the UW Department of Chemistry, and Dr. Charles Fry, of the UW Chemistry Magnetic Resonance Facility, for help with instrumentation and numerous helpful discussions. We also thank Dr. Young Lee for assistance in polyamine speciation calculations. Funding was provided by NSF Grant EAR 020836 and Faculty "Startup Grants" to N. Sahai. NMR Spectrometer funding support: NSF Grant CHE 9629688, NSF Grant CHE 8813550, and NIH Grant 1 S10 RR04981-01.

(49) Manning, G. S. *Q. Rev. Biophys.* **1978**, *11*, 179.