Size Control of Dendrimer-Templated Silica
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One of the most significant challenges facing the biomimetic synthesis of materials is achieving the requisite level of dimensional and spatial control. Typical reaction conditions for biomimetic silica synthesis allow for continued growth and ripening leading to the formation of larger nanospheres on the order of 200–600 nm in diameter. Herein, we have used polyamidoamine and polypropylenimine dendrimers as templates to expand the reaction conditions of biogenic silica production to produce a more robust synthesis leading to size-selective precipitation of silica nanospheres. Through the use of defined concentrations of phosphate buffer and main group metal chloride salts, we have shown that the biomimetic silica growth process is controlled by cationic neutralization of the anionic silica nanosphere surface. Neutralization minimizes electrostatic repulsions, allowing for agglomeration and continued growth of nanospheres. By controlling these concentrations, we can selectively produce silica nanospheres of desired dimensions between 30 and 300 nm without adversely affecting the template’s activity.

Introduction

In contrast to current industrial scale synthesis of silica, biogenic silica synthesis occurs under ambient conditions in aquatic environments.1 Two highly studied examples of biosilicification are the formation of the diatom frustule2 and the defensive silica spicules of sponges.3 Both processes are dominated by species-specific peptide- and protein-mediated formation of ornate nanoparticulate silica structures.4–8 While the mechanisms of silica formation and patterning differ, the final processes result in a vast array of structures and properties over multiple length scales. For instance, the siliceous spicules of the sponge Euplectella have demonstrated critical properties for applications in fiber optics.9

Biosilicification in diatoms is mediated through the interactions of highly post-translationally modified peptide sequences known as silaffins, with a parent peptide sequence of H2N-SSKKGSYSGSKGSKRIL-COOH.5,6 The lysine residues of the peptide have been modified to either ε-N-dimethyl-lysine, phosphorolated ε-N-trimethyl-δ-hydroxylysine, or long-chain polyamine moieties of N-methyl derivatives of polypropyleneimine (PPI), while the serines have been phosphorylated. Phosphorylation of the serine residues promotes aggregation of the peptides in dibasic buffer solutions producing localized areas of high amine concentrations capable of condensing silica.6,9 Silica produced from these peptides, at mildly acidic conditions, is nanospherical with diameters ranging from 400 to 600 nm.5,6

The unmodified R5 peptide sequence is also able to rapidly precipitate silica, but at neutral pH.5 Synthetic site directed mutagenic analysis of the peptide has demonstrated the requirement for peptide self-assembly mediated by the RRLIL motif at the C terminus of the peptide.10 Similar to the silaffins, aggregation of the R5 peptide led to the formation of silica nanospheres with diameters between 200 and 400 nm.10

Recent studies have also investigated the ability of biological and biomimetic amine containing polymers to drive biogenic silica production.7,11–14 Typically, active polyamines are linear polymer chains based upon repeat units possessing amine moieties. Studies of selected polyamines (polyallylamine hydrochloride and biogenic polymers of N-methylpropyleneimine attached to pu-trescine) have demonstrated similar self-aggregation abilities in phosphate buffer solutions under in vitro conditions.11,12 These studies have also shown a link between the phosphate concentration and the yield of the resultant product, with low buffer concentrations leading to decreased production activity and diminished particle diameters.11,12 Because of the phosphate-dependent self-assembly process, it is rather difficult to dissect the particle formation mechanism from the template assembly.

Amine-terminated dendrimers have also been used for biomimetic silica synthesis as models of the self-assembled structures responsible for diatom silica production. Specifically, polyamidoamine (PAMAM) dendrimers and PPI dendrimers display activity that is quite similar to

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that of their biological counterparts. Because dendrimers of higher generations do not self-assemble and remain as discreet globular spheres in solution, they represent unique templates for the study of the biomimetic silica production. Through the use of these dendrimer templates, we have been able to determine key conditions for the controlled in vitro synthesis of silica nanoparticles. Under these conditions, we are able to grow and develop the materials at discrete size regimes without negatively impacting the template’s overall activity.

Materials and Methods

Materials. DAB-Am-4 polypropylenimine tetraamine dendrimer, DAB-Am-8 polypropylenimine octaamine dendrimer, DAB-Am-16 polypropylenimine hexadecamine dendrimer, DAB-Am-32 polypropylenimine dotriacontaamine dendrimer, DAB-Am-64 polypropylenimine tetrahexacontaamine dendrimer (G1–G5), PAMAM dendrimers (G0–G3 and G5), and tetramethyl orthosilicate were purchased from Aldrich Chemical Co. PAMAM dendrimers (G4 and G6) were purchased from Dendritech, Inc. LiCl, NaCl, RbCl, and CsCl were purchased from Acros while KCl and MgCl₂, where purchased from Fisher. All materials were used as received.

Silica Precipitation Assays. Silica precipitation assays were based on protocols of various procedures. To varying concentrations of phosphate buffer (0–100 mM, pH 7.5), a dendrimer reaction solution was prepared to a primary amine concentration of 20 mM. To the dendrimer solutions was added 20 µL of 1 M silicic acid (prepared from the hydrolysis of tetramethyl orthosilicate in 1 M HCl for 15 min at room temperature while stirring), upon which silica precipitation was instantaneous. The solutions were allowed to react while shaking for 5 min. Post-reaction, the assay mixture was centrifuged for 5 min (10K rpm), from which the supernatant was decanted, and the pellet was washed thoroughly with water. The silica pellet was then either quantitated using the β-silico molybdate assay or studied using scanning electron microscopy (SEM).

Silica precipitation was also performed using various concentrations of group metal chloride salts (0.255–400 mM, pH 7.5) in the absence of buffer. The salts included LiCl, NaCl, KCl, RbCl, CsCl, and MgCl₂. Silica precipitation assays were conducted as above.

Silica Quantitation. Silica samples to be quantitated were dissolved in 1 mL of 500 mM NaOH by incubation at 95 °C for 30 min. After complete dissolution, the samples were filtered using molecular-weight-cutoff centrifuge filtration devices (Millipore, Inc.) to remove the liberated dendrimer because it was an interferent with the molybdate assay. As a result of the readily available molecular-weight cutoffs, only silica produced from PAMAM G2 and greater generations 4 and 5 for PPI dendrimers was quantitated. To the filtered samples was added the molybdate reagent, and it was allowed to react to produce the colorimetric product that was monitored at 410 nm (Agilent 8453 UV–vis spectrophotometer) and quantitated using a previously produced standard curve.

SEM. Silica samples were prepared as above. After the final wash, the pellet was resuspended in water and added dropwise to an aluminum SEM puck (Ted Pella, Inc.). After vaporization of the solvent, the samples were sputter coated with a thin layer of gold (Pelco model 3 sputtering instrument) to avoid charging. The samples were analyzed using an Hitachi S4200 scanning electron microscope operating at variable voltages. Particle size distributions were manually determined.

Dynamic Laser Light Scattering. G6 PAMAM and G5 PPI dendrimers templated by light scattering were dissolved in appropriate solutions (0.5, 10, and 40 mM phosphate buffer or NaCl at pH 7.5) to a concentration of 20 mM primary amines. The samples were analyzed using a Zetasizer Nano Series dynamic laser light scattering instrument with a CGS-3 compact goniometer system by Malvern Instruments.

Results and Discussions

Recent efforts in biomimetic silica synthesis have focused on improving the ability to control nanoparticle size and dispersity. Using self-assembled biological and biomimetic polyanine templates, researchers have developed strategies to control the particle size distributions, but these methods result in variable silica production. Ideally, a template would have consistent activity with controllable product size distributions over a wide range of reaction conditions. The concentration of phosphate in solution has been identified as a key factor in the biological control of product size, with the activity and particle size increasing with increasing phosphate concentrations. The degree to which the monomers aggregate has also been correlated to the concentration of the phosphate species. These results suggest that the size and condensation activity of silica can be linked to the degree of aggregation of the monomeric templates to larger active structures in solution. Thus, the observed phosphate concentration dependence may be involved only in template aggregation, while the growth mechanism of silica to its final size may be dependent upon other conditions. Unfortunately, in such a system, it is difficult to separate the self-assembly process from possible silica growth mechanisms. Such questions may be effectively examined by a monomeric amine displaying template.

Previous studies have shown that PAMAM and PPI dendrimers are effective biomimetic analogues of the self-assembled templates used by diatoms for silica production. Dendrimers also remain in an unaggregated state in solution as determined by small-angle X-ray scattering. Similar results from dynamic laser light scattering studies at various concentrations of additives in solution confirm these findings. With such a well-defined template, the silica growth process can be examined independent of the buffer conditions required for self-assembly of biological systems.

Each dendritic template was assayed for silica production and nanosphere characterization at various concentrations of phosphate buffer (0–100 mM, pH 7.5). Previously studied self-assembling amine templates have shown a distinct phosphate concentration dependence in silica production activity. In contrast, dendritic templates (PAMAM G2–G6 and PPI G4 and G5) presented no phosphate concentration dependence on silica formation. Assays performed in the absence of phosphate buffer, however, resulted in no silica produced (Figure 1). Consistent activity over a wide range of buffer concentrations is undoubtedly the result of the monomeric state of the dendritic template.

While the silica production activity was not affected by phosphate-buffered solutions, the silica nanosphere size appeared to be. SEM analysis of particle size distributions demonstrated linear size dependence for silica produced with concentrations below 20 mM phosphate buffer. Above 20 mM phosphate, the silica spheres had a constant diameter between 250 and 350 nm depending on the template. The size-selective effects are likely due to the charge neutralization of cations electrostatically interacting with the negatively charged surface silanol groups on the silicate surface. It has been previously suggested, from light scattering evidence, that the growth process of dendrimer-templated silica demonstrates two dominant phases: the rapid nucleation of silica encapsulating the template followed by a slower ripening phase to the final particle diameter. Electrostatic repulsions between smaller silica nanospheres are minimized by the surface neutralization from cations present in solution, thus, allowing for particle growth. Once a certain critical

particle size is reached, however, the silica sphere precipitates from solution. It is of interest to note the size differences between templates precipitated from PAMAM templates versus those from PPI templates. Previously, Knecht and Wright showed that silica particles precipitated from PPI templates were approximately 60% of the size of particles precipitated from the associated PAMAM template [PPI G(x) vs PAMAM (G(x)-1)], consistent with the ratio of template diameters. These effects were also clearly noted in the size distributions of the silica particles of the associated templates at the corresponding buffer concentrations (Figure 1 and Supporting Information), providing further support for the idea that the small silica-encapsulated dendrimers serve as the basic building block of the growing aggregates.

For lower generation dendrimers (PAMAM G0 and G1, PPI G1), cationic concentrations played a lesser role in the resultant particle size distributions. This was attributed to the increased degree of intercalation between the low generational dendritic templates. Such intercalates could result in assembled and aggregated structures of amines. Because such aggregates present a larger surface for polycondensation activity, larger silica nanoparticles are produced by their extended growth along the aggregated structures.

These results suggest that the phosphate anions, in reality, play little role in the actual size determination of nanosilicates from monomolecular templates. Thus, while phosphate may be critical to the degree of template formation, it is difficult to see how anions, rather than cations, would support silicate growth. In the biogenic system of silica production, cations could mediate the controlled synthesis of size-selective silica particles.

To probe the role of cations, the activity of the G4 PAMAM template was assayed in various concentrations of salt solutions (LiCl, NaCl, KCl, RbCl, CsCl, or MgCl2). Quantitation of the reaction product demonstrated a statistically equivalent activity for each assay. Further, this activity profile paralleled silica condensation reactions run in phosphate. A series of control experiments, studied by varying the dendrimer template concentrations, were conducted at constant salt concentrations which showed linear activity toward silica production based upon primary amine concentrations, similar to previous results (Supporting Information). It is noteworthy to mention that under these reaction conditions, the self-aggregating templates of the biological systems would be inactive as a result of the polyvalent anion requirement for template aggregation.

Assays to determine the cation affects upon particle size displayed a concentration dependence on the salt in the reaction media. Standard reaction conditions with the G4 PAMAM dendrimer template in which phosphate was replaced with individual salt solutions (0.255–400 mM, pH 7.5) yielded silica nanospheres. SEM analysis of the particle size revealed a linear increase in particle diameter as salt concentrations increased to 100 mM. Beyond 100 mM salt, silica sizes stabilized to a constant diameter. Silica precipitated from LiCl, NaCl, and KCl all produced nanospheres with a maximum size of approximately 235 nm at salt concentrations higher than 100 mM. RbCl and CsCl produced nanospheres of smaller maximum diameter of 210 and 195 nm, respectively (Figure 2). The difference in nanosphere sizes may be attributed to the atomic radii of each cation studied. The smaller cations, with radii between 90 and 152 pm, preferentially bind to a single silanol moiety along the surface of the ripening clusters. These smaller cations, having a stronger affinity to the growing silica structures, promote charge neutralization of the surface of the silica. The larger cations, greater

than 166 pm, most likely bridge between multiple silanol groups. Such insufficient surface coverage of the particles leads to less surface charge neutralization. This effect leaves numerous negatively charged silanol groups exposed, thus, increasing the amount of interparticle electrostatic repulsions leading to diminished particle sizes.

Examination of the divalent magnesium cation resulted in the formation of smaller silica nanospheres (115-nm diameter). Similarly, the silica production activity showed a marked decrease (see Supporting Information). These results indicate that the surface stabilization effects of the divalent magnesium are insufficient for silica production. The divalent cation may attempt to bridge multiple singly charged silanol groups along the growing silicate surface, as was seen with Rb\(^+\) and Cs\(^+\), leading to poor surface coverage and neutralization and, thus, leading to diminished particle size distributions.

**Conclusions**

Control of particle size is a principal consideration in nanoscale design and engineering. Particle size distributions dictate many of the properties associated with the material. It is believed that diatoms use highly functionalized organic scaffolds of proteins and polyamines for discrete particle formation and synthesis of highly structured siliceous materials.\(^7,20\)–\(^22\) Atomic force microscopy evidence suggests that these structures are composed of individual silica nanospheres.\(^23\) Currently, synthetic size control of biogenic silica has been challenging. Under in vitro conditions, particle ripening and aggregation leads to the formation of much larger non-natural silica nanospheres. This process can be controlled through charge stabilization of the growing spheres in solution. Particle agglomeration is dictated by the neutralization of negatively charged surface silanol groups. We have shown, through the use of dendrimer templates, that such interactions are extremely important for the size selectivity of silica nanospheres. The neutralized surface is formed through interactions of cations with the growing negatively charged silicate surface. The effective charge neutralization decreases the electrostatic repulsions, thus, permitting the particles to agglomerate and grow to significantly larger sizes. This study is in stark contrast to the results of the biologically derived templates that demonstrate a dependence on polyvalent anions, mainly phosphate for activity. These previous results most likely reflect the buffer requirements for template self-assembly, which is unnecessary for activity from monomolecular dendrimers. This study also invites inquiries into the cation content of the silica deposition vesicle used by the diatom for silica formation. Does the diatom use ions, in concert with other organic molecules, to control the growth and formation of the nanoparticles in vivo? Further research must be conducted to determine if these effects play a role, if any, with in vivo silica production.

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**Supporting Information Available:** PPI dendrimer analysis, SEM micrographs, particle size histograms, amine concentration dependence in NaCl solutions, and MgCl\(_2\) analysis. This material is available free of charge via the Internet at http://pubs.acs.org.


