Nanostructure Assembly

Biomimetic Patterning of Silica by Long-Chain Polyamines**

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The production of species-specific, precisely shaped inorganic structures is a widespread phenomenon among organisms. A large proportion of biogenic silica is formed by diatoms, which are unicellular algae ubiquitously present in marine and freshwater habitats. Diatom biosilica is mainly composed of hydrated SiO_2 (silica) that is in an amorphous state even on an atomic scale.[1] However, diatom biosilica exhibits highly symmetrical patterns in the nano- to micrometer range, as is evident from scanning electron microscopy (SEM) images of diatom cell walls.[2] A new siliceous cell wall is produced in a specialized intracellular compartment, the silica deposition vesicle (SDV).[3] The investigation of the mechanism that assures the precision of reproduction of nanostructured silica in each generation is not only a fascinating problem but also of interest in materials science. In the past decade numerous examples have demonstrated the potential of templating mechanisms to create inorganic structures that resemble, at least to some extent, their biological counterparts.[4,5] Biomimetic approaches are believed to enable the production at ambient temperatures and neutral pH of advanced materials with potential applications in catalysis, separations science, electronics, and photonics.

The nanofabrication of silica in diatoms results from specific interactions between biomolecules and silicic acid derivatives. The biomolecules isolated from diatom biosilica are the silaffin peptides and long-chain polyamines: *N*-methylated poly(propylene imine)s attached to putrescine.[6–8] Silaffins isolated from *Cylindrotheca fusiformis* mainly consist of lysine and serine residues, and all of these residues are

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modified. The latter are phosphorylated, and the lysines are converted into three different derivatives: ε-*N*-dimethyllysine, ε-*N*-trimethyl-δ-hydroxylysine, and lysine residues covalently linked to long-chain polyamines. Both silaffins and long-chain polyamines have been shown to rapidly direct the formation of silica nanospheres from silicic acid in vitro.[6,7] Polyamines are known to affect silica formation in several ways. They catalyze siloxane-bond formation and can act as flocculating agents.[9,10]

Particularly intricate silica patterns including fine structures in the 30 to 50 nm range are found among diatom genera, such as *Stephanopyxis* and *Coscinodiscus* (Figure 1).

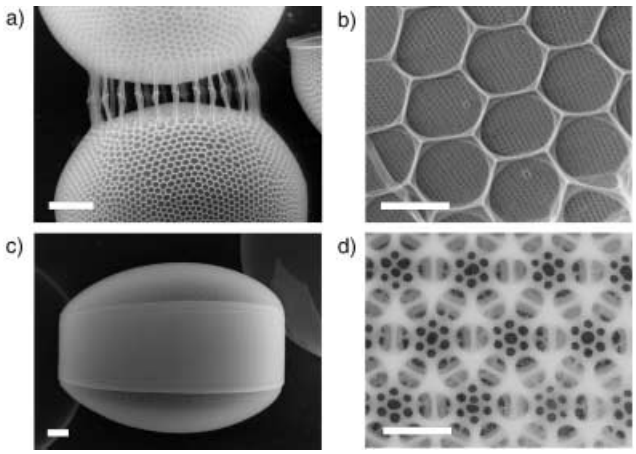

Figure 1. SEM images of the cell walls of the diatoms *Stephanopyxis turris* and *Coscinodiscus granii*. a) Cell walls of *Stephanopyxis turris*; scale bar: 20 μm; b) details of the silica nanoscale architecture; scale bar: 3 μm. c) Cell wall of *Coscinodiscus granii*; scale bar: 20 μm; d) details of the silica nanoscale architecture; scale bar: 1 μm.

The *Coscinodiscus* valve structure can be interpreted as being composed of a hierarchy of hexagonal silica structures, which create the complex but highly symmetrical valve pattern. Surprisingly, when *Coscinodiscus* shells were extracted with hydrogen fluoride, polyamines were found to be the main organic constituent of the extracts.[11] These observations stimulated the development of a model of pattern formation that is based exclusively on the physicochemical properties of polyamines.[11] The underlying concept is the assumption that phase separation occurs within the SDV to form emulsions of microdroplets of polyamines. In a close-packed arrangement, these microdroplets would form a hexagonal monolayer within the flat-walled SDV. The aqueous interface between polyamine droplets, which contains silicic acid derivatives, should promote silica formation (catalyzed by the polyamine surfaces[9]) to give a honeycomb-like framework of silica precipitates. Reiteration of this scenario on smaller and smaller scales would create the patterns observed in *Coscinodiscus*.

Subsequent in vitro experiments demonstrated the phase separation of polyamines in aqueous solutions and led to an understanding of how microdroplet size is controlled.[12] In aqueous solution polyamines form aggregates (microemulsions) with positively charged surfaces, and multivalent anions promote higher-order assemblies of the emulsion droplets. Polyamine-induced silica-nanosphere production from a solution of mono- and disilicic acid was found to depend strictly on microscopic phase separation.[13] Silicic acid derivatives may be adsorbed on and/or dissolved in the polyamine droplets, whereby they form a coacervate (a "liquid precipitate"), which finally hardens by silica formation. This mechanism may explain the observed correlation between polyanion concentration and the size of the resulting silica nanospheres. Defined diameters between 50 and 1000 nm could be obtained and the resulting size distributions were close to monodisperse. However, pattern formation as proposed by the above-mentioned model requires a polyamine-based mechanism that produces a hexagonal framework of silica rather than a population of silica spheres.

Herein I demonstrate the potential of a polyamine/phosphate system to guide the production of silica structures composed of hexagons under slightly different conditions. Monosilicic acid rapidly polymerizes at neutral pH to produce a sol, which then solidifies to a gel.[10] The kinetics of sol-particle growth can be followed readily by dynamic light scattering. Under the conditions used (Figure 2), the average

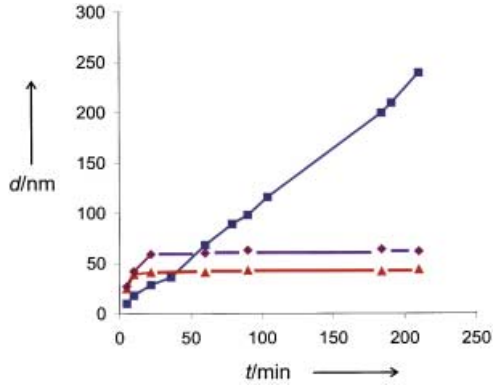

Figure 2. Silica-sol formation followed by dynamic light scattering. A freshly prepared silicic acid solution (70 mM) in Tris-HCl (40 mM, pH 6.8) was incubated at 25 °C for the times indicated. The z-average diameters (*d*) of particles were determined by using a Malvern HPPS 5001 high-performance particle sizer. Blue: no addition of poly(allylamine); violet: in the presence of poly(allylamine) (0.4 mM); red: in the presence of poly(allylamine) (0.4 mM) at pH 6.2.

diameter of poly(silicic acid) particles or aggregates increased continuously over a period of 4 h. A completely different behavior was observed in the presence of a long-chain polyamine, such as poly(allylamine). Particles with defined diameters appeared within a few minutes. These particles were then prevented from further growth. Lowering or increasing the polyamine concentration by a factor of two did not influence the particle size. However, the final particle size was slightly dependent on pH. At pH 6.8, particles with a z-average diameter of about 60 nm (polydispersity index about 0.3) were the stable end product. At pH 6.2 the stable end product had a mean diameter of only 40 nm. These sols were stable for at least 24 h. A stabilizing effect on silica sols has been described for a number of organic bases, such as morpholine and cyclohexylamine, as well as large organic

cations.[10,14] Possibly the strong adsorption of relatively few organic polycations on the surfaces of the colloidal particles serves to keep them apart, thus preventing aggregation. In the following, such a silica sol will be denoted as a polyamine-stabilized sol.

Poly(allylamine)/phosphate-directed silica formation produced a strikingly different morphology when a polyamine-stabilized sol replaced monosilicic acid as the silicon source. Instead of forming a precipitate of nanospheres (as is the case for silicic acid), the stabilized silica sol (particles of 40-nm diameter) assembled within seconds to give a framework of roughly hexagonal silica structures when added to a polyamine/phosphate microemulsion (Figure 3). This dramatic

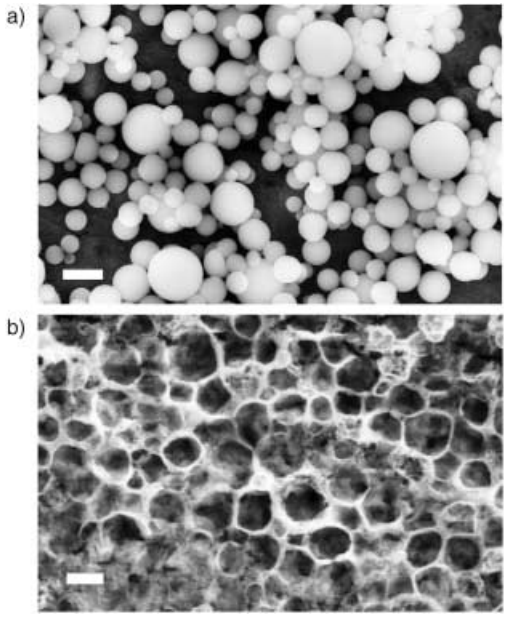

Figure 3. SEM images of silica morphologies formed from monosilicic acid (a) or polyamine-stabilized silica sol (b) as the silicon source in the presence of polyamines. a) A freshly prepared solution of monosilicic acid was added to a phase-separated poly(allylamine)/phosphate mixture (formed by mixing solutions of sodium phosphate (pH 6.8) and poly(allylamine) to yield a mixture 0.2 mM in polyamine and 18 mM in phosphate, which immediately became turbid as a result of microphase separation). The final silicic acid concentration was 40 mM. Silica formation was allowed to proceed for 12 minutes at 25 °C. The precipitate was collected by centrifugation, washed with water, and analyzed by SEM. b) Monosilicic acid was replaced by a polyamine-stabilized silica sol (40-nm particles, final SiO_2 concentration: 40 mM), which was prepared as described in the Experimental Section. Silica started to precipitate within a few seconds. Scale bar: 1 μm.

effect may be interpreted in the following way: The microdroplets formed by the polyamine/phosphate mixture promote silicic acid polymerization to produce silica nanospheres by coacervate formation as described above (Figure 3a). However, the polyamine-stabilized sol used as the silicon source (Figure 3b) carries positive surface charges (charge reversal relative to polysilicic acids) and is therefore unlikely to interact with the positively charged polyamine droplets. This charge-reversed sol becomes concentrated within the aqueous interfaces, and its polymerization to silica is favored by the presence of polyanions (phosphates), which act as the flocculating agent. In this case, clusters of polyamine droplets are assumed to serve as the organic template for silica formation and thereby lead to the formation of a roughly hexagonal network. This network is far from perfectly formed, as a result of the fact that the poly(allylamine) droplets are heterodisperse and not arranged in a two-dimensional close-packed configuration.

This simple scenario may explain a number of observations with respect to silica nanofabrication in diatoms. First, if close-packed polyamine droplets serve as a template, their diameters determine the size of the resulting silica hexagons. We have shown previously that droplet diameters can be precisely controlled simply by tuning the polyamine/polyanion ratio,[12,13] which suggests that diatoms may use the highly phosphorylated proteins present in diatom valves to regulate droplet size.[8,15] Second, it has been demonstrated repeatedly that diatom biosilica is composed of silica particles with diameters of about 30 to 50 nm.[16–18] Little variation in the size range of these silica particles has been found within the same valve, but significant differences have been found among diatom species.[19] A polyamine-stabilized sol (produced at slightly different pH values in different diatom species) that served as the silicon source in vivo would explain these observations. Furthermore, the existence of a polyamine-stabilized sol would solve another enigmatic problem of silica biomineralization in diatoms. As intracellular silicic acid concentrations can exceed micromolar levels, silicic acid would undergo premature condensation in the cytosol unless the silica precursors were stabilized in some way by binding to or association with organic constituents before or during translocation through the SDV membrane.[20–22] Polyamines may turn out to be part of this as yet unknown stabilizing system.

Experimental Section

Poly(allylamine) hydrochloride ($\bar{M}_w = 15000$) was purchased from Aldrich. A stock solution (2 mM) was adjusted with NaOH to pH 6.8. $\bar{M}_w$ = weight-average molecular weight.

A freshly prepared solution of tetramethoxysilane (1M) in HCl (1 mM) was incubated at 25 °C for exactly 15 min and immediately used as a source of mono- and disilicic acid.

Production of hexagonal silica morphologies: Sodium phosphate buffer (pH 6.8) was added to a stock solution of poly(allylamine) to a final concentration of 0.45 mM in polyamine and 40 mM in phosphate. The resulting phase-separated solution of poly(allylamine) (22 μL) was mixed with polyamine-stabilized silica sol (28 μL; prepared by incubating a solution containing poly(allylamine) (0.4 mM, pH 6.8) and monosilicic acid (70 mM) at 25 °C for at least 30 min).

Silica precipitates were collected by centrifugation (2 min, 4.000 rpm) and washed twice with water, then suspended in water, applied to an aluminum sample holder, and air dried. Silica was analyzed (without sputter coating) with a LEO1530 field-emission scanning electron microscope by using energy-dispersive X-ray analysis (EDXA, Oxford instruments).

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