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Silicon biotechnology: harnessing biological silica production to construct new materials

Daniel E. Morse

Silicon, the basis of semiconductors and many advanced materials, is an essential element for higher plants and animals, yet its biology is poorly understood. Many invertebrates produce exquisitely controlled silica structures with a nanoscale precision exceeding present human ability. Biotechnology is starting to reveal the proteins, genes and molecular mechanisms that control this synthesis in marine organisms that produce large amounts of silica. Discovering the mechanisms governing biosilicification offers the prospect of developing environmentally benign routes to synthesize new silicon-based materials and to resolve the biological use of silicon in higher organisms.

Silicon, the second most abundant element on Earth, is widely used in the manufacture of siloxane-based semiconductors, glasses, ceramics, plastics, elastomers, resins, mesoporous molecular sieves and catalysts, optical fibers and coatings, insulators, moisture shields, photoluminescent polymers, and cosmetics[1–3]. The manufacture of these materials typically requires high temperatures, high pressures or the use of caustic chemicals.

By contrast, the biological production of amorphous silica, the simplest siloxane [$(SiO_2)_n$], is accomplished under mild physiological conditions, producing a remarkable diversity of exquisitely structured shells, spines, fibers and granules in many protists, diatoms, sponges, molluscs and higher plants[4,5]. These biologically produced silicas exhibit a genetically controlled precision of nanoscale architecture that, in many cases, exceeds the capabilities of present-day human engineering (Fig. 1). Furthermore, the biological productivity of siloxanes occurs on an enormous scale globally, yielding gigatons per year of silica deposits on the floor of the ocean. Diatomaceous earth (composed of the nanoporous skeletons of diatoms) is mined in great quantities from vast primordial deposits of this biogenic silica.

Biotechnological approaches are now starting to unlock the molecular mechanisms of polysiloxane

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synthesis under physiological conditions, offering the prospect of new, environmentally benign routes to the synthesis and structural control of these important materials.

Silicon transporters and silica-wall proteins

Taking advantage of marine organisms that produce large relative masses of biogenic silica, molecular biologists have begun to isolate the genes and proteins controlling silica biosynthesis and nanofabrication. Hildebrand and colleagues made a significant breakthrough by cloning and characterizing the cDNA encoding the first silicic-acid [$Si(OH)_4$] transporter to be unequivocally identified[6]. They showed, by analysis of the encoded protein and by injection of the mRNA (synthesized *in vitro* from the cloned cDNA) into *Xenopus* eggs, that the transporter protein forms a sodium-dependent transmembrane ion channel that mediates the transport of silicic acid. The action of this protein can account for the uptake of the silica precursor from the dilute pool of silicic acid in oceanic and fresh water, and similar transporters may pump silicic acid (or its conjugates) into the lumen of the silica-deposition vesicle (silicalamella), in which polycondensation (polymerization) is known to occur.

Kröger and colleagues have cloned and characterized cDNAs encoding two families of protein (at least one of which is glycosylated) that contribute to the organic sheath surrounding the silica walls of a diatom[7,8]. The proteins most intimately associated with these silica walls contain regularly repeating hydroxyl-rich domains potentially capable of interacting with the growing silica structure, as previously suggested for the control of biosilicification[9–11]. Hecky *et al.* had proposed that such hydroxyl-rich domains might align silicic-acid monomers, either by condensing with them (with elimination of water) to form covalent adducts or by hydrogen bonding, thus bringing them into favourable juxtaposition for their condensation to form silica[9]. Thermodynamic calculations support the energetic feasibility of such a pathway[12].

Silicateins

We have focused our efforts on the common marine sponge *Tethya aurantia*, taking advantage of the fact that 75% of the dry weight of this organism is composed of simple silica spicules (glassy needles ~2 mm long and 30 μm in diameter) to obtain biochemical quantities of the occluded proteins controlling silicification[13,14]. Each spicule contains an axial filament of protein (~2 mm long and 1–2 μm in diameter) that is fully occluded within the opal-like silica. These filaments can be purified (after dissolving the silica with buffered hydrofluoric acid) and resolved into three very similar subunits, designated silicateins (for silica proteins) α, β and γ[13].

X-ray diffraction reveals a periodically repeating substructure within the macroscopic protein filament, suggesting that it is composed of a regularly repeating subassembly of the silicatein subunits[13]. Cloning and characterization of the cDNAs encoding silicatein α, the most abundant subunit, revealed the surprising fact that this protein is highly homologous to members of the cathepsin L subfamily of papain-like proteases, suggesting a possible catalytic mechanism for its control of silicification[13].

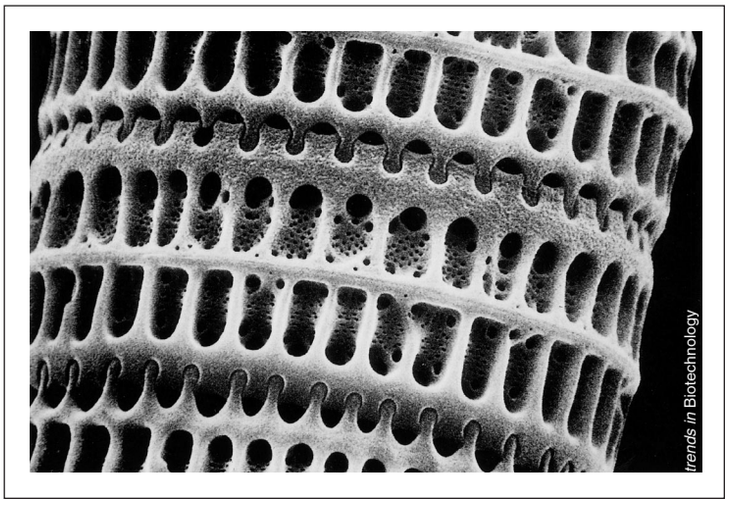

Figure 1

Genetically controlled nanoscale architecture of the silica wall of the marine diatom *Paralia sulcata* (30 μm in diameter). (Electron micrograph by G. Hallegraeff; reproduced with permission from Ref. 22.)

This homology includes close similarities between the cathepsin L and silicatein α in their signal, propeptide and mature peptide sequences, pointing to a shared evolutionary ancestry for these two proteins, both of which function in membrane-enclosed organelles (the lysosome and silicalemma, respectively). These findings suggest that the silicatein–silicalamella system in *T. aurantia* may have evolved from a common ancestor of the protease–lysosome system, and thus may function in an analogous way to the hydrolytic protease in the control of silica synthesis[13–15].

The positions of all six of the cysteine residues forming disulfide bridges in the proteases are highly conserved in silicatein α, suggesting that the two proteins have similar three-dimensional structures. The catalytic cysteine residue of the protease is replaced by a serine in silicatein α, making the silicatein inactive as a protease[13,14]. Because the other two residues of the protease's catalytic triad (histidine and asparagine) are conserved in silicatein α, the putative active-site serine and histidine residues of the silicatein might function in the polycondensation of siloxanes in a manner analogous to the role of the serine–histidine pair and the cysteine–histidine pair in the active sites of the serine- and cysteine-based proteases, respectively[14].

Consistent with these suggestions, it has been found that the silicatein filaments, the dissociated silicatein subunits and the purified and reconstituted silicatein α expressed from a cloned cDNA template in bacteria all catalyse the polycondensation of silica from silicon tetraethoxide at neutral pH *in vitro*[14]. In the absence of the protein, polycondensation of this industrially used precursor typically requires acid or base catalysis of the rate-limiting hydrolysis of the silicon–alkoxide bond. The catalytic activity of the silicateins is abolished by thermal denaturation, demonstrating that this activity is dependent on the native conformation of the protein. Use of the macroscopic silicatein filaments reveals a scaffolding or structure-directing activity in addition to the catalytic activity, with the polymerized silica forming a layer that follows the contours of the underlying protein fibre.

Interestingly, when phenyltriethoxysilane or methyltriethoxysilane are provided as substrates *in vitro*, the silicatein filament catalyses polycondensation of the corresponding phenyl- or methylsilsesquioxanes (organically modified silicone-polymer networks)[14]. Silsesquioxane synthesis from the silicon alkoxides also typically requires acid or base catalysis in the absence of the silicatein, as commonly used in many current industrial syntheses. The proposed mechanism of silicatein-mediated catalysis of the condensation of the silicon alkoxides thus may be closely similar to the mechanism of the homologous proteolytic enzymes, with the protein providing a favourable route for the rate-limiting hydrolysis at neutral pH via the formation of a transitory covalent protein–substrate intermediate[14].

The results of site-directed mutagenesis support the predictions of this mechanism, revealing that the sidechains of both the serine-25 and histidine-165 residues of silicatein α are required for efficient catalysis of polysiloxane synthesis[16]. These results suggest that the silicatein actually functions as a hydrolase with these substrates, converting the silicon alkoxides to their corresponding silanols, which are known to condense rapidly and spontaneously to form polysiloxanes. The immediate precursor for the formation of silica *in vivo* remains to be determined, however. Thus, it is not yet known whether the catalytic activity of the silicatein observed *in vitro* with synthetic alkoxide substrates is of physiological significance in the living sponge or simply a vestigial reflection of the protein's molecular evolution[14–16].

Cloning at the interface between two worlds – future prospects

The dawn of silicon biotechnology is now starting to reveal the proteins, genes and molecular mechanisms controlling the biological nanofabrication of silicon-based materials. The discovery that this interface between the inorganic world of silicon and the biopolymers that control siloxane polycondensation includes dynamic catalysts related to well-known enzymes offers prospects for biotechnological control that were previously unimagined. The finding that the silicateins can be harnessed to produce synthetic silsesquioxane (silicone) polymer networks *in vitro* suggests the possibility of adapting these biomolecular mechanisms to develop new, environmentally benign routes to the synthesis of high-performance materials. Site-directed and combinatorial mutagenesis to produce altered silicateins, in conjunction with the biomimetic synthesis of peptide- and non-peptide-based catalytic and structure-directing analogues, offer the prospect of exploiting the biological mechanisms for enhanced structural control of organically modified siloxanes with new functions[15]. Time will tell, for example, whether the improved interfacial control afforded by these new tailored catalysts can produce polysiloxanes with sufficiently coherent alignment or packing to realize the silicon chemist's dream of more-efficient optoelectronic materials with low-loss coupling to silicon-based semiconductors.

Analysis of the genes and proteins governing silicon metabolism and silica biosynthesis may also help to resolve the poorly understood role of silicon in vertebrate skeletogenesis[17–19] and the mechanisms underlying the apparently essential requirement for silicon in many plants and animals[17,18,20,21]. Armed with increasing knowledge of the protein structures, gene sequences and molecular mechanisms controlling silicon utilization and nanofabrication in simple marine organisms, molecular biologists are now better equipped to search for silicon-processing molecules and mechanisms in other organisms, including humans.

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