Biomineralization in Diatoms Mediated through Peptide- and Polyamine-Assisted Condensation of Silica

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Diatoms are unicellular algae with cell walls of amazing variety of shape. Their aesthetic shapes and elaborate patterns have fascinated scientists since the early years of light microscopy (Figure 1). The cell walls of diatoms are predominantly composed of a biomineral derived from hydrated silica (SiO_2), which is associated with peptides and polyamines. Today we know that precipitation of this amorphous material during biogenesis of cell walls is controlled species-specifically, to nanometer resolution.

Figure 1. Transmission electron micrograph of the cell wall of *Cylindrotheca fusiformis*.[10]

Cell-wall formation occurs primarily in silica deposition vesicles (SDVs); intracellular organelles in which soluble forms of silicate are accumulated by active-transport mechanisms. The directed precipitation and formation of highly differentiated cell walls begins only after accumulation of the silicic acid in the SDV. Different shaping mechanisms are thought to be involved in this process. Silica precipitation may be initiated and controlled by diffusion-limited processes in the SDV,[1] and shaping mechanisms mediated by spatial restrictions in the cellular environment may also be involved.[2, 3] Another proposed mechanism involves the presence of an organic matrix in the SDV that promotes and directs silica precipitation. Detailed investigations of organic components in the silica cell walls were carried out to verify this hypothesis.[4]

Recently, this approach led to the elucidation of universal mechanisms involved in directed biomineralization in diatoms, which are the topic of this highlight. Early on, it was recognized that removal of the mineral content of diatom cell walls by using hydrogen fluoride (HF) vapors leaves behind organic material "like the ghost of the cell".[5] Organic components are found even after pretreatment with ethylenediaminetetraacetate (EDTA) or after oxidative purification of the silica cell wall with NaOCl. This organic material is tightly associated with the remaining SiO_2 and can be separated from the inorganic material only by dissolving it in HF. Purification and characterization of the thus-obtained constituents from cell walls of the diatom *Cylindrotheca fusiformis* resulted in the discovery of two new families of proteins.[6] The lighter components in the HF extract form a dominant fraction with masses of 4 to 17 kD. Because of their affinity for silica, these peptides were termed silaffins.[7] Kröger et al. observed precipitation of hydrated silica within seconds of adding these purified proteins to supersaturated silicic acid solutions, while untreated controls remained stable for several hours. The amount of amorphous material formed was directly proportional to the amount of added silaffin, which indicates that the peptides are directly involved in the mineralization process. Remarkably, the structure of the formed silica nanospheres depends on the nature of the added peptide. While addition of a mixture of all the purified silaffins from *C. fusiformis* induces the formation of SiO_2 particles with average diameters of < 50 nm, addition of the smallest silaffin 1A leads to the formation of particles with diameters between 500 – 700 nm.[7] N-terminal sequencing of silaffin 1B and cloning of the corresponding gene revealed a coding protein with a modular structure. A signal peptide and a highly negatively charged unit are followed by seven repetitive, strongly basic, units. After endoproteolytic cleavage, these form the corresponding silaffins. Characteristic structural elements of these proteins are regularly arranged lysine – lysine clusters which are modified post-translation. ESIMS-based structure elucidation of the 15 and 18 amino acid peptides silaffin $1A_1$ and silaffin $1A_2$ shows that the ε-nitrogen atoms of the lysine residues bear different modifications (shown in Scheme 1).[8]

These modifications of lysine play a central role in the formation of silica under intracellular, acidic conditions. While both silaffins and the corresponding nonmodified peptides promote the precipitation of silicic acid in a neutral medium, this process is exclusively accelerated by the post-translatory-modified silaffins if the surrounding medium has a pH value of < 6.

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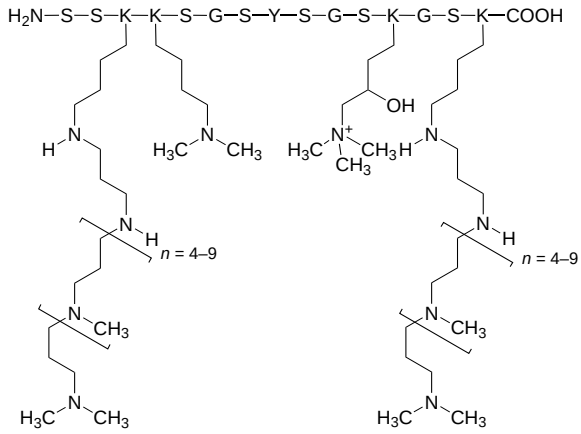
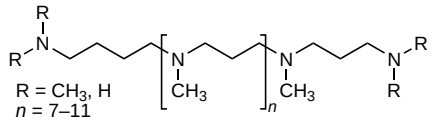


Scheme 1. Structure of silaffin $1A_1$ obtained from *C. fusiformis* cell walls treated with HF. The polypeptide is represented in the one-letter amino acid code (S = serine, K = lysine, G = glycine, Y = tyrosine). Only the structures of the post-translatory modified amino acids are shown.[8]

Newer investigations show that the principles found for the formation of silica in *C. fusiformis* represent, with variations, a general concept for biomineralization in diatoms.[9] Comparison of the organic constituents of cell walls from six different diatom species revealed that silaffins and related molecules are ubiquitous in this algal class, and may be responsible for the complex pattern formation of morphologically different cell walls. Significantly, these silica-precipitating molecules differ, depending on the species; this is a prequisite for such a formation mechanism. After treatment with HF and purification, the remaining organic components exhibited differing characteristics for the six different species. Detailed investigations led not only to the identification of species-specific sets of silaffins but also to the characterization of another, non-proteinogenous compound class, which is incorporated into the diatom silica. These cell-wall constituents are complex mixtures of long-chain polyamines with molecular weights of up to 1500 Da. ESIMS shows different patterns and mass distributions of these macromolecules in all of the diatoms investigated.[9] The amines from *Nitzschia angularis* showed regular mass distributions, which suggests the presence of defined homopolymers. MS-based structure elucidation led to the identification of a polymer formed from *N*-methyl-propylamine monomers (Scheme 2). Comparison of

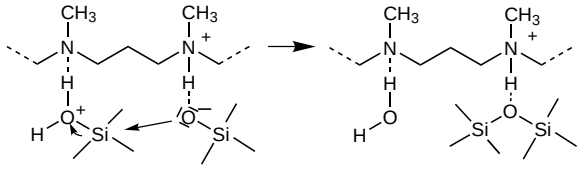


Scheme 2. Polyamines from the diatom *N. angularis*. A family of 25 amines is generated by variation of the chain length and the degree of methylation. Additional variation is achieved by exchange of the butylamine (left) for a propylamine unit.

the mass spectra of macromolecules from other diatom cell walls showed the universal presence of polyamines with an inherently similar structural principle. The amines from *C. fusiformis*, for example, consist of polymers of non-methylated propylamine units of different lengths.[9] These polymers can extend by up to 20 repeat units, which makes them the longest linear polyamines found in nature. These amines often comprise the majority of organic material in diatom cell walls.

If purified amines from *N. angularis* are added to metastable solutions of silicic acid, they induce the rapid precipitation of silica and are simultaneously incorporated into the formed biomineral.[9] As a species-specific influence of the matrix on the silica pattern implies, diameters of the resulting spherical particles depend on the nature of the added amines. If mixtures of silaffins and amines are added to silicic acid solutions, the nature of the precipitate is altered and the composite material forms spherical particles that are arranged in blocklike structures.

A mechanism for the activity of polyamines during silica formation has been suggested, based on the alternating presence of protonated and non-protonated tertiary amine groups in the polyamine chain. These amine groups could form hydrogen bonds to silicic acid and thus facilitate the Si–O bond formation, according to Scheme 3.[10] The resulting biomineral has a relative composition of 1.25:1 SiO_2/polyamine based on weight, which indicates that the amines interact directly with the silanol functional groups of hydrated SiO_2.[9]



Scheme 3. Postulated mechanism of the amine-mediated silicic acid condensation.

An elegant model explains how the above-mentioned polyamines and peptides might also be involved in the shaping process during the formation of diatom cell walls.[11] Based on the assumption that the amphiphilic methylated polyamines form emulsions of microdroplets by phase-separation processes in the SDV, a template mechanism has been suggested that could explain the observed stages of cell-wall development in *Coscinodiscus wailesii* (Figure 2A). In a close-packed arrangement the microdroplets would form a hexagonal monolayer within the flat SDV. Precipitation of silica around this arrangement creates a honeycomb-like framework. The consumption of a part of the polyamine population by coprecipitation could cause dispersion of the original organic droplets that, again, could serve as precipitation templates (Figure 2B). Repetition of this simple iterative mechanism (Figure 2C,D) could explain the pattern observed during electron microscopic investigation of *C. wailesii* cell walls during growth (Figure 2E-H).[11]

The concepts underlying diatom biomineralization have also inspired research into directed bio- and nanotechnological silica formation.[3] While diatoms have the ability to create highly complex and intricate silica structures under physiological conditions, laboratory methods often require elevated pH values or temperatures, with long reaction times. It is thus not surprising that possible applications of biochemical

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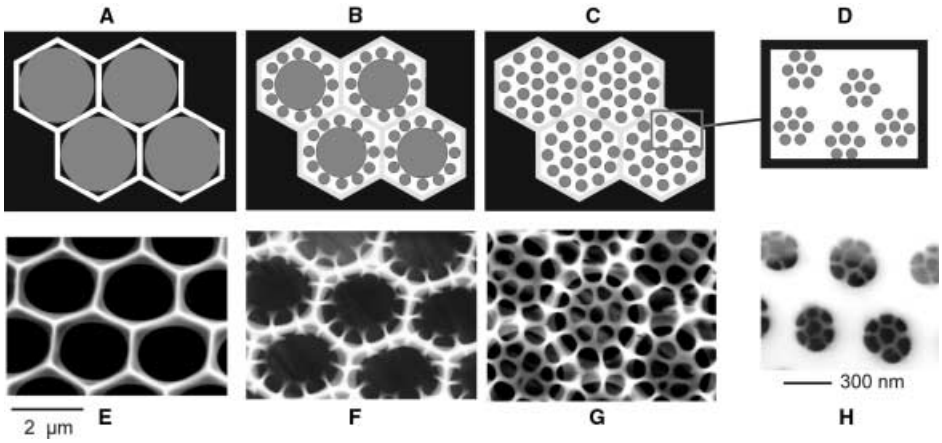

Figure 2. Schematic representation of the phase-separation mediated templating mechanism (A – D) and comparison with different stages of the cell-wall biogenesis of *C. wailesii* (scanning electron micrographs of valves during formation (E – H). Reproduced with permission from ref. [11], copyright (2002), American Association for the Advancement of Science.

processes have been explored during the search for new ways to create sensitive hybrid materials with silicate minerals. Technical applications that allow ordered, peptide-assisted precipitation of silica onto a polymer hologram were developed following the initial identification of silaffins.[12] For this purpose, silaffin A_1 was incorporated into a polymer hologram to form a regularly arranged peptide matrix. Treatment of this hologram with a solution of silicic acid for 10 min resulted in the formation of spherical silica nanospheres arranged in a regular two-dimensional array. This process combines the ease of processability of organic polymers with the improved mechanical properties of a composite material. The directed biomineralization results in structures with increased mechanical stability and improved optical properties (up to 50 times increase in surface refractive index), and may be of practical use in the fabrication of photonic devices.[12]

Only in recent years have we begun to understand the underlying principles of the formation of complex silica structures in diatoms. The identification of the first matrices for silica precipitation and new models for shaping processes leave the field open for in vivo approaches to clarify the detailed interactions leading to the highly differentiated structures of diatom cells.

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