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Silica Biomineralisation in Diatoms: The Model Organism Thalassiosira pseudonana

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After complete genome sequencing, the diatom Thalassiosira pseudonana *has become an attractive model organism for silica biomineralisation studies. Recent progress, especially with respect to intracellular silicic acid processing, as well as to the natures of the biomolecules involved in diatom cell wall formation, is described. On the one hand, considerable progress has been made with respect to silicon uptake by special proteins (SITs) from the surrounding water, as well as to the storage and processing of silicon before cell division. On the other hand, the discovery and characterisation of remarkable biomolecules such as silaffins, polyamines and—quite recently—of silacidins in the siliceous cell walls of diatoms strongly impacts the growing field of biomimetic materials synthesis.*

Diatoms are an abundant and widespread group of eukaryotic algae (class *Bacillariophyceae*). A diatom cell is tightly enclosed in a silica-based cell wall that is constructed like a Petri dish. This rigid cell wall consists of two halves, with the top half (epitheca) slightly overlapping the smaller bottom part (hypotheca). Each half is made up of a valve and several girdle bands. Diatom cell walls are famous for being intricately and ornately structured on the nanometer scale. Their shells, being composed of hierarchically organised silica elements, are among the most beautiful objects for microscopic studies.[1] The cell wall structure is species-specific, so diatom identification is mainly based on analysis of the hierarchically organised silica structures. An estimated number of about 200 000 diatom species exist worldwide.[2] Diatoms can be found in salt- and freshwater habitats, in soils and on nearly all types of damp substrates. Diatoms are a major constituent of oceanic phytoplankton and are assumed to contribute up to 40% of total oceanic primary production.[3] The global impact of diatoms is also documented by the existence of huge deposits of fossil diatom cell walls known as diatomaceous earth, diatomite, or kieselguhr, which are commercially exploited for the production of filters and separation media, as well as explosives.

SEM images from a few representative diatom genera are shown in Figure 1. These organisms precisely reproduce silica formation and patterning, generation by generation. That means that the production of this nano-structured silica is under genetic control, which in turn implies the existence of specific gene products (proteins) guiding these biomineralisation processes. Immediately after cell cleavage, each daughter cell initiates the synthesis of a new hypovalve. This biogenesis takes place in a specialised intracellular compartment, the so-called silica deposition vesicle (SDV).[4] The SDV is considered to be a cellular "reaction vessel" in which all the chemical steps of silica formation and patterning take place. The shape of the SDV must be assumed to be determined by the cytoskeleton, and in turn influences the shape of the developing valves.

Biosilica from all diatom species investigated so far has turned out to be a composite material containing proteins (mainly the silaffins) and long-chain polyamines as organic components. These organic constituents have been recognised as important players participating in silica biomineralisation. Several recent reviews have described the structures and properties of these organic molecules (mainly from the diatom *Cylindrotheca fusiformis*) and their possible function in silica formation and patterning.[5–8] The recent sequencing of the complete genome of *Thalassiosira pseudonana* has greatly simplified biochemical studies on biomineralisation and qualifies this species as a model organism for future research into silica biomineralisation.[9] In addition, diatom cells can be transformed by the classical particle gun method,[10] offering the possibility of genetic studies on biomineralisation.[11,12] This minireview has been exclusively conceived to highlight a few very recent developments and discoveries not yet covered by the previous articles, and in particular focuses on biochemical and biophysical data obtained for the model diatom *T. pseudonana*.

The Enigma of the Intracellular Processing of Silicic Acid

The predominant form of dissolved silicon in marine and freshwater environments is monosilicic acid, $Si(OH)_4$, which also represents the source for the biogenesis of the cell walls of diatoms.[13] Global oceanic average concentration amounts to 70 μM monosilicic acid.[14] In aqueous solution, silicic acid remains in the monomeric state as long as the concentration is less than about 2 mM, but polymerises at higher concentrations to form polymers recognisable as colloidal particles.[15] The predominance of diatoms in phytoplankton communities

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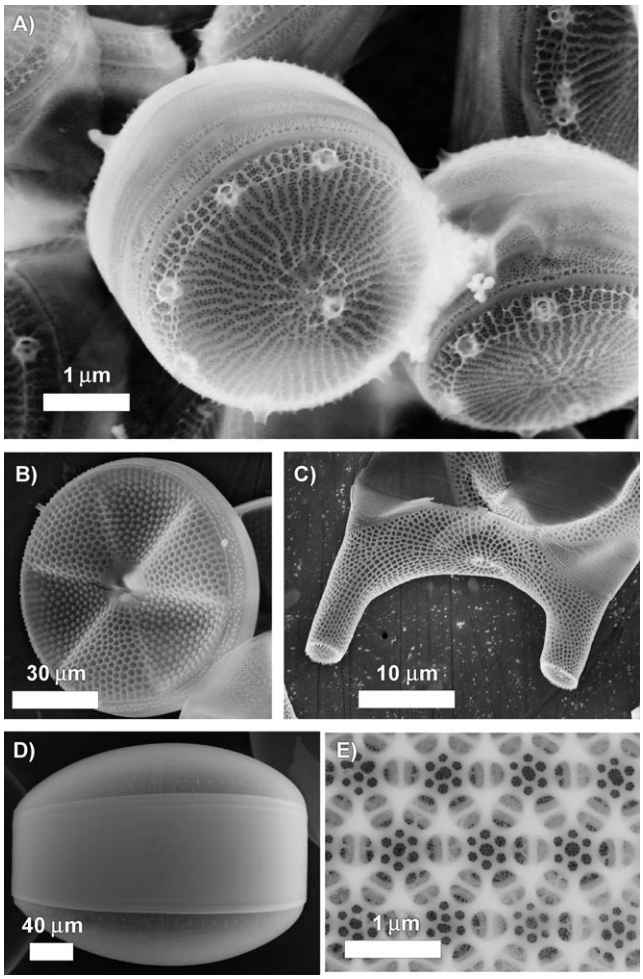

Figure 1. SEM images from a few representative diatom genera. A) *Thalassiosira pseudonana*, B) *Actinoptychus spec.*, C) *Eucampia zodiacus*, D) and E) *Coscinodiscus granii*, complete cell wall and high-resolution image of the valve patterning.

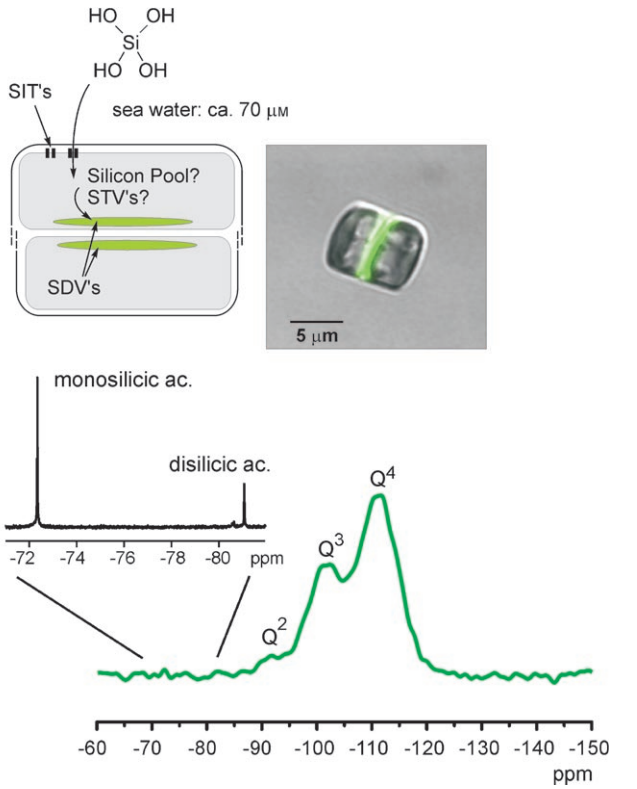

Figure 2. Schematic description of silicon uptake and cell wall formation in diatoms during cell division (top, left: SIT, silicon transporter; STV, silicon transport vesicle; SDV, silica deposition vesicle) and fluorescence microscopic image of a *T. pseudonana* cell during cell division (top, right). The ^{29}Si MAS NMR spectrum below (green) was obtained from cells pulse-labelled with ^{29}Si at the developmental stage shown in the image. Note that both the fluorescing structures in the image and the ^{29}Si NMR signals in the spectrum are exclusively due to freshly synthesised silica. Fluorescence labelling of freshly synthesised silica structures was performed as described by Descles et al.[85] For comparison, a liquid-state ^{29}Si NMR spectrum of a solution containing mono- and disilicic acid is also shown (black).

is directly related to the availability of silicon. Even at silicon concentrations as low as 2 μM, diatoms typically represent more than 70% of the plankton community. The highly efficient silicic acid uptake system operating in diatoms has been the subject of extended investigations. Originally, a gene family of silicon transporters (SITs) was identified in the pennate diatom *Cylindrotheca fusiformis*.[16,17] SITs have been found in all diatom species investigated since then.[17,18] Different SITs exhibit distinct patterns of expression through the cell cycle, suggesting some degree of functional specialisation among paralogues.[19]

The processes underlying the intracellular transport and transfer into the SDV after the uptake of silicic acid by a diatom cell (Figure 2) remain poorly understood. Numerous papers on diatoms report the existence of intracellular silicon storage pools that might represent up to 50% of the total cellular biosilica, depending on the species.[20–23] This silicon storage pool, if present, is believed to accumulate silicon for the production of a new valve.[14] It is important to note in this context that the formation of a valve is a rapid process: the two-dimensional expansion of a valve in the SDV takes place within only 15 min in the case of *Navicula salinarum*, demanding a rapid supply of silicon.[24] The reported concentrations of the intracellular silicon within the storage pool[22,23] strongly exceed the levels necessary for stability of monosilicic acid,[15] excluding this molecule as the intracellular substrate for silica formation. A soluble silica pool of 0.9–4.6 fmol per cell has been reported for *T. pseudonana*,[22] corresponding to concentrations of 20–100 mM for typical cells of about 5 μm diameter and 2.5 μm thickness. Various possibilities have been discussed in the literature. Azam et al.[25] suggest that silicic acid is associated with some kind of organic material or special proteins, thus forming a stable silicic acid pool inside the cell. Another silicon storage mechanism would be some kind of prepolymerisation of silicic acid inside the cells, such as the formation of a so-called polyamine-stabilised silica sol as suggested by Sumper.[26] Schmid and Schulz[27] proposed the existence of special silicon transport vesicles (STVs) on the basis of the observation that certain cytoplasmic vesicles are seen to fuse with the developing SDV. Until now, however, there has been no evidence for the presence of silicon inside these vesicles,

although silicon-containing vesicles were recently identified during the formation of the siliceous spicules of sponges.[28]

A major reason for the uncertainty concerning the silicon storage mechanisms is that the techniques commonly applied for the determination of internal silicon storage pools are not able to distinguish between the different condensation states of silicic acid. This is particularly true for silicon bound to organic matter. The molybdate method (Martin-Jézéquel et al.[22] and references therein) exclusively detects mono- and disilicic acid and requires the prior destruction of the diatom cells and chemical treatment of the remaining compounds. Alternatively, the radioisotopic analogue ^{68}Ge (Martin-Jézéquel et al.[22] and references therein) can be used as a tracer for silicon. This method can be applied to investigation of intact cells but again suffers from the fact that there is no possibility to distinguish between silicic acid, polymerised silicic acid species and silicon bound to organic molecules.

^{29}Si NMR spectroscopy is a powerful and nondestructive tool with which to investigate and distinguish the different silicon species because the chemical shifts are very sensitive to changes in the chemical environment,[29–31] such as if silicon is bound to organic material. Several solid-state ^{29}Si NMR studies have been performed on extracted diatom cell walls or freeze-dried cells,[32–35] but to the best of our knowledge only one ^{29}Si NMR spectroscopic study on intact diatom cells has been carried out so far. In that study, liquid-state ^{29}Si NMR was applied.[36] A dominant signal at −71 ppm corresponding to free monosilicic acid and a weak and transient signal at −131 ppm could be observed. The latter signal has been assigned to a hypercoordinated organosilicon complex that was suggested to be a silica precursor compound. However, liquid-state ^{29}Si NMR spectroscopy is not able to identify slowly tumbling or immobilised species such as silicic acid attached to organic components or polysilicic acid species. This can be achieved, however, by solid-state ^{29}Si NMR spectroscopy, which was used recently in order to study integer cells of a synchronised *T. pseudonana* culture.[37] A characteristic ^{29}Si MAS NMR spectrum of integer diatom cells is shown in Figure 2. This spectrum only represents newly synthesised silica: the cells were initially grown and synchronised[38] in a medium only containing ^{28}Si. After synchronisation (monitored by applying a fluorescence dye that labels newly synthesised silica structures), pure ^{29}Si-containing medium was added in order to pulse-label intracellular silicon pools.

Two interesting observations were made. Firstly, there was no indication of precursor compounds such as well defined and stable hypercoordinated silicon or organosilicon complexes. Instead, all silicon taken up by the cell obviously exists as precondensed silica, showing the common Q-group signals characteristic for silica.[31] Therefore, it is suggested that the silicic acid rapidly forms prepolymerised silicic acid species. Secondly, intact diatom cells always exhibit a lower degree of silica condensation than the extracted cell walls, as reflected in the lower Q^4/Q^3 ratio.[32,37] Therefore, it is concluded that the diatom species *T. pseudonana* does not store silica in the form of well defined and stable organosilicon complexes. Instead, the observed ^{29}Si MAS NMR spectroscopic investigations are consistent with the presence of precondensed silica species. Silica sols are known to be stabilised by polyamines, as has already been demonstrated in vitro,[26] and may act as the substrate for the production of new valves and girdle bands during the cell division cycle, as the presence of polyamines in diatoms has been demonstrated previously.[39] The silica sol nanospheres would only deliver the common signals due to four-coordinated Q-groups in the ^{29}Si NMR spectra, probably with Q^4/Q^3 ratios different from those in pure diatom cell walls depending on their size and condensation state, in agreement with the experimental results.[37] This scenario is completely in line with the observation that diatom biosilica is composed of fused silica nanospheres.[40–43]

Structural Features of Polyamines Found in Diatom Biosilica

In all diatom species investigated so far, biosilica has turned out to contain long-chain polyamines as an organic constituent.[39] Investigated species cover the following genera: *Chaetoceros*, *Coscinodiscus*, *Cylindrotheca*, *Eucampia*, *Navicula*, *Stephanopyxis* and *Thalassiosira*. The polyamines—even from closely related diatom species—exhibit differences and species-specific structural characteristics.[44] Structural variations include the overall chain length, the degree of methylation, the positions of secondary and tertiary amino functionalities and, unexpectedly, even the site-specific incorporation of quaternary ammonium functionalities. A selection of typical polyamine structures covering these features is shown in Figure 3. Most polyamines are based on putrescine, although a subpopulation of polyamines isolated from *T. pseudonana* is formally derived from propane-1,3-diamine.[45] The polyamines extracted from *Coscinodiscus granii* cell walls do not exhibit any methylation at all and consequently contain only primary and secondary amino functionalities. The much shorter polyamines found in *T. pseudonana* display terminally located tertiary amino functionalities generated by methylation. A higher degree of methylation is found in the polyamines from *Coscinodiscus wailesii*. Only four secondary amino groups remain in site-specific positions. Finally, permethylated long-chain polyamines are a typical feature in the diatom *Coscinodiscus concinnus*. Minor polyamine components isolated from *T. pseudonana* as well as from *C. wailesii* even include a quaternary ammonium functionality at a site-specific position, which introduces a permanent positive charge.[44,45] The tentative structure of such a very special polyamine is shown in Figure 3.

The existence of species-specific differences in polyamine structures, even among diatoms within the same genus (compare *Coscinodiscus granii*, *wailesii* and *concinnus* in Figure 3) supports the idea of a specific role of polyamines in creating species-specific silica nanostructures.[46]

Structural Features of Silaffins Found in Diatom Biosilica

Silaffins are highly post-translationally modified peptides occurring in the cell walls of numerous diatom species. Originally,

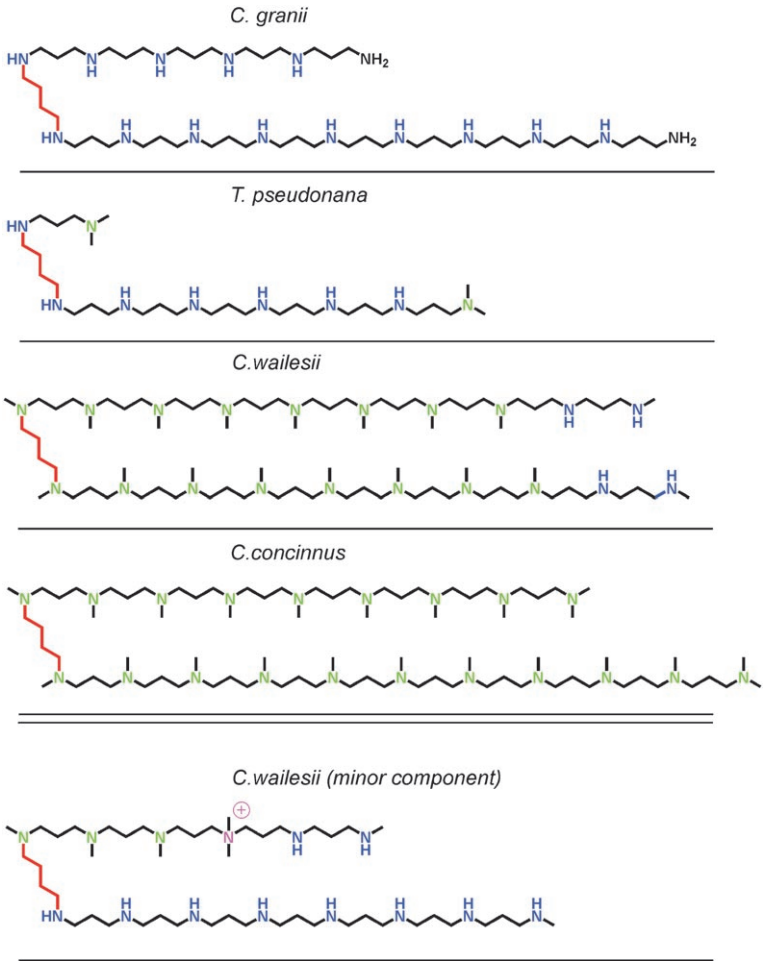

Figure 3. Representative structures of long-chain polyamines associated with biosilica from different diatom species. Primary (black), secondary (blue), tertiary (green) and quaternary (red) amino functionalities are shown in different colours.

silaffins were discovered in the diatom *C. fusiformis*.[47] Silaffin-1 A_1 from this diatom is a peptide (15 amino acid residues) mainly composed of serine and lysine units. All the serine units turned out to be phosphorylated, and all the lysine units are either methylated or covalently linked with polyamines.[48] Another silaffin-1 A variant (-1 A_2) even contains a quaternary ammonium group introduced in the form of an ε-*N,N,N*-trimethyl-δ-hydroxylysine residue.[49] This set of modifications creates highly zwitterionic peptides with the capability for self-assembly driven by ionic interactions. These macromolecular assemblies create matrixes that are able to guide silica formation and precipitation. Silaffin-2 from *C. fusiformis* features additional post-translational modifications such as phospho-hydroxyprolines, glycosylations, and sulfations.[50]

The extremely high degrees of posttranslational modification present in all silaffins very often prevent the collection of amino acid sequence data required for the cloning of novel silaffin genes. Therefore, the recent sequencing of the *T. pseudonana* genome has greatly simplified the identification of three additional silaffin genes, termed *sil1*, *sil2* and *sil3*. By alternative processing, five gene products (Sil-1L, Sil-1H, Sil-2L, Sil-2H and Sil-3) were found to be derived from these genes.[51] All of them are components of the cell wall. Sil-1L and Sil-2L are closely related lysine-rich peptides (Figure 4). Sil-1H and Sil-2H are higher molecular mass isoforms of Sil-1L and Sil-2L with *N*-terminal extensions. Finally, another silaffin gene encodes the much larger Sil-3 protein, while quite recently it has been possible to identify another silaffin gene encoding the silaffin Sil-4 (Wenzl, Hett and M.S., unpublished results; see below).

The chemical characterisation of the numerous posttranslational modifications in silaffins remains a challenge. Quite recently, the structures of nearly all modified lysines within Sil-3 from *T. pseudonana* have been characterised, and it was possible to unveil an amino acid sequence-based code selecting the type of modification.[52] The polypeptide chain of Sil-3 contains 33 lysine residues, 30 of which are embedded in a defined K-(A/S/Q)-X-K tetrapeptide sequence (boxed in Figure 4). This regularity suggested the existence of rules for the introduction of a given type of lysine modification, an idea that has meanwhile been confirmed. For all tetrapeptide motifs sufficiently well separated from each other, the N-terminally located lysine is modified by two aminopropyl units (4,8-diazaoctanyl residue), whereas the *C*-terminally located lysine is converted into ε-*N,N*-dimethyllysine. More complex rules apply if these tetrapeptide motifs are clustered within the polypeptide chain. In this case, the very first and the last lysine residue of a cluster are converted into hydroxylysine residues including quaternary ammonium functionalities (structure shown in Figure 4). The detailed pattern of lysine modifications is schematically summarised in Figure 4, and the tentative positive and negative charges within this polypeptide chain are indicated by red and blue labelling (serines, as possible phosphorylation sites, are also shown in blue).

The existence of such rules implicates the presence in diatoms of an enzymatic machinery that transforms the amino acid sequence information within a silaffin polypeptide into a pattern of positive (and negative) charges that certainly determines the assembly behaviour of these molecules. At least in vitro, only assemblies of silaffins are able to guide hard silica formation (and patterning). This code might, therefore, represent a link between species-specific silica morphologies and their genetic control.

It has recently even been possible to identify a further related silaffin (denoted as Sil-4) as a constituent of *T. pseudonana* biosilica (Wenzl, Hett and M.S., unpublished results). This highly zwitterionic silaffin is similarly organised with respect to its lysine residues (Figure 5). A greater number of clustered tetrapeptide motifs than in Sil-3 are found to be present, and—as predicted by the rules derived for Sil-3—a higher percentage of hydroxylysines with quaternary ammonium functionalities could be determined by mass spectroscopic analysis in acid hydrolysates of Sil-4.

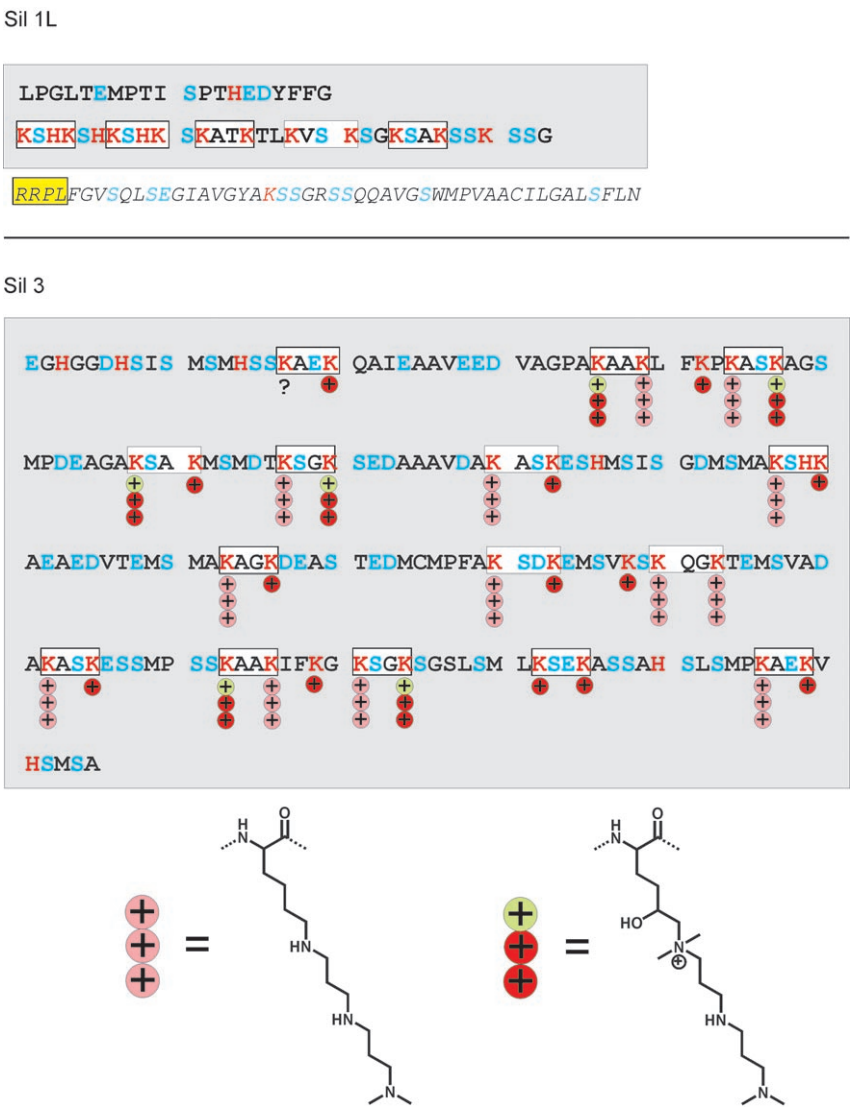

Figure 4. Primary structures of silaffins from *T. pseudonana*. **Sil-1L**: The amino acid sequence as derived from the corresponding gene contains a sequence RRPL that has previously been recognised as a signal for processing of silaffin precursor polypeptides. Therefore, the mature Sil-1L is likely to lack the C-terminal sequence shown in italics. **Sil-3**: The nature and distribution of complex lysine modification within the silaffin-3 polypeptide. The type of lysine modification is indicated by the symbols representing the chemical structures shown. Tentatively positive and negative charge carriers are shown as red and blue letters, respectively.

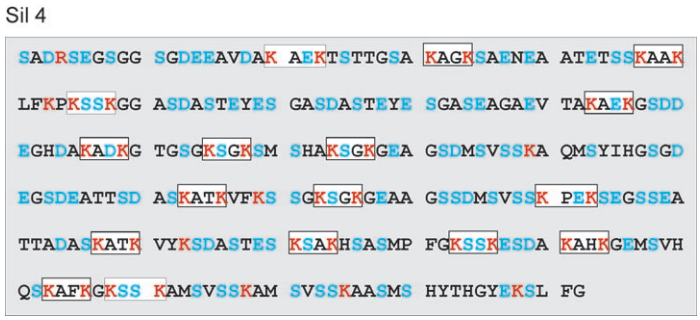

Figure 5. Primary structure of Silaffin-4 from *T. pseudonana*. Lysine-containing tetrapeptide motifs are boxed. Positive and tentatively negative charge carriers (serine phosphate) are shown as red and blue letters, respectively.

A New Class of Phosphopeptides Found in Biosilica: Silacidins

In vitro, polyamines display silica precipitation activities only if polyanions that allow their assembly through electrostatic interactions are provided. Phosphate anions or phosphorylated silaffins are able to fulfil this function in vitro. Quite recently, it was possible to identify three entirely polyanionic peptides as components of biosilica from *T. pseudonana* that may serve as cross-linking agents for the assembly of long-chain polyamines.[53] These remarkably bizarre peptides mainly consist of serine phosphates and the acidic amino acids aspartic and glutamic acid. Because of their presence in silica, combined with their acidic characters, these peptides were named silacidins. The structure of the main silacidin component is shown in Figure 6. A polyamine/silacidin binary system is able to precipitate silica from a silicic acid solution. The efficiency of silacidins as cross-linkers is remarkable: concentrations of phosphate anions at least two to three orders of magnitude higher would be required to induce comparable amounts of silica precipitation. As shown previously for phosphate anions,[54] the silacidin concentration controls the size of the precipitating

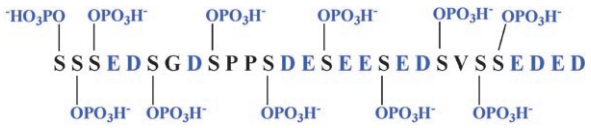

Figure 6. Primary structure and phosphorylation of silacidin A from *T. pseudonana*.

silica nanospheres. Increasing silacidin/polyamine ratios cause the formation of nanospheres with increasing diameters.

Interestingly, phosphopeptides of similar composition have also been found in quite different biomineralisation processes involving (transiently amorphous) inorganic phases: calcium

phosphate[55–57] and calcium carbonate.[58,59] For instance, phosphophoryn, an acidic protein containing numerous aspartates and serine phosphates, is involved in the matrix-mediated processes responsible for dentin formation.[60] Phosphophoryn is believed to play a dual function: it is able to organise matrix assembly by cross-linking fibrillar collagen and also to initiate mineralisation by binding calcium ions.[61] Silacidin, the first highly acidic peptide involved in biosilica formation, probably has similar functions. This puts the concepts of acidic proteins, phosphates and their interactions with inorganic phases in biomineralisation,[57] as well as their role in the hierarchical assembly of composite materials,[62] into a broader evolutionary context.

Mechanistic Studies

The ability of amines and polyamines to accelerate silicic acid polymerisation was originally recognised by Mizutani et al.[63] In addition, they found that the resulting silica gel represents a composite material incorporating the applied polyamine. After the discovery of polyamines acting in biosilica formation, many synthetic polyamines were analyzed with respect to their activities in silicic acid polymerisation. Particularly well investigated polyamines include poly(allylamine),[63–65] poly(amino acids),[66–68] dendrimers,[69] poly(ethyleneimines)[70] and putrescine homologues.[71] A systematic study employing model compounds was carried out in order to investigate the influence of the amine structure upon the ability to accelerate silica condensation.[72] A main conclusion drawn from these experiments was that increased alkylation (methylation) of an amine results in significantly higher reaction rates, confirming earlier observations.[73] In particular, bis(quaternary ammonium) groups displayed the greatest reactivity enhancement. This observation is in agreement with a mechanism proposed by Coradin et al.,[74] suggesting that neighbouring ammonium groups help to preconcentrate anionic oligosilicic acid molecules, thereby enhancing the rate of condensation. These interpretations are interesting in the context of the Sil-3 structure from *T. pseudonana* (Figure 4). In this silaffin, clusters of bis(quaternary ammonium) groups are a characteristic feature that might have evolved to provide a high rate of silica formation.

To understand the mechanism of silica formation, it is important to realise that long-chain polyamines not only accelerate silicic acid polymerisation (gel formation) but are also able to precipitate hard silica within minutes. As mentioned above, this latter property of polyamines depends on the formation of macromolecular assemblies including polyamines and polyanionic components.

In vitro, the binary polyamine/Sil-3 system guides silica precipitation in a very remarkable manner. If increasing amounts of silaffin are added to a given amount of polyamine and monosilicic acid, the resulting degree of silica precipitation follows a bell-shaped curve.[51] This has been attributed to an inhibitory function of Sil-3, which was therefore classified as a "regulatory silaffin". As mentioned above, polyamines initiate hard silica formation only in an aggregated state. On this basis, we propose an alternative interpretation of the behaviour of the binary polyamine/Sil-3 system. The bell-shaped curve resembles a Heidelberger–Kendall curve[75] as observed in antigen/antibody immunoprecipitations: maximum precipitation of antigen/antibody complexes occurs at the point of equivalence, where cross-linking of antigen and antibody is the most likely event. Excess amounts either of the antibody or of the antigen only produce small complexes. If analogous behaviour is assumed for the polyamine/silaffin system, large macromolecular assemblies formed by cross-linking should be observed only at the point of equivalence. In the presence of an excess of polyamine or silaffin, only small silaffin/polyamine complexes exist (Scheme in Figure 7). The latter complexes are less efficient in guiding silica precipitation, which explains the bell-shaped curve observed in silica precipitation experiments. Turbidity measurements (Lehmann and M.S., unpublished results) indeed confirm this interpretation. The addition of increasing amounts of Sil-3 to a given amount of polyamine (in the absence of silicic acid!) causes an increase in turbidity (absorbance at 660 nm) towards a maximum indicating the formation

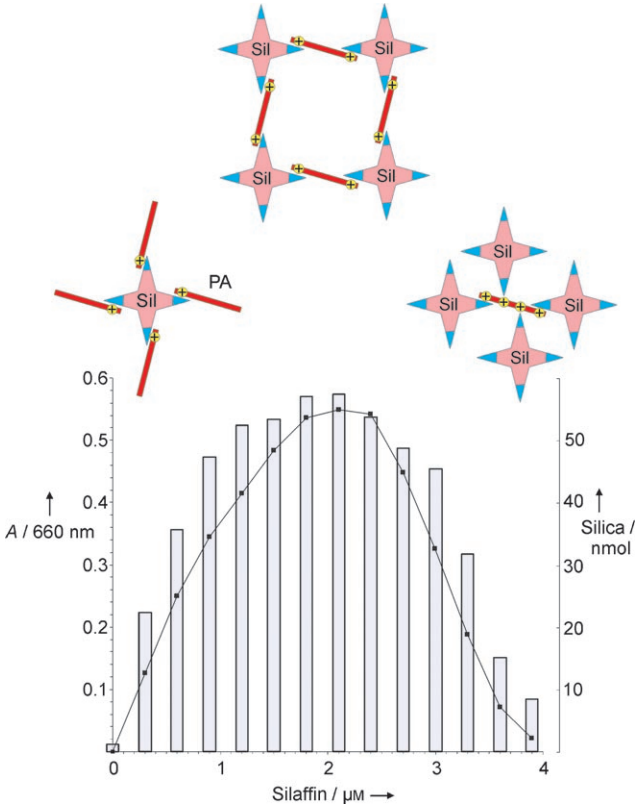

Figure 7. Aggregation behaviour and silica-precipitating activity of polyamine/silaffin-3 mixtures. Increasing amounts of silaffin-3 were added to a constant concentration of polamines (50 μM) buffered at pH 5.8 with sodium acetate. The resulting turbidity was measured at 660 nm. The silica precipitation activities (grey bars, SiO_2 precipitated after 10 min) of different polyamine/silaffin-3 mixtures were determined by the addition of silicic acid to a final concentration of 40 mM. The scheme presents the interpretation of the aggregation behaviour of polyamine/silaffin-3 mixtures according to the Heidelberger–Kendall model of immunoprecipitation. The positively charged polyamines are shown as red bars and the blue patches denote negatively charged clusters in silaffin-3.

of large macromolecular complexes. Remarkably, this maximum of turbidity exactly coincides with the maximum of silica precipitation activity of the polyamine/Sil-3 mixture (Figure 7). Qualitatively identical results could be obtained for the binary system polyamine/Sil-1H.

Summary and Outlook

Silica biomineralisation in diatoms continues to be a challenging field of fundamental research. During the past few years, a number of interesting new observations have been made, especially for the model organism *T. pseudonana*. It has been possible to obtain new insights into the processing of silicon. On the one hand, considerable progress has been made with respect to silicon uptake by special proteins (SITs) from the surrounding water, as well as to the storage and processing of silicon before cell division. On the other hand, the discovery and characterisation of remarkable biomolecules such as silaffins, polyamines and—quite recently—of silacidins in the siliceous cell walls of diatoms strongly impacts the growing field of biomimetic materials synthesis. Recently, numerous papers reporting on biomimetic or bioinspired approaches to silica synthesis have appeared (for selected examples, see refs. [76–79]). In the meantime it has proved possible to synthesise sophisticated materials such as double-walled silica nanotubes,[76] helical silica fibres[77] and many others, apart from silica nanospheres. Hollow silica spheres could be formed in the confined environments of reverse micelles.[78] Three-dimensional polyamine-rich scaffolds have been silicified by the so-called direct ink writing technology.[79] These robotically created structures mimic the shape of naturally occurring diatom frustules amazingly well.

The biomolecules found in biosilica elegantly combine two indispensable functions. On the one hand, they are capable of accelerating silica formation, as well as of stabilising the resulting silica sols. On the other hand, they are capable of forming macromolecular assemblies that are assumed to act as structure-directing templates. Remarkably, all the macromolecular self-assembly processes operating in silica formation are controlled mainly through electrostatic interactions. This concept is corroborated by the silacidins—a new class of super-anionic peptides. In this context, it is tempting to speculate that the observed variety of silaffin species might be required in order to guide the production of different substructures of a diatom cell wall.

It is, furthermore, highly remarkable that the spicule formation in silica sponges more and more appears to rely on principles analogous to those found for diatoms. So far, two striking analogies have been discovered. Firstly, special biomolecules—the so-called silicateins—have been identified in silica sponges.[80] Silicatein filaments are capable of directing silica precipitation in vitro.[81] As it has been possible to show meanwhile,[82,83] the abilities of these biomolecules to self-assemble depend critically on their phosphorylation, in analogy to the silaffins found in diatoms.[48] Secondly, long-chain polyamines with structures similar to those observed previously in diatoms were recently discovered in the silica sponge *Axynissa aculeata*.[84] The sponge polyamines contain sulfate ions as counterions and are capable of inducing silica precipitation in vitro. Moreover, the discovery of the silacidins is an interesting analogy to the observation of similar proteins (such as phosphophoryn) found to be involved in calcium phosphate and calcium carbonate biomineralisation processes. These unexpected analogies give rise to questions about possible evolutionary relationships between rather different biomineralisation processes.

Keywords: biomineralisation · diatoms · polyamines · polyanions · silaffins · silica

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