Biogenic Silica Patterning: Simple Chemistry or Subtle Biology?

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1. Introduction

The importance of solid-state biochemistry was revealed more than twenty years ago by the pioneering work of Lowenstam et al.,[1] and it can now be considered as a field on its own.[2]

Among biogenic minerals, silica appears rather singular. Whereas widespread carbonate and phosphate salts are crystalline iono-covalent solids whose precipitation is dictated by solubility equilibria, silica is an amorphous metal oxide formed by more complex inorganic polymerization processes. Biogenic silica has mainly been studied with regard to the diversity of the species that achieve this biomineralization process, and at the level of diversity in the morphology of silica structures.[3] It is only recently that chemists turned their attention to the formation process. One of the triggers was the possibility of designing mesoporous silica materials with a highly ordered network of pores, similar to the siliceous structure found in unicellular diatom algae.[4] Since then, chemists and biologists have learned how to take advantage of each other's knowledge, not only to get new insight into the biochemical processes involved in the natural systems, but also to design materials inspired by nature.[5–7]

As the frontier between these two fields is vanishing, the question arises whether the art of silica chemistry alone is, or will be, enough to understand and mimic the biosilicification reactions that occur in living organisms.

2. Some Aspects of the Aqueous Chemistry of Silica

2.1. Silica polymerization in aqueous solutions

The occurrence of the neutral monosilicic acid $Si(OH)_4$ is limited to dilute aqueous solutions ($[Si] < 100$ ppm) at room temperature and in neutral or moderately basic media ($2 < pH < 9-10$).[8] At higher pH values, the formation of silicate anions $SiO(OH)_3^-$ and $SiO_2(OH)_2^{2-}$ occurs.[9] The increase of silicic acid concentration leads to the formation of dimeric species.[10] This first condensation reaction involves a nucleophilic substitution (S_N2) of a Si—OH oxygen atom on another silicon atom, which leads to the formation of a Si—O—Si siloxane bond, concomitant with the departure of a water molecule:

$$Si(OH)_4 + HO{-}Si(OH)_3 \rightarrow (OH)_3Si{-}O{-}Si(OH)_3 + H_2O \quad (1)$$

This process could, in principle, take place between two neutral species, but the reaction is very slow as it implies the formation of unfavourable pentacoordinated silicon species. However, the presence of a nucleophilic oxygen atom in the charged species $Si{-}O^-$, speed up the reaction. Trimer, tetramer and oligomer formation proceeds in a similar manner (Figure 1). Within these oligomers, the substitution of a Si—OH group for a Si—O—Si siloxane linkage increases the charge of the silicon atoms, which become more electrophilic and constitute preferential sites for further monomer addition. Condensed rather than chained oligomers are formed, which gives rise to the formation of a sol of colloidal particles (2 – 3 nm). Between pH 2 and pH 7, silicon species are weakly ionized and the primary particles are formed slowly. Above pH 7, they can bear a large negative charge and the process is very fast.

What these nuclei become, in the absence of salts or other additives, is dictated by the same pH/charge parameters that govern their formation. At pH values below 7, particles aggregate because only weak electrostatic repulsions exist. Brownian movement renders interparticle collision possible and, if the contact time is long enough, siloxane linkage between surface silanol groups occurs following Reaction (1). Steric and electrostatic considerations mean that further particle additions take place at the end of the elongating chains, which results in

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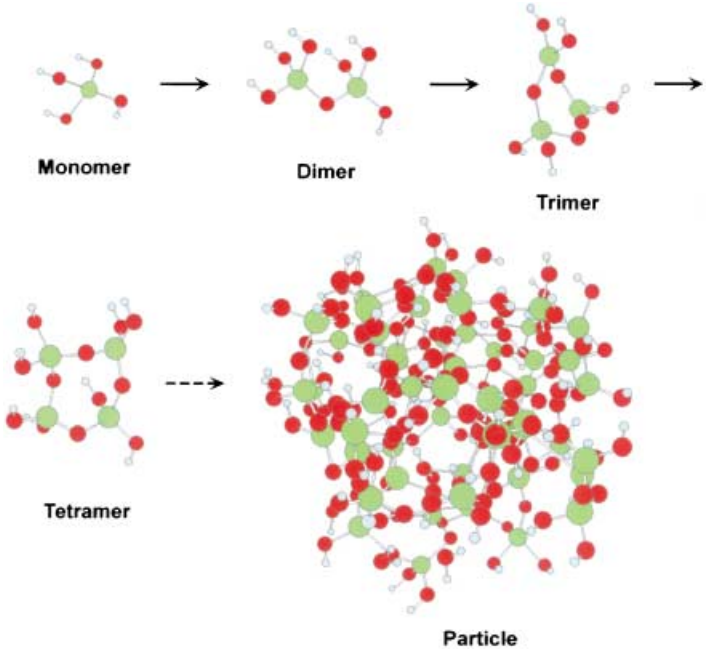

Figure 1. *Polymerization behavior of silica. In aqueous solution monosilicic acid condenses to form dimeric, trimeric, and tetrameric (likely cyclic) structures, which then evolve to form particles with sizes in the nanometre range.*

fibrillar assemblies. As fibrils grow, additions can also occur on the side of the chains and a three-dimensional open network is formed with large water-filled cavities (gel; Figure 2). In contrast,

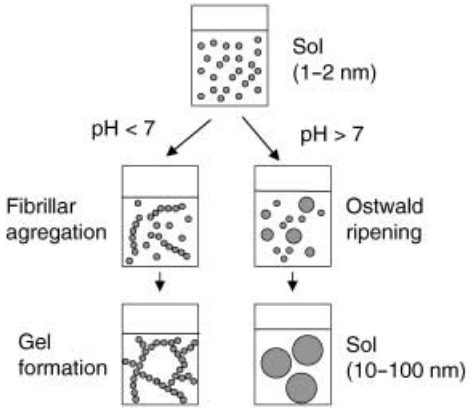

Figure 2. *Sol-gel formation processes. At pH < 7, the initial particles undergo fibrillar aggregation, which leads to gel formation. At pH > 7, the Ostwald ripening process leads to the growth of larger particles, which can form a stable sol.*

at pH values above 7, electrostatic interactions between charged particles limit the aggregation process. Therefore, primary particles increase in size and decrease in number. This is a result of the Ostwald ripening process. The solubility of particles decreases with increasing size. Small particles dissolve and are redeposited onto the larger ones until the difference in solubility between the smallest and the largest particles becomes negligible. A monodispersed sol is obtained whose stability increases with the pH value (Figure 2).

2.2. Controlling the polymerization process

2.2.1. Kinetics

2.2.1.1. Reaction conditions

As mentioned above, the most obvious parameter controlling the polymerization kinetics is the pH value. The minimum rate is obtained at pH 2. This rate increases by two orders of magnitude between pH 3 and 5, and by two more orders between pH 6 and 9. Above pH 2, silicic acid polymerization is a second-order reaction; consequently, the reaction rate rapidly increases with silicic acid concentration. For example, at pH 7 a 0.5 mol L^{-1} silicic acid solution forms a gel in about five minutes at room temperature, whereas a 0.1 mol L^{-1} silicic acid solution under the same conditions shows no gelation after several hours. An increase in temperature also promotes the polymerization process.[8]

2.2.1.2. Additives

Metal cations are known to speed up the condensation process, especially above pH 7.[11] They interact with negatively-charged silicate species and screen the surface charge of the particles, which favors their aggregation (coagulation). Therefore, solutions that lead to stable sols in the absence of salt can form a gel at low concentrations of NaCl.

Cationic polyelectrolytes can also promote silica formation.[12] The obvious mode of action involves polymer adsorption onto the particle surface, which reduces interparticle electrostatic repulsion and then induces coagulation. If more polymers are adsorbed on the surface, the apparent charge on the particles can become positive and the sol is redispersed. Long-chain polymers may also bridge particles together, which leads to flocculation and precipitation of silica (Figure 3a). Recently, activation of the silicic acid polymerization process itself, rather than particle aggregation, has been observed with polyamines such as polyallylamine, polylysine, and polyarginine.[13, 14] A model was proposed for the aggregation that involves the adsorption of monomers or small oligomers onto amino groups along the polymer chains. The inorganic species are brought closer together and their condensation is favored (see Figure 3b). Oligomers formed in this way may serve as nuclei for gel formation. A similar process has been shown to be dependent on pH value and polymer chain length.[15]

Although electrostatic interactions appear to be the major activation pathway, nucleophilic substitution catalyzed by the nitrogen atom of the amino group could also occur. Studies suggested that hydrogen bonding between the silicate species and the polymer backbone could also be involved in activation of silica polymerization. Such activation was reported for polyamides, polysaccharides, and polyethylene oxides.[16, 17]

2.2.1.3. Organosilicons and silicon complexes

Instead of trying to speed up reaction rate, it might be interesting to slow down the polymerization process for better

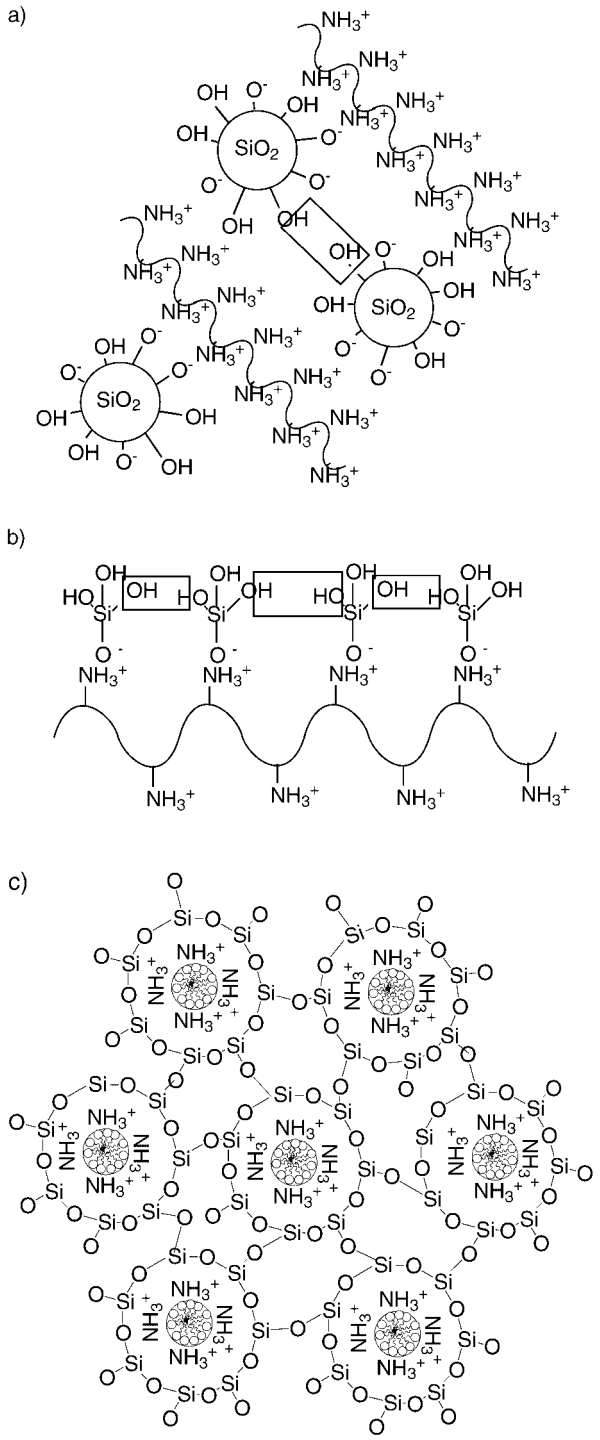

Figure 3. *The influence of proteins on silica formation. a) positively charged proteins bridge silica particles and control their aggregation; b) interaction of silicates with the ammonium groups of a protein chain favors condensation of the silicates; c) self-assembled micelles of amphiphilic cationic proteins act as templates for the formation of mesostructured silica.*

control of the final solid. Therefore, the reactivity of the oxygen atoms that make Si–O bonds must be lowered by linkage to an organic group. The most popular source of organosilicon precursors are silicon alkoxides $Si(OR)_4$, where R is an alkyl chain.[18] These molecules are less reactive than silicic acid since the condensation reaction between two Si–OR silanol ester groups is very slow. The Si–OR bonds must first be hydrolyzed to form Si–OH groups before condensation can occur. Most of these alkoxisilane precursors are not miscible with water, therefore the hydrolysis step can be very slow. Acidic or basic catalysis are usually used to rationally control this process. The reaction rate also depends on the nature of the R group. Overall, the use of silicon alkoxides allows better control of the reaction kinetics and chemical modifications of the silica surface than is otherwise possible. The products obtained can be of great interest for materials chemistry.[19] However, to the best of our knowledge, silicon alkoxides have never been unambiguously identified in natural systems.

Silicates were shown to form water-stable complexes with some polyols[20] and the transport of soluble silicon in plants has been suggested to involve silicon catecholates.[3] The reactivity of such $(M^+)_2[Si(C_6H_4O_2)_3]$ complexes has been studied.[21–23] The silica polymerization occurs by condensation of silicic acid released by the complex breakdown, and the nature of the counterion (M^+) has a significant influence on the reaction kinetics.[21]

2.2.2. Silica morphology

2.2.2.1. Particle size

Above pH 7 and in the absence of an additive, the growth of the silica particles stops when all particles are too large to be redissolved and to take part in the Ostwald ripening process. This is related to the silica solubility and to the reaction temperature. Particles in the 5 – 10 nm size range can be obtained at room temperature, whereas particles with diameters up to 150 nm are formed at 350 °C. The addition of salts at low concentration decreases particle solubility and size. It is also possible to increase particle size by supplementing the sol with additional silica monomers that polymerize on the particle surface.[8]

Primary particles can aggregate, one with another, to form larger particles. This aggregation is very likely to be involved in the Stöber process, a well-known route to calibrated silica particles. The Stöber process is currently used on an industrial scale and involves the polymerization of silicon alkoxides in the presence of ammonia.[24] The aggregation process can also be controlled by polymers. Cellulose was shown to induce the formation of 4-nm silica particles from catecholate complexes, whereas 2 – 14-nm diameters were obtained in the absence of this polymer.[22] Silica spheres of micrometric size have been obtained by using hydrolyzed alkoxide solutions and synthetic co-polypeptides.[25] Recently, it was shown that polymerization of sodium silicate in the presence of bovine serum albumin leads to a mineral – organic gel. The size of the silica particles (50 – 100 nm) is controlled by the protein aggregation.[26] However, despite an increasing number of examples, no conclusive explanation has yet been proposed for the control of particle size by polymers.

Finally, it is possible to design silica spheres by performing polymerization within nanoreactors (vesicles, emulsion droplets,

etc.).[27, 28] Nevertheless, control of monodispersion in confined media is difficult and these approaches are still in their infancy.

2.2.2.2. Structuring silica

Silica formed from molecular precursors at room temperature is amorphous, therefore it can accommodate a large variety of morphologies. This subject has been extensively studied and reviewed in the past few years.[2, 29] Two main strategies can be distinguished. The first one involves templating agents with a well-defined shape that are used as moulds for silica deposition. The second strategy involves the use of self-organized systems that assemble simultaneously in the silica polymerization process and thus create a final morphology according to the template – silica interactions. The morphological control of these self-assembly processes takes place on the nano to micro scale, whereas the molding approaches allow organization in the micro to macro range. It is of course possible to combine these strategies to design hierarchically ordered silica.[30]

Beck et al. were the first to study the formation of an ordered array of silica pores size-controlled by cationic surfactants.[4] Several methodologies have now been examined and a large range of organizational systems (hexagonal, cubic, etc.), pore size (10 – 100 Å), and shape (films, fibers, spheres, etc.) are now available.[31, 32] The mechanism of formation of mesoporous materials is still controversial but it is generally accepted that silica polymerization creates anionic silicate oligomers. These oligomers are supposed to interact with the positively-charged polar heads of the surfactants and these constructs then assemble in an organized network of large micelles.[33] Mineral polymerization around these micellar networks proceeds until silica is formed. Withdrawing the surfactants leaves empty cavities, that is, pores in the silica matrix (Figure 3c and 4).

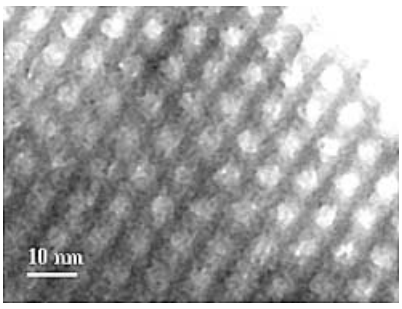

Figure 4. *Synthetic mesoporous silica.*

Shape control at the nanoscale level is possible through the use of synthetic organogelators[34] or proteins such as collagen,[35] which form linear or helical fibers. A larger diversity of structures can be obtained by polymerizing silica at the surface or within preformed templates.[36] Impregnation of wood has been performed and leads, after calcination of the organic template, to a finely detailed replica of the initial structure.[37] The replication speed and accuracy increase with increasing interactions of the surface groups of the mould with the polymerizing silicate species.[38]

It is also possible to induce the organization of preformed silica particles. In solution, particles aggregate following a fractal growth process.[39] However, when deposited on a substrate, their packing can be controlled by the surface structure. Self-assembled monolayers have been widely used to tailor particle organization.[40]

Although two-dimensional packing of particles can be controlled, the building of three-dimensionally organized networks of over a few atomic layers appears more difficult. It might be possible to get some 3D control by diffusion-limited addition of further particles on a prepatterned network. Alternatively, the aggregation process could proceed within a 3D preformed organic matrix, similarly to other mineral formation.

3. Silica Deposition in Natural Systems

It has been shown that bacteria, algae, protozoa, and higher plants use silica, and that silica participates in metazoa development. Since silica sustains different roles in the various cell types, the biochemistry of the underlying silica formation processes is likely to be different. Herein, we will focus on the silica biochemistry of some living organisms and, where they exist, on proposed biopatterning models.

3.1. Bacteria cell surface

A number of silicates have been observed on the surface of bacterial envelopes in different environments. These silicates deposit as thin (≈100 nm) amorphous and often granular crusts that coat the cell wall.[41]

A possible process for the formation of silica in this context involves the direct interaction of soluble anionic silicates with the positively charged groups on the peptidoglycane of the bacterial envelopes, such as the amine side groups on the peptide chain. Hydrogen bonding between silanol groups and the polysaccharide hydroxy groups of the peptidoglycane may also be involved. Finally, if the superficial bacterial layers are predominantly negatively charged, external cations (Al, Fe) can interact with the cell wall and provide nucleation sites for the mineralization. A combination of these processes seems to be involved in the formation of silica at the surface of the Gram-positive bacteria *Bacillus subtilis*[42] and could be relevant for the silicification of the Gram-negative *Thiobacillus* in acid mine tailings.[43]

This coating is often associated with extreme environments, which suggests it may serve as a protecting shell for the bacteria. In this context, silica glasses obtained by the sol-gel methodology were shown to be suitable media for the encapsulation of *Escherichia coli*.[44, 45]

3.2. Sponge spicules

Marine sponges are multicellular organisms that are skilful in generating species-specific amorphous silica structures, from microscopic to macroscopic sizes. Of particular interest is the formation of spicules. These structures consist of glassy rods with diameters ranging from a few microns to several milli-

metres, and lengths reaching up to 3 m, which allows anchoring of the sponge on the seafloor. Spicules are made of concentric layers of hydrated silica (opal) surrounding an axial organic filament (Figure 5). Spicules exhibit high breaking stress and low elastic modulus, which leads to flexibilities of material for which there is no actual equivalent amongst synthetic silica-based materials.[46]

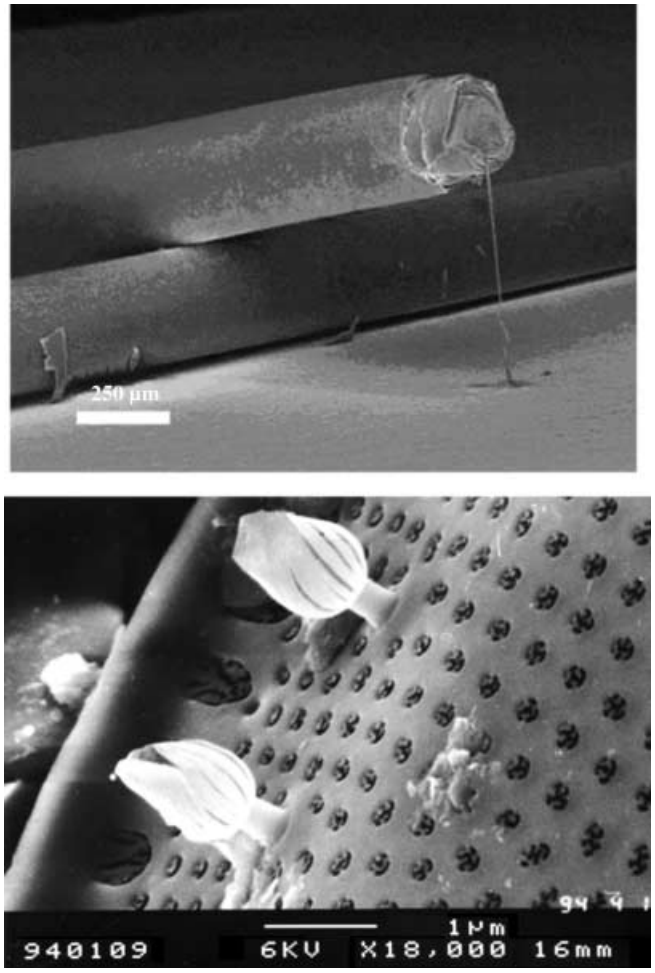

Figure 5. *Biogenic silicas. Top: sponge spicule; bottom: high magnification of pores over the surface of a* Thalassiosira sp. *frustule.*

Formation of these spicules occurs in a differentiated cell type, the sclerocytes. Within these cells, the growing spicule is embedded in a membrane, the silicalemma, which incorporates the axial filament as well as silica-containing specific vesicles termed silica deposition vesicles (SDVs). Silica polycondensation takes place on a protein fiber composed, in the sponge *Tethya aurantia*, of three different proteins, the silicateins;[47, 48] these proteins are also found in *Suberites domuncula*.[49]

These scaffolding silicatein proteins are closely related to the cathepsin L proteolytic enzymes in terms of the amino acid sequences and three-dimensional structure.[47, 49] The ability of silicateins to catalyze the hydrolysis, and hence the polymerization of tetraethoxysilane $Si(OC_2H_5)_4$ appears to closely parallel that of proteases. Experiments performed with *S. domuncula* cells have shown that extracellular silicate is able to up-regulate silicatein gene expression, but this induction is not observed with alkoxysilanes.[49] Since the silica form found in natural aquatic environments is mainly uncondensed monosilicic acid, the nature of the silica precursor inside the sponges remains to be determined.

3.3. Diatom "glass box"

Diatoms are unicellular eukaryotic algae widely distributed around the world. They are the main contributors to the global silica biogenic cycle. The diatom frustule is a finely dedicated and differentiated glass box of opal constitution that consists of two halves overlapping like a petri dish, each half made up of a valve and several girdle bands spanning the circumference of the cell. The hard surface of diatom frustules are structured on the nanoscale, with particles of about 40 nm[50] or 100 – 200 nm.[51] The frustules exhibit a patterned network of pores of nano- to micrometer-scale size that can be quite uniform in shape and are homogeneously distributed throughout the surface (Figure 5).[52] The production of these precisely dedicated structures can take as little as 10 minutes, although the frustules continue to thicken for a few hours or more.[53]

3.3.1. Associated compounds

Whole organic extracts from the cell walls of diatoms have been analyzed. These analyses revealed the presence of soluble glycoproteins enriched in hydroxy amino acids (serine, threonine),[54] which may interact with the Si–OH groups of silicic acids.[55] Some diatom cell-wall proteins have also been isolated from HF-treated extracts and identified. In *Cylindrotheca fusiformis*, the specific silica-associated proteins are named silaffins. The corresponding gene has been cloned and contains repetitive sequence elements.[56] The mature silaffins consist of 15 (silaffin-1A1) and 18 (silaffin-1A2) amino acid residues, rich in lysine and serine, respectively. The lysine residues are the target for post-transcriptional modifications such as introduction of long-chain polyamines, ε-*N*,*N*-dimethyllysine or ε-*N*,*N*,*N*-trimethyl-δ-hydroxylysine.[57] Recently, a softening method for silaffin extraction was used.[58] This technique revealed that all of the hydroxy groups of the silaffin 1A serine residues are phosphorylated. Polyamines also present in the HF-extracted fraction were identified in *C. fusiformis* and in several other diatom species.[59, 60] The complexity of these fractions seems to be species specific but the fractions all show very low molecular weight ($<$ 3.5 kDa). These extracted polyamines are methylated and exhibit amphiphilic properties.[60]

3.3.2. The silicification process

3.3.2.1. Reaction conditions and influencing factors

The concentration of monosilicic acid in natural sea and fresh water is in the micromolar range, but the intracellular free pool concentrations can be as high as the millimolar range. Therefore,

there must be an active transport of silicic acid, and/or transformation of intracellular silica must occur, to allow accumulation above the free diffusion level. A gene named *sit1* (*si*licon *t*ransporter 1), which encodes a polypeptide containing 10 putative transmembrane domains, was cloned from *C. fusiformis*.[61] Later, a gene family encoding five different proteins was discovered in this specie.[62] Expressed sequences showing high homology with *sit* genes have now been identified in two other diatom species: *Phaeodactylum triconutum* (C. Bowler, personal communication) and *Synedra acus* var. *radians* (GenBank accession number AF492011). It has been demonstrated that silica transport is sodium dependent, with a supposed optimal $Si(OH)_4$:Na^+ ratio of 1:1.[61]

As mentioned above for sponges, formation of biogenic silica in diatoms occurs in specialized membrane-bound compartments named SDVs. The origin of these compartments is unknown and could either be the Golgi apparatus, the endoplasmic reticulum, or a specialized entity.[63] The intracompartmental conditions and composition remain mostly unknown. SDVs have been shown to contain silica nanostructures, usually around 30 – 50 nm in size, the smallest with a size of 3 – 20 nm, but also occasionally up to 200 nm.[63] These nanostructures could serve as the elementary building blocks for frustule formation. Dyes that accumulate in acidic compartments when a pH gradient exists were shown to specifically accumulate inside the SDV.[64, 65] During the formation of the new valve, the pH level becomes increasingly acid.[66] It was therefore proposed that acid pH levels facilitate the nucleation and aggregation of the particles and favor the flocculation of the silica sol. The pH value should also influence the global charge of the protein or polyamines mediating silica aggregation. Therefore, it seems that diatom cells might control the structure, the extent, and the overall pattern of silica formation by regulating the acidity inside the SDV.

It is not known whether all the condensation steps occur inside the SDV, or if silica precursors could be formed outside the SDV and transported toward it by silica transport vesicles (STVs). These STVs would provide membranous and proteinaceous material, as well as other components necessary for the enlargement of the SDV, silica mineralization, and pattern formation.

HF-extracted proteins are strongly associated with silica (see above) and have been proposed to play a direct role in the formation and packing of the elementary building blocks. In vitro, silaffins extracted from *C. fusiformis* induce fast precipitation of silica when added to freshly prepared metastable silicic acid solution.[56, 57] The size of the silica particles obtained depends on the modifications and the peptide used; silica nanospheres can be obtained on the order of 500 – 700 nm or below 50 nm in size. It has been proposed that the cationic silaffins play the role of flocculating agent for the negatively charged silica particles. The hydroxy amino acids may also interact with surface silanol groups. The kind of silica particle aggregates obtained from in vitro experiments is also dependent on the exact polyamine fraction used. In the case of *Navicula angularis* at pH 5, the spheres made are 800 nm – 1 μm in diameter for 1 – 1.25 kDa polyamines, and 100 – 200 nm for the 0.6 – 0.7-kDa fraction.[59] A mixture of silaffins with polyamines produces intermediate sizes of precipitated silica structures. Although the size of the silica nanoparticles obtained from these in vitro experiments is of the same order as that of the particles observed for diatom frustules, the arrangements of the particles are quite different. This difference suggests that either the physicochemical conditions of the in vitro experiments only approach the in vivo conditions, or that other undiscovered constituents are involved in the in vivo assembly.

External factors can influence frustule formation. Addition of metal ions and modification of the salinity affect both cell growth and silica formation in diatoms. Several interpretations have been proposed, for example, modification of the cell turgor could influence the overall shape of the frustule, or changes in the physicochemical equilibrium inside the SDV could occur.[67, 68] Alternatively, foreign ions (i.e. Al) may interact with $Si(OH)_4$.

3.3.2.2. Morphogenic models

Chemical and physical paradigms have been used to model frustule patterning. Gordon et al. have proposed that the "initial" space-filling branching patterns are the result of instabilities in the diffusion-limited aggregation of silica particles within the SDV.[69] After their release inside the SDV, particles diffuse until they encounter growing aggregates, to which they bind. Following the "sintering" process, the aggregate tends to reorganize into a dense, thermodynamically stable packing. If further particle addition occurs before this reorganization is achieved, an out-of-equilibrium growth process is obtained, which results in pore formation and complex patterning. It has since been suggested that a surface-stabilizing agent could be introduced within the SDV to slow down the reorganization process and thus enhance the porosity of the aggregates.[70]

More recently, two models for pore formation were proposed that benefit from both the development of synthetic mesoporous materials and the studies of the cell-wall proteins of diatoms. Vrieling and co-workers suggest that, in the presence of silica precursors, short-chain silaffins and polyamines induce rapid precipitation of silica. In this model, larger unknown peptides contribute to the aggregation process by interacting not only with silica particles, but also with each other, which results in an organomineral hybrid mesophase. After two- and three-dimensional growth, the proteinaceous component is removed and can contribute to the organic casing that covers the silica shell.[71] Alternatively, Sumper has postulated the existence of repeated phase separation processes within the SDV that produce emulsions of micro- to nanodroplets consisting of a polyamine-containing organic phase. These droplets could be used as templates for silica patterning.[60] It has also been proposed that the zwitterionic structure of silaffins could lead, through electrostatic interaction, to a self-assembly process in which silica polycondensation occurs.[58] Although the latest models differ in details, these models all rely on the self-organization of the organic matter in the presence of mineral precursors.

In contrast, the possibility of a prepatterned cellular mould for silica deposition has been examined. The organic casing covering the frustule appears to be a good candidate for such a

process. The patterned wall would arise from cytoplasm regions secreting wall material at defined locations and consequently selecting sites of polycondensation. Moreover, these cellular structures could help with the formation of pores within diatom frustules through the formation of spacer vesicles (vesicles, mitochondria, etc.) within the "mother liquor", which create silica-free regions.[63]

4. Silica Constraints and Possibilities in the Biological Chemistry World

Natural systems have evolved to use several of the possibilities offered by silica chemistry to build up their own minerals, while also adapting themselves to the constraints of silica chemistry. As an example, the acidity of the SDVs of diatoms allows the decrease of the charge of silicate species, which slows down the silica polymerization and limits the size of the particles. However, a decrease in the negative charge of silica should also decrease the strength of its interaction with the positively charged ammonium groups of the lysine residues present in templating proteins. In response to this problem, the diatom silaffins are post-translationally modified by alkylation of their amino groups, which increases their basicity (higher pK_a value) and thereby enhances their catalytic activity in acidic media. Furthermore, since the natural availability of silicic acid is too low for spontaneous silica formation, organisms (such as sponges or diatoms) exploit deposition inside vesicles, which allows the confinement and accumulation of silica precursors.

Approaches that make use of current biological knowledge to investigate new chemical systems are certainly of great interest. Silica patterning in diatoms appears to rely on proteins that are able to catalyze silicate polymerization and to act as templating agents through self-assembly process, therefore synthetic models that exhibit both properties have been designed. A first approach involved the synthesis of arginine-based surfactants.[72] These molecules form micelles and liquid-crystalline phases and can therefore be used to synthesize mesoporous materials. Moreover, the ammonium groups of the arginine polar heads of the surfactants are able to activate silica polymerization, therefore this process could occur at pH 7, at room temperature, and at low silicate concentration. Interestingly, the use of unmodified arginine residues does not lead to the formation of silica. This observation suggests that the self-assembly of the surfactants is a key step in the catalytic process. A similar approach has also been reported that uses block copolypeptides poly((amino acid 1)$_n$-b-(amino acid 2$_m$), where n and m are variable large numbers.[25] In the presence of hydrolyzed alkoxide solutions, silica formation was observed when amino acid 2 was lysine. The use of cysteine as amino acid 1 creates a water-insoluble domain so that the poly((cysteine)$_n$-b-(lysine)$_m$) polymers exhibit self-assembly properties, which leads to the formation of silica spheres, globules, or columns, depending on the n and m values. Similarly, silaffins were recently used as structuring agents to produce holographic nanopatterning of silica spheres.[73] Conversely, the principles of silicon chemistry elucidated by relatively simple in vitro experiments can be of great help in understanding biogenic silica formation processes. Biologists studying biosilicifying organisms will benefit from knowledge obtained by chemical methodology. Undoubtedly, recent models of porosity control in diatom frustules have been rendered possible by the investigations of the mechanisms of formation of synthetic mesoporous materials.

Biomimetic strategies such as the ones presented above are within the reach of chemists. However, two main aspects illustrate the gap which, at present, separates biosilicification in organisms from in vitro processes. First, it is possible to reproduce distinct aspects of the silica patterning process in vitro, but cells are able to carry out all aspects of the process in "one pot". The second difference lies in the actual conditions used for such syntheses—silicon alkoxides at high concentration, at extreme pH values, and above room temperature—conditions that are clearly far from the natural ones.

Can these barriers be overcome? Considering the first problem, multicomponent reactions are often difficult to study because of the numerous possibilities of interspecies interactions. Moreover, whereas the organisms can regulate molecular availability and flow on short time scales, such control appears rather tricky for synthetic chemistry. However, one should not aim at reproducing the level of complexity of natural systems but should rather try to extract the leading principles of their strategies and adapt these principles into synthetic possibilities and expected applications. There is, for example, no need for an exact synthetic replica of the diatom frustules, but there is demand for new strategies to control the porosity of material structures on different length scales. Therefore, the discrepancies between the in vivo and in vitro synthesis conditions may not be crucial. It is interesting to note that some areas of silicon chemistry have not yet been discovered by biological systems or appear out of reach. As mentioned above, no silicon alkoxides have yet been identified in natural systems, probably because they are easily hydrolyzed in water. Accordingly, living cells do not seem to be able to form Si–C or Si–N bonds. Synthetic routes use silanes (Si–H) or halogenosilanes (Si–X; X = halogene atom), which are also sensitive to hydrolysis. A comparison with carbon formation by metabolic processes shows that these processes usually involve C=O carboxylate groups and C=C double bonds that do not have equivalents in silicon chemistry. Again, our knowledge on the association of silicic acid with lipids or carbohydrates is very limited and studies may reveal unsuspected capabilities of living organisms. The question is left open as to whether such Si–C bonds are needed in natural systems or if their synthesis has a too high energy cost to be efficiently performed by metabolic pathways. Observations suggest, however, that chemists may develop, with their own tools, new materials that are not biomimetic, but rather bioinspired.

Finally, it is also worth underlining the difficulty of studying silica formation in vivo. The two main limiting factors for the characterization of soluble species are the low concentrations of silicates or silicon complexes involved in the processes and kinetics of silica condensation. As an example, the ^{29}Si isotope probed by NMR studies has less than 5% natural abundance, which implies that the investigation of a 10-mM silicate solution by this technique would involve at least overnight recordings, whereas diatom frustule formation is completed within a few

hours. This is probably why most biomimetic in vitro studies make use of silicon alkoxides at high concentrations; such a procedure allows a better analysis, although it moves these model systems away from the natural conditions of silicification. Accordingly, the study of silica shells of whole cells, for example with regard to porosity, is often hindered by the presence of cellular materials. Removal of silica-associated biomolecules by acidic treatments may modify the condensation state of the silica network. Similarly, the extraction of proteins associated with silica is usually undertaken by dissolution of the mineral phase with HF-buffered solutions, which could also modify these proteins.

Therefore, apart from the combination of concepts, one of the key points that will make the close collaboration of biologists and chemists more fruitful is the application of their respective techniques. For example, solid-state ^{29}Si NMR spectroscopy is widely used in materials chemistry but its application to study natural systems is still in its infancy.[74, 75] Silica chemistry should also benefit from the scaling-down of such techniques, which can then be more easily adapted to the magnitude of biological samples. On the other hand, the development of large-scale methods, such as proteomic experiments in the context of whole genome information about organisms such as diatoms, should change our understanding of silica biomineralization and biopatterning. The development of methods based on the evolution of single-gene, that is, DNA-shuffling experiments starting with the silaffin gene, could be of interest for learning what elementary building blocks are required for silica polycondensation.

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