Silicic Acid Uptake and Storage by Diatoms

Felicitas Kolbe and Eike Brunner

Abstract

A unique structural feature of diatoms is their hierarchically patterned siliceous cell wall. Silicon uptake, storage, and processing are necessary for the intracellular synthesis of these cell walls. Uptake occurs from the natural habitats, that is, salt-water or fresh-water reservoirs. Dissolved silicon is predominantly taken up as monosilicic acid, $Si(OH)_4$. The required intracellular silicon concentrations are much higher than typical environmental concentrations. Therefore, stabilization and storage inside the cell are necessary. Silicic acid transporters (SITs) were identified and studied within the last decades. These proteins are found in all different diatom lineages. They are able to transport silicic acid into the cell interior. SITs are transmembrane proteins and work as silicic acid/sodium symporters. After silicic acid uptake, it must be stabilized against uncontrolled polycondensation and stored in the cell interior. Different models of silicon storage by diatoms are discussed. Moreover, the incorporation of different "foreign" inorganic elements, like iron or aluminum, in diatom biosilica occurs and can influence the structure. This chapter deals with the chemical transformation of silicic acid during uptake and transport before the start of cell wall silicification. Note that the molecular regulation of cell wall biosynthesis is the topic of another chapter.

Keywords

Silicic acid · Diatoms · Transporters · Membrane · Silicon

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List of Abbreviations

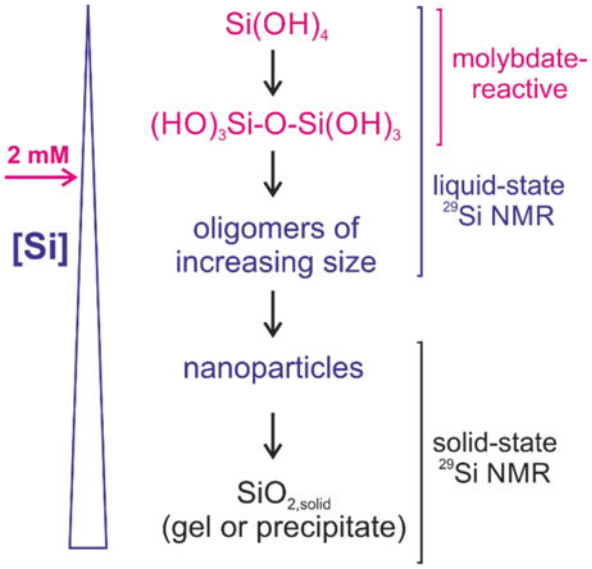
cDNA	Complementary deoxyribonucleic acid
cryo-FIB-SEM	Cryo-focused ion beam scanning electron microscopy
EDX	Energy-dispersive X-ray spectroscopy
EPR	Electron paramagnetic resonance
GXQ (X = Q, G, R, M)	Sequence motif (G: glycine, Q: glutamine, R: arginine, M: methionine)
IR	Infrared
ITC	Isothermal titration calorimetry
K_M	Michaelis constant (in the Michaelis-Menten kinetics)
MAS	Magic angle spinning
Me^{n+}	Exchangeable counter ions like Na^+, K^+ or Ca^{2+}
mRNA	Messenger ribonucleic acid
NMR	Nuclear magnetic resonance
NP	Nanoparticle
Q^n	$Si(OSi)_n(OH)_{4-n}$ moiety ($n = 0, 1, 2, 3, 4$)
SIT	Silicic acid transport protein
SITL	Diatom-like silicic acid transporters
SDV	Silicon deposition vesicle
SSP	Silicon storage pool
STV	Silicon transport vesicle
V_{max}	Maximum reaction rate (in the Michaelis-Menten kinetics)
[Si]	Concentration of silicon

1 Silicic Acid in the World's Water Reservoirs and Its Chemical Properties

About 28 weight-% of the earth's crust consists of silicon, the second most abundant element after oxygen (Encyclopedia Britannica 2020). In nature, silicon is usually oxidized and forms minerals. Examples for purely SiO_2-based minerals are crystalline compounds like quartz, tridymite, and cristobalite. The gemstone opal is an amorphous and partially hydrated compound of the overall composition $SiO_2 \cdot (H_2O)_n$. Typical opal consists of agglomerated, more or less spherical silica particles with water contents of just a few percent. Silica-based minerals dissolve weakly in aqueous solutions at near-neutral pH (Iler 1979):

$$SiO_{2,solid} + 2\ H_2O \leftrightarrow Si(OH)_4 \quad (1)$$

Saturated solutions around pH 7 at 25 °C contain about 120 mg dissolved SiO_2 per liter water corresponding to 2 mM dissolved Si. Usually, the concentration of dissolved silicon found in the world's water reservoirs is considerably lower than the



Scheme 1 Description of the typical polycondensation states of silicic acid observed at increasing Si-concentration, [Si], and near-neutral pH. Selected analytical methods for the identification and quantification of these species are indicated in this scheme on the right.

equilibrium solubility of 2 mM (see below). At such low concentrations, mainly nondissociated ***monosilicic acid, Si(OH)$_4$*** is found in aqueous solutions at near-neutral pH. This very weak acid dissociates into ***silicate ions*** in strongly alkaline solutions beyond pH 9 (Iler 1979).

Spontaneous polycondensation of silicic acid takes place at concentrations beyond 2 mM (see Scheme 1). This reaction is of special chemical importance for biosilica formation in diatoms and will be briefly described in the following. In this context, we will also clarify the definitions and denotations used to describe the chemical states of silicon. For more details, see Iler (1979).

1. ***Silicon (Si)***. The name of the chemical element will be used if the chemical state is not considered or unknown. This is, for example, the case if element compositions, the results of element analyses, are discussed.
2. ***Soluble silica.*** This term is commonly used for ***molybdate-reactive*** silica. ***Monosilicic acid*** transforms into the yellow silicomolybdic acid complex $H_4SiMo_{12}O_{40} \cdot xH_2O$ (Jolles and Neurath 1898; Coradin et al. 2004) after addition of acidified ammonium heptamolybdate or alkali molybdates. Interference with other complex-forming ions, especially phosphate, can be circumvented by the reduction of silicomolybdic acid to molybdenum blue (Iler 1979 and references therein). ***Disilicic acid***, $(HO)_3Si\text{-}O\text{-}Si(OH)_3$, is produced by the OH^--catalyzed condensation of monosilicic acid. It can, however, also rapidly dissociate back into monosilicic acid and thus form the silicomolybdic acid complex. Therefore, mono- and disilicic acid are considered as the molybdate-reactive soluble silica species (Scheme 1).
3. ***Oligomerization***. Depending on the solution pH and concentration, various silicic acid oligomers can be formed from mono- and disilicic acid (Iler 1979). For

example, the reaction of two disilicic acid molecules can produce a linear tetramer. Ring closure is then likely and transforms the linear into a ***cyclic tetramer***. Linear trimers form by the reaction of mono- with disilicic acid. With lower probability than tetramers, the trimers can also adopt a cyclic structure (***cyclic trimer***). Once present, these cyclic species can further react with other mono- and disilicic acid species, resulting in higher oligomers of increasing molecular weight.

4. ***Nanoparticle formation.*** Silica nanoparticles (NPs) of increasing size appear as the result of the further proceeding polycondensation reaction. Their outer surface is covered with silanol groups (SiOH). Depending on the solution pH, these SiOH groups partially dissociate as follows:

$$\mathrm{SiOH} + \mathrm{H_2O} \leftrightarrow \mathrm{SiO^-} + \mathrm{H_3O^+} \tag{2}$$

This causes the ***negative surface charge*** of silica particles, which is increasingly high in basic solutions (pH7–10). The particles then repel each other, preventing them from aggregation in the absence of salts or other positively charged species; a ***silica sol*** is formed. Only less than 10% of the SiOH groups are dissociated at near-neutral pH (Pfeiffer-Laplaud et al. 2015).

5. ***Gel formation and precipitation.*** Below pH7 or in the presence of salts, the silica NPs aggregate and form branched chains, thus making up a loosely packed three-dimensional ***gel network***. Another process denoted as ***coagulation*** occurs at very high salt concentrations or in the presence of certain organic molecules called coagulants. The NPs are then closer packed than in the gel and form dense aggregates, which can separate from the solution and sediment (***precipitates***).

The oceanic average amounts to ca. 70 μM soluble silica mainly present as monosilicic acid (Tréguer et al. 1995), but locally observed concentrations can significantly differ from this average. Diatoms take up and use this monosilicic acid for their silica-based cell walls (cf. Fig. 1). Every year, about 240 ± 40 teramoles of Si are consumed for biological silica production (production rate) in marine habitats, for example, by diatoms (Tréguer et al. 1995; Tréguer and De La Rocha 2013). The estimated net influx amounts to 9.4 ± 4.7 teramoles Si per year. Its largest part of 7.3 ± 2 teramoles Si per year is caused by rivers. The average silicic acid concentration in the world's rivers amounts to about 150 μM. Weathering of SiO_2-based minerals (see Eq. (1)) contributes 6.2 ± 1.8 teramoles Si per year. Dissolution of biogenic silica yields 1.1 ± 0.2 teramoles Si per year. These influxes are more or less counterbalanced by the total net outflux of about 9.9 ± 7.3 teramoles Si per year. Note that diatom sedimentation contributes 6.3 ± 3.6 teramoles Si per year ("burial rate") to this outflux. Despite the uncertainties of these numbers, it can

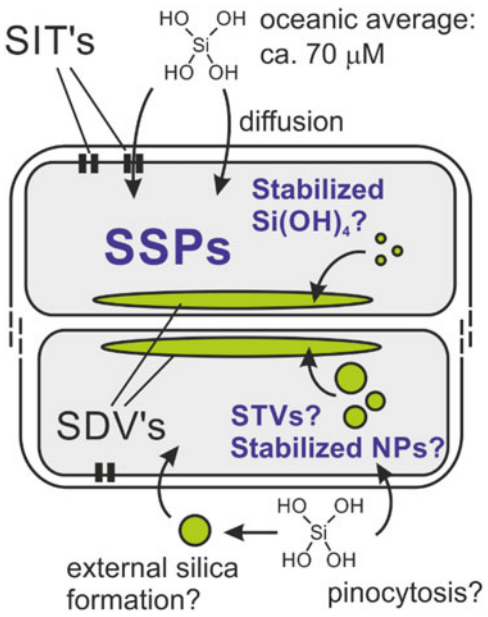


Fig. 1 Scheme of a dividing diatom cell. Cell wall synthesis takes place in a specialized compartment, the silicon deposition vesicle (SDV). Monosilicic acid is taken up from the sea water via silicic acid transport (SIT) proteins and/or direct penetration/pinocytosis across the outer cell membrane (see Sect. 2). Intracellular processing of silicon is not yet understood completely. Several diatom species are reported to exhibit internal silicon storage pools (SSPs, see Sect. 3). Silicon transport vesicles (STVs) are sometimes hypothesized to explain the transport of silicon into the SDV. Adapted from Brunner et al. (2009). Copyright 2009 Springer

be concluded that the "burial rate" is much smaller than the production rate of 240 teramoles per year (Tréguer and De La Rocha 2013).

Many of the biochemical and biophysical processes underlying silicic acid uptake, intracellular stabilization, and processing by diatoms are still enigmatic although various studies have revealed biomolecules involved in silicon bioprocessing and the cellular response to silicic acid including its uptake (Mock et al. 2008; Shrestha et al. 2012; Brembu et al. 2017). Our present knowledge in the field of silicic acid uptake and storage will be summarized and evaluated in the following especially with respect to the underlying chemistry.

2 Silicic Acid Uptake by Diatoms

Intracellular biosilica synthesis in diatoms necessarily requires the uptake of silicon from the environment. As already discussed above, dissolved silicon is predominantly available and taken up as monosilicic acid. Inside cells, much higher concentrations of silicon are reported (Martin-Jézéquel et al. 2000, see below) than

usually present in the environment (70 μM oceanic average, see above). That means, the cells must take up large amounts of silicic acid to meet the high demand for cell wall synthesis. For this purpose, a silicic acid transport mechanism is necessary. Different methods like the measurement of silicic acid depletion in the growth medium or the uptake of isotopic tracers were used to elucidate this mechanism (Paasche 1973; Azam 1974; Sullivan 1976; Bhattacharyya and Volcani 1980; Amo and Brzezinski 1999; Martin-Jézéquel et al. 2000; Milligan et al. 2004; Thamatrakoln et al. 2006). Most studies describe a saturable silicic acid uptake kinetics, indicating the presence of a specific carrier. The measured silicic acid uptake rates follow a Michaelis-Menten kinetics with K_M values between 0.2 and 7.7 μM and V_{max} values between 1.2 and 950 fmol of Si cell^{-1} h^{-1} (Lewin 1954, 1955; Sullivan 1976, 1977; Bhattacharyya and Volcani 1980; Martin-Jézéquel et al. 2000; Tréguer and Pondaven 2000). The presence of a transport protein was assumed, because silicic acid transport is sensitive to protein synthesis inhibitors (Sullivan 1976). Furthermore, the observed inhibition by germanic acid suggests a specific silicic-/germanic-acid-binding site in this tentative protein (Azam 1974).

On the other hand, transmembrane silicic acid transport without the need for transporter proteins must also be considered as a realistic possibility. One suggested mechanism is the direct, diffusive transport of silicic acid through the cell membrane (see, e.g., Raven 1983). An alternative suggestion is pinocytosis, that is, endocytosis of silicic acid from the environment (Vrieling et al. 2007). However, the latter hypothesis so far lacks any experimental evidence. Moreover, silicic acid uptake by pinocytosis would be accompanied by the passage of enormous amounts of water through the cell and requires unrealistically large amounts of cellular membrane material (Thamatrakoln and Kustka 2009). As a possible solution for these problems, Annenkov et al. (2020) suggested pinocytosis of larger silica oligomers/aggregates, which are previously formed at the diatom surface, that is, outside the cell.

Three different silicic acid uptake modes were suggested: (1) surge uptake, (2) externally controlled uptake, and (3) internally controlled uptake (Conway et al. 1976, 1977; Thamatrakoln and Hildebrand 2005). Surge uptake occurs when diatoms experience silicic acid replenishment in the environment after periods of starvation. The intracellular silicon storage pools are then presumably depleted and maximum uptake rates occur due to a strong silicon concentration gradient between environment and cell interior. Note that the chemical nature of the species present in SSPs is not fully understood to date (see Sect. 3). Therefore, all possible intracellular species, namely soluble silica, larger oligomers, or even silica nanoparticles, are referred to as "intracellular silicon" here. When the extracellular concentration of silicic acid is low, silicic acid uptake is controlled by the external substrate concentration (externally controlled uptake). In contrast, internally controlled uptake occurs when the intracellular silicon demand—defined as silica deposition rate in the cell wall—regulates the uptake. Such relations between silicic acid uptake and incorporation were found in most studied diatom species (Chisholm et al. 1978). Interestingly, even an efflux of silicon can be observed under certain conditions (Sullivan 1976). Diatom cells nearly meet the mass balance condition: silicon influx = incorporation + efflux + dissolution. (Milligan et al. 2004). Silicon efflux

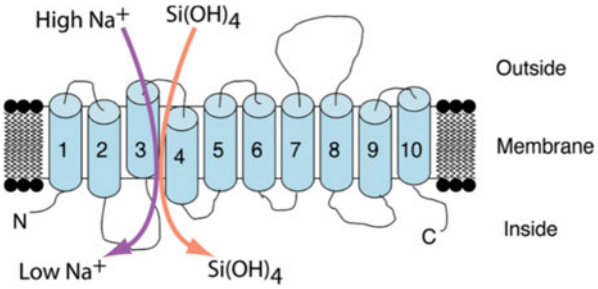


Fig. 2 Schematic illustration of the protein family of SITs with its ten transmembrane domains. SITs are silicic acid/sodium symporters and thus sodium must be present for silicic acid transport (Curnow et al. 2012). Reprinted with permission from Curnow et al. 2012. Copyright (2012) American Chemical Society

was only observed in the presence of extracellular silicon. It increases at high external silicon concentrations. In the absence of external silicon, efflux is inhibited (Sullivan 1976). One explanation is the tendency to keep and bind all available silicon inside the cell in the absence of extracellular silicic acid. In presence of high external silicic acid concentrations, maintenance of the favorable intracellular silicon concentration may even require the efflux of redundant intracellular silicon (Hildebrand 2004). The discovery of a putative silicon efflux protein emphasizes the importance of silicon efflux for diatom cells (Shrestha et al. 2012).

The first proteins directly interacting with silicic acid were found in the 1990s in the diatom *Cylindrotheca fusiformis* (Hildebrand et al. 1997). Five types of silicic acid transporters (SITs) could be identified in *C. fusiformis* based on their cDNA sequences. These integral membrane proteins transport silicic acid from outside across the cell membrane into the cell. SITs occur as multiple gene copies with a highly conserved transmembrane domain. Protein sequence analyses provide evidence for multipass membrane proteins. Twelve transmembrane α-helix segments were initially predicted. This number was later revised to only ten (Hildebrand et al. 1998; Hildebrand 2004). A conserved sequence motif, GXQ with X = Q, G, R, or M, is located in four different regions of the protein, and was proposed to be the coordination site for silicic acid. Site specific mutagenesis of the GXQ motifs supported their importance for silicic acid binding and SIT function (Knight et al. 2016). SITs were identified in all diatom species investigated to date (see, e.g., Thamatrakoln and Hildebrand 2005; Thamatrakoln et al. 2006; Durkin et al. 2016).

In marine diatoms, sodium must be present for silicic acid transport (Bhattacharyya and Volcani 1980; Amo and Brzezinski 1999). It was shown that SITs are 1:1 Na^+:$Si(OH)_4$-symporters (Hildebrand et al. 1997; Curnow et al. 2012) (Fig. 2). Subsequent studies on the uptake kinetics of SITs from *Thalassiosira pseudonana* revealed a change from nonsaturable to saturable silicic acid uptake over time (Thamatrakoln and Hildebrand 2008). For diatom cells after 24 h incubation in a silicic-acid-free medium, saturable uptake kinetics was observed (Fig. 3). In contrast, silicic acid uptake was nonsaturable under silicic acid repletion conditions

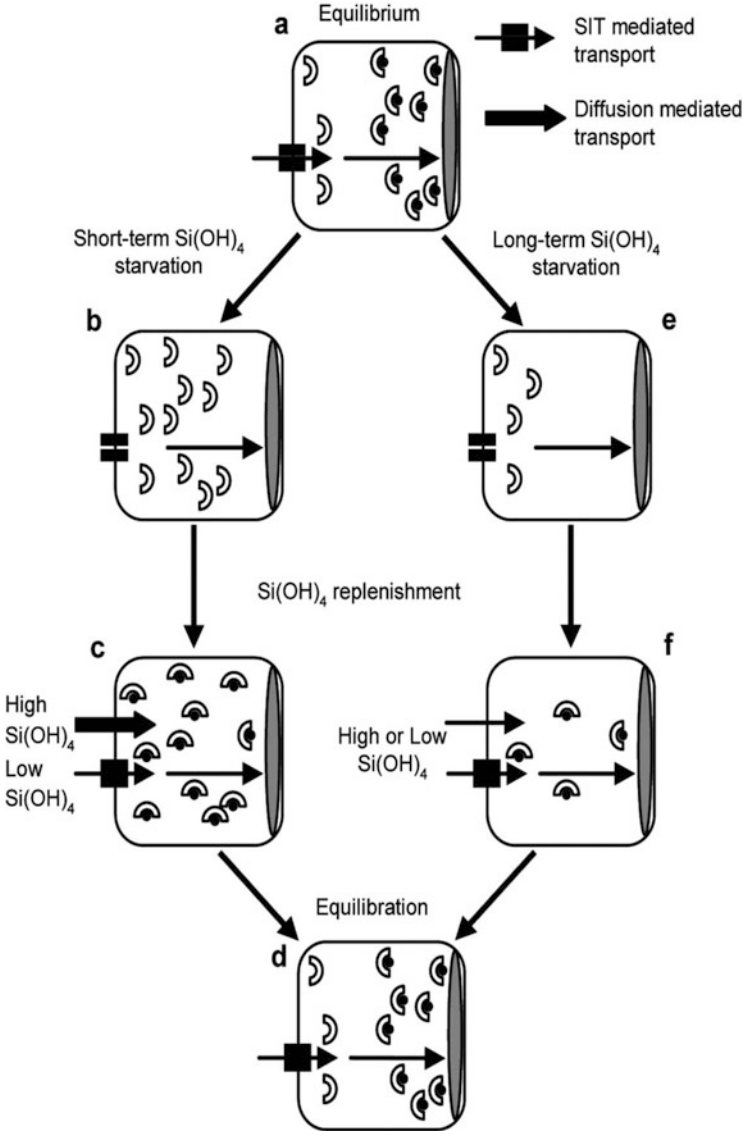


Fig. 3 Model for silicic acid uptake by diatoms. The rounded boxes represent diatom cells. Horseshoe-shaped structures represent intracellular silicon-binding components, and black dots represent $Si(OH)_4$. Black squares represent the SITs. The direction of transport is indicated by arrows, their thickness symbolizes the amount of transported $Si(OH)_4$. SIT-mediated transport is symbolized by arrows, which cross the black squares. (a) During exponential growth, equilibrium between uptake and cell wall incorporation occurs. Most silicon-binding components are loaded with $Si(OH)_4$. The uptake is internally controlled. (b) Under short-term $Si(OH)_4$ starvation, most Si $(OH)_4$ can be found in the SDVs and is used for cell wall incorporation. (c) Under $Si(OH)_4$ replenishment, uptake kinetics depends on the $Si(OH)_4$ concentrations. For concentrations <30 μM saturable SIT-mediated uptake occurs, otherwise nonsaturable diffusional uptake occurs. (d) Over time, equilibration is regained and uptake becomes internally controlled. (e) Under long-term $Si(OH)_4$ starvation, intracellular binding components are reduced. (f) Under $Si(OH)_4$

at the beginning and changed to saturable transport over time. Nonsaturable transport seems to occur when the intracellular silicic acid uptake capacity is high and surge uptake takes place. The mechanism changes to saturable kinetics when the silicon pool is in equilibrium with the silicon demand for cell wall biosynthesis.

Transport by SITs in *T. pseudonana* occurs mainly at low silicic acid concentrations (<30 μM) following sigmoidal kinetics (Thamatrakoln and Hildebrand 2007). Competitive uptake of $Si(OH)_4$ and $Ge(OH)_4$ at low concentrations further supports the idea of an active transport by SITs (Thamatrakoln and Hildebrand 2008). At silicic acid concentrations exceeding 30 μM, competition experiments and the measured nonsaturable transport kinetics revealed that uptake takes place via diffusion and is not transporter-mediated. At these high concentrations, the major role of SITs is probably silicic acid sensing and regulation of cell wall formation. In summary, active silicic acid transport mediated by SITs is probably necessary at the low environmental silicic acid concentrations, which are typical in most diatom habitats today. Passive transmembrane diffusion seems to be the main route for silicic acid uptake at higher concentrations. As discussed above, the world's oceans recently exhibit a low average silicic acid concentration of ca. 70 μM although special habitats can exhibit enhanced concentrations. Note that much higher silicic acid concentrations were present in ancient times when diatom species evolved (Holland 1984). Diffusive transport was then probably the dominating uptake mechanism. This consideration poses the question of the evolutionary history and role of SITs (Shrestha and Hildebrand 2015). It is proposed that SITs were initially used to remove silicon from the cell and prevent toxicity, since silicic acid transport mechanisms probably evolved when the oceanic silicic acid concentrations were much higher than today (Marron et al. 2016).

Several studies deal with the regulation of silicic acid transport (Thamatrakoln and Hildebrand 2007; Sapriel et al. 2009). Protein expression levels and amounts of mRNA were investigated. Thamatrakoln and Hildebrand (2007) have shown that the uptake activity and SIT expression levels are rather uncorrelated. It is suggested that SIT transport activity is mainly regulated on the translational and posttranslational level and by intracellular processes rather than on the mRNA level (Thamatrakoln and Hildebrand 2007). However, besides the regulation of the transport proteins, equilibrium effects of the transported silicic acid also influence the transport activity (Hildebrand 2008).

Curnow et al. reported the recombinant expression, purification, and reconstitution of a silicic acid transporter from *T. pseudonana* in proteoliposomes (Curnow et al. 2012). This paved the way to subsequent in vitro studies of SITs. The three SIT isoforms SIT1, SIT2, and SIT3 from *T. pseudonana* were expressed in *Saccharomyces cerevisiae*. It was shown that silicic acid transport also takes place in

Fig. 3 (continued) replenishment, saturable uptake occurs due to low intracellular capacities (Hildebrand 2008). Reprinted with permission from Hildebrand (2008). Copyright (2008) American Chemical Society

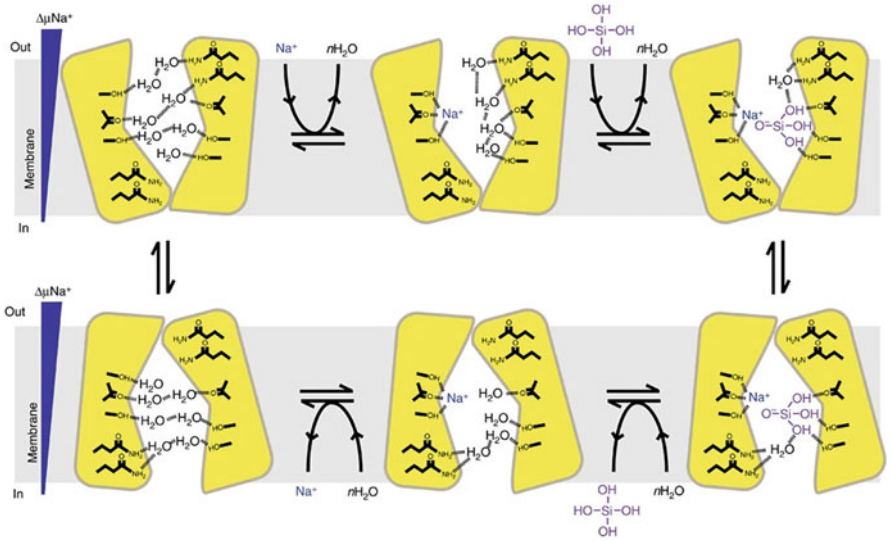


Fig. 4 Proposed model (not to scale) for the SIT transport mechanism. The binding of Na^+ and $Si(OH)_4$ as cosubstrates at 1:1 ratio is driven by an increased solvent entropy. A change in protein conformation is induced, and the binding site switches to the opposite site of the membrane. The substrate dissociates from the binding site, and the protein changes back to the original confirmation. Protein–substrate interactions could be mediated by side chain or main chain groups (Knight et al. 2016). Copyright 2016

reconstituted proteoliposomes. Furthermore, silicic acid uptake depends on the applied sodium gradient. Substrate affinity measurements of the reconstituted transporter SIT3 show similar but slightly higher values of 19.4 ± 1.3 μM compared to 8.0 ± 0.9 μM for SIT-mediated transport in cells. Further studies were performed on other recombinant SITs from different diatom species (Knight et al. 2016). PtSIT1 from *Phaeodactylum tricornutum* showed comparable transport kinetics with K_M of 19.1 ± 4.5 μM for silicic acid. The performed control experiments revealed that silicic acid diffusion was negligible at the applied low silicic acid concentrations.

As already discussed above, sodium is essential for silicic acid transport by SITs and it is necessary for the high-affinity binding of silicic acid. One suggestion for silicic acid transport by SITs is the presence of two functionally identical binding sites at both sides of the membrane. However, isothermal titration calorimetry (ITC) measurements at a $Na:Si(OH)_4$:SIT ratio of 1:1:1 indicate the presence of only one shared binding site at the center of the membrane for both, sodium and silicic acid (Knight et al. 2016). Moreover, silicic acid binding only occurs after preceding sodium binding. It is thus assumed that sodium binding is the initial first step in the transport mechanism of SITs. The proposed mechanism is schematically visualized in Fig. 4.

In addition, the evolution of the SIT gene family was studied for eukaryotes (Durkin et al. 2016; Marron et al. 2016). Sequence homologs encoding SITs were

found for various organisms including the major diatom lineages and other algal protists. The identification of a bacterial gene with homology to SIT sequences may indicate a lateral gene transfer event between bacteria and protists (Durkin et al. 2016). Five different basal clades of SIT genes were found in diatoms. The basic SIT clades are common for the different diatom lineages, whereas several derived clades are lineage-specific. Thus, a variety of different SIT types are combined in the different major diatom lineages. Variations in functional SIT protein domains give rise to the assumption of functional differences for SITs from different clades (Durkin et al. 2016).

Interestingly, even studies on coccolithophores revealed the presence of a family of diatom-like silicic acid transporters (SITLs) for some species, although this group of organisms produces calcium carbonate rather than silica. These SITLs differ in their structure compared to diatom SITs, constituting a novel group of transporter proteins (Durak et al. 2016).

3 Storage of Silicon by Diatoms

As described above, diatoms take up high amounts of monosilicic acid prior to cell division. Intracellular concentrations of "soluble silica" (see Sect. 1) up to hundreds of mM were observed for several species (Werner 1966; Chisholm et al. 1978; Blank and Sullivan 1979; Sullivan 1979; Martin-Jézéquel et al. 2000), as summarized in Table 1. These "soluble silica pools" (SSPs) strongly exceed the solubility of 2 mM and would thus be expected to result in spontaneous and uncontrolled polycondensation, that is, silica formation (Scheme 1). This is, however, not the case. Consequently, diatoms must be able to inhibit uncontrolled polycondensation processes. In other words, a supersaturated silica solution is intracellularly stabilized before cell wall synthesis. But how is that achieved by the cell?

A method to detect and quantify soluble silicon species in diatoms was developed by Sullivan (1979). Diatom cells were extracted following different protocols such as boiling in water or a mixture of H_2SO_4 + KNO_3 at 100 °C, treatment with perchloric acid at 0 °C, 50% ethanol at 20 °C, and others. Quantitative analysis was based on the ^{68}Ge radioisotopic tracer method. It allowed the indirect determination of the amount of soluble silicon species by measuring the amount of ^{68}Ge

Table 1 Concentrations of intracellular SSPs in diatoms. Data in mol/cell were taken from Martin-Jézéquel et al. (2000). The cell volume was either estimated from the typical cell dimensions assuming cylindrical cell shapes (a) or taken from the literature ((b), (Chisholm et al. 1978)). This allowed to estimate $[Si]_{SSP}$ in mM

Diatom species	$[Si]_{SSP}$/mol/cell	Typical cell volume/liter/cell	$[Si]_{SSP}$/mM
Thalassiosira pseudonana	$(0.9 \ldots 4.6) \times 10^{-15}$	$(40 \ldots 60) \times 10^{-15}$ (a)	18 … 115
Ditylum brightwellii	12.3×10^{-12}	$(6 \ldots 175) \times 10^{-12}$ (b)	70 … 2050
Coscinodiscus granii	$(1 \ldots 6) \times 10^{-12}$	$(0.55 \ldots 2.5) \times 10^{-9}$ (a)	0.4 … 11

released from ^{68}Ge-doped cells after the extraction procedure. The presence of soluble species confirmed the existence of SSPs, which are not yet incorporated into the cell walls. Instead of the ^{68}Ge radioisotopic tracer method, the photometric silicomolybdic acid assay is often used (see Sect. 1). However, special care must be taken to establish appropriate lysis and extraction procedures in any case. Such procedures should minimize the redissolution of oligomers, particles, or solid SiO_2 and prevent the unwanted generation of molybdate-reactive species that were absent in vivo. Otherwise, reported SSP concentrations may sometimes overestimate the actual SSP concentrations in intact cells.

Recently, *T. pseudonana* cells were investigated by cryo-focused ion beam scanning electron microscopy (cryo-FIB-SEM) imaging (Kumar et al. 2020). Element analysis by energy-dispersive X-ray (EDX) spectroscopy revealed that the diatom species *T. pseudonana* maintains a significant amount of silicon stored in SSPs. Typical intracellular concentrations of about 150 ± 50 mM silicon could be detected. This concentration range is in agreement with previously reported values (see Table 1). The intracellular silicon is not found as large electron-dense aggregates or compartments. Remarkably, the internal silicon concentration is maintained even under silicon starvation.

Liquid-state ^{29}Si NMR spectroscopy provides another approach for the detection of silicon species in solutions. This method is particularly well-suited and sensitive if ^{29}Si isotope enrichment is feasible. The ^{29}Si chemical shift of $Si(OSi)_n(OH)_{4-n}$ moieties, the so-called Q^n groups ($n = 0, 1, 2, 3, 4$), depends strongly on the number n. For solid silica, solid-state ^{29}Si NMR must be carried out under magic angle spinning (MAS) to reduce the line widths. ^{29}Si NMR spectroscopy thus allows to measure the concentrations of the various Q^n groups, that is, to determine the degree of silica polycondensation (cf. Fig. 5).

Different hypotheses for such intracellularly stabilized silica precursors in diatoms are found in the literature. It is, for example, suggested that the precursor species might be hypercoordinated Si stabilized by the interaction with organic molecules. This was supported by the presence of a weak and transient signal in ^{29}Si NMR studies of integer cells at the chemical shift of hypercoordinated Si species (Kinrade et al. 2002). The existence of stable four-, five-, and six-coordinated silicate complexes has been confirmed by in vitro ^{29}Si liquid-state NMR spectroscopic studies of alkaline silicate solutions containing aliphatic polyols (Kinrade et al. 1999; Vis et al. 2020). The stabilizing influence of various synthetic molecules including polyamines upon silicic acid solutions at near-neutral pH was also studied in vitro. Typically, soluble silica concentrations exceeding the solubility limit by a factor of 2 to 3 could be found over several days (Spinde et al. 2011; Preari et al. 2014; Demadis et al. 2015).

Other authors suggested Si-containing cell inclusions like silicon transport vesicles (STVs, cf. Fig. 1) (Schmid and Schulz 1979; Annenkov et al. 2013). Fluorescence microscopy investigations in combination with EDX analyses (Grachev et al. 2017) indicate the presence of Si-containing inclusions in the cytosol of *Synedra acus*. The inclusions are of yet unknown chemical composition. Such inclusions as well as the presence of STVs (see above) would be compatible with the

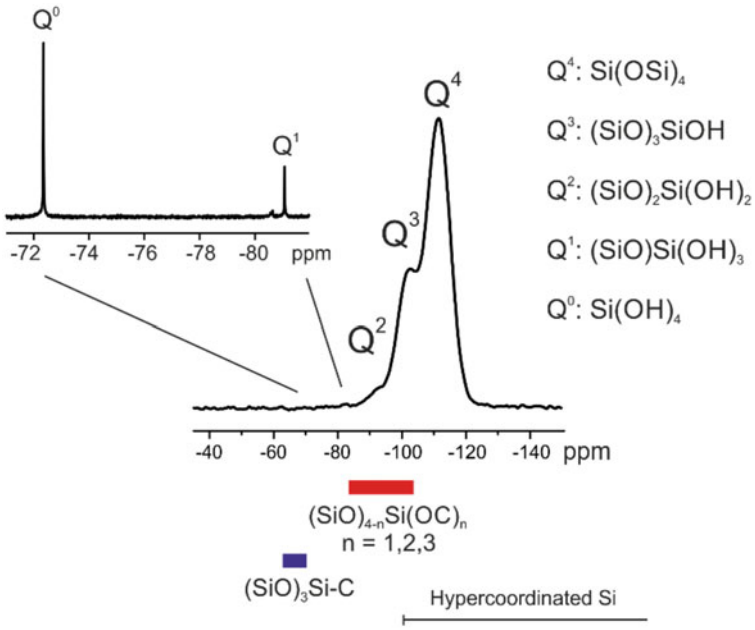


Fig. 5 ^{29}Si MAS NMR spectrum of ^{29}Si-isotope-enriched cell walls isolated from *T. pseudonana*. The insert displays a liquid-state ^{29}Si NMR spectrum with signals characteristic for Q^0 and Q^1 (mono- and disilicic acid). Chemical shift ranges characteristic for ^{29}Si involved in covalent bonds with organic material are given at the bottom for comparison. Reproduced from Brunner et al. (2009). Copyright 2009 Springer

existence of SSPs in the form of silica NPs (see below, Gröger et al. 2008). It should be noted in this context that the delivery mechanism of the tentative silica precursor compound(s) to the SDV (cf. Fig. 1) is also unknown. One hypothetical explanation is the fusion of STVs with the developing SDVs during cell wall synthesis.

To study SSPs without extraction, a ^{29}Si solid-state NMR-method for the investigation of integer, shock-frozen cells was developed and applied to *T. pseudonana* and *Ditylum brightwellii* (Gröger et al. 2008; Brunner et al. 2009). The measured intracellular concentrations of monosilicic acid are about two orders of magnitude smaller than the silica concentrations found in the SSPs (cf. Table 1 and Fig. 6). The presence of signals characteristic for Q^3 and Q^4 groups even before cell wall synthesis supports the suggestion that SSPs mainly contain silica in higher condensation states than mono- and disilicic acid (see Scheme 1), possibly stabilized silica NPs.

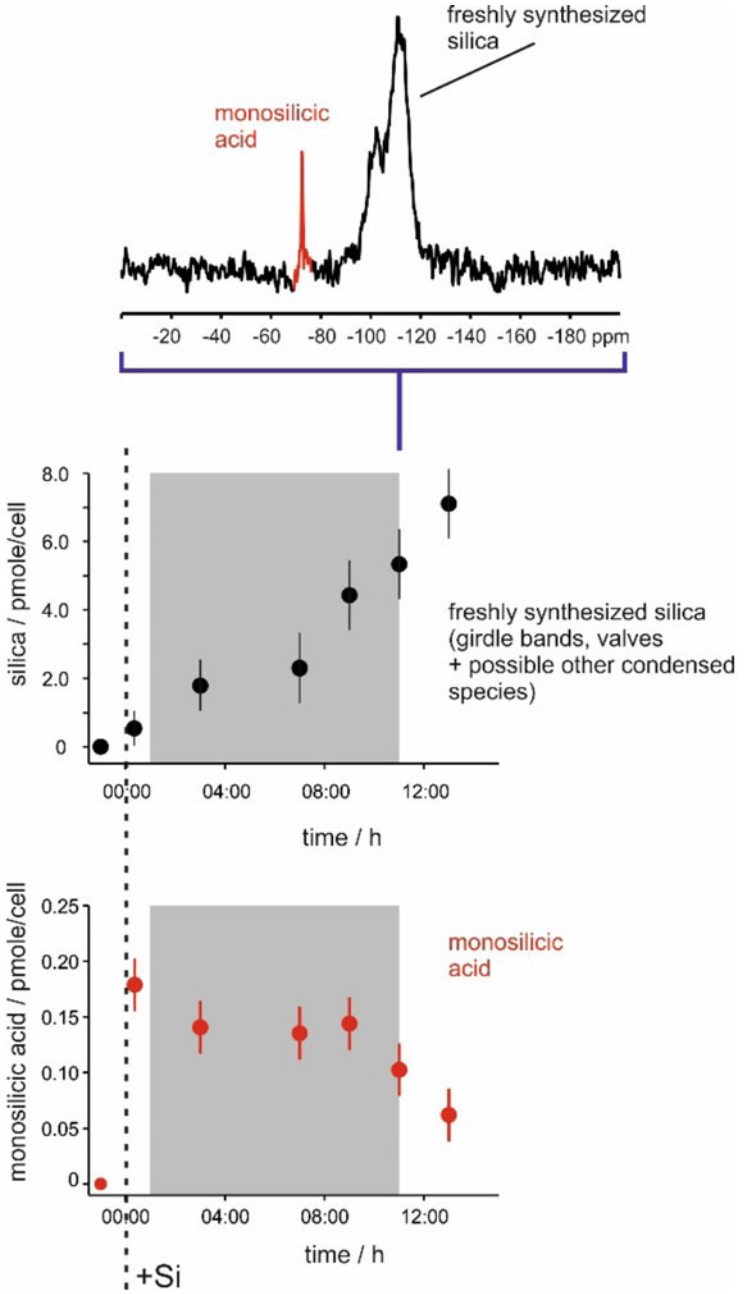


Fig. 6 Top: ^{29}Si solid-state NMR spectrum of intact, frozen *D. brightwellii* cells after silicon starvation and 11:00 h after the addition of ^{29}Si (265 K). The silica content (Q^n group signals, $n = 2$, 3, 4, middle) and the intracellular monosilicic acid content (Q^0 group signal, bottom) of *D. brightwellii* cells determined by ^{29}Si solid-state NMR spectroscopy are shown as a function of time. The vertical grey fields indicate the dark phase. Reproduced with permission from Brunner et al. (2009) Copyright 2009 Springer

4 "Foreign" Inorganic Elements in Diatom Biosilica

Various inorganic elements other than silicon can be incorporated into diatom cell walls. Examples are germanium, aluminum, iron, copper, cadmium, zinc, and others (Azam et al. 1973; Rorrer et al. 2005; Davis and Hildebrand 2008; Qin et al. 2008). Their role as well as the chemical structure of the formed biominerals are not yet completely understood. Germanic acid can be taken up by diatoms and is incorporated into the biosilica due to its silicic acid like chemical properties (Azam et al. 1973; Rorrer et al. 2005; Davis and Hildebrand 2008; Qin et al. 2008).

In the following, two selected elements abundantly present in nature will be considered in more detail, namely, iron and aluminum. The comparison of these two "foreign" elements reveals interesting differences with respect to their amount, distribution, and chemical state in biosilica.

Iron In addition to copper and zinc, iron is an essential nutrient for diatoms (Marchetti et al. 2009). Transcriptomics studies indicate a connection between silicon and iron metabolic pathways in diatoms (Mock et al. 2008; Morrissey and Bowler 2012), but the physiological role of iron for biosilification and the reasons for iron incorporation into biosilica are still unknown (Ingall et al. 2013). Previously, Ellwood and Hunter (2000) demonstrated that the amount of incorporated iron in *T. pseudonana* cell walls is limited. Subsequent studies of Fe(III) uptake (Kaden et al. 2017) confirmed this observation and revealed an iron to silicon mass ratio below 0.003:1 in the biosilica, which is almost independent of the iron concentration in the growth medium. Ingall et al. (2013) studied the iron removal from Antarctic seawater by diatoms and suggested that iron is attached to cell walls in two different states: clustered and dispersed. Combined use of Electron Paramagnetic Resonance (EPR), ^{29}Si MAS NMR, and Infrared (IR) spectroscopy revealed that more than 95% of the iron attached to *Stephanopyxis turris* biosilica forms clusters, probably Fe_2O_3 nanoparticles (Kaden et al. 2017). Only less than 5% are dispersed. That means, iron preferentially forms separate Fe_2O_3 clusters instead of replacing silicon atoms at framework positions in biosilica.

Aluminum Dissolved aluminum present in the oxidation state Al(III) can also be taken up by diatoms and is incorporated into the biosilica (Dixit et al. 2001). Aluminum concentrations below 10 nM are commonly observed in open ocean surface waters, except for heavily impacted regions with atmospheric dust inflow or in coastal regions with arriving rivers (Hydes et al. 1988; Measures and Edmond 1990; Chou and Wollast 1997). Surprisingly, diatom blooms are facilitated by elevated aluminum levels (Ren et al. 2011) although this element is toxic for numerous other organisms (Driscoll and Schecher 1990). Laboratory experiments on various diatom species in Al-spiked growth media revealed different levels of Al incorporation into biosilica (Van Bennekom et al. 1991; Van Beusekom and Weber 1995; Gehlen et al. 2002; Machill et al. 2013). So far, the highest Al content was reported for *S. turris* biosilica. It corresponds to an Si: Al ratio of 10: 1. Spectroscopic investigations (Machill et al. 2013) suggested a dispersed Al incorporation into the

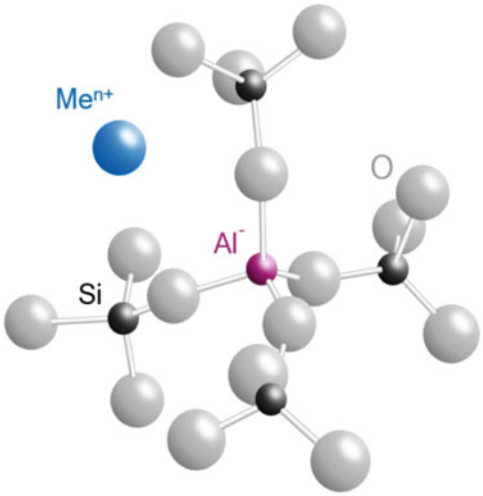


Fig. 7 Structure of aluminum in the biosilica framework. Me^{n+} denotes exchangeable counter ions like Na^+, K^+, or Ca^{2+} (Gehlen et al. 2002; Köhler et al. 2017)

SiO_2, that is, the formation of aluminosilicate structures as shown in Fig. 7, which is in contrast to the above-reported behavior of iron. The negative charge introduced by Al incorporation into the SiO_2 framework is compensated by positively charged metal ions (e.g., Na^+, K^+, Ca^{2+}), which can be easily exchanged (Gehlen et al. 2002; Köhler et al. 2017). Recent investigations on *Pinnularia sp.* demonstrated the tendency to form cell walls with increased inorganic content, resulting in cell walls of increased thickness and decreased pore diameters (Soleimani et al. 2020). Given the fact that aluminosilicates are usually less soluble than silica, it is tempting to speculate that the incorporation of the "foreign" Al atoms into biosilica aids in protecting diatom cell walls against dissolution.

5 Conclusion

Silicic acid uptake and storage inside diatom cells are crucial initial steps for biosilica synthesis in the SDV. An active transport of silicic acid is suggested especially at low silicic acid concentrations in the environment. Alternative possible transport mechanisms like direct diffusion or pinocytosis are also discussed in literature. Active silicic acid uptake via SITs was investigated in detail over the past decades. These transmembrane proteins are silicic acid/sodium symporters with 10 transmembrane segments and exhibit the highly conserved GXQ sequence motif. This motif is predicted to be important for silicic acid uptake. The regulation of SIT activity occurs probably at the translational or posttranslational level (Hildebrand et al. 1997; Thamatrakoln and Hildebrand 2008; Sapriel et al. 2009; Curnow et al. 2012; Knight et al. 2016). SITs were found in all different diatom line clades (Durkin et al. 2016). However, their role and function are not fully clarified to date. Are they working as transporters or is silicic acid sensing their main function? Considering that the environmental silicic acid concentrations were probably close to saturation

when SITs evolved, their initial function was possibly the prevention of high and toxic silicic acid concentrations in the cell interior or even the removal of redundant silicic acid (Marron et al. 2016). SIT-related genes are also present in nonsilicifying organisms (Durak et al. 2016). This raises the question of their function in nonsilicifying organisms. Increasingly powerful genetic tools may in the future deepen our understanding of the silicic acid uptake and especially the role of involved proteins.

The presence of SSPs in diatom cells, which significantly exceed the solubility limit, was demonstrated by various investigations. Consequently, it must be assumed that the cells are able to stabilize either silicic acid or silica to prevent it from uncontrolled polycondensation (cf. Scheme 1). It could already be shown that the species making up the SSPs cannot solely consist of stabilized silicic acid. ^{29}Si NMR investigations rather point toward the existence of stabilized silica nanoparticles. However, fundamental questions remain to be answered in the future. Especially the chemical nature of SSPs is one of the central questions in this context. Are special biomolecules responsible for the intracellular stabilization of silicic acid concentrations far beyond saturation? Where are SSPs located within the cells? The answer to these questions would, on the one hand, be of great interest from the point of view of fundamental research. On the other hand, it would also open new horizons for environment-friendly and energy-efficient, "green" silica materials synthesis approaches at near-neutral pH and ambient temperature.

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