# Milestones in the Biochemistry of Silicon: From Basic Research to Biotechnological Applications

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*Dedicated to Professor Klaus Rühlmann on the occasion of his 70th birthday*

About two decades ago the first comprehensive books dealing with biological aspects of silicon chemistry were published.[1–4] Since that time it has been generally accepted that silicon is an essential element for many biological systems, being required for the production of structural materials and/or for metabolic processes. However, unlike the well established biochemistry and pharmacology of a great number of unnatural organosilicon compounds (compounds containing at least one Si–C bond),[5, 6] little is known at the molecular level about how cells transport, process, and use silicon. For many other essential elements specific binding sites (for example in enzymes), through which they exert their biological effects, are well established,[7] but this is not yet the case for silicon. This, together with the lack of evidence for biologically generated Si–C bonds and the kinetic instability of Si–OC bonds under physiological conditions has prompted the development of alternative ideas for the mode of action of silicon. These (very controversial) concepts include the proposal that the essentiality of silicon may not be a result of the direct action of silicon (in whichever chemical form) on biological functions, but rather may be related to its ability to limit the bioavailability of other elements, such as aluminum, calcium, and iron.[8, 9] Bearing this dilemma in mind, the results of some very recent studies in marine silicon biochemistry make one sit up: biochemists and molecular biologists working with marine organisms that produce large quantities of silicified structures (biosilicification) are now starting to identify the proteins, genes, and molecular mechanisms that control the formation of these structures. These investigations, which are the main subject of this article, can be regarded as milestones in the development of silicon biochemistry.

Dissolved silicon in seawater occurs mostly as the undissociated orthosilicic acid, $Si(OH)_4$, whose total content in the world's oceans amounts to about $10^{17}$ mol (ca. 9.6 teratonnes (Tt); $T \mathrel{\hat{=}} 10^{12}$), and whose concentration averages about 70 $\mu M$.[10] A variety of marine organisms, such as diatoms, silicoflagellates, radiolarians, and sponges, contain silica skeletons ($SiO_2 \cdot n H_2O$) that are built up by taking up orthosilicic acid from seawater. The gross production of biogenic silica in surface waters was estimated to amount to $240 \pm 40$ Tmol of silicon per year. This means, marine biological systems process the gigantic amount of about 6.7 gigatonnes (Gt; $G \mathrel{\hat{=}} 10^9$) of silicon annually.[10] The genetically controlled biosynthesis of silica is accomplished under mild physiological conditions,[3] whereas the geochemical and industrial syntheses of silica typically require extreme conditions of temperature, pH, and/or pressure.

Diatoms constitute the largest class (more than 10000 recent species) of protists living in seawater, brackish water, and freshwater. Of the oceanic planktonic organisms for which silica is an important structural component, diatoms are the dominant source of biogenic silica in the surface layers of the world's oceans. These organisms produce shape-controlled silica structures of breathtaking beauty (Figure 1). The intriguing species-specific design and ornamentation of the cell wall's silica is the basis for diatom systematics and clearly indicates a genetic determination.

The diatom cell wall (frustulum) is made of nanostructured amorphous silica that is associated with polysaccharides and proteins.[3] The frustulum consists of two parts, the epitheca and hypotheca, each being composed of a valve and several silica strips (girdle bands). It is the girdle band region in which epitheca and hypotheca overlap. New cell walls are produced in a specialized vesicle (*s*ilica *d*eposition *v*esicle, SDV): soluble silicon is taken up from the environment and concentrated in the SDV, and here the insoluble silica is formed and subsequently secreted. Recently, the first data were reported that correlate distinct silica elements with specific proteins within the diatom cell wall.[12–14] A new family of calcium-binding glycoproteins, the EDTA-extractable so-called frustulins, were identified as constituents of the cell wall of the marine diatom *Cylindrotheca fusiformis* (EDTA = ethylenediamine tetraacetate).[12, 13] In addition, there is another family of glycoproteins in the cell wall that are resistant to EDTA treatment and therefore appear to be more tightly associated with the silica scaffold.[14] These proteins can be isolated after complete dissolution of the cell wall with

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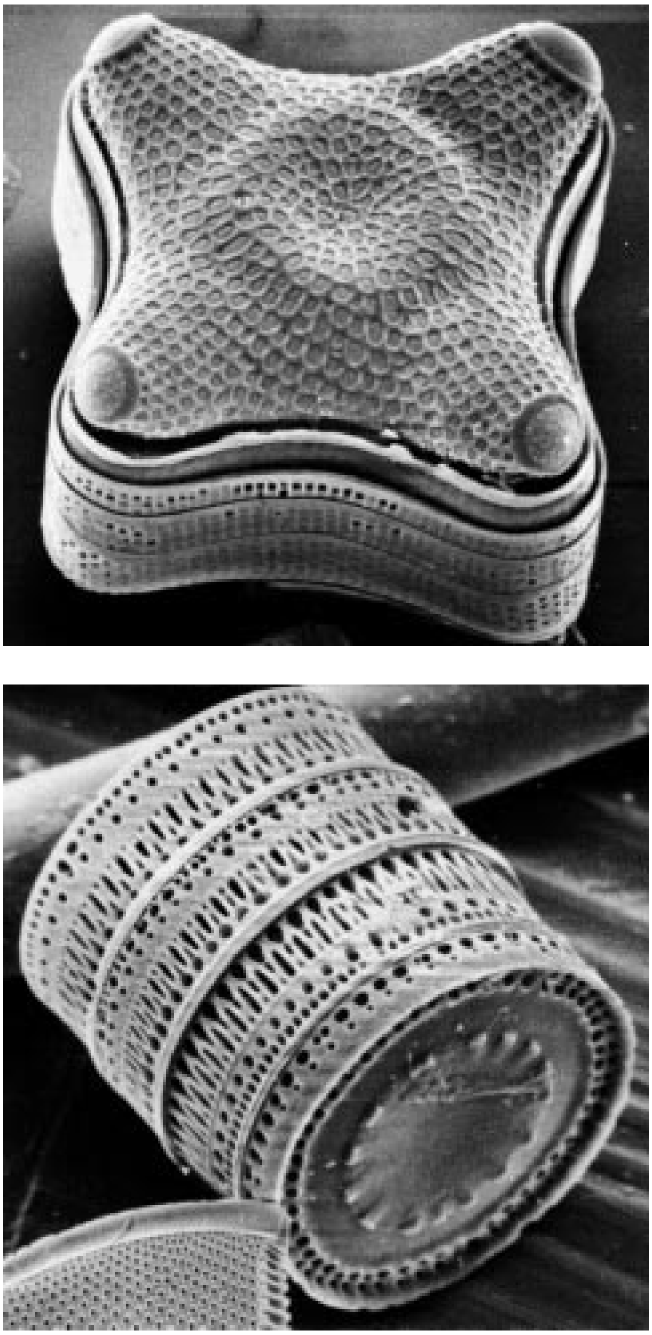

Figure 1. Scanning electron micrographs showing cells of the diatoms *Amphitetras antediluviana* (single cell; above) and *Paralia sulcata* (linked cells, fossil; below). The SEMs are reproduced with permission.[11] ©1990 Cambridge University Press.

anhydrous hydrogen fluoride and are therefore named *H*F-*e*xtractable *p*roteins (HEPs). Extensive studies with one of these proteins, the 200 kDa protein HEP200, have demonstrated that it is specifically located within the girdle band region of the cell wall. As shown by immunolocalization experiments with antibodies against frustulins and HEP200, the frustulins constitute the outer coat of the cell wall and exhibit a ubiquitous distribution, whereas HEP200 is located at a subset of about six out of about 30 silica strips that constitute the girdle band region of the cell wall.[14] These results support the idea that the species-specific architecture of the silica of the diatoms cell walls could be a consequence of differences in the structure of species-specific proteins associated with the silica scaffold.

Diatoms were also investigated for the mechanism of silicon transport that is an integral part of the silicification process.[15, 16] As the environmental concentrations of dissolved silicon are rather low (see above), diatoms must have an efficient transport system. Silicon (as orthosilicic acid or silicate) must not only be transported into the cell, but also transported intracellularly into the SDV where silica formation occurs. The cells maintain pools of dissolved silicon (in whichever chemical form) in relatively high silicon concentrations. Silicon is taken up only during a specific time in the cell cycle (just prior to cell-wall synthesis), and the kinetic parameters for silicon transport were found to vary during the uptake period. Very recently a protein of the diatom *Cylindrotheca fusiformis* was characterized that transports silicon from solution (seawater) into the cell.[15, 16] This discovery was accomplished by cloning and characterizing the DNA that codes for this protein. To test directly whether the isolated DNA encodes a silicon transporter, messenger RNA (obtained from the cloned DNA in vitro) was injected into *Xenopus laevis* oocytes, which were then investigated for the uptake of silicon (germanium) by using $^{68}$Ge as a silicon-analogous tracer.[15] Indeed, synthesis of the transporter protein and a significant silicon (germanium) uptake was observed with the RNA-injected frog cells, with the uptake being sodium-dependent. However, silicon transporter proteins of this particular type are not necessarily involved in intracellular transport.

A further major breakthrough in silicon biochemistry came from recent studies with the common marine sponge *Tethya aurantia*.[17–21] Siliceous sponges deposit silica in needlelike spicules that support the organism and provide defense against predation. About 75 % of the dry weight of *Tethya aurantia* is comprised of silica spicules (length 1–2 mm; diameter 30 μm), each containing an axial central filament of protein (length 1–2 mm; diameter 1–2 μm) that is fully occluded within the silica.[17, 18] After dissolution of the silica with buffered hydrofluoric acid, these filaments can be isolated, purified, and resolved into three very similar silicon-free subunits, named silicatein (*silica* prot*ein*) $\alpha$, $\beta$, and $\gamma$.[17] Analyses of the amino acids demonstrated that the compositions of silicatein $\alpha$ (29 kDa), $\beta$ (28 kDa), and $\gamma$ (27 kDa) are highly similar, the relative proportions in the filaments being $\alpha$:$\beta$:$\gamma$ = 12:6:1. Small-angle X-ray diffraction studies revealed evidence for a regular, repeating structure within the filaments (periodicity 17.245 nm), as would be predicted if a simple repeating subunit structure underlies the macroscopic filaments.[17] Analysis of the DNA sequence for silicatein $\alpha$ (the principal subunit that comprises about 70 % of the mass of the filaments) revealed that this protein is highly similar to members of the cathepsin L and papain family of proteases.[17] The close relationship between silicatein $\alpha$ and cathepsin L is shown by the high similarities between their amino acid sequences, three-dimensional structures, and membrane-enclosed intracellular localizations. These and further findings strongly suggest a common evolutionary origin of these proteins.[17] The fact that both proteins are found within membrane-localized vesicles that are essential for their function (silicatein $\alpha$ in the SDV; cathepsin L in the lysosome) supports this suggestion.

These findings gave rise to the fascinating idea that the silicateins might possess an enzyme-like activity and might catalyze the hydrolysis of silicic acid esters.[18] Since the homologous proteases function catalytically as hydrolases (cleavage of peptide and ester bonds under neutral conditions), related silicatein-catalyzed reactions were studied with substrates of the formula type $RSi(OEt)_3$ ($R = OEt$, Me, Ph). Hydrolysis of such compounds, followed by condensation, should yield silica ($SiO_2 \cdot n\,H_2O$; $R = OEt$) or organyl-substituted silsesquioxanes ($((RSiO_{1.5})_n$; $R = Me$, Ph). The chemical (nonenzymatic) synthesis of such products typically requires acid or base catalysis, with the hydrolytic Si–OC bond cleavage being the rate-limiting step. In contrast, the intact silicatein filaments were found to accelerate the formation of silica (from $Si(OEt)_4$) and silsesquioxanes (from $RSi(OEt)_3$; $R = Me$, Ph) in vitro at neutral pH.[18] In addition, the resolved silicatein subunits ($\alpha$, $\beta$, $\gamma$) and the purified and reconstituted silicatein $\alpha$ subunit (produced in bacteria from a cloned DNA template) were also found to accelerate the formation of silica from $Si(OEt)_4$ under neutral conditions.[18] Little or no product formation was observed in the absence of the silicatein filaments and subunits. Furthermore, the activity of the silicatein filaments and subunits was abolished by thermal denaturation and the related proteolytic enzymes papain and trypsin had no effect. This result clearly indicates the catalytic function of the silicateins (in their native three-dimensional structure) in accelerating the formation of silica and silsesquioxanes.[18]

In addition to their catalytic activity, the macroscopic silicatein filaments also show a structure-directing activity: the macroscopic filaments were found to serve as scaffolds to organize the deposition of the resulting silica and silsesquioxane products; that is, they behave as templates that direct the growth of the product layers over the surface of the protein filaments.[18]

In summary, silicateins can catalyze the formation of silica and organyl-substituted silsequioxanes from the corresponding silicic acid esters $RSi(OEt)_3$ ($R = OEt$, Me, Ph) at neutral pH and ambient temperature, and can direct the structures of the resulting products. However, it is still an open question as to whether the catalytic activity observed in vitro is of any physiological significance for the silicification process in vivo. The biological substrate (free orthosilicic acid or organic conjugates?) for silicification in the sponge still remains to be determined.[18]

As the complete amino acid sequence and the three-dimensional structure of silicatein $\alpha$ are highly homologous to that of cathepsin L, parallels between the silicatein $\alpha$-mediated catalysis and the known mechanism of action of the protease can be assumed. Thus, it has been proposed that silicatein $\alpha$ catalyzes the hydrolysis of $Si(OEt)_4$ at neutral pH through the activity of the serine and histidine residues that occupy positions corresponding to the catalytically active, functionally related residues in the proteolytic enzymes of both the cathepsin L (cysteine–histidine) and trypsin/chymotrypsin (serine–histidine) types.[19] Direct experimental evidence for the proposed role of the specific serine-26 and histidine-165 residue came from site-directed mutagenesis, in which the cloned recombinant DNA that codes for silicatein $\alpha$ was modified in a site-specific manner in vitro, and the mutant DNAs were then used as templates to direct the synthesis of the corresponding proteins in bacteria.[19] In these studies two structural variants of the silicatein $\alpha$ protein were produced, in which the serine-26 residue and histidine-165 residue were specifically replaced by an alanine moiety. Quantitative comparison of the catalytic activities of the resulting protein products with the activity of the original protein supports the suggestion that serine-26 and histidine-165 of silicatein $\alpha$ are indeed required for the efficient catalysis of silica formation from $Si(OEt)_4$ at neutral pH.[19] From these findings a detailed mechanistic model for the silicatein $\alpha$-mediated catalysis was deduced.[19]

In addition to site-directed mutagenesis, further methods of genetic engineering (for example, combinatorial mutagenesis) may be used to identify and optimize the catalytic and structure-directing activity of the silicateins.[20, 21] Genetic engineering, in conjunction with biotechnological methods, offers the prospect of developing new, environmentally benign routes to the synthesis of organyl-substituted siloxanes.[20, 21] It has been suggested "to use the information obtained from the studies of polysiloxane synthesis with the mutationally altered proteins to design synthetic peptide-based catalysts" and "to guide the design of synthetic non-peptide-based catalysts and structure-directing scaffolds that are both less expensive and more robust than the natural and genetically engineered proteins".[21]

The exciting results described in this article clearly demonstrate that significant progress has been made in the biochemistry of silicon. It can be expected that further studies of the proteins, genes, and molecular mechanisms controlling silicon metabolism in diatoms and sponges may also help to reveal the mechanisms for the essential requirement for silicon for optimal development and growth in many plants and animals. Furthermore, these studies may initiate the development of new technological methods for the shape-controlled production of new patterned silicone-based materials.

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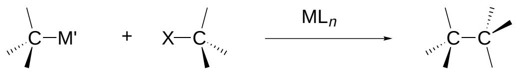
# Towards Efficient and Wide-Scope Metal-Catalyzed Alkyl–Alkyl Cross-Coupling Reactions**

## Diego J. Cárdenas*

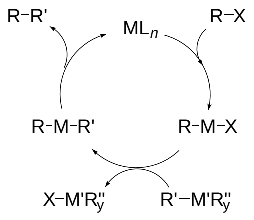
Cross-coupling between organic electrophiles and main group organometallic derivatives constitutes the most direct approach for the formation of carbon–carbon bonds. During the last two decades a great number of methods based mainly on Pd and Ni complexes have been reported.[1, 2] Many different organometallic compounds can be employed as the nucleophile, ranging from the most reactive organolithium and Grignard reagents to the more functional group tolerant tin,[3] boron,[4] and even silicon derivatives.[5] In general C(sp$^3$), C(sp$^2$), and C(sp) substituents may be coupled with suitable electrophiles. Diverse organic electrophiles such as those containing alkynyl, aryl, alkenyl, allyl, benzyl, and acyl groups as well as alkyl halides and pseudohalides have also been used as coupling partners.

Nevertheless, not all the reactions leading to the possible types of carbon–carbon bonds whose formation can be envisaged by crossing all the different nucleophiles and electrophiles have been equally developed. Whereas, for example, coupling between different kinds of C(sp$^2$) centers can be achieved with a variety of reagents and substrates, the development of catalytic methods for the formation of C(sp$^3$)–C(sp$^3$) bonds between alkyl derivatives (Scheme 1) has been much less successful. Cross-couplings between unactivated alkyl halides and organomagnesium compounds catalyzed by Cu salts are probably the most studied of such reactions.[6, 7] Recently it has been demonstrated that alkylcopper–zinc reagents, exhibiting a higher functional group tolerance, can be coupled with primary and benzyl halides.[8]



Scheme 1. A general metal-catalyzed alkyl–alkyl cross-coupling reaction. M' = Li, Mg, Zn, Al, Sn, B, Si, etc.; X = halide, OTf, etc.

However, a general metal-catalyzed cross-coupling reaction of unactivated alkyl halides and alkyl nucleophiles less reactive than organomagnesium compounds, and hence compatible with more functional groups, has not been developed yet. This is due to several possible drawbacks which can be rationalized on the basis of the general mechanism for metal-catalyzed cross-coupling reactions (Scheme 2). A coupling reaction usually starts with the oxidative addition of the electrophile to a coordinatively unsaturated low-valent metal complex, yielding an organometallic compound to which the second organic group is transferred from the nucleophile (transmetalation) in a second step. The resulting diorganometal complex finally gives rise to the coupling product by reductive elimination, regenerating the catalytically active species.[9]



Scheme 2. General mechanism for a metal-catalyzed cross-coupling reaction between an organic electrophile and an organometallic nucleophile.

Difficulties are present in all these steps when unactivated alkyl halides and alkyl electrophiles are employed:

1) Alkyl halides (even $CH_3I$) react slowly with Pd$^0$ complexes, in contrast with the behavior observed for allyl, benzyl, alkenyl, and aryl bromides and iodides.[10] The reaction of $CH_3I$ with phosphane–Ni$^0$ complexes is about $10^7$ faster and follows a complex pathway in which radical species may be involved.[11]

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