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From biogenic to biomimetic silica

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Abstract

Although widespread in nature, the biomineralisation processes of silica formation by living organisms are still poorly understood. Recent advances in the elucidation of biosilicification mechanisms by combining analytical, biomolecular and biomimetic approaches are reviewed, focusing on sponge spicule and diatoms frustule biosynthesis. ***To cite this article: T. Coradin et al., C.R. Palevol 3 (2004)***.
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Résumé

Silice biogénique et silice chimique. Bien que la silice soit très répandue dans la nature, les processus de sa formation par les organismes vivants sont encore mal connus. Les contributions les plus récentes à l'étude de ces mécanismes, qui associent approches analytiques, biomoléculaires et biomimétiques, sont présentées, en s'appuyant sur l'exemple de la biosynthèse des spicules de spongiaires et des frustules de diatomées. ***Pour citer cet article : T. Coradin et al., C.R. Palevol 3 (2004)***.
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1. Introduction

La présence de silice SiO_2 est avérée dans de nombreux organismes : bactéries, algues, plantes, insectes, et, à l'état de traces, chez les mammifères [48]. Tout comme pour les biominéraux les plus répandus (apatites, carbonates de calcium), les processus de biosilicification reposent sur le contrôle de la cinétique de déposition et de la morphologie du minéral par des macromolécules [34]. Cependant, les mécanismes physico-chimiques mis en jeu sont très différents et plus complexes que pour les sels de calcium [21]. De plus, la nature amorphe de la silice biogénique rend les

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techniques usuelles de caractérisation des biominéraux peu adaptées à son étude. Ces remarques expliquent que les mécanismes de la biosilicification sont encore mal connus.

En nous appuyant sur l'exemple de deux organismes, les éponges et les diatomées, nous souhaitons démontrer, dans la suite de cet article, que la combinaison d'approches biologiques, chimiques et analytiques permet de mieux comprendre comment les systèmes naturels utilisent et contrôlent la chimie de la silice afin d'élaborer des matériaux adaptés à leurs besoins [10].

2. Mécanismes cellulaires de la biosilicification

Les éponges marines s'ancrent sur les fonds océaniques à l'aide de spicules, qui peuvent atteindre plusieurs mètres de long. Certaines de ces spicules sont constituées de silice, déposée en couches concentriques autour d'un filament organique [32] (Fig. 1). Lors de la formation de ces spicules, le filament organique est entouré de cellules, appelées sclérocytes, dont les membranes délimitent un compartiment pour la condensation des précurseurs de silice captés dans le milieu extérieur [51].

Les diatomées sont des algues unicellulaires photosynthétiques qui s'entourent d'une coque de silice, appelée frustule, constituée de deux valves dont la morphologie et la porosité sont parfaitement contrôlées, de l'échelle nanométrique aux dimensions micrométriques [45]. Lors de la division cellulaire, les deux cellules filles issues de la mitose doivent élaborer chacune une nouvelle valve afin de compléter leur frustule. Les précurseurs de silice, captés dans le milieu extérieur et/ou extraits d'une réserve intracellulaire, sont transportés, accumulés et condensés au sein d'une vésicule spécialisée, appelée VDS (vésicule de déposition de la silice), qui s'élargit à mesure que la valve se forme [43].

3. Approches analytiques

L'étude de la déposition de la silice par les microorganismes a longtemps été limitée à l'observation ultrastructurale par microscopie optique ou électronique [42,44]. Plus récemment, les spectroscopies utilisant le rayonnement synchrotron (SAXS, EXAFS, XANES) [17,53], la microscopie à force atomique [1,15,19] et la résonance magnétique à l'état solide du ^{29}Si [2,18,39] (Fig. 2) ont permis d'explorer plus avant les silices biogéniques, de leur structure à l'échelle atomique aux propriétés mécaniques des frustules. Des approches de chimie quantique ont aussi été utilisées pour modéliser les interactions protéines/silice [33,46].

4. Approches biomoléculaires

Une approche usuelle pour identifier les macromolécules intervenant dans les processus de biominéralisation consiste à les extraire des organismes par dissolution de la composante minérale, les purifier et les analyser (électrophorèse, séquençage...), puis d'étudier leur effet sur la formation in vitro des minéraux considérés. La description du génome complet de certains organismes est désormais accessible et des approches génomiques sont envisageables.

Dans ce cadre, le traitement par l'acide fluorhydrique de spicules de l'éponge *Tethya auranthia* a permis d'isoler le filament organique central. Il est constitué des trois protéines, appelées silicatéines α, β et γ. La silicatéine α, majoritaire, présente une séquence et une structure tertiaire très proche d'une enzyme protéolytique, la cathepsine L. En fait, ces deux protéines ne diffèrent que par un acide aminé situé au niveau du site actif de l'enzyme [47]. Il a été montré que cette modification permettait à la silicatéine α de catalyser l'hydrolyse d'un alcoxyde de silicium $Si(OR)_4$, et donc d'activer la formation de la silice, à partir de ces précurseurs. De fait, la mise en contact du filament organique avec ces précurseurs conduit à un dépôt immédiat de silice sur sa surface [5].

Dans le cas des diatomées, plusieurs protéines ont pu être isolées, selon le mode d'extraction utilisée [41]. Un traitement à l'EDTA a permis l'identification de glycoprotéines, nommées frustulines, qui semblent interagir faiblement avec la surface de la frustule via des ions calcium [24,25,36]. Un premier traitement avec HF a conduit à l'extraction de protéines appelées pleuralines, riches en proline et présentant une répétition de domaines PSCD [23,26,50]. Ni les frustulines, ni les pleuralines ne semblent activer la formation de la silice. Elles pourraient cependant jouer un rôle dans le contrôle de la morphologie de la frustule.

Des traitements supplémentaires avec HF ont permis d'isoler trois nouvelles protéines, appelées silaffines [27]. Deux d'entre elles, les silaffines 1-A_1 et 1-A_2 sont enrichies en lysine et sérine [29]. Ces groupe-

ments lysine sont modifiés par des polyamines à longue chaîne (Fig. 3). Une fraction supplémentaire contenant des polyamines méthylées a aussi pu être isolée [28]. En présence d'alcoxydes de silicium préhydrolysés, ces différentes molécules conduisent à la formation de nanoparticules de silice, la vitesse du processus et la taille des particules dépendant de la fraction utilisée. Plus récemment, un traitement HF dans des conditions plus douces a montré que les groupements hydroxyles des silaffines sont tous phosphorylés, montrant la possible dégradation des molécules présentes dans les organismes par le procédé d'extraction [30]. Ces silaffines phosphorylées présentent des propriétés d'auto-assemblage qui pourraient être mises en jeu dans la morphogenèse de la frustule [49].

Des informations supplémentaires sur les gènes et les protéines gouvernant la biosynthèse de la silice pourront bientôt être obtenues grâce au séquençage, récemment achevé, du génome de *Thalassiosira pseudonana* et de celui, en cours, de *Phaeodactylum tricornutum* [36].

5. Approches biomimétiques

Même si elle constitue l'approche la plus directe pour étudier les processus de biosilicification, l'étude des interactions entre les molécules extraites des organismes et les précurseurs de silice est rendue difficile par leur diversité et leur complexité. Une approche biomimétique mettant en jeu des biomolécules plus simples et mieux connues peut permettre d'identifier, dans un premier temps, la nature des interactions mises en jeu ainsi que les propriétés des protéines requises pour condenser et organiser la silice.

Parmi les différents précurseurs de silice possibles, les silicates de sodium, constitués d'acides siliciques monomères $(Si(O_n(OH)_{4-n})^{4-n}$ et d'oligomères $(Si_xO_y(OH)_z)^n$ paraissent les mieux adaptés, puisqu'ils correspondent aux précurseurs naturels présents dans les milieux marins. Cependant, leur réactivité dépend fortement de la concentration, du pH et de la salinité du milieu réactionnel, rendant leur étude parfois difficile [21].

Dans un premier temps, des acides aminés et polyacides aminés ont été mis en contact avec des solutions diluées de silicate de sodium [8,35]. Alors que les acides aminés semblent avoir peu d'effet, deux peptides, la polylysine et la polyarginine, favorisent la condensation de la silice. Ces études, menées en fonction du pH et de la longueur de la chaîne polymère, permettent de proposer un modèle d'activation mettant en jeu l'adsorption des silicates négatifs sur les groupements ammonium positifs des peptides, ces silicates servant de sites de nucléation pour la formation du gel de silice [12]. Ces résultats renforcent la validité du modèle choisi, puisque la lysine est présente en quantité importante dans les protéines extraites des diatomées.

L'utilisation de deux protéines, le lysozyme et l'albumine de sérum bovin (ASB) contenant toutes deux une quantité importante de groupements lysine et arginine, a, par la suite, permis de révéler l'importance des propriétés d'auto-assemblage des biomolécules sur le contrôle de la morphologie de la silice [14]. En effet, le lysozyme, petit peptide (MM = 16 kDa) chargé positivement à pH 7, se comporte de façon comparable aux polylysine et polyarginine à ce pH. Au contraire, l'ASB, de taille plus importante (MM = 69 kDa), n'acquiert une charge positive que vers pH 5, valeur à partir de laquelle la chaîne peptidique peut se déployer et former des gels en présence d'agents dénaturants ou de polyanions. De fait, l'ajout de silicates à ce pH conduit à la formation d'un gel composite constitué de nanoparticules de silice de taille contrôlée (50–100 nm), piégées dans une matrice protéique d'ASB (Fig. 4). D'autres travaux mettant en jeu des tensioactifs dérivés de l'arginine [13], ainsi que des copolypeptides à blocs synthétiques [6], ont permis de confirmer l'importance de l'organisation des groupements ammonium des résidus lysine et arginine sur l'activation de la condensation de la silice et le contrôle de sa morphologie.

6. Conclusion

L'étude des processus de biominéralisation est, par essence, un champ de recherche interdisciplinaire. Cela est d'autant plus vrai pour la silice, dont la physico-chimie, très différente de celle des autres biominéraux, nécessite des approches expérimentales et des techniques d'analyse spécifiques.

Outre l'élargissement de la connaissance du fonctionnement des micro-organismes, les études présentées ici peuvent avoir des implications dans l'étude des phénomènes de fossilisation [54], ainsi que dans l'élaboration de nouveaux matériaux pour la microélectronique [4], la catalyse [16], les biotechnologies [37] et les biomatériaux [11].

1. Introduction

Silicon is the second most abundant element in the Earth's crust. When associated to oxygen, it forms silica SiO_2, which can be crystalline (quartz, cristobalite...), or amorphous. In the later case, it is mainly found from biogenic origin. Living organisms that use and/or deposit silica include bacteria, radiolaria, diatoms, plants (rice husks, horsetail shoots), insects (mandibles) and, as trace element, higher animals, in which it was suggested to play a role in bone formation [48].

Similarly to common biominerals (carbonate, phosphate), biosilicification processes involve macromolecules that regulate deposition kinetics as well as mineral morphology [34]. However, while apatite or calcite formation relies on dissolution/precipitation mechanisms, silica builds up via an inorganic polymerisation process of molecular precursors. This process is complex, since both the nature of these precursors and their association strongly depend on reaction conditions such as pH, concentration or salinity [21]. Moreover, because biogenic silica is deposited as a porous, amorphous mineral, traditional X-ray analysis techniques are often not suitable for its study. Overall, if the main principles of biomineralisation elucidated for calcium salts may be relevant for silica formation by living organisms, the macromolecules that are likely to interact with silica, the nature of these interactions as well as the experimental techniques allowing their study may differ significantly.

In this paper, we focus on the two silicifying organisms, i.e. sponges and diatoms. Starting from the present knowledge of the cellular processes of silica deposition, we show that the combination of biological, chemical and analytical approaches allow a better understanding of biosilicification mechanisms, revealing Nature's skills and tricks to control silica chemistry [10].

2. Cellular processes of biosilicification

2.1. Sponge spicules

In order to resist the water streams in the ocean bed, marine sponges anchor on the seafloor thanks to a mineral spicule. Some of these spicules consist of silica rods with diameters reaching several millimetres and lengths up to 3 m. They are made of concentric layers of silica deposited on an axial organic filament [32] (Fig. 1).

At the first stage of spicule formation, the axial filament is surrounded by specialized cells, the sclerocytes, whose membranes delimit a silica-deposition compartment. Silicic acid, the naturally occurring precursor of silica, is collected from the outside media and transported through the sclerocyte cytoplasm, to reach the surface of the filament, where it polymerises [51].

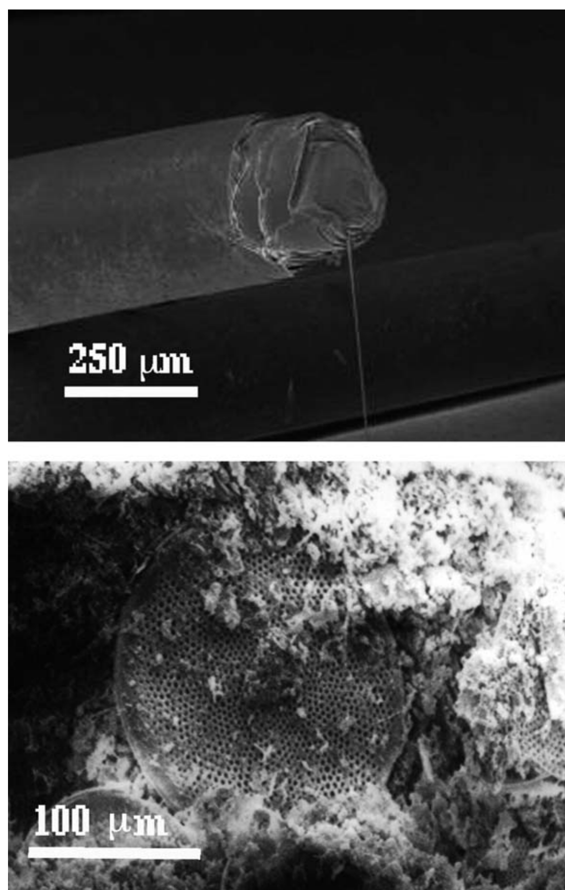

Fig. 1. Biogenic silica electron micrographs of (top) a sponge spicule showing silica concentric layers deposited around a central organic filament and (bottom) a finely dedicated silica structure of diatom frustule.

Fig. 1. Cliché de microscopie électronique (haut) d'un spicule d'éponge, montrant les couches concentriques de silice déposées autour d'un filament organique central, et (bas) de la structure délicate siliceuse d'une frustule de diatomée.

2.2. Diatoms frustule

Diatoms are photosynthetic algae that build up a silica wall named frustule. In most species, the frustule consists of two half valves joined by several girdle bands, fully casing the cell. They exhibit a finely dedicated and differentiated network of pores of nano- to micrometer size [45] (Fig. 1).

Frustule formation occurs during the cell division process [43,45]. After mitosis, two daughter cells are obtained, each of which needing a complementary half case and corresponding girdle bands to separate. At this point, extracellular silicic acid, as well as precursors from an intracellular silica pool, is transported to the non-silicified surface of each cell. Accumulation and polymerisation then occurs in a silica deposition vesicle (SDV) that enlarges as frustule growth proceeds. It was suggested that silicic acid could be transported by specific vesicles that would fuse together to form the SDV. Upon frustule completion, exocytosis of the newly formed valves occurs, allowing daughter cells separation. After girdle band formation, maturation of the silica network proceeds for a few hours.

3. Recent analytic approaches

Investigations of silica deposition by microorganisms have long been limited to ultrastructure observations using light and electron microscopy [42,44]. However, if histological staining or contrasting agents allow the specific labelling of cellular materials, characterisation of the mineral component was more difficult to achieve. Moreover, as mentioned above, in contrast to other biominerals, X-ray diffraction techniques were not suitable to study amorphous silica.

In this context, the development of synchrotron X-ray source opened up new possibilities to study biogenic silica. X-ray scattering techniques (SAXS, WAXS) allowed the characterisation of frustule-pore sizes and structure [53]. X-ray absorption spectroscopy (XANES, EXAFS) was used to investigate the association of aluminium and silica in diatom silica [17].

Atomic force microscopy (AFM) allowed the investigations of the surface of living diatoms, showing that the mineral network is formed of packed silica nanoparticles, covered by a thick mucilaginous organic layer [15]. This technique was also used to investigate the micromechanical and structural properties of a pennate diatom. It shows that the hardness and elasticity of diatom shells show similarity with that of other silica, but regions with different mechanical properties were revealed [1]. In fact, hardness of the silica frustule is not only a consequence of its composite properties, but also results from its architecture [19].

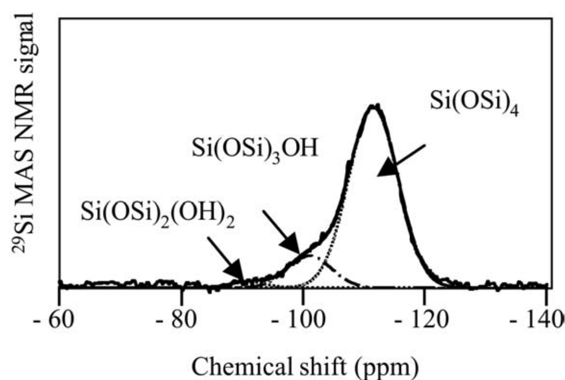

Fig. 2. ^{29}Si solid-state NMR of a mature diatom frustule indicating a partially condensed silica network.
Fig. 2. RMN du ^{29}Si à l'état solide d'une frustule de diatomée parvenue à maturité, indiquant un réseau de silice partiellement condensé.

However, none of these techniques gives access to the silica polymerisation reaction at the atomic scale. This could, in principle, be achieved using ^{29}Si solid-state nuclear magnetic resonance (NMR). Such a technique is sensitive to the environment of silicon atoms, allowing discrimination in silicate degree of condensation (i.e. $Si(OSi)_4$, $Si(OSi)_3OH$, ..., $Si(OH)_4$) (Fig. 2). At this time, it has already been applied to different diatom species, but a great discrepancy between reported data suggests that the conditions of cell culture must be fully controlled before any relevant comparison can be drawn [2,18,39].

Finally, although this does not strictly fit in this section, it is to be noted that computational models have been developed to study the possible interactions between silicic acid and amino acids/proteins [33,46].

4. Using biomolecular tools

In order to understand the biomineralisation processes, a common approach relies on (1) dissolution of the mineral deposit to extract the associated biopoly-

mers (proteins, poly-saccharides, glycoproteins...), (2) purification and characterisation of the extracted molecules, and (3) investigation of mineral formation in the presence of these putative templating agents. In some cases, it might also be possible to identify and then study the genes encoding the extracted proteins. Finally, for some organisms, the description of the full genome becomes accessible and functional genomic approaches can be envisioned [36].

The first approach has been used to study biosilicification in plants as well as in sponges and diatoms as we will show later [41]. Nevertheless, it is worth noting here that, in contrast with calcium salts that can usually be withdrawn in mild conditions (EDTA...), silica only dissolves in hydrofluoric acid HF, which can also degrade the associated biomolecules.

4.1. Sponges

Upon HF treatment of *Tethya aurantia* spicule, the initial axial filaments could be recovered. They were shown to contain three proteins, named silicatein α, β and γ. Determination of amino acid sequence and modelling of the three-dimensional structure of silicatein α revealed a very close similarity with the cathepsin L proteolytic enzymes. Among the three amino acids that compose the catalytic site (cysteine-25, histidine-163 and asparagine-187), the cysteine is replaced by a serine for the sponge α and β proteins [47]. This substitution is interesting, as the hydroxyl group borne by serine is known to interact strongly with silicates and silicon alkoxides. Indeed, silicatein α was shown to catalyse the hydrolysis of tetraethoxysilane $Si(OC_2H_5)_4$ (TEOS) and hence, to induce the polymerisation of silica. When the extracted axial filament was put into contact with TEOS, it was rapidly covered by a silica layer [5]. Moreover, when the active site of silicatein α was modified using combinatorial mutagenesis and the serine group was substitute by an alanine, the catalytic activity was lost [55]. More recently, one silicatein gene was found in *Suberites domuncula* [22] and a partial mRNA sequence was identified from *Halichondria okadai*. However, in the later case, the histidine residue of the active site was replaced by a tryptophane. It could be interesting to check for the consequence of such a substitution on the catalytic activity.

4.2. Diatoms

In the case of diatoms, several proteins could be isolated, depending on the extraction process. Using EDTA, soluble glycoproteins named frustulins were characterized, exhibiting acidic cystein-rich domains with polyproline/hydroxyproline or polyglycine spacers [24,25]. They appear to cover the frustule surface, weakly interacting with silica likely via Asp^{-} Ca^{2+} ^{-}O–Si bonds. This proteins family was initially found in two pennate diatoms species, but recent progress in genomic information revealed that they also exist in another pennate *Phaeodactylum tricornutum* [36] and in the centric *Thalassiosiria pseudonana*. A first extraction using anhydrous HF led to the identification of proteins, originally named HEP (HF-Extracted Proteins) and later on renamed as pleuralins. They exhibit high molecular weights (from 150 to 200 kDa), harbouring a proline-rich stretch followed by several repetitions of a PSCD-domain (composed by Pro 22%, Ser 11%, Cys 11%, Asp 9%) [23,26]. The hydroxy amino acids (Ser) may form hydrogen bonds with Si–OH silanol groups [50]. These proteins, associated to the girdle region, are still unique for *Cylindrotheca fusiformis*. None of these two sets of proteins, the frustulins and the pleuralins, were able to induce silica precipitation in the presence of mineral precursors. However, they were suggested to be involved in the control of frustule morphogenesis.

Further HF extraction experiments allowed the isolation of three silica-associated peptides named silaffins [27]. Two of them, the silaffin-$1A_1$ and -$1A_2$, were sequenced by mass spectrometry and were shown to be encoded by the same *sil1* gene from *C. fusiformis* [29]. These silaffin-1As are enriched in lysine and serine groups. Moreover, amino groups of the lysine residues are modified by introduction of long-chain polyamines, ϵ-*N,N*-dimethyl-lysine or ϵ-*N,N,N*-trimethyl-δ-hydroxy-lysine [29] (Fig. 3). Another fraction of methylated polyamines were also recovered and identified from a HF-extraction procedure [28]. When put in contact with HF-extracts, aqueous solutions of prehydrolysed silicon alkoxides at pH 7 precipitate and form silica nanoparticles. The reaction rate as well as the particle size depends on the extracted fraction [28,29]. Of particular interest is the fact that, at pH 5, unsubstituted polyamines did not induce silica formation, whereas alkylated ones did. This is to be linked

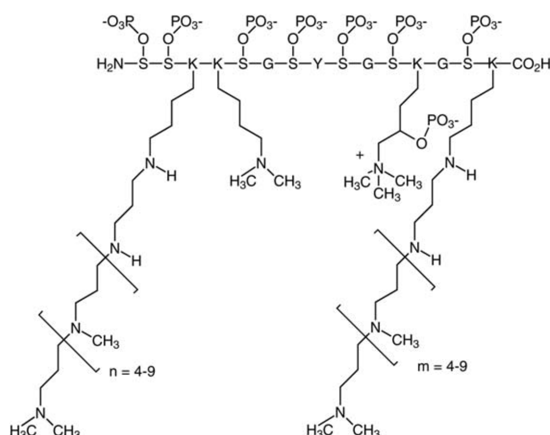

Fig. 3. Scheme of silaffin 1-A1 structure showing modified lysine groups and phosphorylated hydroxyl groups.

Fig. 3. Schéma d'une structure 1-A1 silaffine montrant les groupes lysine modifiés et les groupes hydroxyle phosphorylés.

with previous reports on a possible acid pH within the SDV [52]. More recently, HF treatment in milder conditions revealed that the hydroxy groups of the silaffin serine residues were phosphorylated, illustrating the possible degradation of pristine molecules during the initial extraction process [30]. These zwitterionic proteins exhibit self-assembly properties that may be involved in the frustule morphogenesis [49].

More information on the function and role of genes and proteins that govern silica biosynthesis in diatoms is expected to come from the availability of genomic information and the development of a range of tools used for functional genomics. Indeed, the genome sequence of the centric *Thalassiosira pseudonana* has been completed and its annotation is in progress. The pennate diatom *Phaeodactylum tricornutum* is due to be completely sequenced this year.

5. Biomimetic approaches

Studying the interactions between extracted proteins and silica precursors appears as the most straightforward pathway to understand biosilicification. Nevertheless, because of the complexity and diversity of these proteins, the need arose of biomimetic approaches involving biomolecules whose properties were already well known as well as synthetic peptides.

A first requirement for designing appropriate biomimetic studies is the choice of the silica molecular precursors. Silicon alkoxides $Si(OR)_4$ are widely used in material chemistry but they have never been identified in nature [3]. Moreover, their polymerisation process involves the release of alcohol ROH that may be detrimental to biomolecules [7]. Some silicon complexes have been identified in biosilicifying plants [40]. However, they easily decompose in water and they are difficult to obtain synthetically in large amounts. As a matter of fact, the naturally-occurring sources of silica are silicic acids $(Si(O_n(OH)_{4-n})^{4-n}$. These monomeric species only exist in highly diluted aqueous solutions and tend to condense to form silicate oligomers $(Si_xO_y(OH)_z)^{n-}$ [20]. Silicates are well soluble in water but their degree of polymerisation and acid/base properties strongly depend on concentration, pH, and salinity [21]. Therefore, even if they appear as the more suitable precursors for biomimetic studies, their somehow complex reactivity can sometimes be misleading in the interpretation of experimental data.

Amino acids and poly-amino acids interactions with sodium silicate solutions were investigated [8,35]. No important effects could be observed for different amino acids, but some peptides appear to activate silica formation. This was especially true for polylysine and polyarginine, which allowed silica precipitation in a few minutes whereas, at the same concentration, sodium silicate did not gel over a week. The efficiency of the activation process increases with the poly-amino acid chain length [12]. Since lysine and arginine have common side-chain amino functions, a model involving electrostatic interactions between the negatively charged silicate species and the positively charged ammonium groups was proposed. Once fixed on the peptide chain, silicates are then close enough for their condensation to be favoured, resulting in silica formation. Moreover, for a similar chain length, polyarginine was more efficient than polylysine at activating the condensation process. Since the main difference between the two amino acids is the number of amino groups, possible hydrogen bond formation was also mentioned. However, no activation effect could be observed in the presence of the sole polyserine, meaning that H-bonds alone do not appear responsible for silica precipitation. Accordingly, alginic acid, a polysaccharide bearing a carboxylic acid function is not able to precipitate silica, but can control its morphology [9]. The significance of these results is strengthened by the identification of active lysine residues in the silica-precipitating silaffins.

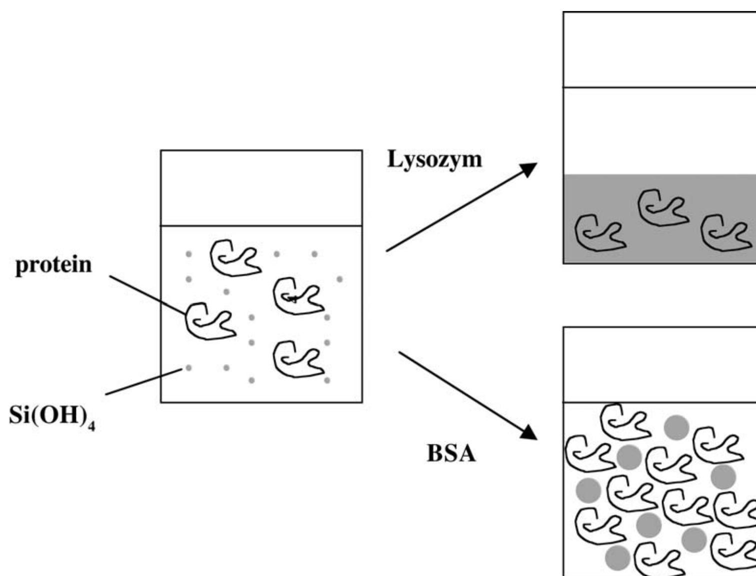

Fig. 4. Biomimetic studies of silicic acid/protein interactions.
Fig. 4. Études biomimétiques des interactions acide silicique/protéine.

An alternative approach using combinatorial phage peptide display was recently proposed [38]. Silica particles precipitated with silaffins were panned against about 10^9 random 12-amino acids peptides fused to a phage. Immunofluorescence microscopy and phage immunoassay allowed the study of peptide binding on particle surfaces. The most effective silica-binding molecules were then used to precipitate silicic acid solutions. Silica binding/precipitation properties of peptides were shown to depend on the presence of histidine and hydroxyl-containing residues as well as a global positive charge. Moreover, a positional effect with respect to residues distribution on the protein backbone was observed.

Preliminary studies involving simple but well-known proteins have also been performed [14]. The lysozyme and bovine serum albumin (BSA) were chosen because of their high content of lysine and arginine. However, lysozyme is a small protein (MW = 16 kDa) with an isoelectric point (pI) of 11, whereas BSA is a large protein (MW = 69 kDa) with a pI of 4.8. As expected, upon mixing with sodium silicate at pH 7, only lysozyme leads to silica formation. The process is less efficient than for poly-amino acids since active amino groups are dispersed along the peptide backbone. At pH 5, lysozyme induces no silica formation, whereas a gel is formed with BSA. As a matter of fact, this composite is not a silica gel containing BSA chains but consists of silica particles incorporated in a BSA gel (Fig. 4). Since this gel is not obtained in the absence of silicate, it was proposed that the inorganic species could decrease the electrostatic repulsion arising between positively charged BSA molecules. Moreover, deposited nanoparticles exhibit a limited size polydispersity in the 50–100-nm range. These studies not only confirm the importance of pH, which dictates the charge of both proteins and silicate and therefore their interaction, but also reveals the role of protein–protein self-assembly properties that can be modified by inorganic precursors. The importance of this self-assembly process was also revealed by the use of surfactants bearing arginine polar heads [13]. Whereas arginine alone does not induce silica precipitation, the self-organisation of the surfactants in a micellar system allows an organized close packing of arginine groups, leading to the formation of mesoporous silica. Accordingly, synthetic block copolypeptides with self-assembly properties were used to generate silica spheres, globules, and columns [6].

6. Conclusion

The study of biomineralisation processes is essentially a multi-disciplinary field of research. It benefits from new experimental and technical approaches and

participates in the unification of knowledge and concepts.

This is especially true for silica, exhibiting an uncommon chemistry for which specific analytical methods have been developed. For instance, ^{29}Si solid-state NMR is essential in comparing diatom frustule structure with silica formed by interaction with extracted proteins or model biomimetic peptides.

Apart from gaining further knowledge on biosilicifying micro-organisms, these studies will contribute to a better understanding of the possible role of silicon in bone formation [31], have implications for fossilization processes [54], as well as provide basis for the elaboration of new materials finding application in microelectronics [4], catalysis [16], biotechnology [37], and biomaterials [11].

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