Beyond the diversity of the species that achieve the biomineralization, the sponges take an outstanding place. The skeletal elements of sponges have the definite archaeological structure and amaze by diversity of morphology. The discrete elements of skeleton known as spicules composed of amorphous silica. This organism could be a good example for studying the biosilicification processes. From the other hand, this knowledge could be used for designing the new materials with high hierarchical structure not only on micro-, but also on nanoscale.

**Keywords:** Sponges, biogenic minerals, biosilicification.

### 1. SILICON AND WATER

Silicon dioxide (SiO$_2$), otherwise known as silica, is one of the most abundant compounds in the Earth’s crust. Large amounts of silicon can be found in various minerals (e.g. feldspar, mica and kaolinite), but in its soluble form as silicic acid it is relatively scarce in oceans and nearly all other waters and presented mostly as the undissociated monomeric silicic acid Si(OH)$_4$. Complex physical, chemical, geological and biological processes influence the presence of this compound in the water. The first way of the inputs of silicic acid to the world ocean is chemical weathering of sedimentary and crystalline rocks by CO$_2$-charged water. The second way of the delivery of Si(OH)$_4$ to the world ocean is possible due to the hydrothermal activity at mid-ocean ridges and low-temperature alterations of the basalts of the oceanic crust [1].

The production of silicic acid is also affected by river runoff. The concentration of Si(OH)$_4$ is ranged from undetectable levels to 1400 µM in eutrophic Danish lakes. The typical ambient fresh water and sea water concentrations are usually lower then 2 µM. Yet the concentration in deep waters is much higher, then in surface water. In the deep and bottom waters the concentration of silicic acid varying from 10 to 40 µM in the North Atlantic to 100 to 160 µM in the Antarctic, and 140 to 180 µM in the North Pacific [1]. The soluble form of silicon, monomeric, undissociated silicic acid, is biologically assimilable. Silification, the capability of living organisms to accumulate silica (amorphous, hydrated, polymerized silicic acid) and build their skeletal elements of this material, is wide spread in the living world. Some of marine organisms, such as diatoms, radiolarians, choanoflagellates and sponges, take part in silica turnover in the oceans. One of the most important species producing biogenic silica (BSi) is diatoms. They are dependent upon silicon for growth and development and use it for their siliceous “shells” or frustules [2]. This group of algae living by the millions in each cubic centimeter of surface ocean water and deplated the concentration of Si(OH)$_4$ in the photic zone. A significant amount of silica is dissolved and remobilized in the top few hundred meters of the water column. This process is dependent on surface area, temperature, and trace metals (for example, aluminium). In the photic zone dissolves approximately 60% of the silica produced by diatoms. The fraction of biogenic material, which escapes dissolution in the surface layer, reaches the floor where dissolution continues. This process besides others causes increasing silicic acid concentration in the ocean floor. In these environments occur sponges (Porifera), the multi-cellular sedentary filter-feeder organisms. They have complex network of water connecting channels, which make them able to process their own volume of water in approximately 5 seconds [3]. By this route silicon enter the organism of the sponge. Opaline sclerites, spicules composed of hydrated silica, comprise all or part of the skeleton of three classes of phylum Porifera, namely, the Demospongiae, Sclerospongiae and Hexactinellida. Minerals formed by this living organisms show unusual properties and have unique high ordered structures. These nanoscale
structures are species-specific and genetically controlled. The biochemical mechanisms of small length scales silica structures under ambient conditions have attracted a great deal of interest, because these knowledge could help in materials engineering in vitro.

2. SOME ASPECTS OF SILICA CHEMISTRY

Orthosilicic acid has never been isolated from systems in vivo due to the complex physiological conditions present in living organisms. Salt concentrations, pH range, temperature, additional chemical species result in autopolycondensation reactions, direct silica nucleation and deposition. The process of silica polymerization can be described as three stages:
1. Oligomerisation: monomers of silicic acid produce dimers and other small oligomers by formation of siloxane bonds (Si-O-Si).
2. Growth of nuclei.
3. Aggregation and structuring of particles.

Oligomerisation. Water-soluble monomers of orthosilicic acid could be stable for a long time, but with increasing the concentration up to 100-200 ppm, silicic acid undergoes autopolycondensation reaction. This reaction leads to the formation of dimeric species. There are two possible mechanisms of this reaction. Two uncharged molecules could react with release of water:

\[(\text{OH})_2\text{Si-OH} + \text{HO-Si(OH)}_3 \leftrightarrow (\text{HO})_2\text{Si-O-Si(OH)}_3 + \text{H}_2\text{O} \quad (1)\]

The reaction is very slow as it implies the formation of unfavourable pentacoordinated silicon species. At the presence of silica anions (Si(OH))\text{3}\text{−} and SiO\text{2}((OH))\text{3}\text{−} the reaction takes place between ionized and un-ionized silicic acid molecule:

\[\text{Si(OH)}_4 + \text{Si(OH)}_3 \leftrightarrow (\text{HO})_2\text{Si-O-Si(OH)}_3 + \text{OH} \quad (2)\]

Presence of siloxane linkage (Si-O-Si) in dimers increases the charge of the silicon atoms. Silicic acid reacts preferentially with these dimers. At the end of the first stage we have the mixture of oligomers Si\text{4}O\text{2}((OH))\text{2}, containing 3-6 silicon atoms, dimers and monomers [4].

Growth of nuclei. Larger oligomers have greater volume to surface area ratio, and therefore they are more energetically favoured than monomers or dimers. Brownian movement renders interparticle collision possible, and if the contact time is long enough to build the siloxane linkage, monomers and small oligomers will form larger structures in order to reach a low energy state. The solubility of the small particle is higher than the solubility of the big particle. This process continues until the difference in solubility of the particles becomes negligible and the system reach monodisperse state. The formed monodispersed sol has the solubility of amorphous silica [5].

The reactions on this stage are strongly dependent on pH, presence of chemical species, etc. The pH range interfere with the concentration of silicate anions. By neutral pH the concentration of silicate anions is very low, the particles do not increase the size of 2-3 nm and the process is very slowly [6]. At higher pH, basic conditions, Si-OH silanol groups are deprotonated. The particle containing Si(OH)\text{3}\text{−} and Si(OH)\text{2}((OH))\text{−} anions could bear a large negative charge. Repulsive forces between charged particles hinder their aggregation. As the result of decondensation/condensation process, the silica formed is built up from highly branched networks forming so-called colloidal sol [6]. At lower pH, more acidic conditions, mainly neutral silanol groups are presented on the silica surfaces. In this case, repulsive forces between the particles is low and the silica formed is built up from highly branched networks forming gel. So, pH of the reactions play consequently a crucial role in the morphology. The presence of chemical species, particularly cations and anions affect biosilica formation [6].

Structuring. Amorphous silica forms ordered opaline aggregates (gel-like material), that are moulded into shape by external factors [7]. Silica formed at room temperature by neutral pH can accommodate a large variety of morphologies. The biomolecules producing by living species can be used as templating agents, affecting microscopic and macroscopic properties of hierarchically ordered inorganic silica structures. Any additives bring influence not only on morphological properties of mineral phase, but also on the kinetics of the reaction. Until now, no clear evidences are known about interaction between organic and inorganic part during biosilification process. Biomolecules including proteins are implicated in the formation of silica. The possible interaction mechanisms are hydrogen bonding, electrostatic forces, aromatic and ionic interactions. Covalent bonds should not be excluded. The second way of morphological control of biosilification is the present of self-organized biomolecules in the system. During the simultaneous assembling of this molecules at the time of silica polymerization the control in the nano to micro scale can be achieved. However, these two strategies can be combined. The biological organisms are the most successful in producing high ordered 3-D silica structure. This makes them a powerful tool for the production of biosilica in vitro and extraordinary for specialists in chemistry, biochemistry, biology, ecology and others.

3. FORMATION OF THE SILICEOUS STRUCTURES IN SPONGES

The biochemistry of silica formation process is different from species to species. Herein, we will focus on the silica biochemistry of sponges. Two of the three extant classes of the phylum Porifera, namely, Demospongiae and Hexactinellida are skillful in generating discrete siliceous skeletal elements called spicules from microscopic to macroscopic sizes. The needle-like spicules composed of inorganic (amorphous silica) and organic matter. After dissolving of silica
with 1 N hydrofluoric acid, a residue remains amounting to 4.3% by weight and including the following: carbon, 0.32-0.83%; silicon, 17.5%; sodium, 13.7%; potassium, 2.6%; aluminium, 3.1% [8].

Demospongiae has monoaxonic or tetraxonic spicules; and Hexactinellida has hexaradiate spicules [9]. The main element of the sponge skeletons siliceous spicules serve as major systematic characters for a given sponge species (Schulze and Lendenfeld, 1889). The size of the spicule in Demospongiae increases up to 450 µm and a diameter of 5 µm [10]. The second type of the spicules, called microscleres, are very variable in size and shape. They have an auxiliary function. Light microscope and scanning electron microscope examination of siliceous spicules reveals concentric layers of amorphous silica, surrounding central canal. At a lower scale, concentric layers of hydrated silica are made of densely packed silica nanoparticles in the 50-100 nm range [11].

The solid part of the skeletal element is amorphous to X-rays and electrons at the angstrom level [9]. To help understand the biochemical mechanisms controlling biosilification, it is essential to characterize the proteins occluded within siliceous structures. For the extraction of the organic part from siliceous skeletal elements, different strategies are used. Schultz first described one of the demineralization methods in 1860 [12]. This method is based on the usage of hydrofluoric acid solutions. During the desilicification with HF, some of the posttranslational modification, such as glycosylation, phosphorylation and others, could be lost [11].

Therefore, the quaternary structure of the proteins, their solubility and enzymatic activity could be changed. Another demineralization method is based on the usage of alkaline solutions of sodium hydroxide, anionic detergents and anionic biosurfactants [13]. During this extraction procedure, the proteins, included in the siliceous structure, incubated by 37°C for 14 days by pH range higher than 9. Under these conditions, the proteins are very likely to loose their native conformation. The first protein extracted from the spicules by HF procedure was called silicatein (the silica proteins). Silicatein was found in the sponge Tethya aurantia [14], Suberites domuncula [15] and Halichondria okadai [16]. This protein has a high homology to cathepsin L class of the papain-like cysteine protease superfamily [17].

The reaction of the condensation of silicic acid monomers (or their conjugates such as alkoxides or esters) is formally similar to the reverse of the hydrolysis reaction catalyzed by the proteases. One of the modern hypotheses of biosilification is based on the structural similarities between the silicatein and protease, proposed silicatein as possible enzyme, included in silica polymerization (Fig.1) [18].

According to the proposed mechanism, the nucleophilicity of the serine oxygen is increased by hydrogen bonding between the imidazole nitrogen of the conserved histidine and the hydroxyl of the active-site serine. This reaction generates a “protein-O-Si” intermediate followed by complete hydrolysis of the first alkoxide bond by water. Condensation of two silicon ethoxides occurs when the oxygen of Si-OH on the first hydrolysed silicon ethoxide attacks the second silicon ethoxide.

In 2006 the group of Mueller et al. found two additional proteins (galectin and collagen) involved in silica formation and collaborated another comprehensive hypotheses. The differential display of transcripts revealed one dominant galectin with two galactose-binding sites and hydrophobic region at its N terminus. It was proposed, that in the mesohyl, galectin molecules associate in the presence of Ca²⁺ to strings/nets that allow binding of silicatein molecules. Collagen fibers orient the silicatein-galectin strings concentrically round the growing spicules (Fig.2) [19]. Anyhow, details of the biosynthetic mechanisms controlling silica polymerization in living systems remain unknown.

**Fig. 1.** Proposed reaction mechanism of silicon ethoxide condensation catalyzed by silicatein a, based on the well characterized mechanism of catalysis by the Ser/His and Cys/His active-site proteases [18].
Fig. 2. Schematic outline of the appositional growth of spicules from demosponges.

In the mesohyl, galectin molecules associate in the presence of Ca$^{2+}$ to strings/nets that allow binding of silicatein molecules. Collagen fibers orient the silicatein-galectin strings concentrically round the growing spicules. In the last step, biosilica deposition is mediated in two directions, originating both from the silicatein-galectin strings and from the surface of the spicules (centripetal and centrifugal). Finally a third silica lamella (3) is formed, which is layered onto the previous two lamellae (1 and 2) [19].

4. THE FUTURE APPLICATION OF THE BIOGENIC SILICA

Over the past two decades, interest in biosilicification has been significantly grown. Biomolecules within the biological machinery of living organisms have the ability to produce inorganic based biominerals from the nanometer to the macroscopic scale in mild conditions. Unlike synthetic silica-based materials, they exhibit superior optical and interesting mechanical properties (high breaking stress and low elastic modulus). Identifying the genes, proteins, and molecular mechanisms controlling the formation of siliceous structures in marine organisms can help in developing environmentally safe production methods for new generation of materials and devices from silicon. In modern technologies, silicon compounds are widely used in steel, chemical and electron industries, where it is processed under extremely high temperatures (in excess of a thousand degrees) and with caustic chemicals, resulting in high production costs. Industrially significant silicon compounds are used as semiconductors in microchips (in transistors and other electronic parts). It occurs in elementary form in optic lenses and prisms for infrared light. Important biotechnological approaches arising from the exploitation of biomolecules involved in biomineralization for the construction of controlled silica nanostructures in vitro. Anyhow, extraction and characterization of biosilicifying molecules from living organisms have still a long way to go. First of all, it is noteworthy to find reproducible and simple way of extracting biomolecules occluded in silica structure in their native state (with numerous post-translation modifications), and to develop suitable biochemical characterization techniques. Another approach will be the development of more functional genomics to check for the consequences of gene deregulation on pattern formation. One more problem should be overcome. In nature, siliceous structures are produced in multicomponent reactions, which are difficult to study because of the numerous possibilities of interspecies interactions. The control control on the reaction could be rather too complicated for synthetic chemistry. One of the solutions of this problem could be the adaptation of the main biological principles to the synthetic chemical routes. The obtained knowledge could help in the future to produce new materials for use by industry and academe alike. The discovered proteins may one day be anchored on silicon chips and other electronically useful platforms to produce semiconductors and solar energy converters.

References

ГУБКІ: КРЕМНІЄВА МІНЕРАЛІЗАЦІЯ В БІОЛОГІЧНИХ СИСТЕМАХ

Борейко А.Л., Мюллер В.Е.Г., Л. І. Остапченко Л.І.

Серед многочисленних видів, які структурні елементи формовані в процесі біомінералізації, особо важливе місце займають губки. Скелет губок утворений кремнієвими структурами – спікулами. В отличі від карбонатних і фосфатних солей, що є обережної зводункових амфіфібрій, кремний представляє собою аморфний металоксид, формуючись в результаті біохімічного полімеризаційного процесу. На прикладі цього виду можуть бути вивчені біохімічні процеси біокремніферизації. Розкриття цих механізмів дозволить в майбутньому проектувати нові матеріали на основі аморфного кремнію за упорядкованою структурою мікро- і нано- розмірів.

Ключові слова: губки, біогенні мінерали, біокремніферизація.

ГУБКІ: КРЕМНІЄВА МІНЕРАЛІЗАЦІЯ У БІОЛОГІЧНИХ СИСТЕМАХ

Борейко А.Л., Мюллер В.Е.Г., Л. І. Остапченко Л.І.

Серед багатьох видів, структурні елементи яких утворені в процесі біомінералізації, особливо на місці займають губки. Скелет губки утворений кремнієвими структурами – спікулами. На відміну від карбонатних і фосфатних солей, які є обережної зводункових амфіфібрій, кремний представляє собою аморфний металоксид, у формування якого здійснюється клітинна полімерізаційна процеси. На прикладі даного виду можуть вивчатися біохімічні процеси біокремніферизації. Розкриття цих механізмів дозволить в майбутньому проектувати нові матеріали на основі аморфного кремнію за упорядкованою структурою мікро- і нано- розмірів.

Ключові слова: губки, біогенні мінерали, біокремніферизація.