Bio-mineralization Process in Sea Environment

Intracellular nanorods have been considered as precursors of mature spicules

sponge

Among the siliceous sponges, the spiculogenesis in the demosponge (*Suberites domuncula*) is best understood. It was suspected that the initial stages of spicule formation proceed intracellularly. Because the synthesis of the spicules is a rapid process, the earliest stages of spicule formation can be studied under in vitro conditions in the cell culture system, the primmorphs.

Investigation of nanorods that appear inside the axial filament in the first stages of spicule formation, has been recently performed. Such nanofeatures are not accessible by X-ray techniques and can be investigated only by electron microscopy. One major advantage of electron microscopy is the potential to collect imaging and diffraction data sequentially from the same sample.

The challenge:	small amount of material inside embedded organic tissues
Solution:	Automated 3D diffraction Tomography, EDX and HRTEM

The nanorods with 20 nm diameter and 200 nm length are present intracellularly in the early and initial stages of axial filament formation. The nanorods are then surrounded by a multifunctional polymeric matrix that is the axial filament and silicatein molecules. These nanorods show a typical size of 300–600 nm in length and 20–50 nm in width and were studied by HRTEM, EDX and 3D electron diffraction tomography / PED methods.

By EDX analysis the following stoichiometry was obtained: $Si_{3.86}Al_{2.00}Mg_{0.34}K_{0.16}\ Fe_{0.16}O_{11}$. 3D diffraction tomography data collection led to the determination of the cell vectors and the space group, C2/c, of the structure. Moreover the electron diffraction and images indicated a two layer phyllosilicate structure. Phyllosilicates are layer silicate made of alternating sheets of Si_2O_5 tetrahedra and $(Al,Mg,Fe)_2(OH)_2$ octahedra. The d-spacing of 20 Å of parameter c indicates a TOT–TOT stacking sequence. In particular, lattice parameters and composition fit the mineral smectite. These types of minerals have been widely identified in marine environments and were occasionally linked to biological activity. The crystalline structure was localized within the organic axial filament of the primordial spicules. It is proposed that this crystalline structure acts as an inorganic template and gives shape and orientation to the newly forming spicule. Thanks to the applied techniques the crystalline nanorods could be detected and analysed, thus moving one step closer to the starting point of spicule biosilicification. These results contribute to the understanding of biosilicification in sponges and might help to provide new insight for bio-nanotechnological applications with potential in the biomimetic and biomedical field.

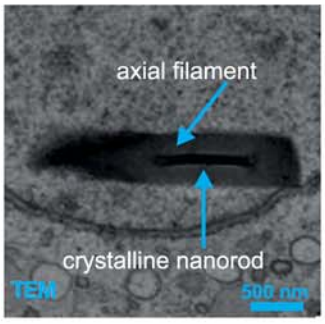
Crystal Structure

$Si_{3.86}Al_{2.00}Mg_{0.34}K_{0.16}\ Fe_{0.16}O_{11}$

Monoclinic C2/c
a= 5.18 Å
b= 9.13 Å
c= 20.20 Å
β= 96°

Experimental data

tilt range: ±30° step: 1°



INITIAL FORMATION OF AN AXIAL FILAMENT THAT COMPRISES A CRYSTALLINE NANOROD

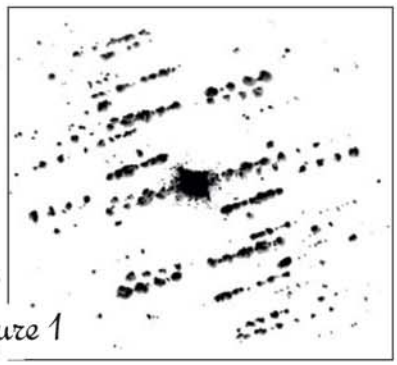
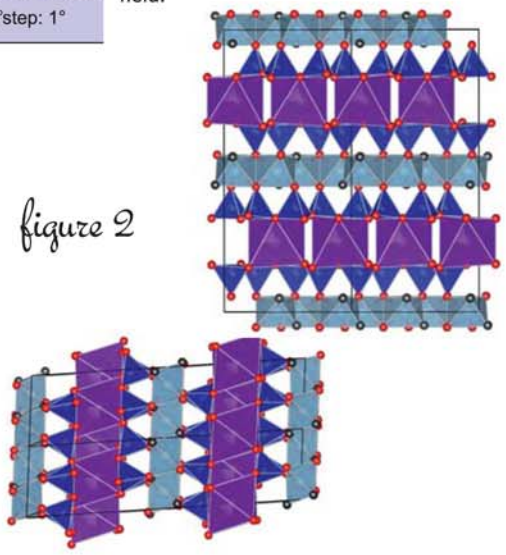


figure 1

3D RECONSTRUCTION MAP FROM THE SELECTED NANOROD

figure 2



CRYSTAL STRUCTURE OF $Si_{3.86}Al_{2.00}Mg_{0.34}K_{0.16}\ Fe_{0.16}O_{11}$

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