Molecular Structures and High Resolution TEM

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Structural analysis of the bio-silica spicule of the sponge *Monorhaphis Chuni*

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Biomineralization is a complex process where organic molecules play an essential role for the formation of a mineral, such as calcite or bio-silica. Such biominerals may have a complex structure of organic/anorganic interfaces and of organic inclusions in a mineral matrix, which can be amorphous as well as crystalline. It was found already more then 100 years ago that biominerals contain structural levels of hierarchy from mm-range to nm-range. We have applied TEM/STEM techniques including high-resolution x-ray microanalysis (EDX) to investigate the structure of the deep-sea glass sponge *"Monorhaphis chuni"*, the largest and oldest bio-silica structure on earth. The cylindrical basal spicule, which acts as an anchor of the sponge tissue, reaches a size of 3 m and a diameter up to 8 mm and consists mainly of silica. Figure 1 shows a SEM picture of the front tip of a basal spicule, which has a tree-trunk structure. It consists of circular lamellas of silica with thin organic interlayers between them. In the center of the spicule an axial filament exists, which has a diameter of about 2 µm. It contains organic molecules, especially the enzyme *silicatein*. The essential role of such molecules for the synthesis of silica has been intensively investigated during the last 5 years (see, e.g., [1-2]). However, questions on structure correlations remained. We concentrated our study on the fine structure of the organic interlayers and the axial filaments. Well-defined samples of the lamella region and of the region of the axial filament in different orientations (longitudinal, plan-view and cross-sections) were prepared by focused ion beam technique (FIB). We combined high-resolution TEM imaging (HR-TEM) with the STEM HAADF technique. The latter one has some advantages for imaging of bio-matter/biominerals. Additionally, specific electron diffraction experiments were carried out.

Figure 2 presents a STEM HAADF longitudinal section of the lamella region. The silica layers are separated by organic interlayers, which are marked by arrows. At higher magnification one could detect a structural transition region between the organic layer and the silica (see Figure 3), which influences the mechanical properties of the spicule [3]. Figure 4 shows a longitudinal cross-section STEM HAADF image of the central axial filament. The organic axial filament (dark) is surrounded by silica (gray). Mineral precipitates (lighter gray) were found in the filament as well as at the interfaces. High-resolution HAADF images as well as high-resolution EDX 2D-mapping yielded that the organic matrix consists of a 3-dimensional lattice of organic and silica structure units. This regular arrangement of these structure units has a periodicity in the range of 5 to 7 nm. This strong periodicity was also confirmed by small-angle X-ray scattering. It might be the result of a self-organization process where the organic molecules, e.g., *silicatein*, play an important role [4]. All information provides a better insight of the complex bio-silica matrix in the nm-range.

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2. X. Wang, et al., J. Experimental Biology 214, 2047 (2011)
3. I. Zlotnikov, et al., Adv. Mat. 25, (2013), in press
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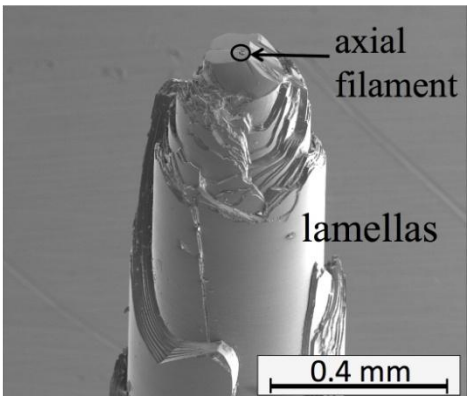

Figure 1. SEM image of the tip of a basal spicule. It consists of a lamellar structure of cylindrical silica shells. In the center an axial filament exits, which has a diameter of about 2 µm. It contains organic material.

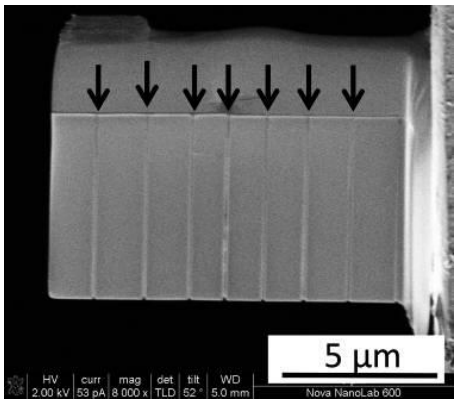

Figure 2. SEM image of a FIB specimen of the lamellar region. This longitudinal section contains 8 silica layers, which are separated by organic interlayers (each 30 nm thick, marked by arrows).

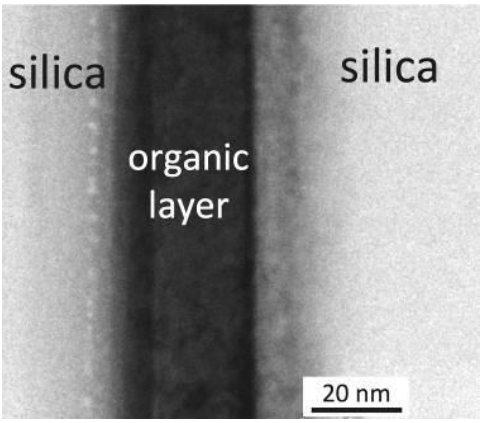

Figure 3. STEM HAADF micrograph of an organic layer at higher magnification (compare Fig.2) showing granular contrast features. Furthermore, the image demonstrates that there is no sharp boundary between the organic layer and the surrounding silica layers, which has an influence on the mechanic properties of the spicule.

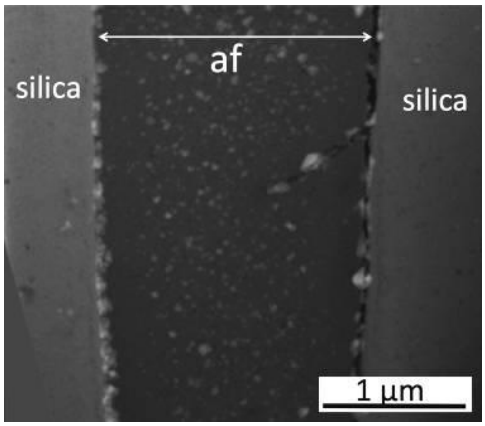

Figure 4. STEM HAADF micrograph of a longitudinal section of the organic axial filament (af), which is embedded in the silica matrix. Mineral precipitates (bright features, mainly NaCl) are embedded in the filament as well as at the interfaces ‘af’/silica.