ESRF	**MICRODIFFRACTION STRUCTURAL ANALYSIS OF SILICEOUS SPICULES BY A NEW TOPOLOGICAL APPROACH**	**Experiment number**: HS 3282
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Report:

Introduction

Sponges are primitive marine animals and consist of organized cells supported by a skeleton of inorganic fibres known as spicules. Spicule are usually composed of calcium carbonate or of silica and their secretion is due to specialised cells called sclerocites. The two most important functions of the spicules are to provide support for the soft part of the sponges and to play a defence role. Their inorganic envelope generally contains a cavity along the elongation axis in which it is possible to identify the presence of an organic proteic axial filament.[1] Proteins from siliceous spicules are called *Silicateins*[2,3] (for SILICA proTEINS) and the biological formation of amorphous hydrated silica, called biosilicification, probably occurs under the control of these specialized organic molecules.[3] This organic matrix is thus suggested to play a critical role in the catalysis of silica polycondensation reactions and in structure direction and the concentric arrangement of the silica is the result of a periodic secretion.[4]

We have already carried out an extensive structural study on a number of siliceous spicules from sponges belonging to different families using a wide variety of experimental and theoretical approaches. [5,6,7,8] The most important results of this study on the structural organization of the organic matter inside the spicules are: *i)* the very high degree of periodic regularity indicated by the very sharp diffraction spots obtained by our SAXS experiments; *ii)* the different arrangement and packing of the protein units in spicules from sponges of two different philogenetic classes; *iii)* the interpretation of the diffraction patterns at different temperatures as due to a regular arrangement of protein units acting as templates in a mesophase silica matrix.

Experimental

Two samples of single needle-like spicules from *T. aurantium* sponges were used: one as such and the other pre-treated at 200°C. They were glued on a glass capillary with a parallel alignment and mounted on the sample holder of the scanning diffractometry set up. They were aligned with their elongation axis perpendicular to the microbeam. Each diffraction pattern was collected for 10 sec. and measurements were carried out in three different ways: *i)* a preliminary set of scans at different Z (Figure 1a) positions indicated a constant behavior at different hights exept near the tip; *ii)* focusing the beam on the central part of the spicule, the sample was rotated of 100° around Z with a step size of 5° and for each rotation angle a complete Y scan with a step size of 1 μm was carried out and a sequence of images collected; *iii)* focusing the beam on the tip of the spicule at an optimal fixed rotation angle, Y scans with a step size of 1 μm were performed at different positions along the Z axis, starting from a point just above the sample and with a downward step size of 1 μm.

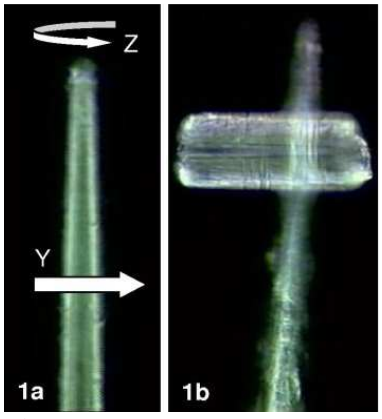

Figure1

In order to analyse the structural arrangement in a direction perpendicular to the Z axis of the sample, a laser cut *T. aurantium* spicule (67 μm long and with 40 μm diameter) was mounted perpendicular to the glass capillary (Figure 1b) and aligned parallel to the beam. Different points of the circular section were scanned, with a step size of 0.6 μm both along the horizontal and the vertical axis.

Result and Discussion

For the untreated sample the analysis of the patterns obtained from the Y scans across the elongation axis of the spicule allowed to identify two different regions: the images from the external parts of the sample showed only a diffuse scattering due to the amourphos silica of the spicule envelope, while the patterns from the central region, where the *Silicateins* are hosted, all showed a certain number of sharp independent diffraction spots. This result is consistent with our previous findings [6,7] that a high degree of structural order is present in the central cavity of the sample (Figure 2). Rotation around Z showed that at all angular positions the central region gave patterns with sharp diffraction spots, thus indicating the presence of a "single crystal" like 3D order. The same scanning and rotation sequenze of pattern collections was repeated for the sample treated at 200°C and showed a similar distribution of the two regions, with an increase both in intensity and in sharpness of the spots from the ordered cavity region. The ordered central part has a dimension of about 10 μm, larger than the reported 3-4 μm of the cavity. This indicated that the partial order extends beyond the cavity towards the amorphous region.

Finally, the scanning of the circular section of the cut spicule confirmed the presence of an hexagonal structural order in the cavity region as indicated by the 6 spots with an hexagonal simmetry in the diffraction patterns (Figure 3). The appearance of three-dimensional higher order reflections not only due to the mesophase pattern normal to the hexagonal axis, but also to the ordered arrangement along the 6 axis opens the possiblity of collecting a sufficient number of "single crystal" diffraction data for a more accurate structural description.

The microdiffraction images confirm that the order can only be indirectly related to the arrangement of the protein units, and strongly support our hypothesis[7,8] of the presence of an ordered mesophase siliceous system inside the spicules, where the proteins act as templates.

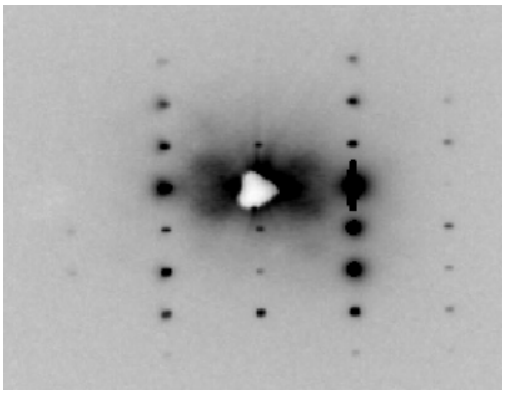

Figure 2.

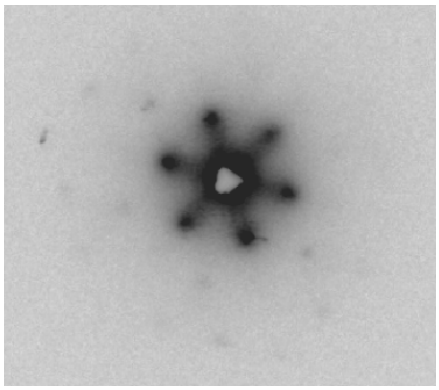

Figure 3.

Publication: Preliminary results of this experiment have been submitted for presentation to two international meetings: *i)* XXI Congress of the International Union of Crystallography in Osaka (Japan), August 2008. *ii)* 6th International Mesotructured Materials Symposium in Namur (Belgium), September 2008.

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