ESRF	**Experiment title:** Analysis of the relationship between the mineral phase and the organic matrix in biominerals	**Experiment number:** CH721
Beamline: ID21	**Date of experiment:** from: 08-09-1999 to: 11-09-1999	**Date of report:** 29-02-2000
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Report:

Among biologically produced minerals, Ca-carbonate prismatic units of Mollusc shells epitomise the paradox of biocrystals. The long-standing recognition of their biologically controlled sizes and shapes has raised questions about the sequence of "molecular tectonic" events [1] that lead to formation of crystalline objects so visibly growing apart from crystallographic rules. The modelling of biomineral growth, controlled from the nanometre to the millimetre scale, requires the understanding of the roles of the mineral and organic matrices and their interaction [2]. However, little information on the organic matrix is available, in particular no correlation has been yet established between its high sulphur content and the mineralising process. Sulphur can be found in both proteins and glucids, the two major organic compounds that compose organic matrices responsible for calcification in carbonate producing organisms [3]. In this prospect, studying the chemical state of sulphur may be used as an indicator of the relationship between the mineralising matrices and the mineral ions. The study was carried out on calcitic units that built the external layer of a Mollusc shell, the Pelecypod species *Pinna nobilis*, rather common in the Mediterranean Sea. These units appear as linear prisms, closely and very regularly side-by-side packed, of 50 to 75 micrometers in transverse sections and up to 3-4 millimetre in length (Figure 1). The prisms of Pinna offer remarkable advantages to investigate the organisation of biocrystals. They are the largest units that globally exhibit a single crystal like organisation, although they are typical polyclinic structures. Chemically, they are characterised by the highest sulphur content ever observed, about 0.3 to 0.4%, a chemical feature that could be related to its exceptional crystallographic coherence. The experiment was carried out on ID21 using the scanning X-ray microscope in fluorescence mode at the sulfur K-edge. The microscope used a Fresnel zone-plate as focussing lens and delivered a microbeam of $0.25 \times 0.25 \mu m^2$. Sulfur speciation analysis was performed in both intra- and inter-prismatic unit matrices. Mapping of these composites at two energies revealed for the first time the existence of at least two different sulphur compounds in the matrices of *Pinna nobilis* (Figure 2). The spectra measured in the organic and mineral phases were compared with XANES spectra of pure standard products (cystin, cystein and chondroitin sulphate). These preliminary results indicate clearly the absence -or the very low concentration- of sulfidic crosslinks, the predominance of sulphate in the intra-

prismatic matrix and the high concentration of "amino-acid" type sulphur in the inter-prismatic matrix, in particular at the nodes of the hexagonal mesh of the matrix. Further analysis must be performed to clarify the exact role of those various sulphur compounds in this organic-inorganic interface, which drives the bio-mineralisation process.

References:

[1] S. Mann, Nature, 365, 499 (1993)

[2] S. Weiner, L. Addadi, J. Mater. Chem. 7(5), 689, (1997)

[3] Y. Dauphin, J.P. Cuif, Annales des Sciences Naturelles, 2, 73, (1999)

Publications:

Y. Dauphin, M, Salomé, J. Susini, J. Doucet , J.P. Cuif, "*In situ* characterization of sulfur chemical state in calcareous biomineral organic matrices by XANES mapping" *in preparation (to be submitted to Nature).*

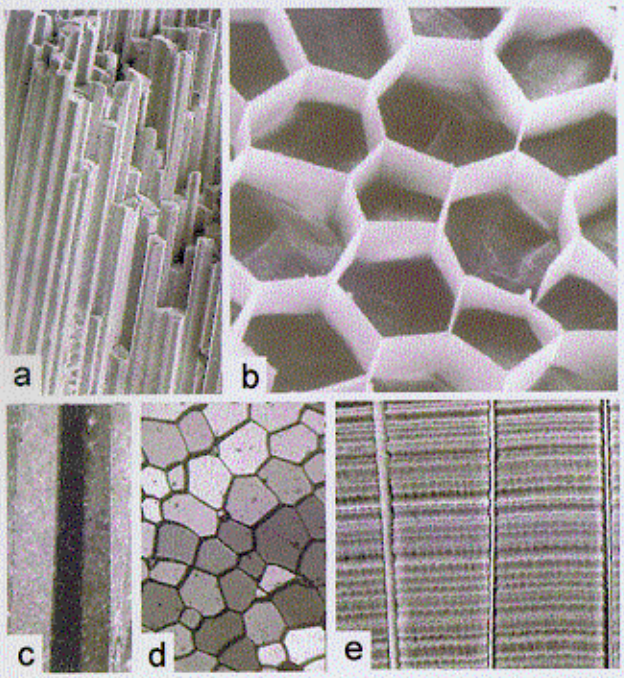

Figure 1: The studied mollusc shell appears as linear prisms, closely and very regularly side-by-side packed (Fig. 1a). The upper level compartment, the organic template that surrounds mineral prisms, can easily be made visible after acidic etching (Fig. 1b). Microscopic observation of thin sections (polarized light) allows the monocrystalline appearance of prisms to be evidenced (Fig. 1c,d). The remarkably rigorous parallelism of prism incremental bandings (Fig. 1e) demonstrates that internal growth surface of the prismatic shell layer is under control of secretory activity of the whole mantle surface.

Figure 2: Fluorescence yield images of a Pinna shell taken at two energies, 2.482keV and 2.473keV, specific of the sulphur in sulphate or amino-acid forms respectively. The pixel size is 0.5x0.5µm2. A visible light microscope image is given for comparison.