ESRF	**Experiment title:** Study of the role of sulfur compounds in biomineralization process : chemical state mapping of sulfur in the organic matrices of various seashells	**Experiment number**: CH948
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Report:

Introduction

The long-standing recognition of a permanent association between mineral phases and organic macromolecules in biominerals, which exhibit taxonomy linked crystallographic features, has suggested that in living organisms, crystallization is a biologically driven process. Modeling of this process requires the understanding of the biochemical interactions between organic matrices and mineral ions. The starting point of the study presented here is the positive correlation which has been evidenced between the sulfur content and the mineralogical features of the prismatic units of seashell outer layer (1). As reported from experiment CH721, spatially resolved XANES performed on *Pinna nobilis* L. at ID 21 showed that ***in situ*** **mapping of sulfur oxidation state** was possible, allowing the different biochemical combinations of sulfur to be identified (2). At least two different sulfur chemical species in the intra-and inter-prismatic organic matrices were evidenced. In particular a difference in sulfate concentrations was observed. This result may be related to the putative opposite roles (respectively catalyst and inhibitor) of the two matrices in the biomineralization process. The aim of this new experiment was to assess the sulfur species distribution in the organic matrices of seashells from different origins, in order to check if the preliminary results obtained on *Pinna nobilis* L. can be generalized.

Experimental setup

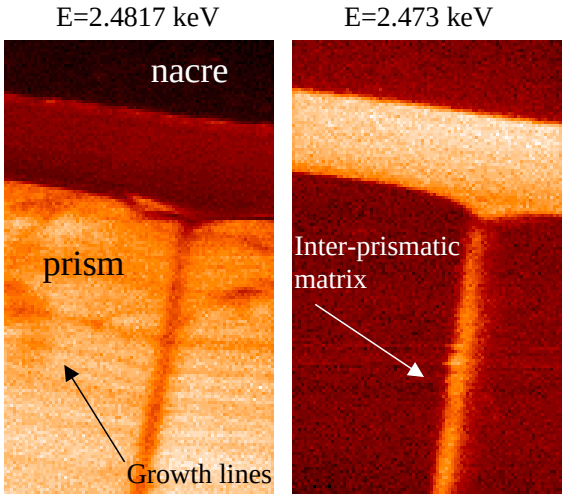
The measurements were performed using the Scanning X-ray Microscope (SXM), operated in fluorescence mode. The beam was focussed to a submicron microprobe (0.25 x 0.25 μm^2) using a Fresnel zone plate. The fluorescence emission of the sample was collected by a Hp Ge detector. XANES spectra were recorded by scanning the primary excitation energy around the sulfur K-edge (2.472 keV), using a Si double crystal fixed exit monochromator ($\Delta E/E = 10^{-4}$). 2D fluorescence images at fixed energies were also performed pixel-by-

pixel by scanning the sample in the beam. The excitation energies for the imaging mode were determined from the XANES spectra of reference compounds to allow discrimination and mapping of different sulfur species in the sample (Sulfate peak position : 2.482 keV, Amino-acid linked sulfur : 2.473 keV). The low energy of the sulfur K-edge required working under vacuum to avoid absorption of the primary excitation beam and the fluorescence emission in the air.

Results

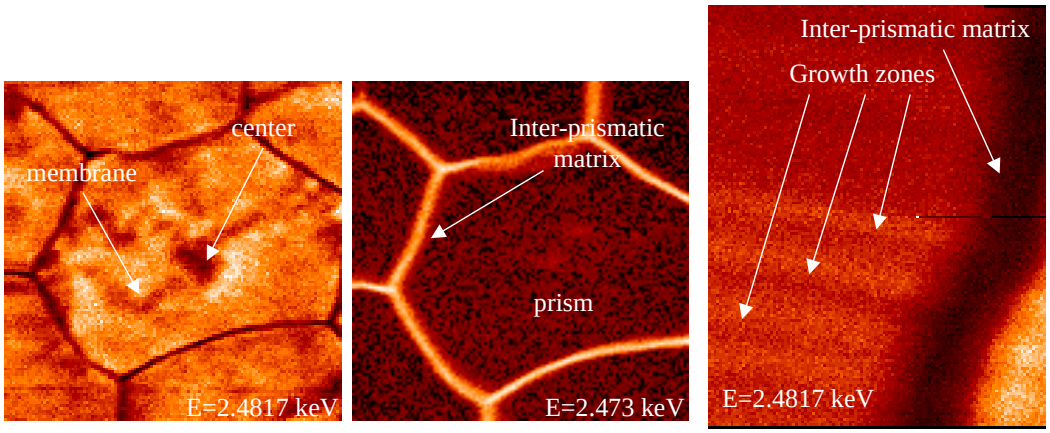
The samples investigated were

- two modern molluscs which shells include both an inner aragonitic nacreous layer and an outer calcitic prismatic layer : *Pinna nobilis* L. (see Fig. 1), *Pinctada margaritifera* L. (see Fig. 2).
- Aragonitic fibres and calcification centers in corals : *Montastrea*.



30 x 50 µm^2, 0.33 µm pixel size

Figure 1: Sulfur XANES mapping of the interface between prismatic and nacreous layers in *Pinna nobilis* L. The contrast inversion observed between the two mapping energies confirms the inversion of concentration in sulfate / amino-acid linked sulfur between intra- and inter-prismatic organic matrices. The lines resulting from the polycyclic growth of the prisms are clearly visible. Interpretation of the chemical speciation of sulfur in the nacreous layer is in progress.



50 x 50 µm^2, 0.5 µm pixel size 40 x 50 µm^2, 0.33 µm pixel size

Figure 2: Sulfur XANES mapping of transversal and longitudinal sections in the prismatic layer of *Pinctada margaritifera* L. *In-situ* XANES spectra performed in the intra- and inter-prismatic organic matrices are similar to those obtained in *Pinna nobilis* L. Intra-prismatic membranes and mineralisation centers are nicely depicted.

(1) Dauphin, Y. & Cuif J.P. (1999) *Ann. Sci. Nat.* **2**, 73-85.

(2) Salomé, M., Dauphin, Y., Susini, J., Doucet, J., Fayard, B. & Cuif, J.P., "The role of sulfur in biomineralization explored by X-ray absorption spectroscopy with submicron resolution: the relationship between sulfur chemical states and shell micro-architecture in *Pinna Nobilis*", submitted to *Proc. Natl. Acad. Sci. USA*.