

Structural control of biominerals by intracrystalline organic macromolecules

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Biominerals, which are inorganic materials formed thorough biological processes, often have strictly-controlled structures and superior properties to geological counterparts. These characteristics are ascribed to organic constituents or macromolecules contained inside the biominerals. Although the intracrystalline organic macromolecules (IOMs) are considered as one of the crucial parts in biomineralization, it is not clear how they interact with the biominerals and control their characters. We have used transmission electron microscopy (TEM) and scanning TEM (STEM) to visualize IOMs, and investigated the influence of the IOMs on the microstructures of biominerals. The goal of this study is to elucidate the interaction between inorganic materials and IOMs in biomineralization. In this paper, we present some of our recent results, to consider the possibility to apply our methodologies to other geoscience fields.

In this study, we compared the influence of IOMs in the prismatic structures of three mollusk shells; a pearl oyster (*Pinctada fucata*); a pen shell (*Atrina pectinata*); and an oyster (*Crassostrea nippona*). These prismatic structures are composed of calcite (CaCO₃) columnar crystals. Thermogravimetric analyses (TG) showed that every shell contains the IOMs of approximately 3 wt.%. TEM observations were conducted for thin specimens prepared using a focused ion beam system (FIB). In under-focused bright-field TEM images, spherular Fresnel contrasts appeared inside the crystals. Since the Fresnel contrasts in the shells are a few dozen nanometers, they were assumed to correspond to IOMs. This speculation was verified using electron energy-loss spectroscopy (EELS) equipped to STEM. The spectra obtained from the Fresnel contrasts exhibited a fingerprint of organic matter in the fine structure of carbon *K*-edge. Thus we can conclude that IOMs are visualized as Fresnel contrasts in defocused TEM images. Next three-dimensional distribution of IOMs was reconstructed using STEM tomography. In *Pinctada* and *Crassostrea* shells, the IOMs are distributed inhomogeneously to separate the crystals into sub-grains. On the contrary, *Atrina* shells show homogeneous distribution of the IOMs. Inhomogeneous distribution of the IOMs in *Pinctada* and *Crassostrea* not only induces small-angle grain boundaries and strain into the crystals but also influences mechanical properties such as suppression of cleavages in the calcite crystals.

Synthetic experiments were also conducted to understand the influence of IOMs on calcite crystals. Calcite crystals were synthesized *in vitro* in the presence of the IOMs extracted from the shells. The IOMs were incorporated into the crystals and observed as Fresnel contrasts in TEM images. The crystals synthesized with the IOMs extracted from *Pinctada* shells have defective microstructures similar to the original shells.

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