

Crystallographic analyses of exoskeletal calcium carbonate in *Armadilidium vulgare* (Crustacea, Isopoda)

YOKOO, Naoki^{1*}, Masako Isobe¹, SUZUKI, Michio¹, Hiromichi Nagasawa², KOGURE, Toshihiro¹

¹Earth & Planetary Science, Univ. Tokyo, ²Agricultural & Life Sciences, Univ. Tokyo

Calcium carbonate (CaCO_3) is one of the most major biominerals, found in many organisms such as mollusks, arthropods, and echinoderms. Previously, it is thought that biotic CaCO_3 crystals are precipitated from a supersaturated solution directly. However, in recent years, existence of amorphous calcium carbonate (ACC) as a precursor phase of calcite or aragonite has been suggested in some organisms, but this is still not certain. In this study, we have investigated a terrestrial isopod, *Armadilidium vulgare*, because previous works indicated by Raman spectroscopy that at least a portion of calcite is transformed from ACC in its exoskeleton. We mainly investigated crystallography of calcium carbonates, using X-ray diffraction (XRD), Scanning Electron Microscopy (SEM) with Electron Backscatter Diffraction (EBSD), Transmission Electron Microscopy (TEM) to understand the characteristics ACC-mediated calcite. Focused Ion Beam (FIB) was used for the preparation of TEM samples.

FIB-TEM observation indicated that the outermost layer (distal layer) of the exocuticle consists of well-crystallized platy calcite single crystals of ~ 0.5 micron thick and several micron in width. SEM-EBSD analyses of the distal layer indicated that scale-like domains on the surface of the exocuticle correspond to calcite single crystals. The *c*-axes of calcite in the distal layer are basically parallel to the anteroposterior axis, but varied from the complete parallelism to standing by $\sim 50^\circ$ from the surface. On the other hand, the inner layer (transition zone) of the exocuticle contains spindle-shaped crystals of ~ 0.5 micron and organic matrices. Electron diffraction (ED) indicated that the crystals are also calcite. TEM imaging, ED and Energy Dispersive X-ray Spectroscopy (EDS) analyses suggested that the endocuticle under the transition zone does not contain crystalline calcium carbonates, but ACC and organic matrices. TEM-EDS analyses indicated that calcite in the distal layer was almost free of phosphorous which can act as an inhibitor of the crystallization of ACC, whereas a certain amount of phosphorous was detected in the transition zone. Phosphorous is the most abundant in the endocuticle, probably to stabilize ACC.

From these results, it is inferred that calcite in the distal layer was precipitated on organic matrices from solution directly, and that in the transition zone was crystallized from the ACC precursor.

Keywords: calcite, ACC, SEM-EBSD, FIB-TEM, phase transition