論文内容の要旨

論文題目

Investigation of amorphous calcium carbonate (ACC) in biomineralization

（生体鉱化作用における非晶質炭酸カルシウム(ACC)の研究）

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Amorphous calcium carbonate (ACC) is the unique inorganic material only found as biomineral in nature. In order to deepen the understanding of biomineralization involved with ACC, two kinds of hard tissues, cuticle of terrestrial isopod and larval shells of mollusks, have been investigated in this thesis. The reason to select the former material is mainly to characterize the mineralogical or crystallographic features of crystalline calcium carbonate (calcite) transformed from ACC. The latter material was selected to elucidate the existence or nonexistence of ACC in the larval shells, through the detailed characterization of the microstructure of the shells at several stages after fertilization.

Previous studies reported that the cuticle of the terrestrial isopod, *Armadillidium vulgare*, at the intermolt stage consists of three calcified layers and one uncalcified layer; distal layer (calcite), transition zone (calcite), endocuticle (ACC), and membranous layer (uncalcified). The present study has revealed that calcite in the transition zone is crystallized from ACC and the calcite crystals have distinct features with respect to their crystal orientations; the crystals in the upper transition zone have the identical orientations to those of calcite in the distal layer, whereas the lower transition zone

consists of nano-crystalline calcites with their ***c***-axis parallel to the organic fibers. Hence, calcite crystals in the upper transition zone are suggested to be formed by epitaxial growth, taking over the crystal orientation from the distal layer. In contrast, crystallization is regulated by organic fibers in the lower transition zone. These results suggest that the calcite transformed from ACC can have various crystallographic features, depending on the substrates or templates. On the other hand, considerable amounts of phosphorus were detected in the transition zone and endocuticle. Hence, as reported in previous studies of ACC formed in crustaceans, phosphorus compounds probably work as the stabilizer of ACC or inhibitor of the crystallization also in *A. vulgare*. Combined with the observations of the cuticle at several molt stages, the calcification and crystallization process in the cuticles of *A. vulgare* can be illustrated as in Figure 1.

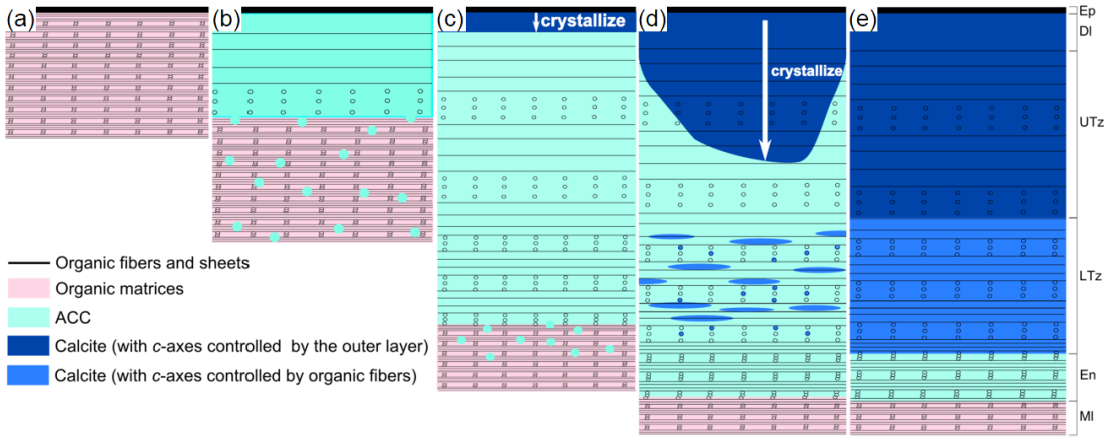

Fig. 1. Schematic illustration of the cuticle formation processes in a molting cycle. (1) Organic membranes are formed under the old cuticle. (2) ACC deposits under the epicuticle. (3) ACC deposits continuously and the crystallization starts from the surface. (4) Organic fibers induce ACC crystallization at the lower transition zone. (5) Both of the crystallization processes from the surface and along the organic fibers are completed.

Larval shells of *Pinctada fucata* and the other species have been investigated to elucidate the shell microstructure and possibility of the existence of ACC during their developments. The shells were mainly investigated using transmission electron microscopy (TEM) with the specimens prepared using the focused-ion-beam (FIB) technique. A three-layered structure has been identified in the

larval shell of *P. fucata,* as reported in previous studies. From detailed TEM analyses, each layer has the following characteristic structure;

(1) The outer prismatic layer contains dense multiple {110} twins and few grain boundaries (Fig. 2).

(2) The middle globular layer is actually the mixture of the areas with multiple {110} twins and with a polycrystalline feature. However, the ***c***-axes of the aragonite crystals are oriented normal to the surface, regardless of such structural difference.

(3) The inner prismatic layer consists of "prismatic crystals" with distinct grain boundaries.

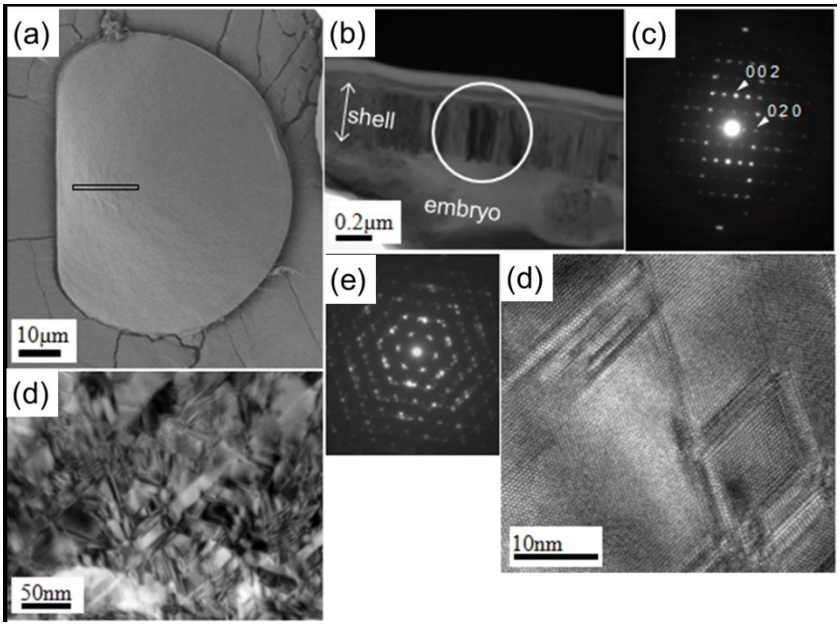

Fig. 2. Results of the observation of 18 h shell by SEM/TEM. (a) Outer appearance. (b) Cross-sectional TEM image of the initial calcified layer. (c) SAED pattern from the circle in (b). (d) Plane-view image of the layer. (e) SAED pattern from the plan-view specimen, indicating dense {110} multiple twins. (f) Magnified image of (d).

Although the analysis of the shell microstructure was not as complete as that for *P. fucata*, I have investigated the larval shells of other three species selected from seawater Gastropoda, freshwater Bivalvia, and freshwater Gastropoda. All of them have a three- layered structure, all of which consist of aragonite whose ***c***-axes are oriented normal to the shell surface. Hence, the basic design of the larval shell is very close among these species, or in Bivalvia and Gastropoda. During these analyses, I did not find ACC or any structures which suggest the existence of ACC as a precursor phase in the

larval shells of *P. fucata* and other species. Considering the results for *A. vulgare* and characterization of synthetic ACC (Appendix), the transformation of ACC to aragonite by the FIB process seems unlikely.