Relation between the organic network and the crystal defects in the calcite of the prismatic layer of *Pinctada fucata*

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Biominerals are biogenic mineralized tissues containing not only inorganic components but also a small amount of organic matrices that play an important role in formation of biominerals. Molluscan shells, which are typical biominerals consisting of calcium carbonate and organic matrices, have various kind of microstructures. The shell of a pearl oyster, *Pinctada fucata*, has two different layers. The outer layer of the shell is a prismatic layer consisting of calcite crystals. Each prism, which is surrounded by the organic framework, is composed of some single crystals of calcite. The single crystal has the small distortion of crystal orientation because this crystal has some small-angel grain boundaries inside of the crystal which cause the minim lattice distortion or defect and divide the crystal into subgrain units of a few hundred nanometers. Such minim lattice distortion and defects may increase the toughness of the shell by inhibiting the cleavage and fracture of the crystals in the prismatic layer. The small-angle grain boundaries were observed by TEM along with the localized organic matrices like networks, indicating that the organic networks may cause the small-angle grain boundaries. TEM observations showed that the organic networks are a few dozen nanometers in thickness and divide the crystal into subgrain units of a few hundred nanometers. However, what the organic networks consist of or how they cause the small angle grain boundaries has not been reported yet.

To reveal the components of the organic networks and formation mechanism of the small angle grain boundaries in the calcite crystal of the prismatic layer, we extracted the organic network from the prismatic layer and tried to identify the components. The IR spectrum of acetic acid-insoluble materials from the prisms revealed that the major component of the organic network was chitin. LC-MS/MS analysis of acetic acid-insoluble and SDS/DTT-soluble fractions showed that the chitinolytic enzymes such as chitinase and chitobiase were involved in the intracrystalline organic matrices of the prismatic layer. These results suggested that the chitinase and chitobiase regulate the formation of chin fibers that interact with the calcium carbonate to make the small-angle grain boundaries. To understand the function of the chitinolytic enzymes in the prismatic layer, calcium carbonate was precipitated in the chitin hydrogel after being treated with the commercially available enzyme. The calcite crystals precipitated in the chitin hydrogel appeared to contained larger crystal defects as the chitinolytic enzyme concentration increase and crystal defects similar to those in the prismatic layer were observed as to some extent concentration. In addition to observation, a variance of lattice spacing was calculated from peak broadening in powder X-ray diffraction and compared among the these calcite crystals. As a result, a variance lattice spacing tended to increase depending on chtinolytic enzyme concentration, implying that chitinolytic enzyme decrease the thickness of the chitin fiber to increase the interaction between chitin fibers and calcium carbonate. Such interaction is probably important to produce the small-angle grain boundaries in the calcite crystal and strengthens the toughness of shell.

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