Studies on the functions of organic matrices to make the molluscan shell microstructures.

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Biominerals are biogenic mineralized tissues containing not only inorganic compounds, but also a small amount of organic matrices that play an important role in biomineral formation. The mollusk shells which are typical biominerals consisting of calcium carbonate and organic matrices, have various microstructures. The organic matrices promote the nano-cluster formation of minerals to regulate the nucleation, crystal growth, crystal orientation and crystal morphology. We want to introduce two molecular formation mechanisms of shell microstructures in two molluscan species using organic matrices.

The shells of gastropods have spiral in shape around the central axis. As the living body grows up, the shell thickness in the internal side of spiral becomes thin to expand interior space. These observations suggested that a dissolution process, working as the remodeling mechanism, changes the shell shape in molluscan shells. The molecular mechanisms of remodelling processes in the vertebrate bone have been studied well. The function of both osteoblast and osteoclast cells orchestrate the bone formation and remodeling. Although various proteins associated with shell calcification have been identified in molluscs, no organic molecules related to the dissolution of molluscan shells have been identified and the remodeling mechanism of molluscan shells is unclear.

To reveal the dissolution mechanism for the remodeling of the spiral shells in gastropod, we used the fresh water snail, *Lymnaea stagnalis*, as an experiment material and focused on chitinases of the organism. Chitinase activity was observed in the acetic acid-soluble fraction from the shell and the buffer extract from the mantle, indicating that the shell and mantle may have a function to degrade the chitin scaffold in the shell. The chitinase activity in both fractions was disappeared by the heat treatment. Allosamidin, a specific inhibitor of family 18 chitinases completely inhibited the chitinase acitivity of both fractions indicating that the enzyme activities in the shell and mantle were from only the family 18 chitinases. Homology cloning and transcriptome analyses from the mantle revealed five genes encoding family 18 chitinases (*chi-I, chi-II, chi-III, chi-IV and chi-V*). GH18 domain for the activity of chitin degradation was conserved in all chitinases. All chitinases were expressed not only in the mantle, but also in other tissues, suggesting that chitinases in the mantle have multiple-functions. We injected the allosamidin into living snails to inhibit the chitinase activity in the mantle. Although the chitinase activity in the mantle was strongly suppressed by allosamidin injection, the shell microstructure before and after injections was not changed. However, treatment of chitinase from *Trichoderma viride* by a commercially available altered the shell microstructure of *L. stagnalis* suggesting that the chitinase was associated with the shell dissolution process.

On the other hand, the “scaly foot” gastropods (*Chrysomallon squamiferumuse*) use the organic matrices to make the scales of iron sulfide on the foot and shell surface. The “scaly foot” was discovered in the Kairei deep-sea hydrothermal field of the Central Indian Ridge. The black scales consist of nano-crystal of iron sulfide minerals within a laminated organic matrix. Although iron sulfide mineralization is known in the metabolic sulfate reduction from prokaryotes, the formation mechanism of iron sulfide nano-crystal is unclear. In this study, we tried to extract the key organic components that interact with iron in the scale. Such organic components may keep the small size of iron sulfide crystals on the foot and shell surface.

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