Mechanisms of inorganic carbon uptake and supply to thioautotrophic bacteria in the gill of deep-sea chemosymbiotic bivalves

［課程博士・論文博士共通］

博士学位論文内容要旨
Abstract

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論文題目 Title	Mechanisms of inorganic carbon uptake and supply to thioautotrophic bacteria in the gill of deep-sea chemosymbiotic bivalves.		

Deep-sea *Calyptogena* clams harbor thioautotrophic bacteria in their gill epithelial cells (bacteriocyte) and nutritionally depend on their symbionts because their digestive tracts are vestigial. Genome analysis of the symbiont of *C. okutanii* has revealed that the symbiont has Rubisco systems to fix CO_2 for synthesis of organic carbon. To rely on the Rubisco systems in this symbiosis, transport of inorganic carbon (Ci; CO_2 and HCO_3^-) from seawater to symbionts via host is important to understand. However, in the equilibrium at seawater pH, the major form of Ci is HCO_3^-. Unlike CO_2, the HCO_3^- is impermeable to the cell membrane and their interconversion ($CO_2 + H_2O \leftrightarrow HCO_3^- + H+$) rate is relatively slow. Carbonic anhydrase (CA) catalyzes the interconversion between HCO_3^- and CO_2. In *Calyptogena* clams, CA activity has been measured in the gill, foot and mantle tissue, and the highest activity been found in the gills by Kochevar and Childress (1996). Therefore, CA is believed to play a major role in taking up and transporting Ci to the symbionts in *Calyptogena* clams. In *Calyptogena* clams, however, the detailed processes of Ci uptake and transport from seawater to symbionts are still mostly unknown. To understand these processes, I examined mRNA sequences, proteins and CA activity in gill tissue of *C. okutanii* using expression sequence tag (EST), SDS-PAGE with LC-MS/MS. It was shown that two acetazolamide-sensitive cytosolic CAs (CokCAg1 and -2), which were co-purified by the affinity chromatography, were abundantly expressed in the gill tissue of *C. okutanii*. Mouse monoclonal antibodies against the both CokCAg1 and -2 were raised and used in Western blot analysis and immunofluorescence staining of the gill tissues of *C. okutanii*. Both of the CAs were exclusively in the cytoplasm but not on the cell membrane of symbiont-harboring cells (bacteriocytes) in gill epithelial cells. These indicate that CA is important in providing the Ci to symbionts in the vacuole of bacteriocytes. However, Ci uptake in bacteriocytes from seawater to cytoplasm remained still unknown.

To clarify the mechanism of Ci uptake from seawater to cytoplasm, pyrosequencing of cDNA in gill tissue was examined by using next-generation sequencings (454 GS FLX Titanium). Total reads from the sequencing, which were 1,174,164 reads, were assembled into 18,640 and 50,699 contigs from Newbler and MIRA, respectively. About forty percent of those contigs were shown to have significant similarities ($E \leq$ 1e–5) to protein-encoding genes in the NCBI nonredundant database. From the protein-coding gene contigs, four genes of bicarbonate transporter belonging to solute carrier family 4 (SLC4co1, -2, -3 and -4) and one gene of membrane associated CA (mCAco1) belonging to α-CA family were identified. These gene products are known to play major roles in taking up Ci for photosynthesis in diatoms and sea anemone-zooxanthellae symbiosis, respectively. From these data, I considered that these gene products participate in taking up Ci from seawater to cytoplasm in the gill tissue of *C. okutanii*.

The gill filament of *C. okutanii* consists of two major zones; 1) the external asymbiotic zone containing ciliated cells and goblet-like cells; and 2) the internal symbiotic zone containing bacteriocytes and asymbiotic cells called intermediate cells. To identify gill epithelial cells transcribed *slc4*s and *mcaco1*, *in situ* hybridization experiments

were performed. The signals of *slc4co1* and *slc4co2* were detected in the ciliated and the intermediate cells that are asymbiotic cells, which those of *mcaco1* and *slc4co1* were detected in the bacteriocytes. Gene expression of *slc4co3* and *slc4co4*, which were shown to be very low in the gill tissue by RT-PCR, were not detected by the *in situ* hybridization.

The results have led me to propose a hypothesis on the process of Ci transport from seawater to the symbionts. The mCAco1 in the cell membrane of the bacteriocyte converts HCO_3^- to CO_2 at the surface of bacteriocyte, and CO_2 penetrates into the bacteriocyte through the cell membrane. Then CokCAg1 and -2 catalyze it to reach Ci equilibrium in the cytoplasm of bacteriocytes. This process makes it possible to store Ci pool in the cytoplasm, because the abundant form is the membrane impermeable form, HCO_3^-, at the equilibrium. On the other hand, the membrane permeable form, CO_2, passes through the symbiosome membrane is consumed by symbionts. SLC4 is generally localized basolateral cell membrane of mammalian kidney and functions to reabsorb HCO_3^- to prevent or avoid acidification of the blood. In the gill tissue of *C. okutanii*, *slc4co1* was shown to be localized in asymbiotic cells and also in bacteriocytes, while *slc4co2* was found only in asymbiotic cells. SLC4co1 may be localized in the basolateral membrane of bacteriocyte, and may take up HCO_3^- from blood to the cytoplasm of bacteriocyte, where CokCAg1 and -2 probably function in supplying CO_2 to the symbionts. Although the physiological roles of the intermediate cells and ciliated cells in symbiosis of *C. okutanii* are still not known, expression of the SLC4co1 and -2 in the ciliated and the intermediate cells suggest their role in maintaining the blood pH in the gill tissue like that in mammalian kidney.

In this study, I performed, a comprehensive molecular biological, biochemical and immuno-histochemical analyses of the host, *C. okutanii* and related bivalves. Two cytoplasmic type CAs, CokCAg1 and -2, were abundantly and exclusively expressed in the bacteriocyte of the gill tissue. Genes of a membrane type CA, mCAco1, and a bicarbonate transporter, SLC4co1, were expressed in the bacteriocyte. From these data, I proposed a hypothesis for the Ci transport system in the *Calyptogena* clam symbiosis that the CokCAg1 and -2 facilitate to supply CO_2 for the symbionts in the cytoplasm, and mCAco1 function in the Ci uptake from seawater to cytoplasm of bacteriocyte. In *Calyptogena* clams, the process of Ci uptake and transport was first limiting step for carbon metabolism. CA of host may help for the symbiont to facilitate synthesis of organic compounds in their symbiosis.