

## Two new carbonic anhydrases from the gastropod *Haliotis gigantea*: Implications for the evolution of molluscan shells

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Carbonic anhydrases (CAs) catalyze the reversible hydration of carbon dioxide. They are ubiquitous enzymes involved in many fundamental biological processes including biomineralization. In molluscs, several CAs have been reported and two of them were extracted from bivalve shells. We now report two novel molluscan CAs, one of them were extracted directly from gastropod shells for the first time. Both CAs, HgCA1 and HgCA2, were expressed in the mantle of the the Siebold's abalone *Haliotis gigantea*, and only HgCA1 was the shell matrix protein. HgCA1 are N-linked glycosylated and probably not a cation-binding protein. The asparagine and glycine-rich repeated structure of some molluscan CAs were believed to be important for the shell formation, but HgCA1 does not have the repeated structure, suggesting that it is not necessarily important to the shell formation. The phylogenetic analyses revealed that the gastropod CAs form a monophyletic clade with the cytosolic and mitochondrial CAs, while bivalve CAs as well as secreted and membrane-associated CAs are placed outside the clade. The result suggests that CAs were co-opted for molluscan biomineralization independently after the divergence of the gastropod and bivalve lineages. It also suggests that the asparagine and glycine-rich repeated structures evolved independently between them. Taken together with the fundamental role of CAs in biomineralization, some parts of the shell formation mechanisms, or perhaps the shells themselves, may not be homologous between gastropods and bivalves.