

Amino acid composition in molluscan shells and its relation to taxa and shell microstructure ~ Toward reconstruction of ancient trophic level using compound specific isotope analysis of amino acids ~

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We may clarify the trophic level of fossils applying “trophic level estimation method using compound specific isotope analysis of amino acids” (Chikaraishi et al., 2009). When applying the method to fossils, we must pay attention to whether amino acids in fossils are original or not, so screening of fossil samples is important. We can apply "amino acid composition conforming to shell microstructure" to the screening of fossil samples. This depends on a study showing the amino acid composition is different for each shell microstructure in the present bivalve (Kobayashi and Samata, 2006). We may determine whether amino acids present in the fossil shell are original or not by confirming “whether there is an amino acid composition specific to the shell microstructure observed in the fossil”. However, Kobayashi and Samata (2006) showed only the difference in amino acid composition each shell microstructures in the bivalvia, and not showed stability in the lineage relationship of the same microstructure. Moreover, there are problems such as the fraction of the amino acid composition, the method used, and the equipment to be used are different for each article, so it is difficult to compare for each article easily. In addition, there are no papers showing amino acid composition of Protobranchia of bivalvia. From the above, the stability of the amino acid composition for each shell microstructures in the lineage is unknown. Therefore, we set the purpose of this research that clarifying the stability of the amino acid composition of each shell microstructure in molluscan from the present raw sample. In this study, we focus on nacreous structure because this structure contain a lot of organic matter and it is primitive structure. We will clarify the stability of the amino acid composition of each taxa and shell microstructure, comparing within each taxa and lineage of bivalve, gastropod, and cephalopod.

As a result of cluster analysis, we found that the amino acid composition can be roughly divided into two groups, "primitive group" and "derivative group", in both the gastropoda and bivalvia. The amino acid composition of *Turbo sazae*, *Turbo mamoratus* and *Lunella coreensis* of gastropoda belonging to the same family were stable. Moreover, in the bivalvia, the amino acid composition is stable in the Pteriomorpha and Palaeoheterodonta, and in the Protobranchia, respectively. We could find the differences in the amount of alanine etc. in the Pteriomorpha- Palaeoheterodonta lineage and Protobranchia lineage. From this, it can be said that the amino acid composition of the nacreous structure is stable in family in gastropoda and stable in subclass in bivalvia. Cluster analysis showed that "gastropoda group" and "bivalvia group" have relatively close relationships, and their amino acid composition is similar in that alanine and glycine are abundantly included. However, it has been suggested that proteins used for shell formation in bivalvia and gastropoda may have been obtained independently in the process of evolution (Mann et al., 2018). Since amino acids which are likely to be included depending on the function of the protein are determined, it is thought that the amino acid composition converges evolutionarily and is similar even if it has different proteins.

Through this study, both bivalvia and gastropoda are roughly divided into two groups, "primitive group" and "derivative group", and furthermore, we could set the amino acid compositions used for screening of

fossil samples of nacreous structure to five, "Nautilus", "primitive of Vetigastropoda", "derivative of Vetigastropoda", "primitive of Bivalvia (Protobranchia)", "derivative of Bivalvia (Pteriomorpha, Palaeoheterodonta)". Therefore, we will be able to screen fossil samples of nacreous structure more precisely by using "amino acid composition conforming to lineage-shell microstructure" for trophic level estimation.

Keywords: amino acids, Phylogenetic relationships, trophic level, molluscs