

Influence of cold seep methane on the forming of echinoderm skeletons

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Recently, a few modern species of echinoderms have been reported as a member of the chemosynthetic community organisms, and at least two fossil species of echinoderms have been found from cold seep deposits (Pawson and Vance, 2004; Gaillard *et al.*, 2011; Landman *et al.*, 2012). In order to understand how these echinoderms from cold seep environment take carbon to form their skeletons, two analyses were done; 1) stable carbon isotope analysis of the fossil skeletons, and 2) stable carbon isotope analysis of a living sea urchins in aquaria under environments of controlled stable carbon isotope ratios ($\delta^{13}\text{C}$).

The fossil echinoderms have been collected and studied from two cold seep areas; the Upper Cretaceous (Campanian) in South Dakota, USA and the coeval formation in Hokkaido, Japan. The both crinoids from South Dakota and Hokkaido have clearly lower $\delta^{13}\text{C}$ values than normal echinoderms, suggesting their skeletons were influenced by cold seep methane which has extremely low $\delta^{13}\text{C}$ values (Kato and Oji, 2015). However, it was still unknown how these crinoids formed their skeletons with such low $\delta^{13}\text{C}$ values.

A lot of marine organisms such as many mollusks, are considered to precipitate their skeletons in equilibrium with the ambient sea water in regards to their carbon isotope (Epstein *et al.*, 1951). For example, bivalves shells from a chemosynthetic community show $\pm 5\%$ $\delta^{13}\text{C}$ values (e.g. Mae *et al.*, 2007). However, echinoderm skeletons, even from a normal (non-seep) environment, are known to be formed not in isotopic equilibrium, and the $\delta^{13}\text{C}$ values of the echinoderms skeletons are different depending on the classes or species (e.g. Weber, 1968). Thus, in echinoderm biomineralization, an isotope fractionation due to vital effect or some carbon sources other than from sea water should be possible.

In order to clarify the cause of inequilibrium of the $\delta^{13}\text{C}$ value in the skeletons of echinoderms and to estimate degree of isotope fractionation in forming skeletons, specimens of modern echinoid (*Strongylocentrotus intermedius*) have been raised in two experiments; 1) the echinoids has been raised under three types of sea water with differently controlled $\delta^{13}\text{C}$ value of DIC (dissolved inorganic carbon), and 2) with two different types diets (a kelp (*Saccharina longissima*) and a land plant (*Fallopia sachalinensis*)) with different $\delta^{13}\text{C}$ value.

Compared with the controlled case of culture with normal sea water with seaweed diet, $\delta^{13}\text{C}$ value of the echinoid test changed in both cases (in waters with high $\delta^{13}\text{C}$ value, and with different diets. Thus, it suggests that skeletons $\delta^{13}\text{C}$ of this echinoid, are influenced by isotope ratio of both sea water and foods. This result clearly shows that the process of isotope fractionation and forming skeletons of echinoderms should be different from those of most of mollusks. It is thought that the echinoderms in cold seep environment depend on bacteria mat for their diet, or detritus that have low $\delta^{13}\text{C}$.

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