Search for deep-sea subterranean biospheres by analyzing phosphatase activities and amino acids

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It has been known that there are vast subterranean biospheres. On the other hand, submarine hydrothermal systems have been considered as possible sites for the origin of life on the earth. Thus biospheres in submarine hydrothermal sub-vents are interesting from the point of view of astrobiology: origins, evolution and distribution of life. As members of Archaean Park Project, we analyzed amino acids and phosphatase activities in core and chimney samples obtained in Suiyo Seamount and South Mariana submarine hydrothermal systems, and discussed possible subterranean biospheres in hydrothermal systems.

Core samples and chimney samples were collected as a part of the Archaean Park Project at the Suiyo Seamount, Izu-Bonin Arc, the Pacific Ocean in 2001 and 2002, and in South Mariana hydrothermal systems in the Pacific Ocean in 2003.

Phospatase activity in solid (rock) samples was measured spectrophotometrically by using 25 mM p-nitrophenyl phosphate (pH 8.0 or pH 6.5) as a substrate: Pulverized sample was incuvated with substrate solution for an hour, and then production rate of p-nitrophenol was calculated with absorbance change at 410 nm. Phosphatase activity in extracts was measured fluorometrically by using 4-methylumberyferryl phosphate as a substrate. Amino acids and their enantiomeric ratio were determined by HPLC after digestion of the samples with HF followed by hydrolysis with 6 M HCl at 383K.

Significant enzymatic activities were revealed in hydrothermal sub-vent systems of Suiyo Seamount, which is crucial evidence of vigorous microbial oasis. It is consistent with the fact that large enantiomeric excess of L-form biogenic amino acids were found in the same core sequences. On the other hand, Mariana core samples showed significant level of phosphatase activities only at the surface. Hydrothermal fluid can go through Suiyo core samples, but it hardly go through Mariana core samples, which seems to made the difference.

The outer part of the chimney samples showed higher phosphatase activities than the inner part. When the activity in extract from the chimney samples both at 310 K and 353 K, both were almost the same while the former showed much higher than the latter in the case of extracts from usual rock samples. Estimated molecular weight of the extracted phosphatase was ca. 100,000, and the activity was inhibited with EDTA. These facts suggested that the activity found in chimney samples are caused by metalloenzymes of thermophiles.

The present results of phosphatase activity and amino acids agreed with the results of organic and microbial analyses. It was shown that phosphatase activity is one of possible biomarkers for extant life.