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復元した祖先型タンパク質の耐熱性と触媒活性の pH 特性
The pH profiles of the catalytic efficiency and thermal stability of resurrected ancestral proteins

笹本 峻弘 [1*] ; 赤沼 哲史 [1] ; 別所 瑞萌 [1] ; 横堀 伸一 [1] ; 山岸 明彦 [1]
SASAMOTO, Takahiro[1*] ; AKANUMA, Satoshi[1] ; BESSHO, Mizumo[1] ; YOKOBORI, Shin-ichi[1] ; YAMAGISHI, Akihiko[1]

[1] 東京薬科大学・生命科学部・応用生命科学科
[1]Dept. of Appl. Life Sci., Tokyo Univ. of Pharm. Life Sci.

Little is known about the geological setting of the early earth. However, the method to estimate the ancient environment with genetic information has been emerging (Akanuma *et al*., 2013). The genetic information of ancestral life has been inherited to its descendants. We can therefore infer the amino acid sequence of an ancestral protein by comparing a huge number of extant amino acid sequences that have evolved from a single common ancestor. Because the amino acid sequence of a protein is encoded by DNA sequence, an ancestral protein's amino acid sequence has been also inherited in its descendants, i.e. extant proteins' sequences. We can estimate the ancient environment by reconstructing an ancestral protein and analyzing its physicochemical properties. In addition, physicochemical properties of a protein have often related to the environment of its host.

We have previously estimate the environment of early life by resurrecting ancestral nucleoside diphosphate kinases (NDKs). The amino acid sequences of ancestral NDKs that might be possessed by the last common ancestors of Archaea and of Bacteria were inferred by phylogenetic analyses. The inferred amino acid sequences were reconstructed by using the genetic engineering techniques. Because the ancestral amino acid sequences fold into extremely thermally stable proteins, we concluded that both archaeal and bacterial ancestors were hyperthermophilic. This conclusion is robust because significantly similar characteristics were observed for the ancestral proteins predicted by several different methods. We also estimated that the last universal common ancestor, the Commonote, was a thermophile or a hyperthermophile that thrived at a temperature above 75 ℃ (Akanuma *et al*., 2013).

In the current study, we have attempted to estimate the surface pH of early earth. We analyzed the pH profiles for catalytic efficiency and thermal stability of the ancestral NDKs that might exist 3.5-4.0 billion years ago. The specific activities at 70 ℃ were determined at pHs ranging from 5.5 to 10.0. All of the ancestral NDKs showed the highest activity at pH 9.5 or 10. The same was true for several NDKs of extant microorganisms that grow optimally at an acidic or neutral pH. Therefore, the optimum pH for catalytic efficiency of a NDK does not reflect its host's environment.

We also analyzed the pH dependence of thermal stability of the ancestral and extant NDKs. The extant NDKs from Sulfolobus tolodaii and Thermoplasma acidophilum, which grow optimally at acidic pHs, are stable at both acid and neutral pHs. In contrast, the NDKs of Thermus thermophilus and Archaeoglobus fulgidus, which grow optimally at neutral pHs, showed the greatest thermal stability at a neural pH (pH 6.0 or 7.6) and less stability at an acidic pH (pH 4.5). Because most of the ancestral NDKs also showed the greatest thermal stability at pH 6.0 or 7.6 and were less stable at pH 4.5, we concluded that the ancient organisms such as the archaeal ancestor, bacterial ancestor and the Commonote lived at neutral pHs. However, we cannot rule out the possibility that the ancient organisms lived at an acidic pH because a few ancestral NDKs showed the greatest thermal stability at pH 4.5.

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