

Elucidation of salt adaptation mechanism of halophilic enzymes
based on the protein structure

Taketomo Fujiwara

(Department of Biology and Geosciences, Faculty of Science, Shizuoka University)

Summary

Some microbes are adapted to living in the extreme saline conditions which are fatal to the common organisms, such as salt lake or salt pond. In such microbes, the proteins generally show the feature of 'halophilicity' which reveals the requirement of high concentration of salts for the stability and/or the functional activity.

Based on the crystal structure of catalase-peroxidase (HmCP) from extremely halophile *Haloarcula marismortui*, we have proposed that the strong hydration and salt-bridge formation by the acidic residues on the protein surface would be important to the stability of the protein¹⁾. In this study, we tried purification, crystallization and structural analysis of additional two sorts of halophilic enzymes, *H. marismortui* nitrite reductase (HmNirK) and *Haloferax volcanii* catalase peroxidase (HvCP). Moreover, we planned the point-mutation experiment targeting the salt-bridge structure in the HmCP molecule.

The HmNirK crystal obtained in this study gave X-ray diffraction image with 4Å resolution. The crystal of HvCP, an orthologue of HmCP, was also obtained. Crystallization condition and anti-freezing reagent composition are now optimizing for both of the enzymes. Furthermore, by utilizing the *Haloferax-Escherichia coli* shuttle vector pWL102, we succeeded in introducing the point-mutation (Tyr218 ->Ala) to the amino acid residue neighboring the catalytic center of HmCP. Molecule mechanism of salt adaptation is expected to become clear by advancing the structural analysis and the mutation experiment of the halophilic proteins.

- 1) Yamada, Y., Fujiwara, T., Sato, T., Igarashi, N. & Tanaka, N. (2002) The 2.0 crystal structure of catalase-peroxidase from *Haloarcula marismortui*. *Nature Structural Biology* 9(9), 691-5.