Structural analysis of the halophilic enzyme which requires a high ionic strength for its stability and activity

Taketomo Fujiwara
Faculty of Science, Shizuoka University

Noriyuki Igarashi
Institute of Materials Structure Science,
High Energy Accelerator Research Organization

Summary

Catalase-peroxidase purified from an extremely halophilic archaeon, *Haloarcula marismortui,* is a typical halophilic enzymes that requires molar salts for its stability and enzymatic activity. We tried and succeeded in the X-ray structural analysis of the *H. marismortui* catalase-peroxidase (HmCP) crystal to understand the 'haloadaptation' based on the three dimensional structure of the halophilic enzymes.

The 2.0 Å crystal structure of HmCP clarified that the molecule is an assembly of two identical subunits, each of which is composed of two homologous halves, and four halves from the two subunits are packed together to make the pseudo-tetramer. As generally known in the halophilic proteins, a content of acidic (Glu and Asp) residues in the sequence of HmCP is very high (19.7 %). The crystal structure revealed that the ratio of the acidic residues on the surface of the HmCP molecule was accounted for more than 50%, in contrast to the value of about 20 % in the non-halophilic catalase-peroxidases. The result suggests that the 'haloadaptation' of the HmCP is achieved by recruiting anions to the protein surface to reduce the electrostatic potential in the high salt concentration. A large number of water molecules (1,360 moles per mole of HmCP) was bound with the acidic residues on the surface of HmCP dimer by the hydrogen-bonding, preventing the salting out and irregular aggregation of the proteins in the extreme salinity. The HmCP dimer also binds several decades of solvent ions, most of them were assigned as Cl-, from the crystallization solution in addition to the water molecules. They bind with basic residues or amide of the peptide chains to stabilize both of the peptide conformation and the association of subunits.