Crystal Structure and Structural Stability of Acylphosphatase from Hyperthermophilic Archaea *Pyrococcus Horikoshii* OT3

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Pyrococcus horikoshii OT3 is a hyperthermophilic archaea that grows at temperatures between 88 ºC and 104 ºC. Proteins produced by this archaea possess high thermostability. To elucidate the structural basis for the high stability of acylphosphatase (AcP) from *P. horikoshii* OT3, we determined its crystal structure at 1.72 Å resolution. *P. horikoshii* AcP possesses high stability despite its approximately 30% sequence identity with eukaryotic enzymes that have moderate thermostability. Comparison with the crystal structure of eukaryotic AcP revealed some significant characteristics in *P. horikoshii* AcP that likely play important roles in structural stability: (i) shortening of the flexible N-terminal region and long loop; (ii) an increased number of ion pairs on the protein surface; (iii) stabilization of the loop structure by hydrogen bonds. In *P. horikoshii* AcP, two ion pair networks were observed, one located in the loop structure positioned near the C-terminus, and other on the β-sheet. The importance of ion pairs for structural stability was confirmed by site-directed mutation and denaturation induced by guanidium chloride.

Keywords: structure and stability of protein, thermostable, x-ray crystallography