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Halophilic Mechanisms and Industrial Application of Enzymes from Halophiles

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Summary

We have studied halophilism of enzymes from extremely halophilic archaea and moderately halophilic bacteria. Extremely halophilic archaea require salt concentrations greater than 2 M for their growth, and almost all enzymes from these organisms require at least 1 M salts for their stability and activity. Moderately halophilic bacteria can grow in a much wider range of salt concentrations, e.g., from 0.2 M NaCl to saturation and enzymes from these bacteria also show the wide range of salt concentration-dependent characteristics.

Most halophilic enzymes are highly acidic and negatively charged under the physiological conditions. In fact, the amino acid composition of nucleoside diphosphate kinase (NDK) from the cytoplasm of extremely halophilic archaeon (HsNDK) and enzymes from extracellular or periplasmic spaces of moderately halophilic bacteria were characterized by an abundant content of acidic amino acids. We have proposed a working hypothesis for the mechanisms of enzymatic halophilicity as follows: the highly acidic molecular nature of halophilic proteins generates a high solubility and hence tolerance against salting out effects of higher concentration of salts. This property also ensures highly efficient renaturation without irreversible aggregation after heat- or urea-denaturation. This denaturation-resistant property as well as higher activity and stability in the presence of high salt concentrations make halophilic enzymes attractive for the industrial application. Following data were obtained in this three years project:

(1) The crystal structure of HsNDK was solved to a resolution of 2.2 angstrom and the electrostatic surface potential of HsNDK was found to be highly negative as expected from its high contents of acidic amino acid residues.

(2) We have isolated NDK and its gene from moderately halophilic bacterium, *Halomonas* sp. 593 (HaNDK) and compared the enzyme to its counterpart from non-halophilic bacterium, *Pseudomonas aeruginosa* PAO1 (PaNDK).

(3) We demonstrated that HaNDK formed a dimeric structure and this is the first isolation of active dimeric subunit structure of NDK.

(4) Charged state of two residues that are located in the C-terminal region (134th and 135th residues) of HaNDK and PaNDK plays a critical role in determining halophilic characteristic, and we succeeded in donation of halophilic properties to the non-halophilic enzyme, PaNDK.