# Protein folding of halophilic enzymes.

Masao Tokunaga, Matsujiro Ishibashi,
Hiroko Tokunaga and Mayumi Miyauchi
Faculty of Agriculture, Kagoshima University

We have attempted to characterize halophilic enzymes, aiming at expansion of their commercial applications. Extremely halophilic archaea require more than 2.5 M NaCl for growth, and accumulate high concentration of compatible solutes inside the cells. Thus, the industrial application of halophilic enzymes is very attractive, since these enzymes can function under the extreme conditions where most of the 'normal' enzymes cannot. However, they always require high salt concentration for its stability, and this basic property is the main reason why the number of halophilic enzymes isolated in pure form is very small. We think the most important points of research are (1) the understanding of folding mechanisms of halophilic enzymes and (2) the establishment of refolding conditions of (partially) unfolded state of these enzymes.

We studied molecular chaperone DnaK from extremely halophilic archaea *Halobacterium cutirubrum*. This halophilic DnaK requires the presence of more than 2 M NaCl for binding to the ATP column. We found that one protein bound to ATP column without high salt concentrations. This protein was identified to be nucleoside diphosphate kinase (NDK), and was stable without high salt. We purified and studied the properties of NDK, and isolated its gene. Nucleotide sequence analysis demonstrates that NDK conserves the amino acid composition characteristic of halophilic enzymes, i.e. high content of acidic amino acid residues, although it does not require high salt for its stability and activity. We found that NDK is a very good model enzyme for the study of halophilic protein folding.