Comparative study on the halophilism of the soluble and membrane proteins in Halobacteria.

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Summary

An extremely halophilic archaebacteria not only tolerate but require NaCl concentrations above 10 to 15 % for survival. In order to adapt to the hypersaline environment, living organisms should acquire mechanisms to prevent the breakdown of the membrane system due to the osmotic pressure. There are two different modes of adaptation. One is the accumulation of organic neutral solutes concomitant with extrusion of the inorganic ions. The other one is the accumulation of inorganic ions to balance the extracellular high salt concentration. The extremely halophilic archaebacteria acquired the latter mechanisms. Therefore, all the cellular components have to be adapted to function at the extremely high salt concentrations. In fact halophilic malate dehydrogenase (hMDH) requires high salt concentrations (1 to 4 M) both for the stability and the activity. Comparison of the 3D structures of hMDH and ferredoxin and their nonhalophilic cognates revealed structural features for adaptation to high salt concentrations (halophilic substitution and halophilic addition).

In contrast, little is known about the halophilism of the membrane systems in the extremely halophilic archaebacteria. In this study we compared the structures of retinal proteins. Bacteriorhodopsin and archaerhodopsin form the 2D crystals in the membrane and are stable at both low and high salt concentrations. On the other hand halorhodopsin does not form the 2D crystals in the membrane and is not stable at low salt concentrations. However, there are no remarkable differences in the distribution of the charged acidic residues (numbers and positions) at the surface loops regions among these retinal proteins. Furthermore, when the haloopsin (*hop*) gene was expressed in *Schizosaccharomyces pombe*, the seven transmembrane α-helices were properly folded in the organelle membranes. *S. pombe* harboring the *hop* gene colored purple when growing in the presence of retinal.

The present results indicated that the high salt concentration was not required for the *in vivo* folding of the integrated membrane proteins. Since there is little correlation between the salt stability and the distribution of acidic amino acids in retinal proteins, it is necessary to study the effects of salt on the stability of another types of membrane proteins.