The effects of salt on the stability of the light-driven chloride pump, halorhodopsin.

Yasuo Sugiyama
Division of Biological Science, Graduate School of Science, Nagoya University

Summary

Extremely halophilic archaebacteria grow in a medium containing 25% (w/v) NaCl and balance the external high salt concentration by accumulating within the cell the inorganic ions such as KCl. Therefore, all the cellular components have to be adapted to function at the extremely high intracellular concentration of salt. X-ray crystallography of halophilic malate dehydrogenase and ferredoxin revealed the structural features that promote the stability of the soluble proteins at the high salt concentrations. On the other hand, littel is known about the stability of the membrane-embedded proteins under the high salt conditions. To address this issue, we compared the stability of halorhodopsin/purple membrane (hR/PM) with that of bacteriorhodopsin/purple membrane (bR/PM). It was known that the chromophore of hR/PM was gradually bleached even at 4 °C. In this work, we studied the effects of chaotropic ions and SDS on hR/PM. Furthermore, the thermal stability of hR/PM and the purified hR was examined.

1. Low concentrations of SDS (<cmc) induced the blue form of bR/PM in a reversible manner. bR/PM was denatured with increasing the concentrations of SDS (>cmc). Denaturation proceeded via an intermediate with $\lambda max = 450$ nm. On the other hand, low concentrations of SDS accelarated the isothermal chromophore bleaching (denaturation) of hR/PM because the 570 nm chromophore changed to the 380 nm species without an intermediate. This indicated that the binding of SDS monomer changed the tertiary structure of hR/PM, which led to the hydrolysis of the retinal Schiff base linkage.

2. Whereas bR/PM was stable at 80 °C, hR/PM irreversibly denatured at room temperature and the denaturation rate increased with increasing the temperature. The denaturation proceeded with two independent processes (fast and slow phases) and the fraction of fast phase increased at higher temperature. Isothermal denaturation of purified hR diminished at high concentrations of salt and low concentrations of MEGA-9. Then the micellar structure of the detergents and/or lipid would affect the stability of hR and salt may restrict the thermal motion of these mixed micelle structure.