9528 Effects of salt on the stability of light-driven chloride pump, halorhodopsin.

Yasuo Sugiyama and Kazuki Nagano
Department of Biology, School of Science, Nagoya University

Summary

Halophilic archaebacteria have the characteristic retinal proteins in their cytoplasmic membrane. Bacteriorhodopsin (bR) is a light-driven proton pump and forms a two-dimensional crystalline patches in the membrane, which is known as purple membrane (PM). bR in PM is stable in a medium containing high concentration of salts, an acidic or alkaline solution and furthermore at high temperature (above 100 °C). In contrast, the amount of halorhodopsin (hR), a light-driven chloride pump, is less than 10% of the bR content and the crystalline membrane domain of hR does not naturally form. It was reported that hR in the membrane was not stable in 0.1 M NaCl and slowly denatured . However, it was difficult to examine the effects of salts and temperature on the stability of hR systematically, since only 3 mg of hR was purified from 80 L culture. Recently, recombinant halobacterium overexpressing hR was constructed from which large amounts of hR were easily isolated. In this study we examined the stability of hR chromophore under the various storage conditions.

hR/PM was prepared from hR overexpressing *Halobacterium salinarium*. SDS-PAGE of hR/PM indicated that the purity of hR was 40-50 %. Since the absorbance at 565 nm decreased by 20 % after one week at 4 °C, hR/PM was stable even in distilled water.

hR was purified by a phenyl-Sepharose column chromatography in the presence of MEGA-9. The absorbance at 570 nm of purified hR in 0.5 % MEGA-9 decreased and became to be 30 % after one week even in the presence of 2 M NaCl (isothermal bleaching). The rate of this bleaching was accelerated in a low salt medium and at a higher temperature. However, when MEGA-9 was removed from the purified hR by dialysis, hR was very stable in the range from 4 °C to 40 °C. Therefore, the stability of hR was related to the concentration of salt, detergent and temperature.