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主　論　文　の　要　旨

論文題目 Crystallographic Studies on Archaeal Light-driven Proton Pumps
（古細菌の光駆動プロトンポンプの結晶構造解析）

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論　文　内　容　の　要　旨

To acquire energy for metabolism, organisms develop different strategies to obtain energy from the environment. One of the most important energy sources is sunlight. Plants and algae convert light energy to chemical energy by photosynthesis process, in which carbon dioxide and water are converted to sugar and oxygen by chlorophyll. On the other hand, archaea and some other microorganism convert light energy to a proton gradient by retinal-containing membrane proteins which function as light-driven proton pumps.

Since the discovery of the first light-driven proton pump -- bacteriorhodopsin (bR) -- in early 1970s, efforts have been made to understand the structures and mechanism of light-driven proton pumps for more than four decades. To date, several proton-pumping rhodopsins from various types of organisms have been crystallized and their structures have been determined. Comparison of these different structures would provide an insight into essential structural motifs that are relevant to the pumping activity. In this thesis we report the crystal structure and spectroscopic properties of cruxrhodopsin-3, which is a newly found member of archaeal light-driven proton pump; we also discuss about the spectroscopic properties of archaerhodopsin-3.

Cruxrhodopsin-3 (cR3) is a new member of archaeal light-driven proton pump that is found in *Haloarcula vallismortis*. Recently we have successfully crystallized cR3 by the membrane fusion method into crystals with space group *P321*, which diffracts x-ray to 2.1 angstrom resolution. Similar to the crystal structures of archaeal rhodopsins (bacteriorhodopisin, archaerhodopsin-2, and deltarhodopsin-3), cR3 consists of seven transmembrane alpha helices, with a beta sheet located at the BC loop region on the extracellular side. The proton-releasing group, which consists of two glutamates facing each other as a low barrier hydrogen bond, is conserved among all the archaeal proton-pumps crystallized by the membrane fusion method.

Detail structural analysis shows that cR3 consists of the following specific structural properties: i) cR3 has a remarkably long DE loop which interacts with a neighboring subunit, strengthening the trimeric structure; ii) three positively charged residues are pointing outward at the cytoplasmics end of helix F, influencing the packing of cR3 assembly; iii) the cytoplasmics part of helix E is bent, such that it opens a possible path for proton uptake; iv) an extra short helix near the C terminal covers the cytoplasmic surface of the protein; v) the retinal binding pocket of cR3 is more rigid than that of bR; and the increase in rigidity of the retinal binding pocket restricts the motional freedom of retinal, affecting formation/decay kinetics of the early reaction intermediates in the proton-pumping photocycle.

Photobleaching experiment showed that the photo-stability of cR3 decreased dramatically when cR3 monomer was dissociated from the trimeric assembly embedded in claret membrane by mixing with an excess amount of detergent. This result suggests that the trimeric structure / protein-lipid assembly increases the photo-stability of cR3.

A carotenoid bacterioruberin was observed at the crevices between subunits of cR3 trimer, surrounded by helices AB and DE of neighboring subunits. The similar binding of carotenoid molecules between subunits within the trimeric structure was also observed in different archaeal rhodopisn. It is possible that such binding strengthens the structural stability to the trimeric structure of microbial rhodopisn.

An homologous protein of archaerhodopsin-2, archaerhodopsin-3 (aR3) was also crystallized by the membrane fusion method. Although the size of aR-3 crystal was too tiny for crystallography study at the moment when this thesis was finished, the spectroscopic properties and the absorption kinetics of aR3 were determined.