Biophysics Interdepartmental Group

Ph.D. Thesis Defence

Wednesday April 21st – 9:00 am - SCIE Rm. 2315

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"BIOPHYSICAL CHARACTERIZATION OF A NEW GROUP OF RHODOPSINS FROM PHOTOSYNTHETIC ORGANISMS"

ABSTRACT

Microbial rhodopsins are ubiquitous light-activated proteins serving as light-driven ion pumps, light-gated ion channels, and photosensors in procaryotic and eucaryotic organisms. The structure of these transmembrane proteins is formed by seven helices which create an interior binding pocket for the retinal chromophore attached by a protonated Schiff base linkage to a lysine in the middle of the seventh helix. Until this century, all microbial rhodopsins were considered to be close homologs of a well-studied prototypical protein, bacteriorhodopsin (BR). In the last decade, another large group of microbial rhodopsins, typified by proteorhodopsin (PR), was discovered in marine bacterial plankton. Very recently, it became clear that the taxonomic and geographic diversity of microbial rhodopsins is even higher, as many more interesting rhodopsin types were discovered. With the sequences intermediate between those of BR and PR, one of the most interesting new groups combines taxonomically diverse proteins structurally similar to recently discovered carotenoid-binding xanthorhodopsin (XR). Besides XR, other members of this family have not been studied until now. To learn more about this new group, we characterized its two members, which come from photosynthetic organisms, biophysically. *Gloeobacter* rhodopsin (GR) and *Pyrocystis* rhodopsin (PyrR) are found in *Bacteria* and *Eukarya* domain, respectively, belonging to cyanobacteria and dinoflagellate algae. We have elucidated the photochemical cycles and pathways of light-driven proton translocation of GR and PyrR using the combination of site-directed mutagenesis, heterologous expression, and three biophysical techniques: Raman, time-resolved FTIR, and time-resolved visible spectroscopies. We did a systematic comparison with the data from the well-known rhodopsins, such as BR and PR, to understand the unique photochemical properties of the new group, often intermediate between those of BR and PR. The proton-pumping function was confirmed for both rhodopsins, and the proton acceptor and proton donor for the active center were identified as Asp121 and Glu132, in GR, and Asp 101 and Glu 112, in PyrR, respectively. We found many interesting features, both common for the group, and unique for each protein. For instance, one of the striking traits of GR is its unusually strong coupling between the cytoplasmic proton donor and the retinal, while PyrR shows two unique protein vibrations in the light-induced FTIR difference spectra.

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