Abstract of Doctoral Dissertation

Degree requested Doctor of Life Science Applicant's name Shariful Haque

Title of Doctoral Dissertation

Driving the Photochemical Reaction Cycle of Proteorhodopsin and Bacteriorhodopsin Analogues by Photoisomerization of Azo Chromophores

(アゾベンゼンクロモフォアによるプロテオロドプシンとバクテリオロドプシンアナログのフォトサイクルの駆動)

Microbial rhodopsins, photochromic retinal proteins are highly efficient and robust molecular machines to transduce the light energy into chemical energy or into physiological signals. Photoisomerization of retinal induces a cascade of photophysical and photochemical phenomena which lead the photofunctional properties of microbial rhodopsins. Due to their various technical application and in particular optical application, numerous studies have been executed to elucidate function and mechanism of these molecular machines. Azobenzene, an artificial chromophore which has unique photochromic properties can be a suitable alternative of retinal to know the molecular mechanism of photofunctional properties of microbial rhodopsins, modulate the photofunctional properties of them and to develop more efficient artificial machine based on microbial opsin protein or other chemical structure. Till now, there was a single report of azo chromophores 4-[[4′-(*N,N*-dimethylamino)phenyl-1′]azo]-benzaldehyde (denoted as Az I) and 3-[4-[[4′-(*N,N*-dimethylamino)phenyl-1′]azo]phenyl-1]prop-2-enal (denoted as Az II) bound bacteriorhodopsin analogues. But, in the previous report, photochemical reactions and photo-induced proton pumping function of azo chromophores bound bacteriorhodopsin analogues are ambiguous.

In this thesis, the author reports the successful substitution of retinal of proteorhodopsin (PR) and bacteriorhodopsin (BR) by azo chromophores. Also, the author reports the photo-induced proton transfer reaction and the characteristic photocycle comprising of multiple steps of azo chromophores bound PR and BR. The author studied the photo-induced proton pumping function of azo chromophores bound proteorhodopsin and bacteriorhodopsin analogues. However, it was found that azo chromophores bound PR and BR analogues cannot pump the proton upon illumination. The author describes through the mutational analysis why azo chromophores bound PR and BR analogues cannot pump the proton upon illumination. The photo-induced proton pumping malfunction of azo chromophores bound PR and BR analogues would be caused by the absence of photo-induced proton transfer reactions through the cytoplasmic side even though photo-induced proton transfer reactions through the extracellular side were observed. Furthermore, the author reports the design and synthesis of a new azo chromophore 4-[[2′,6′-dimethoxy, 4′-(*N,N*-dimethylamino)phenyl-1′]azo]-benzaldehyde (denoted Az III). It was observed that azo chromophore Az III bound BR analogue denoted as BAz III showed higher efficiency in photo-induced proton transfer reactions compared to Az I and Az II chromophores bound bacteriorhodopsin analogues. Also, the author proposes a putative model of photo-induced proton transfer reaction based on experimental results along with other findings.

This study shows the advances for semi-artificial molecular machine and provides some fruitful information about the azo chromophores bound PR and BR analogues. It can be expected that this study can help to design a new azo chromophore bound microbial rhodopsin pigment to drive the ion pumping upon illumination. This study strongly implies that photoisomerization of azo chromophores may induce the conformational changes of proteoopsin and bacterioopsin proteins. In future, the other photofunctional properties of azo chromophores bound PR and BR analogues will be studied.