chromatic adaptation. Unlike the most microbial rhodopsins, the cytoplasmic (CP) half of ASR is unusually hydrophilic due to many polar residues and water molecules. Basically, ASR lacks electrogenic proton transport. However, unusual proton transfer was observed: the Schiff base proton is transferred to Asp217 in the CP domain upon the formation of M photo-intermediate (Shi *et al.*, *J. Mol. Biol.* 2006). Thus, ASR has a potential to move proton in the direction opposite to that of proton-pumping rhodopsins.

In this study, we examined the proton transfer reactions of ASR by using indium-tin oxide electrode, which enables the time-dependent measurements of local pH changes by flash-activated ASR. At the neutral pH, ASR induces the transient alkalization of the local medium, indicating that photolyzed ASR accepts proton from the medium and then releases it at the same side. On the other hand, above pH 8, the sequence of the proton movement is reversed: proton release occurs first, followed by uptake. This suggests that a certain residue acts as proton releasing residue in the alkaline condition. These proton transfers were greatly modified by the mutations of Asp217 and Glu36 locating at the CP domain. Thus, the proton transfers mainly occur through this hydrophilic channel. Based on this analysis, we will discuss the proton transfer reactions of ASR.

2PT178 ウシロドプシンの構造に関する理論研究

A theoretical study on the structure of bovine rhodopsins

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Rhodopsin is a photoreceptor in the retina. It consists of the protein moiety opsin and an all-trans-retinal which is derived from Vitamin A. The photoisomerization of the retinal induces the structural change of the protein and eventually activates the associated G-protein, transducin. The purpose of this study is to elucidate the structures of early intermediates during the photoreactions such as lumirhodopsin. Computationally, the structure around Schiff base of the retinal is difficult to be described with the classical forcefield, although accurate description of the structure around the retinal is critically important for the calculations of vibrational frequencies and the excitation energy. Therefore, QM/MM calculations must be involved to obtain reasonable structure. On the other hand, structural rearrangements/relaxations at finite temperature (not only fluctuations) of the system, which are usually not involved in QM/MM methods, shall not be ignored for the structural relaxation which takes more than nanoseconds. In order to fulfill these two requisites, in this study, we employed QM/MM-RWFE-SCF method [Kosugi and Hayashi, *JCTC*, 2011]. The structures and vibrational frequencies of the Schiff base in rhodopsin, bathorhodopsin, and lumirhodopsin will be discussed.

2PT179 グロエオバクターロドプシンの三量体形成に関する研究

Homo-trimeric assembly of a cyanobacterial ion-pump *Gloeobacter* rhodopsin

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Rhodopsin-like molecules in microorganisms (microbial rhodopsins) play roles as an ion-pump or a photosensor. The four microbial rhodopsins from halophilic archaea have been investigated well so far; bacteriorhodopsin (BR), halorhodopsin (HR), sensory rhodopsin I (SRI) and sensory rhodopsin II (SRII, also called phoborhodopsin). These proteins form intrinsic oligomers depending on their function. The phototaxis sensors SRI and SRII form a tetramer which is composed of two units of receptor-transducer hetero-dimer. On the other hand, the ion-pumps BR and HR form a homo-trimer. This is a unique structural property of them and recent studies have revealed several factors for the trimer formation and stabilization (Tsukamoto *et al.*, *Biophys. J.* 2012). Other than BR and HR, however, little is known about the trimer formation of the ion-pumping rhodopsins.

We have started to investigate the oligomeric structure of *Gloeobacter* rhodopsin (GR), which is an ion-pumping protein discovered from freshwater cyanobacterium, by means of size-exclusion chromatography (SEC) and circular dichroism (CD) spectroscopy. The analysis of SEC gave us a conclusion that GR formed a trimer as well as BR and HR. However, the CD spectrum of GR ((+) 500 nm/(-) 567 nm, crossover point; 540 nm) indicated that the trimeric state of GR was different from that of BR and HR. Interestingly, the trimeric state of GR altered in a pH-dependent manner. Based on these analyses, we will discuss the homo-trimeric structure of GR.

2PT180 パルミトイル修飾とロドプシンのラフト親和性の関係

Relationship between palmitoylations and raftophilicity of rhodopsin

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As we reported in the last meeting of BPSJ, we have found that rhodopsin forms dimer in the complex of retinal G protein transducin (G_t). The rhodopsin dimer favors *l_o* lipids and distributes in the detergent-resistant membrane (DRM) prepared from vertebrate (bovine and frog) rod photoreceptor outer segment membranes. Our single molecule observation of rhodopsin and transducin revealed that the increased affinity to raft of rhodopsin upon dimerization hinders the diffusion of rhodopsin. Here, we studied the contribution of tandem palmitoyls at the C terminal end of helix-8 of rhodopsin to the raftophilicity of rhodopsin dimer. We examined the effect of depalmitoylation of rhodopsin by reducing agent (20 mM dithiothreitol) on the distribution of rhodopsin-transducin complex or IgG-crosslinked (artificially dimerized) rhodopsin to the DRM of frog rod photoreceptor outer segments. Depalmitoylation was confirmed by MALDI-TOF MS. Rhodopsin-transducin complex in DRM fraction was reduced to almost half by depalmitoylation. When we artificially dimerized rhodopsin by anti-rhodopsin monoclonal antibody, significant portion of anti-rhodopsin IgG, which means rhodopsin dimer, was detected in DRM fractions, however, when we used depalmitoylated rhodopsin, much less IgG was detected in DRM fractions. These data support our idea that the palmitoyl modifications on rhodopsin are indispensable for raftophilicity of rhodopsin dimer.

2PT181 海洋性細菌におけるプロテオロドプシンの生理的役割および分子特性

Physiological role and molecular properties of proteorhodopsin from marine bacterium

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Nature created two kinds of light-driven ion pumps, bacteriorhodopsin (BR) and halorhodopsin (HR), functioning as outward proton and inward chloride pumps, respectively [1]. Then, thousands of proteorhodopsin (PR) have been discovered and are widely distributed among marine prokaryotes [2]. But its physiological role was unclear, because very few strains carrying the PR gene have been cultured. Recently, we demonstrated light-driven proton pump activity of PR in native *Flavobacteria* cells [3].

Here we studied the molecular mechanism and the physiological role of PR from *Krokinobacter* sp. in *Flavobacterium* that codes two microbial rhodopsin genes in their genome. We first examined their pump activity in native cells. It was found that PR is expressed in stationary phase, suggesting that PR-mediated electrochemical proton gradient is used to ATP synthesis when nutrition is depleted. We then studied the spectral and photochemical properties of recombinant PR from *Krokinobacter* (KroPR). KroPR shows λ_{max} at 525 nm (pH 7.0), being similar to GPR. On the other hand, pK_a of the counterion (4.3) is lower than that of GPR (6.9), although the amino acid identity between KroPR and GPR is 41 % and His75 (GPR) is conserved. Flash photolysis measurement of KroPR is in progress and the molecular mechanism of KroPR will be discussed.

[1] Kandori, *Mol. Sci.* 5, A0043 (2011).

[2] Beja *et al.* *Science* 289, 1902 (2000).

[3] Yoshizawa *et al.* *Environ. Microbiol.* 14, 1240 (2012).

2PT182 時間分解赤外分光法によるファラオニスハロロドプシンの機能発現過程での水の水素結合ダイナミクス

Water hydrogen-bonding dynamics in the function of *pharaonis* halorhodopsin studied by time-resolved FTIR spectroscopy

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pharaonis Halorhodopsin (pHR) is an inward chloride ion pump protein. pHR has a retinal as the chromophore, which is bound through the protonated Schiff base and all-*trans* to 13-*cis* photoisomerization triggers conformational changes of the protein moiety for pumping a chloride ion. The ion transportation is performed through the sequential formation of some intermediates which are named K, L₁, L₂, N and O. The structural detail of each intermediate state is still