pharaonis phoborhodopsin (ppR, also called *pharaonis* sensory rhodopsin II; pSRII) which has a slower photocycle than BR. We made several constructs of the ppR chimera containing the third cytoplasmic loop of bovine rhodopsin. Molecular properties of the ppR chimera proteins are currently investigated, and we will present the results together with the G-protein activation.

[1] T. Yamashita, A. Terakita and Y. Shichida, J. Biol. Chem. 275, 34272-34279, (2000). [2] Geiser et al. Protein Sci. 15, 1679-1690 (2006).

1P-259 活性中間体の pH 依存的平衡に着目したロドプシンの活性化機構の解析

Elucidation of the activation mechanism of rhodopsin based on the analysis of pH dependent equilibrium between metarhodopsin I and II

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Rhodopsin is a visual pigment present in rod photoreceptor cells and contains a retinal chromophore bound to the protein moiety via a protonated Schiff base linkage. On absorption of a photon, rhodopsin converts to an equilibrium mixture between G protein-activating intermediate metarhodopsin II (MII) and its precursor metarhodopsin I (MI). The pH profile of the equilibrium is opposite to the predicted one from the protonation states of the Schiff base in these intermediates. That is, high pH (low proton concentration) favors MI and low pH (high proton concentration) favors MII, although the MI and MII contain protonated and unprotonated Schiff bases, respectively. This suggests that there is a dissociable residue(s) in the protein moiety, which regulates the pH profile of the equilibrium, and this residue should connect with the retinal Schiff base through a hydrogen bonding network system. Therefore, to identify the residues involved in this system, we have investigated rhodopsin mutants that altered the pH-profile of the MI-MII equilibrium. We previously investigated the constitutively active mutation (CAM) site at position 257 and showed that the mutations at the site caused large shift of the MI-MII equilibrium. In the present study, we have investigated another CAM site at position 134 and some residues that could be candidates of forming the hydrogen bonding network system, which were inferred from our previous study. We discuss candidates that regulate the pH profile of the MI-MII equilibrium.

1P-260 光センサー型ヒスチジinkinナーゼ PYP ドメインの光反応に対する C 末端ドメインの影響

The Photoreaction of the PYP Domain in the Light Sensor Histidine Kinase Ppr is Influenced by the C-terminal Domains

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To study the role of the C-terminal domains in the photocycle of a light sensor histidine kinase (Ppr) having a photoactive yellow protein (PYP) domain as the photosensor domain, we analyzed the photocycles of the PYP domain of Ppr (Ppr-PYP) and full-length Ppr. The gene fragment for Ppr-PYP was expressed in *Escherichia coli*, and it was chemically reconstituted with *p*-coumaric acid; the full-length gene of Ppr was coexpressed with tyrosine ammonia-lyase and *p*-coumaric acid ligase for biosynthesis in cells. The light/dark difference spectra of Ppr-PYP were pH-sensitive. They were represented as linear combination of two independent difference spectra analogous to the PYPL/dark and PYPM/dark difference spectra of PYP from *Halorhodospira halophila*, suggesting that the pH dependence of the difference spectra is explained by the equilibrium shift between the PYPL- and PYPM-like intermediates. The light/dark difference spectrum of Ppr showed the equilibrium shift toward PYPL compared with that of Ppr-PYP. Kinetic measurements of the photocycles of Ppr and Ppr-PYP revealed that the C-terminal domains accelerate the recovery of the dark state. These observations suggest an interaction between the C-terminal domains and the PYP domain during the photocycle, by which light signals captured by the PYP domain are transferred to the C-terminal domains.

1P-261 高度好塩菌フォボロドプシン (センサリーロドプシン II) の光反応サイクル

Photoreaction cycle of phoborhodopsin (sensory rhodopsin II) from *Halobacterium salinarum*

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Although phoborhodopsin (pR or sensory rhodopsin II, sRII) was first found in *Halobacterium salinarum* as a photoreceptor of the negative phototaxis, the publications of photochemical properties of this pigment was not so many because the amounts of this pigment were so small that its purification was difficult. Thus, detailed studies have been done on another pR-like pigment found in *Natronobacterium pharaonis* (ppR, psRII) because a purified form of ppR had been obtained in much higher amount by an expression system in *E. coli*. Recently an expression system of *salinarum* phoborhodopsin (spR, ssRII) in *E. coli* was developed and the photochemical property can be studied with a purified spR.

The photoreactions of spR at -180°C, the thermal reaction of photoproduct(s)

formed at -180°C and the photoreactions at various temperatures up to 20°C were studied. The same photo- and thermal reactions as reported previously were observed with some new findings as follows. 1. The photoreaction at -180°C was more complex than previously reported. 2. On the thermal reaction of the photoproduct(s) formed at -180°C, formations of M-, L- and O-like intermediates were observed. 3. At 20°C we found new photoproduct, which showed absorbance increase at 370 nm and decayed into spR slowly in the dark. Based on our results the similarities and the differences between spR and ppR will be discussed.

1P-261 センサリーロドプシン I の正の走光性のための情報伝達における Tyr の役割

Role of tyrosine in the signal transduction from Sensory Rhodopsin I to the transducer for positive phototaxis

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Sensory Rhodopsin I (SRI) is a light sensor protein both for positive and negative phototaxis in haloarchaea. SRI forms a 2:2 complex with its transducer protein, HtrI, in the membrane region and the signal transduction mechanism between them for dual functions is of particular interest. In our previous FTIR study, we revealed the structural and interaction changes of the SRI-HtrI fusion protein are greatly influence when Asp76 is protonated [1]. We observed transducer-dependent structural changes of SRI in the 1285-1235cm⁻¹ region. Since it is a characteristic frequency of phenol C-O stretching vibration of tyrosine, structural changes of tyrosine(s) appear to be involved in the formation of the S373 intermediate, the active state for the positive phototaxis. Here, we attempted to elucidate the role of tyrosine in the formation of the S373 intermediate by use of isotope labeling and site-directed mutagenesis of tyrosine in SRI and HtrI. Consequently, some bands characteristic to the SRI-HtrI fusion protein were assigned to the C-O stretching vibrations of tyrosine. FTIR spectra of Y210F in SRI and Y18F in TM1 in HtrI show that hydrogen-bonding network near these residues are greatly influenced upon formation of the S373 intermediate when Asp76 is protonated. We will discuss the importance of tyrosine near the interaction surface and molecular mechanism of the intermolecular signal transduction from SRI to HtrI.

[1] Y. Furutani, H. Takahashi et al., Biochemistry 47, 2875-2883 (2008).

1P-262 バクテリアのセンサーロドプシンに対する 1 アミノ酸の置換で実現した内向きのプロトンポンプ

Engineering an inward proton pump from a bacterial sensor rhodopsin

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ATP is synthesized by an enzyme that utilizes proton motive force and thus nature creates various proton pumps. The best understood proton-pump is bacteriorhodopsin (BR), a light-driven proton pump in *Halobacterium salinarum*. Many archaeal-type rhodopsins are now known to show similar outward proton-pump activity as seen for BR. Sasaki et al. previously converted BR into an inward chloride-ion pump [1], but an inward proton pump has been never achieved. This may be reasonable because light-driven inward proton pump is dangerous for the survival of bacteria if some mutations easily convert the proton pump direction. Thus, natural proton pumps must have a specific mechanism to exclude the reversed direction, and in the case of BR, highly hydrophobic cytoplasmic domain constitutes one of the machinery.

Here we report an engineered inward proton pump from a bacterial rhodopsin by a single amino acid replacement. *Anabaena* sensory rhodopsin (ASR) found in a freshwater cyanobacteria is a photochromic sensor [2], possessing little proton pump activity. However, when we replace an amino acid at the cytoplasmic domain (distance ~15 Å from the retinal chromophore), ASR was converted into an inward proton pump. Molecular mechanism of a newly designed inward proton pump from a bacterial sensor protein will be discussed.

[1] J. Sasaki, L. S. Brown, Y.-S. Chon, H. Kandori, A. Maeda, R. Needleman and J. K. Lanyi (1995) *Science* 269, 73-75.

[2] A. Kawanabe, Y. Furutani, K.-H. Jung and H. Kandori (2007) *J. Am. Chem. Soc.* 129, 8644-8649.

1P-263 Rc-PYP における N 末端部位の役割

Role of the N-terminal cap in the Rc-PYP

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Photoactive yellow protein (PYP) is a blue light sensor with *p*-coumaric acid as a chromophore. The Rc-PYP is one of the PYP homologue proteins from a purple photosynthetic bacterium *Rhodobacter capsulatus*. Hh-PYP (a typical PYP from *Halorhodospira halophila*) shows a characteristic absorption peaks at 446nm in its spectrum. On the other hand, Rc-PYP shows two characteristic absorption peaks at 375nm and 436nm.

Structural change at the N-terminal region was observed in the photo-cycle of the Hh-PYP. But Rc-PYP does not show any large conformational change, indicating that the N-terminal cap has different role between Rc-PYP and Hh-PYP. To elucidate the role of the N-terminal cap, the effect of the N-terminal deletion