structure of the chromophore binding region. The N-terminal lacking PYP is also photoactive. These facts suggest that the N-terminal region of PYP does not contain structure element. In order to analyze the effect of N-terminal region to structure formation of PYP as well as the effect of the chromophore binding, we applied systematic alanine insertion to the N-terminal region of PYP.

Wild type apo-PYP takes a unique tertiary structure, although its CD spectrum is slightly different from that of holo-PYP, indicating that the further structure formation by chromophore binding. In apo-form, CD intensities of some alanine insertion mutants decreased from that of wild type. The CD increase upon chromophore binding is commonly observed, even though the CD spectra of holo-form are slightly different from that of wild type. These results indicate that the N-terminal region does not contain structure element, although the effect of chromophore binding to the structure formation is affected by insertion.

[1] R. Shiba, et.al. Biophysics 7, 1-10 (2011)

2PT173 Rc-PYP の相互作用における発色団周辺水素結合の寄与

The role of hydrogen bonding network around the chromophore for the interaction of Rc-PYP

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Photoactive Yellow Protein (PYP) is a blue light receptor firstly isolated from purple photosynthetic bacterium, *Halorhodospira halophila* (Hh-PYP), which has a *p*-coumaric acid as a chromophore. The PYP photoreaction and structural information has been analyzed in detail, but there has been little progress in the analysis of signal-transduction mechanism, since the target protein of Hh-PYP has not been identified. PYP from *Rhodobacter capsulatus* (Rc-PYP) is the only protein that the interaction protein is identified so far. Therefore, Rc-PYP is more suitable for the research of PYP signal-transductions. Rc-PYP shows two absorption maxima at around 370 and 440 nm, and shows reactivity to UV-A light. Rc-PYP's reaction with its interaction protein is light dependent. UV-A illuminated Rc-PYP binds its interaction protein. At the formation of the Rc-PYP complex the absorption maximum of *cis*-chromophore shows red-shift, this is indicating chromophore environment changes in the complex state.

To verify relationship between the chromophore environment and complex formation, we examined spectral difference and complex formation about several amino-acid residues mutants around the chromophore which construct hydrogen bond network and Rc-PYP re-constituted by chromophore analog which lacks chromophore hydrogen bond. All the mutants kept their interaction ability, even though spectral features were different from that of wild-type. But the Rc-PYP in which chromophore substituted with cinnamic-acid did not show complex formation.

2PT174 桂皮酸導入 Photoactive Yellow Protein の発色団由来水素結合の欠損効果

Roles of hydrogen bonds around chromophore in Photoactive Yellow Protein studied by OH-deficient cinnamic acid

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Photoactive Yellow Protein (PYP) is a water-soluble photosensor protein isolated from *Halorhodospira halophila*. It has a covalently attached chromophore, *p*-coumaric acid (*p*CA). Oxygen atom of *p*CA phenol group forms hydrogen bonds with the E46 and Y42. These two hydrogen bonds play crucial roles in the structure formation and photoreaction of PYP. In order to clarify the roles of these hydrogen bonds, we have attempted to remove these hydrogen bonds by using a cinnamic acid as a chromophore(CA-PYP). CA-PYP was successfully reconstituted. Although free cinnamic acid showed its absorption maximum at 265nm, it shifted to 316nm in CA-PYP, suggesting that the importance of hydrogen bonds for visible light absorption. Absorption intensity of CA-PYP decreased by UV illumination, but intensity never recovered to its original state by shutting of the light. CA-PYP is light reactive but not cyclic. FT-IR difference spectrum showed chromophore vibrational mode changes of *trans-cis* photoisomerization by UV-light illumination in CA-PYP. In addition, back bone changes and carboxyl group changes were also observed in FT-IR difference spectrum. CA-PYP did not show radius of gyration change and secondary structural change. These results suggest that hydrogen bonds constructed from phenolic oxygen of *p*CA, Y42 and E46 are required for the proper photoreaction of PYP accompanying with the substantial structural change, although the hydrogen bonds are not essential for the chromophore attachment.

2PT175 青色光センサータンパク質 YtvA の LOV と LOV-linker の光化学反応の比較

Comparison of the photochemical reaction between the LOV domain and the LOV-linker domain of a blue light sensor protein YtvA

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YtvA is a blue light sensor protein which consists of the N-terminal LOV (Light-Oxygen-Voltage) domain and the C-terminal STAS (Sulphate Transporter and Anti Sigma factor antagonists) domain, linked by the linker helix. Physiological studies have reported that YtvA is involved in the initiation of environmental stress response (light, salt, heat etc.) by regulating an activity of transcriptional factor σ B. However, the molecular mechanism of its signal transduction process has not been revealed yet. For understanding this signal transduction mechanism, the photochemical reaction dynamics has to be elucidated. In this study, we investigated the photochemical reaction dynamics of the LOV and LOV-linker domains of YtvA using the time-resolved transient grating (TG) method. In particular, by comparing the photochemical reactions dynamics of the LOV and LOV-linker domain, we try to find the importance of linker domain in the signal transduction. From the concentration dependence, we found that the LOV domain in the ground state exists in the equilibrium between the dimer and the tetramer, whereas the equilibrium is between the dimer and the higher oligomeric form for the LOV-linker domain. For both cases, it was shown that the photoreaction of dimer seemed to be important; that is, the dimer of the LOV domain associates into the tetramer, whereas the dimer of the LOV-linker domain occurs structural change without oligomerization. We will discuss the reaction in more detail in the poster presentation.

2PT176 *Natronomonas pharaonis* 由来のセンサリーロドプシン II の光誘起プロトン移動における塩化物イオンの役割

The role of chloride in the photo-induced proton transfer in sensory rhodopsin II from *Natronomonas pharaonis*

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Natronomonas pharaonis has a sensory rhodopsin II (NpSRII, or called *pharaonis* phoborhodopsin) which functions as a negative phototaxis receptor. This protein has a retinal as a chromophore and illumination induces the photochemical reaction (photocycle) accompanied by the photo-induced proton uptake and release. Previously, it was reported that the early proton release originated from Asp193 in phospholipid-reconstituted NpSRII occurs in the presence of chloride [1, 2]. In this study, the effect of Cl⁻ on this proton release from Asp193 was investigated by detail experiments with the indium-tin oxide (ITO) electrode. With increasing of Cl⁻ concentration in the medium, the pK_a's of Asp193 in both of the dark and excited state increased. From these Cl⁻-dependence of the pK_a's in Asp193, K_d values in both of the photolyzed and unphotolyzed state were estimated. In the unphotolyzed state, two K_d's were estimated. One was estimated to ~24 mM, while the other was estimated to > ~1 M which indicates very weak Cl⁻ binding affinity. On the other hand, K_d was estimated to ~244 mM in the photolyzed state. This implies that the Cl⁻ binding affinity becomes about 10-fold weaker by the conformational change in the excited state. In addition to these estimations, we verified the position of putative Cl⁻ binding sites. Finally, the role of Cl⁻ in the photo-induced proton transfer was discussed.

[1] Iwamoto *et al.* (2004) *Biochemistry* **43**, 3195-3203. [2] Kitade *et al.* (2009) *Biochemistry* **48**, 1595-1603.

2PT177 アナベナセンサリーロドプシンの光誘起プロトン移動反応の解析

Photo-induced proton transfer of *Anabaena* sensory rhodopsin

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Anabaena sensory rhodopsin (ASR), found from freshwater cyanobacterium, is a novel photosensing rhodopsin, which activates the soluble transducer for the