13:10~16:10 Q会場/ Room Q

光生物・視覚・光受容 4

Photobiology: Vision & Photoreception 4

3Q1322 Rhodobacter capsulatus 由来 PYP における相互作用状態の溶液構造**Solution structure of the complex states of Rhodobacter capsulatus PYP with its binding protein**Yoichi Yamazaki, Miki Hayashi, Hironari Kamikubo, Mariko Yamaguchi, Mikio Kataoka (*Nara Institute of Science and Technology*)

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. Interaction mechanism of these proteins is of interest for understanding the signal-transduction process of PYP and also PAS protein family.

In this study, we firstly analyzed the solution structure of the interaction protein and then the complex state with Rc-PYP by use of X-ray small angle scattering (SAXS). The result of SAXS revealed that the complex states is composed of several different forms originated from stoichiometrically different states. Most characteristic state included in the complex states showed a ring shape. Complex formation and the signal transduction mechanism will be discussed in this meeting.

3Q1334 光制御型転写因子 AUREO1 の光反応**Photoreaction of a light-regulated transcription factor, AUREO1**Osamu Hisatomi¹, Ken Takeuchi¹, Takahiro Murakami¹, Fumio Takahashi², Hironao Kataoka³ (¹*Graduate School of Science, Osaka University*, ²*PRESTO, JST*, ³*Botanical Gardens, Tohoku University*)

Aureochrome 1 (AUREO1) is a blue light receptor found in *Vaucheria*. AUREO1 has one basic region/leucine zipper (bZIP) domain and one light-oxygen-voltage (LOV)-sensing domain, and plays a role as a blue light (BL)-regulated transcription factor. To clarify the molecular mechanisms of AUREO1, we expressed AUREO1 and its truncated mutant (A1-ZC), containing bZIP and LOV domains in *E. coli*. Our recombinant proteins showed absorption and emission spectra typical of a flavoprotein, and selectively bound to the target sequence (TGACGT) of AUREO1. The absorption around 450 nm decreased after BL illumination, and recovered to its original level with a half-life of about 6 min at 25°C in the dark. A site-directed mutant (C254S) of A1-ZC lost the ability of spectral change upon illumination, indicating that Cys254 was responsible for photoadduct formation. The addition of DNA showed little effect on its absorption and fluorescence spectra. Translational diffusion coefficients of A1-ZC molecules suggested that A1-ZC underwent a certain structural change upon BL illumination.

3Q1346 青色光受容体 BLUF タンパク質 TePixD の可逆的な光反応; 低温分光による解析**Reversible Photoreaction of Blue-Light Sensor BLUF Protein TePixD; Low Temperature Spectroscopy Analysis**Yoshimasa Fukushima¹, Koji Okajima^{2,3}, Masahiko Ikeuchi³, Shigeru Itoh¹ (¹*Nagoya Univ.*, ²*Osaka Pref. Univ.*, ³*Univ. of Tokyo*)

BLUF domain is a photoreceptor class that binds oxidized flavin (Sensor of Blue Light Using Flavin). BLUF proteins trigger various physiological activities, such as gene expression and cell motility in bacteria and *Euglena*. BLUF proteins exhibit a 10 nm red-shift of the absorption band of flavin upon photon absorption (red-shifted form). This red-shifted form has been proposed to be a signaling state that transmits photic information to other domains/proteins. The photoreaction of this red-shifted form has been assumed to be

important for the physiological function, because many photoreceptors show switching between dark-adapted states and signaling states by photochromism. We investigated the photoreactions of a dark-adapted form and a red-shifted intermediate of TePixD, BLUF protein of *Thermosynechococcus elongatus*, by low temperature spectroscopy. We obtained following results; (i) the dark-adapted form was turned into a red-shifted intermediate by 1st-illumination at 150 K; (ii) By 2nd-illumination at 80 K, a half population of the red-shifted intermediate was turned into a semiquinone form. This semiquinone was trapped at 80 K; (iii) the other half remains the red-shifted intermediate; (iv) the semiquinone form reverted to the dark-adapted form (re-oxidation of flavin) above 120 K. These results indicate that TePixD quenches excess light energy by reverting to the dark-adapted form upon 2nd-photon absorption. We will discuss the reversible photoreaction and the photoreduction mechanism of TePixD.

3Q1358 BLUF ドメインの光反応におけるグルタミンとチロシン残基の水素結合構造**Hydrogen-bonding structures of glutamate and tyrosine residues during the photoreaction of the BLUF domains**Tatsuya Iwata^{1,2}, Mineo Iseki^{3,4}, Masakatsu Watanabe^{5,6}, Hideki Kandori² (¹*Ctr. Fost. Yng. Innov. Res., Nagoya Inst. Tech.*, ²*Dept. Front. Mater., Nagoya Inst. Tech.*, ³*Cent. Promotion of Int. Sci., SOKENDAI*, ⁴*Faculty of Pharm. Sci., Toho Univ.*, ⁵*Sch. Adv. Sci., SOKENDAI*, ⁶*Grad. Sch. Creat. New Photo. Ind.*)

BLUF (sensor of blue-light using FAD) is a flavin-binding photoreceptor domain. In the photoreaction of BLUF domains, a red-shifted intermediate appears, whose hydrogen bond of the C4=O group of FAD becomes stronger. Conserved glutamine and tyrosine residues nearby FAD play crucial roles in the formation of the intermediate state, but it is not easy to experimentally monitor the hydrogen-bonding alterations. Using FTIR spectroscopy, we have shown detailed structural changes in the active center of various photoceptive proteins, where X-H stretching vibrations are particularly useful to study hydrogen bonds. We recently observed an unusually low O-H stretching vibration of tyrosine in the red-shifted intermediate of the BLUF domain of AppA from *Rhodobacter sphaeroides* and PAC (photoactivated adenylyl cyclase) from *Euglena gracilis*, implying strong donation of the hydrogen bond of tyrosine of the active center [1].

Here we attempted comprehensive vibrational analysis of the active center (Gln/Tyr) by monitoring N-H (N-D) and O-H (O-D) stretching vibrations. The light-induced difference FTIR spectrum of AppA-BLUF in D₂O showed a positive band at 2028 cm⁻¹, which is attributed to the O-D stretch of Tyr21 downshifted from about 2800 cm⁻¹. Further analysis using isotope-labeled samples (¹³C- and ¹⁵N-uniform labeling, and Tyr-D₄ and reverse labeling of Gln) is in progress. The environment of the Gln/Tyr and its changes will be discussed.

[1] Iwata, T., *et al.* (2011) *J. Phys. Chem. Lett.* 2, 1015-1019.**3Q1422 FTIR Study of Light-Dependent Activation and DNA Repair Processes of E. coli CPD Photolyase**I Made Mahaputra Wijaya¹, Yu Zhang¹, Tatsuya Iwata², Junpei Yamamoto³, Kenichi Hitomi⁴, Shigenori Iwai³, Elizabeth D Getzoff⁴, Hideki Kandori¹ (¹*Department of Frontier Materials, Nagoya Institute of Technology*, ²*Center for Fostering Young and Innovative Researchers, Nagoya Institute of Technology*, ³*Graduate School of Engineering Science, Osaka University*, ⁴*Department of Molecular Biology and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, USA*)

Photolyases (PHRs) and Cryptochromes (CRYs) have an FAD (flavin adenine dinucleotide) as the chromophore within homologous protein architectures, but their functions are entirely different. CRYs function as photosensors or an element of biological clock. In contrast, PHRs' function is to repair damaged DNA, either cyclobutane pyrimidine dimers (CPD) or (6-4) photoproducts, by use of near UV/blue light. The activation process of PHR/CRY and the photorepair by PHR accompany light-induced electron/proton transfers. Light-induced difference FTIR spectroscopy is a powerful tool to study such protein-mediated chemical reactions. Hydrated film samples have been used for photoceptive proteins such as rhodopsins, LOV and BLUF domains. Since we wanted the PHR/CRY samples with higher water content to facilitate enzyme turnover, we thus established two different methods, re-dissolved samples [1] and concentrated solutions [2]. Here we applied FTIR spectroscopy to *E. coli* CPD PHR, and successfully measured difference spectra of the photoactivation and DNA photorepair using concentrated solutions. During photoactivation, hydrogen-bonding alteration of a protonated carboxylic acid was observed only