the FTIR spectra of the photoactivation of Cry-DASH from *Synechocystis* sp [2] and the activation and photorepair by (6-4) photolyase [3]. In present study, we applied FTIR spectroscopy to the photorepair of Cry-DASH from *Synechocystis* sp. We confirmed that Cry-DASH from *Synechocystis* sp is able to repair CPD in single-strand DNA. Interestingly, it was suggested that the oxidized form of FAD is involved in repair process. Molecular mechanism of photorepair will be discussed on the basis of the FTIR spectroscopic results.

[1] Hitomi et al. Nucleic Acid Res. 28, 2353 (2000)

[2] Iwata et al. To be submitted.

[3] Zhang et al. to be submitted.

3P265 光照射固体 NMR を用いた ppR の M 中間体の観測と光異性化によるレチナール化学シフト変化の解析
Detection of M-intermediate and the chemical shift change of retinal chromophore in ppR by in-situ photo-irradiation solid-state NMR

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Pharaonis phoborhodopsin (ppR, also called *pharaonis* sensory rhodopsin II; SRII) is a photo-receptor for negative phototaxis in *Natronomonas pharaonis*. ppR forms a 2:2 complex with its transducer protein, pHtrII, in the membrane. Photoisomerization of retinal chromophore from all-*trans* to 13-*cis* trigger the signal transduction. To understand molecular mechanism of negative phototaxis, it is important to observe the conformational change of retinal in the photo-activates state. In this study, we uniquely assigned ¹³C₁₅ and ¹³C₂₀ NMR signals of retinal in ppR and ppR-pHtrII complex using in-situ photo-irradiation solid-state NMR. The two peaks of ¹³C₁₅-, ¹³C₂₀-retinal in the M-state of photo intermediates appeared at 162.0 ppm and 22.4 ppm corresponding to 13-*cis*, 15-*anti* configuration in ¹³C CP-MAS NMR spectrum at 273K, respectively. The peak of ¹³C₂₀ in the M-state shifts to lower field (+0.4 ppm) when ppR formed complex with pHtrII. Therefore, our results indicate that retinal-protein interactions in ppR are slightly different from that of the complex in the M-intermediate. Interestingly, we observed multiple peaks of ¹³C₂₀ at 253K but a single peak appeared at 273K in the M-intermediate of ppR. This result indicates the presence of few different M-intermediates, having some different retinal-protein interactions around ¹³C₂₀ of retinal. It is of interest to note that two or three different conformations at lower temperature, may average among them at room temperature to show a single M-intermediate.

3P266 光照射固体 NMR によるファラオニスフォボロドプシンの信号伝達に関わる Tyr174 の局所構造変化の解析
3A1205 In situ photoirradiation solid state NMR study of local conformational change of Tyr174 corresponding to signal transduction in ppR

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Pharaonis phoborhodopsin (ppR) is a negative phototaxis receptor in *Natronomonas pharaonis*. ppR forms a complex with *pharaonis* Halobacterial transducer II (pHtrII), and this complex transmits the photosignal into cytoplasm. However, initial step of the signal transduction mechanism induced by the retinal photoisomerization of ppR, has not well understood yet. Interestingly, Tyr174 and Thr204 in the vicinity of retinal play an essential role in a negative phototaxis of ppR because T204A and Y174F mutants do not show negative phototaxis. In this study, we focused on Tyr174 of F-helix in ppR and investigated dynamic structure of [1-¹³C] Tyr-, [¹⁵N] Pro-labeled ppR and ppR/pHtrII complex in the dark and under photoirradiation by means of solid state NMR. The peaks at 175.5 ppm and 113.8 ppm were assigned to Tyr174 and Pro175 in ppR by REDOR (Rotational Echo Double Resonance) filter experiments for [1-¹³C] Tyr-, [¹⁵N] Pro-ppR. Using in situ photo irradiation solid-state NMR, it was found that the signals of Tyr174 and Pro175 decreased or shifted from Ground state to the M-intermediate in [1-¹³C]Tyr-, [¹⁵N] Pro-ppR/pHtrII complex. This result indicates that dynamics or conformation of Tyr174 moiety changed to more flexible state when retinal changed to M intermediate in ppR. Furthermore, it was observed that a local rather than overall structure was changed in the M intermediate. In summary, we succeeded in developing in situ photoirradiation solid state NMR and observed the behavior of Tyr174 moiety in M intermediate of ppR/pHtrII.

3P267 赤外分光解析を用いた BLUF ドメインの光反応に必須のチロシン残基の構造変化の検出
3A1220 FTIR detection of structural changes of tyrosine residue that are crucial for the photoreaction of the BLUF domain

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BLUF (sensor of blue-light using FAD) domain is a blue-light sensor domain that binds FAD (flavin adenine dinucleotide) as a chromophore. In the photoreaction of BLUF domains, a red-shifted intermediate forms where hydrogen bond of the C4=O group of FAD became stronger. Conserved glutamine and tyrosine residues nearby FAD play crucial roles for the formation of this state. Thus, it is important to know hydrogen-bonding environment of those residues. In this study, we measured difference FTIR spectra of BLUF domains in the higher frequency region (4000-1800 cm⁻¹) to observe N-H and O-H stretches of glutamine and tyrosine residues.

Light-induced difference FTIR spectra exhibited broad positive bands with multiple peaks in the 2800-2400 cm⁻¹ region for both BLUF domains of AppA from *Rhodobacter sphaeroides* (AppA-BLUF) and F2 domain of α -subunit in PAC (photoactivated adenylyl cyclase) from *Euglena gracilis* (PAC α F2). Since the band showed spectral shift by ¹³C-labeled and Tyr-D4-labeled samples of AppA-BLUF, the bands originate from tyrosine. We interpreted that those bands originate from the Fermi resonance of strongly hydrogen-bonded O-H group and overtone of ring mode of tyrosine. FTIR measurements of tyrosine mutants are in progress, and hydrogen-bonding network and its alteration upon activation will be discussed.

3P268 シロイヌナズナ phot の FMN 近傍にある Arg の Lys への置換は S390 状態の寿命を短くする
Replacement of Arg near FMN in LOV2 with Lys lead to shortening of the life time of S390 state in Arabidopsis phototropin

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Phototropins (phot), one of the blue receptors in plant, are involved in phototropism, stomatal opening, chloroplasts movement, etc. In phot, two LOV domains (LOV1, LOV2) in the N-terminal region regulate Ser/Thr kinase in the C-terminal region light-dependently. In the dark, mainly LOV2 inhibits the kinase activity. When LOV domain is irradiated by blue light, a covalent bond is formed (S390) between FMN and conserved Cys. The adduct lead to conformational changes of LOV2 and cancels the inhibition. Activated kinase shows autophosphorylation and substrate phosphorylation. S390 returns to the ground state (D450) thermodynamically. It is thought that the life time of S390 is one of factors regulating light sensitivity. We focused on Arg residue which is next to the photo-reactive Cys residue in LOV and interacts with the phosphate of FMN (R513 and R427 for Arabidopsis phot1 and phot2, respectively). We produced mutated LOV2-kinase peptide whose Arg was replaced to Lys (P1L2K_R513K, P2L2K_R427K) and performed spectroscopy and kinase assay. The life times of S390 in both mutated P1L2K and P2L2K were about 10 times shorter than that in wild type. The mutation caused the reduction for light sensitivity of kinase activity. Regulation mechanisms by LOV2 will be discussed.

3P269 FTIR 法による赤色・緑色光センサータンパク質 AnPixJ の光反応の解析
FTIR analysis of the photoreactions of red/green light sensor protein AnPixJ

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AnPixJ is a novel cyanobacteriochrome found in a cyanobacterium *Anabaena* (*Nostoc*) sp. PCC 7120. AnPixJ possesses phycocyanobilin (PCB) as a chromophore, and shows a reversible photoconversion between green-absorbing (Pg) and red-absorbing (Pr) forms. The X-ray crystallographic structure of Pr has been resolved, although that of Pg is still unknown. Previous studies have shown that the photoconversion processes from Pg to Pr, and Pr to Pg proceed via at least two intermediates, respectively. In this study, we analyzed the photoreaction intermediates using light-induced FTIR difference spectroscopy. FTIR difference spectra were measured upon illumination of Pr or Pg at temperatures of 283-140 K to trap different intermediates or convert to the final