2P254 Photoactive Yellow Protein におけるアルギニン 52 のプロトン化状態**Protonation State of Arginine 52 in Photoactive Yellow Protein**

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Our neutron structural analysis of Photoactive Yellow Protein (PYP) revealed an unusual deprotonated arginine (R52) with a low barrier hydrogen bond (LBHB) between the chromophore and E46. To examine the protonation state of R52 in solution, we performed FTIR spectroscopy. The comparison of PYP_L/PYP_{Dark} difference spectra between WT and R52Q confirmed the substantial differences in the region from 1500 cm⁻¹ to 1600 cm⁻¹, which is assigned to a C-N stretch vibration of the deprotonated guanidino group. The observation indicates that the deprotonated R52 of WT is slightly perturbed during the reaction. The signals show clear isotope effect with ¹⁵N labeled Arg. We conclude that R52 in PYP is surely deprotonated in solution.

2P257 光化学系 II の Mn4 クラスター S0 状態における Mn(II)存在可能性の理論的研究**S0-State Model of the Mn4-cluster in Photosystem II: Possibility of Mn(II)**

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Mn4-cluster in photosystem II protein accumulates oxidizing equivalents generated by the photoreaction-center and catalyzes the water-splitting of photosynthesis. Then, depending on the number of oxidizing equivalents, Mn4-cluster shows different configuration of possible Mn(II)/Mn(III)/Mn(IV) ions. For the Mn4-cluster having no oxidizing equivalent (S0 state), spectroscopic studies have proposed two possible configurations; Mn4(III,III,III,IV) or Mn4(II,III,IV,IV). To elucidate a more possible configuration at S0, we focus on the two factors; (a) the stable Mn4-structure on each possible valence-configuration and (b) Mn-Mn distances shown in EXAFS. We will report QM/MM optimization results for each configuration and the agreements with EXAFS.

2P255 開口数 0.9 の極低温光学顕微鏡の開発とその植物細胞内色素イメージングへの応用**Development of a cryogenic optical microscope with NA of 0.9 and its application to studies of pigment distributions in plant cells**

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Sharpening of spectral bands and reduced photo-damage of samples upon lowering temperature enable us to get detailed information about the functional states and spatial distributions of chlorophyll (Chl)-protein complexes within a plant cell. To realize fluorescence microspectroscopy observations of photosynthetic components at cryogenic temperatures, we developed a laser-scanning cryogenic microscope, in which the objective lens is set in the adiabatic vacuum achieving drastic shortening of the distance between the lens and a sample. Pigment distributions in etiolated *Zea mays* leaves were studied. We could dissolve the distributions of protochlorophyllide, the precursor to Chl, in different spectral forms with a much improved spatial resolution from previous studies.

2P258 フィコエリスリンを有するラン藻における励起エネルギー移動**Excitation energy transfer in cyanobacteria containing phycoerythrin**

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Cyanobacteria are the most primitive oxygenic photosynthetic organisms, in which phycobilisome (PBS) works as major light-harvesting pigments. Cyanobacteria, *Synechococcus* sp. CCMP1334 (*Synechococcus*) and *Phormidium* sp. NIBB1081 (*Phormidium*), show red color, because these two species contain large amount of phycoerythrin (PE) in PBS. We examine excitation energy transfer in *Synechococcus* and *Phormidium* by means of steady-state and time-resolved fluorescence spectroscopies. In steady-state excitation spectra, *Synechococcus* showed R-PE-type spectra, whereas *Phormidium*, C-PE-type. Results of the time-resolved measurements indicated that energy transfer within PE occurred about two times faster in *Synechococcus*.

2P256 ガリウム置換フェレドキシンの結晶構造と PS 1 および FNR との相互作用部位**Crystal Structure of Ga-substituted Ferredoxin and its interaction sites for Photosystem I and Ferredoxin-NADP⁺ reductase**

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Plant-type ferredoxin (Fd) is an electron transfer protein with a [2Fe-2S] cluster, mediating one-electron from photosystem I (PSI) to several Fd-dependent enzymes such as Ferredoxin-NADP⁺ reductase (FNR). For the interaction study between Fd and Fd-dependent enzymes, NMR spectroscopy has used Ga-substituted Fd (Ga-Fd) due to the paramagnetic properties of [2Fe-2S] cluster. To establish how much Ga-Fd can be used as a replacement of native Fd, we solved the crystal structure of Ga-Fd at 1.4-Å resolution, and confirmed the comparable binding affinities of native-Fd and Ga-Fd using an FNR immobilized column. NMR chemical shift perturbation experiments of both Fds also exhibit no significant structural differences in the interaction sites for PSI.

2P259 ホタルルシフェラーゼとの相互作用を考慮したオキシルシフェリンの吸収スペクトルの量子化学計算**Quantum chemical calculation of the absorption spectra of oxyluciferin interacting with firefly luciferase**

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Firefly is most investigated among the bioluminescent species. Its reaction mechanism is oxygenation of substrate luciferin by firefly luciferase (E) under the existence of Mg-ATP and oxygen. Visible light is emitted when final product of this reaction, electronically excited oxyluciferin (OxyLn*), goes down to its ground state. By X-ray diffraction study, tertiary structure of E with OxyLn was already reported (PDB; 2D1R). In this study, we attempted to predict the absorption spectra of OxyLn interacting with E including all water molecules. After correction of missing residues and atoms, QM/MM geometry optimization was performed using Discovery studio (Accelrys). At last, INDO/S calculation was performed for optimized structure of 2D1R by MO-S (Fujitsu).