complexes with CO, NO, and CN ligands were revealed by Muramoto et al. In this study, employing these crystal structures, we performed hybrid *ab initio* quantum mechanics/molecular mechanics (QM/MM) simulations, and analyzed the geometric and electronic structures of the BNC.

As a result of the analysis, we found that the configuration of CO ligand in the crystal structure is different from that of the theoretically-optimized structure. We investigated this discrepancy by mapping the potential energy values of various positions of CO ligand in the BNC. The obtained potential field revealed the major two types of configurations, for which the energy values are close to each other, although the corresponding electron densities obtained by the crystallographic analysis cannot be separated into the two CO configurations. So, we calculated the IR spectrums, and found that they are consistent with the experimental data. In the session, the roles of the Cu_B site in the reaction will be discussed.

2PT168 In-situ 光照射固体 NMR を用いた D96N-bR の M 中間体で生じる構造変化の解析

Conformational change in M-intermediate of D96N-bR as studied by in-situ photo-irradiated solid-state NMR

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Bacteriorhodopsin (bR) is the membrane protein of *H. salinarum*. When bR is photo irradiated, retinal in bR is photoisomerized and bR transfer proton from cytoplasmic side to extracellular side during photo cycle starting from all-*trans* retinal via K, L, M, N and O-intermediates. When M-intermediate generate, Schiff base is deprotonated and occurred large dynamic conformational changes. Since these conformational changes play a key role for proton pump mechanism, we aim to detect the conformational changes occurred in M-intermediate by applying in-situ photo-irradiated solid state NMR (P-SS-NMR) to D96N-bR that has long life time of M-intermediate.

We measured ¹⁵N P-SS-NMR spectrum of [ϵ -¹⁵N] Lys-D96N-bR at the temperature range from 0°C to -60°C. As a result, we assigned new peaks appeared with photo-irradiation at 300 ppm and, 201 and 399 ppm (first spinning side bands) to deprotonated Schiff base of M-intermediate in D96N-bR. In contrast, we assigned the peaks decreased significantly with photo-irradiation at 154 and 147 ppm to the protonated Schiff bases of 13-*cis*, 15-*syn* and all-*trans* retinals in D96N-bR, respectively.

Additionally, we measured ¹³C P-SS-NMR spectrum of [3-¹³C]Ala, [1-¹³C]Val doubly labeled D96N-bR. We observed the changes of ¹³C NMR signal intensities from dark adapted state to photo activated state. Consequently, we detected local conformational changes in the protein side at M-intermediate of D96N-bR.

2PT169 LOV2 ドメインの中間体揺らぎの時間分解検出

Transient fluctuation of phototropin LOV2 domain

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For biological function of proteins, their structural fluctuations could play an important role. Recently we succeeded in detecting such transient volume fluctuations by using TG and TrL techniques for a blue light sensor protein, PixD. Here, we studied the fluctuation of reaction intermediates of phototropin LOV2 domain, which is one of the most versatile blue-light sensor domain. Although the reaction have been investigated extensively so far, it remains still unknown how LOV2 domain transmits its light signal toward the effector domain linked with usually the C-terminal of LOV2 domain, because only a minor structural change around its chromophore is observed upon the blue-light excitation. For example, it has been reported that the LOV2 domain except the chromophore region does not undergo a remarkable structural change upon the light excitation, but it causes the unfolding of the C-terminal linker-helix domain. We consider that the intermediate structural fluctuation of LOV domain should be involved in this step. Therefore in the present study, we detected and compared the intermediate fluctuation of the LOV2-linker and LOV2 domains by extending the TG and TrL measurements. For the LOV2-linker domain, we succeeded in observing the enhanced fluctuation for the unfolding event of the linker-helix. By comparison of the fluctuation between the LOV2 domain and LOV2-linker, we discuss the functional role of the fluctuation of the LOV2 domain in the signal transmission step.

2PT170 Photoactive Yellow Protein 中に存在する低障壁水素結合近傍のアルギニン 52 のプロトン化状態

Protonation State of Arginine 52 near the Low Barrier Hydrogen Bond in Photoactive Yellow Protein

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Low Barrier Hydrogen Bond (LBHB) is a special type of hydrogen bonds (HBs), in which a proton locates near the center between donor and acceptor atoms. Although the bond energy of LBHB (ΔH_{LBHB}) is generally higher than that of a normal HB, ΔH_{LBHB} in a protein has not been evaluated. To estimate ΔH_{LBHB} , we carried out pH titration experiments at various temperatures upon Photoactive Yellow Protein. We clarified that the HB between the chromophore (pCA) and E46 is LBHB. The LBHB is converted to an ordinary HB (NH-O) in E46Q. Neither the LBHB nor the HB can be formed in E46A. Therefore, ΔpK_a (WT-E46A) and ΔpK_a (E46Q-E46A) is assumed to reflect bond free energies of LBHB and HB, respectively. From the temperature dependence of ΔpK_a values, ΔH_{LBHB} and ΔH_{HB} were estimated to be 8 kcal/mol and 7 kcal/mol, respectively. ΔH_{LBHB} is rather smaller than the expected value. We found the substantial difference in Hill's coefficients. The Hill's constant for WT is about 2, while those of E46Q, and E46A are close to 1, suggesting that there exist another protonation site in WT than pCA. To identify the presumed protonation site, we performed the neutron crystallographic analysis on E46Q, revealing that R52 in E46Q is partly protonated. Together with the fact that R52 is deprotonated in WT, it can be proposed R52 was cooperatively protonated upon the protonation of the pCA in WT. The apparent ΔH_{LBHB} reflects both of the energy gain of LBHB and the energy loss due to deprotonation of R52 at the physiological pH, resulting in the relatively small value.

2PT171 PYP Phytochrome Protein Related (Ppr) に存在する PYP ドメインによるフィトクロムドメインの光反応構造変化の制御

Photoreaction of PYP domain regulates the structural change during the photoreaction of phytochrome domain of Ppr

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PYP-Phytochrome Related protein (Ppr) is a multi-domain protein comprised of a PYP domain, a bacteriophytochrome domain (Bph), and a histidine kinase domain. We previously reported that the photo-reactions of PYP and Bph are closely coupled, where the intermediate state of PYP influences the photo-reaction rates of Bph. To investigate the molecular mechanism of the interdependency, we prepared holo-holo-Ppr and apo-holo-Ppr, and performed solution x-ray scattering experiments. The scattering profiles of the holo-holo and apo-holo Pprs were nearly identical, indicating that the overall molecular shapes of holo-holo and apo-holo Pprs are indistinguishable. Red light absorption brings that holo-holo-Ppr changes in the scattering profile, holo-holo-Ppr does not. It can be suggested that the structural change is suppressed by the holo-PYP domain despite that holo-PYP does not absorb red light. Blue light irradiation to holo-holo-Ppr causes the formation of PYP_M. The photointermediate of Bph was slowly accumulated by the following red light irradiation. The scattering profile of the final product is largely different from the original state, the change of which is similar to that of apo-holo-Ppr by red light absorption. From these results, we propose that the photoreaction of PYP domain regulates the domain rearrangement during the Bph photoreaction. The unphotolyzed PYP domain suppresses the structural change, but the PYP_M state enables Ppr to undergo the large structural change.

2PT172 PYP の構造形成に対する N 末端領域の効果

Effect of N-terminal region to the structure formation of PYP

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Structure element is defined as an uninterrupted region on the amino acid sequence which is required for the structure formation of a protein [1]. Photoactive yellow protein (PYP) is a water soluble light absorbing protein. PYP can be separated into the N-terminal region and the chromophore binding region. Upon absorption light, large disorder is brought to the N-terminal region. The deletion of the N-terminal region, however, does not affect the