2P242 In-situ 光照射固体 NMR によるバクテリオロドプシン D96N 変異体の光中間体の捕捉とタンパク質構造変化の解明
Trap of photo-intermediate and structural change of bacteriorhodopsin D96N mutant as revealed by in situ photoirradiation solid-state NMR

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Bacteriorhodopsin (bR) is an integrated membrane protein that functions light-driven proton pump. Upon photoirradiation, all-trans retinal start photocycle through K-, L-, M-, N-, O-intermediates. During the photocycle, proton is transported from cytoplasmic to extracellular side. When the M-intermediate generates, proton moves from retinal to Asp96 accompanied by a large dynamical changes, which may play an important role for proton pump. Because D96N-bR shows a longer half life of the M-intermediate than the WT, we could trap the M-intermediate using in situ photoirradiation solid-state NMR by observing the ¹⁵N NMR signal of [¹⁵N-ε-Lys]-D96N-bR at -60 °C. It was observed that mobility of F-helix and C-terminus in protein was increased in the M-intermediate.

2P243 In situ 光照射固体 NMR による 13-cis, 15-syn バクテリオロドプシンの光励起過程における局所構造変化の解析
Structural changes in the photo excited process in 13-cis, 15-syn retinal of Bacteriorhodopsin studied by in situ photoirradiation SS-NMR

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Bacteriorhodopsin (BR) is an integral membrane protein that functions light-driven proton pump and has retinal chromophore which forms two different configurations of all-trans (AT) and 13-cis, 15-syn (CS) with 1:1 ratio (dark-adapted state (DA)), and changes to ~100% AT under photoirradiation (light-adapted state (LA)). In this study, retinal configuration change in BR was observed using [¹³C]Tyr-, [²⁰-¹³C]Retinal-BR by means of *in situ* photoirradiation SS-NMR. At 20°C, change from DA to LA is seen; however, at -20°C, an CS-like intermediate (CS*) is observed stationary under green light illumination. Large structural change was also observed in protein side in CS*. These results suggest a new photocycle, in which CS may play an essential role in proton pump function.

2P244 光駆動型 Cl⁻ポンプ ファラオニスハロロドプシンの Cl⁻放出・取込み過程の解析
Analysis of Cl⁻ release and uptake steps of light-driven Cl⁻ pump *Natronomonas pharaonis* halorhodopsin

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KM-1 is a mutant of *Natronomonas pharaonis* expressing a large amount of NpHR-bacterioruberin (Brub) complex. At the formation of O-intermediate, flash-photolysis results of the NpHR-Brub complex showed wavy absorbance changes (Wac) that were originated from the potential-modulated Brub. On the other hand, the opposite-directed changes of Wac were observed when Cl⁻ was added to the free NpHR. These indicated that at O, Cl⁻ is released to the cytoplasmic (CP) space. On the other hand, imposition of the interior negative membrane potential of envelope membrane vesicles decreased the O-accumulation and slowed down the O-decay. These indicated that Cl⁻ release and uptake occur during the formation and decay of O, respectively, as proposed by previous works.

2P245 光駆動型 Cl⁻ポンプ ファラオニスハロロドプシンにおける Thr218 の役割
Role of Thr218 in light-driven Cl⁻ pump mechanism of *Natronomonas pharaonis* halorhodopsin

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Thr218 is considered to be functionally important, because this hydrophilic residue is located near the retinal of hydrophobic cytoplasmic (CP) channel. We analyzed the photocycle of various T218 mutants. Wild-type and T218S show 1) the fastest L2 to N transition and 2) the largest dissociation constant of Cl⁻ (*K_d*) to CP. Thus, Thr or Ser at this position is suitable for the Cl⁻ movement which involves the transfer from the binding site in the extracellular channel followed by the release to the CP space. Pressure dependence of *K_d* yielded the volume differences between N and O (Δ*V*). Results suggested the water entry at O, which may increase *K_d*. The relationship between *K_d* and Δ*V* may propose a special coordination of the water molecules with OH group of Thr or Ser.

2P246 アセタブラリアロドプシン I の光化学反応
Photochemical reaction in *Acetabularia* rhodopsin I

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Acetabularia rhodopsin (AR) is a light-driven proton pump found in marine alga, *Acetabularia acetabulum*. Recently, Jung *et al.* cloned two homologous AR opsins, and named them ARI and ARII. Previously we reported details of photochemical properties of ARII prepared by the cell-free synthesis. In this study, we focused on the photochemistry of another homologous one, ARI. The photocycle and photo-induced proton transfer of ARI was investigated by the flash photolysis and photoelectrochemical measurement with indium tin-oxide (ITO) electrodes, respectively. On the basis of these results, the expected photocycle and proton transfer scheme of ARI were suggested, and finally discussed compared with ARII or well-known bacteriorhodopsin.

2P247 in situ 光照射固体 NMR による光受容膜タンパク質 ppR/pHtrII の光励起過程における transducer タンパク質膜貫通領域の構造変化の観測
Photoactivated conformational changes of photoreceptor membrane protein ppR/pHtrII observed by in situ photo irradiation solid-state NMR

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ppR/pHtrII is a complex of photo receptor and signal transducer membrane proteins and functions as negative phototaxis. [¹⁵,²⁰-¹³C] retinal ppR and [¹-¹³C]Val,^{[2}-¹³C]Gly,^{[3}-¹³C]Ala pHtrII was expressed respectively and 2:2 complex was prepared. Conformational changes of ppR/pHtrII complex in a membrane (Egg-PC) were observed using *in situ* photo irradiation NMR. The experimental result demonstrated that transmembrane region of pHtrII changed from α-helix to random coil, while retinal of ppR from all-trans (grand states) to 13-cis (M-intermediates). In addition, we observed the presence of at least 3 types of M-intermediates. These results can provide a clue to clarify the mechanisms of signal transduction of photo-sensor proteins.