2P241 アナベナセンサリーロドプシンの細胞質側で生じる光誘起プロトン移動反応の解析

Light-induced proton transfer reactions at the cytoplasmic half channel of *Anabaena* sensory rhodopsin

Takatashi Hasemi¹, Takashi Kikukawa¹, Masakatsu Kamiya¹, Tomoyasu Aizawa¹, Kwang-Hwan Jung², Naoki Kamo¹, Makoto Demura¹ (¹*Grad. Sch. Life Sci., Hokkaido Univ.*, ²*Dept. Life Sci. & Inst. Biol. Interfaces, Sogang Univ.*)

The photocycle of *Anabaena* sensory rhodopsin (ASR), which is the photoreceptor for the cyanobacterial chromatic adaptation, was examined by focusing on the H⁺ transfer reactions. Photoinduced deprotonation of the Schiff base induces the formation of M intermediate. However, the M formation of ASR was accompanied with the H⁺ uptake from the medium. This indicates the contributions of two residues: a H⁺-accepting residue from the Schiff base and a H⁺-capturing residue from the medium. The mutation experiments suggested that these residues are assigned to Asp217 and Glu36, respectively, and their protonation states influence each other. We will discuss the relationship between the H⁺ transfer reactions and the conformational change of ASR.

2P242 海洋性細菌に含まれる光駆動クロライドポンプの分光研究
Spectroscopic study of light-driven chloride pump from marine bacteria

Arisa Mori^{1,2}, Keiichi Inoue^{2,3}, Faisal Hammand Mekky Koua², Yoshitaka Kato², Rei Abe-Yoshizumi², Michio Homma¹, Hideki Kandori² (¹*Grad. Sch. Sci., Nagoya Univ.*, ²*Nagoya Inst. Tech.*, ³*JST, PRESTO*)

Thousands of microbial rhodopsins have been recently found from marine bacteria. Most of them are light-driven proton pumps, possessing the DTE motif at the corresponding positions of D85, T89 and D96 in bacteriorhodopsin. In contrast, some rhodopsins contain the NDQ or NTQ motif, which functions as light-driven sodium or chloride pump, respectively. The latter is evolutionally far different from halorhodopsin, well-known chloride pump in Halophilic Archaea. Here we present spectroscopic study of the new chloride pump. The results are compared with those of halorhodopsin, and mutant proteins are also used in this study. Molecular mechanism of uni-directional chloride transport will be discussed based on the present results.

2P243 チャネルロドプシンの吸収波長に関する量子化学的研究
Quantum chemical study of the absorption maximum of channelrhodopsin

Nami Yoshino¹, Tomohiko Hayashi¹, Azuma Matsuura², Tadaomi Furuta¹, Minoru Sakurai¹ (¹*Tokyo Tech.*, ²*Fujitsu Lab*)

Herein, the absorption maximum of channelrhodopsin (ChRh) is calculated using our recently developed method in which the whole protein can be treated quantum mechanically at the level of INDO/S-CIS. This calculation successfully reproduced the so-called opsin shift of ChRh. We also applied the same calculation for different mutants, each of which was constructed by replacing any one of the amino acid residues of the wild-type ChRh with Gly. This substitution made it possible to elucidate the extent to which each amino acid contributes to the opsin shift and to estimate the inter-residue synergistic effect. Based on these data, we discuss the spectral tuning mechanism in ChRh.

2P244 固体 ¹³C NMR によるファラオニスフォボロドプシンの機能に重要な Tyr 残基の構造解析

Conformation of functionally important Tyr residues in *pharaonis* phoborhodopsin as studied by Solid-State ¹³C NMR

Ryota Nishikawa¹, Izuru Kawamura¹, Takashi Okitsu², Akimori Wada², Yuki Sudo³, Naoki Kamo⁴, Akira Naito¹ (¹*Grad. Sch. Eng., Yokohama Natl. Univ.*, ²*Kobe Pharm. Univ.*, ³*Okayama Univ.*, ⁴*Grad. Sch. Life Sci, Hokkaido Univ.*)

Pharaonis phoborhodopsin (ppR) has 7 transmembrane α -helices with a retinal. Tyr174 and Tyr199 residues in ppR are important for the signal transduction from ppR to pHtrII [1]. Here we present a Solid-State ¹³C NMR spectra of [¹⁵,20-¹³C]retinal, [¹³C]Tyr and [¹³C]Lys in ppR, T204A and D75N incorporated in the EggPC lipid bilayer. We observed each of the correlated peaks of C ζ -Tyr174 with retinal by proton-driven spin diffusion 2D NMR experiments. Besides, we successfully assigned ¹³C chemical shift of C ζ -Tyr199. These site-specific chemical shifts have revealed interesting information on the Tyr conformation. We will discuss about retinal-protein and ppR-pHtrII interaction by comparing the structure with its mutant. [1]Y. Sudo & J.L. Spudich(2006) *PNAS*. 103, 16129.

2P245 サル青感受性視物質の赤外分光解析
FTIR study of monkey blue-sensitive visual pigment

Yuki Nonaka¹, Kota Katayama¹, Kei Tsutsui², Hiroo Imai², Hideki Kandori¹ (¹*Nagoya Institute of Technology*, ²*Primate Research Institute, Kyoto University*)

Primates including human have three types of color visual pigments; blue, green, and red. Although these pigments contain the same chromophore molecule, 11-cis-retinal, different chromophore-protein interactions allow absorption of different colors. In color tuning, three factors are generally considered; (i) chromophore distortion, (ii) interaction between the protonated Schiff base and the counterion, (iii) polarity around the chromophore.

In this study, structural basis of color tuning in monkey blue-sensitive visual pigment was examined by light-induced FTIR spectroscopy. The color tuning mechanism of blue pigment will be discussed based on the FTIR spectral comparison with the other visual pigments.

2P246 In-situ 光照射固体 NMR による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 photoexcited process in retinal of Bacteriorhodopsin studied by in-situ photoirradiation solid-state NMR

Arisu Shigeta¹, Ryota Miyasa¹, Miyako Horigome¹, Izuru Kawamura¹, Takashi Okitsu², Akimori Wada², Satoru Tuzi³, Akira Naito¹ (¹*Grad. Sch. Eng., Yokohama Natl. Univ.*, ²*Kobe Pharm. Univ.*, ³*Univ. Hyogo*)

Bacteriorhodopsin (BR) is a seven-helical membrane protein that functions as a 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 in the dark state, and changes to ~100% AT under photoirradiation at 20°C. Using in-situ photoirradiation SS-NMR and [¹³C]Tyr-, [20-¹³C]Retinal-BR, CS-like intermediate and N intermediate were trapped under green light illumination at -20°C. Large structural changes between dark and light state were observed not only in retinal configuration but also in protein structure. These results gain insight into an essential role of CS state in proton pump function. At the presentation, trapping yield of N intermediate will be displayed.