1P421

FTIR Study of the O Intermediate in the Complex between *pharaonis* Phoborhodopsin and Its Cognate Transducer

○Motohiro Ito¹, Yuji Furutani¹, Masayo Sumii¹, Naoki Kamo², Hideki Kandori¹

¹Department of Materials Science and Engineering, Nagoya Institute of Technology,

²Graduate School of Pharmaceutical Sciences, Hokkaido University

pharaonis phoborhodopsin (ppR; also called *pharaonis* sensory rhodopsin II, psRII) is a receptor for negative phototaxis in *Natronobacterium pharaonis*. It forms a 2:2 complex with its transducer protein in the membrane region. ppR absorbs maximally at 498 nm, and the light triggers *trans-cis* photoisomerization of the retinal chromophore in its electronically excited state, followed by rapid formation of the ground-state species such as the K intermediate. Relaxation of the K intermediate leads to functional processes during the photocycle, where protein structural changes are transmitted from ppR to pHtrII presumably in the M and/or O state. In the previous FTIR experiments, we found that hydrogen bond of Thr204 is strengthened in the K intermediate. Recently, we also found that hydrogen bond of Asn74 in pHtrII is strengthened, and a temperature-dependent interaction in the cytoplasmic region between ppR and pHtrII occurs upon the formation of the M intermediate [1, 2]. In this study, we will focus on the structural changes in the O intermediate of the ppR/pHtrII complex. We observed difference in the amide I bands between the absence and presence of pHtrII, implying the important role of the O intermediate for the signal transfer. Protein structural changes in the O state will be discussed based on the isotope labeling and mutants results, which are now in progress.

References

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1P423

Local Conformation and Dynamics Changes in the vicinity of the Retinal in Photoactivated *pharaonis* phoborhodopsin by Solid-State NMR

○Takudo Nishio¹, Izuru Kawamura¹, Hazime Saito², Naoki Kamo³, Akira Naito¹

¹Graduate School of Engineering, Yokohama National University, ²Center for Quantum Life Sciences, Hiroshima University, ³Laboratory of Biophysical Chemistry, Graduate School of Pharmaceutical Sciences

pharaonis phoborhodopsin (ppR) is a retinal protein in *Natronomonas pharaonis* and acts as a receptor of negative phototaxis. It forms a complex with its cognate transducer pHtrII in the membranes and transmits light signals through protein-protein interaction. When ppR absorbs light energy at 498 nm, the photoisomerization of retinal in ppR occurs to change K, L, M, and O intermediates. Because M intermediate is known to transmit light signals to pHtrII, it is important to characterize the structure of M intermediate. Solid-state NMR study gains insight into the local structure and dynamics under physiological conditions. We introduced optical fiber to the top part of spinner in the NMR probe to irradiate light during solid-state NMR experiments. Because M intermediate have an absorption maximum at 390 nm and the thermal relaxation time to O intermediate is relatively long, we could trap M intermediate by illuminating the light with the wavelength from 450 nm to 600 nm. Using this apparatus, we could distinguish ppR in the dark from that in the light adapted states. ¹³C CP-MAS NMR spectra of [1-¹³C] Tyr-ppR and ¹⁵N CP-MAS NMR spectra of [¹⁵N] Pro-ppR were measured in the dark and light adapted states, respectively. It turned out that ¹³C and ¹⁵N NMR signals of residues which exist near retinal in ppR changed pronouncedly. These results indicate that the local structure and dynamics near retinal switched as the dark adapted state changes to the light adapted state in ppR, although the overall structure did not change prominently.

1P422

Converting a light-driven ion pump (bacteriorhodopsin) into a photosensory receptor

○Yuki Sudo, John Spudich

The University of Texas at Houston

Some members of the rhodopsin family carry out photoenergy transduction by light-driven ion transport, while others are photosensory receptors that transfer signals to transducer proteins. What changes would be required to convert a proton pump into a sensory receptor? Here we show that three mutations in bacteriorhodopsin: two on its surface (mimicking Thr189 and Tyr199 in SRII [1]) and one between the retinal attachment site and the membrane-embedded surface (mimicking Thr204 in SRII), enable it to bind to the Sensory rhodopsin II (SRII) transducer HtrII and relay the retinal photoisomerization signal to induce HtrII-mediated phototaxis responses [2]. In SRII, the three residues form a chain of hydrogen bonds from the retinal's photoisomerized C13=C14 to residues in the membrane-embedded α -helices of HtrII. The results identify the minimal core mechanism of signal relay from the SRII receptor to the HtrII transducer, which entails initial storage of energy in the hydrogen-bond between Thr204 and Tyr174 [3-5], which is in van der Waals contact with the retinal, followed by transfer of this energy to drive structural transitions in the transducer helices. The results furthermore demonstrate that evolution accomplished an elegant but simple conversion. [1] Sudo, Y., et al. (2006) *J. Mol. Biol.* 357,1274. [2] Sudo, Y., and Spudich, J.L. (2006) in preparation. [3] Sudo, Y., et al. (2006) *J. Biol. Chem.* submitted. [4] Sudo, Y., et al. (2003) *Biochemistry* 42, 14166. [5] Sudo et al. (2005) *J. Am. Chem. Soc.* 127, 16036.

1P424

Pressure induced isomerization of retinal and dynamics change of protein on bacteriorhodopsin in the dark by Fast Magic Angle Spinning NMR

○Izuru Kawamura¹, Yoshiaki Degawa¹, Satoru Yamaguchi², Katsuyuki Nishimura³, Satoru Tuzi², Hazime Saito⁴, Akira Naito¹

¹Grad. Sch. Eng., Yokohama Natl. Univ., ²Dept. Life Sci., Univ. Hyogo, ³Inst. Mol. Sci., ⁴Dept. Quantum Life Sci., Hiroshima Univ.

Bacteriorhodopsin (bR), a retinal protein in purple membrane of *H. salinarum*, shows functions as a light-driven proton pump. We have detected pressure induced isomerization of retinal in bR by ¹⁵N cross polarization-magic angle spinning (CP-MAS) NMR spectra of [ϵ -¹⁵N]Lys-labeled bR. In the ¹⁵N NMR spectra, both all-*trans* and 13-*cis* retinal configurations have been observed at 148.0 and 155.0 ppm, respectively, at the MAS frequency of 4 kHz in the dark. When the MAS frequency was increased up to 12 kHz corresponding to the sample pressure of 80 atm, the ¹⁵N NMR signals of Schiff base of retinal were broadened. The signal intensity of 13-*cis* retinal at 155.0 ppm was increased when the MAS frequency was decreased from 12 kHz to 4 kHz. These results showed that the equilibrium constant of [all-*trans*-bR] / [13-*cis*-bR] decreased by the pressure of 80 atm. It was also revealed that the changes induced by the pressure were quite local. Therefore, microscopically, hydrogen-bond network around retinal would be disrupted or distorted by a constantly applied pressure. It is, therefore, clearly demonstrated that increased pressure induced by fast MAS frequencies generated isomerization of retinal from all-*trans* to 13-*cis* state in the membrane protein bR.