3P236 ファラオニスセンサリ-ロドプシン II の光情報伝達を開始させる特異なタンパク質-発色団相互作用について
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Specific Protein-Chromophore Interaction Initiates Light Signal Transduction of *pharaonis* Sensory Rhodopsin II
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Natronomonas pharaonis sensory rhodopsin II (μ SRII; also called *pharaonis* phoborhodopsin, μ pR) is a light sensor for negative phototaxis. Previous FTIR spectroscopy showed that the C14-D stretching and C14 hydrogen-out-of-plane (HOOP) vibrations of the retinal chromophore appeared at 2244 and 864 cm^{-1} in the K state, respectively [1, 2]. This result suggests the specific protein-retinal interaction near the C14 atom in μ SRII. In this study, we attempted to determine the counterpart of the protein side by use of several mutants. The C14-D stretching and C14-HOOP bands completely disappeared in T204A, S, and C. The intensities were significantly reduced in Y174F, which loses a hydrogen band with T204. On the other hand, they were preserved in T79A and N105D. Interestingly, the band intensities were correlated with the negative phototaxis response. We also applied the same FTIR analysis to a triple bacteriorhodopsin mutant (P200T/V210Y/A215T; BR-T) which can work as a light sensor for negative phototaxis [3, 4]. BR-T exhibits the C14-D and C14-HOOP bands at 2250 and 861 cm^{-1} , respectively, although these bands are not present in wild-type BR. We conclude that the steric constraint between Thr204 and the C14-H group of the retinal chromophore in the earliest photocycle intermediate K is important for signaling by this transduction of archaeal light sensor.

References

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3P238 センサリ-ロドプシン II トランスデューサータンパク質の信号伝達ダイナミクスの研究
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Study on the signal transduction dynamics of Sensory rhodopsin II Transducer protein
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Transducer protein (HtrII) relays the light signal received from activated sensory rhodopsin II (SRII) from the extracellular to cytoplasmic side of the cell. We studied the signal transduction dynamics of HtrII by transient grating (TG) method and HtrIIs which have been truncated at various position of N-terminal domain to identify the position where the conformational change occurs on the signal transduction process. Figure 1 shows TG signals of HtrIIs which have 1-114th (Tr114) (Fig. 1(a)) and 1-157th residues (Tr157) (Fig. 1(b)) of N-terminus part. These HtrIIs are complexed with SRII D75N mutant which transfers identical signal to the one of wildtype SRII and there are significant difference of TG signals between Tr114 and Tr157. Based on the detailed analysis, we clarified that one of the reaction intermediates of SRII/D75N-HtrII complex (K4 intermediate) showed a large reduction of molecular diffusion coefficient (D) from initial state in only the case of Tr157. This result indicates a large structural change occurs during the photo-reaction cycle at the HAMP domain of HtrII which is a linker domain locating between transmembrane and cytoplasmic domain of HtrII. The large reduction of D may be caused by unfolding of α -helices of HAMP domain. This is a first observation of the temporal conformational change of HAMP domain of HtrII and the conservation of this domain over various signaling protein indicates that this change should be a crucial step of signal transduction process among eukaryotic signaling systems.

3P237 ファラオニストランスデューサータンパク質、pHtrII の構造学的研究
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Structural studies of juxtamembrane region of a pharaonis transducer protein, pHtrII by NMR and EPR
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The *pharaonis* halobacterial transducer protein, pHtrII and *pharaonis* phoborhodopsin (also called sensory rhodopsin II), μ pR are phototaxis proteins from *Natronomonas pharaonis*. They form complex and transmits a light signal to the cytoplasm. In transducer proteins, the linker region connecting between membrane region and cytoplasmic region is believed to compose an important region for many taxis signal transductions. But linker region structure of the transducer protein has not been reported. Thus, we focused on the structure of pHtrII linker region. We expressed pHtrII(1-159) and identified a trypsin resistant fragment (residue 100-159) within the linker region. The 3D structure was determined by NMR and it was shown that pHtrII(100-159) formed α -helix partly in an aqueous environment. The CW-EPR spectra represented that this α -helix was preserved in liposome. The distances between pHtrII linker and μ pR E-F loop by pulsed electron-electron double resonance (ELDOR) suggest that pHtrII linker region does not form rigid continuous α -helix in detergent. It was reported that light activation alters the distance between E-F loop of μ pR and juxta-membrane region of pHtrII. Based on this, our data suggests that the flexibility of the linker region may be important at the signal transfer from μ pR to pHtrII. The relationship between the structural changes and signal transduction will be discussed.

3P239 センサリ-ロドプシン II トランスデューサーHAMP ドメインの分子動力学研究
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3aD11 Molecular Dynamics Study of the HAMP domain of Halobacterial Transducer of Rhodopsin II (HtrII)
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Sensory rhodopsin II (SRII) is a member of light-activated sensory proteins in extreme halophiles. It is argued that upon excitation of the retinal by blue-green light SRII changes the conformation and induces a structural change in the cognate transducer HtrII to finally lead to phototactic responses. The HAMP domain of HtrII, which lies between the transmembrane and cytoplasmic domains, plays an important role in signal transduction. The crystal structure of 2:2 complex of SRII and HtrII has a lot of missing parts including the HAMP domain. Here, we tried to have a stable model of the HtrII HAMP domain based on the NMR structure of Af1503 (PDB id:2ASW), and performed molecular dynamics (MD) simulation to confirm the structure stability. The NMR structure of the HAMP domain is a homodimer of a four-helix bundle coiled-coil structure. It is proposed in the original article of Hulko et al. that HAMP domain could interconvert between two packing modes "knobs-to-knobs" and "knobs-into-holes" by rotating the adjacent helices in the coiled-coil structure by 26° in signal transduction. The NMR structure is in "knobs-to-knobs". A homology model of the HtrII HAMP domain was generated based on a pairwise alignment between Af1503 and HtrII. This model, as well as the NMR structure, was subject to 10ns MD simulation in the explicit solvent, and proved the structure stability. We are now trying to model the "knobs-into-holes" structure for both Af1503 and HtrII by rotating helices. We will discuss the results in the poster.