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硝酸イオン結合型ファラオニスハロロドプシンの赤外分光
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FTIR Study of Nitrate-bound *pharaonis* Halorhodopsin
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pharaonis Halorhodopsin (*pHR*) is a light-driven chloride pump and a chloride is bound in the Schiff base region of the retinal chromophore. Recently, we proposed a model for the early stage of the chloride pump [1]. This is based on an FTIR analysis of the X-D stretching region (O-D or N-D stretching vibrations) in D₂O, by which specific hydrogen-bonding interactions were found for the protonated Schiff base and water molecules. In the present study, we investigated the anion pumping mechanism by use of nitrate-bound *pHR*. It is known that *pHR* can also pump nitrate, and the photocycle kinetics are different between *pHR*(NO₃) and (Cl⁻). The present time-resolved UV-visible spectroscopy showed accumulation of the O intermediate for *pHR*(NO₃). Then, we successfully measured the O-minus-*pHR* difference FTIR spectra of *pHR*(NO₃) at 250 K, which was impossible for *pHR*(Cl⁻). A negative band at 1725 cm⁻¹ was observed for the O-minus-*pHR*(NO₃) spectra, but not for the L₂ intermediate. The band was observed for the L₂-minus-*pHR*(Cl⁻) spectra, and identified as the C=O stretching vibration of Glu234 [2]. It seems that deprotonation of Glu234 at the extracellular surface depends on the anion size. Together with the assignment of each vibration by isotope-labeling, protein structural changes in the late intermediates will be discussed.

Reference

[1] M. Shibata et al. (2005) *Biochemistry* **44**, 12279-12286.

[2] M. Shibata et al. (2006) *Chem. Phys. Lett.* **432**, 545-547.

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ハロロドプシンの光異性化初期過程の超高速ポンプ-プローブ分光:ハロゲンイオン依存性と異性化ダイナミクス
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Ultrafast Pump-Probe Study of Primary Reaction Dynamics of Halorhodopsin: Halide Dependence and Isomerization Dynamics
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Halorhodopsin is a retinal protein that acts as a light-driven chloride pump in *Haloarchaeal* cell membrane. A chloride ion is bound near the retinal chromophore, and photoinduced isomerization of the chromophore takes place from all-*trans* to 13-*cis* form. This reaction triggers unidirectional chloride-ion pump that proceeds in the millisecond timescale. This paper reports a 30-fs time-resolved pump-probe study on the primary ultrafast dynamics of *pharaonis* halorhodopsin that contains Cl⁻, Br⁻ or I⁻. All the temporal behaviors of the S₁-S₀ absorption, ground-state bleaching, K-intermediate absorption (13-*cis* form) and stimulated emission consist of three components having 50 fs, 2 ps, and 5 ps time constants. The 30-fs time resolution enabled us to observe a 50-fs component in the S₁-S₀ absorption and stimulated emission signals for the first time, which is assignable to the initial relaxation process from the Franck-Condon region to the reactive (S₁^r) and nonreactive (S₁^{nr}) S₁ states. Referring to previous reports, the 2-ps component was attributed to the isomerization process from the S₁^r state to the K-intermediate via the conical intersection, while the 5-ps component was assigned to the internal conversion from the S₁^{nr} state. We also examined halide-ion dependence of the rate and the yield of the isomerization. On the basis of the observed halide-ion dependence, we discuss the isomerization dynamics and microscopic mechanism of the initial step of the photoinduced halide-ion pumping by using three-state model.

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ファラオニスハロロドプシンのクロライドポンプにおけるプロリン残基の役割
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Role of proline in the chloride pump of *pharaonis* Halorhodopsin
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Halorhodopsin is a light-driven chloride ion pump with a retinal chromophore. Absorption of light by all-*trans* retinal triggers a cyclic reaction that comprises a series of intermediates, designated as the K, L₁, L₂, N, and O states. A chloride ion is bound in the Schiff base region and unidirectional Cl⁻ transport is probably enforced by the specific hydrogen-bonding interaction with the protonated Schiff base and water molecules. Previously, we proposed a mechanism of the early stage of chloride pump by monitoring their FTIR signals in the L₁-intermediate [1]. Then, we found that Glu234 at the extracellular side is deprotonated in the L₂-intermediate, which would contribute to the unidirectional Cl⁻ transport [2]. *pharaonis* Halorhodopsin (*pHR*) possesses three proline residues (Pro75, Pro132 and Pro226) in the transmembrane region, possibly forming a kink in each α-helix. In this study, we investigate the role of proline in the pump cycle of *pHR* by use of the mutants (P75A, P132A and P226A). Time-resolved UV-visible spectroscopy showed that only P132A does not form the O-intermediate. FTIR analysis of the ¹⁵N-labeled proline sample identified the imide C-N stretching vibration, and the mutation study revealed structural changes of Pro75 in the L₁ and L₂-intermediates. In addition, the L₂-formation accompanies structural changes of Pro132. It is thus likely that the kink motions of the B and C helices are important for chloride pump.
[1] M. Shibata et al. *Biochemistry* 2005.
[2] M. Shibata et al. *Chem. Phys. Lett.* 2006.

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ファラオニスハロロドプシンの固体NMR 構造解析：サンプル調製の最適化
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Solid-state NMR of *pharaonis* halorhodopsin: Optimization of sample preparation
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Rhodopsin is a membrane protein having a typical seven-transmembrane-helical structure. This is composed of opsin and retinal chromophore. Archaeal rhodopsin, the structural family of rhodopsin, functions as light-driven ion pump or sensor. Halorhodopsin (HR), one of archaeal rhodopsin, is an inward-directed light-driven chloride pump. In the photo-excited state or chloride-induced state, HR would undergo dynamic structural changes such as helix moving, opening of uptake channel, and re-orientation of the specific side-chain. However, there is no direct evidence for these structural changes of HR. The purpose of this study is structural characterization of HR by using solid-state NMR. ¹³C and ¹⁵N doubly labeled HR from *Natronomonas pharaonis* (*pHR*) was expressed in BL21 (DE3) of *E. coli*, solubilized with n-dodecyl-β-D-maltoside, and purified by using Ni-NTA column. Moreover, the solubilized *pHR* was reconstructed with DMPC-*d₅₇*. The molecular rearrangement of *pHR* in lipid bilayer was evaluated by AFM. By using this sample, we performed various 1D and 2D solid-state NMR experiments of *pHR* for signal assignments and structural analysis.