- 3P216 熱力学振動法によるバクテリオロドプシンの構造変化に関する理論的研究
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Theoretical study on the structural change of bacteriorhodopsin by the thermodynamic perturbation method.
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Bacteriorhodopsin (bR), found in *Halobacterium salinarum*, is a prototype of efficient molecular machine functioning as light-activated proton pump. The photocycle is triggered by the ultrafast photoisomerization reaction of the protonated Schiff base of retinal. Among the five distinct intermediates (K, L, M, N and O), of the photocycle, structural information of the later stages is less available compared to the early intermediates. It has been widely accepted that there are some similarities between the acid blue form and the O intermediate. In this study, we challenge the structure modeling of the O intermediate. Conformational change of bR was simulated by asymptotically changing the protonation state of titratable amino acid residues.

To achieve the accurate molecular modeling, we carefully constructed a realistic system of the purple membrane, which is the most accurate model ever known before. This membrane model is consisting of the trimer of bR molecules and native lipid molecules. Before we perform the modeling of the O intermediate, we tried molecular modeling of the acid blue form of bR. As a result, we succeeded in reproducing the structural features of the acidic state, namely, the global helix arrangement and orientations of some functionally important residues. Furthermore, some implications were obtained about the proton transfer in the later stage photocycle and structural response of bR to the inner charge distribution. The preliminary result of the molecular modeling of the O intermediate is also discussed.

- 3P218 固体 NMR によるバクテリオロドプシンおよびバクテリオオプシンの細胞外ループにおける動的構造解析
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Dynamic aspects of extracellular loop of bacteriorhodopsin and bacterio-opsin as studied by solid-state NMR
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Bacteriorhodopsin(bR) acts as a light-driven proton pump, and is composed of retinal chromophore and its apoprotein, bacterio-opsin(bO). It is important to determine the conformational changes of protein induced by photoisomerization of retinal for elucidation of proton pump activity. In this study, we therefore focus on the interaction between retinal and protein especially at the extracellular side to gain insight into proton release mechanism. Structure and mobility of extracellular region in bR and bO were examined by solid-state NMR using CP-MAS, DD-MAS, REDOR filter and relaxation times measurements in order to investigate the effect of retinal on extracellular region in bR. The ¹⁵N signal of Pro70 was successfully assigned by REDOR filtered experiment. It turned out that Pro70 moiety of BC-loop in the extracellular side appeared to be a rigid β -sheet by T₁ measurement, and BC-loop may possess large amplitude motion as revealed by relatively longer T_{NH} values. In addition, the β -sheet conformation in BC-loop changed to disordered state induced by the removal of retinal. These results explain that retinal influences stabilities of structure and mobility of BC-loop which is located in extracellular side and far from Schiff base region. Thus, hydrogen-bond network formed from Schiff base region to extracellular surface region including BC-loop exists and plays an essential role for the proton transport of bR.

- 3P217 固体高分解能NMRによるバクテリオロドプシンのレチナール異性化に応答するTyr残基の主鎖コンフォメーションの解析
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3aD02 High-Resolution Solid-State NMR Studies of Backbone Conformations at Tyr in Bacteriorhodopsin corresponding to Retinal Configurations.
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Backbone conformations at Tyr in bacteriorhodopsin (BR) corresponding to all-*trans* and 13-*cis*, 15-*syn* retinal configurations in the dark, light, and pressure adapted states were investigated for Tyr-X peptide bonds by using double amino acid isotope labeled [1-¹³C]Tyr; [¹⁵N]X-BR by means of REDOR (rotational echo double resonance) technique in solid-state NMR. The ¹³C NMR signals obtained from the difference spectra between REDOR and full echo experiments allowed selectively detect a Tyr signal for Tyr185, Tyr64, and Tyr26, etc. Two ¹³C NMR peaks at 173.4 and 177.7 ppm were observed for REDOR-filtered spectrum of Tyr185 near retinal in the dark [1]. All-*trans* peak intensity was increased in the light adapted state by *in situ* photo-illumination under MAS condition. In addition, the 13-*cis* peak intensity was increased for pressure-adapted state under a fast MAS [2]. By contrast, the spectra of Tyr26 and Tyr64 show a single peak at 174.0 and 175.2 ppm, respectively, because they are located far from the retinal and their side chains turn outside of the helical bundle. It is, therefore, revealed that these two signals of Tyr185 were attributed to the BR with all-*trans* and 13-*cis* retinal. Our results indicate that the retinal isomerization may induce a significant local change of backbone conformation that is relevant to the regulation of light driven proton pump pathway through retinal-protein interactions in BR.[1] I. Kawamura et al. (2007) *J. Am. Chem. Soc.* 129 1016-1017.[2] I. Kawamura et al. (2007) *Photochem. Photobiol.* 83346-350.

- 3P219 固体NMRによるバクテリオロドプシン中トリプトファン残基の構造分析
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Conformational analysis of tryptophan residues in bacteriorhodopsin by solid-state NMR
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Bacteriorhodopsin (bR) in purple membrane of *H.salinarum* has a light-driven proton pump activity by photoisomerization of retinal. Most Trp residues in bR are located at the membrane surface and transmembrane region in the vicinity of retinal. In particular, Trp182 in F' helix closely lies in the vicinity of retinal. In this study, we will focus attention on characterization of the interaction of Trp residues with retinal for bR by solid-state NMR. We assigned ¹³C NMR signal at 175.9 ppm to the carbonyl carbon of Trp182 using REDOR (rotational echo double resonance) filter experiments for [1-¹³C]Trp-, [¹⁵N]Ser-labeled bR. It is found that a signal of Trp182 appeared as a single peak, whereas Tyr185 in the vicinity of retinal showed a doublet peak corresponding to 13-*cis* and all-*trans* retinal configurations. This is because Tyr185 is located at the extracellular side from retinal and forms hydrogen bond networks including retinal. By contrast, Trp182 is located in the cytoplasmic side from retinal and is not sensitive to conformational change of two retinal configurations in the dark. In addition, ¹³C CP-MAS NMR spectra of [1-¹³C]Trp-, [3-¹³C]Ala-labeled wild-type bR were compared with those of W182F mutant. The result indicates that ¹³C NMR signal at 17.2 ppm of Ala184 in W182F mutant is decreased. It is shown that W182F mutant is more mobile in F' helix around Trp182 than wild-type bR is. It is considered that Trp182 located cytoplasmic side from retinal may regulate dynamics rather than structure near retinal binding pocket.