1P094 バクテリオロドプシンミュータントL93Aの結晶構造解析
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 Crystallographic study of the Leu93 → Ala bacteriorhodopsin mutant.
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Bacteriorhodopsin (bR), a membrane protein found in the plasma membrane of Halobacterium salinarum, functions as a light-driven proton pump. When the all-trans isomer of bR in the light-adapted state absorbs visible light, it undergoes a reaction cycle involving five spectroscopically distinguishable intermediates (K, L, M, N and O). Light-induced structures have been investigated to understand the transport mechanism. We have previously determined the structures of intermediates (K, L and M). Our next targets are late intermediates (N, O). But, it is difficult to capture both intermediates. We tried structure decision of O-intermediate by the L93A mutant that is reported to have very long lifetime of O-intermediate. In this study, we applied membrane fusion method to crystallize the mutant, and got a hexagonal crystal belonging space group P622 (a=b=101.9Å, c=111.9Å). Diffraction data resolution showed that the main-chain structure of L93A is very similar to that of wild-type protein. Replacement of Leucine 93 with alanine is found to create a large cavity near the C13 methyl of retinal, in which three or four water molecules may be accommodated. Absorption kinetics measurements of the hexagonal crystal show that the lifetime of O-intermediate at 296K is 2.6 sec. This value is a little longer than absorbed for a membrane suspension.

1P096 バクテリオロドプシン3次元結晶のM中間体寿命に及ぼす格子力の影響
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 1aC04 Decay kinetics of the M intermediate of bR in 3D crystals: Influence of the crystal lattice force
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Bacteriorhodopsin (bR), the sole membrane protein found in the purple membrane of Halobacterium salinarum, functions as a light-driven proton pump. It contains one molecule of retinal, which is bound via a protonated Schiff base linkage to the ε-amino group of Lys216. When the all-trans isomer of bacteriorhodopsin in the light-adapted state absorbs visible light, it undergoes a reaction cycle involving five spectroscopically distinguishable intermediates (i.e., K, L, M, N and O). In this study, the precipitant concentration in the crystallization mother solution was varied to quantitatively clarify possible effects of the lattice force on the decay kinetics of the M intermediate. We found that once the P622 crystal had been grown by the membrane fusion method, the crystal remained unbroken even when the precipitant concentration was decreased below a critical value for the crystal growth. And it was observed that as the precipitant concentration was lowered from: 3.5 M to 0 M, the half-life of M decreased from 300 ms to 10 ms (at 25 °C). This change correlated with expansion of the unit cell dimension along the c axis, which increased from 112.1 Å to 112.5 Å as the precipitant concentration was decreased from 3.5 M to 1 M. These observations suggested that the elongated lifetime of the M intermediate observed at high precipitant concentrations is due to a limited motional freedom in the protein-protein contact region in an inter-membrane space.

1P095 バクテリオロドプシンの疎水キャビティに結合する希ガスとその効果
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 1aC03 Binding of Noble Gases to a Hydrophobic Cavity in the Cytoplasmic Half of Bacteriorhodopsin
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Bacteriorhodopsin (bR), a light-driven proton pump found in halobacteria, contains retinal as the chromophore. When the all-trans isomer of bR in the light-adapted state absorbs visible light, it undergoes a reaction cycle involving five spectroscopically distinguishable intermediates. Our previous crystallographic studies of the K, L and M intermediates have revealed that water relocation takes place in the early stage of the photocycle. Since the pKa values of the ionizable residues in the proton pathway are dependent on how they interact with water molecules, it is very possible that water relocation plays an important role for unidirectional movement of proton. We hypothesized that there must exist spatial rooms (cavities) for intra-protein movement of water molecules. In this study, we investigated the crystal structure of bR in the presence of xenon, to elucidate the functional role of internal cavities. It was found that xenon binds to a hydrophobic cavity near the CD loop. The occupancy was 20 - 35% when the crystal was exposed to an Xe-pressure of 0.4 - 1.1 MPa before flash cooling. Although xenon binding does not induce any detectable changes in the protein structure, it greatly perturbs the millisecond kinetics of the photocycle; i.e., the decay of M is accelerated, while the decay of N is decelerated. When partial pressure of xenon is under 1.5 MPa, the decay rate of M increases in proportion to partial pressure of xenon. It is suggested that diffusion of Xe is rapid enough to average out the lifetime of M.

1P097 合成脂質膜に再構成したバクテリオロドプシンの光サイクルに対する分子集合状態の影響
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 Effect of molecular assembly on photocycle of bacteriorhodopsin reconstituted into dimyristoylphosphatidylcholine vesicles
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Bacteriorhodopsin (bR), a light-driven proton pump on the plasma membrane of H. salinarum, assembles in the two-dimensional (2D) crystal patches known as the purple membrane (PM). Our recent studies on photobleaching of bR suggested that the 2D molecular assemblies significantly contribute to proton pumping activity by the cyclic photoreaction. In this study, bR reconstituted into dimyristoylphosphatidylcholine (DMPC) vesicles are employed since aggregation state of bR molecules can be changed from the 2D crystals to the monomeric state through the gel-to-liquid crystalline transition of lipid bilayer. The photocycle of reconstituted bR in the gel and the liquid crystalline phase was investigated with transient visible absorption spectroscopy. The measured time-resolved difference spectra indicated remarkable differences near 400 nm between the two phases of DMPC bilayer membranes. In the gel phase at 7 °C, two substates of the M intermediate with almost the same absorption maximum were observed, whereas the spectrum of M showed a splitting into an early and a late component shifted by approximately 10 nm in the liquid crystalline phase at 32 °C. A similar blue-shifted late M intermediate was reported for monomeric bR solubilized with detergents by Varo and Lanyi. For bR molecules in the PM, on the other hand, the absorption maximum of the two substates of M was almost identical in the temperature range. Effect of molecular assembly on photocycle of bR will be discussed with results about protein structure of reconstituted bR.