- 1P098 バクテリオロドプシン再生反応に対するイオン濃度及び温度の影響
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- 1aC05 Effect of Ion Concentration and Temperature on Regeneration of Bacteriorhodopsin
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Bacteriorhodopsin(bR), a light-driven proton pump on the plasma membrane of *Halobacterium salinarum*, consists of an apoprotein, bacterioopsin (bO), and a retinal chromophore. bO spontaneously recovers to bR on adding all-*trans* retinal. Regeneration experiments of bacteriorhodopsin at high temperatures previously showed an existence of two regeneration components with different rate constant. This suggested that bO in the thermally unfolded state regenerates to bR by dynamic structural fluctuation. In this study, we carried out regeneration experiments in the presence of NaCl to investigate the contribution of electrostatic interactions on regeneration of bR. With the kinetic analysis, the regeneration process slows depending on the concentration of NaCl at room temperature. Regeneration experiments at high temperature in the presence of NaCl were also carried out according to the same two protocols as the previous experiments without NaCl. In the first protocol, when regeneration was performed at 25°C after incubation at high temperatures, the addition of NaCl didn't affect the results. In contrast, when incubation and adding retinal was performed at the same elevated temperature (second protocol), regeneration yields at high temperature (50°C-70°C) were lower than that in the absence of NaCl. This suggests that adding NaCl weaken the degree of fluctuation of bO. Based on these experimental results, we propose one schematic model for the regeneration of bR from bO and all-*trans* retinal considering the effect of electrical screening.

- 1P100 再構成バクテリオロドプシンの変性実験から見た、膜タンパク質構造安定性に与えるタンパク質間結合の寄与
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- 1aC06 Effect of inter-molecular interaction for stabilizing membrane protein -Denaturation experiments of reconstituted bacteriorhodopsin-
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Bacteriorhodopsin (bR) is a light-driven proton pump on the plasma membrane of *H. salinarum*, which assembles in the two-dimensional (2D) crystal patches (purple membrane). The environment of bR can be changed by the solubilization with detergents and the reconstitution into the lipid bilayer. Previously, the thermal denaturation and the photobleaching experiments of bR were carried out for purple membrane and solubilized bR. However, the same kind of experiment has not been performed for the reconstituted membrane of bR in dimyristoylphosphatidylcholine (DMPC) bilayer. The solubilized bR and the reconstituted bR are similar in the meaning that the bR molecules do not bind with each other. However, solubilized bR may suffer from the damage by the interaction with detergents. In this work, we performed the experiments of the thermal denaturation and the photobleaching for the reconstituted bR in DMPC bilayer. The thermal denaturation of bR in the reconstituted membrane occurred above 60°C, while the denaturation temperature for bR in purple membrane is 70°C. The temperature of the photobleaching is lower than that of the thermal denaturation in general, indicating that the photointermediates are less stable than the ground state in the dark condition. The temperature of the photobleaching for the reconstituted bR was 30°C and bR in the purple membrane showed the photobleaching above 60°C. This result indicates that the protein-protein interaction with neighboring bR stabilizes the photointermediates rather than the native state.

- 1P099 中性およびアルカリ pH 条件下における光退色が引き起こす紫膜中のバクテリオロドプシンの構造変化
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- Structural changes of bacteriorhodopsin in purple membrane induced by irreversible photobleaching at neutral and high pH conditions
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Bacteriorhodopsin (bR), located in purple membrane (PM) of *Halobacterium salinarum*, is a light-driven proton pump. Although the photo-intermediate states of bR can recover to the initial state through the photocycle at the physiological condition, an irreversible photobleaching occurred at high temperature and at high pH. A recent kinetics study for the photobleaching of bR in PM at high temperature showed that fast- and slow-decayed components were observed below pH 9, whereas only single component was found above pH 10. The structural changes of bR in PM also started to observe at this pH range, suggesting a contribution of the electrostatic interaction on the bR structure and photobleaching behavior. However, it is an open question what kind of structural change occurs in bR in the fast and slow processes. This information should be an aid to consider the photobleaching mechanism. We have herein elucidated structural changes due to the photobleaching at neutral and high pH by attenuated total reflection FTIR spectroscopy. IR spectra of the photobleached bR showed that a α_1 -to- α_2 transition in the helical structure occurred with increasing a degree of the photobleaching and that the α_1 structure diminished in the completely photobleached bR. Time dependences of the helical structural changes were similar to those of the retinal absorption changes, suggesting that the α_1 -to- α_2 transition occurred in the fast and slow components. A model for the photobleaching processes at neutral and high pH will be discussed from these results.

- 1P101 高速AFMによるbR2次元結晶の脱結晶化及び再結晶化のダイナミックイメージング
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- Dynamic imaging of disassembling and recrystallization processes of bR 2D crystals by high-speed AFM
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The light-driven proton pump bacteriorhodopsin oligomerizes into a trimer which naturally assembles into two-dimensional (2D) crystals forming the so called purple membrane (PM) of *Halobacterium salinarum*. The mechanism of the two-dimensional crystallization has been investigated by various techniques for a long time. Electron microscopy, X-ray crystallography and nuclear magnetic resonance have determined that the lipid composition of the membrane, protein density and temperature are essential for the 2D crystallization. On the other hand, atomic force microscopy (AFM) allows imaging single proteins in fluid with molecular resolution; disassembly due to photo-bleaching and then recrystallization have been observed using AFM. These observations indicated that protein-lipid and protein-protein interactions are also relevant for the crystal formation. However, conventional AFM lacks the temporal resolution necessary to monitor the dynamic process of crystallization. While, we have succeeded in developing high-speed AFM which can directly capture the dynamic behavior of individual protein molecules at 80 ms/frame in solutions.

In this study we applied the high-speed AFM to monitor the dynamic process of disassembly induced by photobleaching and subsequent recrystallization. We succeeded in capturing the disassembling process that was spontaneously developing and bacterio opsins were diffusing as a trimer within the membrane patch. Thus, membrane protein dynamics was directly observed at high resolution.