unbound protein, while it was not found for the ATP-bound protein. These findings suggest that binding of ATP controls the opening/closing motion responsible for a structural change from outward-facing into inward-facing, where the drug binding site is accessible from the cell interior.

3P-082 機械受容型イオンチャネルダイナミクスの統計的解析

Statistical analysis of the mechanosensitive ion channel's dynamics

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The mechanosensitive ion channel(MscL) is a membrane protein which functions under the external force (i.e. membrane tension) by the surrounding lipid bilayer. Here we focus on the analysis of MscL dynamics. Patch clamp experiment(PCE) is one of the fundamental techniques to investigate the function of ion channels, and the data from PCE (i.e. the conductance time series) are analyzed in general by the Markov kinetic model with several states. Although this analysis provides us with the information about the free energy landscape, it is somehow difficult to adopt the result as the model of gating dynamics. There are several reasons:(1)we can not avoid specifying the number of states subjectively, (2)when the time series doesn't show the distinct conductance levels (this may be the case of MscL), it is difficult to distinguish the several levels, (3)since the fitting of the model is performed globally, the local dynamical information might be lost sometimes.

We will , therefore, take another kind of statistical approach, so-called the change point analysis(CPA). CPA finds the "change points", defined as the time when the property of the underlying system changes. In CPA, the number of states is not assumed a prior and determined by the objective criteria. We present the result of the fundamental analysis and CPA for the conductance time series of MscL dynamics, and discuss what we can learn of the gating mechanism from these analyses. (This research is collaboration with the Prof. Sokabe's group in Nagoya University.)

3P-083 バクテリオロドプシンミュータント L93A の長寿化させた光反応中間体の結晶学的研究

Crystallographic study of a long-lived photoreaction intermediate of the L93A mutant of bacteriorhodopsin

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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). To understand the transport mechanism, we previously analyzed the structures of several reaction intermediates (K, L and M) of bacteriorhodopsin. We also tried to determine the structures of the intermediates occurring in the late half of the photocycle remains unsuccessful, but our trials remain unsuccessful due to difficulty in efficiently trapping these intermediates. To overcome this difficulty, we crystallized the L93A mutant, which is reported to have a very long lifetime of the O intermediate. Diffraction data showed that replacement of Leu93 with alanine creates a large cavity near the C13 methyl of retinal, in which four water molecules are accommodated. In this study, we tried to obtain structural information of the O intermediate of L93A. In a difference map that was derived from two datasets that were collected from illuminated and un-illuminated crystals, we were not able to observe any clear densities. This seems to reflect a very low efficiency of formation of the O intermediate in the crystal. We are now searching for optimal crystallization conditions for investigation of light-induced structural changes in the L93A mutant.

3P-084 紫膜バクテリオロドプシンの光退色現象におけるタンパク質-脂質間相互作用の影響

Effect of protein-lipid interaction on irreversible photobleaching of bacteriorhodopsin in purple membrane

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Bacteriorhodopsin (bR), which is a light-driven proton pump of *Halobacterium salinarum*, forms a two-dimensional (2D) crystal together with lipid molecules, known as purple membrane (PM). We previously revealed that bR underwent an irreversible photobleaching at high temperature, in which the recovering process of photocycle is hindered eliminating the function. A kinetic study for the photobleaching showed that two kinds of bR species with respect to the structural stability existed in PM at high temperature and that a fraction of less stable species increased with the 2D-crystal melting. This strongly suggests that intermolecular interaction in PM affects the heterogeneous stability against photobleaching. Here, we studied the effect of lipid molecules on the 2D-crystal melting and the functional activity by using delipidated bR. We prepared delipidated bR using a detergent of 3-[(cholamidopropyl)dimethylammonio]propanesulfonate (CHAPS). The results showed that delipidation caused bR destabilization under illumination at temperature range between 60 and 85 °C at

pH 7. Kinetic analysis showed that this destabilization was mainly responsible for an increase of the rate constant of slow-decayed component. We also conducted the same experiments for native PM in the presence of NaCl at high concentration and found that the similar characteristics of photobleaching as delipidated bR were exhibited at above NaCl concentration of 1 M. The effects of lipid removal and an electrostatic shielding on PM will be discussed based on these results.

3P-085 DMPC、DPPC 二層膜に再構成したバクテリオロドプシンの光退色

Photobleaching of bacteriorhodopsin reconstituted in DMPC and DPPC bilayer.

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Reconstituted bacteriorhodopsin (bR) in dimyristoylphosphatidylcholine (DMPC) lipid membrane was previously studied in view of the relationship between the lipid bilayer transition and the irreversible photobleaching of bR. The photobleaching of bR became significant above 30 °C at which DMPC membrane is in the lipid crystalline phase. An interesting question arises from this previous result: At which temperature the photobleaching occur for reconstituted bR in dipalmitoylphosphatidylcholine (DPPC) bilayer whose main transition is 42 °C. In the photobleaching measurement, suspensions of reconstituted bR membrane which were reconstituted in DMPC and DPPC membranes were incubated at high temperature for an hour under illumination or in the dark and then quenched to about 4 °C. The ratio of native bR after incubation to total bR was estimated by the absorbance at 560 nm. The photobleaching of bR in DPPC began at about 30 °C and the temperature dependence of the ratio of photobleached bR in DPPC membrane was the same as that in DMPC membrane. However, small but significant difference was observed between the two types of reconstituted bR below 30 °C. The onset temperature of the photobleaching of reconstituted bR membrane in DMPC bilayer was actually about 20 °C. We will discuss the relationship between the onset temperature of the photobleaching and the three types of phase transitions of lipid bilayer membrane: main, pre- and sub-transitions.

3P-086 バクテリオロドプシンに対するフォースカーブ測定のための細胞質側と細胞外側の識別

Identification of cytoplasmic and extracellular side of bacteriorhodopsin for force spectroscopy

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Recently, force spectroscopy is extensively studied for evaluating intra- and inter-molecular interaction. Bacteriorhodopsin is one of typical membrane protein which has been used for the experiments of force spectroscopy.

We are developing experimental and computational methods for estimating the intra-/inter-interaction in membrane proteins by the force spectroscopy. On one hand, amino acid chemical potentials in membrane and in water are considered in this simulation. The simulation showed the force curves from the cytoplasmic side and from extracellular side. In the present study, we have established a method to identify the cytoplasmic and extracellular sides for force spectroscopy.

AFM is SPA400(SII nanotechnology). Constant of spring of cantilever is 0.08 N/m, and tip diameter is not over 20 nm. Measurements were carried out at the room temperature, and in darkness. Purple membrane suspension which controlled pH was adsorbed onto freshly cleaved mica. Half an hour later, rinsed once with buffer solution. Membranes were observed in buffer, and obtained force spectrum.

The difference of the images of cytoplasmic and extracellular sides is significant in air but insignificant in buffer solution. But we first took the images of bacteriorhodopsin in buffer solution and then those in air. Comparing the images, we found the global characterization of purple membrane in the buffer solution. We used this result to obtain force spectrum from cytoplasmic and extracellular side by AFM.

We will show the comparison between the experimental force spectra and the computational simulation.

3P-087 大腸菌機械受容チャネル MscL のメカノゲーティングにおける水の浸潤と透過の役割

Role of Water Penetration and Permeation in Mechano-Gating of the *E-coli* Mechanosensitive Channel MscL

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The bacterial mechanosensitive channel of large conductance MscL is constituted of homopentamer of a subunit with two transmembrane inner and outer α -helices, and its 3D structure of the closed state has been resolved. The major issue of MscL is to understand the gating mechanism driven by tension in the