the four peaks came from the chemical potential of side chains. The F-D curves of BR for drawing from loop segments other than the N- and C-terminal segments could be calculated. We are planning to compare the F-D curves of simulation and the corresponding experiment at various pH, which will be discussed at presentation in detail.

1B1612 人工リン脂質2層膜に再構成されたバクテリオロドプシンの分子集合・構造安定性における古細菌型脂質の役割

Role of archaeal lipid in molecular assembly and stability of bacteriorhodopsin reconstituted into dipalmitoylphosphatidylcholine bilayer

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Bacteriorhodopsin (bR), which was found in cytoplasmic membrane of *Halobacterium salinarum*, forms a two-dimensional (2D) crystal with a hexagonal packing of bR trimers and archaeal lipids, referred as purple membranes (PM). Although bR originally acts as a light-driven proton pump, irreversible photobleaching, i.e. light-induced denaturation, was observed together with bR disassembly above the main transition temperatures in reconstituted bilayers. However, it was shown that unignorable amounts of endogenous PM lipids were also reconstituted with bR, and that bR cannot form the 2D-crystalline array even under the main transition temperature when all endogenous PM lipids were removed. To elucidate an effect of archaeal lipids on bR assembly and irreversible photobleaching, we performed the photobleaching experiments against bR in the reconstituted system with partial (75 %) removal of PM lipids. Visible circular dichroism spectra showed that the delipidation of PM lipids brought about partial disaggregation at 23 °C at which some kind of lipid phase transition occurred. Flash-photolysis measurements exhibited about 20 % reduction in formation of M-intermediate state at 25 °C in the delipidated samples compared to 11°C, despite no change in the M-formation below 25 °C in the undelipidated samples. Light-induced difference absorption spectra showed significant generation of a blue-shifted M-state at 385 nm above 25 °C in the delipidated samples. The role of archaeal lipids will be discussed based on these experimental results.

1B1624 HAMP ドメインの分子モデルを結合した SRII-HtrII 複合体の分子動力学シミュレーション

Molecular dynamics simulations of SRII-HtrII complex with a modeled HAMP domain

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The complex of sensory rhodopsin II (SRII) and its cognate transducer HtrII (SRII: HtrII/2: 2) is a photosensor associated with phototaxis in extreme halophiles. In the complex, the HAMP domains of HtrII play an important role in transferring the signal from the membrane to the cytoplasmic regions. However, the coordinates of the HAMP domains are missed in the crystal structures of the complex. We present model structures of the complex with the N-terminal HAMP domain of *Natronomonas pharaonis* (SRII:1-239 and HtrII: 1-136), which were built using the crystal structures of the ground state and of the M intermediate, respectively, and a homology model of the HAMP domain, a dimeric four-helical coiled coil. These models were stable during molecular dynamics (MD) simulations for 100 ns, and gave good agreements with the electron paramagnetic resonance (EPR) experiments by Engelhard *et al.* for the “compact” state observed in a higher salt concentration. In the MD simulations, we observed significantly large changes in the relative orientations of the SRII subunits and the dynamic instability in the linker region between the transmembrane region and the HAMP domain in the ground state, not in the M intermediate. We also observed the motions in the E-F loop, the cytoplasmic side of F and G helices, and HtrII dimmer. We will discuss the relationships between these motions and the signal transduction process initiated by the photo-isomerization of retinal.

1B1636 The uptake and export of drugs in a multidrug transporter studied by coarse-grained simulations

Xin-Qiu Yao¹, Shoji Takada^{1,2} (¹Grad. Sch. Sci., Kyoto Univ., ²CREST, Japan Science and Technology Agency)

Multidrug resistance is a serious problem in current chemotherapy. The

tripartite assembly AcrA-AcrB-TolC plays the major role in the drug resistance of *E. coli*. By this system, drug molecules are actively pumped out of the bacterial cell bypassing the periplasm, energized by proton motive force. In the system, the transporter AcrB is the main functional part, which takes the responsibility for drug binding and for the coupling between drug export and proton translocation. The X-ray crystal structure of AcrB suggests a functionally rotating mechanism^{1,2}, which was confirmed by a computer simulation³. However, many details about the function of AcrB are still unclear. In this work, we investigate the pathways by which the drug binds to and then being exported from AcrB, by using coarse-grained theoretical models of proteins and ligands. First, the free energy profiles of passage of drug through different tunnels in AcrB were calculated and compared, which showed evident correlation between conformational change of AcrB and drug translocation. Second, direct simulation of drug uptake and drug export provided insight into the dynamics of transporting by AcrB. The details of results will be presented on the meeting.

1 Murakami, S., *et al.* Nature 419, 587-593 (2002).

2 Murakami, S., *et al.* Nature 443, 173-179 (2006).

3 Yao, X. Q., Kenzaki, H., Murakami, S. & Takada, S. Nat Commun 1, 117 (2010).

1B1648 多剤排出トランスポーター AcrB の全原子分子動力学シミュレーション

All-atom molecular dynamics simulations of drug efflux transporter AcrB

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In *E. coli*, it is known that the tripartite multidrug efflux system (AcrB/ AcrA/ TolC) exists, and AcrB resides in the inner membrane region and take part in substrate recognition and energy transduction for drug export through proton transfer. Recently, x-ray structures provided that AcrB forms trimeric protein where each protomer is different conformation, “binding”, “extrusion” and “access” state. In addition, extrusion state protomer has the unique side chain conformations of residues which are essential for proton translocation, “protonation site” (Asp407, Asp408 and Lys940). These results suggest that drugs are exported by a three-step structural change involved in the proton motive force which related with the conformational change in protonation sites. Because of the protonation state of residues is unknown from x-ray crystallography, the relation between conformational change mechanism and protonation state in protonation site is still unclear. In the present study, to clarify a relation between the structural change and protonation state in protonation site residues, we performed four types of all-atom molecular dynamics (MD) simulations of AcrB-membrane-water system which are different from protonation state of Asp 407 and 408 in protonation site of extrusion state protomer. The results of MD simulations suggested that the protonation state of Asp408 is of importance to control the conformational change of protonation site residues.

13:00~17:00 C 会場 / Room C

核酸

Nucleic acid

1C1312 エントロピー駆動的な DNA の機械的アンズIPPING

Entropic stabilization of DNA structures by hydrogen bonds found in mechanical unzipping

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Unzipping of double-stranded DNA is involved in several biological processes, such as transcription and replication. We have performed a quasi-static mechanical unzipping of DNA using intermolecular force microscopy, modified atomic force microscopy with high resolution. We obtained force-extension curves of two oligonucleotides with a stem-loop containing six tandem repeats of a 6-bp sequence, TCGGAG/TGCCTC and TCTGAG/TGACTC.

To reveal the molecular mechanism, we have carried out the molecular dynamics (MD) simulations of mechanical unzipping of DNA. Cross-correlation analysis of force curves showed the force-peaks of single hydrogen bonds similar to the unzipping experiments. Furthermore, we depicted the two-dimensional (2D) energy landscape, trajectory density map and entropy map to visualize the behavior of DNA unzipping from MD simulations. Trajectory density map revealed that GC base pair tended to maintain the three hydrogen