known about the detailed mechanism of the protein translocation at the atomic level.

In this study, we carried out 100-ns molecular dynamics simulations of SecY translocon from *Methanococcus jannaschii* and other bacterial species with explicit membrane and solvent molecules. From the simulations, it was found that the transmembrane segments of SecY are stable, while larger fluctuations are observed in the cytoplasmic segments. The relationship between conformational changes of SecY and the size of the cytoplasmic pore is discussed.

3P-088 固体 NMR を用いた暗順応状態バクテリオロドプシンの Tyr 主鎖コンフォメーションによるレチナール-タンパク質間相互作用の解析

Solid-state NMR studies of backbone conformations at Tyr as a probe of retinal-protein interaction in the dark-adapted Bacteriorhodopsin

Izuru Kawamura (1), Junko Tanabe (1), Takudo Nishio (1), Satoru Tuzi (2) and Akira Naito (1). (1: Graduate School of Engineering, Yokohama National University; 2: Department of Life Science, University of Hyogo)

The two retinal configurations in the dark-adapted bacteriorhodopsin (bR) coexist as all-*trans* and 13-*cis*, 15-*syn* states with the isomeric ratio close to 1. To understand the retinal-protein interaction, protein backbone conformations at Tyr residues in bR corresponding to two retinal configurations in the dark were investigated for individual Tyr-X peptide bonds by using double amino acid isotopically labeled [^{13}C]Tyr, [^{15}N]X-bR by means of REDOR technique in solid-state NMR. The ^{13}C NMR signals obtained from the difference spectra between REDOR and full echo experiments allowed selectively detection of individual Tyr signal for Tyr26, Tyr57, Tyr64, Tyr79, Tyr133, Tyr150 and Tyr185 at 293K. Two ^{13}C NMR peaks at 173.4 and 177.7 ppm were observed for REDOR-filtered spectrum of Tyr185 near retinal in the dark [1]. Two ^{13}C NMR peaks were also observed for the spectra of Tyr57, 79 and 133, respectively. Therefore, two signals of Tyr57, 79, 133 and 185 were attributed to the bR with all-*trans* and 13-*cis*, 15-*syn* retinal in the dark. By contrast, the ^{13}C NMR spectra of Tyr26, 64 and 150 show a single peak at 174.0, 175.2 and 175.7 ppm, respectively; because they are located far from the retinal and their side chains face outside of the helical bundle. These results indicate that protein structure can be altered in the region between the retinal binding site and extracellular surface through the hydrogen bond network depends on the retinal in the dark at room temperature.

[1] I. Kawamura et al. (2007) *J. Am. Chem. Soc.* 129 1016.

3P-089 KcsA への希ガス麻酔薬(Xe, Kr, Ar)と nonimmobilizer (Ne, He) の結合について

Noble gas anesthetics and immobilizers bind to different site of KcsA

Tomoyoshi Seto(1) and Masayuki Ozaki(1). (1: Dept of Anesthesiology, Shiga University of Medical Science)

Neuronal ion channel is prominent candidate of molecular target anesthesia. Using KcsA ion channel as model, anesthetic-protein interactions are investigated. We choose xenon, krypton and argon as anesthetics, which have simple structure and no electric charge. Neon and helium were studied as nonimmobilizers, which are structurally similar to anesthetics but do not have the anesthetic effects. Using computer simulation these binding sites of KcsA are searched. We discuss binding characteristics of anesthetic and nonimmobilizer. Methods: 1k4c (Protein Data Bank) was used. The coordinates of the associated protein, lipid, potassium ion, water molecule were deleted from 1k4c coordinates except structured water. Cavities in KcsA was searched with alpha-site finder in Molecular Operating Environment 2007.0902 (MOE, Chemical Computing Group, Canada), that is candidates of binding site of noble gas. Obtained dummy atom from alpha-site finder was used as initial position. Noble gas binding position was searched with energy minimization around initial position. MMFF94x was used for forcefield. Results&Discussion: Xe, Kr, Ar bound to gating region first, then bound to inter-helical space of transmembrane region. Ne bound to inter-helical space first, then to the gating region. Anesthetics and nonimmobilizers bound to different sites of KcsA. We speculate that pharmacological difference of anesthetic and nonimmobilizer originates from the interaction difference of these substances.

3P-090 アミロイド前駆体蛋白質の膜貫通部位の二量体構造

The dimer conformation of amyloid β precursor protein fragment in membrane

Naoyuki Miyashita (1), John E. Straub (3), and Yuji Sugita (1) (2) (4). (1: Computational Science Research Program, Riken, Japan; 2: Advanced Science Institute, Riken, Japan; 3: Department of Chemistry, Boston University, USA; 4: Institute for Bioinformatics Research and Development, Japan Science and Technology Agency)

The aggregation/oligomerization A β peptides has been known as an essential element of the pathogenicity of Alzheimer's disease. A β peptide is a product of cleavage of Amyloid Precursor Protein (APP) by β -secretase and γ -secretase. APP is a type I transmembrane (TM) protein composed of 695-770 amino acid residues.

Recent experimental studies showed that APP in membrane exists in a dimeric form, which is important for the cleavage by γ -secretase. Because APP contains three Gly-xxx-Gly (GXXXG) motifs, the dimer conformation is likely stabilized by C $_{\alpha}$ -H...O type hydrogen bonds between two Gly in different segments. A LXXXL mutant, in which two Gly in the second GXXXG are replaced to Leu, exists also in a dimeric form, but the cleavage by γ -secretase is prohibited in the mutant [1].

To obtain structural models of APP dimers, we performed replica-exchange molecular dynamics (REMD) simulations of two APP fragments (D $_{619}$ -K $_{651}$) of

wild type (GXXXG) and LXXXL mutant in implicit membrane [2]. Starting from random configurations, a stable dimeric form was predicted for wild-type APP, whereas two different dimeric forms were found in LXXXL mutant. In one form, two APP segment were crossed and strongly packed at the third Gly. In another form, two segments were aligned in parallel, putting hydrophobic residues inside. These structural models can explain the difference in the cleavage of APP by γ -secretase.

[1] P. Kienlen-Campard et al, *J. Bio. Chem.* **283**, 7733, (2008)

[2] Y. Sugita and Okamoto, *Chem. Phys. Lett.*, **314**, 141, (1999)

3P-091 不均一な膜タンパク質安定性-再構成バクテリオロドプシンの変性挙動に対する DMPC 膜相転移の効果-

Heterogeneous stability of membrane proteins -Denaturation behaviors of reconstituted bacteriorhodopsin around phase transition of DMPC-

Lumi Negishi, Shigeki Mitaku (Dept of Applied Phys, Grad Sch of Engineering, Nagoya Uni)

We performed the kinetic analysis of thermal denaturation and the photobleaching experiments of reconstituted bacteriorhodopsin (bR) in dimyristoylphosphatidylcholine (DMPC) bilayer focusing on the influence of phase transition of lipid bilayer on the stability of the membrane protein. The results of the photobleaching experiments indicated that there are four regions in the denaturation behaviors of reconstituted bR: (1) $T < 15^\circ\text{C}$, (2) $15^\circ\text{C} < T < 30^\circ\text{C}$, (3) $30^\circ\text{C} < T < 50^\circ\text{C}$, (4) $50^\circ\text{C} < T$. Below 15°C , bR in reconstituted membrane was not bleached. Photobleaching of reconstituted bR started in the temperature region of $15 - 30^\circ\text{C}$. In the region of $30 - 50^\circ\text{C}$, about half of bR in reconstituted membrane bleached after the light illumination for 4 hours. Thermal denaturation in dark occurred above 50°C . Circular dichroism measurement at 580 nm showed that the two dimensional crystalline structure of bR in reconstituted membrane melted above 30°C , at which phase transition occurred in reconstituted DMPC bilayer. Kinetic analysis of photobleaching was carried out, leading that fast component of two decay constants was almost constant throughout the temperature region of $30 - 50^\circ\text{C}$. These photobleaching behaviors of reconstituted bR in the temperature region strongly suggested that there are two states of bR in view of the structural stability.

3P-092 ヒト赤血球膜蛋白質バンド3膜貫通ドメインの電子線結晶構造解析

The structure of human erythrocyte band 3 membrane domain determined by electron crystallography

Tomohiro Yamaguchi (1), Yoko Hiroaki (2), Yoshito Abe (3), DongChon Kang (4), Naotaka Hamasaki (5), Yoshinori Fujiyoshi (2), Teruhisa Hirai (1). (1: Three-dimensional Microscopy Research Team, Harima Institute, RIKEN; 2: Dept of Biophysics, Faculty of Science, Kyoto Univ; 3: Dept of Immunology, Graduate School of Pharmaceutical Sciences, Kyushu Univ; 4: Division of Clinical Chemistry and Laboratory Medicine, Faculty of Pharmaceutical Sciences, Nagasaki International Univ; 5: Dept of Clinical Chemistry and Laboratory Medicine, Graduate School of Medical Sciences, Kyushu Univ)

Band 3 mediates electroneutral exchange of several anions, such as chloride and bicarbonate, across erythrocyte plasma membranes. A 55kDa C-terminal membrane domain of band 3 is fully capable of anion exchange activity. It has been revealed that the anion-transporting is carried out by a single conformational change between inward and outward forms in band 3, induced by binding of the substrates to the protein. Although the band 3 has been studied for long time, the structure of its membrane domain has remained at low resolution, since it was shown by electron crystallography using negatively stained two-dimensional crystals at only 20 Å resolution more than ten years ago.

Here, we report the structure of the human erythrocyte band 3 membrane domain determined by electron crystallography using two-dimensional crystals. The band 3 membrane domains were purified from human red blood cells with an intra-molecular cross-linker, 4,4'-diisothiocyanatodihydrostilbene-2,2'-disulfonic acid. And the two-dimensional crystals were obtained from these purified proteins with C $_{15}$ E $_8$ and DOPS. The crystalline sheets grew in large vesicles with over than 2 μm diameter. The projection map perpendicular to the lipid membrane calculated from cryoelectron micrographs of these two-dimensional crystals showed better than 10 Å resolution. From present data, the structural futures and suggested mechanism of anion transport is discussed.

3P-093 多次元固体 NMR 法による膜蛋白質 pHtrII の主鎖構造解析

Main chain structural analysis of transmembrane halobacterial transducer pHtrII by multi-dimensional high-resolution solid-state NMR

Ayako Egawa(1), Miwako Saeki(1), Kokoro Hayashi(2), Chojiro Kojima(2), Hideo Akutsu(1) and Toshimichi Fujiwara(1). (1: Institute for Protein Research, Osaka University; 2: Graduate School of Biological Sciences, Nara Institute for Science and Technology)

Transmembrane protein pHtrII is a transducer which binds to phoborhodopsin. The light excitation of phoborhodopsin is transmitted into the cytoplasm through pHtrII to promote negative photoaxis. We studied uniformly ^{13}C , ^{15}N labeled 159-residues pHtrII by high-resolution solid-state NMR. The pHtrII (1-159) was reconstructed into the deuterated DMPC membranes. High-resolution solid-state 2D NMR was measured under magic-angle spinning at the ^1H resonances frequencies of 500, 600 and 700 MHz. At first, we have assigned ^{13}C signals of pHtrII (1-159) by using ^{13}C - ^{13}C spin diffusion under DARR at 40°C . Intra-residue correlations across 1-2 bonds were observed at short mixing time. At a long mixing time of 200 ms, intra-residue C $^\alpha$ correlations across 3-bonds were obtained. Most amino acids except Tyr and Pro were assigned. The DARR spectra were observed for immobile region of pHtrII. All ^{13}C signals could not be