shift anisotropy of the carbonyl carbon of Leu4, Ala7, Gly12 amino acid residues. These results indicate that LFampinB forms α -helix in the N-terminal region inserting into the membrane and the α -helical axis rotates rapidly about the bilayer normal with the tilt angle of 12 degree to the axis

The association constant (K_a) of LFampinB with the neutral lipid was determined which was smaller than that with the acidic membrane. The difference of the K_a of LFampinB with neutral and acidic membrane explain the reason why the α -helical structure of LFampinB in neutral membrane is more distorted than that in acidic membrane. This also explain that LFampinB may selectively interact with the acidic bacterial membrane and shows the hemolytic activity to the neutral membrane.

3PT115 大腸菌異物排出タンパク AcrB のベリスタポンプ機構の解明

Structures of the multidrug exporter AcrB reveal a proximal multisite drug-binding pocket

Keisuke Sakurai¹, Ryosuke Nakashima¹, Seiji Yamasaki^{1,2}, Kunihiro Nishino¹, Akihito Yamaguchi^{1,2} (¹*Institute of Scientific and Industrial Research, Osaka University*, ²*Graduate school of pharmaceutical sciences, Osaka University*)

AcrB and its homologues are the principal multidrug transporters in Gram-negative bacteria and are important in antibiotic drug tolerance. AcrB is a homotrimer that acts as a tripartite complex with the outer membrane channel TolC and the membrane fusion protein AcrA. Minocycline and doxorubicin have been shown to bind to the phenylalanine cluster region of the binding monomer. Here we report the crystal structures of AcrB bound to the high-molecular-mass drugs rifampicin and erythromycin. These drugs bind to the access monomer, and the binding sites are located in the proximal multisite binding pocket, which is separated from the phenylalanine cluster region (distal pocket) by the Phe-617 loop. Our structures indicate that there are two discrete multisite binding pockets along the intramolecular channel. High-molecular-mass drugs first bind to the proximal pocket in the access state and are then forced into the distal pocket in the binding state by a peristaltic mechanism involving subdomain movements that include a shift of the Phe-617 loop. By contrast, low-molecular-mass drugs, such as minocycline and doxorubicin, travel through the proximal pocket without specific binding and immediately bind to the distal pocket. The presence of two discrete, High-volume multisite binding pockets contributes to the remarkably broad substrate recognition of AcrB.

3PT116 エントロピー力による円筒状容器からの大球の放出

Entropic release of a big sphere from a cylindrical vessel

Hirokazu Mishima¹, Hiraku Oshima¹, Satoshi Yasuda¹, Ken-ichi Amano², Masahiro Kinoshita¹ (¹*Inst. of Adv. Energy, Kyoto Univ.*, ²*Dept. of Chem., Kobe Univ.*)

Insertion of a large solute into a vessel comprising biopolymers followed by release of the same solute from it is a fundamental function in biological systems. A typical example of such insertion/release processes is found in TolC. TolC is a cylindrical vessel possessing an entrance at one end and an exit at the other end for the solute. In earlier work [1], we showed that the solute-vessel potential of mean force (PMF) formed by the solvent plays imperative roles in the processes. The PMF can be decomposed into its entropic and energetic components denoted by Φ_S and Φ_E , respectively. Φ_S is insensitive to the solute and vessel properties (i.e., solvophobic or solvophilic) whereas Φ_E is largely dependent on them. A significant feature of TolC is that a variety of solutes are inserted into and released from the vessel, which is known as "multidrug efflux". This feature can be exhibited when Φ_S with the insensitivity mentioned above is dominant. However, our earlier work [1] suggested that Φ_S acts only for the insertion. Here we explore the possibility of releasing the solute by Φ_S . We calculate the PMF between a big sphere and a cylindrical vessel with open ends immersed in small spheres using the three-dimensional integral equation theory [1] combined with rigid-body models. It is shown that the solute inserted through the entrance can entropically be released by a continuous variation of the vessel geometry to squeeze out the solute from the exit.

[1] K. Amano, H. Oshima, and M. Kinoshita, J. Chem. Phys. **135**, 185101 (2011).

3PT117 2 者タンパク質間を認識するライト抗体を利用したバンド 3-グリコ フォリン A 複合体の精製

Use of a dual specificity Wright antibody for the purification of

structurally unstable band 3-glycophorin A complex

Yohei Ikeda¹, Hinako Hatae², Hiroyuki Kuma², Naotaka Hamasaki², Teruhisa Hirai¹ (¹*Harima Inst., RIKEN*, ²*Fac. Pharm. Sci., Nagasaki International Univ.*)

Band 3 (also known as anion exchanger 1 or SLC4A1) and glycophorin A (GPA) are the most abundant membrane proteins in human erythrocyte and both present at $\sim 1 \times 10^6$ copies per cell. Biochemical and genetic analyses suggested a close relationship between band 3 and GPA during and after biosynthesis (Aufray et al., Blood 97 pp.2872-2878, 2001, Telen and Chasis, Blood 96 pp. 842-848, 1990). Bruce et al. reported that band 3 and GPA were associated though an interaction residues Glu658 of band 3 and Arg61 of GPA (Bruce et al., Blood 85 pp.541-547, 1995). However, it was difficult to maintain their structure as a complex during purification, because of loose binding. In this study, we purified band 3 and GPA (B3-GPA) complex with Wright monoclonal antibody (or Fab fragment), which recognized Wright blood group antigens. We also characterized the biochemical properties of the complex.

After we confirmed the binding of Wright antibody Fab fragment against the erythrocyte membrane, we solubilized that erythrocyte membrane with C12E8 directly, then C12E8-solubilized membrane were loaded on a size-exclusion column. Elution peak of B3-GPA-Fab complex appeared at a position predicted for a dimeric form together with a small uncharacterized shoulder. Two dimensional polyacrylamide gel electrophoresis (blue native/SDS-PAGE) analysis showed that purified complex contains three subunits. We will discuss the polypeptide composition and stoichiometry of the complex.

3PT118 カルシウム活性化クロライドチャネルの *in vitro* でのイオン透過活性測定系の構築

Functional *in vitro* reconstitution of Calcium-activated Chloride channel, TMEM16A/anoctamin1

Hiroyuki Terashima^{1,2}, Alessandra Piccolo¹, Alessio Accardi¹ (¹*Department of Anesthesiology, Weill Cornell Medical College*, ²*Department of Macromolecular Science, Graduate School of Science, Osaka University*)

Calcium-activated chloride channel is widely expressed and involved in various physiological functions, for example, electrolyte secretion in epithelia and glands, contraction of cardiac muscle, olfactory signal transduction and maintain/amplification of membrane potential in nervous system. At 2008, three of independence groups demonstrated TMEM16A/anoctamin1 is a component of calcium-activated chloride channel. TMEM16A protein is thought to have eight transmembrane segments and form at least dimer *in vivo* from results of chemical cross-linking and native PAGE. Although TMEM16A is activated intracellular calcium, there is no well-known calcium-binding motif (e.g., EF hand) in TMEM16 family protein. Recently, mutation of two other glutamate residues dramatically decreased calcium-sensitivity, implying that two glutamate residues are involved in calcium-binding or activation. We expressed the protein in insect cell and solubilized it by digitonin. The reconstituted liposomes showed chloride-conducting activity. The purified TMEM16A protein was activated at sub-micromolar of calcium concentration and inhibited by well-known chloride channel blocker. Chloride flux activity was changed depending on applied voltage, suggesting that it has voltage-dependency. From these results, we could construct *in vitro* flux assay system for TMEM16A.

3PT119 COS7 細胞と酵母細胞を用いた筋型 CPT1 の発現系の比較

Comparison of two expression systems using COS7 cells and yeast cells for expression of heart/muscle-type CPT1

Takuya Hada^{1,2}, Yumiko Kato^{1,2}, Eriko Obana¹, Naoshi Yamazaki², Takenori Yamamoto¹, Yasuo Shinohara^{1,2} (¹*Inst Genome Res, Univ Tokushima*, ²*Fac Pharm Sci, Univ Tokushima*)

Carnitine palmitoyltransferase 1 (CPT1), catalyzing the transfer of the acyl group from acyl-CoA to carnitine to form acylcarnitine, is located at the outer mitochondrial membrane. Because it is easily inactivated by solubilization, expression systems using living cells are essential for its functional characterization. COS7 cells or yeast cells are often utilized for this purpose; however, the advantages/disadvantages of the use of these cells or the question as to how the CPT1 enzyme expressed by these cells differs are still uncertain. In this study, we characterized the heart/muscle-type isozyme of rat CPT1 (CPT1b) expressed by these two cellular expression systems. The mitochondrial fraction prepared from yeast cells expressing CPT1b showed 25% higher CPT1 activity than that obtained from COS7 cells. However, the expression level of CPT1b in the former was 3.8 times lower than that in the latter; and thus, under the present experimental conditions, the specific activity of CPT1b expressed in