membrane thickness and hydrocarbon ordering, but also mesoscopic membrane undulation and thickness fluctuation. Tight packing between DPPC and cholesterol made the membrane rigid. Alamethicin can also affect directly by adjusting adjacent membrane thickness to its hydrophobic length and indirectly by hardening the whole membrane in terms of membrane compressibility and membrane undulation as well as cholesterol. On the other hand, influences of lipids on proteins are cholesterol dependent. Membrane proteins have severe influences directly and indirectly on the surrounding membrane systems. Further investigations about raft and non-raft boundaries will be discussed.

1P-210 抗菌性ペプチド-マガイニン2のポア形成に及ぼす脂質膜の表面電荷密度の効果 (2)

Effects of Surface Charge Density of Lipid Membranes on the Pore Formation Induced by Magainin 2: the Single GUV Method Study (2)

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Interactions of antimicrobial peptides with lipid membranes have been investigated using a LUV suspension, and their details remain unclear. Using the single GUV method, we have succeeded in revealing elementary processes of the pore formation in lipid membranes induced by magainin 2 (1). In this report, to elucidate the mechanism of the magainin 2-induced pore formation, we investigated the effect of surface charge density of membranes on the pore formation.

To change the surface charge density, we controlled negatively charged DOPG concentration in DOPG/DOPC membrane from 30 to 60 mol%. We found that, in all kinds of GUVs, magainin 2 induced a rapid leakage of calcein from single GUVs, showing that magainin 2 formed pores in the membrane. For GUVs with the same charge density, the fraction of leaked GUV, P, increased with time, and P at a fixed time increased with magainin 2 conc. in a buffer. Cf. Cf at P = 0.5 at a fixed time increased with a decrease in the surface charge density. Using the fluorescence spectroscopy and the Gouy-Chapman theory, we obtained intrinsic binding constants of F5W-magainin 2 to DOPG/DOPC membranes containing 30-40% DOPG under the same condition, and as a result, we could determine magainin 2 conc. in the membrane interface, Xb, for various Cf. For GUVs containing 30-40%DOPG, P increased greatly at and above Xb = 60 mmol/mol (ratio of peptide to lipid in the membrane). On the basis of these results, we discuss the mechanism of the magainin 2-induced pore formation.

(1) Biochemistry, 44, 15823, 2005

1P-211 MDシミュレーションとDSC測定によるトレハロースとリン脂質二重膜の相互作用の解析

Interaction between trehalose and phospholipid bilayer as studied by MD simulation and DSC measurements

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Trehalose acts as a good stabilizer of proteins and membranes in both hydrated and dehydrated states. Here, we studied the stabilization effect of this sugar on hydrated liposome composed of 1-palmitoyl 2-oleoyl phosphatidylcholine (POPC). First, we examined how the gel-liquid crystalline transition temperature (T_m) of the liposome is influenced by addition of trehalose (concentration in interbilayer medium = 1.8 wt% or 14 wt%) and similar measurements were performed for POPC liposome/ethyleneglycol (61 wt%) systems. As a result, it was found that trehalose raises T_m by 0.5 and 2.5 °C depending on its concentration, meaning an increase in stability of the lipid bilayer, while ethyleneglycol lowers T_m by 1.5 °C. Next, to elucidate the origin of such an opposite effect between the two solutes, we performed MD simulations, where trehalose (or ethyleneglycol) molecules were dissolved in the interbilayer medium at the same concentrations as those in the above experimental systems. In consequence, it was found that a significant fraction of the trehalose molecules directly hydrogen-bond to the head group of POPC and play a role in increasing the packing density of the bilayer and simultaneously lowering the order parameters of the fatty acid chains of POPC, while the ethyleneglycol molecules penetrate into the bilayer more deeply. These results reasonably account for the above observations of T_m changes.

1P-212 分子動力学法による高比重リポタンパク質(HDL)粒子の構造 Structure of High Density Lipoprotein: A Molecular Dynamics Study

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Lipoprotein is a soluble complex composed of protein and lipid membrane, and it has an important function related to the metabolism of lipid in blood vessel. Lipoprotein is classified into several kinds according to the size, density and the class of protein combined with lipid membrane. In such lipoproteins, it is well known that the high density lipoprotein (HDL) particle adsorbs the cholesterol from blood vessel wall and transfers one to the tissue such as the liver. The study of the structure and dynamics of the HDL particle has attracted attention in both regions of the biological and medical sciences. Jonas et al. successfully synthesized the HDL particle in 1989 and found that HDL particle has two alpha-helix proteins called apolipoprotein (apo A-I) which coil around the side of lipid bilayer, showing the nano-disc structure. Several experiments have been carried out for this HDL particle, and have shown the number and sequence of

residue of apo A-I so far. However, due to the difficulty of experimental observation, the detail of structure has still not shown in the present circumstances. In this study, we carry out the molecular dynamics simulation to reveal the detail of structure of the HDL particle, and show the stability of the apo A-I. We also show the binding effect of apo A-I on the lipid membrane by comparing with the pure lipid bilayer system.

1P-213 細胞透過性ペプチド、トランスポーター10、と単一 GUV 脂質膜の相互作用

Interaction of Cell Penetrating Peptide, Transportan 10, with single GUVs of lipid membrane

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Transportan 10 (TP 10), one of the cell penetrating peptides, is an amphipathic peptide with a sequence of AGYLLGKINLKALAALAKKIL (21-mer), containing the sequences from neuropeptide galanin and from wasp venom, mastoparan [1]. So far the permeation of TP 10 into cells and the interaction of TP 10 with lipid membranes using LUV suspension have been investigated. However, the detail characteristics and mechanism of the interaction and its permeation through membranes remain unclear. In this report, we have investigated the interaction of TP 10 with lipid membranes using the single GUV method [2,3].

First, we investigated the interaction of TP 10 with single GUVs composed of dioleoylphosphatidylcholine (DOPC) and PEG2K-DOPE (molar ratio, 98:2) containing the fluorescent dye, calcein, in a physiological buffer using phase contrast, fluorescence microscopy. Low concentrations (0.2-2 μ M) of TP 10 caused the rapid leakage of calcein from single DOPC-GUVs but did not disrupt the liposomes or change the membrane structure. Once rapid leakage of calcein from a GUV started, the complete leakage occurred rapidly (less than ~1 min). The fraction of completely leaked GUV, PL, increased with time, and also with an increase in TP 10 concentration (e.g., PL at 330 s was 0.2 and 1.0 for 0.2 μ M and 1.0 μ M TP10, respectively).

On the basis of these data and other experimental data, we discuss the mechanism of the permeation of TP 10.

[1] BBA, 1467, 165, 2000, [2] Biochemistry, 44, 15823, 2005, [3] Adv. Planar Lipid Bilayers & Liposomes, 7, 121, 2008

1P-214 リン脂質フォスファチジルグリセロール・コレステロールかならる単分子膜の相挙動について

Phase behavior of lipid monolayers containing phosphoglycerol and cholesterol

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The lateral organization, such as domain formations and rafts in biological membranes, is important to both the structure and function of the biomembranes. We observed domain formations in mixed lipid monolayers of phosphoglycerol (PG), phosphocholine (PC), phosphatidic acid (PA) and cholesterol (Chol) by using an atomic force microscope (AFM). Monolayer was prepared on a Langmuir-Blodgett trough with a required surface pressure by using a Wilhelmy balance method and transferred to freshly cleaved, hydrophilic mica by horizontal deposition method. We demonstrated that both microscopic, submicroscopic and nanoscopic domains coexist in DPPG/Chol monolayers and the DPPG-rich domain is higher than the surrounding Chol-rich membrane areas as a consequence of intrinsic phase differences. DPPG-rich microscopic domains became larger as DPPG concentration increased. In cholesterol-higher concentration mixtures, microscopic DPPG-rich domains were disappeared and nanoscopic DPPG-rich domains existed in Chol-rich membrane areas. By phase balance analyses we demonstrated that phase behavior of PG/Chol monolayers were different from those of PC/Chol and PA/Chol monolayers. We propose phospholipids/Chol phase diagrams relevant to complex membrane phase phenomena in biological systems.

1P-215 バイオサーファクタント含有正電荷リポソームによる優れた遺伝子導入経路

A distinguished pathway for gene transfection by cationic liposomes with a biosurfactant

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Gene transfection is a fundamental technology for molecular and cell biology, and also clinical gene therapy. A variety of non-viral vectors have been investigated for gene transfection, but their gene delivery has been remained in inefficient process. Recently, we found that a biosurfactant, mannosylerythritol lipid (MEL)-A, dramatically increased the efficiency in gene transfection mediated by cationic liposomes. However, these unique properties of MEL-A for gene transfection have not fully examined yet.

Here we examined the mechanism of the gene transfection mediated by cationic liposomes with NBD-conjugated MEL-A. In the process to deliver DNA into the nucleus, MEL-A was found to be distributed in the intracellular membrane including the nuclear membrane, and liposome-DNA complexes were fused with the plasma membrane by time-lapse imaging analysis. That is, DNA was rapidly delivered from liposome-DNA complex to the nucleus of target cells at ~30 min after the addition of liposome-DNA complexes. This indicated that MEL-A was capable to exhibit the significant and rapid promotion of gene transfection efficiency by inducing membrane fusion between liposomes and the plasma