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

membrane of target cells.

It seems that this is a distinguished finding with great potential in the gene transfection and gene therapy using cationic liposomes.

1P-216 ジバルミトイルホスファチジルコリン二分子膜と長鎖脂肪酸の相互作用：分子構造が作用様式におよぼす影響

Interactions of long-chain fatty acids with dipalmitoylphosphatidylcholine bilayer membranes: effects of their molecular structures

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The effects of long-chain fatty acids (four saturated and two unsaturated fatty acids, one derivative) on phase transitions of dipalmitoylphosphatidylcholine (DPPC) bilayer membranes were examined in the low concentration region. The effects of all fatty acids on the DPPC bilayer membranes appeared in the concentration range of μM order, and the appearance modes were significantly different from each other. The three differential partition coefficients of the fatty acids between two phases in the DPPC bilayer membranes were evaluated by applying the thermodynamic equation. The DPPC bilayer membranes showed the different receptivity for the fatty acids, the saturated fatty acids had an affinity for gel phase whereas unsaturated fatty acids had an affinity for liquid-crystalline phase to the contrary. In particular, the receptivity in the gel phase markedly changed depending on kinds of fatty acids. The interaction modes between the DPPC and fatty acid molecules in the gel phase were considered from the hexagonal lattice model. The disappearance compositions of the pretransition by the fatty acids coincided with the compositions that the membrane is all covered by the unit in which two fatty acids molecules are regularly distributed in the hexagonal lattice in a different way, and the distribution depended on the chain length and existence of double bond for the fatty acids. Present study clearly reveals that DPPC bilayer membranes can discriminate among fatty acids with specific interaction by the membrane states.

1P-217 リン脂質 - コレステロール混合二分子膜におけるコレステロール依存性膜結合タンパク質の機能発現

Functional revelation of a cholesterol dependent membrane binding protein in phospholipid-cholesterol binary bilayer membranes

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Recently, microdomain structures formed by the lateral phase transition of membranes containing cholesterol, which is called lipid raft, attract great deal of attention. In order to clarify a role of cholesterol in cellular membranes, we have investigated several phosphatidylcholine (PC)-cholesterol binary bilayer membranes and constructed their phase diagrams. Important information with respect to the interaction between phosphatidylcholine and cholesterol molecules such as the gel-phase immiscibility and formation of liquid ordered phase was obtained from the analysis using a hexagonal lattice model. It is now interesting how the membrane structure affects functions of proteins buried in the membrane in relation to the formation of microdomains. We chose a cholesterol dependent membrane binding protein slytysin (SLY) for this purpose. SLY belongs to a kind of cholesterol dependent cytolytins secreted by *Streptococcus suis*. It binds cell membranes by recognizing cellular cholesterol specifically and breaks the membrane structure from a pore formation due to the ring oligomerization of dozens of the molecules. In the present study, the membrane-breaking activity by SLY in the PC-cholesterol binary bilayer membranes, which is estimated from the release of encapsulated fluorescent uranine in the bilayer membranes, was measured as a function of temperature and cholesterol concentration. Correlation between the functional revelation of SLY and the membrane state is discussed on the basis of the phase diagrams constructed previously.

1P-218 固体 NMR と QCM による Myristoylated Alanine-Rich C Kinase

Substrate と Phosphoinositides の相互作用解析

Interaction of Myristoylated Alanine-Rich C Kinase Substrate with Phosphoinositides in bilayer as studied by QCM and solid-state NMR

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Myristoylated alanine-rich C kinase substrate (MARCKS), a major protein kinase C substrate, contains effector domain (151-175:MARCKS-ED) in the middle of the molecule that interacts with various targets, such as phosphatidylinositol-4,5-bisphosphate (PI(4,5)P₂) molecules, and shows functions as a crosstalk point on several signal transduction pathways. MARCKS-ED consists of 13 basic and 5 aromatic residues. Phosphoinositides play an important role in intracellular signaling cascade as a second-messenger. In this work, association constants (K_a) between MARCKS-ED and phosphatidylcholine (PC) bilayers containing phosphatidylserine (PS) and/or phosphatidylinositol (PI) were measured by quartz crystal microbalance (QCM). The local structures in lipid bilayers were examined using high resolution solid-state NMR. The result of QCM experiments clearly showed that MARCKS-ED had a high affinity to negatively charged lipid molecules (PS and PI), and exhibits low-affinity to PC. ³¹P NMR signal showed the well resolved axially symmetric pattern, indicating that MARCKS-ED stabilizes at the membrane surface by neutralizing negative charge of acidic lipids with Lys residues. ¹³C NMR spectra of [1-¹³C]Phe were observed and the two

chemical shift values indicated that β -strand and random coil conformations co-exist as free and bound state. On the contrary the hydrophobic residues of MARCKS-ED may insert into bilayers as a result of strong electrostatic interaction between acidic lipids and the basic residues in the N- and C- terminal region.

1P-219 ¹³C-NMR によるオピオイドペプチドダイノルフィンとκ-オピオイド受容体 ECL-2 との相互作用解析

Interaction of opioid peptide dynorphin and extracellular loop 2 (ECL-2) of κ-opioid receptor as studied by ¹³C NMR

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The opioid peptide dynorphin specifically interacts with κ-receptor, and it shows the morphine like activity. Therefore, the structural analysis of this system is important to design a new pain relief drug. However, large scale expression system of the receptor was not established. We, therefore, tried to reveal the interaction using fragment of the extracellular loop 2 (ECL-2:196-228) of κ-receptor and dynorphin. Previous solid state NMR study revealed that dynorphin interact with ELC-2 at the location of Val¹⁰-Ala¹⁵. However, ¹³C-NMR signal of [1-¹³C]Val¹⁰ was not separated well from that of lipid in the large bicelle system. Therefore, in this system, we used the small bicelle to obtain higher resolution ¹³C NMR signals. [1-¹³C]Val¹⁰, [3-¹³C]Ala¹⁵-ECL-2 was obtained from solid phase peptide synthesis. These peptides were incorporated into small bicelle system (DMPC:DHPC molar ratio of 1:1) with the lipid:peptide molar ratio of 200:1. ¹³C-NMR of this membrane sample were measured by using Bruker DRX400 NMR spectrometer. In ¹³C-NMR measurements, the spectral resolution of the small bicelle system was improved dramatically, as compared with that of the large bicelle system. The signals of lipid and peptide were well resolved. Further, two carbonyl groups of DMPC were well separated. It turned out that high resolution signals can be observed in the small bicelle system and the system can be used for the interaction analysis of dynorphin and ECL-2 in the lipid bilayer. The interaction site between ECL-2 and dynorphin will be discussed.

1P-220 抗菌ペプチド bovine lactoferrampin と細菌膜との相互作用と抗菌活性の解明

Antimicrobial activity and interaction of bovine lactoferrampin with mimetic bacterial membrane as studied by solid-state NMR

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A new antimicrobial peptide has been identified in bovine lactoferrin (bLF), named bovine lactoferrampin (LFampinB). This novel peptide has a potentially antimicrobial activity because the amino acid sequence is similar to many other antimicrobial peptides. Since antimicrobial peptides works to the specific bacteria, structural information and antimicrobial activity is essential to understand defensive mechanism of such peptides. LFampinB corresponding to bLF (268-284) was synthesized using solid phase method. Lipid components (DMPG:DMPC:CL=65:25:10 w/w) of mimetic bacterial membrane (mixed membrane) was used as a mimic of *staphylococcus aureus* membrane. The results of ³¹P solid-NMR measurements showed that LFampinB formed membrane defects as appeared in the isotropic peak at Oppm. QCM and measurements indicates that LFampinB showed a greater affinity for negatively charged phospholipids (DMPG, CL) than neutral phospholipids (DMPC). The result of K⁺ ISE measurements showed that LFampinB caused leakage of potassium ions across the acidic membrane. On the contrary, neutral phospholipids membrane did not show potassium permeation. Solid-state ¹³C NMR measurements showed that spectral pattern was axially symmetric for [1-¹³C] Leu⁴, [3-¹³C] Ala⁷-LFampinB, indicating that LFampinB undergoes uniaxial rotation around the bilayer normal. It is summarized that LFampinB specifically interacts with acidic lipids to cause membrane defects to lead potassium ion permeation which is related to the antimicrobial activity.

1P-221 周波数変調 AFM によるコレステロール - 脂質二分子膜の分子分解能観察

Molecular-resolution imaging of cholesterol-lipid bilayer by frequency modulation AFM

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Cholesterol is an important constituent of biological membranes. Studies using model membranes have suggested that cholesterol induce the phase separation of a lipid membrane into cholesterol-rich and -poor domains. However, the analysis of molecular-scale structure of cholesterol-lipid complexes in the individual domains has been challenging. Recent advance in frequency modulation AFM (FM-AFM) has enabled atomic- and molecular-resolution imaging of local structures under physiological environments. This capability is ideal for the molecular-scale analysis of heterogeneous biological systems, such as cholesterol-lipid complexes.

In this study, we investigated the molecular structure of cholesterol-lipid bilayers in phosphate buffer solution by FM-AFM. In order to evaluate the cholesterol effect on the phase separation of lipid membrane, cholesterol (concentration range 0 to 50 mol%)-dipalmitoylphosphatidylcholine (DPPC) mixed bilayer were prepared on mica using the vesicle fusion method. Our results suggest that cholesterol-DPPC bilayers with low cholesterol content show the phase separation.