1P-203 糖で重みをつけられた油中水滴エマルションの遠心分離を利用したジャイアント・ベシクルの作成

Giant Vesicles Formed by Centrifugation of Sugar-weighted Water-in-Oil Emulsion

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Giant vesicles (GVs), which are cell-size closed compartments composed of phospholipids, have drawn much attention as cell-like microreactors. As one of techniques for preparing unilamellar GV, a method centrifuging a bi-phase system of a water-in-oil (w/o) emulsion layer on a water layer has recently been focused, but its mechanism has not yet revealed sufficiently. Here we report that the current technique affords a series of morphologies of GV, i.e. spherical, adhered or aggregated GV, when a lipid mixture such as phosphatidylcholine, cholesterol and phosphatidylethanolamine was used for coating the w/o emulsion droplets dissolving sugars. The emulsion-water interface composed of lipids may provide an adhesion force among w/o emulsion droplets stacking on the interface

1P-204 ポリマー脂質二分子膜・流動性脂質二分子膜のハイブリッドモデル生体膜：側方拡散の抑制と相分離の誘起

Hybrid model membrane of polymerized and fluid lipid bilayers: Obstruction of lateral diffusion and induction of phase separation

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We previously developed substrate supported model cellular membranes composed of polymeric and fluid phospholipid bilayers by using lithographic polymerization of diacetylene phospholipid (DynePC). Polymerized bilayers acted as a matrix that confined lipid membranes, whereas embedded bilayers were intended to be the model system of the biological membranes, retaining some essential properties such as fluidity. Herein, we report on the effect of polymeric bilayer domains on the embedded fluid lipid membranes. The amount of polymeric bilayer domains that remain after the detergent treatment could be controlled by changing the UV irradiation dose applied for the photopolymerization. On the other hand, fluid bilayers could be incorporated by the vesicle fusion technique. Lateral diffusion of lipid molecules in fluid bilayers was obstructed by the presence of polymeric bilayer domains, as measured by the fluorescence recovery after photobleaching (FRAP). Furthermore, we observed that patterned polymeric bilayers induced separation of domains in fluid bilayers. From the mixture of dioleoylphosphatidylcholine (DOPC), sphingomyelin, and cholesterol, liquid-ordered domains (rich in sphingomyelin and cholesterol) and liquid-disordered domain (rich in DOPC) could be separated. The fact that polymeric bilayers are forming well-defined domains and regulate the distribution and migration of membrane bound molecules suggest their utility for studying and exploiting the functions of biological membranes.

1P-205 油中水滴法を用いた、浸透圧ストレスに対するリポソームの挙動観察

Direct observation of the behavior of liposome under the desired osmotic environment

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Biological membranes mainly consist of lipid bilayer. Their morphology, topology and features can change in response to various physiological signals and environmental cues. Especially, osmotic pressure is the most familiar and one of the most important environmental factors in considering membrane homeostasis. Therefore, there is a lot of studies had tried to evaluate the resistance of biological membranes against the osmotic pressure utilizing liposome as the simplest lipid bilayer vesicle artificially produced.

However, to the best of our knowledge, it has not been reported that procedure to expose liposomes to a uniform desired osmolarity, so it had been impossible to reveal the exact relationship between osmolarity and membrane stability. In almost previous studies, usually liposomes, which were made by the natural hydration method, have been monitored in a concentration gradient of a solvent.

In this study, we adapted the "spontaneous transfer method" to homogeneously expose liposomes to a desired osmolarity. This method enables us to create liposomes (10-100 μ m) encapsulating water into a solution of certain concentration of sucrose (0-1000 mM). Consequently, we successfully found that individual liposomes show any of five different type behaviors, while the majority of them unexpectedly opens large hole (several micrometers), independent of the outside concentration of sucrose. On the other hand, the lag time between the liposome formation and any response beginning shortens with increasing the outside sucrose concentration.

1P-206 エタノールおよびリモネンを皮膚角層に作用したときの構造変化の精密測定

Highly sensitive detection of characteristic structural changes of stratum corneum on applying ethanol and d-limonene

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We developed a novel method to detect the modifications of the minute x-ray diffraction profile resulting from the structural changes of the intercellular lipid matrix, soft keratin, corneocyte, etc. in mammalian stratum corneum when we applied solution of chemical agent to stratum corneum. The method can be applied to the molecular-level detection of the structural change due to the effects of cosmetics, percutaneous drugs, etc. on stratum corneum. To obtain such evidence is extremely important to develop a new cosmetic, a new percutaneous drug, etc. In this study, we observed the structural changes on applying ethanol or d-limonene on stratum corneum by synchrotron x-ray diffraction. From the results, we obtained the evidence that the partial extraction of lipids from intercellular lipid matrix takes place on the application of the both solutions. In addition the uptake of ethanol molecules into corneocytes was proposed from the growth of the diffraction profiles in extreme small-angle region and as a result the broad diffraction peak due to ethanol which is stored in corneocytes grows. On the other hand the application of d-limonene to stratum corneum results in the swelling of the long lamellar structure. This fact suggests that the pathway of percutaneous absorption is formed within the long lamellar structure.

1P-207 鎖長の異なるジアシルホスファチジルコリン二分子膜相挙動に及ぼすコレステロールの影響

Effect of cholesterol on bilayer phase behavior of diacylphosphatidylcholines with varying acyl-chain lengths

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We investigated the thermotropic phase behavior of several kinds of binary bilayer membranes of cholesterol and diacylphosphatidylcholines with different acyl-chain lengths as a function of the temperature and cholesterol composition by fluorescence spectroscopy using 6-propionyl-2-(dimethylamino)naphthalene (Prodan) and differential scanning calorimetry. The temperature-composition phase diagrams were constructed for their binary bilayer membranes. The pretransitions of the binary bilayers abolished at low cholesterol compositions, 3.5-5 mol %, which depends on the acyl-chain length of the host phosphatidylcholine molecules. Liquid-ordered phase began to appear at ca. 15 mol % independent of the acyl-chain length. These characteristic cholesterol compositions can be reasonably explained in terms of the regular distribution of the cholesterol molecules in the binary membranes using a packing model of hexagonal lattice. This model also provides molecular depictions for the two phase coexistence in the binary bilayers below the main transition temperature, which allows us to understand the phase behavior of the binary bilayer membranes at a molecular level.

1P-208 ジヘキサデシルホスファチジルコリン二重層膜の膜表面水和

On the hydration of membrane surface of dihexadecylphosphatidylcholine bilayers

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The hydration properties of proteins and lipid bilayer membranes are fundamental key factors for various biological functions. It is believed that the hydration of the membrane surface affects the bindings and activities of membrane peripheral proteins. We have been studying the hydration properties of phospholipid model biomembranes by investigating a solvent isotope effect, i.e., the effect of deuterium oxide (D_2O) on the structures and physical properties of the lipid bilayers. At last year's annual meeting (2P256, 2007), we reported that D_2O generally increases the generalized polarization (GP) values calculated from the spectrum of laurdan fluorescent probe when the probe is located in a liquid crystalline phosphatidylcholine (PC) bilayers, as compared with H_2O . However, we also reported that dihexadecyl-PC (DHPC) exhibits a different result. DHPC is an ether-linked PC and forms an interdigitated gel phase at low temperature, i.e., below a ripple phase. To clarify the origin of DHPC's exceptional result, we performed fluorescent studies using prodan probe and measured the interbilayer interaction using Osmotic stress/X-ray diffraction technique. Based upon the results obtained, we will discuss the hydration of membrane surface of DHPC bilayers, paying attention to the bilayer structure of the interdigitated gel phase.

1P-209 タンパク質脂質相互作用におけるコレステロールの効果の分子動力学シミュレーション

Molecular dynamics studies on cholesterol effects for interactions between membranes and proteins

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Interactions between proteins and lipids are crucial for membrane stability and protein function. Heterogeneous phenomena, such as lipids rafts, on biological membrane seem to arise under the protein-lipid interactions. However, atomic interactions are difficult to detect by experimental techniques and most of molecular dynamics simulations of membrane proteins are focused on proteins themselves, surrounding lipids being considered just as background.

We analyzed how proteins influence surrounding lipids, and vice versa. A ternary membrane system of Alamethicin-DPPC-cholesterol and a binary system of Alamethicin-DPPC, both of which include 2,000 membrane molecules and over 300,000 atoms in total, were constructed to perform molecular dynamics simulations.

The cholesterol-dependent differences were observed on not only microscopic

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