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3G0924 微細パターン化モデル生体膜における膜結合タンパク質の濃縮の空間的制御

Spatially controlled accumulation of membrane-bound protein in a micro-patterned model biological membrane

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Lipid rafts play a key role to regulate many cellular functions by recruiting membrane proteins. In a model system, liquid-ordered (*lo*) phases have been used as a model of lipid raft. We previously developed a micro-patterned model membrane on a solid support that can control the size and spatial distribution of *lo* phases (1). The model membranes comprised polymerized and fluid lipid bilayers. They induced phase separation of *lo* and liquid-ordered (*ld*) domains in a designed geometry because *ld* domains preferentially localized at the polymeric bilayer boundaries. The controlled phase separation should provide a means to purposefully concentrate membrane-bound proteins in defined regions. In the present work, we evaluated the accumulation of membrane-bound proteins in spatially defined areas. We used cholera toxin subunit B (CTB) as a model protein. The degree of CTB accumulation was correlated with the progress of phase separation, the ratio of polymeric/ fluid lipid bilayers, the geometries of polymerized bilayer regions, and the composition of fluid lipid bilayer. Spatially controlled accumulation of proteins should provide a novel approach to study lipid raft by separating membrane-bound proteins according to their affinities to lipid rafts. This technique should also pave a way toward sensitive bioassay and biosensor owing to the fact that protein molecules are accumulated in pre-defined regions.

(1) Okazaki et al. *Langmuir* 2010, 26, 4126

3G0936 圧力摂動熱量法によるジミリストイルホスファチジルコリン二分子膜の緩和挙動の解明への試み

An attempt to reveal the relaxation behavior of dimyristoylphosphatidylcholine bilayer by pressure perturbation calorimetry

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Pressure perturbation calorimetry (PPC) is a relatively new calorimetric technique, which allows us to directly quantify the thermal expansion coefficient for solute molecules and colloidal particles in solution. In a PPC scan, the flow of the heat that occurs when a small isothermal pressure change (~ 5 bar) is applied to a sample solution is recorded as a function of time, and the expansion coefficient can be obtained from the total heat quantity transferred into the sample solution during the relaxation process by applying several thermodynamic relations. Therefore, the temperature dependence of the thermal coefficient is easily obtained by performing a series of PPC scans at different temperatures. Usually, this technique is used to study thermotropic volume behavior of solutes and dispersed particles and to estimate the relative volume changes of them with thermotropic physical transformations, such first-order phase transitions and conformational changes, on the basis of the temperature dependence of the thermal expansion coefficient. On the other hand, the time dependence of the heat flow measured in a PPC scan can provide us with the information about the relaxation dynamics of solutes and dispersed particles. On the basis of this idea, we have recently started the investigation on the relaxation process of a phospholipid bilayer membrane using the PPC technique. In this presentation, we will report on our recent results about the relaxation behavior of dimyristoylphosphatidylcholine bilayer membrane.

3G0948 リン脂質二重膜の圧力誘起指組み構造形成：疎水鎖長依存性と形成限界

Pressure-induced interdigitation of phospholipid bilayer membranes: dependence of acyl-chain length and limitation of the formation

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Bilayer membranes of diacylphosphatidylcholines (CnPC) are known to induce the interdigitated gel phase under high pressure. Since the interdigitated gel phase is extended into the low-pressure region with increasing the acyl-chain length, it is expected that the bilayers of CnPC with longer acyl chains induce the interdigitated gel phase under atmospheric pressure. In this study, we

investigated the bilayer interdigitation of CnPC with long acyl chains (Cn = 19-22). The temperature-pressure phase diagrams of four CnPC bilayers were constructed by differential scanning calorimetry and high-pressure light transmittance technique. We observed the interdigitation of the C19PC, C20PC and C21PC bilayers under high pressure, and the interdigitation was induced at lower pressures with the chain elongation. On the other hand, the C22PC bilayer showed the peculiar behavior: there exist two transitions under atmospheric pressure while one transition under high pressure. In order to confirm what the low-temperature phase of the C22PC bilayer is like, we performed measurements of the small angle neutron scattering and fluorescence spectroscopy under atmospheric pressure. These results proved that the phase is the interdigitated one. Therefore, it turned out that the maximum acyl-chain length required for the pressure-induced interdigitation is 21. Present results definitely indicate that the bilayer interdigitation is governed by the hydrophobic interaction as well as the hydrophilic interaction of the PC molecule in the bilayer.

3G1010 均でないリン脂質単分子膜における崩壊現象について

Collapse of nonuniform phospholipid monolayers

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The behavior in the stochastic collapse of lipid monolayers of a mixture of dipalmitoylphosphatidylcholine and palmitoyloleoylphosphatidylglycerol, a model system of pulmonary surfactant assembly, has been studied extensively by using experimental techniques and theoretical models. The monolayers at the air-water interface appear to be phase separated under compression and retain the continuous liquid-expanded phase network surrounding islands of condensed phase even at a surface pressure approaching 70 mN/m. When the two-dimensional monolayers are compressed beyond the equilibrium surface pressure, they collapse and assume a three-dimensional formation. However, these phenomena have been observed under a condition of uniform monolayers. In fact, monolayer is not always uniform at the interface. We can rarely observe that the areas with the coexistence of LE-C phases are contiguous to each other, but distinct from each other by using fluorescence microscopy. The nonuniform surface density in the monolayer has an influence on the collapse. Here we investigate the collapse of compressed nonuniform binary phospholipid monolayers using Langmuir-isotherms, fluorescence microscopy and atomic force microscopy. The monolayers at the air-water interface underwent fast collapse by folding within 100 ms or slow collapse by the formation of Baumkuchen-like structure at high surface pressure. They were composed of bilayers that remained suspended beneath the film.

3G1022 脂質分子の電荷が引き起こす膜構造変化：2次元相分離と3次元曲率

Charge-induced transition in membrane mesoscopic structures: lateral domains and vesicular shapes

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Living cells exhibit various 2D and 3D structural dynamics of lipid membranes. These structural changes are recognized as "membrane dynamics". The 2D membrane structures are represented by phase separation named "lipid raft". The 3D membrane structures are morphological changes, such as endocytosis and intercellular vesicular traffic. The intrinsic properties of constituent lipids should play an important role on such membrane dynamics.

Giant unilamellar vesicles (GUVs) are often used as a model of biological membranes, because they are large enough to be observed directly by optical microscope. Previously, using GUVs, we succeeded in reproducing 2D and 3D membrane dynamics such as phase separation (Hamada et al., *Soft Matter*, 7, 220-224, 2011) and vesicular formation (Hamada et al., *J. Am. Chem. Soc.*, 132, 10528-10532, 2010) by changing the molecular structure of membrane constituents.

To advance our understanding, we here focused on the effect of electric charges of lipid molecules, which is one of intrinsic properties of membrane lipids, on 2D (lateral phase separation) and 3D (membrane curvature) membrane mesoscopic structures.

3G1034 油中水滴エマルション遠心沈降法を用いたミクロスフェア内包型ジャイアントベシクル

Microsphere-containing giant vesicles prepared by water-in-oil emulsion centrifugation method