temperature, the mixture is transparent and has a low viscosity, and its structure is thought to be disk-like micelles, which is often called "bicelles". At high temperature, the mixture is turbid and also has a low viscosity, and its structure is thought to be multi lamellar vesicles (MLV). In intermediate temperature range, the mixture has high viscosity. This phase is called nematic phase because the polarized optical microscopy observation exhibit optical anisotropy. The structure of nematic phase is not yet clear although some models, such as worm-like or disk-like aggregates, had been proposed.

We measured membrane fluidity and fluorescence anisotropy of DMPC/DHPC mixtures, using fluorescence GP imaging with Laurdan or fluorescence spectroscopy with DPH. The results support stability of the structure of bicelles at low temperature and mechanism of generating MLVs at high temperature. However, in intermediate temperature range, we obtained conflicting results between microscopic and macroscopic behavior.

Now, we are observing this system using Muller Matrix microscopy which was developed in our laboratory. Muller Matrix microscopy could detect, in principle, any change of polarization states induced by the sample and therefore it is expected to elucidate structural dynamics around the nematic phase.

3PT146 細胞毒性を有する酸化コレステロールのホスファチジルコリン膜分子充填への影響

Effect of cytotoxic oxysterols on the molecular packing of phosphatidylcholine membranes

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Oxidative stress induces the conversion from cholesterol (CH) to various oxysterols in biomembranes. Some oxysterols are cytotoxic. For example, 7 β -hydroxycholesterol (7 β -OH) and 25-hydroxycholesterol (25-OH) have been reported to be associated with neurodegenerative diseases and arteriosclerosis, respectively.

The oxysterol-related diseases presumably result from the difference between CH and oxysterols on ability to modulate the biophysical properties of the membrane bilayer. In this work, to gain fundamental insight into the effects of oxysterols on biophysical properties of biomembranes, we investigated the molecular packing of binary mixtures of dipalmitoylphosphatidylcholine (DPPC)/CH, DPPC/7 β -OH, and DPPC/25-OH.

The apparent specific volumes estimated by neutral flotation method were same for all three mixtures, at least, from 0 to 40 mol% sterol concentrations. Meanwhile, X-ray diffraction analysis of the mixtures containing 30 mol% sterols showed that the order of the bilayer thickness was DPPC/CH < DPPC/25-OH < DPPC/7 β -OH.

These results indicate that the addition of OH group by oxidation decreases CH's capacity of condensing the bilayer in the lateral direction and the OH group on the middle position more strongly perturbs the molecular packing of bilayers than that on the edge position.

We are also currently studying palmitoyloleoylphosphatidylcholine/oxysterol mixtures.

3PT147 ジヘキサデシルホスファチジルコリンの指組ゲル相二層膜間の氷点下温度における水和斥力

Hydration repulsive force of the interdigitated phase of dihexadecylphosphatidylcholine at subzero temperatures

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The clarification on the interactions of lipid bilayers is one of the most important issue of membrane biophysics, however, many aspects of these interactions still remain mysterious. One of such examples is "hydration repulsive forces" and several different explanations on its origin have been proposed. Here we investigated the hydration repulsive force between the interdigitated ($L_{\beta I}$) phase bilayers of dihexadecylphosphatidylcholine (DHPC) at subzero temperatures. The number of headgroups per an unit area at the bilayer surface of the interdigitated phase is about twice as compared with normal gel (L_{β}) phase. The measurement of the force was carried out by using our previously proposed method [1] based on the combination of ice formation free energy values and structural information obtained by X-ray diffraction. The estimated hydration repulsive force curve of DHPC in the $L_{\beta I}$ phase was different from that of dipalmitoylphosphatidylcholine (DPPC) in the L_{β} phase. This suggests that headgroup (dipole moment) density affects the hydration repulsive force profiles. In addition, we are planning to present the result of dipalmitoylphosphatidylethanolamine (DPPE) and will also discuss the effects

of the difference of headgroup on the hydration repulsive forces.

[1] H. Takahashi & P.J. Quinn, *Mol. Cryst. Liq. Cryst.* **367** (2001) pp.427-434

3PT148 架橋脂質二分子膜における秩序液体相/液晶相の分離

Localization of Liquid-Ordered/Liquid-Crystalline Phase Separation at a Lipid Bilayer Suspended over Microwells

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The localization of liquid-ordered (Lo) and liquid-crystalline (L_{α}) phase domains is investigated on a silicon substrate with a microwell array. The localization of Lo and L_{α} phase separation as a model of a lipid raft plays an important role regarding the arrangement and function of membrane proteins. When we fabricate a nanobiodevice that works with membrane proteins, controlling the function of proteins is a key technique. A microwell array with a sealing lipid bilayer, whose phase separation can be controlled, will be a promising platform for nanobiodevices.

Suspended lipid bilayers were formed by rupturing giant unilamellar vesicles (GUVs) on the substrate. The GUVs were prepared from ternary mixtures of DOPC, DPPC, and cholesterol. We found that the L_{α} phase is preferable at the suspended membrane over microwells because of its curvature. On the other hand, the Lo phase domain disappeared from the suspended membrane. Phase separation and localization are governed by the stiffness and line tension of the membrane. Lateral diffusion at the molecular level occurs quickly, although domain movement is restrained at the supported membrane by the interaction with the substrate. Unsaturated lipid is condensed at the suspended membrane, and saturated lipid and cholesterol are excluded. Additional phase transition and domain rearrangement occurs. By modification of surface roughness, charge, and/or chemical state, the localization of phase separation on the suspended lipid bilayer will be controlled.

3PT149 ラウリル硫酸ナトリウム(SLS)溶液塗布によるヒト皮膚角層の構造変化

Effect of sodium lauryl sulfate on the human stratum corneum structures

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The stratum corneum (SC), a thin envelope surrounding our body, is known to be responsible for the skin barrier function. It consists of corneocyte cells filled with keratin filaments and intercellular lipid layers. In this study we examined the effect of a substance inducing rough skin on the SC structures by electron diffraction measurements to clarify its permeation path in the human SC. Layers of corneocyte cells were stripped off from the surfaces of human forearms of five subjects with glue-coated grids for electron microscopy after administration of a 1% sodium lauryl sulfate (SLS) aqueous solution on the skin surface. Control specimens were collected from the opposite forearms without the SLS treatment. Acquisition of electron diffraction patterns was carried out under very low electron dose conditions (~ 3 e/nm²) with an electron microscope (JEM1400) equipped with a high-sensitivity CCD camera. One-dimensional diffraction profiles were calculated for analysis of the structural change induced by the SLS permeation. Our results suggested that the lipid packing structures remain intact during the SLS permeation though the transepidermal water loss increases significantly. Furthermore, the SLS treatment seemed to slightly reduce the diffraction intensity around 2.0 nm⁻¹, which comes from the structure other than the well-established orthorhombic or hexagonal domains in the intercellular lipid layers. We speculate that the applied SLS molecules penetrated into the corneocyte cell body to affect the keratin filament structure.

3PT150 高感度 DSC によって求めたジトリデカノイルホスファチジルコリン-コレステロール系の詳細な相図

Detailed Phase Diagram of Ditridecanoylphosphatidylcholine-Cholesterol System Determined by High-Sensitivity DSC

Yasushi Kamitani, Fumihiro Okazaki, Yasuo Saruyama, Haruhiko Yao (Gra. Sch. Sci. Tech., Kyoto Inst. of Tech.)

It was recently found that the L_x phase exists in ditridecanoylphosphatidylcholine (DC13PC)[1]. Besides, the L_x phase is the liquid-ordered phase reported in phosphatidylcholine-cholesterol system, because there is no phase line between the L_x phase of pure DC13PC and the liquid-ordered

phase of DC13PC-cholesterol mixtures in the binary phase diagram. However, our previous phase diagram was determined every 5 mol% cholesterol concentration. Thus, there is some risk that we might miss the phase line if it is nearly normal to the concentration axis. Therefore, we have performed heat capacity measurements on DC13PC-cholesterol binary mixtures to make the phase diagram more detailed using high-sensitivity DSC developed in our laboratory. As a result, it is confirmed that phase transition was not observed between the L_x and liquid-ordered phases, and that the L_x phase is the liquid-ordered phase. Moreover, it is found that the temperature range of the liquid-ordered phase drastically increases at cholesterol concentrations higher than 23 mol%. The detailed phase diagram will be presented.

[1] F. Okazaki, Y. Saruyama, H. Yao: *Abstracts of 46th Annual Meeting of the Japan Society of Calorimetry and Thermal Analysis*, (2010)2B1420.

3PT151 高感度 DSC によるヒト皮膚角層の熱容量測定

Heat Capacity Measurement of Human Stratum Corneum using High-Sensitivity DSC

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The outermost layer of skin called stratum corneum (SC) is composed of corneocytes and intercellular lipids. Intercellular lipids have the long and short lamellar structures (LSs). It has been proposed that the short LS plays an important role in control of water content in skin [1]. Therefore, we have performed DSC measurements of human SC at various water contents between 0-40 wt% using high-sensitivity DSC developed in our laboratory. Human SC was dried under vacuum overnight. The water content of the dried SC was defined as 0 wt%. Disk-shaped pieces (3 mm diameter) were punched out from the dried SC and hydrated in a container where relative humidity was controlled with glycerol aqueous solution until they had reached a desired water content. Then, they were sealed into a gold pan. DSC measurements were performed in the temperature range of -20-130 °C. For human SC with a water content of 20 wt%, thermal anomalies were observed at 9, 20-60, 75 and 89 °C on 1st heating. The phase transition at 9 °C, that had not been reported previously, was observed at all water contents between 0-40 wt%. On 1st and successive cooling, thermal anomalies were observed at -2, 12, 32, and 70 °C. On 2nd and successive heating thermal anomalies were observed at 5, 15, 33, and 71 °C. At higher water content, it is found that the anomaly at 70-71 °C splits into two anomalies. To identify the phase transitions, we have performed x-ray diffraction measurements and the results will be presented.

[1] I. Hatta *et al.*, *Biochim. Biophys. Acta* **1758** (2006) 1830.

3PT152 モデル細胞内における相分離とマイクロゲルのパターン形成

Gel pattern formation in model cells coupled with phase separation

Shinpei Nigorikawa, Masayuki Tokita, Miho Yanagisawa (*Grad. Sch. Sci., Kyushu Univ.*)

It is known that huge amount and great variety of macromolecules are encapsulated in cells, and they maintain their appropriate spatial distribution inside of the cellular membranes. We used cell-sized water/oil droplet coated by a lipid layer as a cell model, and studied phase separation of binary polymer solution: gelatin and polyethylene glycol (PEG) aqueous system. This system shows phase separation and gelation of gelatin with decreasing temperature, however, the mechanism to bring the phase separation has been unclear. Using fluorescence-labeled gelatin, we observed the pattern formation of gelatin-rich domains upon phase separation by a fluorescence microscope. At first, the time development of domain size was analyzed in PC (phosphatidylcholine) droplets, and found that the growth rate was decreased with an increase in droplet size. Therefore, at a given time, the number of domains in small droplets <30 μm is larger than that in large droplets > 100 μm. This acceleration of domain growth in smaller droplets may be originated from the depletion effect between rigid gelatin and soft PEG molecules under micro-scale confinements. On the other hand, in the case of PE (phosphatidylethanolamine) droplets, a thin layer of gelatin gel was observed at the interface. It is illustrated that the negatively charged PE membrane has an electrostatic attractive force with electrolyte gelatin gel. Furthermore, shape deformation of droplets coupled with the gel patterns was observed, and was discussed from the Young-Laplace equation.

3PT153 脂質膜ヘテロ界面はナノ物質をサイズ依存的に識別する

Size-dependent selective localization of nano-particles on heterogeneous membrane surfaces

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It is of biological importance that we understand the biophysical mechanisms that govern the interaction between nanoparticles (NPs) and heterogeneous cellular surfaces because of the possible cytotoxicity of engineered nanomaterials. In this study, we investigated the lateral localization of NPs within a biomimetic heterogeneous membrane interface using cell-sized two-phase liposomes. We found that lateral heterogeneity in the membrane mediates the partitioning of NPs in a size-dependent manner: small particles with a diameter of <200 nm were localized in an ordered phase, while large particles preferred a fluidic disordered phase. This partitioning behavior was verified by temperature-controlled membrane miscibility transition and laser-trapping of associated particles. In terms of the membrane elastic energy, we present a physical model that explains this localization preference of NPs. The calculated threshold diameter of particles that separates the particle-partitioning phase was 260 nm, which is in close agreement with our observation (200 nm). These findings may lead to a better understanding of the basic mechanisms that underlie the association of nanomaterials within a cell surface.

3PT154 UV irradiation driven deformation of photoresponsive molecule doped membrane

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The phospholipid bilayer has been widely studied as a model system of biological membrane since its structure is the main component of biomembrane. In particular, the deformation of the vesicles has been intensively investigated in relation to the exocytosis and endocytosis.

In the previous study, we doped the photoresponsive molecule *pseudogem*-Bis (diphenylimidazole)[2.2]paracyclophane (abbreviated as "*pseudogem*-BisDPI [2.2]PC" hereafter) to DOPC multilamellar vesicles, and performed UV light irradiation experiments. The *pseudogem*-BisDPI [2.2] PC has one [2.2] paracyclophane that bridges two diphenylimidazoles and one C-N bond that connects two imidazole rings. The C-N bond is easily cleaved by UV irradiation. It was found that the shape of the doped membrane began to change after about 10 seconds of UV irradiation, and its surface area per volume increased with time. Shape of the vesicle was changed into rippling form. We assumed that increasing of surface area per volume of the vesicle was caused by *pseudogem*-BisDPI[2.2]PC that excluded lipids of around the molecule with UV exposure.

In this study, we improved the experimental condition of forming vesicles so that the unilamellar structure became dominant because we can analyze unilamellar spherical vesicles easier than multilamellar vesicles with irregular form. In this presentation, we discuss the membrane deformation of unilamellar vesicles.

3PT155 フリップフロップのダイナミクスの直接的観察

Direct observations of flip-flops in membranes

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How do lipid molecules in membranes perform a flip-flop? Flip-flop of a lipid molecule plays a crucial role in membranes. However, little is known regarding the way of flip-flop by experiments as well as molecular dynamics simulations. Here, we observe single molecules performing flip-flops in membranes using DPD simulations. There are three different ways of flip-flop, which can be clearly characterised by paths in a free energy surface. Furthermore, we find that interevent times of flip-flop are distributed according to the exponential distribution with cutoff. The rate of flip-flop depends on temperature.

3PT156 Low-frequency dynamics of phospholipid bilayers studied by terahertz time-domain spectroscopy

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Phospholipid is a main component of cell, which forms bilayers by self-assembling. Lipid bilayers, which are usually hydrated, continuously fluctuate due to thermal activation in the terahertz (THz; 1 THz ~ 33 cm⁻¹) frequency region. These fluctuations originate from delocalized vibrational motions of