3PT135 モデル細胞間に形成された脂質 2 分子膜の吸着力測定**Adhesive force of a lipid bilayer between contacted cell-sized droplets**

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Cell-sized water/oil droplet coated by a lipid monolayer has been shown to be a useful system for examining the interaction between lipid membrane and encapsulated proteins [1-2]. The droplets can easily connect with each other and form a continuous structure like a cell tissue where lipid bilayers bound them. In the case of adhesive two droplets, the adhesive force is calculated from $F = 2\gamma(1 - \cos\theta)$, where θ and γ are contact angle and surface tension at the oil/water interface [3]. We have evaluated the lipid dependence on θ and γ independently by using three different types of lipids, unsaturated lipids: DOPE (dioleoylphosphatidylethanolamine), DOPC (dioleoylphosphatidylcholine), and a saturated DMPC (dimyristoylphosphatidylcholine). Then, the contact angle term $2(1 - \cos\theta)$ differs among lipid species and fails in the order: DMPC < DOPC ~ DOPE, which might reflect the molecular structure of hydrophobic tails. On the other hand, γ follows the order: DOPC < DMPC < DOPE, which is consistent with non-lamellar PE unlike PC. Finally, we obtained the lipid dependence on F : DMPC < DOPC < DOPE. Based on these values, we elucidated the effects of membrane or cytoplasmic structures on F by the addition of polyethylene glycol (PEG) conjugated DOPE or encapsulation of gelatin gel. We believe that this system is useful to clarify the effects of proteins on adhesive lipid mono-layers.

[1] A. Kato, et al., 2012 Sci. Rep., 2:283.

[2] M. Yanagisawa, et al., 2011 J. Am. Chem. Soc., 133:11774.

[3] P. Poulin et al., 1997 Phys. Rev. Lett., 79:3290.

3PT136 ジェット流による細胞サイズリボソームの作製**Preparation of cell-sized liposomes by pulsed jet flow**

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Giant liposomes are composed of phospholipid membranes similar to those commonly found in living cells. A giant vesicle is typically ~10 μm in diameter, which is sufficient observation by optical microscopy. Therefore, such artificial liposomal systems have been used in biochemical and biophysical studies such as the microencapsulation of gene expression systems. To effectively form uniformly-sized giant vesicles and encapsulate biological molecules into giant vesicles, in our previous study, we created giant vesicles by the microfluidic-jetting-induced deformation of a planar lipid bilayer including an organic solvent. However, this method has limitations; for instance, the giant vesicle is a W/O/W emulsion having a thin shell of an organic solvent. Further, it is unstable and very large (over 300 μm in diameter). In the present study, we prepared cell-sized lipid vesicles using an improved double-well device and a pulsed jet flow. When the jet was applied for 4.2 ms at 250 kPa, vesicle formation could be observed using a high-speed camera. Vesicles of two different sizes were formed by the jet flow. The vesicles with a diameter of over 100 μm immediately collapsed in the glucose solution. However, the vesicles with the diameter of approximately 5 μm sank in the glucose solution and their shape was maintained. The average diameter of the vesicles formed immediately and after 24 h was 4.4 μm .

3PT137 タンパク質内包リボソームの熱安定性に関する小角・広角 X 線散乱研究**SWAXS study of thermal structural stability of liposomes entrapping proteins**

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[Introduction]

By using synchrotron radiation small-angle and wide-angle X-ray scattering (SR-SWAXS), we studied the structural characteristics of liposomes entrapping proteins. The aim of this study is to clarify the thermal stability of liposomes entrapping proteins that mimic an intracellular environment.

[Experimental]

Liposomes measured were large uni-lamellar vesicles (LUVs) of lipid-mixtures composed of glycosphingolipid, cholesterol and phospholipid lipids (egg-PC or DPPC). The protein occluded in liposomes was myoglobin. LUVs were prepared

as follows. The lipid mixtures dissolved in protein solutions in 10 mM HEPES buffer at pH 7.5 were subjected to natural swelling, ultra-sonic dispersion, freeze-throw, extrusion using a extruder system, and spin-filtration, successively. SR-SWAXS experiments were done Spring-8 and at PF The temperature range of the samples was 25 - 85 °C.

[Results]

In the case of the empty DPPC-LUV, the bilayer structure changed evidently in the range from 40 °C to 45 °C due to the gel-to-liquid crystal transition, whereas, both the diameter and the radius of gyration were mostly unchanged. In the case of the filled DPPC-LUV, the transition temperature became higher in around 5 degrees, suggesting that the thermal stability of liposome was improved by the protein occlusion. Both the diameter and the radius of gyration increased once, and suddenly decreased. Above the transition temperature, both values were mostly constant. Further experimental results and analyses will be shown.

3PT138 溶質含有脂質フィルムから調製した巨大リボソームのサイズ、形態と膜多重度**Size, morphology and lamellarity of giant liposomes generated from solute-containing lipid films**

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Giant liposomes are adopted for studies on cell membrane and microcompartment models, and a lot of methods have been developed to improve various properties of them, including formation efficiency, size distribution, lamellarity, etc. Of course, all protocols are useful; however, the hydration methods, such as the gentle hydration (natural swelling) which is based on the Bangham method, and the electroformation, are often used for preparation of giant liposomes in a buffer saline. Especially, the natural swelling is still popular probably because it is so simple and reasonable. Previously, we and collaborators reported improvement of this method, in which hydration and swelling processes in liposome formation may be enhanced through promoted osmosis due to water- and methanol-soluble solutes such as NaI and monosaccharide sandwiched between lipid lamellae of films [1,2]. In the present work, we further studies detailed properties of such giant liposomes with respect to changes in size, morphology and lamellarity. Here, DOPC films with fructose in various molar ratios were hydrated, resulting in transition of liposome sizes from micron to submicron at the higher ratio of Fru/DOPC (60:1 and more). An NBD quenching assay showed the transition from multi- to unilamellar liposomes. We also tried to investigate if the liposome morphology would be dependent on the kind of solute, using polymer as a model.

[1] Yamada, N.L., et al. EPL, 80, 48002 (2007). [2] Tsumoto, K., et al. Colloids Surf. B, 68, 98-105 (2009)

3PT139 表面修飾ナノ粒子の巨大リボソームへのエンドサイトーシス様取り込み
Endocytosis-like Uptake of Surface-Modified Nanoparticles into a Giant Liposome

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Cell-sized giant unilamellar vesicles (GUVs) have been used as artificial cell systems that mimic various cellular events. We previously reported an artificial exocytosis model by encapsulating small unilamellar vesicles in a GUV.

In the present study, we observed the uptake of surface-modified nanoparticles (NPs). We prepared a cell-sized GUV that mimics a cell membrane to investigate the interaction between cell membranes and NPs. As NPs, we used poly (D,L-lactide-co-glycolide) (PLGA) NPs modified with nonionic surfactant P80 or cationic polymer chitosan (CS) on their surface. To observe interaction between GUV and NPs with a fluorescence microscope, GUV was prepared with rhodamine-conjugated phosphatidylethanolamine, and NPs were labeled with 6-coumarin.

Average particle diameters of unmodified NPs, CS-modified NPs and P80-modified NPs were 221 ± 1.4 nm, 350 ± 17.1 nm, and 210 ± 5.1 nm, respectively. Zeta-potentials of unmodified NPs, CS-modified NPs and P80-modified NPs were -27.4 ± 0.6 mV, $+27.5 \pm 0.6$ mV, and -28.3 ± 0.7 mV, respectively.

Endocytosis-like uptake of NPs into a GUV was observed when the NPs were modified with P80. In contrast, unmodified NPs and those modified with CS were not internalized into a GUV, although large number of NPs with CS adhered to the surface of GUV due to their positive charge. These results