Domain Growth Dynamics on Vesicle[1]

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Summary

We have investigated domain coarsening process on model bio-membrane vesicle composed of ternary mixture of deuterated-saturated (d-DPPC), unsaturated (DOPC) phosphatidylcholine lipids and cholesterol (Chol). The nano-meter-sized domains rich in DPPC and Chol are formed on a vesicle and grow to micro-meter-sized in a diffusion-and-coalescence manner. In the late stage the coarsening processes are classified into two types; normal coarsening and trapped coarsening. For the normal coarsening, the domain size grows obeying a power law of $\sim t^{2/3}$. For the trapped coarsening, on the other hand, the domain coarsening is suppressed at a certain domain size because the repulsive inter-domain interactions obstruct the coalescence of domains. The trapping of domains is caused by the coupling between the phase separation and the membrane elasticity under the incompressibility constraint.

1. Introduction

Biomembranes have characteristic lateral heterogeneities arising from the immiscibility of the lipid components such as sphingomyelin, unsaturated phospholipids, and cholesterol. In the so-called raft-model [1], the heterogeneity coupled with the protein distribution plays important roles in the functionality of the biomembranes. In order to realize the formation of rafts in artificial membranes, a ternary mixture consisting of saturated phospholipid, unsaturated phospholipid and cholesterol ternary mixture has been studied [2]. Such artificial membranes show a lateral phase separation below the miscibility transition temperature and form a liquid-ordered phase rich in saturated phospholipids and cholesterol, and a liquid-disordered phase rich in unsaturated phospholipids. In this study we present a systematic experimental investigation on the growth dynamics of the domains in the ternary fluid vesicles consisting of DPPC (dipalmitoyl-phosphatidylcholine; saturated phospholipid), DOPC (dioleoyl-phosphatidylcholine; unsaturated phospholipid), and Chol.

A nucleation and growth process of nano-sized-domains is investigated using a contrast matching technique of small angle neutron scattering (SANS) and small unilamellar vesicles (SUVs) [3, 4]. After the nucleation, domains grow to micro-meter-sized domains. We follow the coarsening process using a fluorescence microscopy [5].

2. Experiment

Samples:

DOPC, h-DPPC, d-DPPC were obtained from Avanti Polar Lipid, Inc. Chol was purchased from Sigma-Aldrich Co. (St. Louis, MO). In order to dye the target region on a vesicle, Texas Red 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (TR-DPPE) and Perylene were obtained from Molecular Probes (Eugene, OR). All lipids were used without further purification and stored at -20℃ until use.

Sample preparation:

Giant vesicles (GVs) were prepared by a gentle hydration method. First we dissolved the lipids, DPPC, DOPC and Chol in chloroform ($5mM$). In order to dye the DOPC rich phase for the fluorescence

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microscope observation, TR-DPPE was added at the ratio of 0.8/10 (dye/lipid). The solvent was evaporated in a stream of nitrogen gas and then the obtained lipid film was kept under vacuum for one night. Before the hydration we heated the lipid film at 60°C, and then hydrated with 1 ml of pure water of 60°C. During the hydration process, the lipid films spontaneously form GVs with diameters of 10-100 μm. In order to obtain SUVs the suspension of GV was sonicated using ultrasonic homogenizer for ∼20min. By the sonication the MLVs transformed to SUVs with radius of ∼100$\mathring{A}$.

Instruments:

The nucleation and growth process of nano-sized-domains were examined using the SANS-U instrument of the Institute for Solid State Physics, The University of Tokyo at JRR-3M of the Japan Atomic Energy Agency. We followed the maicro-meter-sized domain coarsening process using an inverted conformal microscope (Carl Zeiss, LSM 5).

3. Results and Discussions

Formation of nano-meter-sized domains:

We investigated the nano-meter-sized domain formation on the SUVs with the radius R∼110$\mathring{A}$ using the contrast matching technique of SANS experiments. The SANS profiles of the SUV with *d*-DPPC/DOPC/Chol = 4/4/2 at the matching point after the subtraction of the solvent contribution are shown in Fig. 1. At 44℃ where the SUVs are in one phase homogeneous region, we can not observe any significant scattering from the vesicles, indicating that the mean scattering length density of vesicles agrees with that of the solvent. When we decrease the temperature to the phase separation region (26°C), excess scattering intensity due to the phase separation is observed as shown in Fig. 1. The scattering intensity profile have a maximum in the vicinity of $q = 0.03\mathring{A}^{-1}$. In order to examine whether the obtained SANS profiles represent the equilibrium structure or not, we measured time dependence of SANS profiles at 26°C. The obtained scattering profiles are independent of time as shown in Fig. 1, indicating that the obtained SANS profile represents the equilibrium phase separating structure. For the profile analysis, we adopted a Monte Carlo simulation method. In this simulation we calculated the form factor of vesicle with n domains as follows. Since a vesicle is characterized

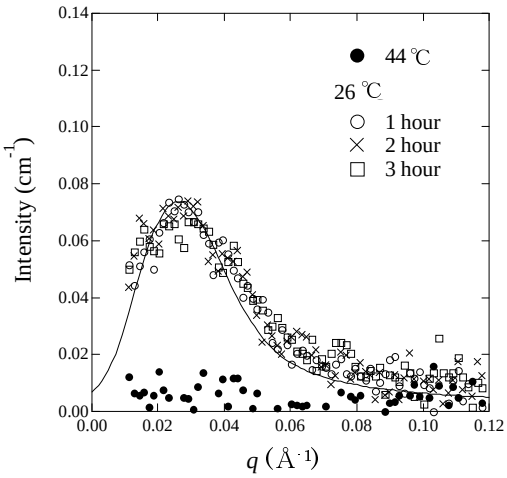

Fig.1 SANS profiles of SUVs composed of *d*-DPPC/DOPC/Chol=4/4/2 under contrast matching condition at 44℃ (closed circle) and 26℃ (time evolution). Solid line is fitting result using mono-domain model.

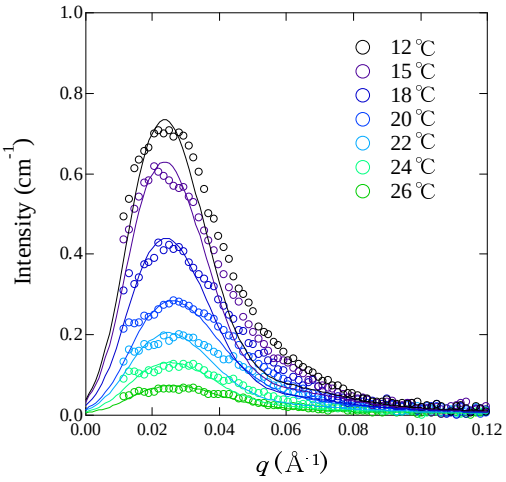

Fig.2 Temperature dependence of SANS profiles of SUVs composed of *d*-DPPC/DOPC/Chol=4/4/2 under contrast matching condition. Solid lines indicate fitting results of mono-domain model.

by two kinds of scattering length density, so we locates two kinds of particles p1 and p2 on a spherical shell. We produced n domains having the same size, and the matrix using p1 particles and p2 particles, respectively. Here the n domains are randomly distributed on the vesicle. Then, the radial distribution functions of particles p1 and p2 are calculated. The form factor of a vesicle with n domains is expressed by

$$P^{mult}(q) = \sum_{\alpha,\beta} \rho_\alpha \rho_\beta S_{\alpha\beta}(q) \qquad (1)$$

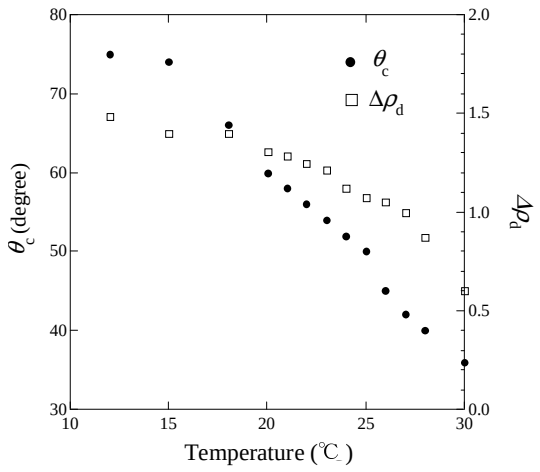

Fig.3 The temperature dependence of domain size θ_c and scattering contrast between domain and solvent,$\Delta\rho_d$.

$$S_{\alpha\beta}(q) = \frac{N_\alpha}{N}\delta_{\alpha\beta} + \frac{N_\alpha N_\beta}{N^2} 4\pi\rho \int_0^{r_{max}} r^2 g_{\alpha\beta}(r) \frac{\sin(qr)}{qr} dr \quad (2)$$

where, α and β indicate the kind of particles, N_α and N_β is the numbers of p1 and p2, respectively, N is the total number of particle and $g_{\alpha\beta}(r)$ is the partial radial distribution function of $\alpha\beta$ component. We averaged the scattering functions over all possible orientations and domains configurations. The model scattering function describes well the observed SANS profile as shown by solid line in Fig. 1.

Next we examined temperature dependence of the nano-meter-sized domain on the vesicle. We shows the temperature dependence of SANS profiles from 26℃ to 12℃ in the two phase region in Fig. 2. With decreasing the temperature the peak intensity increases and the width of the peak profile sharpens while the peak position keeps constant. These SANS profiles are well fitted by the mono-domain model. The obtained the domain size characterized by the bounding angle θ_c and the scattering contrast between the domain and the solvent $\Delta\rho_d$ are plotted against the temperature in Fig. 3. The domain size θ_c increases with decreasing of the temperature and reaches an equilibrium value.

On the other hand, the scattering contrast $\Delta\rho_d$ increases with decreasing the temperature, indicating that the compositions of domain phase and matrix phase depend on the temperature. The observed temperature dependence may be attributed to the transfer of *d*-DPPC molecules from the matrix to the domain,

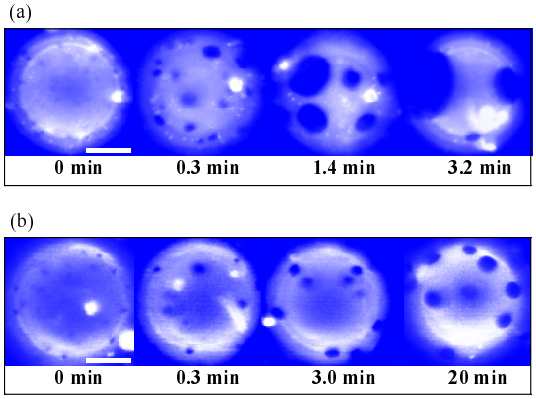

Fig.4 Time evolution of domain growth on vesicle at 30℃ observed by the fluorescence microscope, (a) normal coarsening and (b) trap coarsening mechanisms.

which results in the increase of size of the domain.

Coarsening of micro-meter-sized domains:

The nano-meter-sized domains formed on a GV develop to the micro-meter-sized domains in a diffusion-and-coalescence manner. First we show the coarsening processes of the domains on the fluid giant vesicles. When we decrease the temperature from the homogeneous one phase, the lateral phase separation between the liquid-ordered (L_o) and the liquid - disordered phases (L_d) takes place at 34℃ (the miscibility transition temperature: T_{mix}) with the nucleation-growth mechanism. Figure 4 shows the two types of the domain growth at 30℃ . For the both cases, we set $t = 0$ when the domain size reaches to an optical resolution of the microscope ($\sim 0.4\mu m$). In Fig. 4 (a), the circular domains (L_o phase) grow in a diffusion-and-coalescence manner while keeping the circular shape, and reach to the size of $\sim 10\mu m$ within several minutes. We call this coarsening process as the normal coarsening. In Fig. 4 (b), on the other hand, the circular domains grow to the size of $\sim 2\mu m$ by the normal coarsening process, but then the domain coarsening is trapped for $\sim$ 100 minutes. During the trapping period, the domains having almost the same domain size migrate freely on the vesicle. We call this coarsening process as the trapped coarsening. In order to make clear the difference between the normal coarsening and the trapped coarsening, we plot the time evolution of the mean domain diameter $D(t)$ for the both cases in Fig. 5. For the normal coarsening, the mean domain diameter $D(t)$ grows with a power law of $D(t) \sim t^a (a \sim 2/3)$ until the domains develop into a single large domain. For the trapped coarsening, the

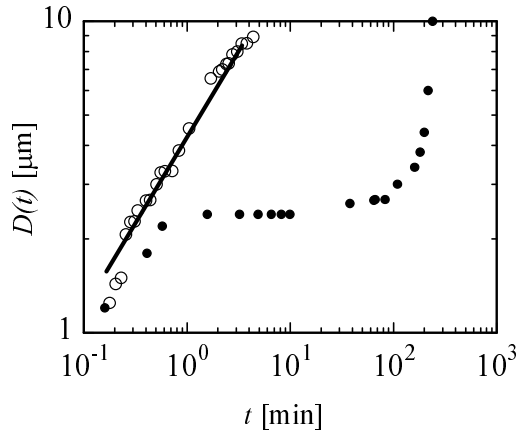

Fig.5 Time dependence of mean domain diameter $D(t)$ on a vesicle shown in Fig. 4. The open and closed circles denote normal and trap coarsening, respectively. A solid line for normal coarsening indicates the power law $D(t)\sim t^a$ with $a = 2/3$.

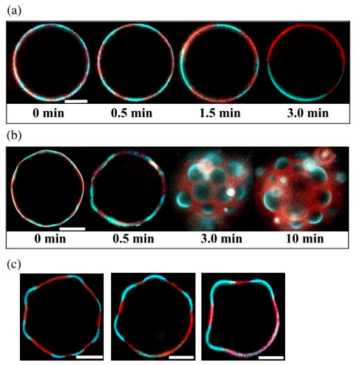

Fig.6 Time evolution of two color (TR-DHPE and Perylene) cross section images for normal coarsening vesicle (a) and trapped coarsening vesicle (b). In order to show the budding domains clearly, the focusing points are at the top of vesicles for 3.0 and 10 minutes images in (b). (c) Two color cross section images for budding domains in the trapped coarsening vesicle. Scale bar in figures is 50 μm.

mean domain size grows to $\sim 2\mu m$ with a power law of $D(t)\sim t^a (a \sim 2/3)$, and then the coarsening ceases for $\sim$100min. After the trapping period, the domains restart to grow towards a mono-domain structure.

In order to elucidate the difference between the normal and the trapped coarsening, we dyed not only the matrix (L_d phase) but also the domains (L_o phase). TR-DHPE localizes in the L_d phase and emits red fluorescence, whereas the Perylene prefers the L_o phase and emits blue fluorescence. Figures 6 (a) and (b) are the time evolution of the two color cross-section images for the normal coarsening and the trapped coarsening domains, respectively. For the normal coarsening, the domains do not bud and have the average curvature that is equal to that of the surrounding matrix. In this case the coarsening proceeds straightway and the vesicle keeps its spherical shape during the coarsening. On the other hand, in the case of the trapped coarsening, the domains have the same curvatures as the surrounding matrix in the initial coarsening stage, although the cross-section of the vesicle is flaccid. With the elapse of time, the domains grow to a certain size and then start to bud to a cap-like shape. This observation indicates that the line energy of domains starts to play a dominant role on the domain morphology. It is important to note that the onset time of the domain budding coincides with that of the domain trapping.

The simplest model to explain the observed trapped coarsening is the bending penalty of the matrix membrane between the two approaching cap-shaped domains. When the domains slightly bud towards the outside of the vesicle (cap-shaped domains), the matrix membrane surrounding the cap-shaped domain is deformed to skirt-like shape accordingly. A cross-section image of the vesicle with the trapped domains (Fig. 6(c)) supports the fact that the geometry of the surrounding matrix is the skirt-like shape. When the two cap-shaped domains approach, the curvature of the skirt-like region becomes large, which results in a repulsive potential between the two domains. In contrast, if the domains are not bud and have the same curvature with the matrix membrane, the approaching two domains are not blocked by the matrix membrane, which gives rise to the coalescence of the domains. Thus we conclude that the bending of the matrix membrane between the two approaching cap-shaped domains induces the repulsive interaction, which in turn results in the trapped coarsening. Here it should be noted that in order to form the cap-shaped domains, the vesicle must have enough excess area. Thus the trapped coarsening is a result of a strong coupling between the phase separation and the elastic nature of the membrane under the area-to-volume constraint.

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