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Description	

Domain-growth Kinetics in a Cell-sized Liposome

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Abstract

We investigated the kinetics of domain growth on liposomes caused by decreasing the temperature; the liposomes consisted of a ternary mixture (unsaturated phospholipid, saturated phospholipid, and cholesterol). The domain-growth process was monitored by fluorescence microscopy, and the growth was mediated by fusing the domains through collisions. It was found that an average domain size r develops with time t as $r \sim t^{0.15}$. This indicates that the exponent is about half of that deduced from the theoretical analysis of a model of the Brownian motion on a two-dimensional membrane. We discuss the mechanism of the experimental scaling behavior by considering the elasticity of the membrane.

KEYWORDS: giant vesicle, cell-sized liposome, microphase separation, domain growth, time development, scaling law, raft

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Text

During the last decade, a raft model has often been adopted in order to describe the dynamical feature of cell membranes.^{1,2)} It was suggested that a mixture of cholesterol and sphingolipids, which are rich in saturated acyl chains, forms dynamic clusters called “rafts,” which exhibit Brownian motion within a bilayer membrane.^{3,4)} Rafts are expected to function as platforms on which proteins are attached during signal transduction and membrane trafficking.¹⁾ In the context of rafts, the microdomain structure is regarded as a form of phase separation that develops due to the interaction between lipid molecules. To investigate this structure, the phase separation in monolayers with different lipid compositions at the air-water interface was studied by fluorescence microscopy.⁵⁾ Recently, cell-sized lipid vesicles with a diameter of the order of 10 μm have frequently been used as models for living cellular structures (e.g., protein crystallization and gene expression encapsulated inside them).⁶⁻⁹⁾ They have also been used as models for raft formation.^{10,11)} Further, some studies have been performed: the relation between the membrane curvature and the domain patterns and the phase diagram in a mixture of lipids and cholesterol.¹²⁻¹⁶⁾ Regarding the domain formation on a membrane, theoretical reports on microphase separation in cell-sized liposomes have been published.^{17,18)} Both experimental and theoretical studies have focused on domain structures under equilibrium. Contrary to the static stable structure of the phase separation in these studies, it is now clear that rafts in living cell membranes exhibit significant time-dependent changes, including the repetitive cycle of generation and disappearance.¹⁹⁾ Some studies by computer simulations have reported the kinetics of domain growth on a liposome.^{20,21)} Recently, time-dependent changes of a model membrane have been investigated through direct microscopic observation and scattering experiments.²²⁻²⁴⁾ However, to our knowledge, no study has experimentally investigated the kinetic aspect of microphase separation in a cell-sized liposome. To elucidate the dynamic property of rafts, kinetic studies on the phase separation in a simple vesicular system are invaluable. In this study, we conducted a microscopic observation of domain growth on a cell-sized liposome consisting of a ternary mixture of saturated and unsaturated phospholipids along with cholesterol. We measured the time development of the average size of domains and then determined the experimental scaling behavior.

Liposomes were prepared using an unsaturated phospholipid, dioleoyl L- α -phosphatidylcholine (DOPC; Sigma, USA), a saturated phospholipid, dipalmitoyl L- α -

phosphatidylcholine (DPPC; Wako, Japan), and cholesterol (Sigma, USA). N-(rhodamine red-X)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (rhodamine red-X DHPE, triethylammonium salt; Molecular Probes, USA) was used as a fluorescent probe. Cell-sized liposomes were prepared by the natural swelling method from dry lipid films through the following procedure: 10 mM lipids dissolved in chloroform/methanol (=2:1) along with 50 mM D(+)-glucose (Nacalai Tesque, Japan) in methanol were dried under vacuum for 2 h to form a thin lipid film. Next, the films were hydrated with distilled water at 60 °C for several hours. The final lipid concentration for the liposomes was 0.1 mM DOPC, 0.1 mM DPPC, 30 mol% cholesterol, and 0.1 μ M rhodamine red-X DHPE, and the concentration of D(+)-glucose was 0.25 mM. In this three-component bilayer membrane, phase separation between the liquid-ordered (L_o) and liquid-disordered (L_d) phases occurs below the transition temperature (~ 31 °C).²⁵⁾ Since the fluorescent probe is localized at the L_d phase, the two phases are clearly distinguished as bright (L_d phase) and dark (L_o phase) zones by fluorescence microscopy.¹²⁾ 20 μ L of the liposome solution was placed on a glass plate and covered with a slip at a spacing of 100 μ m, after which it was sealed. Just before the microscopic observation, we increased the temperature at 60 °C for the period more than 20 s in order to attain a homogenous phase. Then, the samples were cooled down to the room temperature (21 °C); the time to cool down was ca. 10 s. The process of domain growth on a liposome was observed by using a confocal laser scanning microscope (LSM 510, Carl Zeiss).

Typical confocal microscopic images of domain growth on a cell-sized liposome are shown in Fig. 1, where the two phases are distinguished as bright (L_d phase) and dark (L_o phase) zones. Immediately after the temperature shifts below the miscibility transition temperature, many small domains appear all over the membrane. The generated domains exhibit random thermal motions, and the smaller ones exhibit greater agitation.²⁶⁾ The domains become larger through collision and fusion during the thermal motions. Finally, after several tens of minutes, the entire membrane surface is covered with the two different phase regions.

We measured the radii of all the visible domains on individual liposomes by acquiring 9–11 sliced images along the vertical z axis (slice depth < 2.5 μ m, interval = 3 μ m) at every 30 s. Based on the reconstructed three-dimensional image, we determined the radius r as the mean value around the domain boundary on individual domains. The

time-dependent changes in the average domain radius r on individual liposomes during the domain-growth process are exemplified in Fig. 2, where $t = 0$ is the time required to cross the transition temperature. The mean domain radius increases linearly in the double-logarithmic plot; the average of the gradient on seven samples is found to be 0.15 ± 0.06 , i.e., $r \sim t^{0.15}$.

Next, we discuss the scaling behavior in the domain growth by considering the effect of the Brownian motion of each domain.²⁷⁾ The temporal change in the number of domains, n , decreases linearly with the frequency of collisions as follows:

$$\frac{dn}{dt} \sim -n \left(\frac{1}{\tau} \right), \quad (1)$$

where τ is the mean time in which one domain collides with others. In the later stage of the phase separation, the total area of the domains remains almost constant. This indicates that n is inversely proportional to the mean domain area s , i.e., $n \sim 1/s$. We introduce a diffusion coefficient for domains moving on the two-dimensional membrane: $D \sim s^{\alpha/2}$ (by assuming the Stokes-Einstein relation). Substituting the characteristic time $\tau = x^2/4D$ together with the assumption of $x^2 \sim 1/n$ (where x is the mean distance between the domains) into eq. (1), the scaling relationship for r on a plain surface ($s \sim r^2$) is deduced as follows:

$$r \sim t^{\frac{1}{3}} \tau^{0.33}. \quad (2)$$

Although this simple theoretical argument suggests the existence of scaling behavior, the expected value of the exponent, 0.33, is much larger than that (0.15 ± 0.06) observed in our experiment. Such a large discrepancy is probably due to the fact that the above treatment is applicable only in the case of a flat membrane or an infinitely large liposome. On the other hand, it has been reported that a budded morphology with large out-of-plane curvatures along the domain boundaries is generated in phase-separated liposomes.¹²⁾ We have confirmed the appearance of this morphology (data not shown). The budding on the domain is attributable to a competition between the line and bending energies. The line energy between the two phases causes the budding of domains to decrease the boundary length; on the contrary, the bending energy prevents extensive deformation of the membranes. Previous numerical studies have suggested that a coupling between the domain patterns and membrane morphologies changes the domain-growth scaling.^{20,21)} Thus, we will consider the effect of the out-of-plane

curvatures in the next paragraph.

Regarding that the curvature of the domain portion is approximately proportional to $1/r$, the domain surface area s is given as $s \sim \frac{\kappa}{\sigma} r^3$, where κ and σ are the bending rigidity and line tension, respectively.²¹⁾ Through an argument similar to eq. (2), we obtain the following scaling law:

$$r \sim t^{\frac{2}{9}} \kappa t^{0.22}. \quad (3)$$

It is evident that the coupling with the membrane curvature reduces the exponent for domain-growth scaling. Based on numerical simulation with dissipative particle dynamics, Laradji et al. reported that the exponent for the total boundary length of all domains, nr , was $-4/9$, corresponding to $nr \sim t^{\frac{4}{9}}$, which can be transformed from eq. (3).²¹⁾ However our experimental value of 0.15 ± 0.06 is still slightly smaller than the theoretical value of 0.22. Several additional factors may be considered. It should be noted that the hydrodynamic friction of domains on a liposome was not described accurately by the abovementioned Stokes relation for an ideal sphere. During the experiments, we noticed the appearance of geometric membrane deformations around the two phase boundaries. We should consider the friction involved in the movements of the domains due to the effect of out-of-plane curvatures in a two-dimensional fluid membrane.²⁸⁾ Additionally, we should consider the effect of effective repulsive interaction between neighboring domains (e.g., long-range dipolar interaction).²⁹⁾ It should also be mentioned that convection on the membrane surface can affect the apparent mobility during thermal fluctuation. Further theoretical studies together with additional experimental studies are awaited in order to understand the essential processes involved in domain-growth kinetics.

Acknowledgements

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Figure captions

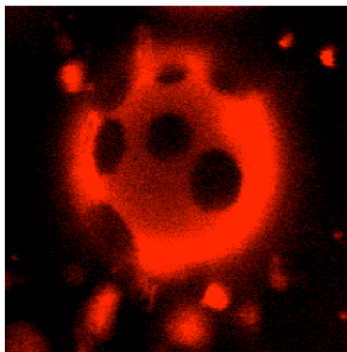
Figure 1. Typical fluorescence microscopic images of domain growth on a cell-sized liposome, obtained by using a confocal laser scanning microscope. (a), (b), and (c) are images obtained 10 s, 1 min, and 10 min, respectively, after the miscibility transition temperature was reached. The numbers of domains on the liposome are (a) 13, (b) 8, and (c) 3. The domains fused when they collided with each other under thermal fluctuation. The scale bar is 10 μm .

Figure 2. Three examples of the double-logarithmic plot of the average domain radius r (μm) on individual liposomes versus time t (s). The slopes are (A) 0.18, (B) 0.15, and (C) 0.17, and the diameters of the liposomes are 24, 19, and 22 μm , respectively.

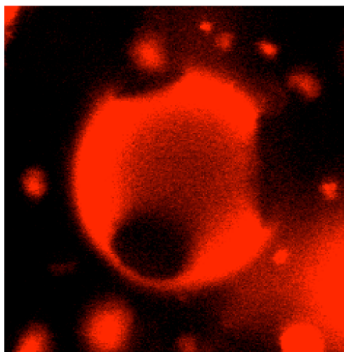
Figures

Figure 1.

(a)



(b)



(c)

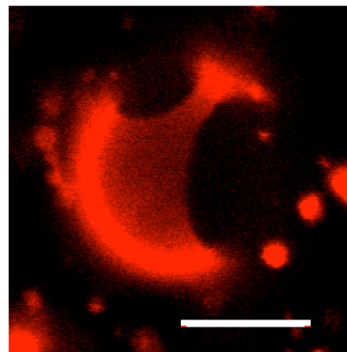


Figure 2.

