Nano-meter–sized domain formation in lipid membranes observed by small angle neutron scattering

T. Masui¹,a, N. Urakami², and M. Imai¹,b

¹ Department of Physics, Ochanomizu University, Bunkyo, Tokyo 112-8610, Japan
² Department of Physics and Information Sciences, Yamaguchi University, Yoshida, Yamaguchi 753-8512, Japan

Received 16 February 2008 and Received in final form 4 August 2008
Published online: 2 December 2008 – c⃝ EDP Sciences / Società Italiana di Fisica / Springer-Verlag 2008

Abstract. Using a contrast matching technique of small angle neutron scattering (SANS), we have investigated a phase separation to liquid-disordered and liquid-ordered phases on ternary small unilamellar vesicles (SUVs) composed of deuterated-saturated, hydrogenated-unsaturated phosphatidylcholine lipids and cholesterol, where the equilibrium size of these domains is constrained to less than 10 nm by the system size. Below a miscibility temperature, we observed characteristic scattering profiles with a maximum, indicating the formation of nano-meter–sized domains on the SUVs. The observed profiles can be described by a multi-domain model rather than a mono-domain model. The nano-meter–sized domain is agitated by thermal fluctuations and eventually ruptured, which may result in the multi-domain state. The kinetically trapped nano-meter–sized domains grow to a mono-domain state by decreasing temperature. Furthermore, between the miscibility and disorder-order transition temperature of saturated lipid, the integrated SANS intensity increased slightly, indicating the formation of nano-meter–sized heterogeneity prior to the domain nucleation.

PACS. 61.05.fg Neutron scattering (including small-angle scattering) – 87.16.D- Membranes, bilayers, and vesicles – 87.16.dt Structure, static correlations, domains, and rafts

1 Introduction

In recent years, lateral phase separation in cell membranes, the so-called lipid raft, has gathered much attention in relation to the biological functionalities [1–3]. Lipid rafts are liquid domains rich in cholesterol, sphingolipid and specific proteins, and they play important roles in the important biological processes such as endocytosis, adhesion, signaling, protein transport and apoptosis [4–7]. Due to the wealth of biological significance of the lipid raft model, lipid rafts in cell membranes have been investigated by various techniques such as fluorescence resonance energy transfer (FRET), single-particle tracking (SPT), detergent solubility, multi-photon laser-scanning microscopy, nuclear magnetic resonance (NMR) and atomic force microscopy (AFM) [3,7–14]. In spite of the extensive investigations, statical and dynamical structures of lipid rafts in cell membranes are still controversial. There are two major hypotheses on raft structures: 1) rafts are relatively large (> 50 nm) cholesterol and sphingolipid-rich structures [1, 10,15], and 2) rafts are dynamical assemblies of small size (< 10 nm), constituted by components that are preferentially associated with lipids [9,11,12,16]. Then, it is desirable to measure domains with size of 10–20 nm on vesicles.

Since a lateral phase separation of lipids to liquid-ordered (Lo) phase and liquid-disordered (Ld) phase might be responsible for the formation of rafts in cell membranes, the model giant vesicle (GV) consisting of saturated lipids, unsaturated lipids and cholesterol have been investigated to understand physicochemical points of the raft formation [17–20]. One of the advantages of the model GV systems is that the domains have the size of micrometer length scale, which is easily observed by fluorescence microscopy. Taking this advantage, the behaviors of micrometer–sized liquid domains in the GVs, such as phase diagrams [18,21,22], domain growth kinetics [18,23], and domain dynamics [23,24] have been well understood.

On the other hand, little is known about behaviors of nano-meter–sized domains and to our knowledge two kinds of nano-meter–sized heterogeneities were reported for the model biomembrane systems. One is the nano-meter–sized heterogeneity observed in homogeneous one-phase region of multi-lamellar vesicles (MLVs) by the FRET method [25,26]. The other is nano-meter–sized domains on vesicles observed below the miscibility
transition temperature, $T_{\text{mix}}$. Keller and colleagues found smaller ($\sim 80 \text{ nm}$) domains just below $T_{\text{mix}}$ in the MLVs composed of dipalmitoylphosphatidylcholine (DPPC)/dioleoylphosphatidylcholine (DOPC)/cholesterol (Chol) by NMR [22]. Pencer and colleagues revealed the existence of multi-domains with nano-meter length scale on the large unilamellar vesicles (LUVs) composed of DPPC/DOPC/Chol by a small angle neutron scattering (SANS) technique [27]. Thus coarsening of nano-meter-sized domains is kinetically suppressed, which may have a relation to the dynamical lipid raft model [9,11,12,16].

Based on these findings, in this study we have systematically investigated the nature of nano-meter-sized domains in model biomembranes to fill up a gap between the lipid rafts in cell membranes and micro-meter-sized domains in the model membranes. We focus on the Ld/Lo phase separation of the ternary model biomembranes but the equilibrium size of domains is constrained to less than 10 nm by the vesicle size. For this purpose we prepared small unilamellar vesicles (SUVs) with nano-meter length scale for the spatial constraint. To obtain the distribution of the different kinds of lipids in nano-meter length scale, we employed the SANS technique [27–29]. The scattering intensity from a vesicle is expressed by

$$I(q) = \left[ \int_r (\rho(r) - \rho_{\text{solv}}) \exp(i\mathbf{q} \cdot \mathbf{r}) \, \text{d}r \right]^2,$$

(1)

where $\rho(r)$ is the scattering length density (SLD) of the lipid membranes at position $r$, $\rho_{\text{solv}}$ is the SLD of solvent, and $\mathbf{q} = 4\pi \sin \theta / \lambda$, where $2\theta$ is the scattering angle and $\lambda$ the wavelength, is the magnitude of the scattering vector. The SLD of a lipid $\rho_{\text{lip}}$ is expressed by

$$\rho_{\text{lip}} = \frac{1}{V} \sum_{j=1}^{n_m} b_j,$$

(2)

where $V$ is the volume of a lipid molecule consisting of $n_m$ atoms and $b_j$ is the coherent scattering length of the $j$-th atom. A unique feature of the neutron scattering is that a hydrogen atom and a deuterium atom have different scattering lengths $b_H = -3.739 \times 10^{-13} \text{ cm}$ and $b_D = 6.671 \times 10^{-13} \text{ cm}$, respectively, which makes it possible to mark domains rich in deuterated lipids. In addition, we used a differential scanning calorimetry (DSC) technique to reveal the phase transitions kinetics from the thermogram point of view. By combining the SANS and DSC results, we elucidate the formation of nano-meter-sized domains on SUVs.

2 Materials and methods

2.1 Commercial reagents

1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) ($> 99\%$ purity), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) ($> 99\%$ purity) and 1,2-dipalmitoyl-1,6-dioleoyl-sn-glycero-3-phosphocholine (d-DPPC) ($> 99\%$ purity) were obtained in a powder form from Avanti Polar Lipid, Inc. Cholesterol (Chol) ($> 99\%$ purity) was purchased from Sigma-Aldrich Co. and Texas Red 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (TR-DHPE) was obtained from Molecular Probes. All lipids were used without further purification and stored at $-20^\circ \text{C}$ until use. Scattering length densities of lipids and solvent were estimated from the molecular volumes of DPPC, DOPC [30], cholesterol [31], water [32] and heavy water [33], and known neutron scattering lengths [34]. The order-disorder transition temperature of the acyl chain for each phospholipid was obtained from the DSC measurement. These values are summarized in Table 1.

2.2 Sample preparation

2.2.1 Giant unilamellar vesicles

Giant unilamellar vesicles (GUVs) were prepared by a gentle hydration method [35]. First we dissolved each lipids (DPPC, DOPC and Chol) in chloroform (5 mM). Then the lipid solutions were mixed at the prescribed ratio of DPPC, DOPC and Chol in a vial. In order to dye the Ld phase for the fluorescence microscope observation, TR-DHPE was added at the ratio of 0.008/1 (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 to remove the remaining organic solvent completely. Before the hydration we heated the lipid film at $60^\circ \text{C}$, and then hydrated with pure water of 1 ml at $60^\circ \text{C}$. During the hydration process, the lipid films spontaneously form GUVs with diameters of 10–100 μm.

2.2.2 Small unilamellar vesicles

We prepared SUVs by the following procedure. We put the prescribed amounts of lipids in a vial. The lipids were dissolved in chloroform and then the chloroform was removed under a stream of nitrogen gas and an overnight vacuum treatment. The dried lipid film was dispersed in pure water with vortexing at $60^\circ \text{C}$, which results in the formation of MLVs with milky appearance. This suspension was sonicated using an ultrasonic homogenizer with 20 kHz frequency (Yamato, Powersonic Model 50) for ~20 min [36]. After the sonication we obtained SUVs with radius of ~100 Å and the suspension became transparent.

2.3 Instruments

2.3.1 Fluorescent microscope

We examined the miscibility transition temperature ($T_{\text{mix}}$) of the ternary GUVs by the fluorescence microscopy observation (Zeiss, Axio SKOP) with a high-resolution CCD camera (Zeiss, Axio Cam). The sample temperature was controlled by using a FP90 system (Mettler Toledo) within the deviation of ±0.1°C. To prevent the domain formation before observations, we kept the sample temperature at ~60°C > $T_{\text{mix}}$. Then we decreased the temperature gradually from ca. 60°C (one-phase region). When