Local Condensation of Artificial Raft Domains under Light Irradiation in Supported Lipid Bilayer of PSM-DOPC-Cholesterol System

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Lipid bilayers supported on solid substrates are considered to offer a potential as biological devices utilizing biological membranes and membrane proteins. In particular, highly patterned bilayers hold a promise for the development of biological devices. Previously, we proposed the concept of “active patterning” and demonstrated the gel-phase domain patterning in the supported bilayers of the DMPC-DOPC system using the light irradiation technique. The essence of this technique is the control of the spatial distribution of bilayer composition due to the local temperature increase. In this study, we applied this technique to the assembly of artificial raft domains in the supported lipid bilayers of the PSM-DOPC-cholesterol ternary system. After the light irradiation, the raft domain size increased in the irradiated region. This result means that the local PSM condensation enhanced the formation of the rafts, and it implies the possibility of the active patterning of the raft domain.

Key words: supported lipid bilayer, raft, phase separation, patterning

1. INTRODUCTION

Biological membranes are composed of continuous fluid phospholipid bilayers with several membrane proteins. These phospholipid bilayers are multi-component systems, and so they exhibit lateral heterogeneity through spontaneous phase separation phenomena under the given conditions of temperature, composition, ion concentration of the environment and others [1, 2]. In particular, the domains called “rafts” play a role of the stage where certain types of the membrane proteins concentrate and cooperatively express important cell functions [3].

Recently, planar lipid bilayers supported on solid substrates (SLB: Supported Lipid Bilayers) are attracting attention because they are relevant in the model system of bio-membrane and the design of synthetic biocompatible surface, biological devices utilizing biological membranes and membrane proteins [4-10]. The SLBs maintain many of the physical and biological characteristics of membranes including lateral fluidity and display similar thermodynamic phase separation phenomena including raft formation [4, 11].

For such applications, there has been a great interest in spatially patterning techniques because it is often important to control the spatial distribution of composition in the SLB for localizing membrane functions. Previously, patterning of the SLBs has been achieved by various methods, such as the use of stamping [12, 13], pre-patterned substrates [14-21], and photo-lithography [13, 22, 23] among others. However, the patterns made by these techniques are static and the membrane fluidity is not utilized.

Contrary to those, we aim to actively control formation, erasing, shape and transfer of phase-separated domains by external physical stimulations as an “active patterning” approach. This technique is for controlling spatial lipid compositions and domain structures in the SLB and has a potential of controlling membrane proteins concentrated in raft domains. In the previous study [24], we demonstrated an example of the active patterning by using a light irradiation. In this experiment, we controlled the spatial distribution of the lipid composition in the SLB of the DMPC-DOPC binary system. Since domain formation depends on the lipid composition, the spatial distribution of the composition can be observed as that of the domains. This technique could make the gel-phase domains condense in the light-irradiated region. The essence of this technique is a strain in membrane due to local temperature increase caused by photo-induced activation of non-radiative molecular vibration of fluorescent molecules doped in the SLB. The detail of this mechanism will be explained later.

Next target is that active patterning is applied to the control of the “raft” domains, which are composed of Lα micro-domains. In plasma membranes, the raft domains are observed in detergent-resistant membranes (DRM) of liquid-order phase (Lα) enriched in cholesterol.
and sphingomyelin (SM) [25, 26]. The L₀ micro-domains have been detected and visualized by fluorescent experiments [27, 28] and atomic force microscopy [29] in the artificial membrane composed of a combination of unsaturated phosphatidylcholine (PC)-SM-cholesterol.

In this study, the light induced active patterning of the raft domains was demonstrated as well as that of the gel phase domains. Raft domains were artificially assembled in the SLB of PSM(palmitoyl SM)-DOPC(dioleoyl PC)-cholesterol ternary system fabricated on a SiO₂/Si substrate, and the effect of light irradiation on the raft domain formation was investigated.

2. EXPERIMENTAL DETAILS
2.1 Materials
The ternary system of PSM-DOPC-cholesterol was used according to the phase diagram of the raft domain formation in giant uni-lamellar vesicles (GUVs) of the same system reported by Veatch et al. [28]. PSM and DOPC were purchased from Avanti Polar Lipids (Birmingham, AL). Cholesterol was purchased from Sigma (Tokyo, JAPAN). The fluorescent lipid probe dipalmitoyl phosphatidylcholine–Texas Red (TR–DPPE) was obtained from Molecular Probes (Eugene, OR). All lipids were suspended and stored in chloroform (HPLC grade) in the freezer (-20 °C) until use. Organic-free deionized water of high resistivity (18.0 M·cm) was obtained by using an ultra-pure water production system (Eugene, OR). A Si (001) wafer with a native oxide layer was used as a substrate. The substrate size was 8 mm × 8 mm. As-delivered Si wafers were cleaned by treatment of the substrates as follows; sonication in acetone, methanol and purified water for 5 min in turn, and UV radiation for 20 min just before the SLB preparation.

2.2 Preparation of SLBs
Lipid bilayers were formed on SiO₂/Si using a vesicle fusion method [30, 31]. The lipid mixtures with various PSM-DOPC-cholesterol molar ratios (27:54:20, 40:40:20, 54:27:20) were used. All samples were doped with 1 mol% TR–DPPE. The detail of the preparation is described in the literature [24].

2.3 Epifluorescence microscopy observation
Fluorescence images were obtained using an epifluorescence microscope (OLYMPUS BX51, Tokyo, Japan) with the CoolSNAP ES charge-coupled device camera (Photometrics, Huntington Beach, CA) and the MetaVue (Molecular Devices, CA) acquisition software. Illumination was provided by a mercury arc light source. A filter cube (OLYMPUS U-MWIV2, Tokyo, Japan, BP:545–580 BA:610IF, DM:600) was used to examine fluorescence from the Texas Red fluorophore (excitation band 530–580 nm; emission band 605–675 nm). To observe the phase separation phenomena of SLB, the temperature of the sample in the glass-bottom dish was controlled using a stage consisting of a Peltier element and a Pt resistance thermometer (Netsudenshi Kogyo, Tokyo, JAPAN) installed on the microscopy. The sample temperature was controlled in the range from 5 – 50 °C. The domain area was determined by measuring the area of the black region using the MetaVue software.

2.4 Light irradiation for photo-induced activation of molecular vibration
In our technique for domain condensation, the local temperature increase via non-radiative vibration of the fluorescent molecules due to photo-induced activation is utilized. The mercury arc light source used to stimulate TR-DPPE was filtered to allow only the range from 530 – 580 nm, which covers the excitation band of Texas Red. Light irradiation fallings was kept at 7.4 × 10⁻³ W/m². The irradiation area was 100 μm² or 100 × 100 m². The light irradiation sequence is shown in Fig. 1. Samples were heated from 13 °C and kept at temperatures above the raft formation temperature to homogenize the composition of the SLB. Light irradiation was applied to the sample to allow photo-induced activation of molecular vibrations for 1 min, and then the sample was cooled to 13 °C. The light irradiation was applied before the re-distribution of composition required the relatively high diffusivity at high temperature.

3. RESULTS
3.1 Phase-separated structure
Figure 2(a) shows the fluorescent image of the phase-separated SLB with the molar ratio of 27:54:20. The gray matrix is the liquid-crystalline phase and the many white spots are likely to be non-fused vesicles and/or junks. In the magnified image, many small domains were observed within the gray matrix. As listed in Table I, the formation temperatures of the small domains were determined from the fluorescent image observations at the several temperatures. These temperatures are almost equal to those of the raft formation in the GUV systems [28]. The small domains were considered to be the raft domains. The large black domains were observed too. The formation temperature of the black domains was lower than that of the raft domains. The black domain density decreased

<table>
<thead>
<tr>
<th>PSM:DOPC:Chol.</th>
<th>Formation temperature (°C)</th>
<th>Present results</th>
<th>GUV [28]</th>
</tr>
</thead>
<tbody>
<tr>
<td>27:53:20</td>
<td>34±2</td>
<td>37±2</td>
<td></td>
</tr>
<tr>
<td>40:40:20</td>
<td>42±3</td>
<td>40±5</td>
<td></td>
</tr>
<tr>
<td>53:27:20</td>
<td>43±2</td>
<td>48±5</td>
<td></td>
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Fig. 1: Sequence of the light irradiation experiments (for the sample with the PSM-DOPC-cholesterol molar ratios: 27:54:20)
3.2 Light irradiation

Using the light irradiation for photo-induced activation of molecular vibration, we tried to localize the raft domains. Following the observation of the phase-separated structure as shown in Fig. 2(a), the light irradiation was applied to the same sample. Figure 2(b) shows the fluorescent images of the sample after the light irradiation. The irradiated region is indicated by the broken line. Both the gel-phase domains and the raft domains precipitated. Moreover, a lot of the many gel-phase domains were preferentially formed in the irradiated region. This phenomenon is similar to the gel-phase domain condensation in the DMPC-DOPC system [24]. In the magnified image, many raft domains were observed, but the density or size of domains was almost same as that before the light irradiation.

In our previous study of the gel domain formation in the DMPC-DOPC system, we noticed that the gel domain distribution was often inhomogeneous spatially. We tried again to apply the light irradiation in the region where the gel phase domain density is relatively low as shown in Fig. 3. They are largely different form those of Fig. 2. Both of size and density of the gel domain are small. After the light irradiation, the area of the raft domain increases as shown in the magnified images. On the other hand, few small gel-phase domains were observed. The raft domain density along the broken line in Fig. 3(b) is shown in Fig. 4. The difference of the domain density is slight. This implies the increase of the raft domain area observed in these images is due to the increase of the domain size. It is noticed that the domain density decreased in the region adjacent to the light-irradiated region.

4. DISCUSSION

This condensation phenomenon can be explained by considering thermal stress due to the light irradiation for photo-induced activation of fluorescent molecular vibration [24]. The temperature increased due to the photo-induced activation of Texas Red dye and the membrane expanded thermally in the light-irradiated region, while the membrane did not expand in the non-irradiated region. The irradiated region is constrained by the non-irradiated region, and consequently, a compressive stress is applied to the membrane around the boundary between the irradiated region and non-irradiated region. To relax this stress, the volume in the irradiated region must decrease. Looking at the difference in molecular size among the constituent molecules, the smaller molecules concentrating in the light-irradiated region can reduce the membrane volume to relax the stress. In the present case, the size of the unsaturated DOPC lipid is...
relatively larger than that of the saturated PSM lipid since the DOPC possesses double bonds at the fat chain. In addition, the molecular size of cholesterol is much smaller than those of these lipid molecules. Thus, the volume of the membrane decreases with increasing PSM and cholesterol composition. Hence the PSM and cholesterol may be enriched in the light-irradiated region and enhance the raft formation.

In Fig. 2, however, the enhancement of the raft formation was not observed. It may be because the PSM is enriched in not only the raft domain but also the gel-phase domain. In the PSM-rich region, both the gel-phase domains and the raft domains can preferentially form. The present results in Figs. 2 and 3 indicate the competition between the raft domain formation and the gel domain formation. If the raft domain formation is enhanced, the gel-phase domain formation is not enhanced, and vice versa. Unfortunately, we cannot answer the question that which domain preferentially forms due to the light irradiation. It is necessary to study further in the future.

The decrease of the domain density in the region adjacent to the irradiated region as shown in Fig. 4 supports the condensation mechanism: The spatial distribution of the domain density corresponds to the PSM concentration and this PSM distribution is considered to form via the re-distribution of the small and large molecules between the irradiated region and non-irradiated region.

5. SUMMARY

We tried to apply the active patterning technique by using the light irradiation to the raft domain formation in the SLB of the PSM-DOPC-cholesterol ternary system. The preferential raft domain formation due to the light irradiation could be observed. However, this condensation did not occur when the gel-phase domain formation was preferentially enhanced. It is because both the gel-phase domain and the raft domain are the PSM-rich phase. Thus, it is possible to enhance the local formation of the raft domains by avoiding the formation of the gel phase domains. The present result implies the possibility of the active patterning of the raft domains.

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