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Effect of Nanogap Structure on Dynamics of Supported Lipid Bilayer

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1. Introduction

Cell membranes play an important role in the activity of bio-molecules, for example, as a natural host for transmembrane proteins. A lipid bilayer, which is a fundamental component of a cell membrane, can be fabricated artificially on a hydrophilic surface and it retains fluidity based on lateral diffusion. Recently such supported membranes have attracted a lot of attention in the field of model cell membranes and biosensor applications.

Supported lipid bilayers are typically prepared by vesicle fusion or the Langmuir-Blodgett method. Various important properties of lipid bilayers have been revealed using these methods. In addition to these studies, some reports have been undertaken related to a self-spreading method, which results from the spontaneous growing characteristic of lipid bilayers [1-4]. This method is especially suitable for investigating the dynamic properties of supported lipid bilayers in contrast to the conventional techniques. We have used this method to demonstrate some interesting features of supported lipid bilayers, including the observation of fluorescence resonance energy transfer (FRET) [5-7].

In this study, we investigated the way in which a single sub-100-nm scale nanogap affects the dynamics of lipid bilayers. For this purpose, we fabricated patterned surfaces with a nanogap in a microchannel. Using these devices, we observed the self-spreading behavior of a lipid bilayer passing through a nanogap. We also investigated the molecular transport using the lipid bilayer as a molecule carrier under various conditions.

2. Experimental

Figure 1(a) shows a schematic view of the device structure used in this study. A nanogap structure with a separation of 10-200 nm was fabricated by electron beam lithography and the liftoff technique using Au/Ti (30 nm/5 nm) on a silicon wafer with a thermally oxidized SiO_2 layer. Microchannels that were 10 μm wide and that had wells at both ends were fabricated on this nanogap structure using an organic photoresist. The devices were treated at 200°C to harden the photoresist.

We prepared a mixture of L-α-phosphatidylcholine (extracted from egg yolk) containing 1-5 mol% of dye-conjugated lipid [7]. The dye-conjugated lipids used were Texas Red-DHPE, fluorescein-DHPE and NBD-DHPE as shown in Fig. 2. A small amount of the solid was attached to the tip of a glass capillary and transferred inside the well. The self-spreading of the lipid bilayer was initiated by immersing the device in a buffer solution (100 mM NaCl + 10 mM Tris-HCl (pH = 7.6)). Fluorescence from the lipid bilayer labeled with dye-conjugated lipid was observed using an Olympus BX51-FV300 confocal laser scanning microscope. All the observations were performed in a buffer solution at room temperature.

3. Results and Discussion

Figure 3 shows the typical time evolution of a self-spreading lipid bilayer before and after passage through a nanogap, where $t = t_0$ is the time at which the advancing lipid bilayer reaches the nanogap. A single lipid bilayer developed along a microchannel and successfully passed through a nanogap with a semicircular shape even when the nanogap was only 10 nm wide. We confirmed that the lipid bilayer did not develop on the photoresist or gold pattern but only on the hydrophilic SiO_2 surface.

Figure 4 shows fluorescence images and corresponding fluorescence intensity profiles obtained after a sufficient time interval for devices using 1, 3 and 5 mol% of Texas Red-DHPE with a 15 nm nanogap. Interestingly, at 5 mol%, the fluorescence intensity decreases discontinuously in the vicinity of the nanogap. The rate of this decrease is about 40%. A similar phenomenon was also observed using other dye-conjugated lipids and nanogap width. The tendency of the decrease became conspicuous when bulkier dye moieties and narrower nanogaps were used. The decrease in the fluorescence intensity depends strongly on both the dye-conjugated lipid and the nanogap width as summarized in Fig. 5. It should be mentioned that dye molecules experience interference when they pass through a nanogap even though the nanogap width is much larger than the size of the dye moiety (at most 3 nm).

On the other hand, the lipid bilayer containing 1 mol% of dye-conjugated lipid molecules exhibited a jump in fluorescence intensity in the vicinity of the nanogap (Fig. 4(c)). This behavior is almost independent of the dye-conjugated lipid and nanogap width in contrast with the result for 5 mol%. Although the origin of this phenomenon remains unclear, we assume that it results from the formation of corrals partitioned by the nanogap, where the lateral diffusion generally occurs not between neighboring corrals but within individual corrals leading to the redistribution of the dye molecules. At the nanogap position, the unidirectional flow to the self-spreading direction is dominant, and the lateral diffusion through the gap to the opposite direction is significantly restrained. Thus it is dependent strongly on the pristine fluorescence profiles, which are varied by the concentration and kinds of dye-conjugated lipids, before the self-spreading supported lipid bilayer reaches the nanogap structure. At 3 mol%, the behavior fell between that observed 1 and 5 mol%, namely there was little decrease or increase in the fluorescence

intensity (Fig. 4(b)).

4. Conclusion

We investigated the self-spreading behavior of a lipid bilayer containing dye-conjugated lipid molecules supported on patterned substrates with a single nanogap structure. We confirmed that the self-spreading lipid bilayers passed through the nanogap. We found that the fluorescence intensity varied discontinuously before and after passing through the nanogap structure depending on the dye concentration. We concluded that at high dye concentration, embedded dye molecules experience interference when they pass through a nanogap. At low dye concentration, the movement of embedded dye molecules is mainly controlled by the lateral diffusion in corrals partitioned by the nanogap, which acts as an effective barrier to lateral diffusion between the corrals leading to the redistribution of the dye molecules.

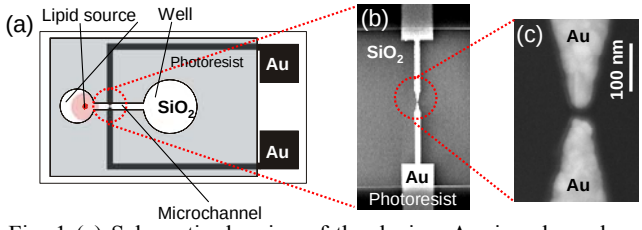

Fig. 1 (a) Schematic drawing of the device. A microchannel and wells are formed on a gold nanogap structure using a photoresist. At the beginning of the experiments, a lipid source is fixed inside the well. (b) Magnified view of the device around a nanogap. (c) SEM image of a 15 nm nanogap.

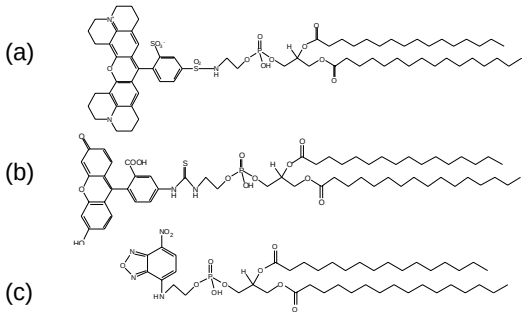

Fig. 2 Chemical structures of dye-conjugated lipids used in this study. (a) Texas Red-DHPE, (b) fluorescein-DHPE, and (c) NBD-DHPE.

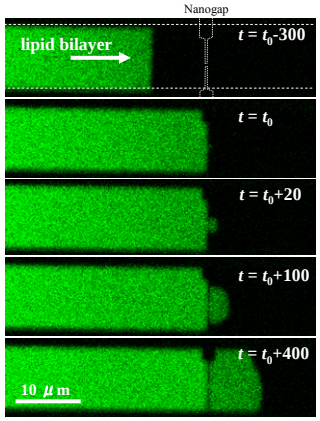

Fig. 3 Typical time evolution of a self-spreading lipid bilayer before and after passing through a nanogap. The green areas are the fluorescence from fluorescein-DHPE. The lipid bilayer grows from left to right along the microchannel. The time at which the advancing lipid bilayer reaches the nanogap is set at $t = t_0$.

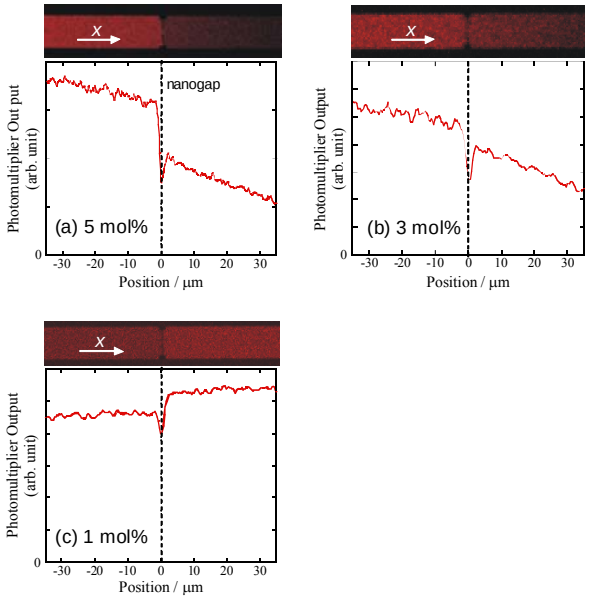

Fig. 4 Fluorescence image and fluorescence intensity profile for samples containing Texas Red-DHPE after a sufficient time interval. The position at a nanogap is set at 0. (a) 5 mol%, (b) 3 mol%, (c) 1 mol%.

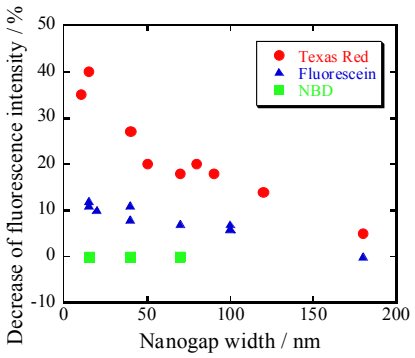

Fig. 5 Decrease in the fluorescence intensity at the nanogap as a function of the nanogap width. (●) Texas Red-DHPE, (▲) fluorescein-DHPE, (■) NBD-DHPE.

Acknowledgement

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