Electrostatic Control Mechanism of Lipid Bilayer Self-Spreading Using Nanogap as Molecule Gate

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1. Introduction
Solid supported lipid bilayers (SLBs) have been studied over recent years as a promising interface between semiconducting substrates and biomaterials such as membrane proteins [1]. One of the key technologies for the development of nano-bio devices is a method for forming reliable SLB membranes. However, SLB formation at a preferred time and place is still an issue of great interest as regards device application despite the fact that many SLB fabrication techniques have been reported including the vesicle fusion method [2] and the Langmuir-Blodgett method [3], which have sometimes been combined with lithographically patterned surfaces and an electric field.

Self-spreading, which results from the spontaneous growing nature of an SLB at a solid-liquid interface, is a characteristic feature of an SLB [4]. Using this phenomenon, we achieved spatial control of the formation of SLBs on a patterned substrate [5]. We also investigated the dynamics of an SLB passing through a single nanogap spacing (< 100 nm) [6,7]. Furthermore, in our previous work, we reported for the first time that the self-spreading of an SLB could be controlled electrostatically by the temporal switching of an electric field applied between nanogap electrodes, where we concluded that the electric double layer played a significant role in the trapping/detrapping of the SLBs [8]. In this study, we describe the mechanism of the electrostatic control of SLB self-spreading in detail, and we investigate the dependence of the ionic concentration of an electrolyte and the nanogap width under finely controlled conditions.

2. Experimental procedure
Figure 1 shows a schematic diagram of the device used in this study. A pair of gold electrodes with a separation of ≤ 200 nm was fabricated on a SiO2 surface. A 10 µm wide microchannel with wells at both ends was fabricated on this nanogap structure using an organic photoresist.

A lipid mixture (molar ratio 7:3) consisting of uncharged L-α-phosphatidylcholine (Egg-PC) and negatively charged L-α-phosphatidylglycerol (Egg-PG) containing 1 mol% Texas Red-DHPE was prepared. A small amount of the solid was attached to one of the wells. The self-spreading of an SLB was initiated by immersing the device in a 10 mM Tris-Cl (pH = 7.6) buffer solution including 1-100 mM NaCl as an electrolyte. Fluorescence from the SLB was observed with a confocal laser scanning microscope. A DC voltage was applied between nanogap electrodes during the observation. All the observations were performed in a buffer solution at room temperature.

3. Results and Discussion
Figure 2 shows typical trapping/detrapping behavior of a self-spreading SLB induced by the temporal switching of an applied voltage. A red fluorescent single SLB developed along a microchannel from the left side. Before the SLB passed through the nanogap, no voltage dependent change in the self-spreading was observed [Fig. 2(a) and (b)]. However, when the SLB reached the nanogap, the self-spreading was forcibly prevented by the voltage application (-50 mV) [Fig. 2(c)]. This continued for 720 s corresponding to the voltage application interval [Fig. 2(d)]. The SLB started to develop again after the applied voltage was returned to 0 V [Fig. 2(e)-(g)]. Figure 3 shows the mechanism of the electrostatic control of a self-spreading SLB. When the nanogap width (d) is much greater than the width of the electric double layer (D), the electric field was shielded by counterions outside the electric double layers, which induced no significant change in the self-spreading SLB. In contrast, when d ≈ D, the effect of the voltage application became different from the above. The electric field could be effectively applied over the nanogap spacing without being shielded by counterions as a result of the overlap of the two electric double layers from both electrode surfaces. In such a situation, the electric field in the nanogap becomes large (typically ~10V/cm).

Thermodynamics implies that the energetic balance of a lipid molecule between diffusion within an SLB and the electrostatic force can be expressed as 2kBT = qEdB, where kB is the Boltzmann constant, T is the absolute temperature, q is the charge of the lipid molecule, E is the maximum electric field, and dB is the amplitude of the Brownian motion within the SLB. Under our experimental conditions, where typically q = e and E ≈ 10V/cm, this gives dB ≈ 5 nm. This indicates that the lipid molecules are confined in the vicinity of the nanogap. Thus, the nanogap acts as a molecule gate, leading to the electrostatic trapping of the SLB.

To enable us to understand the above discussion more clearly, we calculated the electric potentials in the nanogap as a function of the distance from one side of the electrode surfaces using the Debye-Hückel equation as shown in Fig. 4. We found that both electric double layers overlapped when d ≤ 5D. Furthermore, we performed experiments to determine whether SLB self-spreading could be controlled at each ionic concentration of NaCl as a function of nanogap width under a constant voltage (-50 mV). Based on the results of more than 30 experiments under controlled conditions, the effect of an electric field on a self-spreading SLB was divided into two cases depending on the relationship between the nanogap width and the width of...
the electric double layer, which depends on the ionic concentration of the electrolyte. The results are summarized in Fig. 5. The circles and triangles, respectively, show cases where self-spreading could and could not be controlled by applying a voltage. This result is suggestive of a certain threshold for controlling the self-spreading. For NaCl solution, it is expressed as $D \approx 0.304\alpha/\sqrt{c}$ (mol/L), where $c$ is the ionic concentration of NaCl [9]. Therefore, the maximum nanogap width for controlling the self-spreading at a certain ionic concentration is given by $d_{\text{max}} \approx \alpha D \approx 0.304\alpha/\sqrt{c}$, where $\alpha$ is the threshold factor. According to the above equation, dotted lines with a slope of -0.5 were drawn in Fig. 5 for each $\alpha$ value and the line for $\alpha = 5.5$ provided the best fit with the experiments. This is in good agreement with the prediction obtained from the electric potential calculation in Fig. 4, where $d = 5D$ is a threshold for the control of a self-spreading SLB.

4. Conclusion

We described our proposal for the electrostatic control mechanism of SLB self-spreading, in which a nanogap acts as a molecule gate. The validity of the mechanism was confirmed in terms of the dependence of the nanogap width and the ionic concentration of an electrolyte, which is closely related to the electric double layer. The technique and concept described here can provide a new strategy for the creation of nano-bio devices and the fundamental study of nanofluidics.

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References


Fig. 1 (a) Schematic diagram of the device. (b) Magnified view of the device around a nanogap. (c) SEM image of a nanogap.

Fig. 2 Time evolution of a self-spreading SLB induced by the temporal switching of the applied voltage. A 50 nm nanogap and 10 mM NaCl solution were used. The red areas are the fluorescence from the SLB. A record of the voltage application is shown on the right. The time at which the advancing SLB reaches the nanogap is set at $t = t_0$.

Fig. 3 Mechanism of electrostatic control of a self-spreading SLB. (a) $d >> D$. (b) $d \approx D$.

Fig. 4 Calculated electric potential in the nanogap. Dotted lines show the positions at the electric double layer. (a) $d = 10D$. (b) $d = 5D$. (c) $d = 2D$.

Fig. 5 Result showing whether the self-spreading could be controlled at various ionic concentrations. The circles indicate ‘controllable’ and the triangles indicate ‘not controllable’.