

Kinetic Process of Phase Transition of a Lipid/Salt Mixture Film on a Si Substrate

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We have measured specular neutron reflectivity for a mixture film of dimyristoylphosphatidylcholine (DMPC) and NaI, spin-coated on a Si substrate. The repeat distance of bilayer stacking was drastically decreased at high temperature above chain-melting transition temperature, about 60°C, and the evaluated value was consistent with that in an “interdigitated gel” phase. Also, we performed the time-resolved measurement of neutron reflectivity, and it was shown that the Bragg peak corresponding to the “interdigitated gel” phase grows linearly with time when temperature is jumped to 120°C. This result is different from the time evolution of the transition observed in an aqueous solution systems, in which the “interdigitated gel” phase grows exponentially with time on a pressure jump.

Key words: Lipid, Phase transition, Neutron reflectivity

1. INTRODUCTION

It has been well known that phospholipid molecules, the basic constituent molecules of biomembranes, usually form multi-lamellar vesicles in water. These mimic biomembranes produced by phospholipids have been extensively studied to understand the actual behavior of real biomembranes. Such vesicles are typically obtained by hydrating a dry lipid film on a solid surface [1]. In contrast, effective methods to create uni-lamellar vesicles (ULVs) with the phospholipids have been also pursued since living cells and their organelle exist as the ULVs [2-6]. It was recently shown that the addition of sugar or salt promotes the formation of ULVs only when they are mixed with lipid films before hydration [7]. The mechanism of the ULV formation induced by additives, however, has not been clarified yet.

To understand the mechanism of the vesicle formation through hydration of lipid films, it is important to investigate the stacking structure of lipid bilayers on a substrate before hydration. Hishida *et al.* observed the structure of both the pure lipid films before hydration and the hydrated products by phase-contrast microscopy and atomic-force microscopy [8]. Their results indicated that the vesicle formation strongly depends on the initial structure of the lipid film on a substrate as well as the conformation of the hydrocarbon chains, that is, the chains are melting or not. Since generally vesicles can be hardly fused or split once they are formed, the hydration process from the lipid film could govern the vesicle formation.

We previously investigated the structure of thin lipid films with and without salt as an additive on solid substrates by neutron reflectivity measurement to clarify the mechanism of the ULV formation induced by additives [9]. This result showed that the added salt is preferentially intercalated between the stack of lipid bilayers, and supported our SAXS results that osmotic pressure due to the additive drives neighboring bilayers apart, and the isolated bilayer forms ULV [10]. On the other hand, temperature

effect is another key factor to create ULVs, since vesicles are effectively formed only above the chain-melting temperature [8]. It was shown that the addition of NaI induced an “interdigitated gel” phase above the chain-melting transition temperature [11]. Although this phase transition was often observed in the aqueous solutions with alcohol or at high pressure [12,13], the mechanism of the structural formation at the phase transition to the “interdigitated gel” phase has not been clarified well. Therefore, the difference of the transition between the aqueous solution and the dry lipid film is interesting to understand the mechanism. In this study, we investigated the kinetic process of the phase transition to the “interdigitated gel” phase by neutron reflectivity.

2. EXPERIMENTAL

Dimyristoylphosphatidylcholine (DMPC), whose hydrocarbon chains were fully deuterated, and sodium iodide (NaI) were purchased from Avanti polar lipids Inc. and Sigma-Aldrich Corp., respectively. NaI was chosen as an additive because it is easily soluble in the organic solvent used for spin-coating the lipid films. These ingredients were used as purchased without further purification. The DMPC was dissolved in a 1:2(v/v) methanol/chloroform mixture at the DMPC concentration of 5 mg/ml. For the lipid film with additive, NaI was dissolved in methanol at the equi-molar fraction to the DMPC before mixed with chloroform. Si wafers with 3 inch diameter and 3 mm thickness were cleaned by UV/ozone cleaning system. The solution of the mixture of lipid and NaI was spin-coated on the cleaned surfaces of Si wafers at 3000 rpm. The obtained films were dried in a vacuum at room temperature overnight.

The stacking structure of lipid bilayers on Si substrates was observed by neutron reflectivity measurement using the SPEAR reflectometer at the pulsed-neutron source of LANSCE, Los Alamos National Laboratory, USA [14]. The reflectivity profiles at 30°C were taken at different θ_s , 0.40 and 2.60 degrees, with the incident neutrons of 1.5 -

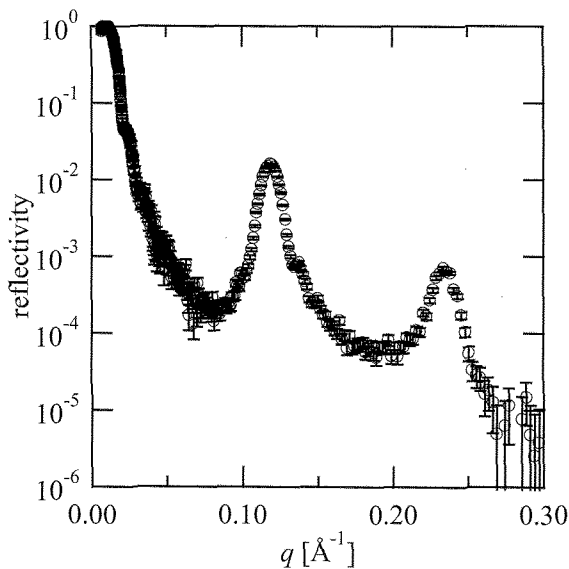


Fig. 1: Reflectivity profile of a dry DMPC/NaI film at 30°C.

16 Å wavelength, and combined into one specular reflectivity profile in the range of $0.007 < q < 0.3 \text{ \AA}^{-1}$. An evacuable cell was used for the neutron reflectivity measurement, since the experiments were performed in a vacuum to avoid the effect of humidity. On the other hand, the time-resolved measurement was performed keeping θ 2.60 degrees at 120°C, which is well above the chain-melting transition temperature of DMPC, about 60°C, when water content around the lipid molecules is low enough [15]. In this experiment, temperature was jumped up to 120°C, and the data were taken every 15 minutes.

3. RESULTS AND DISCUSSION

Figure 1 shows a reflectivity profile for the DMPC/NaI film at 30°C in a vacuum, where the two distinct Bragg peaks appeared with the Kiessig fringes. According to our previous study [11], the Bragg peaks at $q \sim 0.12$ and 0.235 \AA^{-1} are attributed to the bilayer stacking oriented preferentially parallel to the substrate surface, and the ambiguousness of the fringes to the relatively rough surface of the lipid film.

When temperature was jumped from 30°C to 120°C, these Bragg peaks gradually disappeared while a new Bragg peak grew at $q \sim 0.165 \text{ \AA}^{-1}$ as shown in Fig. 2. It indicates that the two phases with different repeat distance of the bilayers co-exist in the process of the phase transition. The drastic decrease of the repeat distance is consistent with our previous result on the phase transition to the “interdigitated gel” phase at high temperature [11]. It took very long time (over two days!) to finish the evolution of the profiles in case that annealing temperature was kept at 120°C.

To evaluate the time dependence of the structural parameters on the transition, the fitting of the reflectivity profiles was performed in the range of $0.10 < q < 0.18 \text{ \AA}^{-1}$. Since the structure factor of a lamellar phase can be approximated to the Lorentzian [16], the following equation was used for the fitting:

$$R(q) = \frac{R_1^0}{(q - q_1^0)^2 \xi_1^2 + 1} + \frac{R_2^0}{(q - q_2^0)^2 \xi_2^2 + 1},$$

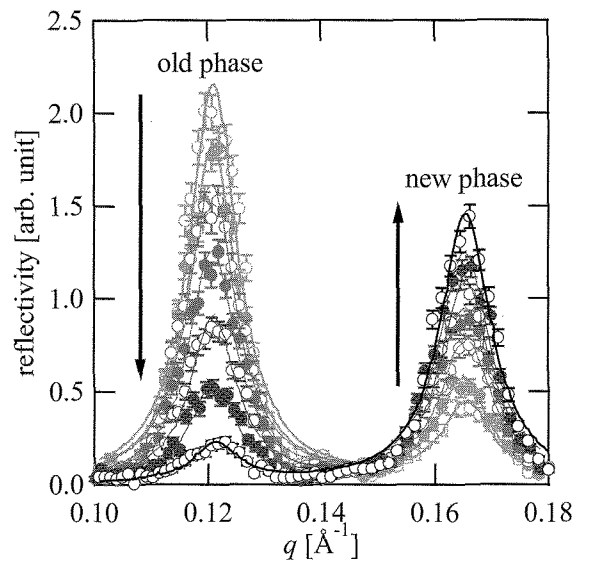


Fig. 2: The time evolution of reflectivity profiles after a temperature jump to 120°C. The symbols and the solid lines are the measured data and the fitting results, respectively. (Not all the measured data were shown here for clarity.)

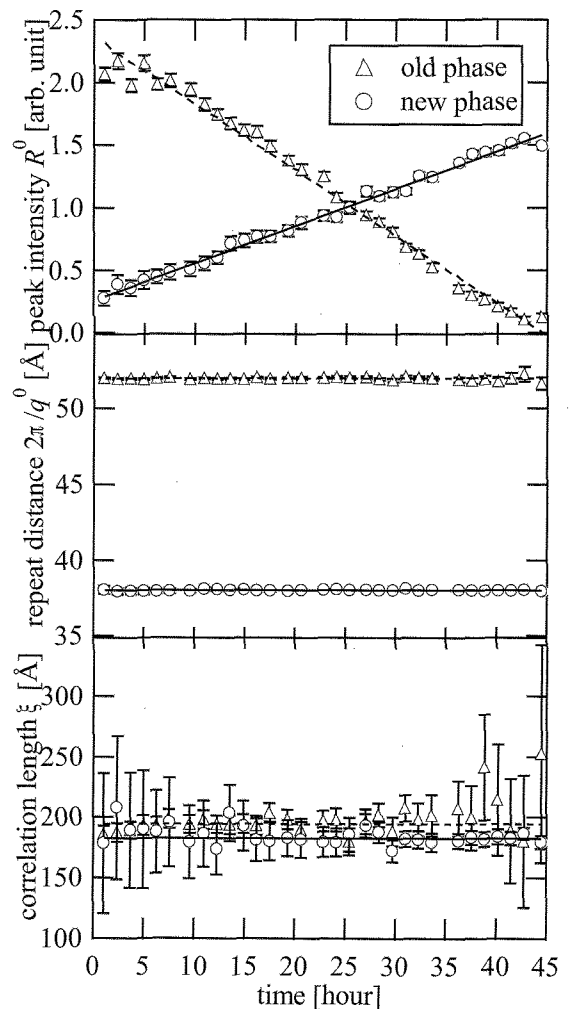


Fig. 3: The dependence of the structural parameters on annealing time after the temperature jump.

where R^0 , q^0 , ξ are a peak intensity, a peak position, and a correlation length of lamellar stacking structure, and the subscripts 1 and 2 indicate old and new phases, respectively. Figure 3 shows the time dependence of the obtained structural parameters. First, the intensity of the Bragg peak linearly depends on annealing time. Generally, the peak intensity is related with an occupied area of the corresponding phase, the number and the degree of ordering of bilayer stacking. Next, the repeat distance of each phase did not change for all the time: 52.0 and 38.0 Å for the old and new phases, respectively. Furthermore, the correlation lengths were almost constant, about 190 Å, for both the phases during the transition as the repeat distance. They indicate that the number and the degree of ordering of the bilayer stacking do not change during the phase transition. Therefore, the time dependence of the peak intensity directly reflects the occupied area of the “interdigitated gel” phase in the thin film. In other words, the domain of the “interdigitated gel” phase for the thin lipid film system would grow two-dimensionally as shown in Fig. 4. This is different from the phase transition in the aqueous solution, in which the “interdigitated gel” phase grows exponentially [12]. To understand the difference between them, a further study about the chain packing of the lipid would be required since it may be drastically changed by the transition to the “interdigitated gel” phase.

4. CONCLUSION

In this study, we performed neutron reflectivity measurement in a DMPC/NaI mixture film. The observed structure of the mixture film was consistent with our previous studies. To investigate the phase transition to an “interdigitated gel” phase, the time evolution of the reflectivity profile was also observed after temperature was jumped to 120°C. From the time dependence of the structural parameters, it was clarified that the occupied area of the “interdigitated gel” phase grew linearly with time in thin film. This result is different from the transition in the aqueous solution system.

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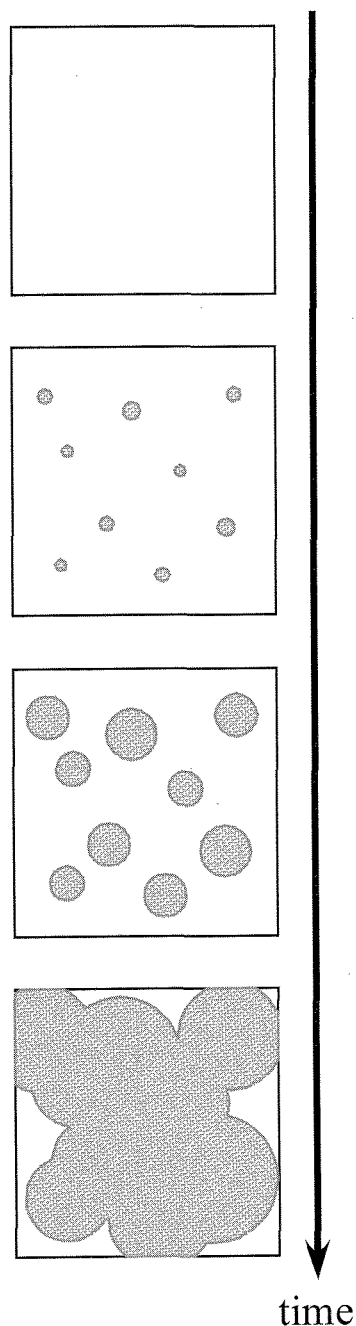


Fig. 4: A schematic illustration of domain growth of an “interdigitated gel” phase in the thin film of DMPC/NaI with time after a temperature jump. The shaded areas indicate the domains of the “interdigitated gel” phase.

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