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Short Communication

Growth of Molecular Superlattice in Fully Hydrated Dipalmitoylphosphatidylcholine during Subgel Phase Formation Process

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Abstract. — Recently, it has been reported that the structure of the subgel phase of dipalmitoylphosphatidylcholine (DPPC) is characterized by two lattices, i.e., a hydrocarbon lattice and a molecular superlattice [1]. On the basis of the results, the domain growth of the molecular superlattice in fully hydrated DPPC multilamellar vesicles at 2.5 °C was studied by means of X-ray diffraction. The change of the intensities of the (11) diffraction peak originated from the molecular superlattice was analyzed by the Kolmogorov-Avrami theory. We found that the effective dimensionality of the growing domains is 2.3±0.4. This result indicates that the domains of the molecular superlattice grow two-dimensionally during the subgel phase formation process, i.e., the domain grows in a single bilayer and is independent between the domains in adjacent bilayers.

1. Introduction

Phospholipid molecules in water form bilayers that are the basic structures of biomembranes. Therefore, the study of phospholipids is one of main subject in biological science. In addition, from the viewpoint of physical science, the phospholipid-water systems are of interest because of their two-dimensional nature [1,2] and the other various novel features [3–6].

In various phospholipid molecules, dipalmitoylphosphatidylcholine (DPPC) has been extensively studied. It is well-known that a DPPC-water system exhibits polymorphic phase behavior depending on temperature or water content [7–12]. In a variety of phases for fully hydrated DPPC vesicles, the subgel phase was discovered in 1980 by Chen et al. [7]. The subgel phase has lower enthalpy [7,9], lower volume [10], and smaller number of water molecules per lipid [11,12] in comparison with the other phases such as the gel and the liquid-crystalline phases. In addition, the DPPC multilamellar vesicle in the subgel phase gives rise to more X-ray diffraction peaks than those in the gel phase [11–17]. One of the most remarkable feature of the subgel phase is that the formation of the phase requires to incubate at ~0 °C for more than several days [7,11–16].

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Owing to the slow transformation, the structural change during the subgel phase formation of DPPC is able to be detected by X-ray diffraction technique that is generally poor in the study of rapid changing phenomena. In fact, many time-dependent X-ray diffraction studies on the subgel phase formation have been carried out for DPPC multilamellar systems [11-15]. However, the detailed structure of the subgel phase had been unclear until recent. Lately, from the X-ray diffraction study on oriented multilamellar sample [1,17], it has been revealed that there are two commensurate lattices in the subgel phase DPPC multilamellar system. One is a two-dimensional molecular superlattice \((a_s = 0.94, b_s = 0.100 \text{ nm}, \gamma_s = 90^\circ)\) and the other is a hydrocarbon chain lattice \((a = 0.53, b = 0.88 \text{ nm}, \gamma = 94^\circ)\) [1,17]. The former lattice is associated with the arrangement of the headgroups. The unit cell of the molecular superlattice has twice the area of that of the hydrocarbon lattice.

Therefore, it is of interest to reexamine the subgel phase formation by means of X-ray diffraction, based upon the new revealed structure [1,17]. In previous X-ray diffraction studies for the subgel phase formation [11-15], only kinetics for change of the lamellar and the hydrocarbon chain lattice spacings has been investigated. In the present study, the growth of the molecular superlattice in the subgel phase formation process in the DPPC multilamellar system was studied, using X-ray diffraction technique. The X-ray diffraction intensity caused from the molecular superlattice were analyzed with a domain growth theory called the Kolmogorov-Avrami theory [18,19]. We will discuss the dimensionality in domain growing during the transformation from the gel phase to the subgel phase.

2. Experimental Methods

L-\(\alpha\)-dipalmitoylphosphatidylcholine (DPPC) used in this study was obtained as a chloroform solution from Avanti Polar Lipids Inc. (Alabaster, Al, USA). The purity of this sample was examined by thin layer chromatography (TLC). A single spot on the silica gel TLC plate was found. Therefore, the sample was used without further purification. By exposing the chloroform solution of DPPC to a stream dry nitrogen, the solvent was evaporated, and then the sample was stored over 16 hours in vacuum to remove the remaining traces of solvent. Multilamellar vesicles were prepared by adding about 20 mg of dried DPPC to about 30 mg of pure water that was prepared with a water purification system (Milli-Q, Millipore, MA, USA). The DPPC and water were mixed by stirring. The mixture was incubated at 60 °C for 3 hours, and then was stirred again. The sample was sealed in an aluminum cell with a window of polyimide film. The thickness of the sample cell was 1.5 mm.

X-ray diffraction data were obtained by using a double mirror focusing camera with nickel filtered CuK\(\alpha\) radiation from a rotating anode RU200BEH X-ray generator (Rigaku, Tokyo, Japan) with a power of 1.2 kW. The sample cell was set to a brass hollow holder. Temperature of the sample was controlled within \(±0.1 \text{ °C}\) by circulating water to the sample holder from a temperature-controlled water bath (B. Braun, Melsungen, Germany). The temperature of the sample was monitored with a chromel-alumel thermocouple set near the sample. The sample to detector distance was 225.5 mm. Each exposure time was 4 hours. X-ray diffraction patterns were recorded on imaging plates of 20×25 cm\(^2\) size (BAS-III, Fuji Photo Film Co. Ltd., Tokyo, Japan). Data sampling on imaging plates were performed on a BAS2000 system (Fuji Photo Film Co., Ltd., Tokyo, Japan).

3. Experimental Results and Discussion

Figures 1a and 1b show the diffraction profiles of the DPPC multilamellar vesicles in the gel phase recorded at 20 °C and in the subgel phase at 2.5 °C, respectively. In these figures,
the horizontal axis represents the reciprocal space ($S$). It is defined as $S = 2 \sin \theta / \lambda$ ($2\theta$ = scattering angle, $\lambda$ = X-ray wavelength). The lamellar repeat spacing of the subgel phase (5.93 nm) was smaller than that of the gel phase (6.32 nm). These values agree with the previously reported values [1,11-17]. Several diffraction peaks originated from the molecular superlattice were observed for the subgel phase in the range of $S > 1.0$ nm$^{-1}$. The spacings that are assumed to be the (01), (10), and (11) diffraction peaks were 1.010 nm, 0.946 nm, and 0.685 nm, respectively. These spacings are consistent with the previously reported values [1,17].

Paying attention to these diffraction peaks which originated from the molecular superlattice, we investigated the time course of the change of X-ray diffraction profiles after rapid cooling from 20 °C to 2.5 °C. Figure 2 presents the series of X-ray diffraction profiles in the range of $S = 0.8 - 2.0$ nm$^{-1}$ at various times after cooling. It can be seen that the intensities of the diffraction peaks reflecting the molecular superlattice increase with the incubation times.

We analyzed this intensity change by the Kolmogorov-Avrami theory [18,19]. This theory indicates that the phase transformation begins with nucleation distributing randomly and proceeds by domain growth. The fraction at the new phase, $f$, is given by

$$f = 1 - \exp \left( - \left( \frac{t}{\tau} \right)^n \right), \quad (1)$$

where $t$ is time, $\tau$ is the relaxation time, and $n$ is the effective dimensionality in growing the domain. If the domain grows spherically, $n$ is 3. In the analysis, we followed the peak intensity...
Fig. 2. — Middle angle diffraction profiles ($S = 0.8 - 2.0 \text{ nm}^{-1}$) for DPPC multilamellar vesicles at 2.5 °C after cooling. The numerals displayed at the right side of each profile indicate the time in days after cooling. In figure, "5/d", "7/d" and "9/d" indicate the 5th, 7th and 9th orders of lamellar diffraction, respectively.

of the (11) diffraction of the molecular superlattice, because it is difficult to distinguish the intensities of the (01) and (10) diffraction peaks (1.01 nm and 0.946 nm, respectively) from those of the 6th order lamellar diffraction peak (0.988 nm) owing to small differences in the spacings among these diffraction peaks. The change of peak intensity of the (11) diffraction peak originating from the molecular superlattice is displayed as a function of time in Figure 3 and the fitting curve to equation (1) is also shown. From this analysis, we obtained $\tau$ and $n$ to be $2.4 \pm 0.3$ days and $2.3 \pm 0.4$, respectively. This result indicates that the domains of the molecular superlattice grow two-dimensionally during the subgel phase formation process, i.e., there is no coupling between interbilayer domains.

However, a different result for the same phenomenon has been reported by Yang and Nagle [3]. In their dilatometric study on kinetic process of the gel phase to the subgel phase of DPPC, a smaller effective dimensionality (1.0 ~ 1.3) has been obtained from the analysis by the same Kolmogorov-Avrami theory.

In order to make clear this discrepancy, let us consider what is measured by X-ray diffraction and dilatometry. In the dilatometric studies, the volume of the bilayers is obtained by subtracting the volume of the water layer from that of whole system. This procedure is based on the assumption that the density of the water layer between adjoining phospholipid bilayers is equal to that of bulk water. There is no evidence supporting this fact. A recent X-ray diffraction study [6] has rather suggested that the density of water is altered when water molecules are incorporated into the inverted hexagonal phase a phospholipid-water system from the bulk water. In the dilatometric measurements on the DPPC-water system, strictly speaking, the
measurable quantity is not the volume of a DPPC bilayer but that of a DPPC-water system including the water layer between adjoining DPPC bilayers. In contrast, in the X-ray diffraction measurements, we can get directly evidence for the state in the DPPC bilayers independently of the water layers.

It has been reported that the hydration in the subgel phase is lower than that in the gel phase [11, 12]. Consequently, water molecules diffuse through the lipid bilayer during a dehydration process associated with the subgel phase formation. Therefore, the kinetics of the dehydration process may be measured in the dilatometric study, because the measurable quantity is the total volume of the DPPC-water system including the water layers in dilatometric studies. We concluded that the discrepancy between the dilatometric study and the present study is due to the difference of the measurable subjects. The small value ($1.0 \sim 1.3$) [3] for the effective dimensionality might be due to the fact that the water permeation through the lipid bilayers accompanied by the dehydration, i.e., one-dimensional diffusion of water molecules perpendicularly to the lipid bilayer plane in the subgel phase formation.

In summary, it is concluded that the domain of the molecular superlattice grows two-dimensionally in each bilayer, i.e., an in-plane domain growth occurs during the subgel phase formation process. Recently, using calorimetry, the similar result has been reported by Ye et al. [20] at the main transition (the transition from the ripple to liquid-crystalline phases) of the fully hydrated DPPC vesicles. They have concluded that the kinetics of the main transition are represented as a single relaxation process of dimensionality of $\sim 2$ by applying the Kolmogorov-Avrami theory.

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