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Effects of poly(L-lysine) on the structural and thermotropic properties of dipalmitoylphosphatidylglycerol bilayers

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The effects of poly(L-lysine) on the structural and thermotropic properties of dipalmitoylphosphatidylglycerol (DPPG) bilayers were studied with differential scanning calorimetry (DSC), X-ray diffraction and freeze-fracture electron microscopy. For thermal behavior, in the DPPG/poly(L-lysine) system the main transition temperature rises to 45.7°C and the pretransition disappears in opposition to pure DPPG vesicles. An additional transition appears approximately at 36°C for the DPPG/poly-(L-lysine) system after incubation at 4°C for two months. The incubated sample gives a X-ray diffraction pattern having several additional reflections in the range of 0.2–0.9 nm at 15°C. These results suggest that even in the presence of poly(L-lysine) the DPPG bilayers form the subgel (L_c) phase after the long incubation at a low temperature. The X-ray diffraction measurements indicate that the structure of the L_c phase for DPPG/poly(L-lysine) system is different from that of pure DPPG bilayers. On the other hand, in the gel ($L_{\beta'}$) phase, the wide-angle X-ray diffraction pattern suggests that the presence of poly(L-lysine) hardly affects the packing of hydrocarbon chains in the DPPG bilayers. The small-angle X-ray diffraction and freeze-fracture electron microscopy exhibit that the DPPG/poly(L-lysine) system forms a tightly packed multilamellar structure in which the poly(L-lysine) is intercalated between the subsequent DPPG bilayers.

Introduction

Binding of proteins to a biomembrane surface alters the physical properties of the membrane lipids and influences the functional properties of the membrane itself. In studies on the interaction between water-soluble proteins and biomembranes, poly(L-lysine) has frequently been used as a typical model for basic proteins, because the poly(L-lysine) binds electrostatically to the surface of negatively-charged lipid bilayers. Thus many researchers have investigated the interaction between poly(L-lysine) and an acidic lipid bilayer with various techniques.

Using circular dichroism [1–3] and Raman spectroscopy [4–6], ordered conformations, that is, α-helix and β-sheet, of poly(L-lysine) were reported on the surface of negatively-charged lipid bilayers. Poly-(L-lysine) adopts a β-sheet conformation when it binds to phosphatidic acid bilayers [2,5,6]. However, phosphatidylserine vesicles induce α-helix conformation of poly(L-lysine) [1]. On the other hand, α-helix and β-sheet structures of poly(L-lysine) coexists on the surface of dimyristoylphosphatidylglycerol bilayers [3]. These are dependent on the degree of polymerization of poly(L-lysine) [5,6].

The interaction between poly(L-lysine) and negatively charged phospholipids affects the phase transition behavior of the lipid bilayers. Generally the addition of poly(L-lysine) to acidic lipid bilayers raises the main transition temperature [4–8]. The effect of poly(L-lysine) on the phase transition behavior of dipalmitoylphosphatidylglycerol (DPPG) has been investigated by Carrier et al. [8], using fluorescence polarization technique. According to their study, the thermotropic properties of DPPG/poly(L-lysine) system also depend on the degree of polymerization of poly-(L-lysine).

A negatively-charged lipid, DPPG, is known to form subgel (L_c) phase after a long incubation at a low

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Abbreviations: DPPG, dipalmitoylphosphatidylglycerol; DSC, differential scanning calorimetry; L_α phase, liquid-crystalline phase; $L_{\beta'}$ phase, gel phase; L_c phase, subgel phase.

temperature (near 0°C) [9,10]. In contrast with dipalmitoylphosphatidylcholine vesicles in the L_c phase, the small-angle lamellar spacing of DPPG vesicles in the L_c phase is influenced by water content, but the spacings and relative intensities of the wide-angle refections are not altered by water content [9]. This result suggests that the multilamellar vesicles of DPPG do not exhibit a three-dimensional lattice but the DPPG molecules are crystallized in each bilayer without the correlation between the neighboring bilayers [9]. In addition to the above experiments, the two-dimensional iattice for each bilayer of DPPG in the L_c phase was analyzed from X-ray diffraction for the oriented sample [10]. There are several studies for pure DPPG in the L_c phase, however, in phospholipid-polypeptide systems, the existence of the subgel (L_c) phase has not been reported as far as we know. In this study, the effects of poly(L-lysine) on the thermotropic and structural properties of DPPG bilayers are investigated by differential scanning calorimetry (DSC), X-ray diffraction and freeze-fracture electron microscopy. In particular, we focus on the subtransition or $L_c$phase of DPPG/poly(L-lysine) system. Furthermore, we investigate the structure of each phase in DPPG/poly-(L-lysine) system by X-ray diffraction. It is confirmed that the DPPG bilayers undergo the phase transition into the L_c phase under the presence of poly(L-lysine) after incubating at a low temperature for a long time. In the former study [11], the structure of dipalmitoylphosphatidic acid bilayer in the presence of poly(L-lysine) has been studied: dipalmitoylphosphatidic acid/poly(L-lysine) complex forms a highly ordered multilamellar structure and on the surface of the bilayers the poly(L-lysine) molecules adopt a β-sheet conformation [3]. On the other hand, on the surface of dimyristoylphosphatidylglycerol bilayer the poly(L-lysine) molecules adopt not only β-sheet but also α-helix conformation and therefore, DPPG/poly(L-lysine) system is expected to offer evidence for much generalized characteristic of their interaction.

Materials and Methods

Materials

An ammonium salt of dipalmitoyl-L-α-phosphatidyl-DL-glycerol (DPPG) and a hydrobromide salt of poly(L-lysine) (15000–30000 mol.wt.) were purchased from Sigma (St. Louis, MO, USA), and they were used without further purification. Acids, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes) and ethylenediaminetetraacetic acid (EDTA), were purchased from Katayama (Osaka, Japan).

Sample preparation

Samples were prepared as follows. A chloroform solution of DPPG was kept into a small test tube. The solvent was evaporated first under a nitrogen stream and then, under reduced pressure overnight. The lipid was dispersed into 50 mM Hepes buffer (pH 7.3) containing 1 mM EDTA and treated in a bath-type sonicator (Sine Sonic 100, Kokusai Denki, Tokyo, Japan), at 70°C for 30 min. After that, DPPG/poly(L-lysine) vesicles were formed by mixing the DPPG dispersion and poly(L-lysine) solution in 50 mM Hepes/1 mM EDTA buffer (pH 7.3) with a vortex mixer. These were incubated at 80°C for 10 min and cooled down to room temperature. Such cycles of heating and cooling were repeated at least three times to get a homogeneous sample. When the mixture of the DPPG dispersion and poly(L-lysine) solution without the above heat cycle was performed DSC measurement, the DSC thermogram at 2nd scan differed from that at 1st scan but after 3rd scan, all DSC thermogram gave the same curve. Thus, a homogeneous sample could be obtained by repeating cycles of heating and cooling through the main transition temperature for DPPG/poly(L-lysine) system. A ratio (R) of lysine residues/ DPPG molecules was initially set to be 2 for the sample used in this study so as include an excess of poly(L-lysine) and incubated it further. The reason why this ratio was selected that DPPG/poly(L-lysine) systems of $R < 1$ undergo multi-step complex phase transitions, however, for the systems of $R \geqq 2$, only one simple transition takes place [8]. The lipid concentration was 3.8 wt% for DSC measurements and 1 to 10 wt% for freeze-fracture electron microscopy. For X-ray diffraction measurements, in order to obtain sufficient intensities, the sample was concentrated with evaporating water; the lipid concentration was 20–30 wt%.

Differential scanning calorimetry

Differential scanning calorimetric measurements were performed with a calorimeter DSC10 with SSC580 (SEIKO I&E, Tokyo, Japan), at a heating scan rate of 1.0 K/min. The sample dispersion of 15 μl was put into a sample pan (P/N 50–023P, SEIKO I&E, Tokyo, Japan), and then the pan were sealed with a cover (P/N 50-023C, SEIKO I&E, Tokyo, Japan) hermetically. Transition enthalpies were evaluated by integrating the area under the transition curve, where the scale of DSC thermogram was calibrated with a standard sample of gallium.

Freeze-fracture electron microscopy

The freeze-fracture electron microscopy is a useful method for observing the microscopic structure of lipid membranes. The sample prepared by the above procedure was sucked into a glass capillary whose temperature was kept within the accuracy of ± 0.2 K by circulating temperature-controlled water. A part of the sample dispersion (3–5 μl) was transfered from the capillary onto a small copper block in a temperature con-

trolled chamber and incubated for about 1 min before being quenched into Freon 22 slush. The frozen sample was fixed on a sample holder and cut in a FD-3 freeze-fracture apparatus (Eiko Engineering, Mito, Japan). After shadowing with Pt-C from angle 40°, carbon was further evaporated from the top to reinforce the replica film. The replica film was washed in hypochlorous acid solution and furthermore in water before putting on a 400 mesh for electron microscopy. The sample was observed with a 100C electron microscope (JEOL, Tokyo, Japan).

X-ray diffraction

X-ray diffraction measurements were carried out at station 15A of the Photon Factory in the National Laboratory for High Energy Physics (Tsukuba, Japan). A monochromatic (0.1504 nm) and horizontally focused X-ray beam was used for the experiments. The optical system was described in Ref. 12 in detail. X-ray diffraction patterns were recorded with a storage phosphor detector called an imaging plate (Type BA-III, Fuji Photon Film, Japan) [13]. Exposure times were between 1 and 3 s. The sample-to-detector distance was about 200 mm, and the diffraction spacings were calibrated with a powder pattern of synthetic fluorophlogopite mica (National Bureau of Standards, Washington, DC, USA). The sample cell was composed of an aluminium plate (1.5 mm in thickness) with a rectangular hole of 4×6 mm. The window of the sample cell was sealed by a pair of polyimide films. This sample cell was set on a brass hollow holder. Temperature was kept constant by circulating water to the hollow holder with a computer-controlled water bath (RCS-20D, Messgeräte Lauda, FRG). The background caused by the scattering of water, the diffraction from polyimide film, etc. were subtracted from each diffraction pattern. Two-dimensional Debye-Scherrer patterns on the imaging plate were transformed into one-dimensional data by the method described in our previous paper [11].

Results

Thermotropic behavior of DPPG/poly(L-lysine) system

Fig. 1A shows a DSC thermogram of pure DPPG vesicles that were incubated at 4°C for two months. The endotherm with three peaks is attributed to the subtransition (25.5°C, $\Delta H = 7.4$ kcal/mol), the pretransition (35.5°C, $\Delta H = 0.4$ kcal/mol) and the main transition (41.3°C, $\Delta H = 8.3$ kcal/mol). These values are agreed with previous reported values [9,14]. On the subtransition temperature of DPPG, Wilkinson and McIntosh [9] reported about 25°C for the sample dispersed in phosphate buffer and Eklund et al. [14] also reported 25.1 ± 0.5°C in the presence of 50 mM NaCl for the incubation of 15–16 days at 6°C. When a

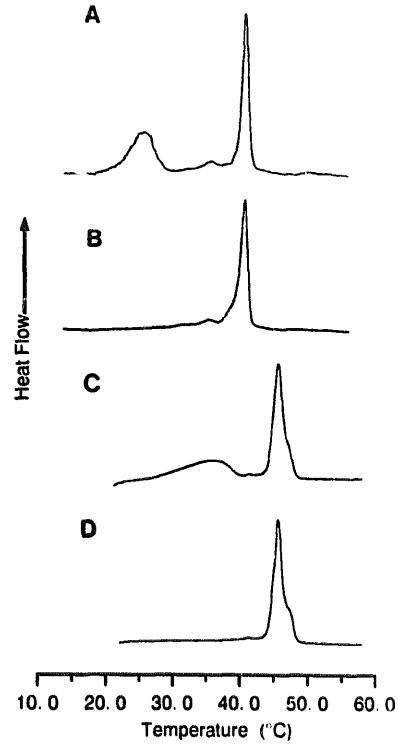

Fig. 1. DSC thermograms of pure DPPG vesicles (A, B) and DPPG/poly(L-lysine) system ($R = 2$) (C, D). (A) First scan of pure DPPG after storage at 4°C for two months; (B) second scan performed successively; (C) first scan of DPPG/poly(L-lysine) after storage at 4°C for two months; (D) second scan performed successively. In the all above measurements, heating rate was 1.0 K/min.

successive heating scan is performed immediately after the first scan, the subtransition peak disappears because the formation of the L_c phase demands a long time incubation (Fig. 1B). A DSC thermogram of DPPG/poly(L-lysine) system of $R = 2$ was obtained after incubation at 4°C for two months (Fig. 1C). The curve has two peaks; a broad peak centered approximately at 36°C with enthalpy of 3.7 kcal/mol, and a sharp peak at 45.7°C accompanied with a shoulder on the higher-temperature side in which the total enthalpy is 8.3 kcal/mol. On the second heating scan performed immediately after the above measurement, only a single transition peak is observed at 45.7°C in the thermogram (Fig. 1D). The low-temperature peak of DPPG/poly(L-lysine) systems disappears in the successive heating scan. Therefore, the nature of this transition is also of subtransition. These results suggest that the long incubation at low temperature induces the L_c phase even in the DPPG bilayers binding poly(L-lysine). As we shall see later, the formation of the L_c phase in this system is confirmed by X-ray diffraction.

Structural characteristics of DPPG/poly(L-lysine) system

Freeze-fracture electron microscopy

As a preliminary experiment, pure DPPG vesicles were observed by the freeze-fracture electron mi-

Fig. 2. Freeze-fracture electron micrograph of DPPG/poly(L-lysine) system ($R = 2$) quenched from 50°C. The bar indicates 200 nm.

croscopy. As the result, pure DPPG sample formed multilamellar vesicles, in which the distance between each lamellar was not correlated and therefore, seems to be fluttered. Sometimes small unilamellar vesicles were observed (data not shown). In contrast, the freeze-fracture electron microscopic study of DPPG/poly(L-lysine) system ($R = 2$) shows a tightly packed multilamellar structure (see Fig. 2). The period of the packed lamellar is about 6 nm. This period agrees with the lamellar spacing obtained by X-ray diffraction (see next section). Such a structure was observed in the both samples quenched from not only gel but also liquid-crystalline phase. As seem at the top of the left-hand side of Fig. 2, the multilamellar vesicle is markedly deformed. The tightly packed multilamellar structure is terminated, i.e., the multilamellar structure is not enclosed at the end. The ripple structure has never observed in DPPG/poly(L-lysine) system of $R = 2$, while the ripple structure could be found for the case of $R < 0.5$ (data not shown).

Small-angle X-ray diffraction

The small-angle X-ray diffraction patterns of DPPG/poly(L-lysine) systems are shown in Fig. 3. This DPPG/poly(L-lysine) system of $R = 2$ was incubated at 4°C for two months. The lamellar spacings of DPPG/poly(L-lysine) complexes are 5.93, 6.13 and 5.45 nm at 15°C, 40°C and 60°C, respectively. The spacing in the $L_{\beta'}$ phase observed at 40°C is consistent with the value reported by Carrier and Pézolet [4]. The peak (denoted by arrowhead in Fig. 3) observed around

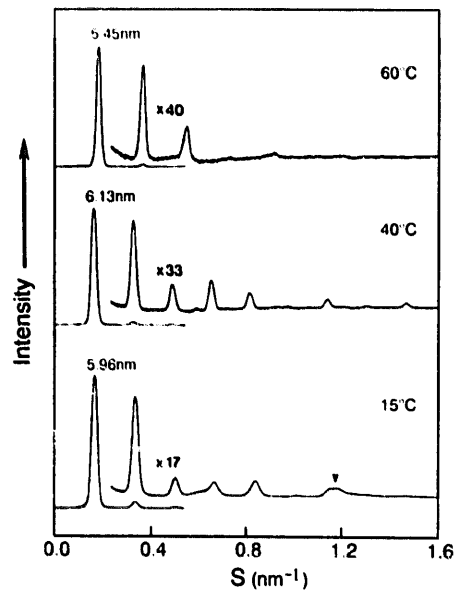

Fig. 3. Temperature-dependent behavior of the small-angle X-ray diffraction patterns of DPPG/poly(L-lysine) system ($R = 2$) after storage at 4°C for two months. The patterns above the second-order lamellar refections are inserted in an expanded scale in the ordinate. For the broad reflection around $S = 1.2$ nm^{-1} at 15°C, see Fig. 5 in detail.

$S = 1.2$ nm^{-1} is not higher order lamellar reflections but the reflections due to the characteristic lattice of the L_c phase. At each temperature, the sharp lamellar diffraction peaks appear. The higher-order lamellar refections up to the 9th order reflection were observed at 40°C. This result indicates that a highly-ordered multilamellar structure appears like that observed in dipalmitoylphosphatidic acid/poly(L-lysine) [11] or cardiolipin/poly(L-lysine) [15]. In contrast, pure DPPG vesicles only give rise to a broad scattering profile in the small-angle region (pattern not shown), which suggests either a lack of ordered lamellar spacing or a small number of layers in DPPG vesicles. This result is in agreement with the above freeze-fracture electron microscopic observation.

Wide-angle X-ray diffraction in the $L_{\beta'}$ phase

The wide-angle X-ray diffraction patterns of pure DPPG vesicles and DPPG/poly(L-lysine) systems at 30°C are shown in Figs. 4 A and B, respectively. Prior to the X-ray diffraction measurements, the samples were heated up to 60°C and then cooled down to 30°C. This procedure is necessary in order to obtain an intrinsic $L_{\beta'}$ phase and to avoid mixing of the L_c phase. There appears a reflection at 1/0.42 nm^{-1} with a broad shoulder around 1/0.41 nm^{-1} in both pure DPPG vesicles and DPPG/poly(L-lysine) system indicating that these samples are in the $L_{\beta'}$ phase at 30°C. The present result is identical with the results on DPPG bilayers in 1.5 M KCl/50 mM Tris (pH 8.0) [16]. It is also consistent with the fact that hydrocarbon chains are packed in a distorted orthorhombic lattice [17]. At 60°C both of pure DPPG vesicles and

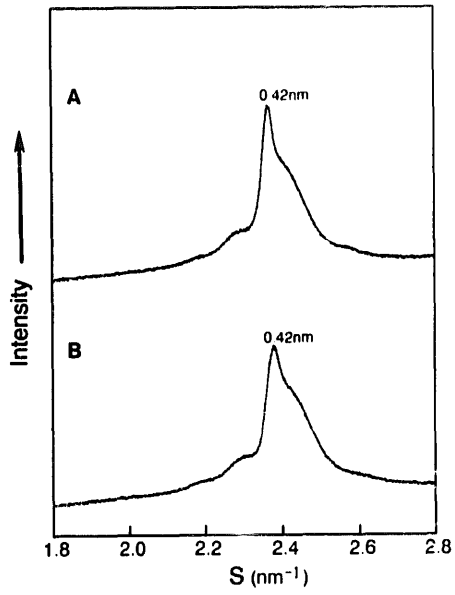

Fig. 4. Wide-angle X-ray diffraction patterns of (A) pure DPPG vesicles and (B) DPPG/poly(L-lysine) system ($R = 2$) at 30°C in the $L_{\beta'}$ phase. Both samples were heated up to 60°C before the measurements.

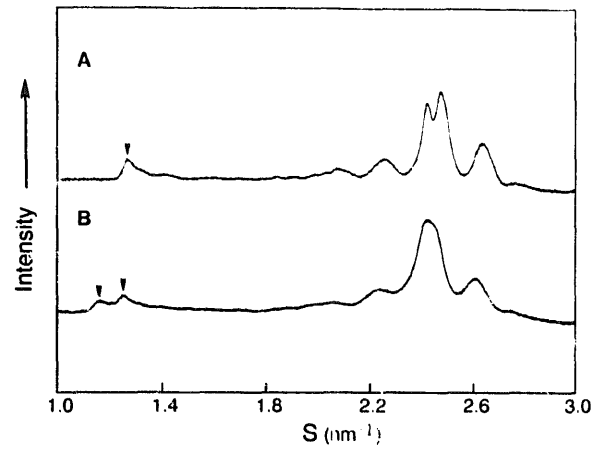

Fig. 5. Wide-angle X-ray diffraction patterns of (A) pure DPPG vesicles and (B) DPPG/poly(L-lysine) system ($R = 2$) at 15°C after storage at 4°C for two months.

DPPG/poly(L-lysine) systems are in the liquid-crystalline (L_α) phase, since the diffuse profile, which is a characteristic diffraction with melted hydrocarbon chains, was observed at 0.46 nm (data not shown). The spacings and shapes of the wide angle reflections of DPPG/poly(L-lysine) system are not significantly different from those of pure DPPG vesicles. These results indicate that the presence of poly(L-lysine) hardly affects the hydrocarbon chain packing in the DPPG bilayers in the $L_{\beta'}$ and the L_α phases. The same tendency was observed in dipalmitoylphosphatidic acid/poly(L-lysine) [11].

Wide-angle X-ray diffraction in the L_c phase

In the L_c phase, in addition to the reflections corresponding to the lamellar spacing and the spacing of hydrocarbon chain packing, several reflections emerge in the range of 1/0.9–1/0.2 nm^{-1}. Therefore, the L_c phase is more ordered than the $L_{\beta'}$ phase, namely exhibits the characteristic lattice [9,10,19,20]. The wide-angle diffraction patterns for pure DPPG vesicles and DPPG/poly(L-lysine) systems shown in Figs. 5 A and B, respectively. These were obtained at 15°C after incubation at 4°C for two months. The both patterns contain a series of additional sharp and broad reflections different from lamellar reflections. These results strongly suggest that the L_c phase is formed not only in pure DPPG vesicles but also in DPPG/poly(L-lysine) system. These detailed structures, however, are not identical with each other: The reflections were observed at 1/0.789 (denoted by arrowhead), 1/0.708, 1/0.542, 1/0.526, 1/0.505, 1/0.492, 1/0.482, 1/0.444, 1/0.413, 1/0.404, 1/0.379 and 1/0.360 nm^{-1} for pure DPPG vesicles (Fig. 5A): The reflection were observed at 1/0.856 (denoted by arrowhead), 1/0.796 (denoted by arrowhead), 1/0.509, 1/0.486, 1/0.447, 1/0.413 and 1/0.383 nm^{-1} for DPPG/poly(L-lysine) system (Fig. 5B). These spacings for pure DPPG vesicles agree

with the data reported by Wilkinson and McIntosh [9]. These spacings are different between pure DPPG vesicles and DPPG/poly(L-lysine) system. These results reveal the following facts; even under a condition of the presence of poly(L-lysine), the L_c phase is formed in the DPPG bilayers after a long incubation at a low temperature.

Discussion

The present results of DSC and X-ray diffraction studies demonstrate that the $L_{\beta'}$ phase converts to the L_c phase after a long incubating at a low temperature for not only the pure DPPG bilayers but also the DPPG bilayers with poly(L-lysine). Both of the subtransition and main transition temperatures of DPPG/poly(L-lysine) system ($R = 2$) are higher than those of pure DPPG vesicles. But the enthalpy at the main transition is not affected by the addition of poly(L-lysine). It is well-known that the main transition enthalpy is largely due to the melting of hydrocarbon chain [21]. The transition enthalpy at the main transition suggests that poly(L-lysine) hardly affects the nature of hydrocarbon chain packing of DPPG bilayers in the $L_{\beta'}$ phase: In fact, there are no difference between pure DPPG and DPPG/poly(L-lysine) on the wide-angle X-ray diffraction patterns in the both $L_{\beta'}$ and L_α phase. Therefore, the addition of poly(L-lysine) like sodium ion [22] might raise the main transition temperature in consequence of electric shielding effect. The enthaply at the subtransition for DPPG/poly(L-lysine) system is smaller than that for pure DPPG vesicles.

It may be due to the fact that the structure of the L_c phase of DPPG/poly(L-lysine) system is different from that of pure DPPG bilayers, i.e., the wide-angle X-ray diffraction pattern of DPPG/poly(L-lysine) system in the L_c phase is not exactly identical with the that of pure DPPG vesicles. The conformation of poly(L-lysine) molecule changes from random coil to ordered structures (α-helix and β-sheet) when it binds to the surface of DPPG bilayers [3,4]. Suppose poly(L-lysine) in the ordered structure has a regular distribution of the positively charged lysine residues, it causes that a regular electrical potential is formed on the surface of DPPG bilayers. This periodic potential of the positive charges will alter the lateral packing of DPPG molecules in the L_c phase because the headgroup of DPPG molecule has a net negative charge. The additional reflections observed in the range 1/0.9–1/0.2 nm^{-1} might be caused by the characteristic lattice of DPPG/poly(L-lysine) system in the L_c phase. However, within the limited information available from the Debye-Scherrer patterns, the structure of the lattice cannot be determined.

On the other hand, the wide-angle X-ray diffraction indicates that there are no difference in the packing of hydrocarbon chains on the $L_{\beta'}$ phase between pure DPPG and DPPG/poly(L-lysine) system. It might be related to the lateral diffusion of phospholipid molecule in the bilayer. The diffusion coefficient is about 10^{-10} cm^2/s for dimyristoylphosphatidycholine in the $L_{\beta'}$ phase [23]. The lateral arrangement of the DPPG molecules in the $L_{\beta'}$ phase is independent of the regular electrostatic potential of poly(L-lysine). Since the lysine residues might also exert thermal fluctuation, the one-to-one correspondence between a DPPG headgroup and a lysine residue is not expected. Poly(L-lysine) forms ordered conformation, that is, α-helix and β-sheet, on the surface of the DPPG bilayers, however, one DPPG headgroup interacts more than one lysine residues electrostatically and the two-dimensional arrangement of the lysine residues is incommensurate with that of the DPPG headgroups in the $L_{\beta'}$ phase.

In this X-ray experiment we observed sharp lamellar reflections for DPPG/poly(L-lysine) system for each phase. In order to the electrostatic repulsion force between adjacent bilayers, negatively charged lipid bilayers at low ionic strength have no correlation between neighboring bilayers, and thus lamellar refections are not observed. Therefore, generally negatively charged lipid vesicles give rise to a broad X-ray scattering pattern in the small-angle region. On the other hand, the small-angle X-ray diffraction patterns of DPPG/poly(L-lysine) system exhibit the formation of a highly ordered multilamellar structure. Also, the freeze-fracture electron microscopy results indicate that DPPG/poly(L-lysine) forms stacked multilamellar structure. One layer of a poly(L-lysine) molecule might be intercalated in between subsequent bilayers of DPPG in DPPG/poly(L-lysine) system similar to dipalmitoylphosphatidic acid/poly(L-lysine) [11] or cardiolipin/poly(L-lysine) [15].

In order to confirm the above mentioned structure, we calculated the electron density profile of DPPG/poly(L-lysine) system at 40°C in the $L_{\beta'}$ phase (Fig. 6). The phase angles were determined from a comparison between the observed data and calculated data with the Gaussian model introduced by Mitsui [24] and Wiener et al. [25]. DPPG/poly(L-lysine) system is assumed to consist of four parts; poly(L-lysine), headgroup, hydrocarbon and terminal methyl regions. In this Gaussians model, the headgroup and terminal methyl regions are represented by Gaussians. They are interconnected by a bridging function that was given by half the period of a cosine function. The amplitude of the cosine function is equal to the difference in electron densities of the poly(L-lysine) and hydrocarbon regions (see Fig. 1 in Ref. 25). In this paper, the electron densities of poly(L-lysine) and hydrocarbon regions were assumed 320 e/nm^3 and 360 e/nm^3, respectively. The latter value was estimated from that

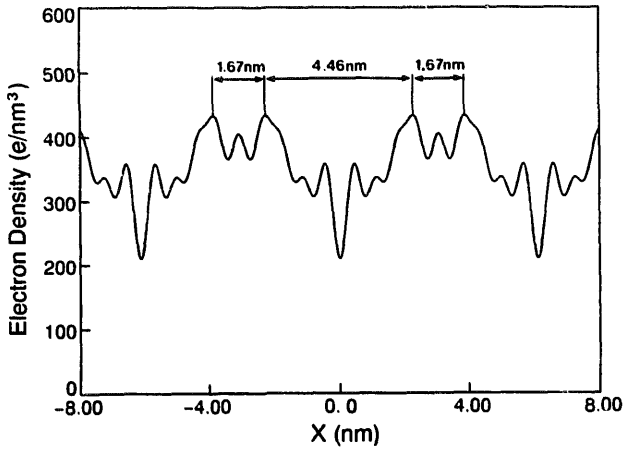

Fig. 6. An electron density profile for DPPG/poly(L-lysine) system in the $L_{\beta'}$ phase at 40°C. The headgroup separation in a bilayer is 4.46 nm. The separation between adjacent bilayers is 1.67 nm.

of dipalmitoylphosphatidylcholine in the $L_{\beta'}$ phase [25], because the hydrocarbon chain packing of DPPG/poly(L-lysine) system is similar to that of dipalmitoylphosphatidylcholine bilayers. The former value was estimated from the data of poly(L-lysine) crystal [26], by taking account of the coexistence of β-sheet and α-helix of poly(L-lysine) on DPPG bilayers [3]. This model requires five parameters. The Gaussian form of the headgroup has three parameters; position, amplitude and width, and the Gaussian from of the terminal methyl has two parameters; amplitude and width. These parameters were determined by fitting to experimental data. This analysis was performed by using the least-squares fitting program developed by Nakagawa and Oyanagi [27]. From the optimum condition for this Gausian model, the phase angle set (π,π,0,π,π,π,π, π,π) was determined. The electron density profile of Fig. 6 was calculated for this set of the phase angles. The value of R-factor obtained was 0.24. Blaurock and McIntosh [10] proposed the procedure that the phases for higher order reflection are determined so as to give the most uniform electron density distribution for the portion of hydrocarbon chains. We adopted this way for the phase determination for dipalmitoylphosphatidic acid/poly(L-lysine) system in the previous paper [13]. But, the set of the phase angles determined by the above straightforward way is not necessarily satisfactory. The reason why the authors adopted the method of the Gaussian model in this study was that the absolute electron density value could be evaluated in the Gaussian model.

The electron density profile shows that the separation between the DPPG headgroups in both surfaces of a bilayer is 4.46 nm, while the distance of headgroups between neighboring bilayers is 1.67 nm. From the size of poly(L-lysine) [26], it is obvious that only one layer of poly(L-lysine), regardless of α-helix or β-sheet conformation, can exist between the neighboring DPPG bilayers. Let us consider the tilt angle of hydrocarbon chain to the bilayer normal. Blaurock and McIntosh have estimated that the bilayer thickness for pure DPPG in $L_{\beta'}$ phase is 4.2 nm from electron density profile [10]. From CPK molecular packing models, the bilayer thickness reaches about 5 nm if the hydrocarbon chains are not tilted. From these facts Blaurock and McIntosh have concluded that the hydrocarbon chain tilt angle to be about 30° for pure DPPG in the $L_{\beta'}$ phase [10]. Watts et al. [16] also have determined the tilt angle of DPPG to be 32° at pH 8 at 20°C. On the other hand, since the result in this work the bilayer thickness is 4.46 nm for DPPG/poly(L-lysine) system, the tilt angle is estimated to be about 25°. The hydrocarbon chain tilt induces the increase of the occupied area per a headgroup and then, the decrease of the surface electrostatic energy, without changing the chain-chain spacing. Watts et al. [16] reported that the hydrocarbon chains of DPPG are not tilted at pH 1.5 at 20°C in the $L_{\beta'}$ phase. Under the condition of pH 1.5, since DPPG headgroups has no charge, the electrostatic repulsion between headgroups does not exist and as a result, the chain tilt is no longer needed. In consistent with these reasons, the decrease of tilt angle for DPPG/poly(L-lysine) system can be explained in terms of neutralizing the headgroup's charge by positive charges of poly(L-lysine). However, the headgroup's charge might be not perfectly neutralized in DPPG/poly(L-lysine) system, and therefore, the tilt angle might not be zero.

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