DIFFERENCES IN HYDROCARBON CHAIN TILT BETWEEN HYDRATED PHOSPHATIDYLETHANOLAMINE AND PHOSPHATIDYLCHOLINE BILAYERS

A Molecular Packing Model

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ABSTRACT Both wide-angle and lamellar x-ray diffraction data are interpreted in terms of a difference in hydrocarbon chain tilt between fully hydrated dipalmitoyl phosphatidylcholine (DPPC) and dipalmitoyl phosphatidylethanolamine (DPPE). Although the hydrocarbon chains of multilayers of DPPC tilt by ~ 30° relative to the normal to the plane of the bilayer, as previously reported by others, the hydrocarbon chains of DPPE appear to be oriented approximately normal to the plane of the bilayer. It is found that the chain tilt in DPPC bilayers can be reduced by either: (a) adding an *n*-alkane to the bilayer interiors or (b) adding lanthanum ions to the fluid layers between bilayers. A molecular packing model is presented which accounts for these data. According to this model, DPPC chains tilt because of the size and conformation of the PC polar head group.

INTRODUCTION

The polymorphic phase behavior of synthetic phosphatidylcholine (PC) has been extensively studied. In particular, Tardieu et al. (1) and Ranck et al. (2) have shown that for fully hydrated saturated PC, below the pretransition temperature, the hydrocarbon chains are tilted with respect to the normal to the plane of the bilayers. In oriented multilayers of hydrated dipalmitoyl phosphatidylcholine (DPPC), this chain tilt has been detected by spin-label experiments (3), as well as by x-ray diffraction techniques (4–6). Low-water content (5% water content or less) DPPC bilayers have no chain tilt (1, 2, 4, 5).

In this paper, we deal with two questions concerning this hydrocarbon chain tilt. First of all, is this chain tilt a general phenomenon associated with all hydrated gel state phospholipids? Second, what are the lipid packing parameters which cause chain tilt? To answer the first question, we have performed x-ray diffraction studies on synthetic, hydrated dipalmitoyl phosphatidylethanolamine (DPPE). Single crystals of PE have been thoroughly analyzed (7), but the orientation of the hydrocarbon chains of fully hydrated PE is not known. To investigate the second question, we have selectively introduced perturbations to different regions of the DPPC bilayer. The hydrocarbon chain region was perturbed by adding *n*-alkanes to the bilayer, whereas the head group of the lipid was modified by adding lanthanum ions to the fluid layers between bilayers. The *n*-alkanes are known to enter the hydrocarbon region of the bilayer, whereas lanthanum has been shown to bind to the

phosphate group of PC (8) and change the conformation of the phosphocholine polar head group (9).

MATERIALS AND METHODS

Saturated, synthetic dipalmitoyl phosphatidylcholine (DPPC) and dipalmitoyl phosphatidylethanolamine (DPPE) were obtained from Calbiochem-Behring Corp. (San Diego, Calif.) or Applied Science Labs, Inc. (State College, Pa.). The lipids were used without further purification, and the same lipids from different commercial sources gave identical diffraction patterns. Decane and tetradecane were obtained from Sigma Chemical Co. (St. Louis, Mo.)

Fully hydrated lipid specimens were made by adding an excess amount (>90% by weight) of triply distilled water or 0.1 M NaCl adjusted to pH 7.4 to the dry lipid. The suspensions were vortexed until thoroughly mixed, allowed to equilibrate above the lipid phase transition temperature, concentrated by a brief centrifugation which a bench centrifuge, and then sealed in quartz-glass, x-ray capillary tubes. Identical results were obtained if the lipids were mixed with 70% water, vortexed, allowed to equilibrate, and directly sealed in the capillary tubes. For experiments with tetradecane and decane in the bilayer, the particular *n*-alkane was mixed with the dry lipid in appropriate amounts before the water was added. For the experiments testing the structural effects of lanthanum, $LaCl_3$ was added to the water in appropriate concentrations under the conditions described in the NMR studies of the conformation of the PC head group (8, 9). The x-ray capillary tubes were mounted in an x-ray camera with either a pinhole collimator or a mirror-monochromator system (10). Either a rotating anode x-ray generator designed by Dr. William Longley of this laboratory (11) or a stationary anode Jarrel-Ash generator (Jarrel-Ash Div., Fisher Scientific Co., Waltham, Mass.) was used to produce copper $K\alpha$ x radiation. Diffraction patterns were recorded with a flat plate film cassette loaded with three or more sheets of Ilford Industrial G x-ray film, Ilford, Ilford, England). Specimen-to-film distances were between 4 and 12 cm, and exposure times were between 1 and 5 h. All diffraction patterns were recorded at room temperature, 20°C ± 2°C. This temperature is below both the main and pretransition temperatures of DPPC, and below the main transition temperature of DPPE (12). DPPE does not have a pretransition (12).

Diffraction data were processed by standard methods. The discrete lamellar low-angle reflections obeyed Bragg's law: $2d \sin \theta = h\lambda$, where d is the repeat period; θ is the Bragg angle; h is the diffraction order; and λ is the wavelength of the incident radiation. Densitometer traces were recorded on a Joyce, Loebl microdensitometer, model MK III C (Joyce, Loebl and Co., Gatesherd-on-Tyne, England), the background curve was subtracted, and integrated intensities $I(h)$ were measured. Electron density distributions, $\rho(x)$, were calculated by use of the formula:

$$\rho(x) \alpha \sum_h \sqrt{h^2 I(h)}\, \phi(h) \cos \frac{2\pi \times h}{d},$$

where $\phi(h)$ is the phase information for each order h. For a centrosymmetric system $\phi(h)$ must be either positive or negative for each order h. The phase information has previously been determined for hydrated DPPC (13, 14) and for low-water content multilayers of PE (15). For calculations of electron density profiles in this paper we have used the phase choices previously determined for hydrated DPPC (13, 14). For the cases of hydrated DPPE, DPPC:tetradecane, and DPPC:lanthanum we have assumed that the electron density profiles should be consistent with a bilayer structure. In support of this assumption, it is noted that the lamellar repeat periods and wide-angle diffraction from these specimens (see Results) have the defining characteristics of bilayer phases (1). For this relatively low-resolution data (15 Å) it was found that this assumption was sufficient to choose the correct phase combination uniquely. All phase combinations were systematically tested for each data set

TABLE I

STRUCTURE AMPLITUDE ($\sqrt{h^2 I[h]}$) DATA

h	DPPC (d = 65 Å)	DPPE (d = 63 Å)	DPPC:tetradecane (d = 73 Å)	DPPC:lanthanum (d = 79 Å)
1	1.00	1.00	1.00	1.00
2	0.94	0.11	0.76	1.62
3	0.53	0.13	0.47	0.25
4	0.55	0.55	0.28	~0
5	0.42	~0	0.27	0.67
6	~0	0.15	~0	~0

and only one combination gave a profile that was consistent with a bilayer structure. All other phase choices gave anomalous results, such as large positive electron density peaks in the center of the bilayer. All profiles are on relative electron density scales.

RESULTS

For all specimens, discrete lamellar reflections were recorded to $(15\ \text{Å})^{-1}$ in reciprocal space. Identical results were obtained for lipids hydrated in water or 0.1 M NaCl. Intensities and repeat periods are given in Table I. The wide-angle diffraction, caused by the lipid hydrocarbon chains, consisted in all cases of a strong reflection near 4.2 Å. However, the shape and precise spacing of this reflection depended on the composition of the specimen. Typical wide-angle patterns from hydrated DPPC, DPPE, and DPPC with added tetradecane are shown in Fig. 1. The wide-angle reflection from DPPC (Fig. 1 A) consists of a sharp

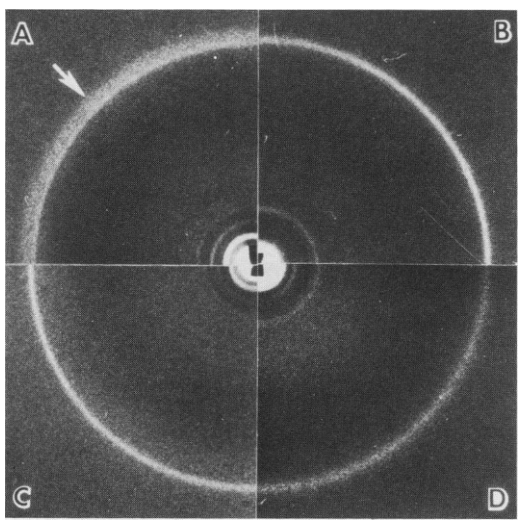

FIGURE 1 Wide-angle, x-ray diffraction patterns from fully hydrated (A) DPPC, (B) DPPE, (C) 1:1 molar ratio DPPC:tetradecane, and (D) 3:1 molar ratio DPPC:lanthanum. The wide-angle reflection for DPPC (arrow) consists of a sharp reflection at 4.24 Å, surrounded by a diffuse band. The wide-angle patterns from DPPE, DPPC:tetradecane, and DPPC:lanthanum consist of a sharp symmetrical reflection at 4.15 Å. Some of the overexposed, low-angle lamellar reflections are seen in the middle of the figure.

reflection at 4.24 ± 0.04 Å surrounded by a diffuse band centered at ~ 4.1 Å. This type of pattern was first recorded by Tardieu et al. (1) and interpreted in terms of a chain tilt of the lipid hydrocarbon chains with respect to the normal to the plane of the bilayer. However, the wide-angle reflections from both hydrated DPPE (Fig. 1 B) and DPPC:tetradecane (Fig. 1 C) consist of a sharp, symmetrical peak centered at 4.15 ± 0.4 Å. Three molar ratios of DPPC:tetradecane were used, 2:1, 1:1, and 1:3, and all gave wide-angle patterns like the one shown in Fig. 1 C. Identical wide-angle patterns were also recorded from DPPC with added decane at 1:1 and 1:3 lipid:alkane molar ratios. Tardieu et al. (1) and Brady and Fein (16) have interpreted this type of pattern in terms of hydrocarbon chains which are very nearly perpendicular to the surface of the bilayer. The differences in the spacing and shape of the wide-angle diffraction from these specimens can also be seen in densitometer traces across these reflections (Fig. 2). The symmetric wide-angle reflections from DPPE (Figs. 1 B and 2 B) and DPPC:tetradecane (Figs. 1 C and 2 C) appear to be identical. Both are quite different from the asymmetric reflection from hydrated DPPC (Figs. 1 A and 2 A).

Electron density profiles from hydrated DPPC, DPPE, and DPPC:tetradecane are shown in Fig. 3. We note that the profile of hydrated DPPC of Fig. 3 is very similar to profiles

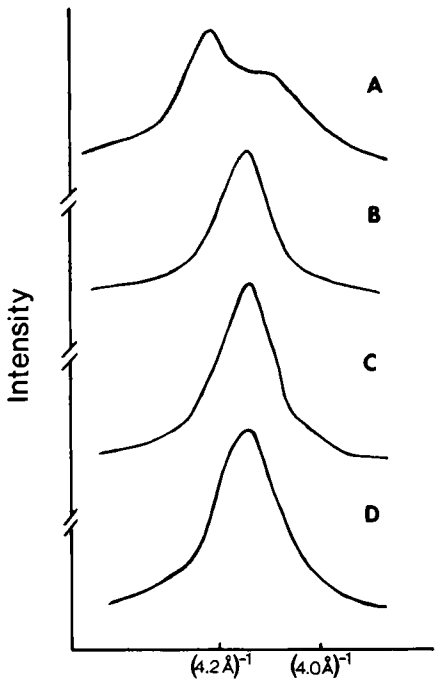

FIGURE 2

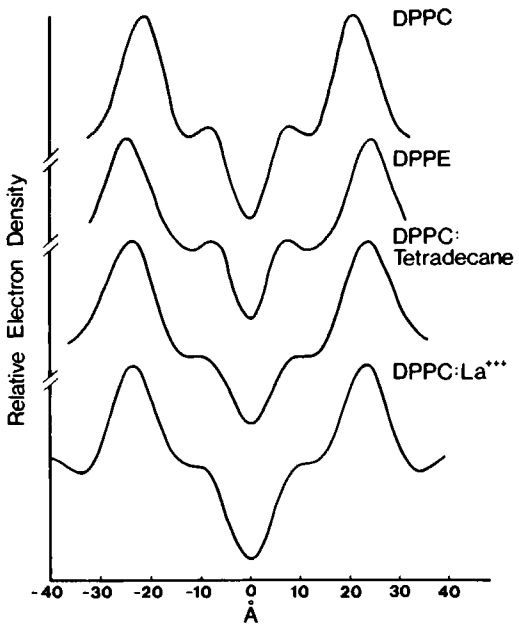

FIGURE 3

FIGURE 2 Densitometer traces across wide-angle diffraction patterns similar to those of Fig. 1. The asymmetric reflection of DPPC, which consists of a sharp reflection at 4.24 Å and a broad band centered at ~ 4.1 Å, is shown in A. Densitometer traces of the sharp symmetric reflections of DPPE, DPPC:tetradecane, and DPPC: lanthanum are shown in B, C, and D, respectively.

FIGURE 3 Electron density profiles of DPPC, DPPE, 1:1 molar ratio DPPC:tetradecane, and DPPC in 200 mM $LaCl_3$ at a lipid:ion ratio of ~ 3:1. The center of each bilayer is at 0 Å. Bilayer profiles were computed using the data of Table I.

obtained by Lesslauer et al. (13) and Levine (17). As might be expected, these profiles of Fig. 3 have many features in common. At the center of each bilayer at 0 Å is a low density trough corresponding to the terminal methyl groups of the hydrocarbon chains (13). The width of this trough is fairly broad due to the low resolution of these profiles. The terminal methyl trough is narrower in higher resolution profiles (13). At either side of this trough is a medium density region (centered at ~ ± 10 Å) that corresponds to the lipid methylene groups. The high density peaks, centered at ± 21 Å for DPPC, at ± 24 1/2 Å for DPPE, and at ± 24 Å for DPPC:tetradecane, correspond to the lipid head groups, primarily the high density phosphate groups. The relatively low density region at the outer edge of each bilayer corresponds to the fluid layers between bilayers. The fluid layers are somewhat larger for PC than for PE. The most important parameter that is to be obtained from these profiles is the bilayer width. As measured by head group peak-to-head group peak separation, DPPE is 7 Å wider and DPPC:tetradecane is 6 Å wider than DPPC. This 6 or 7-Å difference is accounted for by the difference in chain tilt between these bilayer systems (see Discussion).

The introduction of lanthanum ions to the fluid space between bilayers also modifies both the wide-angle and low-angle lamellar diffraction patterns. At low lanthanum contents in the fluid spaces (molar ratio of lanthanum:DPPC of < 1:20) the diffraction patterns looked like DPPC in water (Fig. 1 A). However, at lanthanum concentrations > 1:10 mole ratio lanthanum:DPPC the wide-angle pattern changed from this asymmetric reflection of Fig. 1 A to a symmetric reflection as in Fig. 1 D. The low-angle lamellar diffraction pattern also changed with increasing lanthanum concentration. In the range from approximately 1:10 to 1:5 lanthanum:DPPC, three broad diffraction bands centered at ~ $(50\ Å)^{-1}$, $(23\ Å)^{-1}$, and $(16\ Å)^{-1}$ were recorded. These patterns resemble the continuous diffraction from dispersions of single-walled DPPC vesicles (13). This type of pattern has also been recorded for hydrated DPPC treated with calcium chloride (18) or uranyl acetate (19). At higher lanthanum concentrations sharp, low-angle patterns were recorded. Inoko et al. (18) have recorded similar transitions from sharp reflections to continuous transform back to sharp reflections for DPPC treated with increasing concentrations of $CaCl_2$. The diffraction pattern from a molar ratio of lanthanum:DPPC of 1:3 consists of discrete lamellar reflections with a periodicity of 79 Å along with a symmetrical wide-angle reflection centered at 4.15 ± 0.04 Å (Fig. 1 D). No analysis was performed on the intermediate stage, continuous transform data. However, an electron density profile calculated from the 1:3 lanthanum:DPPC data (Table I) is shown in Fig. 3. Note that the head group-to-head group separation of this bilayer is ~ 6 Å wider than the pure DPPC bilayer.

DISCUSSION

Both the wide-angle reflections (Fig. 1 and 2) and the electron density profiles (Fig. 3) provide evidence that the hydrocarbon chains of hydrated DPPC are tilted relative to the normal to the plane of the bilayer, whereas the hydrocarbon chains of hydrated DPPE are approximately normal to the plane of the bilayer. As Tardieu et al. (1), Ranck et al. (2), and Brady and Fein (16) have discussed, asymmetric reflections such as shown in Fig. 1 A can be interpreted in terms of tilted chains, whereas sharp symmetric reflections like those of Fig. 1 B, C and D are characteristic of chains oriented perpendicular to the bilayer plane. Both Tardieu et al. (1) and Brady and Fein (16) have calculated angles of tilt of ~30° for fully

hydrated DPPC. Levine (4) and McIntosh (5) have recorded diffraction patterns from oriented, partially hydrated (~15% water content) DPPC bilayers. In these cases, the position on the x-ray film of the oriented wide-angle reflection directly shows the chain tilt. There have been extensive studies of crystals and low-water content multilayers of PE (7, 15). However, to the best of my knowledge, the pattern of Fig. 1 B, together with the electron density profile of Fig. 3, is the first evidence that hydrated DPPE has no chain tilt.

The electron density profiles of Fig. 3 confirm the interpretation of the wide-angle data. The difference in bilayer width between hydrated DPPC and DPPE, as measured by head group peak-to-head group peak distance across the profile, can be fully accounted for by the difference in chain tilt. The head group separations are 42 Å for DPPC and 49 Å DPPE. Taking the chain tilt for DPPC to be 30°, we note that 49 Å cos 30° $\simeq$ 42 Å. Space filling molecular models show that for fully extended hydrocarbon chains oriented perpendicular to the plane of the bilayer, the distance between the high density phosphate groups across the DPPE bilayer is ~ 49 Å. There may be other factors, such as possible differences in head group conformation, which could contribute to the differences in bilayer width between DPPC and DPPE. However, molecular models show that the possible increase in profile width caused solely by head group conformational changes is small compared to the effects of chain tilt. Moreover, in the case of DPPE, the molecular models show that the 49-Å separation between phosphate groups can only be accounted for if the acyl chains are oriented approximately perpendicular to the bilayer plane—regardless of conformation of the head group.

These data provide a response to the first question we sought to answer. That is, it is evident that not all hydrated phospholipids have tilted hydrocarbon chains. The second question concerns the packing parameters that cause chain tilt. The data from DPPC bilayers with added tetradecane and lanthanum shed light on this question. Both tetradecane, which is known to partition into the hydrocarbon region of the bilayer, and lanthanum, which is known to bind to the lipid head group and change the head-group conformation (9), significantly change the wide-angle and low-angle diffraction patterns. The shape of the wide-angle reflection (Fig. 1) and the head-group separations in the electron density profiles (Fig. 3) provide strong evidence that the lipid chains are oriented perpendicular to the bilayer plane in DPPC bilayers with added tetradecane or lanthanum ions. Tardieu et al. (1) have previously noted that small amounts of decane remove chain tilt from PC bilayers. The profile of DPPC:lanthanum (Fig. 3) is not at high enough resolution to localize the lanthanum or observe the change in head group conformation. The profile is simply intended to show that lanthanum increases the bilayer width by ~ 6 Å, the amount of increase expected for a loss of chain tilt. The lanthanum binding and resulting change in head group conformation could also be contributing factors in the change of head-group position in the profile. The medium density regions at the edges of the bilayer, outside the high density peaks at $\pm$ 24 Å, are considerably wider for DPPC:lanthanum than for DPPC or DPPC:tetradecane. This could be explained by a more extended polar head-group conformation, with the added lanthanum causing the choline group to extend farther from the bilayer center than the phosphate group.

One possible explanation of all of this data is a simple model of lipid packing, which is similar to a model first proposed by Nagle (20). In Nagle's model, it was assumed that the lipid's glycerol backbone has a larger excluded area in the plane of the bilayer than the hydrocarbon chains. Thus, according to Nagle, it is energetically favorable for the chains to

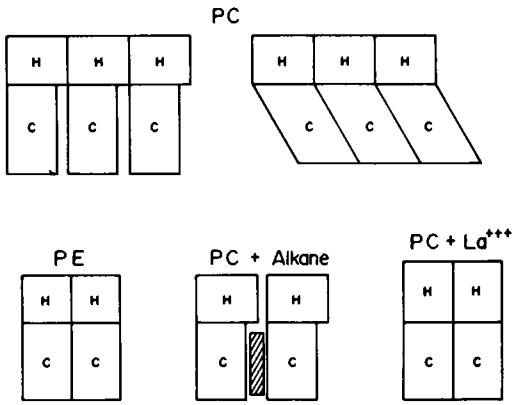

FIGURE 4 A schematic representation of the packing of lipid molecules with a head group region H and a chain region C. This model is similar to one proposed by Nagle (20). Since the head group of PC is oriented approximately parallel to the bilayer plane (5, 21–23), it is assumed in the drawing on the top left that the head groups prevent further lateral compression and prevent the chains from coming in close contact. Thus, to maximize the attractive van der Waals force, the chains of hydrated PC tilt (top right). For PE (bottom left), the case is somewhat different as the head group is approximately the same radius as the chain region, so the chains maximize the van der Waals interaction without tilting. For PC, the introduction of tetradecane (shaded rectangle, bottom center), fills in potential voids in the hydrocarbon region and thus make it energetically favorable for the chains to straighten and become perpendicular to the bilayer plane. Lanthanum also straightens the hydrocarbon chains (bottom right), by changing the conformation of the PC head group and reducing its excluded area in the plane of the bilayer.

tilt and thus increase their attractive van der Waals force. This is a very reasonable model which appears to be basically correct. However, it does not explain why DPPE has no chain tilt, unless one assumes that the glycerol groups of DPPE have a smaller excluded area than the glycerol groups of DPPC. A more likely explanation for the differences between DPPC and DPPE is the fact that the hydrated phosphocholine polar head group is larger than the polar head group of PE. In addition, it is now known that the phosphocholine dipole is oriented approximately parallel to the plane of the bilayer (5, 21, 23) (Nagle assumed that the head group was oriented perpendicular to the bilayer plane—a configuration that produces a smaller excluded area in the plane of the bilayer). A schematic representation is shown in Fig. 4. The DPPC chains tilt because the PC head group, in its position parallel to the bilayer plane, has a larger excluded area in the plane of the bilayer than the hydrocarbon chains. To "fill in" this potential void in the hydrocarbon region, the chains tilt. Note that this chain tilt is not necessary in case of PE, which has a smaller head group. Phillips and Chapman (24) have shown that PE molecules pack more closely together in condensed monolayers than do PC molecules. Space filling molecular models indicate that the head group of PE has approximately the same excluded area as the lipid chains. The representation of Fig. 4 also can explain why both tetradecane and lanthanum reduce chain tilt. Tetradecane partitions into the hydrocarbon region of the bilayer and fills the potential void created by the bulky PC head group. Thus, in the presence of tetradecane, van der Waals forces can be maximized without having a chain tilt. In this model, lanthanum (Fig. 4) reduces the chain tilt by reducing the excluded head group area in the plane of the bilayer by changing the conformation of the PC head group to a more extended position.

The loss of chain tilt at low-water content for DPPC may be due to a conformational change in the head group, or perhaps a reduction in the effective size of the head group as the hydration shell of the head group is lost. The electrostatic interactions between lipid head groups will also be affected by the loss of water. As judged by the relative widths of the fluid layers in Fig. 3, hydrated DPPE multilayers do not contain as much water as hydrated DPPC multilayers do. This may be an important factor in the differences in chain tilt between hydrated DPPE and DPPC.

Tilted hydrocarbon chains may be associated with the "pretransition" observed in saturated PC bilayers. Both Rand et al. (25) and Brady and Fein (16) conclude that the tilted DPPC chains become perpendicular to the bilayer at the pretransition. Janiak et al. (26) also note a change in chain tilt at the pretransition, but calculate a much smaller tilt change. Janiak et al. (26) also show that there is a structural transformation at the pretransition, from a one-dimensional lamellar to a two-dimensional monoclinic lattice. Gaber et al. (27), using Raman difference spectrscopy, show that there is an increase in the number of gauche bonds at the pretransition and an alteration in the lateral order of the bilayer. Clearly, there is more occurring at the pretransition than just a change in chain tilt. However, chain tilt may be a prerequisite for the pretransition. Pretransitions are not observed in bilayers with chains perpendicular to the bilayer plane, such as DPPE (12), DPPC with added tetradecane (28), and DPPC at low-water contents (26).

Since the time that this manuscript was submitted for publication, Jahnig et al. (29) have shown that the chain tilt of dihexadecylphosphatidic acid can be modified by varying the surface charge. They show that the chain tilt of that lipid increases with increasing surface charge and, therefore, with increasing area per lipid molecule. Their data from phosphatidic acid are completely consistent with the model of Fig. 4, and clearly show that hydrocarbon chain tilt depends on the surface area per lipid head group.

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