Fatty-acid chain tilt angles and directions in dipalmitoyl phosphatidylcholine bilayers

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ABSTRACT   X-ray diffraction has been applied to determine the various tilt angles and directions (if any) which can be assumed by oriented gel phase multilayers of dipalmitoyl phosphatidylcholine (DPPC) as a function of hydration. We report for the first time that oriented DPPC multilayers with a repeat spacing (d-spacing) of 55.2 Å at 25°C and 0% relative humidity (RH) have hydrocarbon chains tilted at an angle θ of 21.5° with respect to the bilayer normal. In addition, the chains are tilted along one of the bisectors ($\omega = 0°$) of the hexagonal lattice (8 wide-angle maxima, 2 unique), a phase not previously reported in DPPC studies. At 100% RH, the chain tilt angle and d-spacing increased to $\approx 29.0°$ and 58.9 Å, respectively. Since at 100% RH only 4 wide-angle maxima are observed, we analyze the data on the assumption that the hydrocarbon chains may rotate independently of the hexagonal lattice ($\omega = 0$–$30°$), at a fixed chain tilt angle θ (Stamatoff, J.B., et al. 1979. *Biophys. J.* 25:253–262). The largest observed angle ϕ made by the wide-angle maxima with the equator is 29.5° corresponding to a θ of $\approx 32.6°$ ($\omega_{avg.} = 24°$) and the sample having a d-spacing of 64.0 Å (excess water condition). Finally, θ remains relatively constant ($\approx 21.5°$) up to a RH of $\approx 45\%$ and a d-spacing of 57.8 Å, after which, with increases in RH, θ increases to a maximum of 32.6°.

INTRODUCTION

In 1967, Chapman et al. (1) using x-ray diffraction predicted a limiting tilt angle (θ) of 32° with the bilayer normal for the hydrocarbon chains of a nonoriented 1,2-dipalmitoyl-DL-phosphatidylcholine/water system. The value for the tilt angle θ was calculated assuming hexagonally packed hydrocarbon chains, from the expression $2A = A_0 \cos\theta$, where $A = 20.3$ Å^2 is the cross-sectional area per hydrocarbon chain and A_0, the area occupied by the polar group (glycero-phosphorylcholine and fatty acid carbonyl groups) which increases with increasing hydration. Using oriented samples of 1,2-dipalmitoyl-L-phosphatidylcholine (DPPC) at various relative humidities (RHs), Levine (2–4) observed that at 0% RH (2% water content by weight), the $[4.2 \text{ Å}]^{-1}$ reflection due to the hexagonally packed hydrocarbon chains was oriented on the equator, indicative of 0° chain tilt. However, at 20% water content, a limiting value of $\sim 28°$ was obtained for θ.

Since the studies of Chapman et al. (1) and Levine (2–4), the chain tilt angle of oriented and nonoriented DPPC multilayers under various temperature and hydration regimes has been further studied by use of x-ray diffraction and other physical techniques. Using x-ray diffraction and nonoriented samples of DPPC/water, Tardieu et al. (5) found that θ varied from 17° at $c = 0.94$ ($c = $ wt of DPPC/[wt of DPPC + wt of water]) to 33° at $c = 0.75$ and 20°C. In similar experiments to Tardieu et al., Janiak et al. (6) using multilamellar dispersions of DPPC in excess water found that θ varied as a function of temperature from about 35° at 5°C to a 30° chain tilt at 40°C. At 20°C the angle of tilt was found to be slightly greater than 33°, in agreement with the result obtained by Tardieu et al. (5).

In the case of nonoriented multilayers in excess water, the literature values seem to be in good agreement with

each other. However, using oriented samples of DPPC and water contents up to ≈ 23 wt%, the results do not always seem to agree with each other or with the powder sample data. Using electron diffraction and oriented multilayers or single bilayers of DPPC, Hui (7) found that a 16° ± 1° tilt existed in hydrated multilayers but not in single bilayers. In addition, Hui also observed a chain tilt of 0° in the dehydrated multilayers. Birrell and Griffith (8) using electron spin resonance observed a θ of 33° at the C_5 position in oriented DPPC multilayers at 93% RH. They also observed a decrease in the chain tilt angle (21°) at the C_{16} position of the fatty acid chain (8). McIntosh (9) measured the $[4.2 \text{ Å}]^{-1}$ reflection to be 24° off of the equator in oriented DPPC multilayers at 100% RH, same as Levine (2–4). Stamatoff et al. (10) using "freestanding" monodomain DPPC samples at room temperature, calculated θ to be 12.5° for a sample having 10 ± 1 wt% water and a repeat-spacing (d-space) of 57.4 Å using x-ray diffraction. Hentschel et al. (11) using oriented bilayers of DPPC containing ≈ 23 wt% water, measured chain tilt angle as a function of temperature. At 20°C they obtained a θ of $\approx 13°$ and a d-spacing of ≈ 70 Å.

In this study using x-ray diffraction and a two-dimensional detector, we observe a chain tilt angle of 21.5° at 0% RH and 25°C, corresponding to a d-spacing of 55.2 Å. In addition, the number of reflections observed (8 reflections) indicate that the chains are tilted along one of the bisectors of the hexagonal lattice (11–13). At 100% RH, the chain tilt angle θ increased to $\approx 29.0°$ with the sample having a d-spacing of 58.9 Å. Since, at 100% RH only 4 maxima are observed, instead of the 6 or 8 corresponding to the chains tilted either along one of the basic vectors ($\omega = 30°$) or along one of the bisectors ($\omega_{avg.} = 0°$) of a hexagonal lattice formed by the fatty

acid chains, respectively (11–13), we analyze the data on the assumption that all tilt directions ($0° < \omega < 30°$) are equally probable for hydrocarbon chains of a fixed tilt angle θ (10). The largest tilt angle of $\approx 32.6°$ ($\omega = 24°$) corresponded with a sample having a d-spacing of 64.0 Å at excess water conditions.

MATERIALS AND METHODS

L-α-Dipalmitoyl phosphatidylcholine (DPPC) was purchased from Avanti Polar Lipids, Inc. (Birmingham, AL.) and used as supplied. DPPC was dissolved in a minimum amount of methanol at room temperature and heated to $\approx 50°C$, after which point it was pipetted onto the outside surface of a 30-ml Pyrex beaker (No. 1000). After evaporation of the methanol, a clear film of lipid was left adhering to the outside of the glass beaker. The remainder of the methanol was removed by placing the samples under vacuum for 24 h, after which time they were hydrated in a 100% relative humidity (RH) environment for about a week. 0% RH humidity samples were obtained by placing hydrated samples under vacuum for 48 h.

A point source (0.3 mm^2) of Cu K$_\alpha$ radiation was obtained using a Rigaku Rotaflex RU-200B series rotating anode x-ray generator (Rigaku Corp., Japan) operating at 4 kW (50 kV and 80 mA) of power. Monochromation of the Cu radiation was achieved using a graphite crystal. Diffraction patterns were obtained with an R-Axis IIC two-dimensional image plate detector (Rigaku Corp., Japan) with a pixel size of 105 × 105 μm^2 and an effective detection area of 200 × 200 mm^2. Sample to film distance and calibration of the detector were determined by using the diffraction pattern of potassium hydrogen pthalate as a reference.

The sample holder was designed to control both the temperature and humidity of the sample. The holder was positioned so that the x-ray beam passed through the sample tangent to the vertical side of the beaker. As such, only half of all of the possible reflections were detected. 0% RH was obtained by flowing N$_2$ through the sample holder. All other RHs (except excess water) were achieved by adjusting the flow rate of N$_2$ through water in a Fisher-Milligan gas washer before passage through the sample holder. The sample at excess hydration was obtained from the placement of oriented multilayers in a 100% RH environment exposed to water condensation. The RH was monitored by a digital hygrometer (Vaisala HMI 31; Helsinki, Finland) which has a resolution of 0.1% RH and an accuracy of ±2% RH. Temperature was controlled by a Lauda RC3 water bath (West Germany).

Finally, the background due to the windows of the sample holder and the glass beaker itself was subtracted from all of the diffraction patterns. This was achieved by collecting a diffraction pattern using the same setup as described above, minus the sample.

RESULTS AND DISCUSSION

In Fig. 1, two-dimensional x-ray diffraction patterns of oriented DPPC multilayers at 25°C and various hydration levels are presented. The diffraction pattern obtained from a sample at 0% RH (Fig. 1a) having a d-spacing of 55.2 Å and water content of $\sim$2 wt% (2, 10), contains 8 maxima in the wide-angle region with a spacing of ≈ 4.2 Å (Fig. 1 a, inset). Since only half of the diffraction pattern was recorded (see Materials and Methods), only 4 maxima are observed, and of those four, only two are unique (Fig. 2 d). This is consistent with the chains being tilted along one of the bisectors ($\omega = 0°$) of the hexagonal lattice (Fig. 2 a and Fig. 3 b) formed by the hydrocarbon chains (11–13). In this case, the angles made by the two wide-angle reflections result-

ing from the 110 (ψ) and 200 (ϕ) lattice planes (Fig. 2 d and Fig. 3 b) can be related to the tilt angle θ by

$$\sin \phi = \pm\cos 0° \sin \theta$$
$$= \pm\sin \theta$$
$$\sin \psi = \pm\cos 60° \sin \theta \quad (11, 12).$$

From the x-ray diffraction pattern (Fig. 1 a), we measure ϕ to be 21.5° and ψ, 11.4°. Substitution of ψ into the above equation yields a calculated θ of 23.3°, in good agreement with the measured value ($\phi = \theta$) but also, indicative of a slightly distorted hexagonal lattice. This phase, although known to exist in smectic liquid crystals such as terephthalydenebis-4-n-pentylaniline (13), has not to our knowledge ever been observed in DPPC. In addition, the number (11) and widths of the lamellar reflections recorded along the c^* axis are indicative of the sample having a high degree of order.

The existence of a chain tilt angle at 0% RH in oriented DPPC multilayers has not been previously reported using x-ray diffraction. Levine, in his Ph.D. thesis (2), reported that diffraction patterns of oriented DPPC multilayers with 2 wt% water (0% RH) contained a very sharp $[4.2 \text{ Å}]^{-1}$ reflection oriented on the equator, along with some fainter reflections. Levine interpreted these diffraction patterns as being indicative of multilayers with no chain tilt. However, Stamatoff et al. (10) presented an intensity map of the wide-angle region ($[4.2 \text{ Å}]^{-1}$) for a highly oriented "freestanding" sample (4° mosaic spread) of DPPC at 22 ± 3°C containing 2% water by weight and having a d-space of 55.7 Å. Upon analysis of their contour map (Stamatoff et al.), one notices that there are a total of 6 reflections (only 3 shown), 2 equatorial and 4 off equatorial (Fig. 2 e), implying that the hydrocarbon chains are tilted along one of the basic ($\omega = 30°$) hexagonal lattice vectors (Fig. 2 b and Fig. 3 a). In this case the angle ϕ made by the off equatorial reflections (110 lattice planes) is related to the tilt angle θ by

$$\sin \phi = \cos 30° \sin \theta \quad (2-4, 11, 12).$$

From the intensity contour map of the wide-angle region presented by Stamatoff et al. (10), we measured a ϕ of $\approx 18°$ which corresponds to tilt angle θ of $\approx 21°$. This result compares favorably both in d-spacing and chain tilt angle with our 0% RH sample, but differs in the direction of the chain tilt. The reflection due to the 020 lattice planes is centered on the equator since $\psi = 0°$ ($\sin \psi = \cos 90° \sin \theta$).

Further evidence for the existence of chain tilt at 0% RH is provided by the electron density profiles of DPPC bilayers at 23°C and various RHs obtained by Torbet and Wilkins in 1976 (14). It is well known that increases in water concentration lead to a decrease in the bilayer thickness of DPPC multilayer dispersions and oriented films (5, 14, 15). DPPC vesicles in excess water attain a
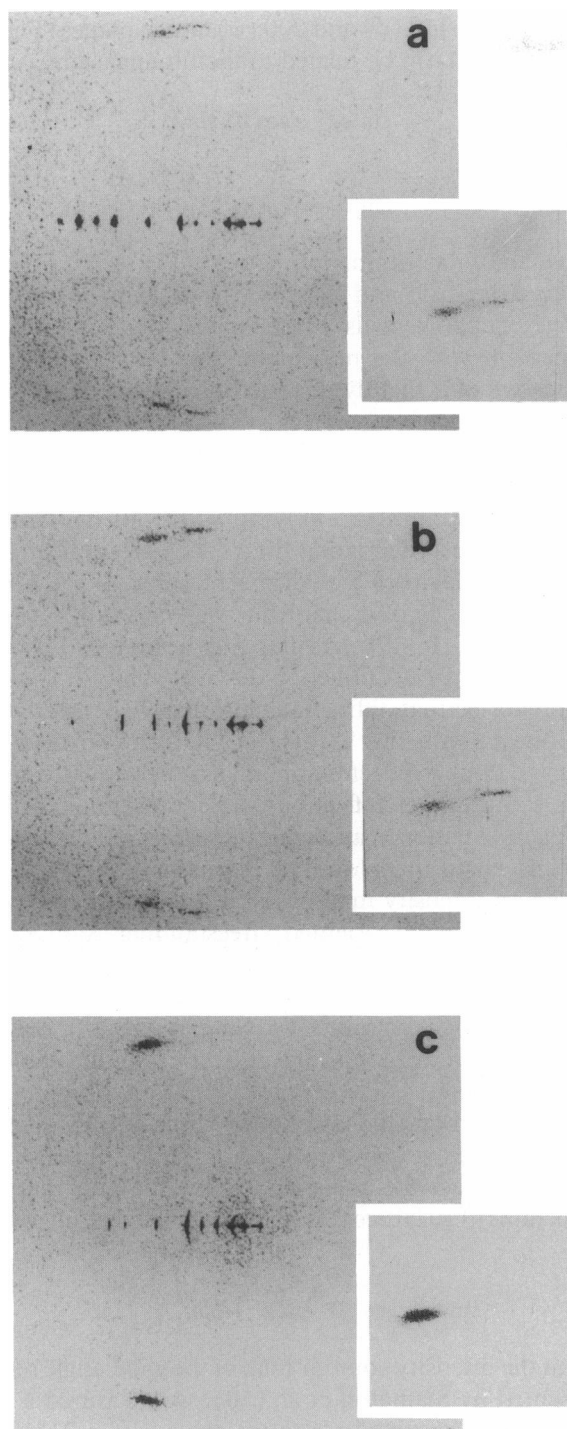
FIGURE 1   X-ray diffraction patterns at 25°C of oriented DPPC multi-bilayers at (a) 0% RH having a d-spacing of 55.2 Å and φ and ψ angles of 21.5° and 11.4°, respectively, corresponding to a chain tilt angle θ of 21.5°. (b) 70% RH bilayers (d-spacing of 57.9 Å) with φ and ψ angles of 25.5° and 13.1°, respectively, implying a θ of 25.5°. (c) 100% RH sample with a d-spacing of 58.9 Å and a θ of 29.0° (φ = 26.3°).

≈45.7 Å (phosphate peak-to-phosphate peak). If the chains in this sample are tilted by ≈33°, then the bilayer thickness in the 0° tilt case should be ≈54.5 Å, assuming that the decrease in the thickness of the bilayer at increased levels of hydration can be attributed totally to the chains being tilted (5). However, when measuring the bilayer thickness of oriented multilayers under vacuum (56.6 Å d-spacing), we observe a thickness of only 51.4 Å, which can be made to equal the thickness expected from the fully hydrated bilayers by introducing a 19.5° chain tilt (51.4 Å/cos 19.5°). A more accurate determination of the chain tilt could have been derived from the electron density map if we measured the hydrocarbon thickness (carbonyl peak-to-carbonyl peak) instead of the bilayer thickness. This was not possible in the case of the electron density map derived from the vesicles in excess water, since the peak due to the fatty acid carbonyl groups was not sufficiently resolved. Also, Tardieu et al. observed that multilamellar dispersions of DPPC at 20°C and 4 wt% water contained an angle of tilt of 17° (5).

From Table 1 we observe that the tilt angle θ remains relatively constant at ~21.5° up to a RH of ≈45% (after which point it increases), independent of an increasing d-space. This is of interest since it has been assumed that the tilt angle in DPPC multilayers increases monotonically with increased levels of hydration (2–4).

At 70% RH and 25°C (Fig. 1 b), the d-spacing increased to 57.9 Å along with the chain tilt angle (φ = 25.5° and ψ = 13.1°), while still maintaining a chain tilt direction along one of the bisectors of the lattice (ω = 0°). Again, the two θs obtained from the φ and ψ angles differ by 1.4°, indicative for the most part of distortions in the hexagonal lattice (11–13).

In Fig. 1 c, we present a diffraction pattern of the DPPC multilayers at 100% RH having a d-spacing of 59.1 Å. However, the number of wide-angle reflections have decreased from 8 to 4 (1 unique reflection, Fig. 2 f), implying that the chains may assume any tilt direction with respect to a fixed hexagonal lattice (Fig. 2 c) while maintaining a fixed angle of tilt (10). The angle φ, made by the wide-angle reflections with the equator is 26.3°. Stamatoff et al. (10) obtained a tilt angle θ of 12.5°, which they calculated by modeling the intensity distribution in the [4.2 Å]$^{-1}$ region with 20 Å rods. From a measurement of φ = 11.4°, which we obtained from their intensity contour map, we get an ω_{avg} of 24°. We assign an average value for ω in this case, since, the chains may rotate independent of the fixed hexagonal lattice. Using the equation sin φ = cos ω_{avg} sin θ (11, 12) we obtain a θ of ~29° for the sample at 100% RH having a φ of 26.3°, comparing favorably with a θ of 28°, obtained by Levine (2–4) for a similar sample (100% RH, ω = 30°). Although ω_{avg} may vary slightly with changes in hydration (we used same ω_{avg} for 100% RH and excess water samples), its effect on θ are minimal. Hent-

maximum d-spacing of ≈64.0 Å and a θ of 33° (5, 6). From the electron density distribution of DPPC vesicles in excess water (d-space = 64.0 Å) calculated by Torbet and Wilkins, we can measure a bilayer thickness of
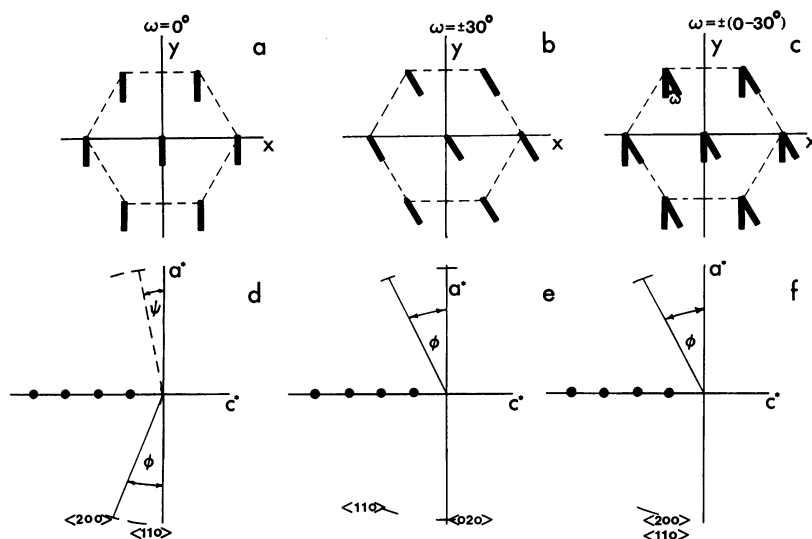
FIGURE 2 (a–c) Schematic of real lattices showing the tilt directions (ω) of the hexagonally packed hydrocarbon chains in DPPC bilayers and their resulting diffraction patterns (d–f). The c* axis is normal to the plane of the bilayers and contains the so-called small-angle reflections which are present due to the periodic stacking of the bilayers and allow for the direct measurement of bilayer spacing. In addition, the (hkl) planes giving rise to particular wide-angle maxima are indicated. All of the diffraction patterns (d–f) due to the different phases (a–c) are indicative of poor in-plane correlations between bilayers (e.g., hexagonal net shift distortions between bilayers) in addition to the presence of a monodomain (see reference 13).

schel et al. (11) working with oriented DPPC multilayers containing ≈20 wt% water, observed 6 maxima in the [4.2 Å]$^{-1}$ region (Fig. 2 e) for all temperatures (0–40°), except near room temperature where no central maxima were present (4 maxima). They attributed the extinction of the central maxima (020 reflections, Fig. 2 e) to a different or undefined tilt direction and used an ω of 30° in calculating θ (11, 12).

Stamatoff et al. (10), Hentschel et al. (11), and ourselves observe only 4 wide-angle maxima for oriented DPPC multilayers at 100% RH and room temperatures. Levine (2) on the other hand observes 6 maxima in the

wide-angle region. Stamatoff et al. (10) attributed the lack of maxima at c* = 0 in the [4.2 Å]$^{-1}$ region to the absence of a backing block to support their bilayers, hence the name "freestanding" sample. They argued that the freestanding samples diminished absorption effects due to the sample, and eliminated scattering and absorption due to the supporting block (mica substrate in Levine's case) which are maximized on the equator and probably accounted for the observed peaks at c* = 0 by Levine (2). We believe that this argument is to a great extent incorrect since our sample were not freestanding and yet we do not observe maxima centered on the equa-
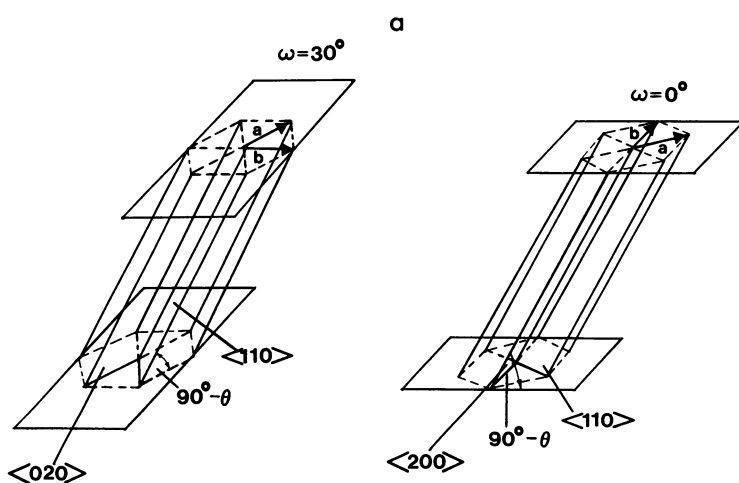

FIGURE 3 Three-dimensional representation of hexagonally packed hydrocarbon chains tilted (a) along one of the basic hexagonal lattice vectors (ω = 30°), or (b) along one of the bisectors (ω = 0°) of the hexagonal lattice. From the schematic, one can observe that the 200 and 110 planes are tilted with respect to the planes of the bilayer while the 020 is perpendicular.

TABLE 1 Dimensions of oriented DPPC multilayers at 25°C

% RH	d-spacing	Observed tilt	Calculated tilt	No. of maxima in $[4.2 \text{ Å}]^{-1}$ region
	Å	ϕ, ψ	θ	
0	55.2	21.5°, 11.4°	21.5°	8 (2 unique)
10	55.4	21.2°, 11.3°	21.2°	8 (2 unique)
30	57.1	21.9°, 11.7°	21.9°	8 (2 unique)
45	57.8	21.7°, 11.5°	21.7°	8 (2 unique)
70	57.9	25.5°, 13.1°	25.5°	8 (2 unique)
100	58.9	26.3°, ——	29.0°	4 (1 unique)
>100	64.0	29.5°, ——	32.6°	4 (1 unique)
40	57.5	21.8°, 11.4°	21.8°	8 (2 unique)*

* Annealed for 1 wk at room temperature and ≈40% RH.

tor. In addition, Levine's argument (2) that the equatorial reflections are attenuated in regions close to the equator is also not totally correct, since Hentschel et al. (11, 12) observed 6 maxima in nonfreestanding samples (mylar substrate) at temperatures 10°C above and below room temperature which had approximately equal intensities and widths. From the above, one can draw the conclusion that the hydrocarbon chains of gel phase DPPC multilayers at various hydration and temperature combinations exist in three distinct structural phases, $\omega = 0°$ (8 maxima), $0° < \omega < 30°$ (4 maxima), and finally, $\omega = 30°$ (6 maxima).

From Torbet and Wilkins' (14) electron density distributions for DPPC bilayers, we measured an approximate hydrocarbon thickness (carbonyl-to-carbonyl separation) of 37.8 Å for a 100% RH sample with a d-spacing of 58.8 Å. Using our tilt angle of 29.0° obtained from a similar sample (100% RH, 58.9 Å d-spacing), we calculate at 0° tilt, a hydrocarbon thickness of 43.2 Å. Again, when we measure the hydrocarbon thickness of their sample (14) under vacuum (mentioned previously, d-space = 56.6 Å), we observe a hydrocarbon thickness of only 40.4 Å. This can only be made to equal 43.2 Å by assigning a tilt of 20.7°. This result agrees favorably with a tilt angle of 21.5° which we observe in our 0% RH sample (Fig. 1 a).

In Fig. 4, we present the diffraction pattern of an oriented DPPC sample annealed for 1 wk at room temperature and ≈40% RH. Although this sample had undergone a gel-to-liquid crystalline phase transition, after the annealing process its hydrocarbon chains assumed a tilt direction of $\omega = 0°$, similar to the samples in Figs. 1 a and b indicating that these are equilibrium structures.

In Table 1, we present a summary of the dimensions for the various systems that were studied. The maximum d-spacing of 64.0 Å also provided the largest tilt angle θ of 32.6° (Table 1). Aside from the initial work by Levine (2), one of the difficulties with studies of oriented samples of DPPC (7, 10, 11), is that the tilt angle θ reported has been much smaller than the θ obtained from powder samples under similar conditions of temperature and hu-

midity (5, 6). However, the tilt angle θ at 100% RH in oriented samples has either been found to be in the high twenties (2, 9) or low teens (7, 10, 11), leading us to believe that this discrepency may be attributed to the samples being in slightly different thermodynamic states giving rise to two different populations and may depend on a combination of sample preparation, history, and geometry (curved vs. flat sample). Previously, Levine (2–4) obtained a tilt angle of 28.0° for oriented DPPC multilayers containing 20 wt% of water. Here, we provide for the first time a tilt angle derived from oriented multilayers that is within 1° of tilt angles calculated for multilamellar dispersions of the lipid (1, 5, 6).

CONCLUSIONS

Using oriented gel phase DPPC multilayers, we have directly determined the chain tilt angle as a function of hydration at 25°C. For the first time, we report DPPC multilayers having chain tilts of 21.5° at 0% RH. In addition, the tilt angle θ remains relatively unchanged at ≈21.5° up to a RH of ≈45% even though the sample is experiencing an increase in d-spacing. This result differs from the assumption that DPPC multilayers undergo a monotonic increase in chain tilt with increasing levels of hydration.

From the diffraction patterns obtained, we were not only able to directly determine the angle of tilt for the various conditions (0–100% RH) but also the direction of the chain tilt. If 8 maxima are observed, then the chains are tilted along one of the bisectors of the hexagonal lattice ($\omega = 0°$). This was the case for multilayers up until a RH of 70% after which only 4 maxima were observed, implying that at higher hydrations the chains
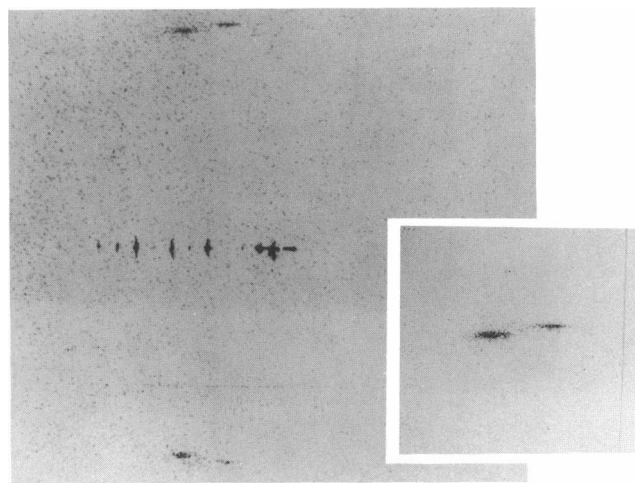

FIGURE 4 X-ray diffraction pattern of oriented DPPC multilayers annealed for 1 wk at room temperature and ≈40% RH after exposure to the liquid-crystalline phase. The measured ϕ and ψ angles are 21.8° and 11.4°, respectively, with the bilayers having a d-spacing of 57.5 Å.

were tilted in a direction $\omega_{avg.} = 24°$ with respect to a fixed hexagonal lattice.

Finally, we were able to record diffraction patterns from oriented samples in excess water conditions which contained hydrocarbon chains tilted to within 1° of tilt angles obtained from multilamellar dispersions.

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