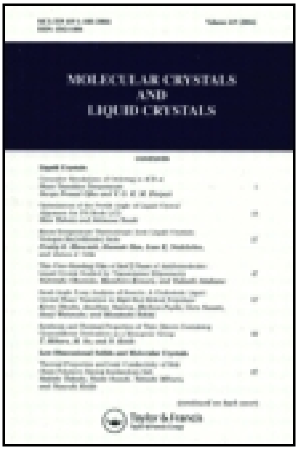

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Structural Parameters of Phosphatidylcholine Bilayer Membranes

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Structural Parameters of Phosphatidylcholine Bilayer Membranes

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We present x-ray diffraction results of dimyristoylphosphatidylcholine(DMPC),-dipalmitoylphosphatidylcholine(DPPC) and distearoylphosphatidylcholine(DSPC) multilayers in three structural phases (L_α, P_β, $L_{\beta'}$). Using a high angular resolution method, we have determined the interlamellar repeat distance, the fractional completion of the phase transition, the bilayer thickness, the interbilayer water thickness and the linear thermal expansion coefficient of lipid bilayer.

INTRODUCTION

Phospholipid bilayer membranes have importance both in membrane biophysics and in the physics of liquid crystals. In order to understand the structure-function relationships in biological membranes there is special interest in the structural transitions of model membranes composed of lecithin molecules.[1-5]

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In this study, we have examined DSPC bilayers using high angular resolution x-ray diffraction method. Results obtained with DSPC bilayers are compared to experiments performed with DMPC and DPPC bilayers. The experimental results permit determination of the interlamellar repeat distance, the bilayer thickness, the interbilayer water thickness, the linear thermal expansion coefficient of lipid bilayer and the fractional completion of the phase transition.

MATERIALS AND METHODS

DMPC, DPPC and DSPC were purchased from Fluka (Switzerland) and were used without further purification. A known weight of phosphatidylcholine and water was incubated in a sealed glass test tube for 40 hours above the respective main transition temperature, T_m. For x-ray measurements samples were contained in thin-walled quartz capillary tubes (internal diameter 0.7mm), and diffraction patterns were recorded from 5 to 70°C with 2–5°C steps. Temperature was controlled to ±0.5°C. At every temperature, before the 1000 sec exposition, the sample was equilibrated for 1500 sec. The position sensitive small-angle x-ray diffractometer was made in the Institute of Crystallography of the Soviet Academy of Science. Nickel filtered Cu Kα x-ray radiation (λ = 1.54 Å) of BSV-22 x-ray tube (operating at 30kV and 30mA) was collimated by a three slit collimator. The path of incident beam and scattered beam was under vacuum. The sample to detector distance was 74.0 cm. This is unusually long and provided significantly improved angular resolution. The detector sensitivity is 0.12 mm and the width of x-ray beam is taken to be 0.02°. Stomatoff et al.[4] applied similar high resolution on DPPC samples.

RESULTS

In Figure 1 typical small angle diffraction patterns of DMPC are shown. Reflections due to stacking of the lipid bilayers are apparent. These reflections do not indicate disorder. Higher diffraction orders remain sharp. Applying the Bragg formula one can determine the average interlamellar repeat distance, *d*. For the first-order diffraction peak:

$$d = \lambda/(2 \cdot \sin \Theta) \qquad (1)$$

where 2Θ is the scattering angle.

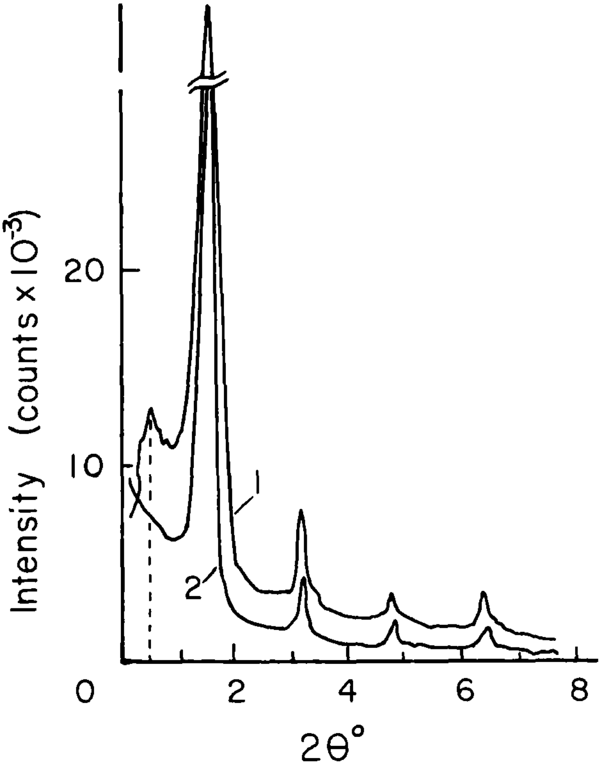

FIGURE 1 Small-angle x-ray diffraction patterns of DMPC. The unoriented multilayer sample contains 60% water. 1: 18°C, exposition 2000 sec. 2: 30°C, exposition 1000 sec. 2Θ°: is the scattering angle.

In Figure 2, the temperature dependence of the interlamellar repeat distances of DMPC, DPPC and DSPC are shown. The small-angle x-ray information indicates that two structural transitions take place in each case. The first transition, belonging to the lower temperature, T_p, is called pretransition. This involves a $L_{\beta'} \rightarrow P_\beta$ structural transition,[4] where a one-dimensional lamellar lattice of tilted crystalline-like chains transforms into a two-dimensional monoclinic lattice of straightened crystalline-like hydrocarbon chains. The second transition at higher temperature, T_m, is called main transition, and involves a $P_\beta \rightarrow L_\alpha$ transition, where L_α marks a one-dimensional lamellar lattice of liquid paraffin-like hydrocarbon chains.[1] Our DPPC data agree excellently with the previous data of Inoko and Mitsui,[3] and DMPC, DPPC data partly agree with the data of Janiak et al.[2] There are 4–5Å disagreements in the values obtained in the P_β phases. The

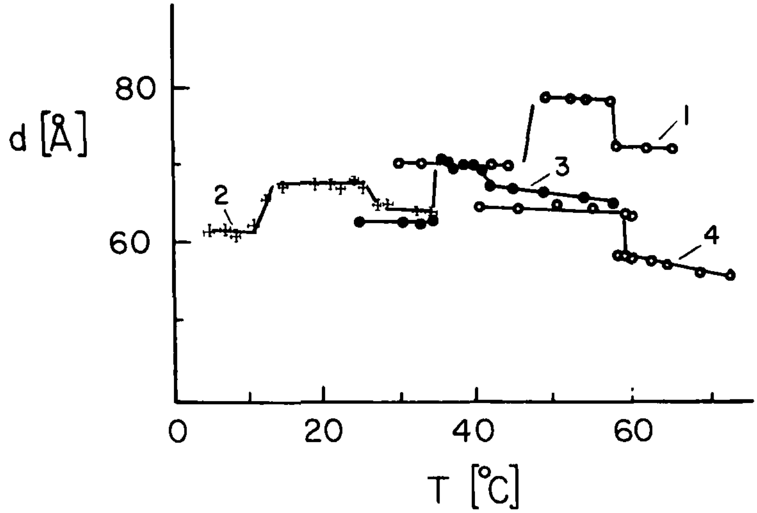

FIGURE 2 Temperature dependence of average interlamellar repeat distance, *d*. 1:DSPC, 2:DMPC, 3:DPPC, 4:DSPC. Samples 1, 2 and 3 contain excess water, while sample 4 contains 30% water.

interlamellar repeat distance of DSPC is determined at low- and high water content too.

In Figure 3 the compositional dependence of the interlamellar repeat distance of DSPC is shown in each phase of the bilayer. The interlamellar repeat distance increases with increasing water concentration reaching a maximum value at maximum hydration. According to our data the maximum hydration of DSPC is at 40–60% for each phase.

In Figure 4 small-angle x-ray diffraction intensity curves of DSPC at different temperatures around T_m are shown. Near T_m the simultaneous presence of two sharp peaks is direct evidence for the coexistence of P_β and L_α phase. By determining the relative areas of the sharp peaks at each temperature, one obtains the fractional completion of the phase transition. We note, that the DSPC main transition does not show hysteresis in the presence of excess water.

In Figure 5 high-angle diffraction patterns of DMPC at each phase are shown. Similar curves were obtained for DPPC and DSPC bilayers (not shown). Asymmetry of the peak in $L_{\beta'}$ phase indicates hydrocarbon chain tilting (see double peak in Figure 5). In the P_β phase the reflection has become symmetric indicating that the hydrocarbon chains have straightened. In L_α phase the broad diffraction band indicates that the hydrocarbon chains have melted.

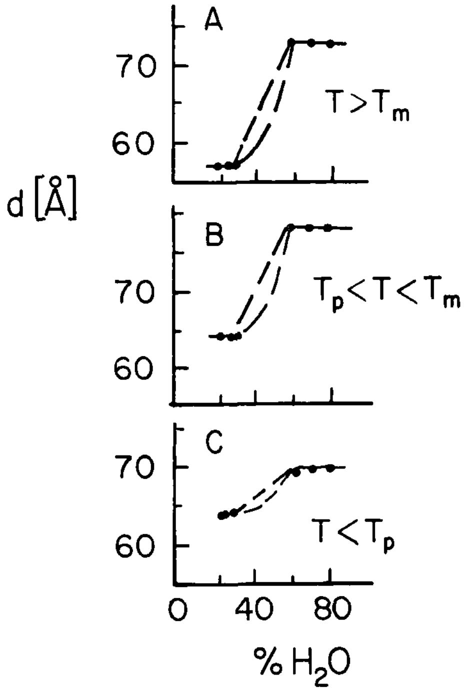

FIGURE 3 Compositional dependence of DSPC interlamellar repeat distance in a) $T_{\beta'}$, b) P_{β} and c) T_{α} phase.

DISCUSSION

The average interlamellar repeat distance, *d*, is the sum of two terms:

$$d = d_l + d_w \tag{2}$$

where d_l is the lipid bilayer thickness and d_w is the thickness of interbilayer water. By means of the maximum hydration data one can deconvolute *d* into d_l and d_w as follows. First we determine the lipid volume ratio c_v at maximum hydration:

$$c_v = c\ v_l\{cv_l + (1 - c)\ v_w\} \tag{3}$$

where *c* is the lipid weight ratio at maximum hydration, v_l and v_w is the lipid and water specific volume respectively. Thus the bilayer thickness is:

$$d_l = d \cdot c_v. \tag{4}$$

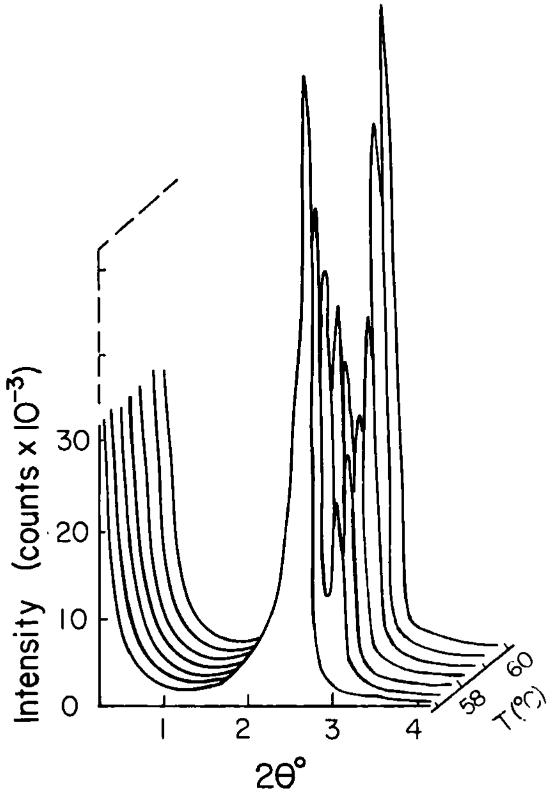

FIGURE 4 Small-angle x-ray diffraction patterns of DSPC (30% water content) at different temperatures around T_m. 2Θ°: is the scattering angle.

The d_l and d_w values calculated by means of the available d and c data and Eqns. (1–3) are collected in Table I. Because of the inaccuracy of DSPC maximum hydration data we did not calculate d_l as described above. Rather we assumed that the thickness of interbilayer water, d_w, depends mainly on the surface structure of the bilayer and is independent of the hydrocarbon chain length. Thus in the case of DSPC we used the d_w data of DPPC, and applying Eqn.1 we got the d_l data.

By means of the bilayer thickness data one can determine the hydrocarbon chain tilting, φ, in the $L_{\beta'}$ phase:

$$\varphi = \cos^{-1}\{d_{l(g)}/d_{l(i)}\} \qquad (5)$$

where $d_{l(g)}$ and $d_{l(i)}$ is the bilayer thickness in the $L_{\beta'}$ (gel) and P_β

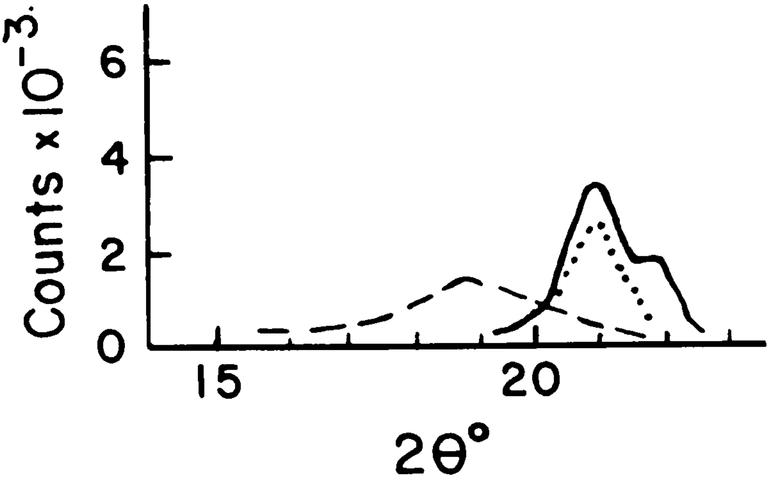

FIGURE 5 High-angle x-ray diffraction patterns of DMPC. Solid line: $T_{\beta'}$ phase, dotted line: P_β phase, dashed line: T_α phase. 2Θ°: is the scattering angle.

(intermediate) phase respectively. This formula is valid because of the symmetry of the high-angle diffraction pattern. Thus the hydrocarbon chains are not tilted in the P_β phase (see Figure 5). The obtained tilt angles are shown in the last column of Table I. These angles are smaller by ~10° than the results of Janiak et al.[2] They determined the bilayer thickness for the phospholipid with hydrocarbon chains fully extended and normal to the bilayer plane by CPK model, d_{CPK}, and used this value in Eqn.4 instead of $d_{l(i)}$. However, between the polar heads there are water molecules and consequently the water and lipid layer overlap. Thus the bilayer thickness obtained from the x-ray P_β phase data are always smaller than the CPK data, $d_{\mathrm{CPK}} > d_{l(i)}$. Therefore in Janiak's formula, the numerator is not consistent with the denominator resulting in larger tilt angles.

One can determine the linear thermal expansion coefficient of the bilayer in L_α phase by the following formula:

$$\alpha_l = \{(\partial d/\partial T)_{T>T_m} - \alpha_w \cdot d_w\}/\, d_l \qquad (6)$$

where $(\partial d/\partial T)$ is the slope of the interlamellar repeat distance, α_w is the linear thermal expansion coefficient of water ($\alpha_w = 1.3 \cdot 10^{-4}\mathrm{deg}^{-1}$ at 41°C and $\alpha_w = 1.6 \cdot 10^{-4}\mathrm{deg}^{-1}$ at 55°C (6)). By means of the measured interlamellar repeat distances and Eqn.5 $\alpha_l = -2.4 \cdot 10^{-3} \pm 5 \cdot 10^{-4}\mathrm{deg}^{-1}$ for DPPC and $\alpha_l = -3.2 \cdot 10^{-3} \pm 6 \cdot 10^{-4}\mathrm{deg}^{-1}$ for DSPC.

TABLE I

Structural parameters of phosphatidylcholine bilayers

lipid	phase	c(wt/wt)	v_l(cm^3/g)	v_w(cm^3/g)	d (Å)	d_l(Å)	d_w(Å)	φ(deg)
DMPC	$T_{\beta'}$(10°C)	0.71	0.924	1.000	61	42.3	18.7	24.8
	P_β(20°C)	0.71	0.937	1.002	67	46.6	20.4	0.0
	T_α(37°C)	0.60	0.980	1.007	65	38.6	26.4	-
DPPC	$T_{\beta'}$(32°C)	0.73	0.947	1.005	63	45.3	17.7	17.3
	P_β(38°C)	0.69	0.955	1.007	70	47.5	22.5	0.0
	T_α(45°C)	0.64	1.005	1.010	67	42.8	24.2	-
DSPC	$T_{\beta'}$(40°C)				70	52.3	17.7	24.4
	P_β(53°C)				80	57.5	22.5	0.0
	T_α(60°C)				73	48.8	24.2	-

c(wt/wt): is the weight ratio of the lipid at maximum hydration, data for DMPC and DPPC are from Ref. 2 and Ref. 3 respectively. v_l (cm^3/g): is the specific volume of lipid from Ref. 7. v_w(cm^3/g): is the specific volume of water from Ref. 6. d (Å): is the interlamellar repeat distance. d_w(Å): is the thickness of interbilayer water. d_l(Å): is the thickness of lipid bilayer. φ(deg): is the tilt angle of the hydrocarbon chains relative to the bilayer normal.

Finally the small angle x-ray diffraction patterns in Figure 5 clearly show the coexistence of P_{β} and L_{α} phases (within 2–3°C temperature range) during the main phase transition. This result is not in contradiction with the Gibbs phase rule due to the constant presence of physical defects in the two-dimensional array of condensed lipid aggregates. These defects are considered to be an extra component in the system. We note that these phases coexist in chemically highly pure systems too.[8]

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ABBREVIATIONS

DMPC: $L - \alpha$ dimyristoylphosphatidylcholine
DPPC: $L - \alpha$ dipalmitoylphosphatidylcholine
DSPC: $L - \alpha$ distearoylphosphatidylcholine
T_{β},: one-dimensional lamellar lattice of tilted crystalline-like hydrocarbon chains
P_{β}: two-dimensional monoclinic lattice of straightened crystalline-like hydrocarbon chains
T_{α}: one-dimensional lattice of liquid paraffin-like hydrocarbon chains

References

1. A. Tardieu, V. Luzzati and F. C. Reman, *J. Mol. Biol.*, **75**, 711 (1973).
2. M. J. Janiak, D. M. Small and G. G. Shipley, *Biochemistry*, **15**, 4580 (1976).
3. Y. Inoko and T. Mitsui, *J. Phys. Soc. of Japan*, **44**, 1918 (1978).
4. J. Stamatoff, B. Feuer, H. J. Guggenheim, G. Tellez and T. Yamane, *Biophys. J.*, **38**, 217 (1982).
5. B. K. Vainshtein in: Diffraction of X-ray by Chain Molecules. Elsevier, Amsterdam (1966).
6. Handbook of Chemistry and Physics 49th edition p. F-5.
7. J. F. Nagle and D. A. Wilkinson, *Biophys. J.*, **23**, 159 (1978).
8. N. Albon and J. M. Sturtevant, *Proc. Natl. Acad. Sci. USA*, **75**, 2258 (1978).