LETTER TO THE EDITOR

Dimensions of the "Intermediate" Phase of Dipalmitoylphosphatidylcholine

Dear Sir:

Stamatoff et al.'s (1) determination of ripple amplitude in the "intermediate" or "ripple" or P_β phase (2–5) of dipalmitoylphosphatidylcholine (DPPC) allows a striking-comparison between the structural parameters of this phase and its neighboring "gel" or $L_{\beta'}$, and "liquid-crystal" or L_α phases. The intermediate P_β phase shows virtually the same molecular cross-sectional area as the higher-temperature liquid crystal, L_α, despite maintaining the rigid hydrocarbon-chain packing and mean bilayer thickness of the lower temperature gel, $L_{\beta'}$ phase. The intermediate P_β phase structure seems to be the system's way of compromising the need of polar groups to achieve a laterally expanded state even though the nonpolar hydrocarbon chains remain rigid.

To see how molecular area and bilayer thickness are extracted from the ripple structural dimensions, consider Fig. 1. The surface-to-volume ratio for a rippling bilayer is equal to the ratio of the (here sinusoidal) ripple contours to the area between the lines $y = \pm (d_l/2) + \sin(2\pi x/d_r)$. (I follow the notation of reference 1 where A is ripple amplitude and d_r ripple repeat; in addition, d_l is bilayer thickness and d_w bilayer separation.) After some algebra, one finds the length of the two lines in Fig. 1 can be reduced to $4\, d_r/\pi \sqrt{1 + p^2}\, E(m)$ where $E(m)$ is the complete elliptic integral of the second kind (6), $m = p^2/(1 + p^2)$ and $p = (2\pi A/d_r)$. The area between the lines is $d_l \times d_r$. Thus, the area per phospholipid molecule $A_l = [4v_l \sqrt{1 + p^2}\, E(m)]/(d_l\pi)$, where v_l is molecular volume.

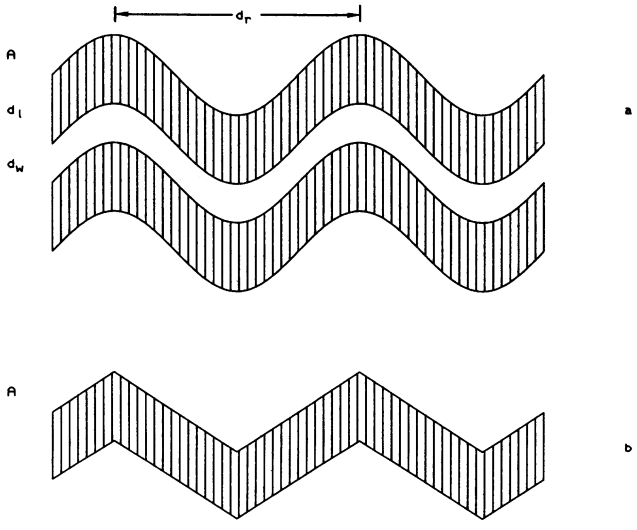

FIGURE 1 Schematic diagram showing parameters characterizing the intermediate phase.

Using the convention (2) that a bilayer contains all the lipid and only lipid and taking 1.045 g/ml (7) for the density of the whole DPPC molecule in the ordered hydrocarbon phase, one obtains $v_l = 1.17 \times 10^3$ Å^3 (733 mol wt). At the repeat spacing of 67 Å in 35% water reported by Stamatoff et al. (1) at 37°C, $d_l = 0.64 \times 67 = 42.9$ Å and $d_w = 24.1$ Å. Given $A = 25$ Å and $d_r = 145$ Å, we have $p = 2\pi 25/145$, $m = 0.54$, $E(m) = 1.33$ (6), and $A_l = 68$ Å^2. This area compares well with the 71 Å^2 cross-sectional area of DPPC in the liquid crystal phase at 50°C but poorly with the 52.3 Å^2 area found for the gel state at 25°C using the densities of reference 7 and the convention for dividing lipid from water with the multilayer dimensions given in reference 8 (see Table I). The bilayer thickness $d_l = 42.8$ Å is close to the thickness $d_l = 44.1$ Å found in the gel state but differs strongly from the $d_l = 34.2$ Å observed in the liquid crystal (8). (The small difference between $d_l = 42.8$ and 44.1 Å may indicate some chain tilting [4] in the ripple structure.) All of these comparisons have been made for conditions of excess water wherein the multilayer lattices are not distorted under stress (8–12) due to removal of water. Similar comparisons for bilayer thickness, but not area, were reported from neutron diffraction studies in limited amounts of water (13); it is difficult to compare calculations based on data from reference 2 with those from reference 1 since reference 2 does not give results taken under conditions of excess water.

The ripple need not be imagined as sinusoidal but rather a zig-zag, as argued by Larsson (14) from considerations of $-CH_2$-packing (14–15). Then, with Stamatoff et al.'s (1) A and d_r retained (Fig. 1 *b*), the area A_l is 66 Å^2 while d_l remains unchanged. The comparisons with gel and liquid

TABLE I
LATTICE DIMENSIONS

Phase	Temperature	d_{repeat}	d_{lipid}	d_w	Area
		Å	Å	Å	Å^2
$L_{\beta'}$ (reference 7)	25°	63.8	44.1	19.7	52.3
P_β (reference 1)	37°	67.0	42.9	24.2	68
L_α (reference 7)	50°	67.0	34.2	32.8	71

crystal states made above still hold nearly as well; the bilayer thickness is similar to that in the gel state but the polar group area is similar to that of the liquid crystal phase. Note that these area estimates are relatively insensitive to experimental error in A or d_r. For example, a 10% error in either A or d_r causes a change of only 3 or 2% in A (for sinusoidal and zig-zag models, respectively).

Early suggestions from calorimetric data that the 34°C "pretransition" showed a polar group rotation (16, 17) disagreed with deuterium and phosphorous magnetic resonance measurements on the conformation and motion of the choline head group (18). In that magnetic resonance study, no change in signal was detected at 34°C, but a strong discontinuity was seen at 41°C where the hydrocarbon chains experienced a order-disorder transition.

However, findings with spin labels (19) and deuterium NMR (20) suggest that there can be rotation about the molecular long axis in a frozen chain system (19, 20). In their deuterium NMR study of the nonpolar regions, Westerman et al. (21) also conclude that there is long-axis rotation of the whole phosphatidylcholine molecule even though the chains are stiff. In agreement with Marsh (19), they find that this rotation is extensive in the region of the intermediate phase, but that it is almost, but not completely, frozen out in the gel state below the pretransition. The rotation is, in fact, indicated by two spectral patterns ascribed (22) either to two differently rotating populations in one ripple structure or to two different, coexisting, ripple structures (which, if carried to full definition as two different ripple phases, would, of course, violate the phase rule). Boroske and Trahms (22) use ^1H and ^{13}C NMR to argue that long-chain rotation is oscillatory motion in the gel phase but that there is "quasi-free chain rotation" in the intermediate phase.

Any reasonable picture of the intermediate phase should be able to combine these resonance data with the polar group area discontinuity of DPPC at 34°C revealed by x-ray diffraction. It seems possible that the motion of polar groups in the intermediate phase is severely restricted by being attached to rigid hydrocarbon chains but that a sudden disordering of the polar groups' orientation with respect to each other can occur with increased whole-molecule rotation at the pretransition. It is to accommodate this disordering, while maintaining dipoles parallel to the membrane surface (13), that the cross-sectional area per molecule can increase, presumably requiring rippling or zig-zagging to occur . The latter requirement may well be met by tendencies to spontaneous curvature (23, 24) or the relaxation of accumulated strain (14). Indeed, Scott (25) proposed a model wherein long-molecule rotations are proposed to be the central feature of the pretransition (although he now appears [26] to emphasize the role of next nearest neighbor interactions).

All models so far seem unable to incorporate Stamatoff et al.'s observation (1) that the ripple repeat period, d_r, increases from 145 Å to 164 Å when multilayer water content is dropped from 35 to 25%; ripple amplitude, A, decreases slightly, from 25 to 23 Å. Bilayer interaction must be influencing these parameters.

An important factor, only beginning to be recognized (8, 27, 28) for its role in lipid aggregate dimensions and phase transitions is the action of hydration forces virtually always seen between bilayers (7, 8, 10, 11). H. Wennerstrom (personal communication) has used the present values of amplitude A and repeat period d_r to compute the effect of ripple structure on the order parameter for water. He finds a change by a factor of two upon transition, comparable with what he has observed experimentally. Rippling, with its increase in molecular area, also entails an increase in bilayer separation (Table I and reference 7). In many cases interbilayer hydration forces appear to increase with molecular area (8). It is hard to imagine that this correlation between the swelling of multilayers and the strengthening of repulsive forces is fortuitous.

Despite the unlikelihood that it will be seen in a biological system, the intermediate phase has been the object of much theoretical and experimental attention. This is due in part to the unusual opportunity it presents to elucidate conflicting tendencies of polar and nonpolar parts of membrane phospholipids.

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