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Small Phosphatidylcholine Vesicles Appear to be Faceted Below the Thermal Phase Transition

A.E. Blaurock* and R.C. Gamble

Arthur A. Noyes Laboratory of Chemical Physics, Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125

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Summary. We have studied the main thermal transition in dipalmitoyl phosphatidylcholine (DPPC) multilayers and a similar transition in small (~300 Å diameter), single-walled vesicles by X-ray diffraction. As judged by the large-angle diffraction, the transition in the multilayers is narrow; aside from small tails, the transition occurs over a range of 0.5 °C. In contrast, the transition in the vesicles is quite broad; the range is about 7 °C. These observations are in agreement with recently published data.

Referring to the small vesicles below the thermal transition, a bilayer structure in which the C_{16} chains are all straight and pointed radially is inconsistent with the large-angle diffraction. Assuming instead that the chains are packed in a regular, planar array, it is clear from their small size that the vesicles can have only limited regions of planar packing. The X-ray data indicate that the planar regions are 75 Å across on the average. In view of the 75-Å size and the average vesicle diameter of about 300 Å, we propose that the small vesicles are faceted below the transition, i.e., that the vesicles are polygonal. The small-angle diffraction pattern from the vesicles below the transition provides support for the faceted structure.

Dipalmitoyl phosphatidylcholine (DPPC) multilayers undergo two transitions as the temperature is raised from 30 to 45 °C. These thermal transitions have been studied by various physical techniques (for a review, *see* Melchior & Steim, 1976). As studied by calorimetry, the main transition occurs at $T_2 = 41.4$ °C, and there is a smaller, "pre-transition" at $T_1 = 35.3$ °C (Mabrey & Sturtevant, 1976). In the case of X-ray diffraction, the large-angle pattern is quite sensitive to both thermal transitions. Below the pretransition, there are at least three rings with Bragg spacings near 4 Å (Tardieu, Luzzati & Reman, 1973; Janiak, Small & Shipley, 1976). Between the pretransition and the main transition, there is a single, fairly sharp, ring with a Bragg spacing of 4.2 Å. Above the main

* *Present address:* Anatomy Department, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514.

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transition, there is a single ring, which is centered at a Bragg spacing of 4.6 Å and which is considerably broader than just below the transition. Our own observations have confirmed this series of large-angle patterns (A.E. Blaurock, *unpublished*). The large-angle patterns indicate that the C_{16} chains rearrange somewhat at the pretransition, transforming from a closer packed, more nearly crystalline array into a somewhat looser, apparently hexagonal, array and that they rearrange considerably at the main transition, transforming into a packing similar to that in the liquid hydrocarbons (Tardieu *et al.*, 1973).

In the present study our main interest is in a similar thermal transition in small, single-walled vesicles. Below the transition we find a large-angle reflection at 4.2 Å which is similar to the reflection from the multilayers just below the main transition. As the temperature is raised in steps, the 4.2 Å reflection from the vesicles evolves, over a range of about 7 °C, into a reflection which is similar to the 4.6 Å reflection from multilayers. In contrast, at the main transition in multilayers the 4.2 Å reflection is converted into the 4.6 Å reflection over a range of only 0.5 °C. The broader temperature range for the small vesicles indicates that the size of the cooperative unit is an order of magnitude smaller than for the multilayers.

The radial width of the 4.2 Å reflection from the small vesicles below the transition is not consistent with a spherical shape for the vesicles; the reflection is too narrow. Instead we interpret this reflection in terms of regular, planar packing of the C_{16} chains in the bilayer. We suggest that the small vesicles have facets, i.e., are polygonal below the transition. Based on the radial width of the 4.2 Å ring, the facets are roughly 75 Å across. For a vesicle 300 Å in diameter, there will be up to 45 facets; fewer if some of the DPPC molecules pack irregularly in edge regions between facets.

The small-angle X-ray diffraction pattern supports our model of a polygonal shape. Below the transition, the pattern from the vesicles in dilute $Ca(NO_3)_2$ shows the effects of cross interference between bilayers; the bilayers are 68 Å apart, center to center. On this basis we suggest that the vesicles in dilute $Ca(NO_3)_2$ tend to aggregate below the transition with bilayer facets parallel. About 15% of the total bilayer area is paired in this way. The cross-interference effects disappear above the transition, indicating that the bilayers are no longer paired. Vesicles in dilute $La(NO_3)_3$ do not show the cross-interference effects either below or above the transition temperature.

Materials and Methods

Dipalmitoyl phosphatidylcholine (DPPC) was purchased from Calbiochem (San Diego, Calif.). Multilayer specimens were prepared by sealing some of the DPPC together with excess water ($\sim 1:1$ wt/wt DPPC to water) in a temperature-controlled metal chamber having 25 μm-thick Al windows to transmit the X-rays. Small vesicles were prepared as reported (Petersen & Chan, 1978) by sonicating a mixture of 10% DPPC in dilute $Ca(NO_3)_2$ or $La(NO_3)_3$. A 150 W sonicator (Measuring and Scientific Equipment, Ltd., Crawley, England) with a Ti microtip was used at the highest power. The tube holding the suspension was immersed in glycerol and the contents were sonicated continuously for 15 min. At the end of sonication the temperature was well above T_2, effectively annealing the vesicles (Lawaczeck, Kainosho & Chan, 1976). The sonicated specimens characteristically looked hazy and somewhat blue. The Ca $(NO_3)_2$ or $La(NO_3)_3$ was added to avoid having the vesicles aggregate and revert to multilayers. On one occasion the suspension changed from the original hazy blue to a milky white. This change was correlated with a series of sharp rings in the small-angle diffraction pattern, indicating that multilayers had formed. The reason for the vesicles reverting to multilayers in this one case is not known.

Multilayer specimens and some vesicle suspensions were exposed in a Franks-type small-angle X-ray camera (Blaurock, 1973) on a microfocus X-ray generator (Jarrell-Ash, Cleveland, Ohio) having a Cu target (300 W maximum tube power). The small-angle (large Bragg spacings) diffraction was recorded on X-ray film (Ilford Ind. G). The large-angle (small Bragg spacings) diffraction was recorded both on film and with a position-sensitive detector (*see* below). Large-angle patterns from the small vesicles were obtained using the Caltech focusing monochromator (Webb *et al.*, 1977) at the Stanford Synchrotron Radiation Laboratory (SSRL). The high flux of synchrotron radiation, about 3×10^9 photons/sec, was useful in obtaining accurate data in a reasonable time. (In fact, it was necessary to attenuate the beam a few-fold to keep within the maximum count rate of the detector). Two temperature series were recorded using the Franks camera mounted on a rotating-anode generator (Elliott GX6, Borehamwood, England) with Cu target (1600 W maximum tube power), but these data were less accurate owing to the smaller number of counts recorded by the detector.

Large-angle diffraction patterns were recorded using a linear, position-sensitive detector with a resolution of 0.4 mm (Blaurock, 1978). This resolution would not be adequate to study the shapes of the 4-Å reflections from the multilayers below the pretransition; however, preliminary observations using X-ray film established that the 4.2-Å reflection from the small vesicles was broadened to the extent that it could be studied satisfactorily with the detector. Much the same radial width was found for the 4.2Å reflection by the two methods, film and the detector. The detector was placed with its axis perpendicular to the beam diffracted at 4.2 Å. This was done to avoid the effects of parallax in the 6 mm-thick detector, which would tend to broaden the reflection. The diffraction pattern was recorded for 5–15 min at each temperature.

The temperature of the specimen was controlled by a thermostated, circulating bath. A temperature-sensitive integrated circuit was embedded in the specimen chamber, which made it possible to read the temperature directly using a digital voltmeter. A small correction was applied, which was determined by calibrating the temperature sensor with an accurate mercury thermometer. The sensor showed that the temperature remained constant to less than 0.1 °C while the diffraction pattern was recorded.

The radial width of an X-ray reflection is a measure of the size of the array giving rise to the reflection (*see*, e.g., Guinier, 1963); both the size of the incident X-ray beam and the point-to-point resolution of the film or detector need to be allowed for. The full width at half the maximum height (FWHM) of the 4.2-Å reflection was determined

as follows. First, the diffraction angles of the two half-heights of the peak were determined, both on densitometer tracings and on detector scans. These angles were then used to calculate the two corresponding Bragg spacings. Finally, the width of the peak is defined as the difference between the reciprocals of the two spacings; this definition conveniently permits direct comparison with widths calculated from model shapes for the array.

Results

The change in the large-angle diffraction pattern at the main thermal transition in the DPPC multilayers is illustrated in Fig. 1. Just below the transition ($T=40.0$ °C) a sharp peak is seen. Near the upper end of the transition ($T=40.5$ °C), there is predominantly a broader peak

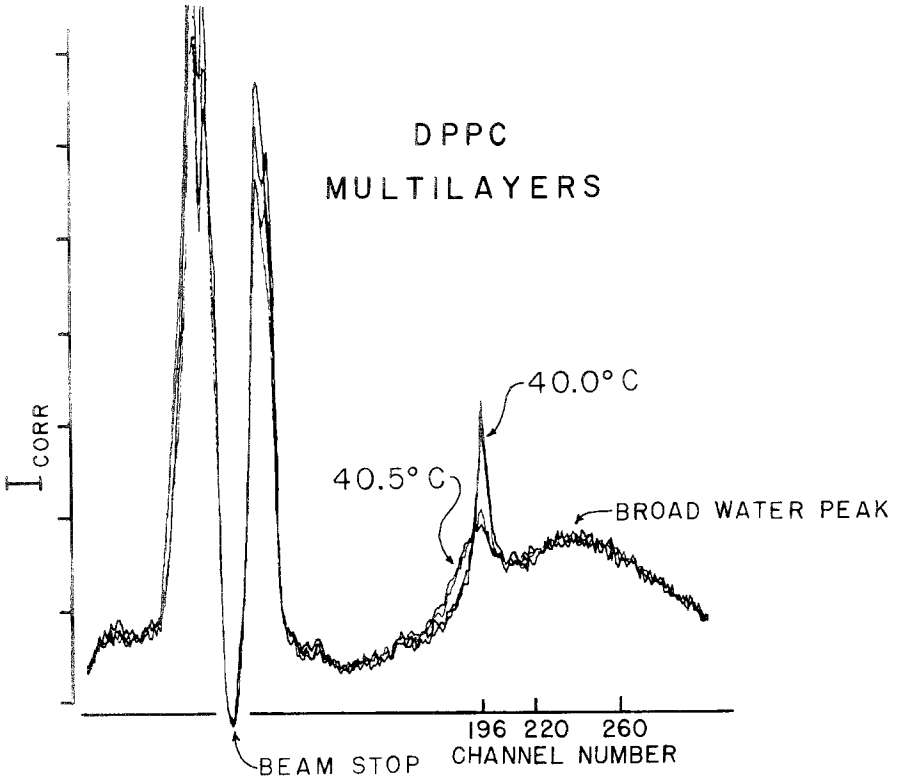

Fig. 1. Large-angle diffraction patterns from multilayers in excess water. The patterns have been corrected for detector efficiency as in Blaurock (1978); abscissae are on an arbitrary linear scale. A sharp peak at 4.2 Å in the patterns recorded at $T=40.0$ °C is largely replaced by a lower, broader peak centered at 4.6 Å when T is raised to 40.5 °C, defining the main transition. A Franks'-type X-ray camera (Blaurock, 1973) was used on a micro-focus generator (Jarrell-Ash). The diffraction patterns were recorded with the axis of the linear, position-sensitive detector normal to the 4.2-Å diffraction and 8 cm from the specimen; 500 sec per pattern

at a somewhat smaller diffraction angle (hence somewhat larger Bragg spacing). Two pairs of patterns are shown; for one pair the temperature was raised, while for the other pair it was lowered, between patterns. Thus, Fig. 1 illustrates the absence of hysteresis, and also the narrowness of the transition.

The counts at the peak of the 4.2-Å reflection (Fig. 1) were normalized by dividing them by the integrated counts in a region largely free of diffraction from the DPPC multilayers. The latter region lay within the broad water peak (channels 220–260 in Fig. 1). The region did not change appreciably with temperature. This was shown by dividing a pattern recorded above T_2 by a pattern recorded below, point for point: the water peak was flat to within the errors of measurement. Figure 2 shows the normalized counts in the single channel at the peak of the 4.2-Å reflection (usually channel 196) as a function of temperature. The decline of the 4.2Å peak indicates that fewer of the C_{16} chains are packed in regular array as the temperature rises, while more of them contribute to the broader peak at 4.6 Å. Thus the large-angle diffraction is a direct measure of the degree of transition from regular to liquid-like packing of the chains.[1] When the linear part of the transition is extrapolated to the plateaus above and below it (dashed lines in Fig. 2), the full width of the transition is 0.45 °C. The midpoint lies between 40.3 and 40.4 °C. Figure 2 also illustrates the absence of hysteresis since it is a composite of temperature scans both ascending and descending with time.

We note that we have also integrated the counts over a range sufficiently broad to include both the 4.2-Å and the 4.6-Å reflections (channels 150–220). We find that the total count depends little, if at all, on temperature.

The transition in the small, single-walled vesicles (Fig. 3) is quite different from the main transition in the multilayers. In contrast to the multilayers, the shape of the large-angle peak from the vesicles evolved over a broad range of temperatures. Representative patterns are shown in Fig. 3. It can be seen that the shape of the large-angle peak changes only slightly for each 1 °C change in temperature. The 4.2-Å peak, to the right of the vertical line in Fig. 3, is gradually replaced by a lower, broader peak to the left of the vertical line as T increases.

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[1] The 4.2-Å reflection disappears at the upper end of the main transition. I_{196} does not go to zero in Fig. 2 because there are residual contributions from water and from the 4.6-Å reflection.

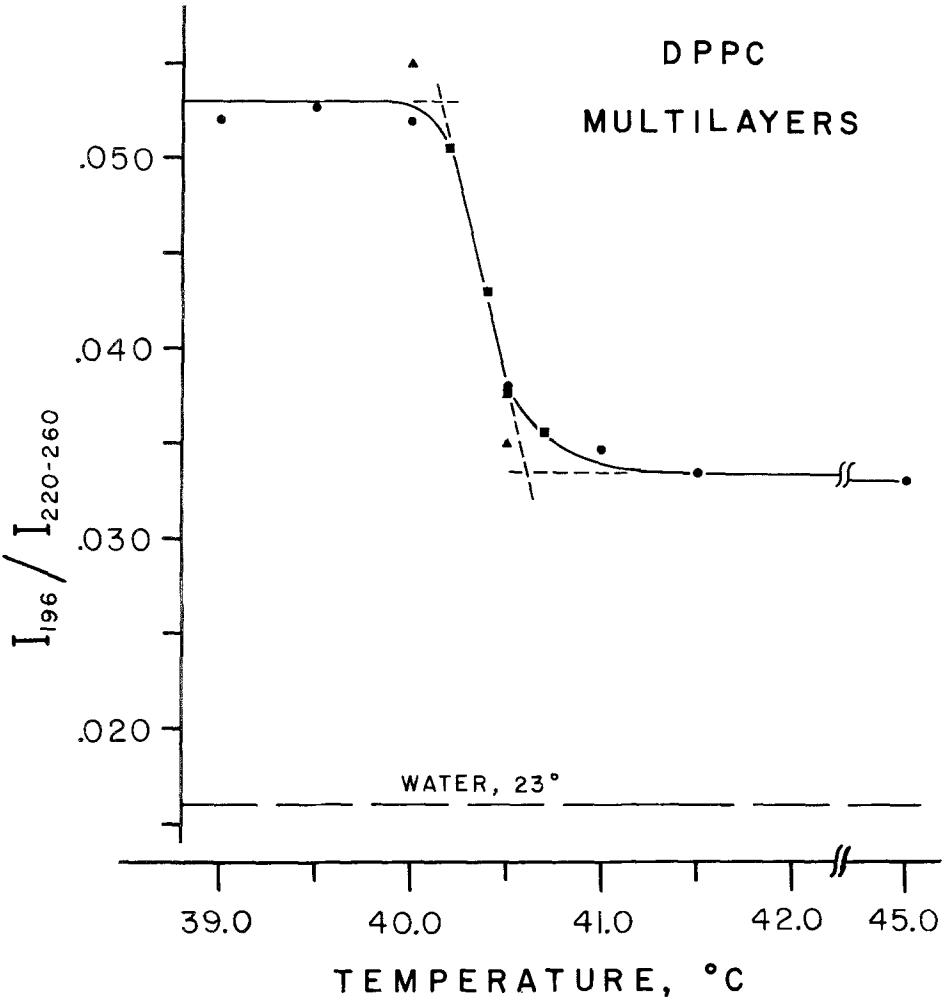

Fig. 2. Height of the 4.2-Å diffraction *vs.* temperature. The counts at the peak of the 4.2-Å reflection (usually channel 196) were normalized by integrating channels 220–260, using data like those in Fig. 1. The dashed line below shows the contribution by water at $T=23$ °C to channel 196. ●, ■ : ascending scans; ▲ : descending scan

The transition in the vesicles was determined as follows. The intensity of the 4.2-Å reflection was measured somewhat arbitrarily by integrating the counts in channels 136–146. No attempt was made to separate a probable contribution from the broad 4.6-Å reflection, nor was a shift of the reflection to the right at lower temperatures (Fig. 3) taken into account (*see Discussion*). The scattering by water was eliminated by subtracting the counts below a sloping baseline (continuous lines beneath the diffraction patterns in Fig. 3). The intensity of the 4.6-Å reflection

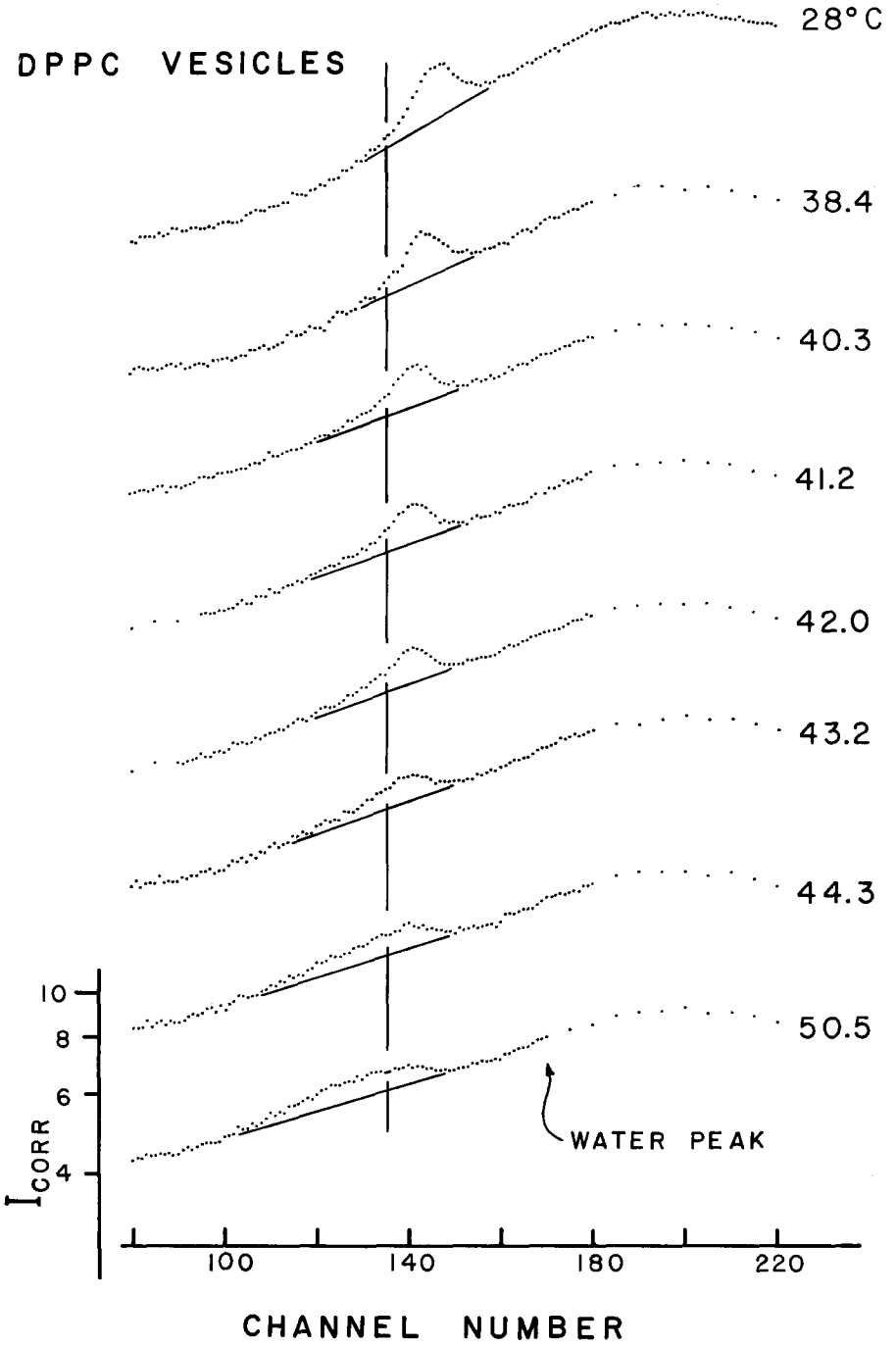

Fig. 3. Variation with temperature of the large-angle diffraction pattern from small vesicles. The patterns have been corrected for detector efficiency as in Blaurock (1978). Ten percent DPPC in 40 mM $Ca(NO_3)_2$ (wt/vol) was sonicated as described in Petersen and Chan (1978). The patterns were recorded as in Fig. 1 except that the Caltech focusing monochromator (Webb *et al.*, 1977) was used at SSRL and the axis of the detector was 14 cm from the specimen; 300 sec per pattern. The Bragg spacing of the verical line is about 4.5 Å

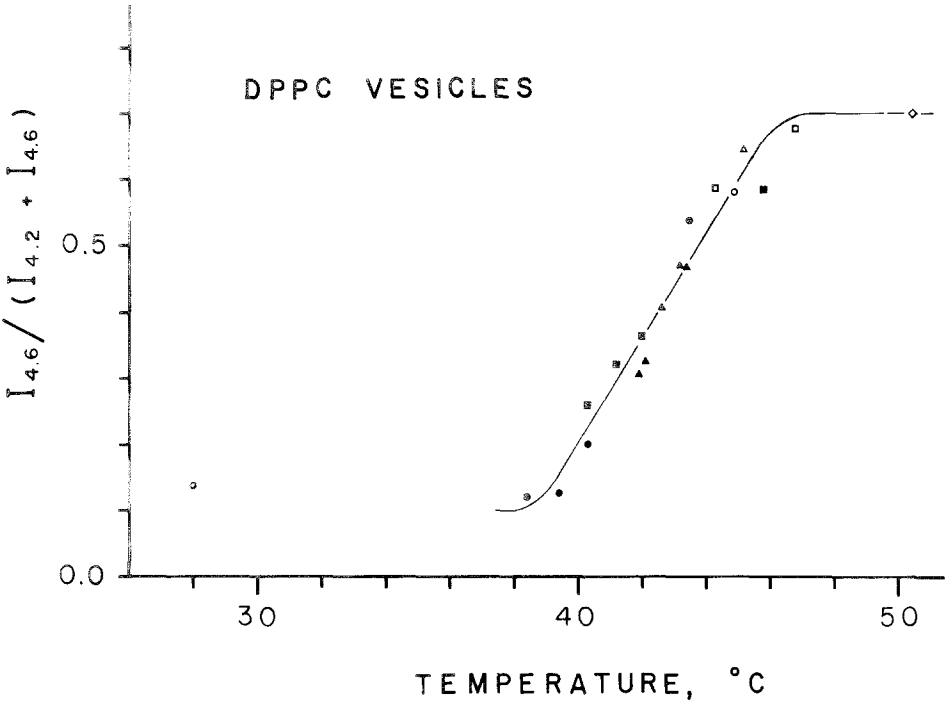

Fig. 4. Ratio of the 4.6-Å to the (4.2-Å + 4.6-Å) diffraction from small vesicles *vs.* temperature. Counts were integrated as described in *Results* using data like those in Fig. 3. ●, ■, ▲, ○, ◇: ascending scans; □, △: descending scans. Inspection of similar large-angle patterns recorded on the rotating-anode generator (*see Results*) indicates that the curve levels off below 38 °C. The plateaus below and above the transition are 0.1 and 0.7, respectively; that these are not 0 and 1, respectively, appears to be simply an artifact of the way in which the 4.2 and 4.6-Å reflections were measured

was measured in a similar way by integrating channels 114–135. Figure 4 shows the ratio of the 4.6-Å reflection to the sum of the 4.2 plus 4.6-Å reflections as a function of temperature. We note that similarly to the multilayers, the latter sum was constant to within a few percent. The transition illustrated by Fig. 4 is broad; it extends from below 39 to above 46 °C with the mid-point near 42.5 °C.

As noted in *Materials and Methods*, the large-angle diffraction was similarly recorded from small vesicles using a rotating-anode generator. Temperature series were recorded for vesicles in both 40 mM $Ca(NO_3)_2$ and 2 mM $La(NO_3)_3$. Although the data were statistically less accurate, the shape of the large-angle peak evolved in much the same way as in Fig. 3. The change in the shape of the peak, and the range of temperature over which the change occurred, were both similar to the series in Fig. 3.

The radial width (FWHM) of the 4.2-Å reflection from the small vesicles is 0.0135 ± 0.0025 Å^{-1}. The reciprocal of this value, 74 Å (range: 63–91 Å), is a measure of the size of the facets postulated for the vesicles. Choosing an exact shape for the facets has, in fact, only a small effect on this estimate (*see Discussion*). At the low-temperature end of the respective transitions, the 4.2-Å reflection from the small vesicles was 2–3 times as wide as that from the multilayers, as measured on X-ray film. This observation indicates that there are larger regions of regular packing of the C_{16} chains in the multilayers and/or more nearly crystalline packing.

The pretransition in DPPC multilayers is characterized by a comparatively small but clear-cut change in the large-angle diffraction pattern (Tardieu *et al.*, 1973; Janiak *et al.*, 1976; A.E. Blaurock, *unpublished*). It is difficult to say whether the pretransition occurs in the small vesicles; our data do not give clear evidence of it (*see Discussion*).

The *small-angle* diffraction pattern from the suspension of small, single-walled vesicles above the transition is illustrated by the lowermost curve in Fig. 5(*a*). The broad band in the corrected densitometer tracing is characteristic of isolated lipid bilayers generally, i.e., when they are not stacked in multilayers (Wilkins, Blaurock & Engelman, 1971). Multilayers would, if they were present in the specimen, concentrate the diffracted X-rays into a few sharp rings (*see*, e.g., Janiak *et al.*, 1976), giving narrow peaks in the tracing. Hence even a small proportion of them would be detected. The absence of such sharp rings from the small-angle pattern therefore shows that multilayers generally were not present in our preparations of small vesicles.

Below the thermal transition in the small vesicles, we have sometimes seen an interesting interference effect. The uppermost curve in Fig. 5(*a*) illustrates a small-angle diffraction pattern from the vesicles below the transition. In this pattern, from vesicles at $T = 23$ °C in 2 mM $Ca(NO_3)_2$, the bilayer band shows two maxima. This effect suggested to us the pairing of planar bilayers, i.e., two bilayers lying parallel and at a fixed distance from one another. From the frequency of the cross-interference effect, the center-to-center distance between the two bilayers is estimated to be 68 Å (Fig. 5(*b*)). In contrast, the small-angle pattern from vesicles at $T = 23$ °C in 2 mM $La(NO_3)_3$, the middle curve in Fig. 5(*a*), has the simple form characteristic of an isolated bilayer (Wilkins *et al.*, 1971) and is without any apparent effect of cross-interference between bilayers.

As noted above, the lowermost curve in Fig. 5(*a*) is characteristic of an isolated bilayer and hence indicates that there is no pairing of

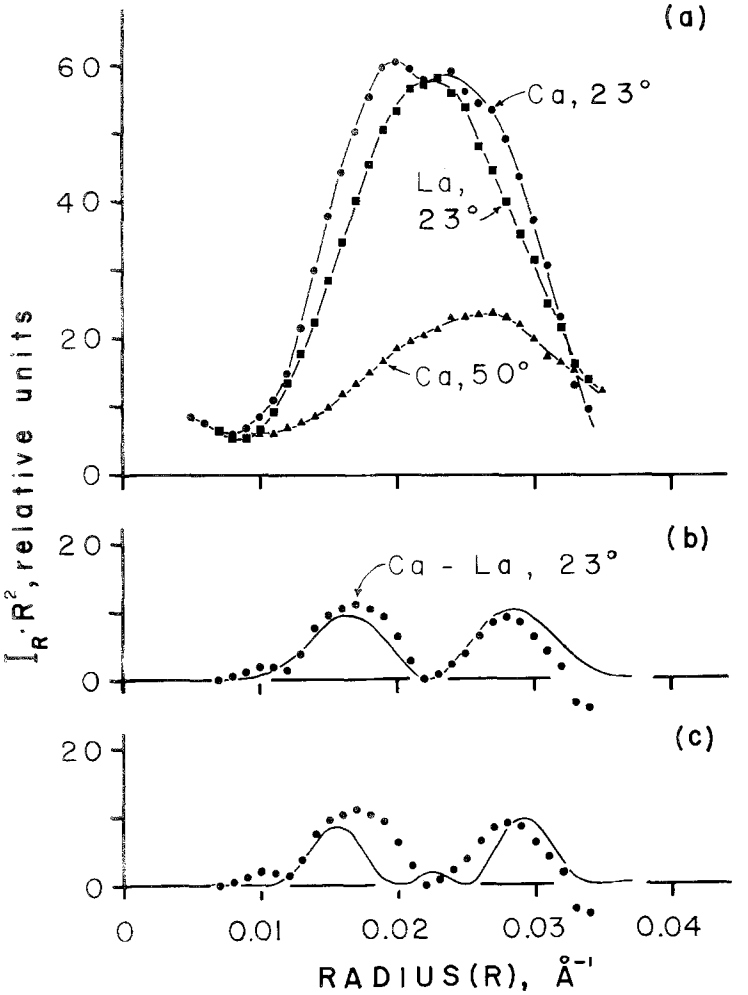

Fig. 5. Small-angle diffraction from small vesicles. Patterns were recorded on X-ray film using a Franks camera; specimen-to-film distance was 6.5 cm. Densitometer traces of the film were made using a microdensitometer (Joyce-Loebl Mark III C). (*a*): The corrected (Wilkins *et al.*, 1971) radial densitometer traces from vesicles: (i) in 2 mM $Ca(NO_3)_2$ at 23 °C (upper curve); (ii) in 2 mM $La(NO_3)_3$ at 23 °C (middle curve; scaled to (i) as in Results); (iii) in 2 mM $Ca(NO_3)_2$ at $T = 50$ °C (lower curve; scaled down for clarity). The lower curve appears shifted to the right, indicating that the bilayer is *thinner* above the transition; a similar result holds for the main transition in DPPC multilayers (*see*, e.g., Inoko & Mitsui, 1978). (*b*): The difference between the upper and middle curves in (*a*). The full circles are the observed difference, and the continuous curve was calculated assuming two bilayers centered 67.5 Å apart. (*c*): As for (*b*) except that the continuous curve was calculated assuming three bilayers spaced by 67.5 Å, center to center

bilayers above the transition. When the temperature was lowered below the transition, the cross-interference effect once again became visible in the diffraction pattern, indicating that the pairing is reversible. It

is possible that the pairing is lost above the transition because the vesicles, which appear to be faceted below the transition, become round above it (*see Discussion*). When $La(NO_3)_3$ was substituted for $Ca(NO_3)_2$, the cross interference effect was never seen. In this case we conclude that pairing over an appreciable surface area did not occur at any temperature.

To test the idea of a pairing of bilayers, we have calculated the diffraction to be expected from parallel bilayers 68 Å apart (Fig. 5(*b*)). Assuming that the bilayer has a symmetric profile of electron density, the diffraction from the pair of bilayers will be the product of the diffraction from an isolated bilayer multiplied by a $\cos^2$ function (*see*, e.g., Guinier, 1963). For a center-to-center distance of 68 Å, the diffraction pattern from a pair of symmetric bilayer profiles will have a zero of intensity at a Bragg spacing of 45 Å. (This spacing lies at the peak of the middle curve in Fig. 5(*a*).) The uppermost curve in Fig. 5(*a*) does indeed show a minimum there; however, the intensity is far from zero. Hence we are obliged to assume that most of the diffraction in this case is as though from isolated bilayers, but with a contribution from paired bilayers (Fig. 5(*b*)) which amounts to about 15% of the total intensity. Accordingly, the upper and middle curves have been scaled to one another at the position of the expected zero ($0.022\ Å^{-1}$ in Fig. 5(*a*)). The solid curve in (*b*) represents the predicted diffraction from the pair of bilayers while the circles show the difference between the upper and middle curves in (*a*). The fit of the solid curve to the difference data in (*b*) is sufficiently good to confirm our hypothesis of paired bilayers. The failure to obtain a perfect fit is considered in the *Discussion*.

The predicted diffraction for three parallel bilayers spaced 68 Å apart, center to center, is also presented (solid curve, Fig. 5(*c*)). The two major peaks in the solid curve in (*c*) are narrower than in (*b*); this is the effect of increasing the number of repeating units (Guinier, 1963). A further increase in the number of parallel bilayers would result in a further narrowing of the two major peaks, making the discrepancy between predicted diffraction and the difference data even worse. Hence our results show that the cross-interference effect from the suspension cannot be attributed to stacks containing more than two bilayers.

The assumption that the bilayer profile is symmetric appears reasonable in view of the faceted structure proposed for the vesicles below the transition. We note that the diffraction from a small, spherical vesicle has been considered (Moody, 1975). For a small spherical vesicle, the bilayer profile may in fact be somewhat asymmetric (Chrzeszczyk, Wishnia

& Springer, 1977; N.G. Webb, *unpublished*), but even in this case the effects of curvature and asymmetry on the bilayer diffraction pattern are small (Moody, 1975; Wilkins *et al.*, 1971).

Discussion

As characterized by changes in the X-ray diffraction pattern at 4–5 Å, the main thermal transition in DPPC multilayers in excess water occurs largely in a range of 0.5 °C (Figs. 1 and 2). This result surprised us because the earlier literature indicated a broader transition (*see*, e.g., Chapman, Williams & Ladbrooke, 1967). Since doing the experiment, however, we have found recent reports that the range of the transition is well under 1 °C; for example, Albon and Sturtevant (1978) have observed, by micro-calorimetry, a transition of about 0.1 °C in exceptionally pure DPPC.

We note that our value for T_2, 40.4 °C, is about 1 °C below the value reported by Albon and Sturtevant (1978). A possible reason for the discrepancy is that our DPPC had a minor contaminant. If our specimen had partially dehydrated, T_2 would have been higher, not lower (Tardieu *et al.*, 1973). Contamination by a simple salt could lower T_2 (*see*, e.g., Chapman *et al.*, 1977), but we do not suspect such contamination.

Our main interest has been in a similar thermal transition for small, single-walled vesicles of DPPC ($\sim$300 Å outside diameter; Sheetz & Chan, 1972). The transition has been characterized by changes in the diffraction pattern in the region of 4–5 Å (Figs. 3 and 4). The transition is much broader than in the multilayers, the range being about 7 °C. A similarly large range has been found by other physical techniques. The reported mid-points (Gaber & Peticolas, 1977; Marsh, Watts & Knowles, 1977; Melchior & Steim, 1976; Suurkuusk *et al.*, 1976) generally are lower than we observe, however. In our case the midpoint of the transition may have been raised by the 40 mM $Ca(NO_3)_2$. Petersen and Chan (1978) found that this solution increases the temperature of the main transition in multilayers by 2.5 °C. The midpoint of the transition for our small vesicles, 42.5 °C is, however, about 5 °C higher than in the studies just cited, rather than 2.5 °C.

We note that our study was aided by using the intense synchrotron radiation available at SSRL, as our 10% suspension of DPPC was comparatively dilute for X-ray study.

We have considered two possible arrangements for the C_{16} chains in the bilayer wall of small vesicles below the thermal transition. The most regular arrangement would be one having every chain all-trans and pointed radially. In this case all DPPC molecules could be in environments differing only slightly from one molecule to the next. We note that a similar problem has been discussed in regard to the packing of protein subunits in the spherical viruses, and the solution put forward by Caspar and Klug (1962), "quasi-equivalence", could serve here. A serious weakness of the radial arrangement, however, is that the distance between neighboring chains would vary considerably from one end of the chain to the other; for vesicles 300 Å in outside diameter and chains 20 Å long, the distance would vary by 16%. Thus, for chains 4.8 Å apart (Tardieu *et al.*, 1973) the distance would vary by 0.7 Å. In comparison, the distance between chains in the multilayers changes by only 0.2 Å at the main transition: from 4.85 Å just below (Tardieu *et al.*, 1973) to 5.0 Å just above (Warren, 1933; Zachariasen, 1935). To move the chains from the equilibrium distance by 0.7 Å would require that a good deal of work be done against the van der Waals' forces, i.e., the free energy of the radial arrangement would be much larger than for a flat bilayer.

An experimental test of the radial arrangement is the radial width of the 4.2-Å ring: if the distance between neighboring chains varied by 16%, then the 4.2-Å reflection, which is attributed to the side-by-side packing of the chains (Dervichian, 1964; Levine, Bailey & Wilkins, 1968; Tardieu *et al.*, 1973), should show a corresponding width. In fact, if it were interpreted in this way, the measured width of the 4.2-Å peak, 0.0135 $Å^{-1}$ (FWHM), would correspond to the distance varying by only 6%. Hence the radial arrangement is not consistent with the diffraction data as well as being unlikely on grounds of the internal energy required. For a spherically symmetric structure in which each C_{16} chain is tilted at a fixed angle to the radius, the distance between chains will vary along the length of the chain in a way similar to the radial model. Hence we rule out this possibility also. We have turned therefore to an alternative model in which the chains are in different environments.

The arrangement which we believe to be the most likely for the small vesicles below the transition is one having regions where the C_{16} chains are packed in planar, regular array. For a sizeable array, this arrangement would result in much lower free energy of packing than for the radial model. We doubt, however, that all the DPPC molecules would be packed in such arrays. It seems more likely that there would be interstitial,

"edge", regions where the C_{16} chains are packed irregularly in such a way as to fill in gaps between facets and hence to prevent small ions from permeating the vesicle bilayer (Lawaczeck *et al.*, 1976). The maximum size possible for a facet would, of course, be limited by the overall size of the vesicle. The dihedral angle between adjacent facets probably sets the more stringent limit on facet size, however: relatively large facets would require that there be large dihedral angles, with correspondingly large distortions of the chain packing in the edge regions, and such large distortions would require an unfavorably large free energy. If we assume that the radial width of the 4.2-Å reflection, 0.0135 $Å^{-1}$, is due to limited size of facet, then we can estimate the average size. Assuming a circular facet, the reflection profile will be proportional to $[J_1(\chi)/\chi]^2$; a diameter of 76 Å is calculated from the observed width. This value is almost the same as the estimate in the *Results*. Each facet will then be tilted 30° from its neighbors (*see* below). Facets about 75 Å across thus are in reasonable relation to the vesicle diameter of 300 Å.

The average facet size can be used to estimate approximately the number of facets per vesicle. For circular facets which are 75 Å in diameter, about 45 facets will account for all the surface area. We have examined the five "regular" polyhedra (4, 6, 8, 12, and 20 facets) and find that none of them gives the observed ratio of the diameter of a facet (75 Å) to the overall diameter of the polyhedron ($\sim$ 300 Å): in all cases the ratio tends to be too large. Examining other fairly regular polyhedra (Cundy & Rollett, 1952), we find that one with 32 facets, a truncated isosahedron, gives a reasonable value for the ratio; polyhedra with 62 and 92 facets give too small a ratio. We see no compelling reason to assume that the vesicles have a very regular shape. Nonetheless, we suggest that the vesicles have, below the transition, a maximum of approximately 45 facets. This number will be less if an appreciable fraction of the DPPC molecules are in the edge regions. For the case of 45 similar facets, the normals to neighboring facets make an angle of about 30° with one another.

The small-angle diffraction patterns support the model of faceted vesicles below the transition. There is a cross-interference effect (Fig. 5) which can be explained by assuming that the vesicles in 2 mM $Ca(NO_3)_2$ tend to stick to one another with neighboring facets parallel. The center-to-center distance between bilayers is estimated to be 68 Å. This value is in good agreement with the maximum periodicity of DPPC multilayers in excess water, which is 65–70 Å just below the main transition (Chap-

man *et al.*, 1967; Tardieu *et al.*, 1973; Janiak *et al.*, 1976; Inoko & Mitsui, 1978). Based on the area between the upper and middle curves in Fig. 5(*a*), we estimate that 15% of the total bilayer area is paired. Thus for a vesicle with 45 facets, there would be 7 neighboring vesicles on average. We note that this is a plausible number since a maximum of 12 spheres can be packed about a central sphere if the spheres are all of uniform size.

That the cross-interference effect could be due to the small vesicle collapsing upon itself below the thermal transition has been ruled out on the basis of work by Lawaczeck *et al.*, (1976). They have shown that, once fully annealed, the vesicles are impermeable below, at, and above the phase transition. Accordingly, we assume that the vesicles do not collapse, and we conclude that intact vesicles come into contact with one another.

The fit between predicted and observed intensities (Fig. 5(*b*)) is good but not perfect. The imperfect fit may be due to the distance between paired bilayers varying somewhat about the average value of 68 Å. In this case both peaks in the difference curve (Fig. 5(*b*)) would be smaller as a result of the variability, but the second peak would be attenuated more than the first (*see*, e.g., Blaurock & Nelander, 1976), as observed. There is evidence of a similar disorder of the stacking in highly hydrated DPPC multilayers (A.E. Blaurock, *unpublished*).

Above the thermal transition, it seems likely that the small vesicles will be round: the chains are packed more irregularly than below the transition (Tardieu *et al.*, 1973) and with the considerable freedom in chain conformation (Levine & Wilkins, 1971), it is easier to suggest a plausible packing of the chains. Assuming that the vesicles are very nearly spherical, then two vesicles can only be in contact over an area small compared to the facet size, and the binding energy of one vesicle to another would be correspondingly smaller. Judging from the lower light scattering above the transition (Petersen & Chan, 1978; Marsh *et al.*, 1977), it appears that the vesicles do in fact disaggregate.

From the absence of the cross-interference effect when the vesicles are in $La(NO_3)_3$ (Fig. 5(*a*)), we can suggest that La^{3+} binds well to the DPPC bilayer below the transition and hence holds the vesicles apart by forces based on electrostatic repulsion. In contrast, Ca^{2+} appears not to bind very well to the bilayer below the transition.

The broad temperature range over which the transition occurs in a suspension of the small vesicles (Fig. 4) may be related to the limited size of the facets. At thermodynamic equilibrium, a simple mathematical

equation (Tsong & Kanehisa, 1977) relates the size of the "cooperative unit" to the slope of the transition. Applying the equation to Fig. 4, we have estimated the size to be 12 molecules (ΔH as in Mabrey and Sturtevant (1976)), or 16 molecules (ΔH as in Suurkuusk *et al.*, (1976)). In comparison, the cooperative unit in small vesicles has been estimated previously to number from 18 to 46 molecules, depending on the physical method used (Marsh *et al.*, 1977; Tsong & Kanehisa, 1977). We note that our salt solution is not the same as in the other cases, and Marsh *et al.* (1977) report that salt affects the size of the cooperative unit. Assuming circular facets 75 Å in diameter and a cross-sectional area of 40–50 Å^2 per DPPC molecule (the area will depend on the angle of tilt assumed for the C_{16} chains relative to the plane of the facet), a facet will contain 90–110 molecules. Thus our estimate of the number of molecules per facet is nearly ten times the estimated number in the cooperative unit.

As noted in *Results*, we have no clear evidence of a pretransition in the small vesicles. We have recorded the 4.2-Å peak at temperatures down to 7.5 °C. This peak shifts to the right (to slightly smaller Bragg spacings),[2] and also broadens somewhat, at temperatures well below the main transition. Using either X-ray film or the detector, we have not seen the three components which are present in the large-angle diffraction pattern from the multilayers below the pretransition. Insofar as the pretransition in multilayers involves a large-scale structural change (Tardieu *et al.*, 1973; Janiak *et al.*, 1976) with a characteristic dimension considerably larger than the average facet size (but *cf.* Inoko & Mitsui, 1978), this transition may not be possible in the case of the small vesicles.

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[2] The shift results in the data point for 28 °C in Fig. 4 being too high. No correction was made, however. We note that a slight decrease in distance, as the temperature decreases, is characteristic of the chain separation in crystals of the normal paraffins. Thus Muller (1932) observed both gradual shifts and discrete jumps in the chain separation as the temperature was varied. Reports too numerous to cite here have amply confirmed the behavior discovered by Muller. Crystals of the normal paraffins also show a transition to an hexagonal form, often a few degrees below melting (Muller, 1932; McClure, 1968). The pretransition in DPPC multilayers appears to be analogous, although the mulilayers are not proper crystals.

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