Role of the Position of Unsaturation on the Phase Behavior and Intrinsic Curvature of Phosphatidylethanolamines

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ABSTRACT The bilayer-to-hexagonal phase transition temperatures (T_H) of di-18:1$_c$ phosphatidylethanolamine with double bonds at positions 6, 9, and 11 are 37°C, 8°C, and 28°C, respectively, as measured by differential scanning calorimetry and x-ray diffraction. Thus T_H exhibits a minimum when the C=C is around position 9, similar to what has been found for the gel-to-liquid crystalline phase transition temperature in other lipids. Factors that may contribute to the dependence of T_H on double bond position were studied by x-ray diffraction of the hexagonal phases in the presence and absence of added alkane, with or without the osmotic stress of polyethylene glycol, and over a wide temperature range. The lattice dimensions show that the intrinsic radius of lipid monolayer curvature increases as the double bond is moved toward the tail ends. A measure of the bending moduli of these lipid monolayers shows a higher value for the 9 position, and lower values for the other two. Consideration of the bilayer-to-hexagonal transition in terms of bending and interstitial energies provides a rationale for the relative values of T_H.

INTRODUCTION

Phospholipids in biological membranes are arranged primarily as planar bilayers. However, a substantial number of these phospholipids, when isolated, will form high-curvature, inverted structures (Luzzati and Husson, 1962; Cullis and DeKruijff, 1979; Lindblom and Rilfors, 1989; Seddon, 1990; Gruner, 1989). The fact that the lipid composition of several biological membranes appears to be regulated so that the membrane is close to, but below, the bilayer-to-hexagonal phase transition temperature (T_H) (Lindblom et al., 1993; Rilfors et al., 1994; Rietveld et al., 1994) suggests that this physical property of the membrane plays an important role in the modulation of membrane function (Epand, 1996). This is supported by the finding that the activity of a number of enzymes is increased (Senisterra and Epand, 1993; Cornell, 1991; McCallum and Epand, 1995), and membrane channel gating changed (Keller et al., 1993) in the presence of non-lamellae-forming lipids. Membrane fusion must also involve changes in membrane curvature during the formation of kinetic intermediates (Siegel, 1993; Chernomordik et al., 1995).

The introduction of unsaturation into the acyl chain greatly reduces the lamellar-hexagonal transition temperature, T_H. However, there has not been a systematic study of the relationship between the position of the double bond in the acyl chain and the formation of nonlamellar phases. Such a study has been reported for the gel-to-liquid crystalline phase transition temperature, T_M, of positional isomers of 1,2-dioctadecenoyl-*sn*-glycero-3-phosphocholine (Barton and Gunstone, 1975). In that work it was found that T_M had a minimum when the unsaturation was in the center of the acyl chain. This result was explained on the basis of differences in hydrocarbon chain packing (Berde et al., 1980). In the present work, we study the phase transition behavior and measure the intrinsic curvature and monolayer bending moduli of three 1,2-dioctadecenoyl-*sn*-glycero-3-phosphoethanolamines. There is a change in monolayer intrinsic curvature with bond position, but monolayer bending moduli show a maximum for the bond position in the middle of the chain. The role of curvature in lamellar-hexagonal transitions has recently been interpreted in terms of several different contributions to the free energies of these phases (Rand et al., 1990; Kozlov et al., 1994). The relative transition temperatures measured here are rationalized on the basis of the balance of bending and interstitial energies of these two phases.

MATERIALS AND METHODS

The following di-18:1$_c$ phosphatidylethanolamines were purchased from Avanti Polar Lipids (Alabaster, AL): dipetroselinoyl-phosphatidylethanolamine (Δ6 PE), dioleoyl-phosphatidylethanolamine (Δ9 PE), and divaccenoyl-phosphatidylethanolamine (Δ11 PE). These lipids showed a single spot in thin-layer chromatography at 100 μg loading.

Differential scanning calorimetry

A high-sensitivity scanning calorimeter (model MC-2; Microcal Co., Amherst, MA) was used to measure excess heat capacity as a function of increasing temperature. The lipid film was dispersed in 20 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid buffer, 150 mM NaCl, 1 mM EDTA, pH 7.4. Sodium azide (0.02 mg/ml) was added to prevent bacterial growth. This buffer is the same as that used previously in the calorimetric studies of phosphatidylethanolamine phase transitions; omission of the NaCl has almost no effect on the phase transition behavior (Epand and Bryszewska, 1988). Lipid suspensions or buffer were loaded into the sample or reference cells, respectively. Heating scan rates between 0.2 and 1°C/min were used,

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and T_H was independent of scan rate over this range. Sequential differential scanning calorimetry (DSC) heating scans performed after recooling and equilibrating the sample at low temperature gave identical T_H. The precision of T_H for replicate samples is <0.1°C. With batch-to-batch variations and small calibration errors, the accuracy of the transition temperatures is estimated as ±0.5°. Transition temperatures are reported to the nearest whole degree (Table 1). Enthalpy values are precise to ±10% for T_M and ±15% for T_H.

X-ray diffraction

Dry lipid was weighed into small weighing bottles, and when tetradecane was used, it was added to the dry lipid and equilibrated for 48 h. Hydration was done by adding excess (to approximately 50 wt%) amounts of 2.0 mM *N*-tris-(hydroxymethyl) methyl-2-aminoethanesulfonic acid buffer (pH 7.4) solutions containing various concentrations of polyethylene glycol (MW 2000) of known osmotic pressure (http://aqueous.labs.brocku.ca/). Samples were sealed and equilibrated in the dark, at room temperature, for 48 h. Before mounting, each sample was combined with some powdered Teflon as an x-ray calibration standard, and then sealed between mica windows 1 mm apart. No samples showed gradients of x-ray spacing, indicating complete equilibrium. Samples were stable over several days and under identical conditions gave x-ray spacings to within 0.5 Å.

X-ray diffraction was used to characterize the structures formed by the hydrated lipid and to measure their dimensions, as previously described (Rand and Fuller, 1994). The CuKα_1 line (λ = 1.540 Å), from a Rigaku rotating anode generator, was isolated using a bent quartz crystal monochromator, and diffraction patterns were recorded photographically with Guinier x-ray cameras operating in vacuo. Temperature was controlled with thermoelectric elements to approximately ±0.2°C. All samples formed either hexagonal phases characterized by at least three x-ray spacings bearing ratios to the dimension of the first order, d_{hex}, of 1, $1/\sqrt{3}$, 1/2, $1/\sqrt{7}$, 1/3, etc., and/or lamellar phases characterized by x-ray spacings bearing ratios to the dimension of the first order, d_{lam}, of 1, 1/2, 1/3, etc. All x-ray repeat spacings are measured with a precision of ±0.1 Å. Because all lipids were equilibrated with excess aqueous solution, there was no experimental error in measuring sample composition. Under identical experimental conditions and with internal Teflon calibration, sample-to-sample variations fall within ±0.2 Å.

From measured d_{hex}, the interaxial spacing between hexagonal cylinders, s, can be determined from geometry (Fig. 1). We estimated the relative radii of curvature, R_p, of the H_{II} phase monolayers by subtracting from s the hydrocarbon chain length dimensions of these lipids, 20 Å, as previously determined for Δ9 PE (Rand and Fuller, 1994). This places the position for measuring the radius of curvature of lipid layers at the polar group/hydrocarbon interface, a pivotal plane where molecular area is constant. We assume that such a position is the same for all of these lipids. This assumption is supported by the observation that even for the addition of as much as 30% 1,2 diolein to Δ9 PE, such a position changes by only a fraction of an angstrom (Leikin et al., 1996). We believe simply shifting the position of the double bond would cause even less change. In addition, for example, a shift of as much as 1 Å would cause only a 20% change in derived bending modulus, well within the changes we observe in this study. In all of these systems, the polar group/hydrocarbon interface, defined by d_{hc}, is a pivotal plane whose molecular area is practically constant and against which the curvature of the monolayer is measured (Rand et al., 1990). Consequently, we estimate radii of curvature, R_p, of the hexagonal monolayers of the three lipids in this study to be equal to (s − 20)/2 Å (Rand et al., 1990).

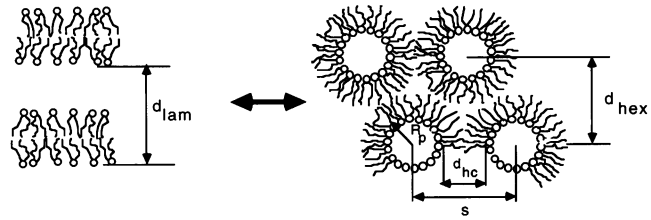

FIGURE 1 Schematic representation of the lamellar and hexagonal phases. X-ray diffraction measures lattice dimensions d_{lam} and d_{hex}. From previous studies of sample composition, structural dimensions for DOPE have been determined, including bilayer and hydrocarbon thickness, d_{hc}, and monolayer curvature, $1/R_p$. Several previous studies have shown that the radius of curvature should be measured from the center of the water cylinder to the polar group/hydrocarbon chain interface, a position where molecular area practically does not change with curvature. For the present study, as described in the text, we have taken the curvature of the hexagonal phase monolayer as $2/(s - d_{hc})$, where d_{hc} is 20 Å.

Assuming that all of the osmotic work on the hexagonal phase goes into elastic bending of the monolayers as previously shown for Δ9 PE, the relation between measured osmotic pressure Π and the R_p is given by

$$R_p^2\Pi = 2K_c(1/R_o - 1/R_p), \quad (1)$$

where K_c is the bending modulus of one lipid monolayer, and R_o is its intrinsic radius of curvature, i.e., the curvature of the unstressed monolayer.

RESULTS

Transition temperatures, T_M and T_H, and enthalpies determined using DSC are shown in Table 1. A minimum in both transition temperatures is found for Δ9 PE.

The temperature dependence of the hexagonal and lamellar lattice spacings, both with and without 16 wt% added tetradecane, for the three lipids is shown in Fig. 2. Only hexagonal phases formed in the presence of tetradecane. In its absence lamellar and hexagonal phases can coexist. The lowest temperature at which a single hexagonal phase was detected correlates closely with T_H, as determined by DSC.

We have measured the relation between osmotic pressure, Π, and hexagonal lattice dimension, d_{hex}, over a wide range of temperatures for all three lipids. d_{hex} itself is sensitively dependent on temperature. To maintain maximum experimental control, all three lipids were mixed in

TABLE 1 Measured parameters for di-18:1$_c$ PE lipids

C=C position	T_M (°C)	ΔH (kcal/mol)	T_H (°C)	ΔH (cal/mol)	R_o (T_H) (Å ± SE)	K_c (kT ± SE)	$\Delta G_i = \Delta G_b$ (ergs/cm^2)
6 (dplpe)	16	3	37	300	26.0 ± 0.2	10.0 ± 0.8	2.95
9 (dope)	−8*	4.5*	8#	290#	28.3 ± 0.2	15.5 ± 1.5	3.85
11 (dvpe)	−1§	ND	28	110	29.2 ± 0.2	10.0 ± 0.8	2.14

*From Cullis, P., P. Van Dijck, B. deKruijff, and J. DeGier. 1978. *Biochim. Biophys. Acta.* 513:21–30.
#From Epand, R. M. 1985. *Chem. Phys. Lipids.* 36:387–393.
§Determined by x-ray diffraction.

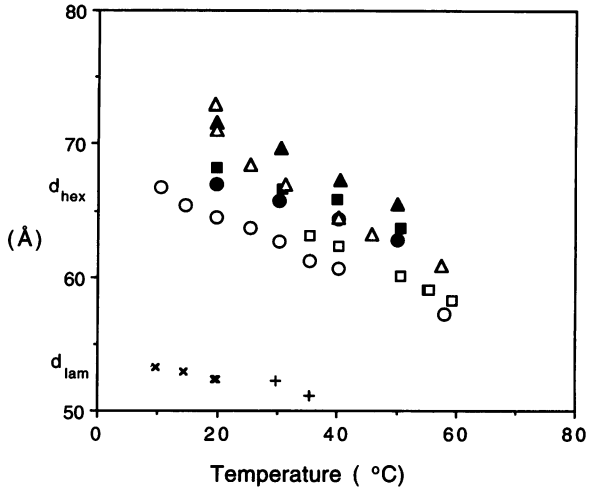

FIGURE 2 The temperature dependence of the hexagonal (○, ●, □, ■, △, ▲) and lamellar (+, ×) lattice spacings, both with (●, ■, ▲) and without (○, □, △) 16 wt% added tetradecane, for the three lipids in excess water. □, ■, +, Δ6 PE; ○, ●, Δ9 PE; △, ▲, ×, Δ11 PE. Without tetradecane, the lowest experimental temperature at which a single hexagonal phase exists coincides closely with T_H for each lipid, as measured by DSC (Table 1).

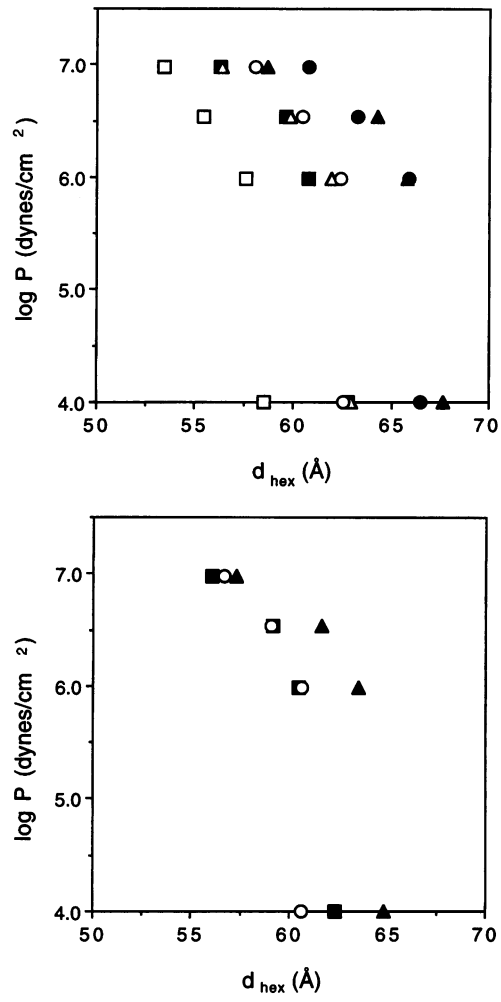

FIGURE 3 Plots of the relation between lattice dimension of the hexagonal phases and the osmotic pressure of the PEG solution with which they are equilibrated. Data are shown for a variety of temperatures, as indicated for the three lipids Δ6 PE (□, ■), Δ9 PE (○, ●), and Δ11 PE (△, ▲). (Upper panel) T_H (●, ■, ▲) and T_H + 20° (○, □, △). (Lower panel) T = 40°C.

identical osmotic stressing solutions, and the temperature dependence of d_{hex} was determined for each. Best fits of d_{hex} with temperature were then used to construct a family of Π versus d_{hex} curves at different temperatures to compare the lipids at defined temperatures. Examples of Π versus d_{hex} curves at fixed intervals of 10°C and 20°C above their respective T_H, as well as those at a constant temperature of 40°C, are shown in Fig. 3.

We have analyzed the osmotic pressure relationships according to Eq. 1, to determine the intrinsic radii of curvature R_o and monolayer bending moduli K_c. An example for T_H + 10 is shown in Fig. 4. R_o, K_c, and their changes with temperature are shown for all three lipids in Fig. 5. The specific values of R_o and K_c at T_H are given in Table 1.

Because the hexagonal lattice dimension is strongly dependent on temperature, we compare these parameters at reduced temperatures, i.e., at temperatures at fixed intervals above T_H. The relative values for the different lipids are independent of the interval selected. On that basis, the intrinsic radius of curvature increases as the double bond moves toward the hydrocarbon chain terminals, although the difference between Δ9 and Δ11 is not significant. At any one fixed temperature, on the other hand, R_o follows a different order, Δ11 > Δ6 > Δ9.

Monolayers of Δ9 PE are less "bendable" than the other two lipids, with a bending modulus significantly higher than that of Δ6 and Δ11. This is qualitatively evident in all of the curves in Fig. 3, by the relative resistance of Δ9 PE to decreasing dimension as osmotic pressure increases.

DISCUSSION

We have measured T_M and T_H for the series of di-18:1_c phosphatidylethanolamines (Table 1). We find that both transition temperatures are lower for the Δ9 PE than for either Δ6 or Δ11. With regard to T_M, a similar result obtained for positional isomers of 1,2-dioctadecenoyl-*sn*-glycero-3-phosphocholine (Barton and Gunstone, 1975) has been explained on the basis of differences in hydrocarbon chain packing (Berde et al., 1980). In the following we attempt to explain the differences in T_H among these lipids on the basis of the energetics of the lamellar and hexagonal phases, using our previous studies on the Δ9 PE (Kozlov et al., 1994).

The monolayer intrinsic radius of curvature is sensitively dependent on temperature. When compared at T_H, or at any fixed interval above T_H, R_o shows an increase as the double bond moves toward the hydrocarbon chain terminals, par-

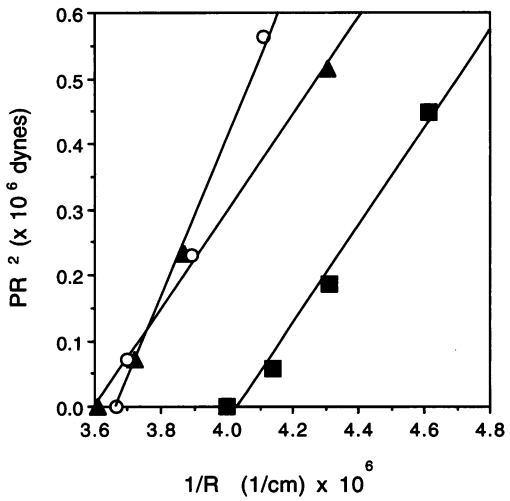

FIGURE 4 Fit of the osmotic stress data of Fig. 3 to the bending elasticity relation $R_p^2 p = 2K_c (1/R_o - 1/R_p)$. Best-fit linear plots yield the values of K_c and R_o, shown in the table, for the three different lipids Δ6 PE (■), Δ9 PE (○), and Δ11 PE (▲). The x axis intercepts indicate the significantly different R_o for Δ6 PE; the slopes reflect the significantly different value of K_c for Δ9 PE.

ticularly from position 6 to position 9. This change in R_o with double bond position might be understood in terms of the effect of that bond on chain packing, particularly as described by the orientational order parameter measured by NMR (Sternin et al., 1988). For Δ11 PE, the double bond is inserted in a position where the order parameter is already low (or the chain disorder is high), even for saturated chains. As the double bond is moved closer to the polar group, into regions of increasing chain order, it could have an increasing disordering effect. At position Δ9 it is placed in the region where the order parameter is decreasing most rapidly. At position Δ6, it is introduced into the plateau region of a higher orientational order parameter and has its biggest effect. R_o therefore appears to decrease in proportion to the length of hydrocarbon chain between the double bond and the terminal methyl group. It is as if the introduction of the double bond into regions of higher chain order disorders the chain from that position toward the terminal end and endows the monolayer with higher intrinsic curvature.

The bending modulus does not show a systematic change with position of the double bond, showing a maximum for Δ9 PE. T_H also does not exhibit a systematic variation with double bond position, showing a minimum for Δ9. We suggest that these two remarkable changes are connected by the free energy changes in the lamellar hexagonal transition. The lamellar-hexagonal transition, in excess water, is dominated by a balance of the change in bending (ΔG_b) and interstitial (ΔG_i) free energies (Kozlov et al., 1994), where the interstitial energy can be viewed as the requirement for the hydrocarbon chains to fill different chain-length environments around the hexagonal cylinder (Kozlov et al., 1994). T_H is the temperature at which the free energies of the lamellar and hexagonal phases are equal, i.e., where, in forming the lamellar phase, the energy gained by the removal of the interstitial energy in the hexagonal phase, ΔG_i, is balanced by the cost of unbending the monolayer from its intrinsic curvature, $\Delta G_b = 0.5 K_c / R_o (T_H)^2$.

We have listed the relative unbending energies ΔG_b in Table 1. For Δ9 PE it is higher than for the other lipids.

The higher unbending energy ΔG_b arises almost entirely from the higher bending modulus and not from the differences in curvature.

With no change in ΔG_i, an increase in bending modulus alone would lower T_H. This is because one would have to lower the temperature to uncurl the monolayer to larger R_o, which would compensate the higher K_c and thereby maintain the equality $\Delta G_b = \Delta G_i$.

Why focus on the detail of these parameters? The bending modulus and the intrinsic radius of curvature are two characteristics of the state of the lipid in a planar membrane that are often invoked as affecting membrane properties. In natural membranes, one expects lipid-protein interactions and the topological changes involved in membrane fusion to be sensitive to these parameters. R_o is one measure of how stressed monolayers are when constrained to the plane, and proteins might respond to that stress. K_c will affect how easy or hard it is to form high-curvature structures, such as fusion intermediates. This work demonstrates that these two pa-

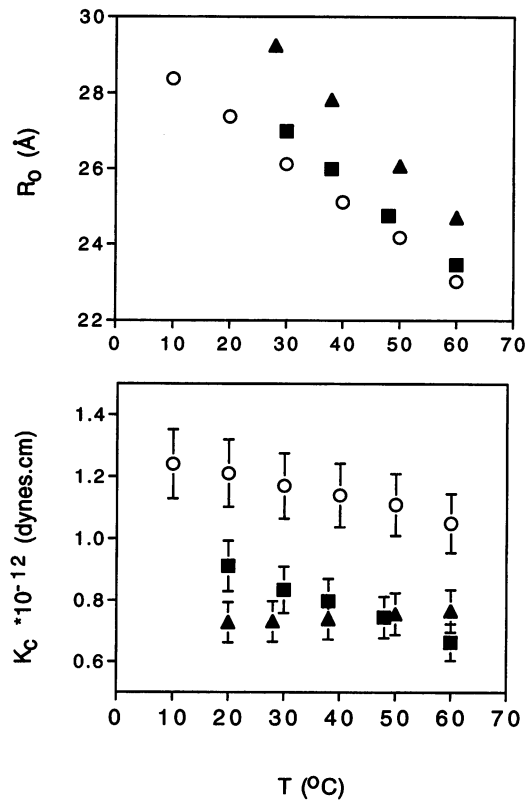

FIGURE 5 Temperature dependence of R_o and K_c. ■, Δ6 PE; ○, Δ9 PE; ▲, Δ11 PE. Standard error bars are estimated by best fit linear analysis of data as shown in Fig. 4. For R_o, error bars fall within the symbols.

rameters vary differently with double bond position and provide an opportunity to relate these changes to specific membrane activities.

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