Lipid Polymorphism of Mixtures of Dioleoylphosphatidylethanolamine and Saturated and Monounsaturated Phosphatidylcholines of Various Chain Lengths†

Mark W. Tate and Sol M. Gruner*
Department of Physics, Joseph Henry Laboratories, Princeton University, Princeton, New Jersey 08544
Received June 16, 1986; Revised Manuscript Received September 11, 1986

ABSTRACT: The L_α–H_{II} phase transition behavior of many lipid–water liquid crystals is dominated by the competition between the tendency to curl the lipid layers to an intrinsic radius of curvature and opposing hydrocarbon packing constraints. In particular, packing constraints can increase the free energy of the inverted hexagonal (H_{II}) phase as compared to that of the lamellar (L_α) phase. This is especially true where the lipid molecule is not long enough to reach into the corners of the lattice in large hexagonal structures necessitated by a large intrinsic radius of curvature. In this paper it is shown that the addition of a minor fraction long-chain lipid to a system of otherwise uniform chain composition can also relax packing constraints, thereby lowering the lamellar to hexagonal transition temperature. For the specific systems used, dioleoylphosphatidylethanolamine (di-18:1_c-PE) with minor fractions of 1,2-diacyl-*sn*-glycero-3-phosphocholines [di-*n*:1_c-PC (n = 14, 18, 22, and 24)], the observed H_{II} lattices systematically increased in size with increasing chain length, suggesting that the chain length also may affect the intrinsic curvature of the mixture. These experiments demonstrate that the lipid "shape concept", which is a qualitative expression of the concept quantitatively described by the intrinsic radius of curvature, is insufficient to understand the L_α–H_{II} transition. It is necessary to, at least, consider the competition between curvature and packing.

Understanding the mechanism underlying the L_α[1]–H_{II} phase transition in neutral phospholipids may give a better understanding of many biological processes (Cullis et al., 1985; Gruner et al., 1985; Gruner, 1985). Unfortunately, this task presents quite a challenge due to the complex nature of the interactions in the lipid–water system. If, however, one considers only those terms that dominate the free energy of the system, the problem becomes manageable, and it is possible to gain insights into the nature of the transition. A model has been presented using this approach (Kirk, 1984; Kirk et al., 1984; Gruner et al., 1985; Gruner, 1985). In this paper, we wish to vary the hydrocarbon packing term of the model in a test of its validity.

In the L_α phase, the lipid molecules are arranged in a series of stacked bilayers with a layer of water separating each bilayer from the next (Figure 1a). Each bilayer consists of two monolayers of lipids, with the hydrophilic polar head groups at the water interface and the hydrophobic acyl chains in the interior of the bilayer, away from any water. These hydrophilic and hydrophobic regions also exist in the H_{II} phase (Figure 1b). Here, tubes of water arranged on a hexagonal lattice are surrounded by the lipid head groups. The hydrocarbon chains point away from the water cores, filling the space between the water cores with a hydrophobic matrix.

To form a phase in which the lipid layers are curved, a bending force must be present. In a lipid monolayer, an asymmetry may exist in the lateral forces within the polar and nonpolar regions. Differences in the binding of the head groups with one another vs. that of the forces between hydrocarbon chains contribute to the asymmetry. Polymer-like qualities of the chains give them an entropic splay in their melted state that increases with temperature. The net result of this asymmetry is that each monolayer has an intrinsic curvature, sometimes called an equilibrium or spontaneous curvature. Any deformation of the monolayer from this value of curvature has a cost in the free energy of the system.

Another important contribution to the free energy comes from the mutual repulsion between lipid head groups across the water region of the liquid crystal. It has been proposed that the observed repulsion arises from polarization of water at the lipid–water interface. For lamellar systems, this repulsion grows exponentially as the lipid head group surfaces get closer to each other, making very small water regions unfavorable (Parsegian et al., 1979; Lis et al., 1982). Since this repulsion is due to the interactions between the lipids and water, the head group composition should play a major role in determining its strength.

The final term in the model for zwitterionic lipids comes from a geometrical consideration of the structures involved. In the H_{II} phase, the lattice consists of hexagonal cells (Figure 1b). Since the corners of the hexagon are further from the central water core than the sides, the lipid must have a distribution of lengths ranging from $d_{H_{II}}$ to d_{max} if it is to fill the cell. Here the water core is assumed to be circular since shapes that are not roughly circular will not be favored by the curvature term (the exact cross-sectional shape of an H_{II}-phase water core is not known). The distribution of lengths is possible since the lipids are not rigid molecules above T_c, the chain melt temperature. Above this temperature, many *gauche* rotamers exist within the acyl chain, allowing the chains to exist in many

† This research is supported by Department of Energy Contract DE-AC-02-76EV03120, National Institutes of Health Grant GM32614, and a Liposome Co. Fellowship to M.W.T.
* Author to whom correspondence should be addressed.

[1] Abbreviations: L_α, lamellar; H_{II}, inverse hexagonal; PE, phosphatidylethanolamine; PC, phosphatidylcholine; di-18:1-PE, dioleoyl-PE; di-18:1-PC, dioleoyl-PC; di-14:0-PC, dimyristoyl-PC; di-18:0-PC, distearoyl-PC; di-22:0-PC, dibehenoyl-PC; di-14:1-PC, dimyristoleoyl-PC; di-22:1-PC, dierucoyl-PC; di-24:1-PC, dinervonoyl-PC; T_c, gel to liquid-crystalline transition temperature; T_{bb}, lamellar to hexagonal transition temperature; d, lattice basis length; d_l, thickness of lipid monolayer in L_α phase; $d_{H_{II}}$, minimum thickness of lipid region in H_{II} phase; d_{max}, maximum length lipid must assume in H_{II} phase; d_w, thickness of water layer in L_α phase; R_0, intrinsic radius of curvature of a monolayer; R_w, radius of water cylinder in H_{II} phase; DSC, differential scanning calorimetry; TLC, thin-layer chromatography.

0006-2960/87/0426-0231$01.50/0

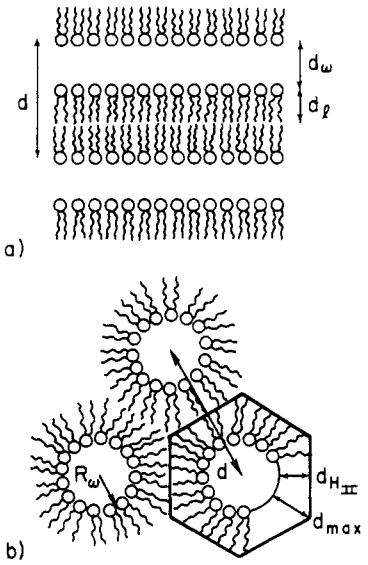

FIGURE 1: Cross sections of the lamellar (L_α) and inverted hexagonal (H_{II}) phases. The lipid head groups are represented by small circles and the melted acyl chains by wavy lines. (a) The L_α phase consists of alternating planar sheets, one of water with thickness d_w and the other of lipid with thickness $2d_l$. (b) The H_{II} phase consists of cylinders of water (radius R_w) on a hexagonal lattice surrounded by the lipid. The lipids must take on lengths from $d_{H_{II}}$ to d_{max} if they are to fill the lattice. In both phases, d is the lattice basis length. In a real system there would, of course, be more interdigitation of the chain ends than is shown in this cartoon.

possible configurations, providing the distribution of lengths necessary for the H_{II} phase. The equilibrium length of the lipid molecules will be shorter than the maximum length, which occurs when no rotamers exist within the hydrocarbon chains. As the H_{II} lattice parameter grows, the difference in $d_{H_{II}}$ and d_{max} increases. This necessitates having chain lengths that are further from the equilibrium value to be able to fill the lattice. The value for $d_{H_{II}}$ has been seen to be fairly constant for a given chain length over a wide range of lattice sizes (Kirk & Gruner, 1985). Thus the size of the H_{II} lattice is a strong function of the intrinsic curvature. However, d_{max} is a function of the geometry, and with a larger radius of the water cylinder, d_{max} increases. Insofar as it costs free energy to stretch or compress chains from the equilibrium length, larger water cores entail greater free energy costs in packing the hydrocarbon chains. Thus, as the intrinsic radius of curvature grows, the lamellar phase becomes more favored since fewer geometrical packing constraints exist for the L_α phase. This is the case at low temperatures, because the equilibrium radius of curvature increases as the temperature decreases (Gruner, 1985; Gruner et al., 1986).

If one could reduce the constraints the packing of hydrocarbon puts on the system, one would expect the H_{II} phase to be favored at these larger intrinsic radii. The packing constraints could be relieved by adding appropriate hydrocarbon to the system which could partition into the corners of the hexagon. Since the intrinsic radius falls with temperature, one would expect that adding hydrocarbon would lower the L_α–H_{II} transition temperature T_{bh}. Added alkanes have been shown to do this, resulting in dramatic reductions in T_{bh} (Kirk, 1984; Kirk & Gruner, 1985; Gruner, 1985; Gruner et al., 1986). Alternatively, if one changes the distribution of acyl chain lengths, replacing some of the chains with longer ones, then, again, the corner regions of the hexagon could be filled at a lower cost in energy, allowing the H_{II} phase to form at a lower temperature. Thus, one may predict that the addition of a small fraction of long-chain lipids to a system of otherwise homogeneous chain lengths would lower T_{bh}.

Experimental Procedures

X-ray diffraction was used to determine the lipid phases and lattice dimensions. A Rigaku RU-200 microfocus rotating anode X-ray machine generating copper Kα radiation was used in conjunction with Franks-type small-angle cameras on two independent beam lines. The Princeton SIV and SIT area detectors (Gruner, 1977; Reynolds et al., 1978; Gruner et al., 1982a; Milch, 1983) were used to record the powder diffraction patterns from the samples. Radial densitometrization on the digital image was performed by computer as described previously (Gruner et al., 1982b; Tilcock et al., 1984; Pascolini et al., 1984). Lamellar phases are characterized by diffraction peaks that correspond to integer multiples of the reciprocal lattice basis, while in the H_{II} phase, the peaks occur at ratios of $1:\sqrt{3}:2:\sqrt{7}$, etc. in reciprocal space. When T_{bh} is reported as a range of temperatures in this paper, that range is from the lowest temperature at which a hexagonal lattice is unambiguously seen to the highest temperature at which a lamellar lattice can be unambiguously distinguished.

Data are presented as the basis vector length d of the lamellar or hexagonal lattice vs. temperature T. Note that the H_{II} phase basis, d, vs. T for dioleoylphosphatidylethanolamine:dioleoylphosphatidylcholine = 3.17:1 with 5% dodecane differs from that presented in the paper by Kirk and Gruner (1985). Although the difference is small, amounting to about 2 Å at the extreme temperatures, it is outside experimental error. We are unable to reproduce the Kirk and Gruner (1985) curve; the present curve has been reproduced many times with a variety of specimen preparation procedures and with different batches of lipid. We do not know the reason the curves differ but suspect a slight contamination of the lipid used by Kirk and Gruner (1985). A few percent of degraded lipid has strong effect on the H_{II} vs. T curves.

Each sample was ramped in temperature from 0 to 85 °C; nominally in 5 °C steps, with a 15-min equilibration time at each temperature. Temperature was controlled thermoelectrically within ±0.3 °C from the set temperature. The diffraction pattern was collected at each step in temperature with a typical exposure time of 60–400 s. Repeat spacings of the lattice were calibrated against lead nitrate and lead stearate, with an uncertainty in the spacings of ±1.0 Å.

Differential scanning calorimetry data were taken on a Perkin-Elmer DSC-4 scanning calorimeter. Scan rates of 10 and 20 °C/min were used. Samples contained about 9 mg of lipid with 60% water by weight.

Samples were prepared by using lipids obtained from Avanti Polar Lipids, Inc. (Birmingham, AL) and checked by TLC for purity. The lipid samples consisted of dioleoylphosphatidylethanolamine with minor fractions of either unsaturated or monounsaturated 1,2-diacyl-*sn*-glycero-3-phosphocholines. The monounsaturated chains were 9-*cis*-tetradecenoic (14:1), 9-*cis*-octadecenoic (18:1), 13-*cis*-docosenoic (22:1), and 15-*cis*-tetracosenoic (24:1). Ideally, we would have preferred to use monounsaturated lipids all with a cis double bond fixed at the 9 position, but these were not readily available. Lipids were mixed in fixed molar ratios, as specified in the text. Samples were mixed volumetrically from chloroform solutions of known concentrations. The chloroform was then evaporated, and the lipid was resolubilized in cyclohexane for lyophilization. About 5 mg of lipid was lyophilized directly in a glass X-ray capillary. A volume of 60–65% water by weight was then added to the lipid and was mixed into the sample mechanically. Lyophilization from cyclohexane forms a fluffy white mass that is readily hydrated.

This is important because the X-ray capillaries are very fragile. Epoxy was used to seal the capillary. In the preparation of samples with dodecane, the appropriate amount of dodecane was added before the water. More mechanical mixing was needed with these samples to assure that it was homogeneous. Samples were thermally cycled at least once between −30 and 85 °C before data were taken. DSC samples were prepared in the same manner. The lyophilized lipid was transferred to the sample pan, the water added, and the pan sealed.

Derivatives of the lattice spacing curves with respect to temperature were found by fitting the data piecewise over the temperature range with low-order polynomials. Linear functions were used when five to eight points could be fit in this manner. Quadratic and cubic terms were added as needed to the polynomial for those regions that could not be adequately fit to a line.

A comment on the equilibration times used in this study is appropriate. The 15-min equilibration time that was used between temperature steps was used because it was deemed adequate to detect the onset of the L_α–H_{II} transition. This does not imply that 15 min is sufficient time for the system to attain true equilibrium. Even for pure di-18:1-PE in excess water, L_α–H_{II} phase coexistence is observed over a temperature span that is several degrees Celsius wide, with the relative proportions of the two phases changing slowly on the time scale of days (E. Shyamsunder and S. M. Gruner, unpublished observations). Our experience with these systems indicates, however, that for small temperature steps even a few minutes equilibration is generally adequate to detect the first appearance of the H_{II} phase to within 5 °C. In the present study, this was verified by observing the behavior of two representative samples (di-18:1-PE:di-18:1-PC and di-18:1-PE:di-22:0-PC mixtures), using equilibration times of many hours.

Also note that the central question addressed by this study is the relative depths of the free energy minima of L_α and H_{II} phases of identical lipid compositions. The conclusions are not altered if the true equilibrium state is another, deeper, difficult-to-access minimum associated with, for instance, another phase or with macroscopic lipid demixing. For example, in the presence of excess water and 5% tetradecane at 25 °C, the H_{II} phase of di-18:1-PE:di-18:1-PC = 3:1 will, over the course of days, phase separate into coexisting L_α and H_{II} phases, whereas 20% tetradecane stabilizes the H_{II} phase for at least weeks. In this case, we suspect that lipid demixing is occurring in the H_{II} phase to optimize the radius of the H_{II} tubes to the amount of available alkane, i.e., that the ratios of the PE to PC are different in the L_α and H_{II} phases (R. P. Rand, N. Fuller, and S. M. Gruner, unpublished observations). Such lipid demixing may be readily understood on the basis of the competition between curvature and hydrocarbon packing.

Results and Discussion

The chain distribution of PE:PC mixtures was varied by combining di-18:1-PE with different diacyl-PC's. The PC acyl chains were 14, 18, 22, and 24 carbons in length, with both saturated and unsaturated chains being used.

In the cases of monounsaturated PC's (Figure 2a), a molar ratio of PE:PC = 3.17:1 was chosen (this is a w/w ratio of 3:1 for di-18:1-PE:di-18:1-PC), since this produced values of T_{bh} that were roughly midway between 0 and 100 °C. T_{bh} for a di-18:1-PE:di-18:1-PC mixture was 50–55 °C, consistent with earlier measurements (Kirk & Gruner, 1985). In the presence of di-24:1- or di-22:1-PC, T_{bh} was 40–45 and 40–50 °C, respectively, thereby confirming the predicted drop in T_{bh} upon the addition of long chains. If, however, di-14:1-PC was used, no unequivocal H_{II} phase was observed up to the maximum measured temperature of 85 °C. The diffraction from the lamellar lattice of this latter case did reduce in intensity beginning at 70 °C, concomitant with an increase in the diffuse diffraction. This suggests that above 70 °C the lamellar lattice was not stable.

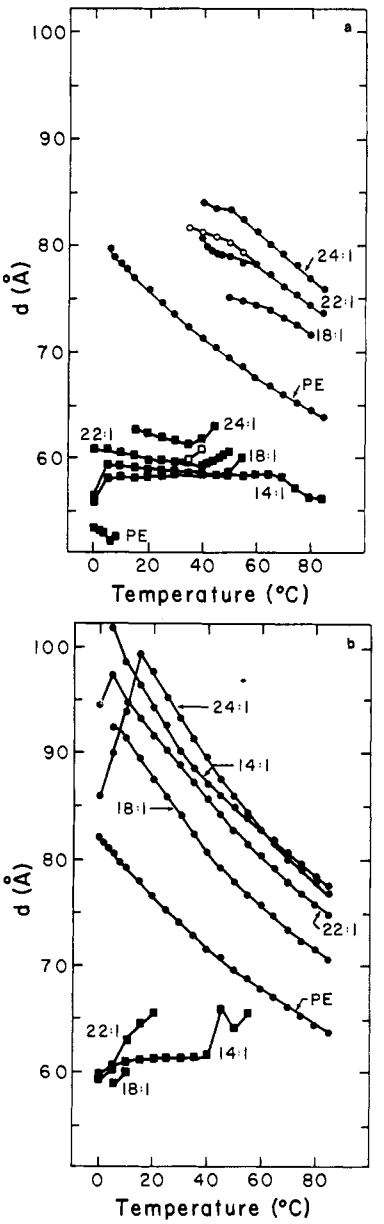

FIGURE 2: (a) Lattice spacings vs. temperature for di-18:1-PE:di-acyl-PC = 3.17:1 mixtures. Curves are labeled by the acyl chain of the PC. Curves labeled as PE are pure di-18:1. Squares indicate the L_α phase, while circles represent the H_{II} phase. Open symbols are data points obtained while cooling; all other points are obtained upon heating. (b) Lattice spacings for the samples in (a) but with the addition of 5% dodecane.

When fully saturated PC's were used (Figure 3a), phase separations between lamellar and hexagonal lattices were observed at low temperatures, even at the molar ratio of PE:PC = 23:1 that was used. Consider, for instance, the case of di-18:1-PE:di-22:0-PC = 23:1 (Figure 3a). T_{bh} occurs within a few degrees of T_{bh} for pure di-18:1-PE. The dimensions of the PE–PC specimen lattice closely tracks those of pure di-18:1-PE up to about 30 °C. Between 30 and 65 °C, the basis vector length, d, of the hexagonal lattice diverges from that of pure di-18:1-PE. Above 65 °C, the lattice dimensions run parallel to that of pure di-18:1-PE, but with a larger repeat

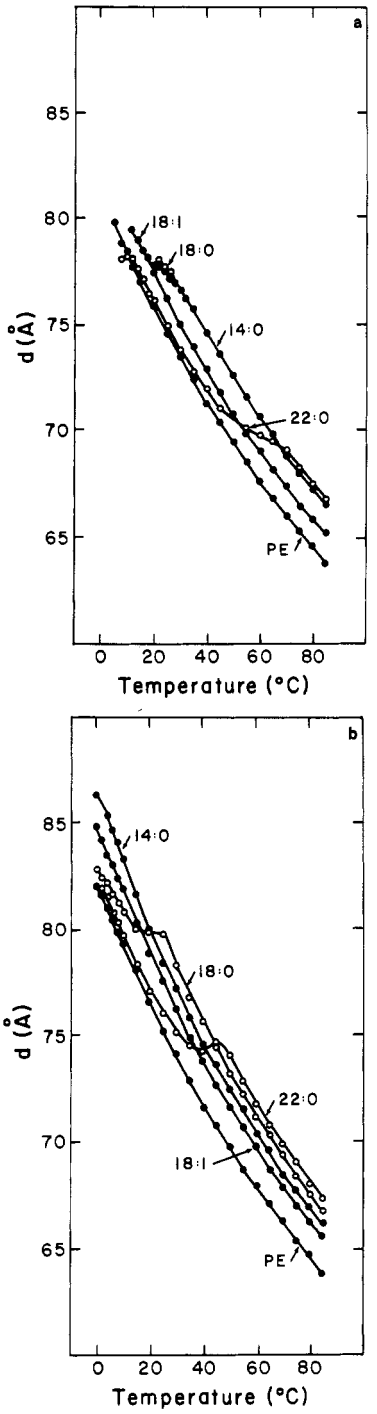

FIGURE 3: Hexagonal lattice spacings for fully saturated diacyl-PC's added to di-18:1-PE in the molar ratio PE:PC = 23:1. Curves are labeled by the acyl chains of the PC. Open circles are for clarity only as all data are taken upon heating. (a) Data from lamellar lattices are not shown. A lamellar lattice was seen to 8 °C for di-18:1-PE, to 14 °C for the mixture with di-18:1-PC, to 10 °C for di-22:0-PC, and to 30 and 32 °C, respectively, for di-18:0-PC and di-14:0-PC. Curves for the mixtures containing di-14:0-PC and di-18:0-PC coincide over much of the temperature range and are shown as one. (b) These samples differ from those in (a) by the addition of 5% dodecane to each sample.

spacing. These results are consistent with the following scenario: At low temperature the di-18:1-PE and the di-22:0-PC do not mix significantly but rather separate into an almost pure di-18:1-PE H_{II} phase and an almost pure lamellar di-22:0-PC phase. In fact, high-resolution diffraction showed a weak lamellar lattice at 40 °C with a lamellar basis length of 73.9 Å. The lamellar basis of pure di-22:0-PC in excess

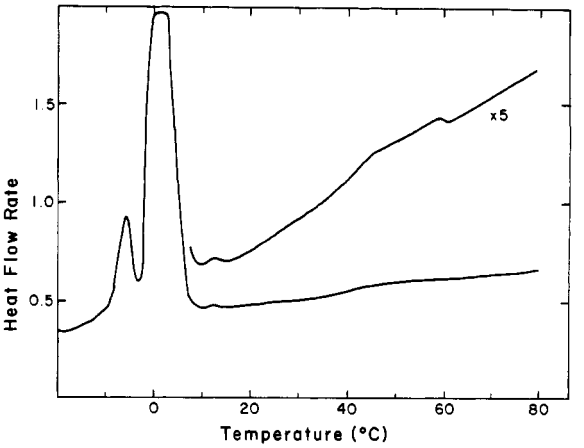

FIGURE 4: Differential scanning calorimeter trace of di-18:1-PE:di-22:0-PC = 23:1 molar ratio sample. Heat flow is in arbitrary units. The peak at the lowest temperature is due to the gel to liquid-crystalline transition, and the largest peak (which has saturated the detector) is due to the melting of the bulk water. The small peak at 12 °C is characteristic of the lamellar to hexagonal transition, and the broad peak from 35 to 60 °C is due to the melting of the chains of the di-22:0-PC in the environment of the di-18:1-PE.

water at 40 °C was measured to be 73.7 Å. Between 30 and 65 °C, entropy causes the di-22:0-PC to begin to mix, or alloy, into the H_{II} phase, thereby altering its curvature. This is why d deviates from the pure di-18:1-PE curve. Note that other experiments in which the ratios of di-18:1-PE to di-18:1-PC were changed systematically generated a set of nearly parallel curves of d vs. T (Kirk & Gruner, 1985).

Differential scanning calorimetry of the di-18:1-PE:di-22:0-PC = 23:1 sample (Figure 4) showed a peak at about 12 °C, corresponding to T_{bh}, and then a broad peak from 35 to 60 °C due to the melting of the acyl chains of di-22:0-PC. The broadness of this peak is a characteristic of a mixture, as is the depression of the melting point from 75 °C, the chain melt temperature for di-22:0-PC (Silvius, 1982). The alloying is also seen with di-18:0-PC when dodecane is added to extend the H_{II} region to 0 °C (Figure 3b). In the presence of dodecane, notice that the full mixing of di-22:0-PC occurs at a lower temperature than without dodecane, a feature observed when low molecular weight soluble compounds are added to crystalline polymers (Olabisi et al., 1979).

Phase separation has been observed previously in lamellar phases when saturated PC's that have chains differing by four or more carbons in length are mixed together (Silvius, 1982). It is also observed here when one of the species is monounsaturated. However, no phase separation of this type was observed in the region of interest for the mixtures in which both lipids were monounsaturated. In each of our unsaturated lipid systems, a large temperature range exists above T_{bh} of pure di-18:1-PE in which only one lamellar lattice is seen. Also, the repeat spacings of both the lamellar and hexagonal lattices are markedly different than the spacings obtained from the pure PE or PC, again suggesting that the PE and PC are mixed in each phase. Two different lamellar lattices were only seen at temperatures below the region of interest. This separation occurred at temperatures that were intermediate to the two constituents' chain melt temperatures, as is also the case for the mixture with saturated PC's, which have a much larger T_c than the corresponding unsaturated chain. Thus it seems that a large difference in chain melt temperatures hampers the mixing of lipids at intermediate temperatures.

The phase transition from L_α to H_{II} in these monounsaturated mixtures is not as sharp as that of pure di-18:1-PE. A phase coexistence region occurs in all cases, with the ratio of

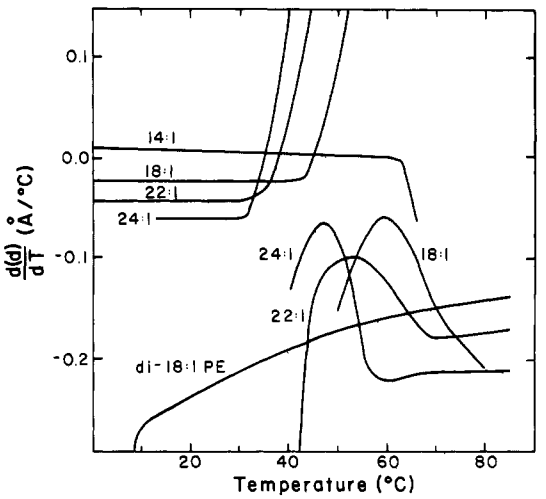

FIGURE 5: First derivatives of the curves shown in Figure 1a. Curves are labeled by the acyl chains of the PC.

lipid in the H_{II} to L_α phase increasing with temperature, as can be seen by comparing the intensities of the L_α and H_{II} diffraction. Also the lattice repeat spacing curves are not as smooth over the phase coexistence temperature region, as is characteristic with a one-component lipid system over the same temperature range. The first derivative of these spacing curves shows this clearly, as well as showing a definite depression in the T_{bh} as chain length increases (Figure 5). If the PE:PC ratio in each phase changed, with the PE going into the H_{II} phase more readily, then the lamellar phase would become PC rich, causing an increase in the L_α spacing. The H_{II} phase would be PE rich, depressing its spacings from a smooth curve. One sees a smooth curve upon cooling, when the L_α phase is not present for the PC to partition into (Figure 2a). It is emphasized that the phase coexistence does not demonstrate total separation of the PE from the PC but, rather, suggests variations in the ratio of PE to PC in both phases at the transition.

Changing the acyl chains may have changed parameters other than the hydrocarbon packing term. As discussed before, the hydration repulsion is largely a function of head group composition, as is the overall curvature (Kirk & Gruner, 1985). The ratio of PE:PC head groups was kept constant to help minimize head group dependent effects.

The intrinsic curvature of the monolayer also could have changed because of chain effects. A melted longer chain may have a tendency for more entropic splay than a shorter one, thereby increasing the curvature. This would also decrease T_{bh} since smaller water cores are favored by packing considerations. Notice, however, that the repeat spacing of the H_{II} lattice systematically increases with chain length of the PC. The larger spacings in the H_{II} phase immediately show that the difference between $d_{H_{II}}$ and d_{max} has increased, producing a more unfavorable geometry for a uniform average chain length. This indicates that the packing energies have been reduced by the change in chain distribution, allowing this larger geometry to form.

Some swelling of the lattice can be expected from the added bulk of hydrocarbon. If one assumes the dimensions of the water regions and the area per lipid at the water interface to be the same as chain length varies, then for the lamellar case, the lattice will swell by

$$\Delta d = 2d_l\Delta V/V_l$$

where d_l is the thickness of the monolayer, ΔV is the added volume per molecule due to the increase in chain length, and V_l is the volume of a molecule. Assuming the lipids to be of constant density, changing the PC's by four carbons will result in an averaged change of Δd = 1.1–1.2 Å in the basis vector length, d, of the lamellar PE:PC = 3.17:1 mixture. A value of 16–17 Å was taken for the thickness of the di-18:1-PE:di-18:1-PC = 3.17:1 monolayer, of the order measured for both egg PE at 25 °C (Lis et al., 1982) and di-18:1-PC at 10 °C (Kirk, 1984). A change of 1–1.2 Å is seen at 10 °C for addition and subtraction of chain length from the di-18:1-PE:di-18:1-PC sample, of the magnitude expected for the lamellae.

In the hexagonal case, the swelling expected due to the extra chain hydrocarbon has the value of

$$\Delta d \cong (d^2 - 2\pi R_w^2/\sqrt{3})\Delta V/2dV_l$$

where d is the H_{II} lattice spacing and R_w is the radius of the water core. For the same change in chain length, Δd = 0.9 Å for d = 73 Å and R_w = 22 Å, the values for the H_{II} lattice of di-18:1-PE:di-18:1-PC = 3.17:1 at 65 °C (Kirk & Gruner, 1985). Δd is a factor of 4 smaller than observed. The large increase in spacing is due to either an increase in the water core size, an increase in the thickness of the lipid annulus surrounding the water core, or both.

An increase in R_w could come about from a combination of an increase in the intrinsic radius of curvature and the relaxation of hydrocarbon packing constraints that allow R_w to relax toward the larger intrinsic radius. To further relieve hydrocarbon packing constraints, dodecane was added to each of the PE:PC combinations (Figure 2b). Dodecane had only a small effect on the lattice spacings of pure di-18:1-PE (Gruner et al., 1986), suggesting that the water core has a radius close to the intrinsic radius of the monolayer in the absence of dodecane for PE. This was confirmed in Gruner et al. (1986). When dodecane was added to the PE:PC systems, the spacings increase more than for the pure PE, but the increase is still on the order of that expected due to the added volume of hydrocarbon (Figure 2a). Moreover, the sequence of H_{II} sizes (d at fixed T) is the same with as without dodecane: 18:1 < 22:1 < 24:1. This strongly suggests that the intrinsic radius of curvature increases with the chain length. Thus, comparing the H_{II} phase dimensions of the di-18:1 and di-22:1 cases of Figure 2a, one sees a drop in the L_α–H_{II} transition temperature concomitant with an increase in the intrinsic radius of curvature. This is entirely reasonable given the curvature vs. hydrocarbon packing competition: The curvature has shifted slightly away from favoring the formation of an H_{II} phase, but the opposing packing constraint has shifted strongly in favor of the H_{II} phase. The resultant sum favors the H_{II} phase and the transition temperature drops. Note, however, that a simultaneous increase in the H_{II} phase radius and a drop in the transition temperature cannot be explained by consideration of shape (intrinsic curvature) arguments alone.

Hydrocarbon may be added to the H_{II} phase either in the form of a free alkane, such as dodecane, or in the form of longer lipid chains. For a given mass of added hydrocarbon, dodecane is more effective at lowering T_{bh}. Adding 5% dodecane by weight adds hydrocarbon which, by mass, is equivalent to about six carbons per chain for each of the PC molecules in the di-18:1-PE:di-18:1-PC = 3.17:1 mixture. This lowers T_{bh} down to 0 °C. Adding six carbons per chain by replacing the di-18:1-PC with di-24:1-PC (i.e., 5% added hydrocarbon chains, by weight) lowers T_{bh} only to 40 °C. Apparently, dodecane diffuses about in the hydrophobic region of the H_{II} lattice and removes chain stress while exerting only

small effects on the overall geometry, that is, on the intrinsic curvature. However, if the extra hydrocarbon is bound to the lipids in the form of longer chains, there appears to be less freedom to independently remove chain packing stress without affecting the intrinsic curvature.

The data presented above support the model of the L_α–H_{II} transition in which an intrinsic monolayer curvature competes with hydrocarbon packing (Kirk et al., 1984; Gruner, 1985). As with prior experimental studies of the L_α–H_{II} model (Kirk & Gruner, 1985; Gruner, 1985; Gruner et al., 1986), it is especially noteworthy that the experimental confirmation of the qualitative phase behavior followed from predictions, not the other way around. These experiments highlight the essential role of hydrocarbon packing in understanding the L_α–H_{II} transition. Unfortunately, there is still a tendency to think about the transition wholly in terms of "molecular shape" arguments. "Molecular shape" is a qualitative cartoon that may be useful in understanding trends that may be quantiatively expressed as an intrinsic radius of curvature. Unless the intrinsic radius is sufficiently small, there is no driving force toward the formation of a H_{II} phase. However, a small intrinsic radius of curvature is a necessary but *insufficient condition* for occurrence of the H_{II} phase. It is also necessary to consider other free energies, most notably the competing free energy associated with the packing of chains in the H_{II} lattice.

Biological membranes are known to contain a mixture of lipids that exhibit an overall bilayer characteristic. Many of these lipids are H_{II}-forming by themselves, and it is thought that these are necessary for some biological processes to occur. The distribution of chains that occurs within the membrane may also contribute to the formation of curved structures for biologically important functions.

ACKNOWLEDGMENTS

We thank Dave Turner and Erramilli Shyamsunder for much help in acquiring data. We also thank Colin Tilcock and Walt Shaw for advice and help with the lipid analysis. In addition, we thank Jeff Koberstein, Adam Galambus, and John Owens for their assistance in acquiring the DSC data.

Registry No. Di(14:1)-PC, 56750-90-4; di(18:1)-PC, 4235-95-4; di(22:1)-PC, 51779-95-4; di(24:1)-PC, 51779-96-5; di(18:0)-PC, 816-94-4; di(14:0)-PC, 18194-24-6; di(22:0)-PC, 37070-48-7; di-(18:1)-PE, 2462-63-7.

REFERENCES

Cullis, P. R., Hope, M. J., de Kruijff, B., Verkleij, A. J., & Tilcock, C. P. S. (1985) in *Phospholipids and Cellular Regulations* (Kuo, J. F., Ed.) Vol. I, pp 1–59, CRC Press, Boca Raton, FL.

Gruner, S. M. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 3665–3669.

Gruner, S. M., Milch, J. R., & Reynolds, G. T. (1982a) *Rev. Sci. Instrum. 53*, 1770–1778.

Gruner, S. M., Rothschild, K. J., & Clark, N. A. (1982b) *Biophys. J. 39*, 241–251.

Gruner, S. M., Cullis, P. R., Hope, M. J., & Tilcock, C. P. S. (1985) *Annu. Rev. Biophys. Biophys. Chem. 14*, 211–238.

Gruner, S. M., Parsegian, V. A., & Rand, R. P. (1986) *Faraday Discuss. Chem. Soc.* (in press).

Kirk, G. L. (1984) Ph.D. Thesis, Princeton University, Princeton, NJ.

Kirk, G. L., & Gruner, S. M. (1985) *J. Phys. (Les Ulis, Fr.) 46*, 761–769.

Kirk, G. L., Gruner, S. M., & Stein, D. L. (1984) *Biochemistry 23*, 1093–1102.

Lis, L. J., McAlister, M., Fuller, N., Rand, R. P., & Parsegian, V. A. (1982) *Biophys. J. 37*, 657–665.

Milch, J. R. (1983) *J. Appl. Crystallogr. 16*, 198–203.

Olabisi, O., Robeson, L. M., & Shaw, M. T. (1979) in *Polymer–Polymer Miscibility*, Academic, New York.

Parsegian, V. A., Fuller, N., & Rand, R. P. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 2750–2754.

Pascolini, D., Blasie, J. K., & Gruner, S. M. (1984) *Biochim. Biophys. Acta 777*, 9–20.

Reynolds, G. T., Milch, J. R., & Gruner, S. M. (1978) *Rev. Sci. Instrum. 49*, 1241–1249.

Silvius, J. R. (1982) in *Lipid–Protein Interactions* (Jost, P. C., & Griffith, O. H., Eds.) pp 239–281, Wiley, New York.

Tilcock, C. P. S., Bally, M. B., Farren, S. B., Cullis, P. R., & Gruner, S. M. (1984) *Biochemistry 23*, 2696–2703.