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Reversed Hexagonal Phase Formation in Lecithin–Alkane–Water Systems with Different Acyl Chain Unsaturation and Alkane Length†

Mats Sjölund,* Leif Rilfors, and Göran Lindblom
Department of Physical Chemistry, University of Umeå, S-901 87 Umeå, Sweden
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ABSTRACT: Investigations of lipid–alkane systems are important for an understanding of the interactions between lipids and hydrophobic/amphiphilic peptides or other hydrophobic biological molecules. A study of the formation of nonlamellar phases in several phosphatidylcholine (PC)–alkane–^2H_2O systems has been performed. The PC molecules chosen in this work are dipalmitoyl-PC (DPPC), 1-palmitoyl-2-oleoyl-PC (POPC), dioleoyl-PC (DOPC), and dilinoleoyl-PC (DLiPC), lipids that in excess water form just a lamellar liquid–crystalline phase up to at least 90 °C. The addition of *n*-alkanes (C_8–C_{20}) to these PC–^2H_2O systems induces the formation of reversed hexagonal (H_{II}) and isotropic phases. The water and dodecane concentrations required to form these phases depend on the degree of acyl chain unsaturation of the PC molecules and increase in the order DLiPC ≈ DOPC < POPC < DPPC. The most likely explanation to this result is that the diameter of the lipid–water cylinders in the H_{II} phase grows gradually larger with increased acyl chain saturation and more water and dodecane are consequently needed to fill the water cylinders and the void volumes between the cylinders, respectively. The ability of the alkanes to promote the formation of an H_{II} phase is strongly chain length dependent. Although the number of alkane carbon atoms added per DOPC molecule in the DOPC–*n*-alkane–^2H_2O mixtures was kept constant, this ability decreased on going from octane to eicosane. The thermal history of a DPPC–*n*-dodecane–^2H_2O sample was important for its phase behavior. A large fraction of the sample formed an isotropic phase at 65 °C when heated at 10 °C/24 h, while an H_{II} phase dominated at this temperature when the sample was heated at 10 °C/h. The results obtained are consistent with the theories presented by Gruner [Gruner, S. M. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 3655–3669] and Siegel [Siegel, D. P. (1986) *Chem. Phys. Lipids 42*, 279–301].

Studies of phase transitions between lamellar and nonlamellar phases in biological membrane lipid–water systems are important for several reasons: (1) Such transitions are induced by a number of biologically relevant factors (Rilfors et al., 1984). (2) Most biological membranes contain at least one lipid species (Ansell et al., 1973; Goldfine, 1982) which does not form a lamellar phase under physiological conditions. Moreover, the balance between lipids forming a lamellar phase and lipids forming a nonlamellar phase is metabolically regulated in some bacterial membranes (Wieslander et al., 1980; Lindblom et al., 1986; Goldfine et al., 1987). (3) Many processes are associated with biological membranes during which the lipid bilayer structure most probably is transiently disrupted (e.g., fusion and exo- and endocytosis) (Lucy, 1970; Rilfors et al., 1984; Cullis et al., 1985; Bentz & Ellens, 1988). (4) The activity of some membrane-bound enzymes or protein assemblies is greatly enhanced in the presence of lipids forming nonbilayer structures (Jensen & Schutzbach, 1984; Navarro et al., 1984; Cheng & Hui, 1986; Siefermann-Harms et al., 1987; Pick et al., 1987).

Some investigations have been performed with phosphatidylethanolamine (PE)[1]–*n*-alkane–H_2O, phosphatidylcholine (PC)–*n*-alkane–H_2O, and PE–PC–*n*-alkane–H_2O systems to elucidate the principles underlying the transition from a la-

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[1] Abbreviations: L_α, lamellar liquid-crystalline phase; H_{II}, reversed hexagonal phase; DO, dioleoyl; PO, 1-palmitoyl-2-oleoyl; DP, dipalmitoyl; DLi, dilinoleoyl; PC, phosphatidylcholine; PE, phosphatidylethanolamine; T_{LH}, temperature interval for the transition from an L_α to an H_{II} phase.

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mellar to a nonlamellar phase (Hornby & Cullis, 1981; Epand, 1985; Kirk & Gruner, 1985; Tate & Gruner, 1987; Sjölund et al., 1987). Studies of lipid–alkane interactions can also be seen as a first step toward studies of the interaction between lipids and hydrophobic peptides and membrane protein segments (Lindblom et al., 1988). In all the above-mentioned systems the addition of *n*-alkanes (C_6–C_{20}) promotes or facilitates the formation of a cubic or a reversed hexagonal (H_{II}) phase. The water content is also important for the phase equilibria. In the dioleoyl-PC (DOPC)–*n*-dodecane–^2H_2O system two different H_{II} phases are formed, one at high and one at low water contents (Sjölund et al., 1987). It has also been shown that the presence of alkanes in the membrane of the bacterium *Acholeplasma laidlawii* drastically alters the lipid composition (Wieslander et al., 1986); the relative amount of monoglucosyldiglyceride, forming an H_{II} phase, decreases, while the relative amount of diglucosyldiglyceride, forming a lamellar phase, increases.

The above-mentioned results have been interpreted by use of theoretical models for the self-assembly of amphiphiles (Israelachvili et al., 1980; Gruner, 1985). It is concluded by Israelachvili et al. that the effective geometry of the amphiphilic molecules in the system determines the lipid aggregate shape. Alkane molecules incorporated into a lipid aggregate can be viewed as altering the effective shape of the lipid molecules, and thus are able to induce phase transitions in the lipid–water system. Gruner proposed that the reason for the ability of the *n*-alkanes to induce an H_{II} phase in lipid–water mixtures is that the alkane molecules fill out the volumes between the lipid–water cylinders and relieve the hydrocarbon packing constraints that prevent the formation of the H_{II} phase. It should also be noted that an interesting implication of this behavior of lipid monolayers to curl was recently suggested by Gruner (Gruner, 1985; Gruner et al., 1988). He presented the hypothesis that biomembranes adjust their intrinsic curvatures to fall into an optimum range. Thus the regulation of the membrane lipid composition in *A. laidlawii* (Wieslander et al., 1980; Lindblom et al., 1986) suggests that the value of the spontaneous curvature of the membrane is actively held constant by the organism (Gruner et al., 1988). Studies of this matter are currently being performed in our laboratories.

In this paper we present the results from further investigations of phospholipid–alkane interactions setting out from the theoretical model by Gruner (1985). We have studied the effect on the phase equilibria in PC–alkane–^2H_2O systems of (1) the degree of unsaturation of the acyl chains of PC, (2) the chain length of the *n*-alkane, (3) the structural isomerization of the alkane, and (4) the water concentration. The influence on the phase equilibria of the thermal history of the samples was also investigated in the DPPC–*n*-dodecane–^2H_2O system.

Materials and Methods

Sample Preparation. The different phosphatidylcholines were obtained from Avanti Polar Lipids, Inc., Birmingham, AL. The purity was given as >99%. Thin-layer chromatography analysis of the lipids showed only trace amounts of contamination, and no further purification was done. The *n*-alkane solvents, adamantane, 2,3,4-trimethylpentane, 2,2,3,3-tetramethylbutane, cyclooctane, and benzene were purchased from Sigma Chemical Co., St. Louis, MO. The purity of the hydrocarbons was given as 99%, and they were used without further purification.

Samples were prepared as described by Sjölund et al. (1987). ^{31}P NMR spectra were recorded repeatedly from all samples during a period of 1–2 months, and the spectra were in all cases reproducible. The actual molar ratio of alkane present within the lipid aggregates was not determined. After the NMR measurements some samples were analyzed for lipid degradation by thin-layer chromatography. The amount of decomposition products was found to be less than 3 mol %.

^{31}P NMR Spectroscopy. ^{31}P NMR spectra were obtained with a Bruker WM-250 Fourier transform spectrometer at 101.3 MHz. Inverse gated high-power proton decoupling was applied. The spectral width was 30 kHz, pulse length 6 μs, relaxation delay 1 s, and dead time 10 μs. For a typical spectrum 500–2000 transients were accumulated, and an exponential multiplication corresponding to 20-Hz line broadening was applied before Fourier transformation. It has been shown in previous works that ^{31}P NMR can be conveniently used to determine the phase diagrams of lipid–water systems (Arvidson et al., 1985; Eriksson et al., 1985; Lindblom et al., 1986; Brentel et al., 1987; Sjölund et al., 1987).

The amount of lipid in the different phases in two- and three-phase samples was determined by spectra simulation. The parameters used in the simulation were chemical shift anisotropy, line width, and relative composition (Eriksson et al., 1985). The accuracy in the determination of the amount of lipid in the different phases is within 3–5%.

Results

PC–n-Dodecane–^2H_2O Phase Equilibria. The phase equilibria in the system DOPC–*n*-dodecane–^2H_2O have been dealt with in detail in a previous paper (Sjölund et al., 1987). The formation of an H_{II} phase at *high* water contents, and with dodecane/DOPC molar ratios between 1.0 and 2.5, was the most interesting observation made with this system. According to a model presented by Gruner (1985) concerning the aggregation of membrane lipids in the presence of hydrophobic molecules, an H_{II} phase should form at different water and dodecane concentrations in PC–dodecane–^2H_2O systems containing PC with different degrees of acyl chain unsaturation. This hypothesis was tested by exchanging DOPC for dipalmitoylphosphatidylcholine (DPPC), 1-palmitoyl-2-oleoylphosphatidylcholine (POPC), or dilinoleoylphosphatidylcholine (DLiPC). ^{31}P NMR measurements were performed in the temperature range 25–55 °C at water concentrations between 10 and 80 wt %. Figure 1 shows the ability of different PC–*n*-dodecane–^2H_2O mixtures, with a dodecane/PC molar ratio of 2:1, to form an H_{II} phase or an isotropic phase at different water concentrations at 45 °C. An increased temperature displaces the phase equilibria toward the H_{II} phase. Replacing the monounsaturated oleoyl chains of PC with the diunsaturated linoleoyl chains only slightly affected the phase equilibria. This similarity between mono- and diunsaturated acyl chains was also found when comparing the formation of a cubic phase at low water concentrations. DOPC containing 4 wt % ^2H_2O formed a cubic phase above 70 °C (Sjölund et al., 1987), and DLiPC with the same water content formed a cubic phase above 55 °C. To form an H_{II} phase with mixtures containing POPC required water concentrations well above those needed for mixtures containing DOPC (Figure 1). The system containing only saturated acyl chains (DPPC) required the highest water concentrations to form a nonlamellar phase. It should also be noted that the amount of dodecane required to induce the formation of a nonlamellar phase in PC–*n*-dodecane–^2H_2O mixtures with a fixed water content is increased in the order DLiPC ≈ DOPC < POPC < DPPC.

The phase equilibria in the system DPPC–*n*-dodecane–^2H_2O are dependent on the thermal history of the sample. At room temperature DPPC is in the gel phase, and above 41 °C the

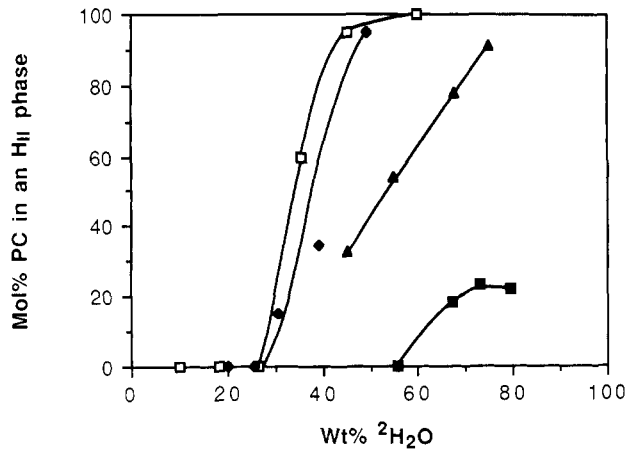

FIGURE 1: Phase equilibria at 45 °C in different PC–*n*-dodecane–^2H_2O systems as a function of the water content. The molar ratio of dodecane/PC is 2:1. (◆) DLiPC; (□) DOPC; (▲) POPC; (■) DPPC. DPPC formed an isotropic phase at this temperature, but at higher temperatures an H_{II} phase was formed. The amount of lipid in an H_{II} phase was determined by spectra simulation for DLiPC, DOPC, and POPC. In DPPC the amount of nonlamellar phase was estimated by cutting out the spectral components and weighing on an analytical balance.

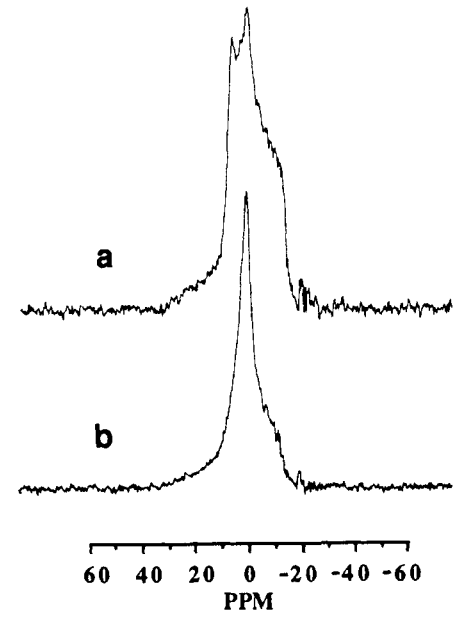

FIGURE 2: ^{31}P NMR spectra recorded at 101.3 MHz. (a) DPPC with 2 mol of *n*-dodecane/mol of lipid and 80 wt % ^2H_2O at 65 °C. The sample was heated about 10 °C in 1 h. (b) The same sample as in (a) but heated about 10 °C in 24 h.

lipid forms a lamellar liquid-crystalline phase in excess water. An isotropic phase appeared at 40 °C in a sample containing 80 wt % ^2H_2O and 2 mol of dodecane/mol of DPPC that had been heated slowly, about 10 °C in 24 h. The amount of the isotropic phase increased further up to at least 75 °C if the sample was heated slowly (Figure 2b). However, if the sample was heated faster, about 10 °C in 1 h, the ^{31}P NMR spectra show, in addition to the signal derived from a lamellar phase, a broad signal that is difficult to interpret. At 65 and 75 °C the spectra are characteristic for a lamellar liquid-crystalline phase in equilibrium with an H_{II} phase and a small amount of an isotropic phase (Figure 2a). At 85 °C almost a pure H_{II} phase was formed.

DOPC–n-Alkane–^2H_2O Phase Equilibria. The effect of alkanes with different chain length on the phase equilibria in DOPC–*n*-alkane–^2H_2O mixtures was also studied. All alkanes from octane (C_8) to eicosane (C_{20}) with an even number of carbon atoms were used. The amount of alkane added was adjusted to give the same number of carbon atoms per DOPC molecule for the different alkanes. Figure 3 shows the phase equilibria in different DOPC–*n*-alkane–^2H_2O systems. The effect on the phase equilibria of three different manipulations can be seen: (1) changes in the temperature; (2) changes in the water concentration; (3) changes in the alkane concentration. Figure 3a shows the result from mixtures containing 39 wt % ^2H_2O (31 mol/mol of DOPC) and 12 mol of alkane carbon atoms/mol of DOPC. Although the added mass of alkane was kept constant, the ability of the alkanes to promote the formation of an H_{II} phase is chain length dependent. On going from octane to eicosane, the amount of H_{II} phase decreases from 33% for octane down to 0% for octadecane at 25 °C. At higher temperatures the phase equilibria are slightly displaced toward the H_{II} phase. At temperatures above 35 and 45 °C octadecane and eicosane, respectively, start to induce a nonlamellar phase. These temperatures seem to be related to the melting points of the hydrocarbons, which are 28.2 °C for octadecane and 36.8 °C for eicosane (Weast, 1974).

If the alkane concentration is increased to 24 mol of alkane carbon atoms/mol of DOPC, the phase equilibria are shifted toward the H_{II} phase (Figure 3c) with the largest effect for the shorter alkanes. In this case there is a very strong chain length dependence with a monotonic decrease in the amount of H_{II} phase from octane to eicosane.

An increase in the water concentration has also a pronounced effect on the phase equilibria. Panels b and d of Figure 3 show the result from mixtures containing 49 wt % ^2H_2O and 12 and 24 mol of alkane carbon atoms per mole of DOPC, respectively. The phase equilibria are generally shifted toward the H_{II} phase as compared to those of the lower water concentrations. However, at 49 wt % ^2H_2O the ability of the alkanes to promote the formation of an H_{II} phase is less chain length dependent at higher temperatures, and even the longer alkanes facilitate the organization of the lipids in an H_{II} phase structure.

POPC–n-Alkane–^2H_2O Phase Equilibria. We also studied the effect of the *n*-alkanes on the phase equilibria in the POPC–water system. Figure 4 shows the result from mixtures containing 49 wt % ^2H_2O (54 mol/mol of lipid) and 24 mol of alkane carbon atoms/mol of POPC. A smaller fraction of the samples containing POPC formed an H_{II} phase compared to the samples containing DOPC (Figure 3d). At 25 °C there is a monotonic decrease in the amount of H_{II} phase from octane to tetradecane. The longer alkanes do not induce nonlamellar phases at all at this temperature. At 55 °C even hexadecane induces an H_{II} phase.

DOPC–^2H_2O with Structurally Isomerized Alkanes. To further study the effect of hydrophobic molecules on the phase equilibria in DOPC–^2H_2O systems, five hydrocarbons bulkier than the linear *n*-alkanes were incorporated. The hydrocarbons used were adamantane, 2,3,4-trimethylpentane, 2,2,3,3-tetramethylbutane, cyclooctane, and benzene. Adamantane and 2,2,3,3-tetramethylbutane, which are solid up to 268 °C (sealed tube) and 100.7 °C, respectively, had no effect at all on the phase equilibria in the DOPC–^2H_2O system in the temperature range 25–55 °C when 12 mol of carbon atoms/mol of DOPC was incorporated and the water concentration was 39 wt % (compare Figure 3a). However, at 90 °C 2,2,3,3-tetramethylbutane induced a phase transition, and 19 mol % of the lipids formed an H_{II} phase. At 39 wt % ^2H_2O 1.5 mol of the liquid hydrocarbon 2,3,4-trimethylpentane per mole of DOPC induced a phase transition, and 26 mol % of

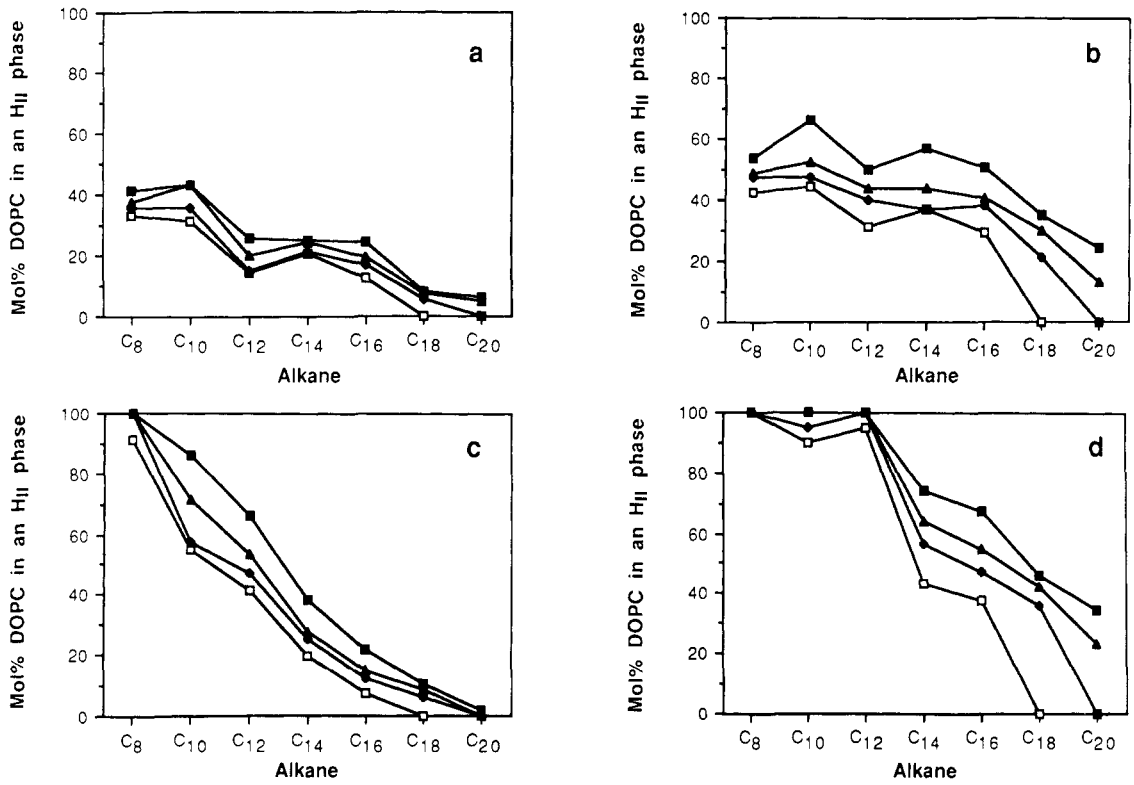

FIGURE 3: Phase equilibria in DOPC–*n*-alkane–^2H_2O systems as a function of the alkane chain length at four different temperatures: (□) 25, (◆) 35, (▲) 45, and (■) 55 °C. The water concentration was 39 wt % (31 mol/mol of DOPC) in (a), 49 wt % (46 mol/mol of lipid) in (b), 35 wt % (31 mol/mol of DOPC) in (c), and 49 wt % (54 mol/mol of DOPC) in (d). The alkane concentration corresponds to 12 carbon atoms per lipid in (a) and (b) and 24 carbon atoms per lipid in (c) and (d). Eicosane (C_{20}) induced the formation of a phase giving rise to an isotropic ^{31}P NMR signal.

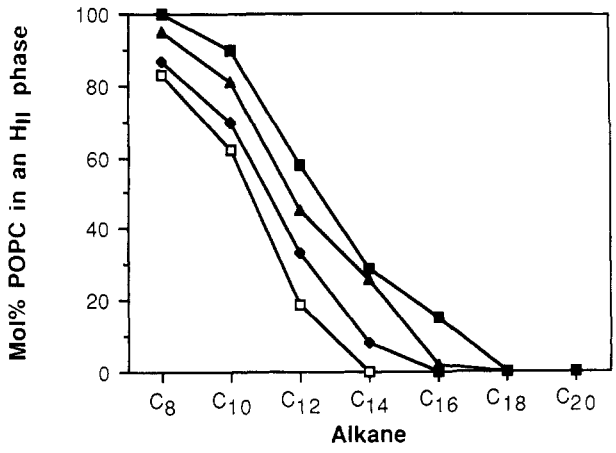

FIGURE 4: Phase equilibria in POPC–*n*-alkane–^2H_2O systems as a function of the alkane chain length at four different temperatures: (□) 25, (◆) 35, (▲) 45, and (■) 55 °C. The water concentration was 49 wt % (54 mol/mol of POPC). The alkane concentration corresponds to 24 carbon atoms per lipid.

the lipids was organized in an H_{II} phase at 25 °C. This is slightly less than the amount of H_{II} phase induced by octane (see Figure 3a). Cyclooctane, another liquid alkane with eight carbon atoms, also induced the formation of an H_{II} phase. At 39 wt % ^2H_2O and a molar ratio of cyclooctane/DOPC of 1.5, 23 mol % of the lipids was organized in an H_{II} phase at 25 °C. Benzene had no effect on the phase equilibria in a sample containing 39 wt % ^2H_2O and 2 mol of benzene/mol of DOPC.

Discussion

One of the main reasons for performing our systematic studies of the effect of alkanes on the phase behavior of different lecithins is to get a better understanding of the formation of nonlamellar phases in membrane lipid–water systems, i.e., cubic and hexagonal liquid-crystalline phases. Here we have concentrated our efforts on the reversed hexagonal phase.

In recent years several workers have reported on theories describing lipid aggregation, the formation of different aggregate structures, and the construction of phase diagrams. Israelachvili et al. (1980) derived a simple theory of self-assembly, which could be very easily grasped by the nonexpert on surface physical chemistry or membrane biophysics. In their model the molecular shape plays the dominant role so that a transition from a lamellar to a hexagonal structure is determined by a change from a cylindrical-like to a wedge-shaped lipid molecule. This first-order theory has been shown to work astonishingly well in a number of situations (Wieslander et al., 1980, 1981, 1986; Khan et al., 1981; Rilfors et al., 1982, 1984; Rilfors, 1985; Lindblom et al., 1986). The main drawback with this approach is that it does not explicitly take all the forces between all the molecules involved into account. But if it is used with care, it has a strong potentiality. Gruner and co-workers (Kirk et al., 1984; Kirk & Gruner, 1985; Gruner, 1985; Gruner et al., 1988; Anderson et al., 1988) developed a theory based on a phenomenological mechanics approach, where the molecules themselves are not explicitly handled, to explain the formation of the H_{II} phase. Here it is assumed that the lipid monolayer, which builds up the aggregate, is characterized by an intrinsic radius of curvature, R_0, and a bending stiffness coefficient. R_0 is proposed not to be dependent on any particular phase structure but only to be a property of the monolayer. This theory predicts that a small R_0 will lead to the formation of an H_{II} phase, while a lamellar phase has a very large value of R_0. A third approach, and the most rigorous one, has been taken by Wennerström and co-workers, who use a thermodynamic model and quantitatively calculate the phase diagram. This has been done

for both two- and three-component systems (Guldbrand et al., 1982; Jönsson & Wennerström, 1987). In their model the electrostatics play a dominant role, and most of their studies are performed on ionic detergents. The H_{II} phase is also here one of the most difficult phases to handle. In many cases we will have to use the more simple descriptions. This will be the case also here, where the experimental information we have got about the systems studied is rather limited.

In a previous report (Sjölund et al., 1987) part of the ternary phase diagram of the system DOPC–*n*-dodecane–^2H_2O was determined, and it was found that with increasing water concentration the following phases form at dodecane/DOPC molar ratios between 1.0 and 2.5: reversed hexagonal (H_{II}^I)–lamellar (L_α)–reversed hexagonal (H_{II}^{II}). This finding is difficult to explain. In the theory by Israelachvili the value of the packing parameter $v(al)^{-1}$ of a lipid molecule can vary; the effective hydrocarbon–water interfacial area (a) can be changed by the degree of hydration of the polar head group (Rilfors et al., 1984), and the hydrocarbon chain length (l) can be changed by the temperature (Wieslander et al., 1980). The L_α–H_{II}^I phase transition can thus be explained by the molecular shape model since the value of a decreases with a decreasing water concentration (Sjölund et al., 1987). However, it is not possible to explain the L_α–H_{II}^{II} phase transition by this model since the value of a ought to increase with an increasing water concentration and this should not lead to the formation of an H_{II} phase (Israelachvili et al., 1980).

Gruner's model on the other hand nicely explains the L_α–H_{II}^{II} phase transition since, when enough water and alkane are available to fill the cylinders and the volumes between the cylinders, respectively, an H_{II} phase will form. The L_α–H_{II}^I phase transition, occurring at low water contents, cannot be explained without further development of this model since it is valid only over a limited range of curvatures. R_0 and the elastic constant are dependent on the water content and the temperature. It has however been shown that the bending energy is accurately described near full hydration, but when the hydration falls below roughly 50% of full hydration, this energy is overestimated (Gruner et al., 1986). Thus, other energies which are negligible near full hydration will dominate the phase behavior at low water contents.

In this work we have investigated the phase equilibria for four different PC molecules at high water contents only. The lipids are, with increasing acyl chain unsaturation, DPPC, POPC, DOPC, and DLiPC. At high water contents they all form a lamellar phase at the temperatures studied (Ulmius et al., 1977; Dekker et al., 1983). Since the molecular shape should change from a cylindrical-like to a wedge-like one and the monolayer curvature should decrease, as the degree of unsaturation increases, one should also expect nonlamellar phases to form. The change in shape is supported by experimental results showing that the area per polar head group increases with unsaturation, where the largest step is taken between saturated and monounsaturated acyl chains (Ulmius et al., 1977; Demel et al., 1972; Lis et al., 1982). The increase in head group area means that a larger part of the hydrophobic region is in contact with water, which should be unfavorable. If an H_{II} phase formed instead, this unfavorable situation could be circumvented, but the problem is that the water cylinders will be large and the bending of the lipid monolayer will create void volumes in the hydrophobic region (see Figure 5; Gruner, 1985). Thus, lipid packing constraints and low water contents in the system will prevent the formation of lipid aggregates with large radii of curvature. However, the addition of water and hydrophobic molecules like alkanes can remove these

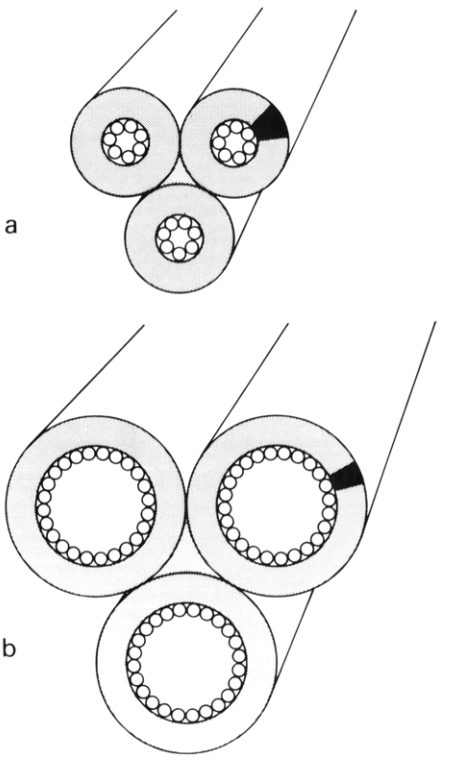

FIGURE 5: Schematic representation showing (a) an H_{II} phase with high degree of unsaturated acyl chains and (b) an H_{II} phase with low degree of unsaturation.

constraints by allowing the formation of large water cylinders and by filling the void volumes between the cylinders in the H_{II} phase (Gruner, 1985; Sjölund et al., 1987). Unfortunately, the H_{II} phase most probably will have to be formed before the R_0 value can be experimentally determined. However, undoubtly the beauty with this model is that it offers a simple and useful way to interpret the experimental findings, although without deeply explaining the phase transitions in terms of molecular interactions.

According to Gruner's model, it is expected that the larger the packing constraints are, i.e., the more saturated the acyl chains are, the larger must the water cylinders in the H_{II} phase be and the higher must the water content be for the formation of this phase. The model indicates that, *if* the PC–dodecane–^2H_2O systems are able to form an H_{II} phase at all, this ability will increase in the following order: DPPC < POPC < DOPC < DLiPC. This was also in principle found (Figure 1). The largest difference in the H_{II} phase forming ability, as a function of the water concentration, occurs between DPPC and POPC. A smaller difference is noted between POPC and DOPC, while practically the same water concentrations are needed for DOPC and DLiPC. The lipid–water cylinder diameter can be estimated if the lipid bilayer thickness and the specific volumes of the different components are known. If the bilayer thickness is assumed to be 50 Å and the specific volumes 1.0, 0.98, and 1.34 $cm^3\ g^{-1}$, for water, lipid, and alkane, respectively, then calculations give the following results. A sample containing DOPC with 2 mol of dodecane/mol of DOPC and 60 wt % ^2H_2O should form cylinders with a diameter of the order of 250 Å, and a sample containing POPC with 2 mol of dodecane/mol of POPC and 75 wt % ^2H_2O should form cylinders with a diameter of the order of 500 Å. It has been shown in a preliminary X-ray diffraction study that the DOPC–water cylinders may grow very large with a diameter of about 200 Å (S. M. Gruner, G. Lindblom, M. Sjölund, and L. Rilfors, unpublished results).

The phase equilibria in the DPPC–*n*-dodecane–^2H_2O system are dependent on the way the thermal equilibration before the measurements was done. A number of samples heated at 10 °C/24 h formed an isotropic phase at 65 °C while an H_{II} phase was the dominating one at this temperature in samples heated at 10 °C/h (Figure 2). These observations can be viewed in light of a theory recently presented by Siegel (1984, 1986a,b,c, 1987) that describes the kinetics in the L_α–H_{II} and L_α–cubic phase transitions. According to Siegel the initial step in the transitions is the formation of very short-lived inverted micelles between apposed bilayers. The inverted micelles may then transform either to an H_{II} phase or to a new intermediate, interlamellar attachments (ILA), which are very long lived compared with the inverted micelles. The ILA is suggested to have a structure similar to the unit cell in some bicontinuous cubic phases (Lindblom & Rilfors, 1989), and it has been proposed to be involved in membrane fusion processes (Siegel, 1986c, 1987; Ellens et al., 1988; Siegel et al., 1988). The conditions for the formation of ILA's can be given in a very simple form in Siegel's theory. The probability for an ILA to form largely depends upon the experimental parameter $Z = a_L/a_H$, where a_L is the average polar head group area in the L_α phase just below the L_α–H_{II} phase transition temperature (T_{LH}) and a_H is the average area per molecule in the H_{II} phase just above T_{LH}. Obviously, the parameter Z is closely related to R_0 in Gruner's theory and to the packing parameter $v(al)^{-1}$ in Israelachvili's theory. For large values of Z the H_{II} phase is formed, and for Z values decreasing toward approximately 1.2 the system will form isotropic phases. Generally, the larger the H_{II} tube diameter in a system at equilibrium, the smaller the values of Z. For the lecithins we have studied the Z value should increase with unsaturation from DPPC to DLiPC, and long-lived isotropic intermediates are expected to occur only with lecithins with a low degree of unsaturation; this was in fact observed. Moreover, according to Siegel's theory the thermal history of a sample may influence the formation of ILA's (Siegel, 1986c). Incubation of a sample for a long time some degrees below T_{LH} may lead to the accumulation of ILA's which are stable enough to endure even after the temperature is raised above T_{LH}. If the sample is heated much faster, fewer ILA's accumulate, and the phase equilibrium is shifted toward the H_{II} phase. This hypothesis is supported by our results obtained with the DPPC–*n*-dodecane–^2H_2O system (Figure 2).

In Figure 3 it can be seen that the chain length of the alkane plays an important role in the formation of the H_{II} phase, and a certain amount of CH_2 groups is not sufficient for the phase transition to occur. At 31 mol of water/mol of DOPC and 25 °C the ability of an alkane to induce an H_{II} phase is abolished if the number of methylene groups in the hydrocarbon chain is larger than 16 (Figure 3a,c). The longest alkanes, C_{18} and C_{20}, are solid at this temperature, so the reason for their inability to induce an H_{II} phase is most probably a question of solubility. However, even at temperatures where the longest alkanes are melted and thus probably are solubilized to some extent (see below) in the hydrophobic region, their tendency is lower to induce an H_{II} phase. To explain the results summarized in Figure 3, there are several factors that must be considered: (1) the location of the alkanes in the lipid aggregates; (2) the solubility of alkanes with different chain lengths; (3) the variation of the solubility with temperature. It has been shown that the location of *n*-alkanes in a bilayer varies with the chain length. Alkanes with less than 12 carbon atoms are probably situated mainly in the center of the bilayer at low concentrations, and the longer ones (C_{12}–C_{16}) seem to align parallel to the lipid acyl chains (McIntosh et al., 1980; White et al., 1981; Pope et al., 1984). The chain length dependence observed in this work may thus partly be due to the fact that the location of the alkanes varies with the chain length so that the shorter ones are situated between the lipid–water cylinders while the longer ones are, at least partly, extended between the lipid acyl chains. Interstice filling might not be the only role of alkanes in stabilization of the H_{II} phase. This phase may also be stabilized by a reduction of the value of R_0. Since the alkanes must partition into the lipid monolayer to change R_0, the solubility of the alkanes will affect the effectiveness in stabilizing the H_{II} phase. The solubility has been shown to be length dependent and decreases with increasing chain length (Pope & Dubro, 1986). The longer chain alkanes are therefore expected to have less tendency to induce an H_{II} phase. At increased temperatures the value of R_0 should decrease (Gruner, 1985; Gruner et al., 1988), and the solubility is supposed to increase. If R_0 decreases, less alkane is needed for interstice filling and/or R_0 adjustment. This is consistent with the observation that the effectiveness of the alkanes in stabilizing the H_{II} phases increases with temperature.

As shown in Figure 3c an increased alkane concentration from 12 to 24 alkane carbon atoms per lipid displaces the phase equilibria toward the H_{II} phase if the alkane length is shorter than 16 carbons. An increased concentration of the longer ones (C_{16}–C_{20}) has small effects on the phase equilibria. An increased water concentration has a large effect on the DOPC–*n*-alkane–^2H_2O systems. As shown in Figure 3b,d, even the longer alkanes facilitate the formation of an H_{II} phase at higher temperatures. The larger water content will not influence the solubility of these alkanes but will probably increase the lipid–water cylinder radius and therefore also the volumes between the cylinders. The latter effect could be a reason for the reduced chain length dependence when the water concentration is increased.

Cyclooctane and 2,3,4-trimethylpentane, two structural isomers of *n*-octane, induce the formation of an amount of H_{II} phase that is almost the same as the amount induced by octane. The difference in packing properties between *n*-octane and the two structural isomers is probably not large enough to give an effect on the phase equilibria. Benzene, a hydrocarbon that is more polar than the alkanes and slightly soluble in water, is probably located both in the hydrocarbon region and at the lipid–water interface (Lindblom et al., 1973; Nagarajan et al., 1984), and therefore, it will not induce an H_{II} phase.

Conclusions

Phosphatidylcholines are examples of biological membrane lipids that form only a bilayer structure, and thus a lamellar liquid-crystalline phase under physiological conditions. However, we have shown that a reversed hexagonal phase can be formed at room temperature at both low and high water contents in phosphatidylcholine–water systems if hydrophobic molecules like *n*-alkanes are added to the systems. It is of great biological interest that nonlamellar phases can also be formed when hydrophobic peptides are added to phosphatidylcholine–water systems. Examples of such peptides are gramicidin (Killian & de Kruijff, 1985; Lindblom et al., 1988) and a peptide consisting of 15 L-leucines (Sjölund, Rilfors, and Lindblom, unpublished results).

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