

# Comparative Study of the Effects of Several *n*-Alkanes on Phospholipid Hexagonal Phases

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**ABSTRACT** The effects of a series of normal alkanes (decane, dodecane, tetradecane, hexadecane, and octadecane) on the hexagonal  $H_{II}$  structures containing dioleoylphosphatidylethanolamine (DOPE) and dioleoylphosphatidylcholine (DOPC) were studied using x-ray diffraction and osmotic stress. The alkanes affect structural dimensions and the monolayer intrinsic curvature and bending modulus. The alkane effects are chain-length dependent and are attributed to their different distribution within the  $H_{II}$  structure. The data suggest that short-chain alkanes are more uniformly distributed within the  $H_{II}$  hydrocarbon regions and change the curvature and bending modulus of the monolayer, whereas longer-chain alkanes appear confined more to the interstitial region and do not change the curvature and bending modulus.

## INTRODUCTION

Considerable interest has been focused on the structures and properties of nonlamellar phases, including the inverted hexagonal phases ( $H_{II}$ ). A number of membrane functions are found to be modulated by  $H_{II}$ -promoting lipids such as dioleoylphosphatidylethanolamine (DOPE), cholesterol, and diacylglycerol (Epand, 1996, and references cited therein; Cullis and DeKruijff, 1979; Seddon, 1990). A particularly lucid study relates membrane curvature stress and channel gating (Lundbaek et al., 1997). In addition, new applications of the  $H_{II}$  phase are being developed (Perkins et al., 1996).

Alkanes are known to promote the formation of the inverted hexagonal phase  $H_{II}$ . Gruner et al. have shown that dodecane lowers the lamellar-hexagonal transition temperature  $T_H$  of many lipids (Gruner, 1985, 1989; Tate and Gruner, 1987). The addition of hexane, octane, decane, or dodecane to egg phosphatidylethanolamine lowers  $T_H$  (Hornby and Cullis, 1981). Dodecane and tetradecane lower the  $T_H$  for DOPE-dioleoylphosphatidylcholine (DOPC) mixtures (Rand et al., 1990). A comparatively long-chain alkane, eicosane, lowers the  $T_H$  for dielaidoyl phosphatidylethanolamine (DEPE) and POPE (Epand, 1985). Alkanes can even induce  $H_{II}$  formation in DOPC, which normally forms lamellar phases (Sjolund et al., 1987, 1989). A recent study showed that the addition of dodecane into a palmitoyloleoyl phosphatidylcholine (POPC)/palmitoyloleoyl phosphatidylethanolamine (POPE) system induces a decrease in chain order of  $H_{II}$  phase (Lafleur et al., 1996).

The alkanes are considered to decrease the free energy of  $H_{II}$  as they fill the interstitial region and relieve the hydrocarbon chain packing stress (Gruner, 1985; Rand et al.,

1990). For this reason alkanes have been used extensively to measure the intrinsic curvature of phospholipid monolayers. It is generally assumed that when the  $H_{II}$  phase is saturated with water and alkane the monolayer takes up the intrinsic curvature of the unstressed lipids (Rand et al., 1990). However, it was clear that tetradecane did not saturate the hexagonal phase of DOPE or DOPE/DOPC mixtures and at higher levels caused disorder. Although much work has been concerned with the effects of alkanes on the order parameters and dynamics of host phospholipid bilayers (Pope and Dubro, 1986; Pope et al., 1984; Jacobs and White, 1984), less attention has been paid either to the structural effects of different alkanes on the  $H_{II}$  phase or to the effects of different amounts of alkanes on the hexagonal structure. It has been shown directly by x-ray diffraction that decane partitions largely but not exclusively into the interstitial space (Turner et al., 1992). Sjolund et al. (1987, 1989) and Seigel et al. (1989), on the basis of nuclear magnetic resonance (NMR) studies, have interpreted their data as showing that alkanes are located in the more disordered regions peripheral to the phospholipid  $H_{II}$  tubes and in the interstices between tubes. Taken together, these latter studies suggest that partitioning into these regions is higher for longer-chain alkanes than for shorter but that higher levels of alkanes may lead to enough alkane partitioning between phospholipid chains as to increase monolayer curvature. In this study we show that alkanes cause continuous increases in  $H_{II}$  lattice dimensions and to different extents depending both on host lipid and on the alkane chain length. We attempt to discern how and where the alkanes act in these structures. In applying the criteria of monolayer curvature based on a pivotal plane we show that decane increases the curvature of DOPE monolayers, but tetradecane does not.

## MATERIALS AND METHODS

### Sample preparation

Synthetic L- $\alpha$ -dioleoylphosphatidylethanolamine (DOPE) and L- $\alpha$ -dioleoylphosphatidylcholine (DOPC) were purchased from Avanti Polar

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Lipids (Birmingham, AL) and used without further purification. The lipid was checked for impurities by thin layer chromatography and judged to be at least 98% pure. Alkanes were products of Sigma Chemical Co. (St. Louis, MO).

The lipids were stored under nitrogen at  $-18^{\circ}\text{C}$  until used. Lipid mixtures were produced by combining the appropriate amounts of DOPE or DOPC, using stock solutions in chloroform, and then removing the solvent first by rotary evaporation and then under vacuum. Tetradecane was added to the dry lipid by weighing directly. The concentration of alkanes is quoted as a percentage of the total dry weight of the sample. After 48 h of equilibration, the dry lipid mixtures were hydrated by adding either known weights of double-distilled water or excess amounts of polyethylene glycol solutions of known osmotic pressure, sealing, and equilibrating them at room temperature for another 48 h. Each sample was reweighed, combined with some powdered Teflon to use its  $4.87\text{-}\text{\AA}$  line as an internal x-ray calibration standard, and then sealed between mica windows 1 mm apart.

### X-ray diffraction

X-ray diffraction was used to characterize the structures formed by the hydrated lipid. The  $\text{CuK}\alpha_1$  line ( $\lambda = 1.540\text{ \AA}$ ), from a Rigaku rotating anode generator, was isolated using a bent quartz crystal monochromator, and diffraction patterns were recorded photographically using Guinier x-ray cameras operating in vacuo. Sample temperature was controlled with thermoelectric elements to approximately  $\pm 0.5^{\circ}\text{C}$ . All samples formed hexagonal phases characterized by at least three x-ray spacings bearing ratios to the dimension of the first order,  $d_{\text{hex}}$ , of 1,  $1/\sqrt{3}$ ,  $1/\sqrt{4}$ ,  $1/\sqrt{7}$ ,  $1/\sqrt{9}$ ,  $1/\sqrt{12}$ , etc.  $d_{\text{hex}}$  is measured to  $\pm 0.1\text{ \AA}$  on any one sample; sample to sample variation, approximately  $\pm 2\%$ , represents experimental error in sample composition.

### Structure analysis

$H_{II}$  phases are two-dimensional hexagonal lattices formed by the axes of indefinitely long, parallel, regular prisms (Fig. 1). Water cores, centered on

the prism axes, are lined with the lipid polar groups, and the rest of the lattice is filled with the hydrocarbon chains. Here, the cross-sectional shape of the water core prism within that lattice is assumed to be circular, although it has been shown that the cross section can be distorted from circularity (Turner et al., 1992).

For a hexagonal phase of known composition, the measured lattice can be divided into compartments as shown in Fig. 1, each containing defined volume fractions of the lipid and water. This volume average division follows the method originally introduced by Luzzati (e.g., see Luzzati and Husson, 1962) and depends only on a knowledge of the specific volumes of the molecular components and their relative amounts and on the assumption of their linear addition. The water and lipid compartments are divided by an idealized cylindrical interface that encloses a volume equal to the volume of water in the  $H_{II}$  phase. We refer to the surface of this cylinder as the Luzzati plane. For the lipid components in this study some physicochemical and structural parameters are listed in Table 1.

The radius of the water cylinder,  $R_w$ , is related to the first-order Bragg spacing in the hexagonal phase,  $d_{\text{hex}}$ , and to the volume fraction of water in the sample,  $\phi_w$ , as follows:

$$R_w = d_{\text{hex}} \sqrt{\frac{2\phi_w}{\pi\sqrt{3}}}. \quad (1)$$

The area per lipid molecule at the Luzzati plane is given by

$$A_w = \frac{2\phi_w V_l}{(1 - \phi_w)R_w}, \quad (2)$$

where  $V_l$  is the volume of an effective lipid molecule, and  $\phi_w = V_{\text{water}}/(V_{\text{water}} + V_{\text{PL}} + V_{\text{alkane}})$ .

We use the notion of an effective lipid molecule that is one phospholipid (DOPE or DOPC) plus  $x$  alkanes, where  $x$  is the molar ratio of alkane to phospholipid in the samples. The effective molecular volume  $V_L = V_{\text{PL}} + xV_{\text{alkane}}$ .

### Elastic energy of the hexagonal phase

The  $H_{II}$  phase can be described in terms of the curvature and molecular area of a pivotal plane where the molecular area remains constant (Rand et al., 1990; Leikin et al., 1996).

Using the radius of curvature at the pivotal plane,  $R_p$ , the elastic free energy,  $F$ , of the hexagonal phase (normalized per lipid molecule) can be approximated by the energy of bending (Helfrich, 1973; Kirk et al., 1984):

$$F = \frac{1}{2} k_{\text{cp}} A_p \left( \frac{1}{R_p} - \frac{1}{R_{0p}} \right)^2 \quad (3)$$

where  $k_{\text{cp}}$  is the bending modulus and  $A_p$  and  $R_{0p}$  are the molecular area and the spontaneous radius of curvature at the pivotal plane, respectively.

We have described a recipe for determining the position of the pivotal plane, spontaneous curvature, molecular area, and bending moduli (Leikin et al., 1996) in the  $H_{II}$  phase.

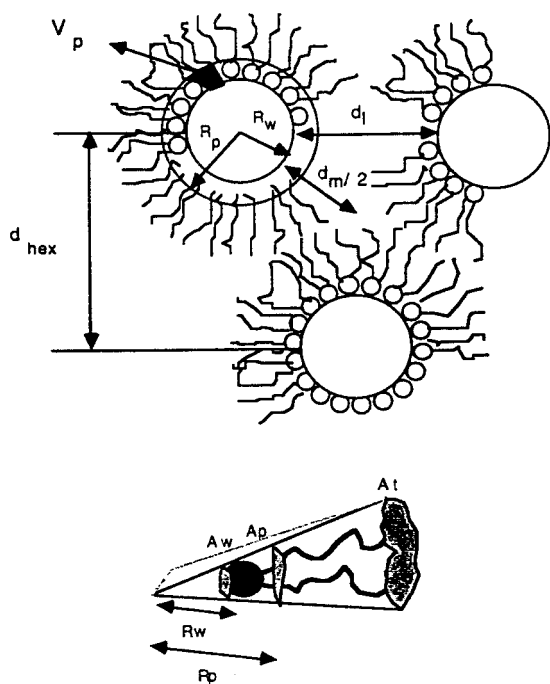


FIGURE 1 Schematic diagram of the structure of the hexagonal phase showing the dimensions determined as described in the text and used for the structural and energetic analysis.

TABLE 1 Physicochemical and structural parameters

	Molecular weight	Density (g/cm <sup>3</sup> )	Molecular volume (Å <sup>3</sup> )
DOPE	744	1	1235
DOPC	786	1.01	1292
Decane	142.3	0.730	324
Dodecane	170.3	0.749	378
Tetradecane	198.4	0.763	431
Hexadecane	226.4	0.773	486
Octadecane	254.5	0.777	544

The molecular area  $A$  and radius of curvature  $R$  at any cylindrical dividing surface, separated by a volume  $V$  per lipid molecule from the Luzzati plane (Fig. 1), are given by

$$A^2 = A_w^2 + 2V \frac{A_w}{R_w} \quad \text{or} \quad A = A_w \sqrt{1 + \frac{1 - \phi_w}{\phi_w} \frac{V}{V_1}} \quad (4)$$

$$R = R_w \sqrt{1 + \frac{1 - \phi_w}{\phi_w} \frac{V}{V_1}}. \quad (5)$$

We first verify whether the system has a well defined pivotal plane. From Eq. 4, in the form that uses normalized areas (Leikin et al., 1996),

$$\frac{A_w^2}{V_1^2} = \frac{A_p^2}{V_1^2} - 2 \frac{V_p}{V_1} \left( \frac{A_w}{V_1 R_w} \right). \quad (6)$$

We establish whether the plot  $(A_w/V_1)^2$  versus  $(A_w/V_1)R_w$  is a straight line. If so, the system has a dividing surface of constant area, which is the pivotal plane. From the slope and the intercept of the plot we determine both the location of the pivotal plane, through  $V_p$ , the volume separating it and the Luzzati plane, and its molecular area ( $A_p$ ).

From the value of  $V_p$  we calculate the radii of curvature ( $R_p$ ) at the pivotal plane using Eq. 5. We use these radii and follow the previously suggested procedure (Gruner et al., 1986; Rand et al., 1990) for determining the elastic parameters of the lipid mixture from osmotic stress experiments. Specifically, comparing the elastic energy given by Eq. 3 with the osmotic work done by the osmotic stress ( $\Pi$ ), we find the following relationship:

$$\Pi R_p^2 = 2k_{cp} \left( \frac{1}{R_p} - \frac{1}{R_{0p}} \right). \quad (7)$$

The plot of  $(\Pi R_p^2)$  versus  $(1/R_p)$  gives, simultaneously from the slope and the intercept, the monolayer bending modulus ( $k_{cp}$ ) and the spontaneous curvature ( $1/R_{0p}$ ) (Gruner et al., 1986; Rand et al., 1990). We use the subscript  $p$  to be consistent with our previous notation and particularly to emphasize that these measurements are at the pivotal plane and not at the neutral plane of the monolayer (Leikin et al., 1996).

## RESULTS

### Alkanes and the hexagonal lattice dimension

The equilibrium lattice dimensions of DOPE  $H_{II}$  phases with increasing alkane content are shown in Fig. 2. Decane has little effect on  $d_{\text{hex}}$  of DOPE, whereas dodecane, tetradecane, hexadecane, and octadecane all increased  $d_{\text{hex}}$  systematically with alkane content, and to extents that systematically increased with chain length.  $d_{\text{hex}}$  increases linearly with increasing alkane in the range of 0–15%, indicating that the alkanes are not forming separate phases within this concentration range.

At any fixed alkane content,  $d_{\text{hex}}$  increases with alkane chain length. Limited amounts of alkane could be incorporated into the hexagonal phase. Above that limit, extra lines, disorder, or maximal spacings were observed indicating either phase separation or phase transition from the single hexagonal phase. We restrict all of our analysis here to the single-phase region.

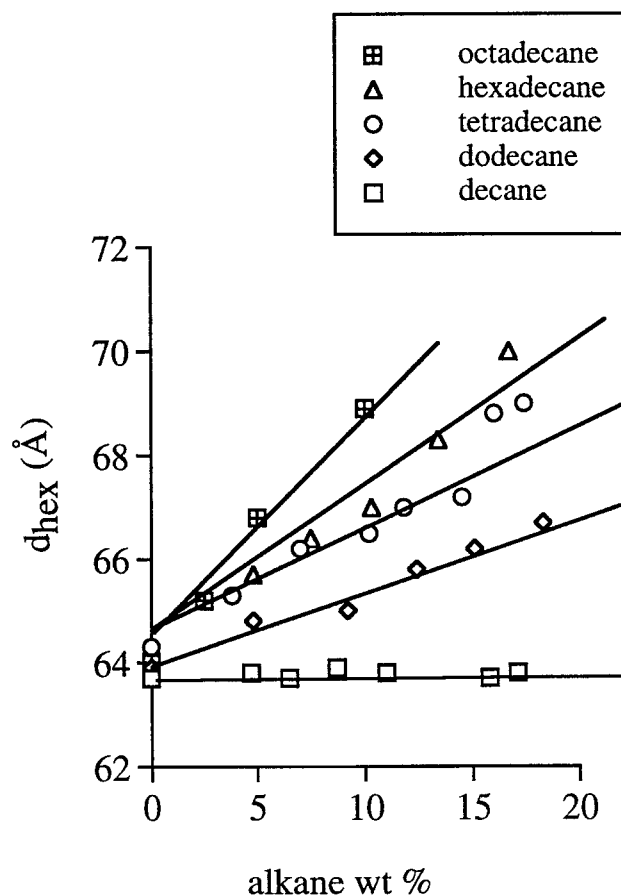


FIGURE 2 Lattice dimension  $d_{\text{hex}}$  of the hexagonal phases formed by dioleoylphosphatidylethanolamine containing increasing amounts of the indicated alkane in excess water. Higher alkane contents result in disorder of the hexagonal phase.

Similar trends for the large hexagonal phases formed by DOPE/DOPC (3:1, mol/mol) are shown in Fig. 3. Here all alkanes including decane increased  $d_{\text{hex}}$  systematically.

### Changes in structural dimensions induced by decane or tetradecane.

The structural effects of alkanes on DOPE were examined.  $d_{\text{hex}}-\phi_w$  phase diagrams were constructed for DOPE/water, DOPE/16%decane/water, and DOPE/16%tetradecane/water and are shown in Fig. 4.

$d_{\text{hex}}$  for the three systems increased with addition of water to a limiting swelling value where the system becomes saturated with water. Remarkably, that limiting value is the same for DOPE with and without decane but increases with tetradecane, consistent with the data of Fig. 3. The limiting values of  $\phi_w^0$  and  $d_{\text{hex}}$  were determined as previously described (Chen and Rand, 1997) by the intersection of the average dimension in excess water with a line fitted to the change in dimension with water content. These lines are shown in Fig. 4 and show the different qualitative effects of these two alkanes. Although the differences are small, they

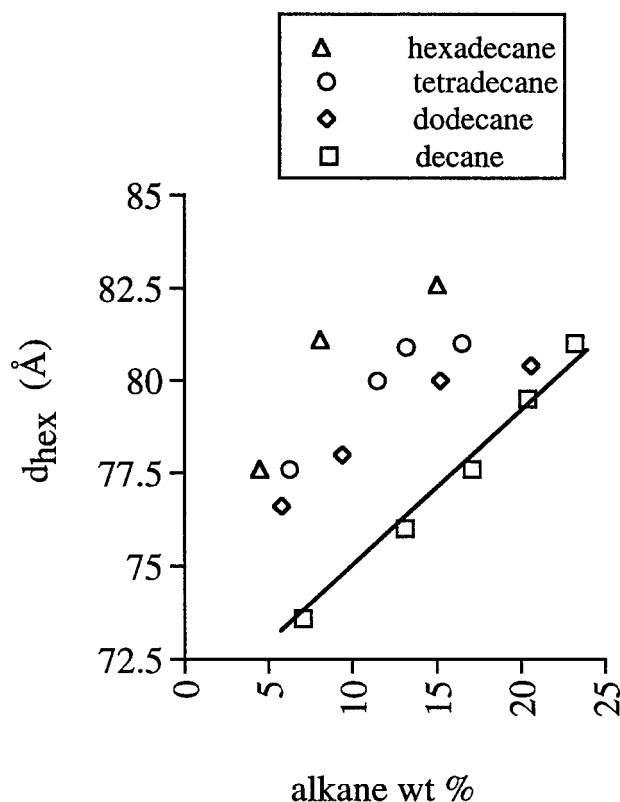


FIGURE 3 Lattice dimension  $d_{\text{hex}}$  of the single hexagonal phases formed by DOPE/DOPC 3/1 with increasing amounts of added alkane and in excess water. Higher alkane contents result in disorder of the hexagonal phase.

are systematic. Decane results in a lower limiting water content than DOPE at the same hexagonal lattice dimension. Tetradecane on the other hand results in an equivalent limiting water content as decane but at a larger lattice dimension. On this basis, these data yield the various structural dimensions, defined in Fig. 1, of the hexagonal phases in excess water that are shown in Table 2.

The striking effect of equivalent volumes of alkane is that the lattice dimensions of both DOPE/decane and DOPE/tetradecane in limited water are larger than that of pure DOPE and increase with alkane size. This effect is qualitatively different from that of dioleoylglycerol (DOG) and cholesterol added to DOPE, both of which cause no increase in  $d_{\text{hex}}$  at constant  $\phi_w$  no matter how much cholesterol or DOG was added (Chen et al., 1997; Leikin et al., 1996). We analyze these data later in terms of a pivotal plane within the hexagonal phase monolayer.

Several structural parameters of the hexagonal phase were measured in an attempt to determine how these hydrocarbons affect lattice dimension. In a completely separate series of samples, a comparison was made for DOPE with and without two levels of decane and tetradecane, where all samples have a constant water content of 14.1 water molecules per polar group. Some characteristic parameters are shown in Fig. 5. With the same number of water molecules per effective molecule,  $d_{\text{hex}}$  of DOPE/

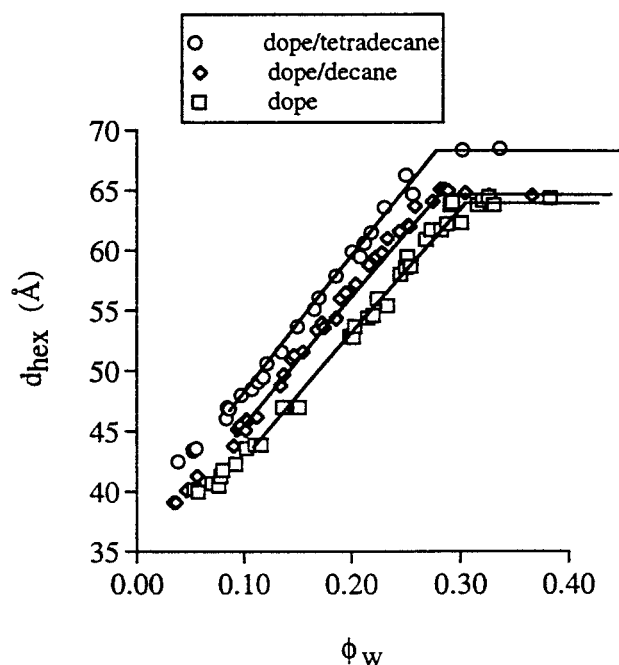


FIGURE 4 Lattice dimension  $d_{\text{hex}}$  of the phases formed by DOPE, DOPE/decane, and DOPE/tetradecane (16% dry weight alkane) as a function of volume fraction water. In less than excess water, the lattice dimensions for each system were detectably different. The maximal lattice dimension, indicated by the horizontal lines, was not detectably different with added decane but was for added tetradecane. The limiting water fraction is determined by the intersection of the horizontal and fitted lines. Structural dimensions for excess water are shown in Table 1.

decane is almost identical to that of pure DOPE whereas  $d_{\text{hex}}$  of DOPE/tetradecane increases with alkane content. Although  $d_{\text{hex}}$  barely changed with addition of decane,  $R_w$  decreased with addition of decane much more than with tetradecane. Both lipid layer dimensions,  $d_l$  and  $d_m$ , increase more in the case of tetradecane than that of decane, as is also the case in excess water (Table 2). These differences between decane and tetradecane are consistent with the data of Figs. 2 and 4.

#### Influence of decane or tetradecane on the pivotal plane of DOPE

The phase data have been used to determine 1) whether there is a pivotal plane within the monolayer and 2) where that pivotal plane is. The diagnostic plots (Leikin et al., 1996) of  $(A_w/V_L)^2$  versus  $A_w/(V_L R_w)$  for DOPE, DOPE/16 wt% decane, and DOPE/16 wt% tetradecane are shown in Fig. 6. All three plots are straight lines, which indicates that there is a well-defined pivotal plane in all three cases. These linear fits yield the values shown in Table 3 where the errors are 95% confidence limits.

These results show that the absolute area  $A_p$  of the pivotal plane increases from  $64 \text{ \AA}^2$  for DOPE to  $73 \text{ \AA}^2$  with added decane and tetradecane. The position of the pivotal plane, however, shifts with added alkanes to include more hydro-

TABLE 2 Some structural parameters of the hexagonal phases in excess water

	$\phi_w^0$	$d_{\text{hex}}$ (Å)	$N_w$	$R_w$ (Å)	$d_l$ (Å)	$d_m$ (Å)	$A_w$ (Å) <sup>2</sup>	$A_t$ (Å) <sup>2</sup>
DOPE	0.708	64.0	17	21.0	32.0	43.4	49	94
DOPE/d	0.718	64.5	19	20.3	33.9	45.4	57	115
DOPE/td	0.713	68.4	20	21.8	35.6	47.9	54	109

$\phi_w^0$  is the volume fraction water in the hexagonal phase in excess water yielding  $N_w$ , the number of water molecules per effective molecule.

carbon volume. The volume of the DOPE polar group is 312 Å<sup>3</sup>;  $V_p$  includes 380 Å<sup>3</sup> for DOPE, 408 Å<sup>3</sup> for decane, and 470 Å<sup>3</sup> for tetradecane.

Osmotically stressed hexagonal phases with or without decane or tetradecane

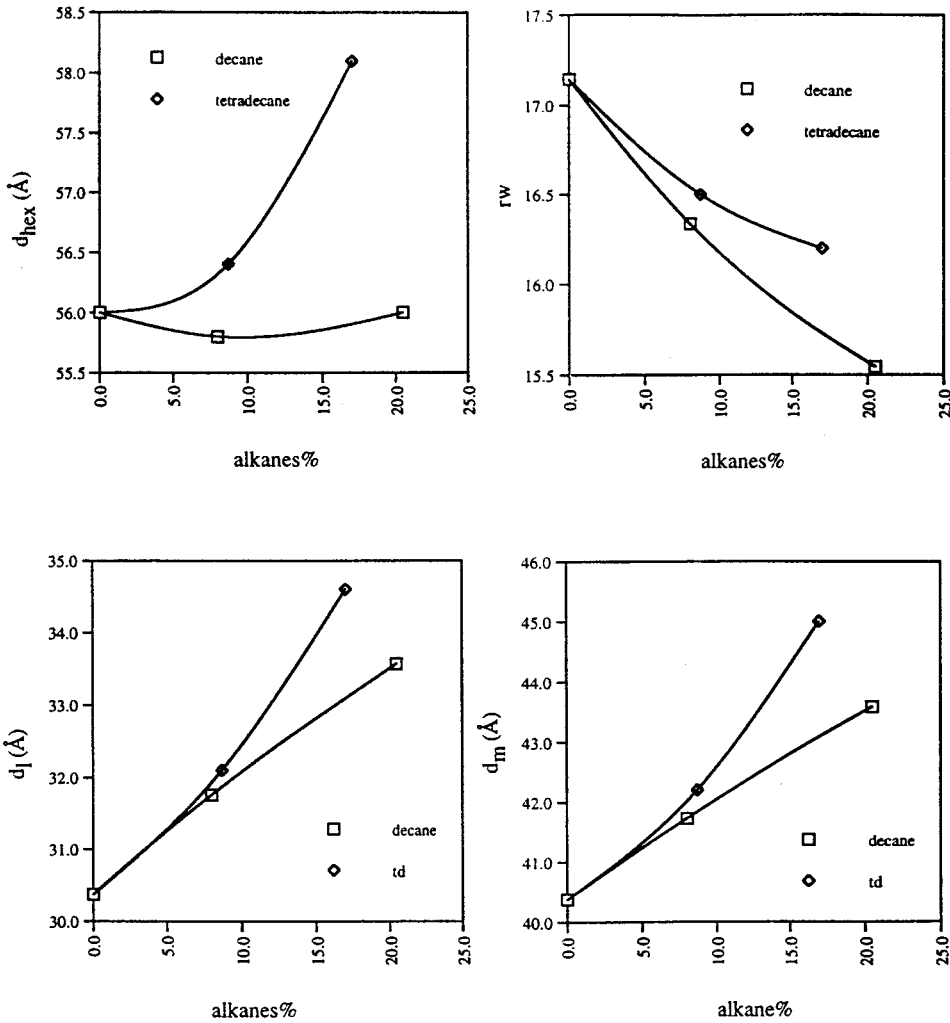
The lattice dimensions of osmotically stressed hexagonal phases of DOPE, with and without 16% of the various alkanes are shown in Fig. 7. The dimensions maintain their relative values throughout the osmotic stress range but appear to converge at high pressures or low water content. For decane and tetradecane, the water content of each of these phases ( $\phi_w$ ) is determined from the  $\phi_w$  versus  $d_{\text{hex}}$

dependence (Fig. 4) and structural dimensions determined as previously described.

Effect of decane and tetradecane on intrinsic curvature  $R_{op}$  and bending modulus  $k_{cp}$

We determine the radius of curvature at the pivotal plane,  $R_p$ , and the monolayer bending modulus  $k_{cp}$  for the osmotically stressed hexagonal phases, using Eq. 6 with  $R_w$  and  $V_p$  and using the  $\phi_w$  as determined from Fig. 4. Figure 8 shows the plots of  $\Pi R_p^2$  versus  $1/R_p$  for DOPE, DOPE/decane, and DOPE/tetradecane, where  $\Pi$  is the osmotic pressure of the equilibrating solution. The intercepts at  $\Pi = 0$ , and the slopes yield the radii of spontaneous curvature  $R_{op}$  and

FIGURE 5 Dimensions, defined in Fig. 1, in the hexagonal phases formed by DOPE with added decane or tetradecane where all samples contain 14.1 water molecules per ethanolamine group, this being less than excess water. For these dimensions, decane is seen to have a smaller effect on the hexagonal lattice of DOPE than tetradecane.





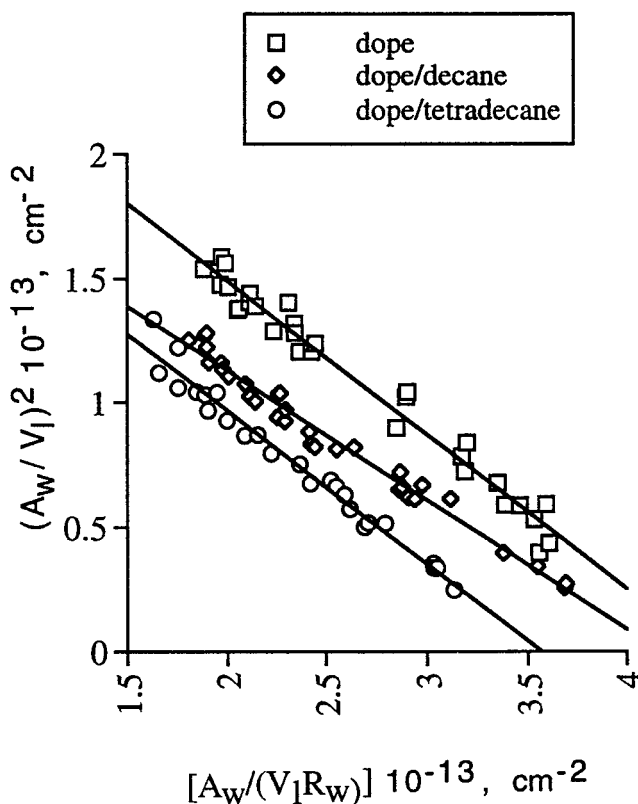


FIGURE 6 Diagnostic plots, as described in Eqs. 6 and applied to the data of Fig. 4, relating molecular area at the Luzzati plane  $A_w$  with the radius of the water cylinder  $R_w$ . When the area is normalized by the volume of an effective molecule ( $V_l$ ) as described in the text, a systematic deviation from pure DOPE can be seen both with decane and tetradecane. However, each system yielded linear plots indicating the presence of a pivotal plane of constant area. Linear least squares fits of these plots give the slope ( $2V_p/V_l$ ), which defines the fraction of molecular lipid volume included inside the pivotal surface, and the intercept, which gives its molecular area. Data are shown in Table 2.

bending moduli  $k_{cp}$  shown in Table 4 where the errors are 95% confidence limits. We describe the position of the pivotal plane in Table 4 in terms of  $\delta_{pol}$  and  $\delta_{hc}$ , where  $\delta_{pol} = R_{op} - R_w$  and  $\delta_{hc} = d_l - \delta_{pol}$ .

## DISCUSSION

The idea that one could saturate the hexagonal lattice with hydrocarbon and water and thereby relieve hydration and hydrocarbon chain stress and allow the phospholipid mono-

layers to relax to their intrinsic or spontaneous curvature has been used to measure that curvature. Alkanes are generally considered to partition into the interstitial part of  $H_{II}$  phases and not to change the radius of the curvature significantly (Kirk and Gruner, 1985; Siegel et al., 1989). It has been shown directly by x-ray diffraction (Turner et al., 1992) that decane partitions largely but not exclusively into the interstitial space. However, these current results indicate that several dimensions within the fully hydrated DOPE hexagonal phase change continuously with alkane content and do not plateau, for example, at the level used to measure intrinsic curvature. Higher alkane contents cause phase transitions. Both of these effects are inconsistent with the concept of saturation of the hexagonal structure with hydrocarbon. Furthermore, these results show that the dimension of the fully hydrated DOPE hexagonal phase increases differently with hydrocarbons of different chain lengths, i.e., the hydrocarbons do not act equally as indifferent solvents. Finally, these results show that these effects are different for DOPE and DOPC/DOPE hexagonal phases. These data raise the question as to what extent the measured  $R_{op}$  is intrinsic or spontaneous or is that of the fully unstressed unmodified phospholipid monolayer.

The more detailed structural dimensions, measured curvatures and bending moduli for decane and tetradecane provide some indications for their different effects. The dimensions in Table 5 summarize the major effects of decane and tetradecane, with values in bold indicating singular significant difference where it exists. All suggest that decane, more than tetradecane, partitions into the hydrocarbon space between the phospholipid chains, and tetradecane is more confined to the spaces between  $H_{II}$  tubes, at the ends of the phospholipid chains. This interpretation is shown schematically in Fig. 9.

The fully hydrated hexagonal lattice dimension is unchanged with added decane but increases with tetradecane. It is clear from this work that the simple lattice dimension and its changes are deceptive in reflecting curvature changes when curvature is defined at the pivotal plane.  $R_{op}$  is unchanged with tetradecane and decreases with decane. This would be expected if decane partitions or intercalates between the hydrocarbon chains of the phospholipids, splaying them, effectively increasing lipid curvature. Tetradecane on the other hand has no effect on curvature but increases  $d_l$  and  $d_m$  more than decane, suggesting that it is more restricted to the regions external to the lipid chains. Similar differential disposition of the alkanes is suggested

TABLE 3 Fits to the data of Figure 6 to determine the position of the pivotal plane as defined by its area  $A_p$  and volume between the pivotal and Luzzati planes  $V_p$

	$A_p/V_l (\text{\AA})^{-1}$	$V_p/V_l$	$V_l (\text{\AA})^3$	$V_{pol} (\text{\AA})^3$	$A_p (\text{\AA})^2$	$V_p (\text{\AA})^3$
DOPE	$0.052 \pm 0.002$	$0.31 \pm 0.02$	1235	312	$64.2 \pm 2.5$	$380 \pm 25$
DOPE-decane	$0.047 \pm 0.002$	$0.26 \pm 0.02$	1569	312	$73.7 \pm 3.2$	$408 \pm 27$
DOPE-tetradecane	$0.047 \pm 0.002$	$0.30 \pm 0.02$	1567	312	$73.6 \pm 3.2$	$470 \pm 30$

$V_{pol}$  is the volume of the polar group of DOPE. Errors are 95% confidence limits.

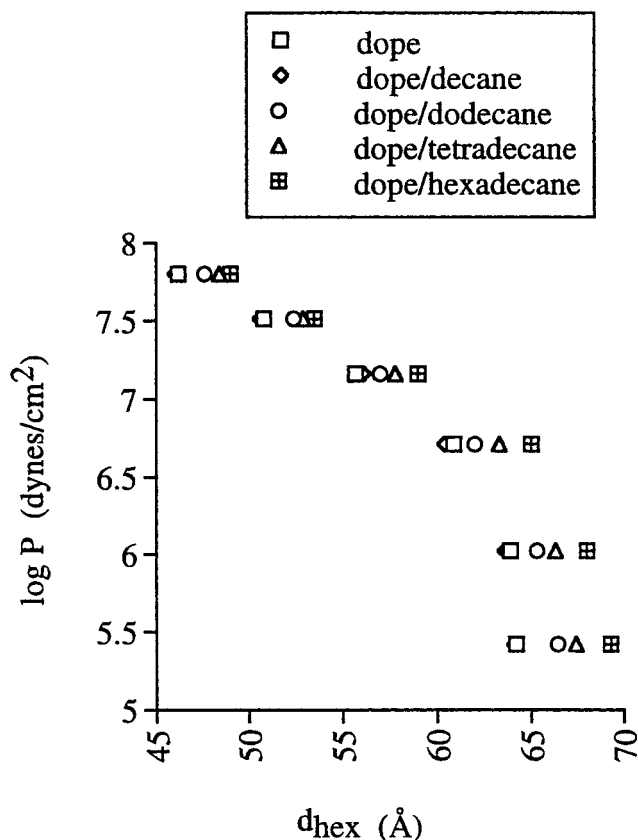


FIGURE 7 Experimental data relating the osmotic pressure,  $\Pi$ , of the equilibrating solutions and the hexagonal lattice dimension  $d_{\text{hex}}$  formed by DOPE with and without 16 wt% of the indicated alkane. With increasing alkane size, the lattice dimensions start from ever larger lattice size at low pressures but converge at higher pressures.

by the structural dimensions at limited and constant numbers of water per polar group seen in Fig. 4. Here decane again has a smaller effect than tetradecane on the hydrocarbon thicknesses  $d_h$  and  $d_m$ , as if it is more distributed among the hydrocarbon chains of the lipids, and tetradecane between  $H_{II}$  tubes.

With both decane and tetradecane the pivotal plane has moved toward the polar group layer, as seen by the decrease in the distance between the Luzzati and pivotal planes,  $\delta_{\text{pol}}$  and increase in the distance between pivotal plane and chain terminals  $\delta_{\text{hc}}$ . However, decane causes a greater relative shift than tetradecane. Such a greater shift is seen also as a greater decrease in  $V_p/V_l$  (Table 3).

Decane, and not tetradecane, causes a decrease in the monolayer bending modulus. In fact, this is the only decrease in the modulus we have ever observed; both cholesterol and diacylglycerol for example cause approximately a 30% increase in bending modulus at equimolar concentrations (Chen and Rand, 1997; Leikin et al., 1996). Each of these latter additives is restricted to the monolayer, constrained to be parallel to the phospholipid chains. One might speculate that if decane, and not tetradecane, increasingly

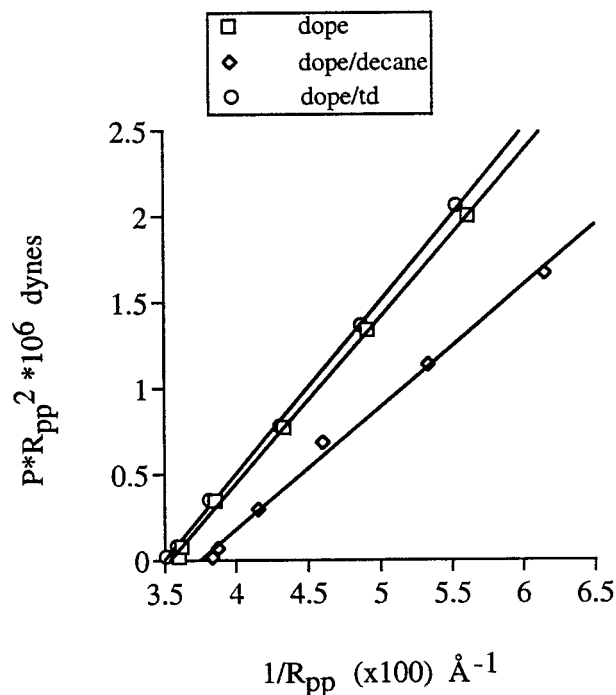


FIGURE 8 Plot, following Eq. 7, to determine the intrinsic radius of curvature  $R_o$  and the bending modulus  $k_{cp}$  for these plots of DOPE and DOPE with 16% decane or tetradecane. Data are shown in Table 3.

partitions into the hydrocarbons of the phospholipids during monolayer bending, that might ease bending, reducing the effective modulus.

The differential partitioning of these alkanes is consistent with several NMR and other previous studies of alkanes in hexagonal phases and their ability to cause lamellar-hexagonal transitions. Sjolund et al. (1987, 1989) and Seigel et al. (1989), on the basis of NMR studies, have interpreted their data as showing that alkanes are located in the more disordered regions peripheral to the phospholipid  $H_{II}$  tubes. Alkanes at higher concentrations were thought to partition between phospholipid chains and to increase monolayer curvature. However, that interpretation relied on the assumption that lattice dimension and intrinsic radius of curvature were directly connected. However, if curvature is measured at the pivotal plane, our results show that even at the high levels of tetradecane such partitioning does not result in curvature change, whereas it does with decane. One

TABLE 4 Fits to the data of Figure 8 giving the intrinsic curvature  $R_{op}$  and bending modulus  $k_{cp}$

	$R_{op}$ (Å)	$k_{cp}$ (kT)	$\delta_{\text{pol}}$ (Å)	$\delta_{\text{hc}}$ (Å)
DOPE	$28.3 \pm 0.3$	$12 \pm 0.5$	$7.3 \pm 0.5$	$8.7 \pm 0.5$
DOPE-decane	$26.7 \pm 0.3$	$9 \pm 0.8$	$6.4 \pm 0.5$	$10.6 \pm 0.5$
DOPE-tetradecane	$28.7 \pm 0.3$	$12 \pm 0.6$	$6.9 \pm 0.5$	$10.9 \pm 0.5$

$\delta_{\text{pol}}$  and  $\delta_{\text{hc}}$  are the distances, shown in Figure 9, from the pivotal plane to the Luzzati plane and to the chain terminals in the interaxial direction, respectively. Errors are 95% confidence limits.

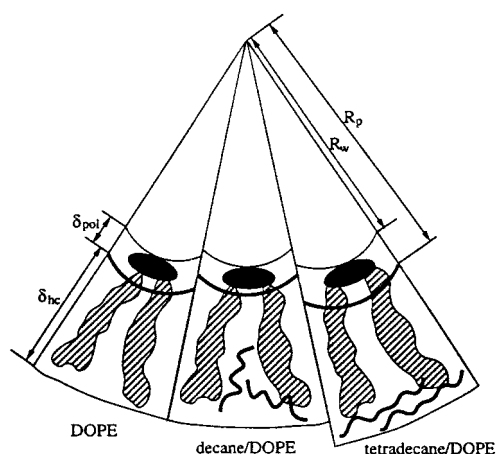
**TABLE 5** Major effects of decane and tetradecane

	$d_{\text{hex}}$ (Å)	$R_{\text{op}}$ (Å)	$\delta_{\text{pol}}$	$A_p$ (Å) <sup>2</sup>	$V_p$ (Å) <sup>3</sup>	$d_l$ (Å)	$\delta_{\text{hc}}$	$k_c$ (kT)
DOPE	64.0	28.3	7.3	<b>64.2</b>	380	32.0	<b>8.7</b>	12
DOPE-decane	64.5	<b>26.7</b>	6.4	73.7	408	33.9	10.6	<b>9</b>
DOPE-tetradecane	<b>68.4</b>	28.7	6.9	73.6	470	35.6	10.9	12

might expect the longer-chain hydrocarbons, on the basis of their higher entropic cost of uncoiling, to be more confined to the more isotropic space external to the lipid chains and the shorter chains to be able to partition more among the lipid chains. Marqusee and Dill (1986) have formulated a mean field theory of solute partitioning in such phases of amphiphiles that depends on such entropy considerations and on gradients in hydrocarbon chain order.

In addition to these more general considerations of partitioning, our empirical results suggest rather specific effects; that tetradecane, for example, is the more appropriate hydrocarbon to use in measuring intrinsic curvature of DOPE as it intercalates less into the lipid chains. However, one needs to confirm any independence of measured curvature on chain length with more studies. One can see from Figs. 2 and 3 that the hydrocarbon effects are different for DOPE/DOPC than for DOPE itself. Until the details of the former effects are studied, it is difficult to judge which hydrocarbon is most appropriate to use to measure these smaller intrinsic curvatures.

Although these results qualify the strategy of measuring intrinsic curvature as we have used it in the past, they do not negate measuring large differences in intrinsic curvature. The effects of alkane content and the differences among the alkanes are relatively small compared with the large changes in curvature induced by, for example, the choline head group (Rand et al., 1990), cholesterol (Chen and Rand, 1997), and lysolipids (Chen et al., 1997).



**FIGURE 9** Schematic diagram of the dimensions occupied by the indicated equivalent molecules in the hexagonal phases. Results suggest that decane partitions more into the hydrocarbon chain regions of the phospholipids, and tetradecane is restricted more to the interstitial regions and the regions between hexagonal tubes.

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