Dependence of lipid chain and headgroup packing of the inverted hexagonal phase on hydration

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ABSTRACT A model which positions the hydrophobic/hydrophilic boundary in phosphatidylethanolamine lipids at the first CH_2 group in the acyl or alkyl chain is used to calculate the surface area per lipid, the mean chain and headgroup dimensions and diameters of the hydrophilic tubes of the inverted hexagonal phase of didodecylphosphatidylethanolamine. The calculated surface areas compare favorably with areas obtained for the lamellar liquid crystal phase of the same lipid using the same boundary. Placement of the boundary within the lipid structure permits a determination of the maximum headgroup packing at hydration levels down to complete dehydration. The headgroup dimensions are consistent with a 5 Å diam void at the center of a hydrophilic tube at zero hydration. The calculated mean fluid chain length is ~2 Å smaller than the mean chain length of the lamellar phase at comparable levels of hydration. Comparison of the calculated mean fluid chain length and distances between hydrophobic boundaries shows that the fluid chains are interdigitated between adjacent tubes, and not interdigitated in the central space between three tubes. At low hydration the chains interdigitate in both spaces. The number of lipids packed around a tube at low hydration is only a function of the headgroup geometry, whereas at high hydration, it is a function of the number of carbon atoms in the chains.

INTRODUCTION

The interaction of water with lipids has been studied using a variety of techniques (Hauser, 1975). Generally, water affects the structural integrity and biological function of lipid membranes in complex ways. Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) lipids, primary constituents of many biological membranes, exhibit differences in water affinity (Elworthy, 1961; Jendrasiak and Hasty, 1974) that are remarkable in view of the seemingly minor replacement of three methyl groups for three hydrogen atoms.

Besides having an apparent lower affinity for water, PE membranes have a tendency to form nonlamellar structures that seem antithetic to the bilayer structure which is so common in biological membranes (Cullis and DeKruijff, 1979). One such structure is the inverted hexagonal phase H_{II} (Verkleij, 1984). Unlike the bilayer, where hydrophilic headgroups cover both surfaces of a hydrocarbon blanket, the headgroups of the H_{II} phase line the surfaces of a hexagonal array of tubes that extend through a hydrocarbon filled volume (Luzzati, 1968). Also in contrast, the lamellar to H_{II} phase transition temperature is lowered by a decrease in the hydration of the headgroups or lengthening of the acyl chains (Seddon et al., 1983), and phase stability is increased by disorder in the acyl chains (Mantsch et al., 1981).

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Recent work by White and King (1985), McIntosh and Simon (1986), and Scherer (1987, 1989) has clearly shown that the position of the hydrophobic/hydrophilic boundary of phospholipids in the lamellar phases should be placed near the average position of the first CH_2 groups of the hydrocarbon chain instead of the outer edge of the lipid headgroup, as assumed in the Luzzati formalism (1968). The position of the boundary has been shown to affect the dependence of the head and chain length dimensions on hydration.

In the following, the headgroup and chain length dimensions of the H_{II} phase of didodecylphosphatidylethanolamine (DDPE) and the surface area, S, are determined from the x-ray data of Seddon et al. (1984), using the same boundary assumed for the lamellar phase of DDPE (Scherer, 1989). It will be shown that the calculated dimensions of the fluid chains at maximum hydration require that they be interdigitated in the regions between adjacent tubes and not interdigitated in the central region between three tubes. At zero hydration the chains are interdigitated in both regions. The headgroup packing at maximum and minimum hydration may be obtained from calculated surface area and shape.

Volumetrics of the H_{II} phase

As for the lamellar phase, the boundary between the hydrocarbon and hydrophilic regions is set at the average position of the C-2 carbon atom of the acyl chain of an ester PE or the first CH_2 group in the alkyl chain for an

ether PE. The total volume (per lipid) is divided into a hydrophobic and hydrophilic part.

$$V = V_{hc} + V_H. \quad (1)$$

By defining the area per lipid, S, at the hc/H boundary, S may be compared with S for the bilayer at all hydrations. The approach introduced by Luzzati (1968), places the boundary at the end of the lipid headgroup, and leads to values of S that are progressively smaller than the value of S for the bilayer at maximum hydration, and 0 Å^2 at complete dehydration (Seddon, 1984). S is related to the volumes by

$$V = 2d^2S/\sqrt{3}\pi d_H \quad (2)$$

$$V_H = d_H S/4 \quad (3)$$

and

$$V_{hc} = d_* S, \quad (4)$$

where d is the x-ray long spacing, d_H the diameter of the hydrophilic tube, and

$$d_* = 2d^2/\sqrt{3}\pi d_H - d_H/4. \quad (5)$$

d_* represents the average (fluid) chain length if all chains were fit into extended volumes with uniform cross sectional areas $S/2$. The value of d_* for chains that are not extensively interdigitated should be between $b \equiv 2d/3 - d_H/2$ and $c \equiv d/\sqrt{3} - d_H/2$. Rewriting Eq. 5, we obtain

$$d_H/2 = -d_* + (d_*^2 + 2d^2/\sqrt{3}\pi)^{1/2}. \quad (6)$$

Assuming that the volume of the hydrocarbon phase is independent of hydration, $\delta V_{hc}/\delta n = 0$, where n is the number of water molecules per lipid,

$$\delta d_*/\delta n = -(d_*/S)\delta S/\delta n \quad (7)$$

and

$$\bar{V}_w = \delta V_H/\delta n = 2(1/V + 1/V_H)^{-1}(\delta \ln d/\delta n + \delta \ln S/\delta n), \quad (8)$$

where $\bar{V}_w$ is the partial molar volume of water. $\bar{V}_w$ and the partial specific volume of lipid $\bar{v}_l$ are assumed to be independent of hydration. Consequently, the total volume V is given by

$$V = \bar{v}_l M_r 10^{24}/\phi N \quad (9)$$

or

$$V = V_{PE} + V_{hc} + nV_w, \quad (10)$$

where ϕ is the volume fraction of lipid, $(1 + [1-c]\bar{v}_w/c\bar{v}_l)^{-1}$, c is the weight fraction of lipid, and V_w is the volume of a water molecule. V_{PE} is the volume of a nonhydrated PE group. Wilkinson and Nagle (1981) have found the value of V_{PE} for acyl chain lipids to be 246 Å^3. In the case of DDPE, the value of V_{PE} is smaller by the volume of two carbonyl groups which, according to Bondi (1964), is 39 Å^3. Subtracting this from the volume of V_{PE} for an acyl chain lipid gives an estimate of 207 Å^3 for V_{PE} of a diether PE. V_H is obtained by adding the volume of the PE portion of the headgroup to the volume of water in the headgroup,

$$V_H = V(1 - \phi) + V_{PE} = nV_w + V_{PE} \quad (11)$$

and S is obtained from

$$S = (2\sqrt{3}\pi V V_H)^{1/2}/d. \quad (12)$$

The change in d spacing of DDPE with hydration was determined by Seddon et al., 1984. The dependence of the calculated values of S, d_*, b, c, and $d_H/2$ on hydration is shown in Figs. 1–3. For comparison, the corresponding dimensions of S, $d_{hc}/2$, and $d_H/2$ for the lamellar phases of dismyristoyl phosphatidylcholine (DMPC), egg phosphatidylcholine (EPC) and DDPE are also shown.[1] An illustration of the PE bilayer and the PE H_{II} phase (enveloped by a bilayer) at minimum and maximum hydration is shown in Fig. 4. The average hydrocarbon chain lengths ($d_{hc}/2$ and d_*), hydrophilic tube diameters (d_H), and lipid packing around the tubes are drawn scaled to the calculated dimensions. Head and chain packing shown in this figure will be discussed in the following section.

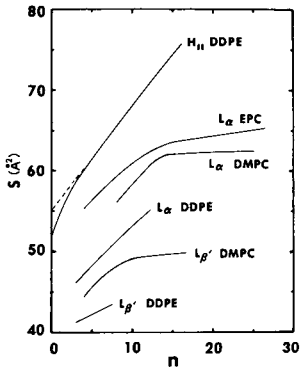

FIGURE 1 The calculated variation of S with hydration for the H_{II} phase of DDPE (135°C); the L_α phases of DMPC (37°C), EPC (room temperature), and DDPE (40°C); and the $L_{\beta'}$ phases of DMPC (10°C) and DDPE (29°C). The dashed curve is obtained by extrapolation of the curve above $n = 4$ and is preferred from a consideration of headgroup packing below $n = 4$ (see text).

[1]Labeling of the polymorphic phases of lipids follows the accepted conventions defined by Tardieu et al., 1973.

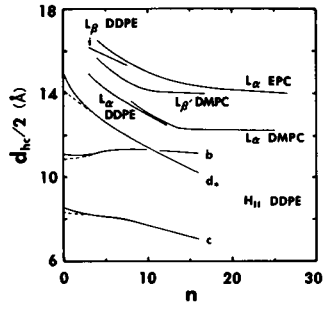

FIGURE 2 The calculated variation of $d_{hc}/2$, d_*, b, and c with hydration for the H_{II} phase of DDPE, and lamellar phases of DMPC, EPC, and DDPE. Conditions are consistent with those listed in Fig. 1. The dashed line for the H_{II} phase of DDPE shows the result of assuming that $\delta S/\delta n$ has a constant value equal to that indicated by the dashed line in Fig. 1.

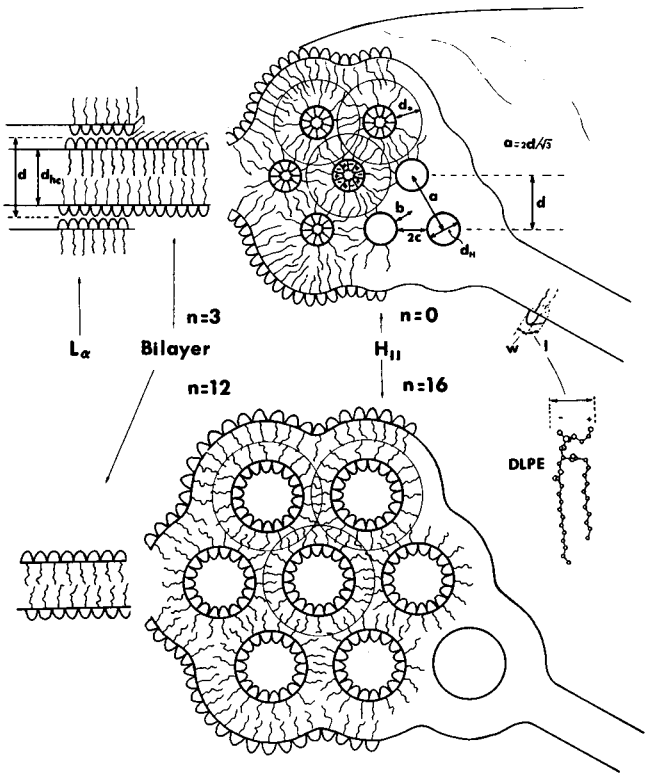

FIGURE 4 Illustration of the calculated structure for the H_{II} phase of DDPE at zero (*above*) and maximum (*below*) hydration and the bilayer structures $L_{\beta'}$ ($n = 3$) and L_α ($n = 12$) for DDPE. The illustration is drawn to the scale of the calculated dimensions in Figs. 2 and 3. Only one of the chains of the lipid is shown. The dimensions are only valid for the pure multilayer and H_{II} phases. Although the H_{II} phase is shown sandwiched between two halves of a bilayer, the calculation predicts interdigitation only for the pure H_{II} phase. The interdigitation shown for the covering lipid layer is due to artistic licence. Similarly, nothing is implied about the mechanisms for interconversion of the bilayer and H_{II} phases.

RESULTS AND DISCUSSION

CH_2 chain dimensions

We begin by considering the calculated dimensions of the CH_2 chains given by the solid lines in Fig. 2. We note that the values of c (half the hydrocarbon distance between adjacent tubes) increase by little more than 1 Å going from maximum to zero hydration. The values for b are essentially invariant over the same range. This behavior deviates from the behavior of lamellar fluid phases where the CH_2 chains extend with dehydration. However, the values of d_* systematically increase with dehydration and parallel the behavior of $d_{hc}/2$ for the lamellar fluid phases at hydrations down to $n = 3$. Chains can be said to interdigitate when their mean chain length exceeds half the distance between chain boundaries, in this case b or c. At maximum hydration, the average chain length is close to the value of the b lattice dimension. However, chains in the c direction are on the average 3 Å longer than the c lattice dimension and must be interdigitated. At zero hydration, the chains are highly interdigitated along both the c and b lattice dimensions. Seddon et al. (1984) used the Luzzati boundary definition and calculated that $d_1/2$ (which they defined to be $[a - d_w]/2$ and is analogous to our c) increases from 10.1 Å at maximum hydration to 16.6 Å at zero hydration. Although not discussed, the corresponding lipid dimension in the b lattice direction increases from 14.2 to 19.2 Å. These large increases in the calculated lipid length are reminiscent of the anomalous increases in the values of d_1 for dehydrating lamellar structures (Janiak et al., 1979; White and King, 1985) and should be contrasted with the small variations in the hydrocarbon core dimensions b and c given by the present analysis.

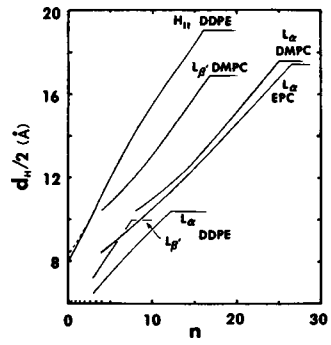

FIGURE 3 The calculated variation of $d_H/2$ for the H_{II} phase of DDPE, and lamellar phases of DMPC, EPC, and DDPE. The dashed lines show the effect of assuming that $\delta S/\delta n$ has a constant value equal to that indicated by the dashed line in Fig. 1.

Gruner (1985) has proposed a theory of "intrinsic curvature" which allows lipid monolayers to curl into tight cylinders that form the H_{II} phase. An underlying assumption of his hypothesis is that the CH_2 chains of the lipids around a hydrophilic tube are not interdigitated, thereby leading to strain in those chains that are pointed in the *b* lattice direction. He proposes that the strain can be alleviated by introduction of hydrophobic molecules into the stressed region. However, the addition of small amounts of tetradecane to DOPE in the H_{II} phase (Gruner et al., 1986) has little effect on the observed dimensions at any level of hydration, which casts doubt on the importance of the presumed stress in controlling the structure.

The present analysis suggests that stress in the hydrocarbon region from low density packing is avoided by chain interdigitation. The reduced strain hypothesis has been invoked to explain observed increases in tube radii from incorporation of relatively longer chain PC lipids into the H_{II} lattice (Gruner et al., 1986; Tate and Gruner, 1987). However, as we shall see in the following section, the tube radii are simply explained on the basis of average fluid chain length and interdigitation.

H_{II} surface area

The calculated dependence of surface area on hydration is given by the solid line in Fig. 1. Using the Luzzati equations, Seddon et al. (1984) calculate the surface area per lipid to be 63.1 $Å^2$ at 135°C and maximum hydration and 0 $Å^2$ at minimum hydration. The present analysis gives 75.6 and 51.9 $Å^2$ at the same limits. The wide range of values obtained with the Luzzati equations stems from the changing molecular surface at the lipid water boundary. However, the C-2 carbon boundary, anchored within the lipid structure, has physical significance at all hydrations and leads to values of the surface area that should be comparable between different phases. At all but the lowest hydrations, the surface areas are larger than those for the $L\alpha$ phase. This result is in agreement with the greater disorder of the CH_2 chains (Mantsch, 1981) as well as the higher temperature of measurement (135°C versus 40°C).

Below $n = 4$, the surface area deviates from the linear dependence shown at higher hydrations. This unexpected deviation is caused by removal of water with $V_w = 32.1$ $Å^3$ in order to achieve the volume of the anhydrous PE group at zero hydration. The value of V_{PE} was determined from a C=O adjusted PE volume derived from crystallographic dimensions of a lamellar phase where molecular headgroup packing occurs within a box shaped volume. The wedge shape of the headgroup volume in the tube at zero hydration leaves little room in the small angle region for occupancy by atoms with normal radii. Consequently, we should expect the headgroup volume at zero hydration to be slightly larger than the value of V_{PE}. Introduction of such a void volume would raise the value of S and cause the value of $\bar{V}_w$ to be $< V_w$.

We test whether such a crowding effect could be responsible for the nonlinear behavior of S below $n = 4$ by extrapolating the curve above $n = 4$ to a limiting value for S of 55.0 Å at $n = 0$ (*dashed line,* Fig. 2) and calculating the change in the values of d_*, b, c, and $d_H/2$. The results are shown as dashed lines in Figs. 2 and 3. The values of $\bar{V}_w$, calculated with Eq. 8, are 30, 27, 22, and 19 $Å^3$ for $n = 3$, 2, 1, and 0. The value of V_H at $n = 0$ is 227 $Å^3$, which implies a void space of 20 $Å^3$ per headgroup at its apex. Note that at $n \leq 1$, a number of water molecules, depending on the lipid packing around the hydrophilic tube, might reside in the central section of tube with normal bulk water volumes. The dashed curve for d_* does not show increased curvature in the region below $n = 4$ and the curves for b and c are largely unaffected. The effect of this proposed void space on the values for $d_H/2$ is considered in the next section.

Headgroup dimensions

The dependence of $d_H/2$ on hydration is shown in Fig. 4. The maximum and minimum values of $d_H/2$ are 19.0 and 8.0 Å, respectively (assuming $\bar{V}_w = V_w$). With the additional void space at minimum hydration discussed in the previous section, the minimum value of $d_H/2$ is 8.3 Å. This value is 2.2 Å larger than the minimum dimension of 6.1 Å for the same headgroup in the L_c phase (Scherer, 1989). We now explore whether this small proposed void space could account for the difference in the headgroup dimensions. In Fig. 5, the wedge shaped cross-section of one headgroup in a hydrophilic tube with diameter d_H is represented with base width w (at the average position of the first CH_2 carbon of the chains) and headgroup height z. Projections of approximate van der Waals radii (1.5 Å)

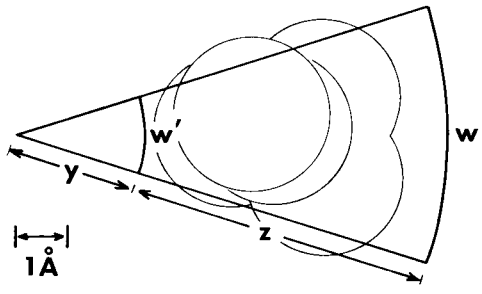

FIGURE 5 Cross section of the PE headgroup in the H_{II} phase defining the y, z, w, and w' dimensions. Projections of the van der Waals volumes of atoms of the phosphate group oriented as in DLPE (Hitchcock et al., 1974) are indicated by circles.

of the *P* and *O* atoms are indicated by circles drawn at the approximate position of the PO_4 group (Hauser et al., 1981). It may be easily shown that z is given by

$$z = d_H [1 - (4 V_{void}/S d_H)^{1/2}]/2, \quad (13)$$

where $V_{void} = V - V_{hc} - V_{PE}$. For $V_{void} = 20$ Å^3, $S = 55$ Å^2, $d_H/2 = 8.3$ Å, we find that $z = 5.8$ Å which is within 0.3 Å of the value for $d_H/2$ for the L_c phase. As we shall see in the next section, an acceptable estimate for w is ~5 Å. The central void radius, y, is 2.5 Å and the width of the top of the headgroup $w' = 2yw/d_H$ or 1.5 Å, which seems a reasonable width to accommodate the upper portions of the atoms along the top of the headgroup.

Headgroup packing at zero hydration

The small values for V_H and $d_H/2$ observed at minimum hydration leads one to suspect favored geometries for the PE headgroup even though the CH_2 chains are disordered. Hitchcock et al. (1974) have determined the crystal structure of dilauroyl phosphatidylethanolamine (DLPE) acetic acid. They find that the PE headgroup in this multi-lamellar crystal is extended parallel to the headgroup surface and has a length, l, of 7.8 Å, width, w, of 5.0 Å, a l/w ratio of 1.56, and a surface area of 38.6 Å^2. Adjacent lipids are packed with their zwitterionic dipoles alternating and two of the hydrogens on each NH_3^+ group are laterally hydrogen bonded to adjacent PO_4 oxygens. Interestingly, the top of the headgroup is ~6 Å above the average carbonyl position. The rectangular profile of the headgroup prompts us to assume that optimal packing of the headgroups should be with their long dimension along, and their short dimension perpendicular to, the tube axis.

Consideration of both dipole/dipole interactions and lateral hydrogen-bonding leads us to assume that maximum packing is achieved by stacking an even number of headgroups side by side around the perimeter of the tube, see fig. 4 for example. We determine w, l, and the l/w ratio for each case where the perimeter ($\Pi d_H = 52$ Å) is divided evenly by n_l groups around the tube and the area/lipid ($S = wl$), is fixed at 55 Å^2. The results are given in Table 1. The values of l/w for $n_l = 10$ and 8 bracket the value observed for the DLPE crystal. Examination of the crystal structure (Hauser, 1975; Hitchcock et al., 1974) leads one to expect that compression on top of the headgroup could be alleviated by slight rotation about the glycerol-phosphate bond (and other minor angular changes) which would increase the l/w ratio slightly above that observed for a lamellar phase. Thus, the $n_l = 10$ packing is preferred with $w = 5.2$ Å and $l = 10.6$ Å.

Seddon et al. (1984) have also obtained a limited amount of data for the dehydration of diarachinoyl phosphatidylethanolamine (DAPE) in the H_{II} phase. Applying the same analysis to the DAPE data at 99°C, the calculated parameters for the H_{II} phase at minimum ($n \approx 2$) hydration are listed in Table 1. Again we note that d_* is larger than the b lattice dimension indicating extensive interdigitation of extended fluid chains. It is not clear whether 12 or 14 lipids surround this incompletely dehydrated tube. However, $n_l = 12$ is preferable because of less crowding is expected at the top of the headgroup at $n = 2$ which would make the value of l/w less than that for DDPE at $n = 0$. These results indicate that a maximum number of 10 PE lipids (diacyl or dialkyl) surround a tube at zero hydration and that the number is independent of CH_2 chain length.

TABLE 1 Observed *d* spacings and calculated lipid headgroup dimensions of DDPE and DAPE for packing n_l lipids around a hydrophilic tube at maximum and minimum hydration

n_l	w	l	l/w
DDPE, $d = 28.7$, 135°C, $n = 0$, $S = 55.0$, $d_H/2 = 8.3$, $d_* = 14.1$, $b = 10.8$, $c = 8.3$			
12	4.3	12.7	2.9
10	5.2	10.6	2.0
8	6.5	8.5	1.3
DDPE, $d = 45.3$, 135°C, $n = 16$, $S = 75.6$, $d_H/2 = 19.1[17.6]$, $d_* = 10.3[12.5]$, $b = 11.1$, c = 7.1[8.6]			
20	6.0	12.6	2.1
18	6.6	11.4	1.7
16	7.5	10.1	1.4
DAPE, $d = 40.0$, 99°C, $n \approx 2$, $S \approx 55.2$, $d_H/2 = 11.2$, $d_* = 20.8$, $b = 15.5$, $c = 11.9$			
14	5.0	11.0	2.2
12	5.8	9.4	1.6
10	7.0	7.9	1.1
DAPE, $d = 68.2$, 99°C, $n = 17.4$, $S = 59.7$, $d_H/2 = 26.4[26.6]$, $d_* = 19.2[18.9]$, $b = 19.1$, c = 13.0[12.8]			
30	5.5	10.8	2.0
28	5.9	10.1	1.7
26	6.4	9.4	1.5

Units: area, Å^2; distance, Å. Values in brackets were calculated using Eq. 14 and the assumption $d_* \equiv b$.

Molecular packing at maximum hydration

Choices for the maximum headgroup packing of DDPE and DAPE at full hydration are shown in Table I. The number of lipids packed around a tube is greater for DAPE ($n_l = 28$) compared with that for DDPE ($n_l = 18$). These estimates for n_l are probably good to within ±2.

The CH_2 chains of DDPE and DAPE are negligibly interdigitated in the b direction ($d_* \approx b$) but are decidedly so in the c direction. This prompts us to propose a general rule that hydrocarbon chain strain is alleviated by requir-

ing the average fluid chain length to match the length b and allowing the chains between adjacent tubes to interdigitate to accommodate this length. This condition and Eq. 5 leads to the general relationship

$$d_H/2 = 2d[1 - (1 - 3\sqrt{3}/2\pi)^{1/2}]/3 = 0.3894d, \quad (14)$$

from which the H_{II} phase dimensions $d_* = b$ and c may be calculated without the use of specific volumes or volume fractions. The values of $d_H/2$, d_*, and c calculated with Eq. 14 are given in brackets in Table I. As expected, the agreement with the full calculation is worse for the shorter chain lipid because the average chain length d_* is slightly less than b. This simple relationship is not valid at reduced hydration because d_* is greater than b, indicating that the chains in the b lattice direction also interdigitate.

It is instructive to compare the average chain lengths d_* calculated from Eq. 14 for a number of diacyl and dialkyl PEs. d spacings of $C_{22,20,18,16}$ diacyl- and $C_{18,16,14,12}$ dialkyl-PEs at 3°C above the $L \rightarrow H_{II}$ phase transition temperature have been measured by Seddon et al. (1984). The calculated values of d_* are plotted against the number of carbons in the hydrophobic part of the CH_2 chain (excluding the C = O carbon) in Fig. 6. We note that all the values of d_* fall on a single curve, which lends additional credence to the simple interdigitation model.

Isothermal dehydration

A final comment should be made about the expected dimensional changes of the hydrophilic tubes under conditions of isothermal dehydration. The above packing arguments indicate that the number of lipids surrounding a tube depends on the level of hydration and the length of the CH_2 chains. At zero hydration the headgroup packing depends only on the geometry of the anhydrous headgroup. A simple way for a tube to conserve lipid mass during dehydration is to move the excess lipid down the tube axis. This would lead to a growth in the tube length of 80% for DDPE, where $n_1 = 18 \rightarrow 10$ ($n = 0$) and 133% for DAPE where $n_1 = 28 \rightarrow 12$ ($n = 2$).

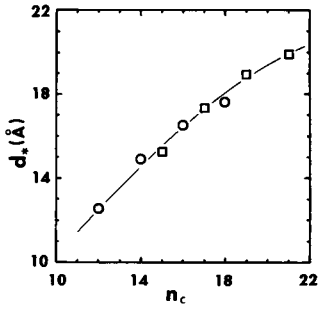

FIGURE 6 Dependence of the values of d_*, calculated with Eq. 14 for the simple interdigitation model at maximum hydration for $C_{22,20,18,16}$ diacyl-, □, and $C_{18,16-14,12}$ dialkyl-PEs, O, on the number of carbons in the hydrophobic part of the chain (excluding the C=O carbon).

CONCLUSIONS

Extension of a "hydrocarbon surface boundary" model to the PE Hexagonal phase eliminates ambiguities about the physical concept of lipid surface area in the H_{II} phase at low hydration and facilitates comparison with surface areas for the lamellar phases.

The present calculations provide evidence for concluding that the fluid hydrocarbon chains between adjacent tubes are interdigitated at all hydration levels. At maximum hydration, the assumption that the average chain length d_* is equal to the b lattice dimension, leads to simple relationship between $d_H/2$ and the hexagonal long spacing d. At minimum hydration the chains in the b lattice direction are also interdigitated. We note that these conclusions do not depend on any *a priori* assumptions about chain packing but only on the assumed position of the hydrophilic/hydrophobic boundary and the calculated values of d_*. The calculated molecular dimensions of the headgroup indicate that a maximum of 10 PE lipids (independent of chain length) can pack in a cross-section of a hydrophilic tube at zero hydration. At maximum hydration, the number depends on the length of the CH_2 chains. The calculated dimensions at zero hydration are consistent with a 5 Å diam void space at the center of a dehydrated tube which alleviates crowding at the center of the tube and accounts for a 2 Å difference in the value of $d_H/2$ between the H_{II} and L phases.

Received for publication 26 July 1988 and in final form 23 January 1989.

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