On the position of the hydrophobic/hydrophilic boundary in lipid bilayers

James R. Scherer

Western Regional Research Center, Agricultural Research Service, United States Department of Agriculture, Albany, California 94710; and Laboratory of Connective Tissue Biochemistry, School of Dentistry, University of California, San Francisco, California 94143-0515

ABSTRACT The sensitivity of calculated structural dimensions of hydrated lipids to the position of the hydrophobic/hydrophilic boundary is reviewed. The position of this boundary is critical in determining the extent of hydration and location of water in the bilayer. A calculation of the dimensions of the hydrophilic and hydrophobic parts of the phosphatidylcholine and ethanolamine bilayer from literature values of the x-ray long spacing shows that the choice of boundary in phospholipids is not arbitrary and is best placed at the average position of the first CH_2 group in the hydrocarbon chains. Calculated dimensions of the hydrocarbon core and the water accessible regions agree with neutron and x-ray diffraction measurements. Hydration differences between phosphatidylcholines and phosphatidylethanolamines are readily explained from derived estimates of the layers of water which cover these headgroups.

INTRODUCTION

Of the various of methods used to study the hydration of lipids, x-ray diffraction has played a unique and central role in efforts to understand the structural basis for lipid-water interactions (Hauser et al., 1981; Hauser, 1975; Shipley, 1973; Luzzati, 1968). While the phase behavior of model lipids is reasonably well understood, there is much to be learned about the relationship between hydration and the structure of the interface between the hydrophobic and hydrophilic parts of the lipid-water system. The position and actual structure of this boundary must strongly influence the interactions of water and lipid in their various polymorphic forms. Indeed, attempts to gain insight into the water-lipid interaction, particularly at low hydration, must focus on the structural interplay at the boundary. It is the author's opinion that much of the controversy around the effect of water on lipid structure can be traced to resistance to modify and extend early concepts of the structure of this interface. Because the research that has shaped our present view of the lipid-water interface has been somewhat contradictory, a brief review of the historical events seems in order.

The pioneering work of Luzzati and colleagues on amphiphilic soaps in the early 1960's established much of the groundwork for interpretation of x-ray data from water-lipid systems (Luzzati et al., 1960; Husson et al., 1960; Luzzati and Husson, 1962; Reiss-Husson and Luzzati, 1964). In their analysis of multilayers, the water and lipid were assigned to separate volumes (V_w and V_l) with common interfacial area S. d is the lamellar long spacing and d_l and d_w are the spacings of the lipid and water layers such that $d = d_l + d_w$ (see Fig. 1 *a*). The lipid volume is given by

$$V_l = \bar{v}_l M_r 10^{24}/N, \quad (1)$$

where $\bar{v}_l$ is the partial specific volume of the lipid, M_r is the molecular weight of the lipid and N is Avogadro's number. The surface area per lipid is given by

$$S = 2V/d = 2V_l/d_l = 2V_w/d_w. \quad (2)$$

It follows that

$$d_l = (V_l/V)d = \phi d, \quad (3)$$

where the volume fraction, ϕ, is given by

$$\phi = (1 + \bar{v}_w(1 - c)/\bar{v}_l c)^{-1} \quad (4)$$

$\bar{v}_w$ is the partial specific volume of water and c is the weight fraction of lipid. It was usually assumed that $\bar{v}_l$ and $\bar{v}_w$ were independent of concentration. In the early work on soaps in the liquid-crystal phase, the calculated values of d_l increased slightly with dehydration and d_w decreased in the expected manner. Analysis of a complex phospholipid isolated from human brain (Luzzati and Husson, 1962) showed similar behavior for d_l and d_w.

In 1967, two distinctly different approaches were used to analyze the long spacing x-ray diffraction data from egg lecithin. Reiss-Husson used the Luzzati model (Fig. 1 *b*), and Small (1967) moved the hydrophilic/hydrophobic boundary to include the phosphorylcholine group (pc) in the hydrophilic volume (Fig. 1 *c*). Small (1967) used the relationship

$$d_L = (V_L/V)d \quad (5)$$

Address correspondence to 1309 Arch St., Berkeley, CA 94708.

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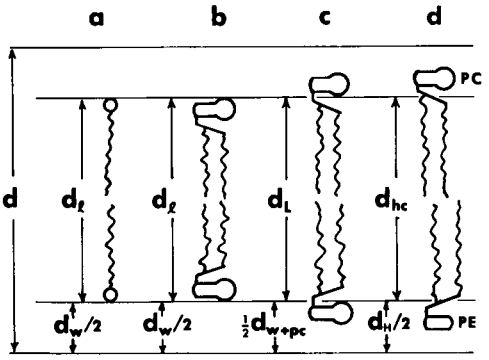

FIGURE 1 Illustration of boundary choices and definitions of decomposed repeat spacings for hydrophilic/hydrophobic boundary of lipids. (*a*) Luzzati, edge of the COO group in fatty acid soaps; (*b*) Luzzati, edge of the headgroup of phospholipids; (*c*) Small, at the connection of glycerol and phosphate groups for EPC; (*d*) first CH_2 in hydrocarbon chain in diacyl or dialkyl phospholipids. *d* Is the interlamellar long spacing.

to calculate the thickness of the lipid layer, where $V_L = V_1 - V_{pc}$. At minimum hydration (15% water, d = 51.0 Å), he found the thickness of the water phosphoryl layer, d_{w+pc}, to be 15.2 Å, whereas Reiss-Husson found d_w to be ~8 Å. At a later point in time, Worcester (1976) found, from neutron diffraction measurements, that water spanned a distance of 21–25 Å at this d spacing. Unfortunately, this result was not available in 1967 to show the advantage of shifting the hydrophobic/hydrophilic boundary to the interior of the lipid. Differences were also noted in the water concentration at maximum hydration. Small's measurements indicated a water concentration of 34 water molecules per lipid, whereas Reiss-Husson found 26. Later measurements by LeNevue et al. (1977) supported the higher hydration value whereas measurements by Shipley (1973) and McIntosh and Simon (1986) favored the lower value. It has been shown that the degree of hydration is dependent on the level of acyl chain unsaturation (Taylor et al., 1978) and variability in the unsaturation of this natural product might have been responsible for the observed differences at maximum hydration. Small's higher value for maximum water concentration also produced a larger value for S (72 Å^2) than that obtained by Reiss-Husson (68 Å^2) and the lower value was subsequently supported by Shipley (1973). It is unfortunate that the disagreement with Small's maximum hydration and surface area calculations overshadowed the significance of his shift of the hydrophilic/hydrophobic boundary.

Luzzati's classic review of x-ray diffraction studies of lipid-water systems (1968) and the elegant work of Tardieu et al. (1973) on the structure of the polymorphic phases of hydrated lecithin do not mention Small's earlier work on egg lecithin, and subsequent analytical decompositions of d spacing used the Luzzati boundary. However, in 1979 Janiak et al. found that the values of d_1 for the $L_{\beta'}$[1] phase of dimyristoyl phosphatidyl-choline (DMPC) increased anomalously at low hydration, even though the distance between phosphate groups on opposite sides of the bilayer, d_{pp} (obtained from electron density profiles), did not change. This might have been rationalized by an extension of the phosphatidylcholine (PC) group perpendicular to the bilayer surface, except that neutron diffraction measurements by Büldt et al. (1978, 1979) unambiguously showed that the PC group is extended parallel to the bilayer at low hydration.

In 1978 Nagle and Wilkinson showed that the expansion characteristics of the bilayer in a phase transition could be attributed to the volume changes in the hydrocarbon part of the lipid beginning at the first CH_2 group in the acyl chain (the C-2 carbon, Fig. 1 *d*) and that volume changes in the headgroup could be neglected. Lewis and Engelman (1983) also showed that the thickness of the hydrocarbon region in the L_α phase is a linear function of the number of carbon atoms in the chain starting at the C-2 carbon atom. They determined the fluid hydrocarbon thickness by subtracting an estimated 11 Å (twice the phosphate to C-2 distance) from the observed values of d_{pp} for a series of saturated and unsaturated PC's. Unfortunately, concurrent with the growing awareness that the hydrophobic boundary of the lipid is better considered at the beginning of the acyl chain, many workers preferred to interpret x-ray dehydration experiments from the Luzzati viewpoint (LeNeveu et al., 1977; Cowley et al., 1978; Parsegian et al., 1979; Lis et al., 1981; Lis et al., 1982; Rand and Parsegian, 1985).

The question of the sensitivity of the hydrocarbon core dimensions to hydration was explored by White and King in 1985. They reanalyzed the x-ray data of Torbet and Wilkins (1976) for egg phosphatidylcholine (EPC) and showed that d_{pp} changed only by 3.7 Å over the whole L_α hydration range, whereas the variation in d_1, using the Luzzati boundary, is 14 Å. Interestingly, Small's earlier result showed a total variation in d_L of 6.2 Å. White and King estimated the change in total volume with hydration from changes in $2S/d$. S was determined from $S = 2V_{hc}/d_{hc}$, where V_{hc} was fixed at 907 Å^3 from literature values for the volumes of palmitic and oleic chains. d_{hc}, the average distance between C-2 carbon atoms across the bilayer, was obtained by subtracting a constant value of 14 Å from d_{pp} obtained from electron density profiles. The accumulation of uncertainties in estimating values for S led to an anomalously low value for the partial molar

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

volume of water, $\bar{V}_w = \delta V/\delta n$, in the region below $n = 10$.

McIntosh and Simon (1986) reinvestigated the effects of osmotic dehydration of lipid bilayers by estimating molecular dimensions from electron density profiles. They define the fluid (aqueous) layer thickness as $d - d_b$, where d_b is the distance between electron-density peaks across the bilayer, d_{pp}, plus two times the distance between the junction of the glycerol and phosphate groups to the outer edge of the PC group, which they estimate at 10 Å for PC or 8 Å for phosphatidylethanolamine (PE). They find that the free fluid space in EPC at $d = 51.5$ Å is only 4 Å instead of 10.8 Å (d_w) as calculated by Parsegian et al. (1979) using the Luzzati boundary. In addition they also found that the bilayer, d_b, is relatively incompressible with dehydration.

In 1987, Scherer reanalyzed x-ray data for the L_α and $L_{\beta'}$ phases of DMPC (Janiak et al., 1979) and the L_α phase of EPC (Reiss-Husson, 1977; McIntosh and Simon, 1986; Parsegian et al., 1979), changing the boundary between the hydrophobic and hydrophilic phases to the C-2 carbon of the acyl chain. The decomposition of d was given by

$$d = d_{hc} + d_H, \tag{6}$$

where d_H is the average distance between C-2 carbon atoms in adjacent bilayers. The change in surface area with number of water molecules, $\delta S/\delta n$, was evaluated at maximum hydration from the slope of the curve of d versus n and the equation

$$\bar{V}_w = \delta V/\delta n = S\,\delta(d/2)/\delta n + (d/2)\,\delta S/\delta n.$$

Scherer made the additional assumption that $\delta S/\delta n$, which is small for PC lipids at maximum hdyration, be constant over the hydration range of the L phases. This assumption led to a reduction in the value of $\bar{V}_w$ from that of bulk water in the hydration range below $n = 15$, a result which was at variance with the results of subsequent calculations for PE lipids using the same assumption (Scherer, unpublished work), and more importantly, the recent experimental determination of $\bar{V}_w$ by White et al. (1987), which showed that $\bar{V}_w$ maintains the bulk water value at least down to hydration levels of 12% water ($n = 6$).

In the following, we show the results of a recalculation of the hydration dependence of $d_H/2$ and $d_{hc}/2$ in DMPC and EPC lamellar phases using the same C-2 boundary and the experimentally justified assumption that the value of $\bar{V}_w$ is the same as that for bulk water. In addition, new results are presented for the hydration dependence of $d_H/2$ and $d_{hc}/2$ for the lamellar phases of didodecylphosphatidylethanolamine (DDPE). It will be shown that the choice of the C-2 boundary and the partial molar volume assumption yield values of $d_H/2$ and $d_{hc}/2$ that are in agreement with neutron and x-ray diffraction experiments and that new insights can be obtained about the location of water in the headgroup as a function of hydration.

Decomposition of *d*

The decomposition of d defined by Eq. 6 is given by

$$d_{hc} = (V_{hc}/V)d, \tag{8}$$

where $V_{hc} = V_l - V_{PC\text{ or }PE}$. Nagel and Wilkinson (1978) have determined that V_{PC} and V_{PE} (Wilkinson and Nagel, 1981) for acyl chain phospholipids are 344 Å^3 and 246 Å^3, respectively. In the case of DDPE, the value of V_{PE} is smaller by the volume of two carbonyl groups (39 Å^3, Bondi, 1964), or 207 Å^3.

The x-ray data for DMPC were taken from Janiak et al. (1979) and the data for EPC from Reiss-Husson (1967) and McIntosh (1986) and are the same data used in the prior analysis (Scherer, 1987). The d spacings for the L_α and $L_{\beta'}$ phases of DDPE were taken from work by Seddon et al. (1984) and are shown in Fig. 2. The dashed line for the $L_{\beta'}$ curve in this figure represents the curve drawn by Seddon et al. through five data points below $n = 6$. The slope of the curve approaching maximum hydration is related to $\delta S/\delta n$ and $\bar{V}_w$ through Eq. 7. Using Seddon's value of 41.5 Å^2 for S and 50.6 Å for d, we find that $\delta S/\delta n = -0.37$ Å^2/w. This negative value, which implies that water added to the headgroup region will decrease the lipid surface area, is untenable. A closer examination of the data in Seddon's Fig. 1 reveals that, with the exception of one point at $n = 5$, a curve with a less steep slope (*solid line*, Fig. 2) can be drawn through the seven data points below a maximum hydration at $n = 7.5$. The value of $\delta S/\delta n$ from this curve is 0.46 Å^2/w. This example shows the utility of using Eq. 7 to insure consistency between $\delta S/\delta n$, $\delta d/\delta n$, and $\bar{V}_w$.

Seddon et al. (1984) find that their value of S (41.5 Å^2 at $n_{max} = 5.8$), when combined with their wide angle reflection data, leads to a small value (7°) for the angle of

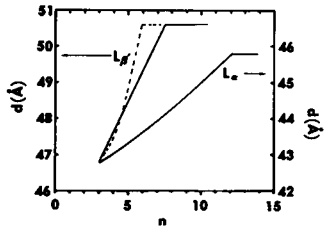

FIGURE 2 Dependence of the observed d spacings of the L phases of DDPE on hydration, Seddon et al. (1984). The dashed line for the $L_{\beta'}$ spacings represents the curve indicated by Seddon et al. (1984).

chain tilt (a β structure). Their measurements for diarachonoyl phosphatidylethanolamine (DAPE) indicate a chain tilt of 29° (a β' structure). Changing n_{max} from 5.5 to 7.5 changes the value of S to 43.5 which gives a chain tilt of 19°. The following analysis is based on a β' structure. The diether PE was chosen for analysis because of more numerous determinations of d for both lamellar and H_{II} phases at all hydrations compared with diester PEs. Calculations for the H_{II} phase will be presented in the following paper.

The surface area of the lamellar phases is not dependent on the position of the hydrophobic/hydrophilic boundary and the dependence of S on hydration is the same as that found by previous workers.[2] Nevertheless, it is informative to compare the variation of S of the PC's and PE's in comparable regions of hydration and the data are shown in Fig. 3. The variation of $d_{hc}/2$ and $d_H/2$ with hydration is shown in Figs. 4 and 5, respectively.

RESULTS AND DISCUSSION

Variation of S

Fig. 3 shows similarities in the hydration dependence of S for PCs and PEs at comparable levels of hydration. $\delta S/\delta n$ is quite small down to $n = 14$ for L_α of DMPC and EPC and $n = 10$ for $L_{\beta'}$ of DMPC.[3] The slight dependence of n

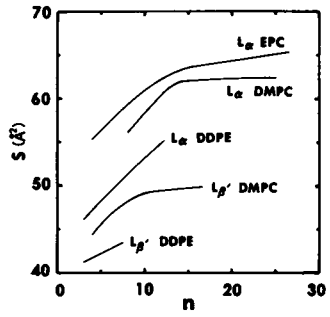

FIGURE 3 The calculated variation of S with hydration, assuming that $\overline{V}_w$ has a value equal to V_w for bulk water, for the L_α phases of DMPC (37°C), EPC (room temperature), and DDPE (40°C) and the $L_{\beta'}$ phases of DMPC (10°C) and DDPE (29°C).

[2]A graphical presentation of the hydration dependence of S for the $L_{\beta'}$, phase of DMPC was not given by Janiak et al. (1979).

[3]For equivalent water density in the PC headgroup region of the L_α and $L_{\beta'}$, phases, the smaller surface area of the β' phase necessarily gives rise to less water per lipid than the α phase. The reduction should be in the ratio of the surface areas. Thus $n = 14$ in the α phase corresponds to $n = 11$ in the β' phase.

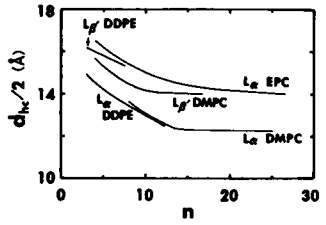

FIGURE 4 The calculated variation of $d_{hc}/2$ with hydration, assuming that $\overline{V}_w$ has a value equal to V_w for bulk water and that the hydrophilic/hydrophobic boundary is at the first CH_2 carbon in the hydrocarbon chain.

is reasonable if the water molecules being removed are located between the hydrated $N(CH_3)_3$ groups of opposed bilayers. These waters are primarily hydrogen-bonded to other water molecules, and their removal should not appreciably influence waters deeper in the headgroup which affect lipid chain spacing or surface area. Below $n = 10$ to 14, $\delta S/\delta n$ increases to ~1 Å²/w. The values of d for DMPC in the $L_{\beta'}$ phase do not change at minimum hydration and application of Eq. 7 gives a limiting value of 1.15 Å²/w for $\delta S/\delta n$. The larger values for $\delta S/\delta n$ below $n = 14$ are also in qualitative agreement with (DSC) measurements which show that the gel/liquid-crystal phase transition moves rapidly to higher temperatures when n is reduced below $n = 14$ (Kodama et al., 1982). The increase in temperature is consistent with greater chain packing or smaller surface area.

The value of $\delta S/\delta n$ for the $L_{\beta'}$ phase of DDPE is smaller than the value obtained for the L_α phase and may be the result of uncertainty in determining the point of maximum hydration mentioned earlier.

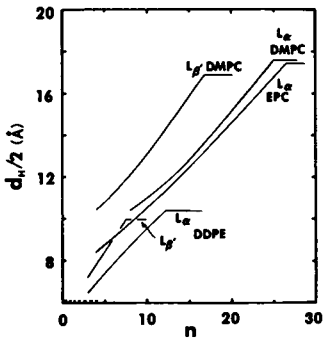

FIGURE 5. The calculated variation of $d_H/2$ with hydration assuming that $\overline{V}_w$ has a value equal to V_w for bulk water and that the hydrophilic/hydrophobic boundary is at the first CH_2 carbon in the hydrocarbon chain.

Variation of $d_{hc}/2$

Fig. 4 shows that $d_{hc}/2$ has a small hydration dependence which is inversely related to the hydration behavior of S. The cholines show very small increases in $d_{hc}/2$ with dehydration down to $n = 14 - 11$. Below this hydration the PCs and PEs show similar rates of increase. White and King (1985) have determined the distance between phosphate groups across the EPC bilayer and found it to vary by ~3.4 Å from maximum hydration to $n = 4$. Assuming that the distance between phosphate groups is primarily regulated by thickness change of the hydrocarbon core, the present analysis yields a total variation of 2.5 Å. McIntosh and Simon (1986) find that the distance between electron density peaks of EPC bilayers is constant ±0.8 Å over a range of d spacings of 63.2 to 51.7 Å. Over the same range of d, this analysis gives a total variation of 0.8 Å, whereas Small's analysis gives a variation of ~5 Å. Thus, moving the hydrophilic/hydrophobic boundary to the outermost CH_2 group of the hydrocarbon core accounts for the change in bilayer expansion with hydration much better than placing the boundary at the end of the lipid headgroup, or between the glycerol and phosphate groups.

Variation of $d_H/2$

The hydration dependence of $d_H/2$ is shown in Fig. 5. The minimum values of $d_H/2$ for both L phases of DMPCs are 10.4 Å. The author has previously estimated (1987) a minimum $d_H/2$ distance of 11.2 Å from neutron diffraction data of Büldt et al. (1979) for DPPC. The value of 10.4 Å agrees well with this estimate.

As was found in the earlier analysis, the value of $d_H/2$ for EPC at minimum hydration is almost 2 Å smaller than the corresponding value for DMPC. Part of this difference results from the lower hydration level ($n = 4$) for EPC. At comparable hydration levels, the surface area for EPC is larger than for DMPC. The increased area might allow the hydrophobic $N(CH_3)_3$ group to move closer to the hydrocarbon core boundary and displace water from within the headgroup. Lateral interactions of the PC headgroup dipoles have been shown to drive the CH_3 groups around the N^+ atom towards the hydrocarbon core when the temperature is increased (Dill and Stigter, 1988; stigter and Dill, 1988). It is conceivable that a reduced water content and larger surface area might facilitate this process and thereby lower the value of $d_H/2$ below that found for PCs with completely saturated acyl chains. Alternatively, an increased value of S caused by CH_2 chain unsaturation might allow the headgroups to partially interdigitate at very low hydration and cause the calculated values of $d_H/2$ to be smaller than those for saturated chain molecules.

The span of the aqueous phase, d_H, in the EPC bilayer at a d spacing of 51.0 Å is 21 Å, which compares well with the 21–25 Å distribution of water as measured by neutron diffraction (Worcester, 1976). This aqueous span may be compared with values of 8 Å (Reiss-Husson, 1967) using Luzzati's boundary, and 15.2 Å given by Small's (1967) boundary.

The value of $d_H/2$ for the hydration independent crystalline L_c phase of DDPE is indicated by the dashed line at 6.1 Å. It was determined by subtracting the length of 13 CH_2s (13[1.27] = 16.5 Å) from an observed $d/2$ spacing of 22.6 Å (Seddon et al., 1984). The values of $d_H/2$ for the L phases of DDPE at maximum hydration are close to 10.2 Å and approach 6.1 Å at minimum hydration. Büldt and Seelig (1980) have studied DPPE in the gel phase at low hydration with neutron diffraction. The distance from the average C(2) carbon position of the acyl chains to the CH_2 next to the NH_3^+ group is 5.8 Å. To compare this value with our result for DDPE we must subtract the length of the (C═O) – O bond and add the distance from the CH_2 group to the edge of the NH_3^+ group. These distances are not known but are probably the same within 1 Å. Thus we confidently adopt 6.1 Å as the minimum value of $d_H/2$ in either PEs.

It is interesting to compare the number of water molecules inside and outside the headgroup volume of a lipid, at both minimum and maximum hydration. The number of layers of water between opposed headgroups is obtained from the dimensions in Fig. 4 by dividing the difference between d_H and a minimum value of d_H, by the thickness of a tetrahedrally hydrogen-bonded water layer (3.6 Å, Scherer, 1987). The indication from Fig. 5 is that estimates of 10.0 , 8.0, and 6.1 Å may be adopted for the minimum values of $d_H/2$ in DMPC, EPC, and DDPE. The results are shown in Fig. 6. The number of water molecules in the volume outside the headgroup is obtained by multiplying the surface area by half the number of layers of water between opposed headgroups and dividing the result by the surface area of a water molecule, 8.3 Å^2.[4] We can estimate the number of water molecules inside by subtracting this number from the total number of water molecules per lipid.[5] The results for minimum and maximum hydration are summarized in Table 1.

[4]This number is independent of the estimated thickness of a water layer because the product of thickness and area per water must be constant (30 Å^3/molecule).

[5]Marra and Israelachvili (1985) have suggested that the aqueous layers are not occupied by pure water because the thermally mobile headgroups can extend into this region. This would make our estimate of the "inside" water low and the estimate of "outside" water high. However, analyses of pressure/area isotherm data (Stigter and Dill, 1988; Dill and Stigter, 1988) indicates that the headgroup lies essentially parallel to the plane of the monolayer and that fluctuations from the bilayer plane are also small.

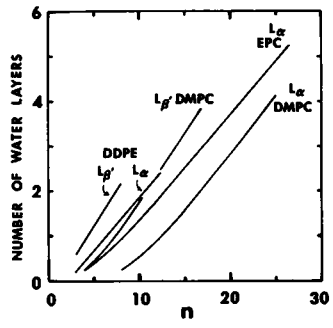

FIGURE 6 Maximum number of layers of water in the fluid region between opposed headgroups of adjacent bilayers as a function of the degree of lipid hydration. The headgroups are assumed to be in their minimum hydration orientation.

At maximum hydration in the L_α phase, the ratio of the water inside to water outside the headgroup for DMPC is 0.6, whereas for EPC it is 0.3. As mentioned earlier, this may reflect displacement of waters in the headgroup by trimethyl groups preferring to occupy space closer to the hydrophobic phase. The same ratio for the $L_{\beta'}$ phase of DMPC is 0.5 and comparable with that obtained from the L_α phase. However, four fewer waters are inside the headgroup because of increased chain packing in the $L_{\beta'}$ phase.

We note that there are generally two fewer water molecules within the headgroup region at minimum hydration than there are at maximum hydration and that the amount of water inside the headgroup does not increase appreciably above the point of intermediate hydration. From maximum → intermediate hydration, water is removed from layers outside of the PC headgroup. From intermediate → low hydration, ~¾ of the removed water is from the first hydration shell covering the PC headgroup and ¼ is from within the headgroup. For PEs from maximum → low hydration, 0.8 to 0.9 of the water being removed is from the first hydration shell covering both the phosphate and NH_3^+ groups and 0.1 to 0.2 of the water is from within the headgroup. There is a rough correlation between the number of waters remaining in the PC and PE headgroups at minimum hydration and the surface areas shown in Fig. 3.

TABLE 1 Number of water molecules/lipid outside and inside the head group at minimum, [intermediate], and (maximum) hydration

Molecule	phase	Hydration			Number outside		Number inside	
DMPC	L_α	min	[n = 14]	(max)	0.7	[5.0] (15.6)	7.3	[9.0] (9.5)
EPC	L_α	min	[n = 14]	(max)	0.7	[8.6] (20.6)	3.3	[5.4] (6.0)
DMPC	$L_{\beta'}$	min	[n = 10]	(max)	0.6	[5.1] (11.4)	3.4	[4.9] (5.2)
DDPE	L_α	min		(max)	0.6	(8.0)	2.4	(4.2)
DDPE	$L_{\beta'}$	min		(max)	1.5	(5.6)	1.5	(1.9)

McIntosh et al. (1987) have measured d spacings and electron density profiles of EPC multilayers exposed to nitrogen atmospheres of controlled humidity. They find that steric repulsion between multilayers begins when the "fluid space" $(d-d_p)$ between adjacent bilayers is <5 Å or a hydration level below 13 waters per lipid. This is the region where $\delta S/\delta n$ increases (Fig. 3) and $\delta(d_{hc}/2)/\delta n$ becomes more negative (Fig. 4), and also where there are fewer than two layers of water between opposed head groups (Fig. 6). It seems reasonable that the effects of steric interaction should be felt at the point where the choline groups begin to lose their own hydration shell of water.

McIntosh and Simon (1986) have estimated that the volume of the fluid space outside the lipid headgroup of dilauroylphosphatidylethanolamine (DLPE) bilayers in the L_α phase at maximum hydration is large enough to accommodate 4.3 waters per lipid (correction, 1986). The total number of waters per lipid (10.3) was obtained by subtracting an anhydrous DLPE volume (856 Å^3) from the total volume of the fully hydrated lipid (1,166 Å^3) and dividing by the volume of a water molecule (30 Å^3). This yields about six waters that lie within the headgroup volume. The number of waters within the headgroup of DDPE in the L_α phase at maximum hydration (n = 12.2) is 4.2. The agreement with McIntosh and Simon's value is acceptable in view of the absence of C=O hydrogen bonding acceptor groups in DDPE.

It is interesting to speculate why PE multilayers don't hold as much water as PC multilayers at maximum hydration. A possible answer to this question lies in the realization that the water in multilayers below maximum hydration can't have the same optimal hydrogen-bonding characteristics as bulk water. A water molecule adjacent to a hydrophobic surface is not expected to form as strong a hydrogen bond to a neighboring water molecule as one which is already hydrogen-bonded to one or more other hydrogen-bonding sites. Consequently, water adjacent to the hydrophobic choline group would have to hydrogen-bond to an additional layer of water before being capable of forming a hydrogen-bond network between opposed bilayers which is strong enough to be in equilibrium with a separate water phase. However, water in the PE headgroup can hydrogen-bond to the NH_3^+ group as well as the phosphate group, giving rise to strong bonding in the first layer of water, and thereby eliminate the additional layers needed to achieve strong hydrogen-bonding across the headgroup region. This viewpoint suggests that the hydrophilicity of the headgroup should not be equated with the ability of the headgroup region hold more water to the point of phase separation. A similar view has been expressed by Nagle (1986).

CONCLUSIONS

The sensitivity of calculated dimensions of the lipid and aqueous parts of the lipid bilayer to placement of the hydrophobic/hydrophilic boundary has been reviewed. Criteria for a suitable boundary are that it correctly predict the span of water in the headgroup region and hydrocarbon core thickness as measured by neutron diffraction, and the compressibility of the bilayer as measured by x-ray diffraction. It has been shown (White and King [1985], McIntosh and Simon [1986], McIntosh et al. [1987], and Scherer [1987]) that the hydrophobic/hydrophilic boundary is near the first CH_2 group of the hydrocarbon chain in PC and PE lipids. The present work demonstrates that classical decompositions of d (Luzzati et al., 1960; Small, 1967), assuming that the partial molar volume of water equals that of bulk water, can yield accurate dimensions for the hydrophobic and hydrophilic parts of lamellar PC and PE lipid phases and account for the distribution of water inside and outside the headgroup region, provided that the hydrophobic/hydrophilic boundary is placed at the first CH_2 group of the hydrocarbon chain. The calculated values for these dimensions are in good agreement with dimensions obtained from neutron diffraction measurements and x-ray electron density profiles.

The minimum dimensions of the PC and PE headgroups obtained here may be used to estimate the number of layers of water in the fluid space above the edge of the lipid headgroup. These estimates, in combination with the surface areas and total water per lipid, establish the number of waters inside and outside the headgroup volume at minimum, intermediate, and maximum hydration. The sensitivity of the surface area to water removal below intermediate levels of hydration for PCs, and below maximum levels of hydration for PEs, is linked to removal of water in the first hydration layer covering the choline or ethanolamine group.

This analysis supports the view that as the bilayer approaches minimum hydration, increasingly less water remains in the space between opposed headgroups and that the reduction of water within the headgroup allows tighter packing of the hydrocarbon chains and reduction of the surface area. At minimum hydration, there is substantially more water inside the headgroup volume than what remains between opposed headgroups. In this limit, the repulsive forces that exist between opposed bilayers at low hydration (Parsegian et al., 1979) must have their origins in steric hinderance and thermal fluctuations. This conclusion is in qualitative agreement with recent work by McIntosh and Simon (1986), and McIntosh et al. (1987) who conclude that steric hinderance between opposed headgroups becomes the dominating factor in dehydrating EPC multilayers at hydrations <13 water molecules per lipid.

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