Variation in Hydration Forces between Neutral Phospholipid Bilayers: Evidence for Hydration Attraction

R. P. Rand*,‡ and Nola Fuller
Department of Biological Sciences, Brock University, St. Catharines, Ontario L2S 3A1, Canada
V. A. Parsegian and D. C. Rau
National Institutes of Health, Bethesda, Maryland 20892
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ABSTRACT: It is now generally recognized that hydration forces dominate close interactions of lipid hydrophilic surfaces. The commonality of their characteristics has been reasonably established. However, differences in measured net repulsion, particularly evident when phosphatidylethanolamine (PE) and phosphatidylcholine (PC) bilayers are compared, suggest there exists a variety of behavior wider than expected from earlier models of hydration and fluctuation repulsion balanced by van der Waals attraction. To find a basis for this diverse behavior, we have looked more closely at measured structural parameters, degrees of hydration, and interbilayer repulsive forces for the lamellar phases of the following lipids: 1-palmitoyl-2-oleoyl-PE (POPE), egg PE, transphosphatidylated egg PE (egg PE-T), mono- and dimethylated egg PE-T (MMPE and DMPE), 1-stearoyl-2-oleoyl-PC (SOPC), and mixtures of POPE and SOPC. POPE and SOPC bilayers differ not only in their maximum degrees of hydration but also in the empirical hydration force coefficients and decay lengths that characterize their interaction. When mixed with POPE, SOPC effects sudden and disproportionate increases in hydration. POPE, egg PE, and egg PE-T differ in their degree of hydration, molecular area, and hydration repulsion. A single methylation of egg PE-T almost completely converts its hydration and bilayer repulsive properties to those of egg PC; little progression of hydration is seen with successive methylations. In order to reconcile these observations with the conventional scheme of balancing interbilayer hydration and fluctuation-enhanced repulsion with van der Waals attraction, it is necessary to relinquish the fundamental idea that the decay of hydration forces is a constant determined by the properties of the aqueous medium. Alternatively, one can retain that fundamental idea if one recognizes the possibility that polar group hydration has an attractive component to it. In the latter view, that attractive component originates from interbilayer hydrogen-bonded water bridges between apposing bilayer surfaces, arising from correlation of zwitterionic or other complementary polar groups or from factors that affect polar group solubility. The same Marcelja and Radic formalism that accounts so well for the repulsive component also leads to an estimate of the attractive one. We suggest that the full range of degrees of hydration and of interbilayer spacings observed for different neutral bilayers results in part from variable contributions of the attractive and repulsive hydration components. The extremes are exemplified by the dominance of attraction between PE bilayers with crystalline hydrocarbon chains and the dominance of repulsion and full hydration of melted PC bilayers. Within the variable hydration of the PE's with melted chains, one sees evidence of variable hydration attraction. With or without the postulated attraction though, good estimates of adhesion energy can be obtained. It is necessary therefore to make other critical tests to distinguish the alternatives.

Interactions between all large molecules, molecular aggregates, and surfaces in aqueous media are subject to electrostatic interactions, van der Waals attraction, and hydration repulsion. The requirement for nonelectrostatic repulsion became evident when it was demonstrated that neutral components take up water against van der Waals attraction and that very strong forces, exceeding electrostatic repulsion between charged surfaces, occur when surfaces approach within about 50 Å of each other [Derjaguin and Churaev (1986) and references cited therein, Churaev and Derjaguin (1985), Barclay and Ottewill (1970) and references cited therein, Callaghan and Ottewill (1974), Clunie et al. (1967), and Parsegian (1967)]. The extraordinary repulsion was sometimes attributed, in an ill-defined way, to "hydration" or "structural" or "solvation" forces, thought to result from dynamic structural perturbation of boundary water. In 1976 the form of this repulsion was determined through the development of an osmotic stress method used to control the degree of hydration of interacting species (LeNeveu et al., 1976). It yielded a measure of the pressure–distance relationship between interacting neutral phospholipid bilayers. Within about 25-Å separation, that hydration repulsion could be described as

$$P_h = P_o \exp(-\text{distance}/\lambda)$$

a pressure that decays rapidly with interbilayer distance, characterized by a hydration coefficient P_o and a decay distance λ. Subsequently, similar measurements on many different phospholipid systems [for a review, see Rand (1981)], both charged (Cowley et al., 1978; Lis et al., 1981) and neutral (Lis et al., 1982), suggested that the hydration force dominates the interactions at separations less than about 30 Å, with decay distances of 2–3 Å.

Since these measurements were made, a number of issues have emerged. Confirmation of the form of the hdyration force came from an entirely different method of measurement. A surface force apparatus (Israelachvili & Adams, 1978) measures the force between curved mica sheets as they are allowed

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to approach. Uncoated, and taking into account electrostatic repulsion, mica surfaces show hydration repulsion resulting from the dehydration of adsorbed ions, though often with longer decay distances (Pashley & Israelachvili, 1981). More recently, repulsion between phospholipid-coated mica sheets (Marra & Israelachvili, 1985) appears to agree pleasingly well (Horn et al., 1988) with the earlier osmotic stress measurements (Lis et al., 1981; Rand, 1981). Now, with the osmotic stress method, similar forces have been measured between xanthans (Rau & Parsegian, 1987), between DNA molecules (Rau et al., 1984), and between myelin membranes (Rand et al., 1979). The decay distances in all these systems are about the same (2.5–3.5 Å) and independent of ionic strength or composition. The energetics of aqueous cavities, such as membrane channels (Zimmerberg & Parsegian, 1986) and the cylinders formed in the nonlamellar reverse hexagonal phase formed by lipids (Gruner et al., 1986), have been probed with osmotic stress, and hydration clearly plays a role in their behavior. We have come to believe that hydration repulsion contributes significantly to the energetics of all large interacting species in water. Its extension to other solvents (Bergenstahl & Persson, 1985; Christenson & Horn, 1985) generalizes it to a solvation repulsion.

The mechanism of hydration repulsion and the meaning of its coefficient and decay distance have stimulated theoretical studies (Marcejla & Radic, 1976; Radic & Marcejla, 1978; Cevc et al., 1982). According to early formulations, the coefficient P_0 reflects the degree to which the surface orders the boundary water, and the decay length λ reflects the way that ordering is propagated through water. Different magnitudes of force have been attributed to different degrees of surface polarization and the common decay distance to a property of water alone. Such formulation has been considerably successful in estimating the effects of polar groups on many properties of phospholipid bilayers (Cevc et al., 1982; 1986; Cevc, 1985). It does not however appear to describe the results of a computer simulation of perturbed hydrogen bonding (Kjellander & Marcelja, 1985).

The contribution to bilayer interactions of a steric repulsion caused by thermally excited mechanical undulations of free bilayers has been developed by Helfrich (Helfrich, 1978; Harbich & Helfrich, 1984). It has been extended recently (Evans & Parsegian, 1986; Evans & Needham, 1987) to include the actions of long-range forces. Undulations contribute significantly to interactions at large separations where electrostatic repulsion dominates; their decay accounts for the anomalous Debye lengths measured in such systems (Cowley et al., 1978; Lis et al., 1981). They contribute significantly to net repulsion between neutral bilayers only near full hydration and likely account for the anomously high compressibilities originally attributed to bilayers on dehydration. They may account for the small differences seen between the free bilayers used in the osmotic stress technique and the fixed bilayers attached to mica (Marra & Israelachvili, 1985; Horn et al., 1988). They result in some correction to the apparent hydration coefficient and decay distance determined from the empirical measurements (Evans & Needham, 1986).

More recently (McIntosh & Simon, 1986a) bilayer thickness has been defined by using low-resolution electron density profiles of the osmotically stressed lamellar phases. Their use results in hydration decay distances, λ, less than those given by the original method. Although this may be partly due to necessarily low resolution, the difference may simply stem from different definitions of the bilayer–water interface in the way pointed out by Janiak et al. (1979). In any case, for consistency we have used both the original method of analysis and a modification suggested by E. Evans (personal communication) in order to compare the several lipids in this study.

Hydration forces can be attractive. Most surfaces polarize water in the same direction with respect to themselves, and it is the overlap of these oppositely oriented fields between apposing bilayers that results in repulsion. However, it has recently been shown that the repulsive hydration between DNA strands (Rau et al., 1984) can be overcome with the addition of certain divalent or polyvalent cations resulting in the condensation of DNA (Parsegian et al., 1985). The condensate is still hydrated, and its further osmotic dehydration is characterized as predicted (Parsegian et al., 1985) by a decay distance half that of the original. The interpretation is that water is polarized in opposite directions around different groups on the same surface. Net attraction results when such arrays on apposing surfaces complement each other spatially on mutual approach with water aligned in the same direction bridging opposite groups from one surface to the other. We suggest here that such attractive hydration forces may exist between some phospholipid bilayers.

There are many differences in the degree of hydration of the lamellar phases of phosphatidylethanolamines (PE) and phosphatidylcholines (PC) with melted hydrocarbon chains. This was shown in the early measurements (Jendrasiak & Mendible, 1976) of water adsorption isotherms of egg PC, egg PE, their 1:1 mixtures, and monomethylated egg PE. PE's extracted from natural sources hydrate to different extents, from 27–30 wt % (blowfly larvae, pig erythrocytes) (Rand et al., 1971) to about 40 wt % (egg PE). Diakyldidodecyl-PE and diacyldiarachinoyl-PE maximally hydrate to 31 and 17 wt %, respectively (Seddon et al., 1984), although only a small dependence of maximum hydration on chain length exists for diacyl-PE's (Cevc & Marsh, 1985). Hydration of dilauryl-PE, measured indirectly, is very low (McIntosh & Simon, 1986b). In this study we have attempted to determine the reasons for some of these differences. First, we have found that hydrations for POPE and SOPC differ both in degree in excess water and in the empirical hydration coefficients and decay distances that characterize their repulsion. Second, hydration of their mixtures is disproportionately dominated by SOPC. Third, we have found that PE's with small differences in hydrocarbon chain composition have different degrees of hydration. Finally, using methylated derivatives of PE, we have found that a single methylation of the PE polar group changes both the degree and form of hydration to be nearly the same as those of PC. A match of these measurements and of complementary adhesion force measurements (Evans & Needham, 1986, 1987) with the theory of hydration forces (Radic & Marcejla, 1978) suggests to us that attractive hydration forces may exist between bilayers, as between DNA molecules (Parsegian et al., 1985). We propose that both attractive and repulsive hydration forces contribute to interbilayer interactions, the attractive contribution affected by the ability of the two apposing polar group layers to form interbilayer hydrogen-bonded water bridges. This attractive contribution is in the same spirit as the complementary surfaces proposed by Kolber and Haynes (1979), seen in the crystal structures of PE and PC (Hauser et al., 1981) and recently described as contributing to the low hydration of DLPE bilayers (Seddon et al., 1984; McIntosh & Simon, 1986b).

Materials and Methods

Materials. Lipids were purchased from Avanti Polar Lipids (Birmingham, AL) and checked for purity by thin-layer chromatography both before and occasionally after the ex-

periments. They always showed less than 1% contamination. The lipids were 1-stearoyl-2-oleoylphosphatidylcholine (SOPC), 1-palmitoyl-2-oleoylphosphatidylethanolamine (POPE), PE extracted from hen eggs (egg-PE), PE produced from transphosphatidylation onto egg PC chains (egg PE-T), and mono- and dimethylated derivatives of egg PE-T (MMPE and DMPE). The egg PE-T was shown by GLC to have the same chain composition as egg PC. These lipids were stored under nitrogen at −18 °C until used. Lipid mixtures were formed by combining the components in organic solvent and then removing the solvent under vacuum. Dextran and poly(ethylene glycol) (PEG) were obtained from Pharmacia (Canada) and used without further treatment. Solutions of these polymers were made with 2 mM TES buffer, pH 7.0.

Lamellar Phase Structural Parameters. Water content was established by weighing dry lipid and 2 mM TES buffer into small weighing bottles. These were then sealed and equilibrated for approximately 2 days at room temperature (about 20 °C) or, in the case of POPE and SOPC, at 30 °C. No water loss was detected before mounting the hydrated lipid into X-ray sample holders at the equilibrating temperature. The lipid was combined with a little powdered Teflon for calibration and sealed between mica windows 1 mm apart. We used X-ray diffraction to characterize the structures that they formed and to measure their dimensions. Only lamellar phases were observed in this study, characterized by diffraction lines of a single dimension representing the repeat spacing d (±0.1 Å) of a one-dimensional crystal. The X-ray camera was of the Guinier type operating in vacuo, using the Cu $K\alpha_1$ line (λ = 1.540 Å) isolated by a bent quartz crystal monochrometer, and diffraction was recorded photographically. Temperature different from room temperature was maintained to ±0.5 °C with a thermoelectric control.

Model-independent presentation of the data is in the form of the relation between the measured water content of the lamellar phase and both the X-ray repeat spacing d and unit cell cross-sectional area A. That area is completely defined by the lamellar repeat spacing, d, and the volume of a unit cell and is independent of the distribution of lipid and water in that cell. We have chosen the unit cell to contain one phospholipid molecule, of volume L, plus the measured volume of water W per lipid molecule in the lamellar phase. Thus

$$(d/2)A = L + W$$

where A is the cross-sectional area *available* per phospholipid molecule projected onto a plane perpendicular to the axis of lamellar repeat. Then

$$A = (2 \times 10^{24})\mathrm{MW}_l\nu_l/\phi d N_o$$

where

$$\phi = 1/[1 + (1 - c)\nu_w/c\nu_l]$$

is the volume fraction of lipid, c is the weight fraction of lipid in the phase, MW_l is the molecular weight of the lipid, N_o is Avogadro's number, and ν_l and ν_w are the partial specific volumes of water and phospholipid, respectively, each taken as 1.0, accurate to within 1.5% of those measured over the entire hydration range for egg PC (White et al., 1987). The volume of water associated with each lipid molecule is

$$V_w = (1 - \phi)Ad/2$$

It is important to recognize that the effective average cross-sectional area determinations and the volume of water per lipid molecule are independent of any assumptions about the internal structure within the unit cell of the lamellar phase.

Maximum hydration of the lipid is determined from the relation between d and c. The limiting concentration c_0 at maximum amount of water uptake (Tables III and IV) is estimated from the intersection of the horizontal line representing the average repeat spacing in excess water with the best-fit quadratic relation between d and c in the region of restricted water.

Model-dependent presentation of the data will continue to vex the field until high resolution of the lamellar structure is achieved and one can more clearly define the bilayer–water interface. Recently, electron-density profiles of the bilayer have been used to estimate bilayer thickness (McIntosh & Simon, 1986a). At the 15-Å resolution available, these estimated thicknesses rely on assumptions about the polar group dimensions and conformational changes with dehydration in order to define the zero of interbilayer dimension, or bilayer "contact". Area changes determined from the unit cell dimensions as described above are sometimes not seen as changes in the electron-density profiles. At this point therefore, measured unit cell contents appear to us to be a more sensitive measure of bilayer dimensions.

Here we make model-dependent presentation of the data in the form of the relation between the measured water content of the lamellar phase, the thickness of the bilayer d_l, taken as a layer that contains all and only the lipid in the sample, and the distance between the bilayers d_w, equal to the thickness of a layer that contains all the water. This division of the repeat spacing follows the Luzzati tradition (Luzzati & Husson, 1962; Luzzati, 1968) of using mass-average thicknesses based on measured sample composition and sidesteps the difficult issue of defining polar group–water interfaces. Without good knowledge of the distribution of water in the polar group region, there are many other ways d can be partitioned. A hypothetical hydrocarbon–polar interface separating a hydrocarbon layer from a layer that contains both polar groups and water might be better than the hypothetical polar group–water interface we have used because the barrier to hydrocarbon–polar group mixing is greater than polar group–water interactions. Nevertheless, we stay with our traditional partition of d but provide the data necessary for the reader to construct other partitions as judged appropriate. Whatever scheme used affects little the conclusions reached in this study.

Bilayer thickness, d_l, and water layer thickness or bilayer separation, d_w, are

$$d_l = \phi d \qquad d_w = (1 - \phi)d$$

A hydrocarbon layer thickness d_{hc} that contains all and only the hydrocarbon chains of the lipid is

$$d_{hc} = \phi_{hc}\phi d$$

where

$$\phi_{hc} = \mathrm{MW}_{hc}\nu_{hc}/\mathrm{MW}_l\nu_l$$

and MW_{hc} is the molecular weight of the hydrocarbon portion of the lipid molecule and ν_{hc} is the partial specific volume of that hydrocarbon, taken to be 1.25 cm^3/g, measured for diacylglycerol (Das & Rand, 1986) and calculated for lipids with melted hydrocarbon chains (Luzzati, 1968). Then

$$d_{wp} = d - d_{hc}$$

represents the distance between hydrocarbon layers, or the thickness of the polar group plus water layer.

The molecular weights used for the lipids and their hydrocarbon and polar parts in these calculations are given in Table I.

Table I: Molecular Weights of the Phospholipid Molecules and Their Hydrocarbon and Polar Parts Used in the Derivation of the Structural Parameters of Their Lamellar Phases

lipid	MW_l	MW_{hc}	MW_{pol}
POPE	712	499	213
egg PE	733	520	213
egg PE-T	733	520	213
MMPE	747	520	227
DMPE	761	520	241
SOPC	786	531	255
egg PC	775	520	255

Dehydration and Interbilayer Forces. We have measured the energetics of dehydrating the lamellar phases and forcing the bilayers closer together by the osmotic pressure technique as previously described in some detail (Parsegian et al., 1979, 1986). Briefly, lipid samples are equilibrated with excess solutions of dextran or PEG, whose osmotic pressures have been directly measured, or with known vapor pressures. X-ray diffraction of these samples yields their structure, dimension, and, by reference to the gravimetrically prepared samples, their composition.

Model-independent data relate this pressure, a direct measure of net interbilayer repulsive pressure (Parsegian et al., 1979), to the volume of water per polar headgroup mass, V_w. To compare lipids, we have usually normalized the volume of water to that associated with a polar mass equal to the molecular weight of the PE polar group (see Table I).

Model-dependent presentation of these data is in a form that relates interbilayer pressure to the interbilayer distance d_w. Theoretical correlations with the data can be made by use of the sum of van der Waals attraction, hydration repulsion, and steric repulsion P_{fl} due to mechanical fluctuations (Evans & Parsegian, 1986; Evans & Needham, 1987). Specifically, interbilayer pressures have been accounted for by

$$P(d_w) = P_o \exp(-d_w/\lambda) - A_h/[6\pi(d_w - 2d_v)^3] + P_{fl} \quad (1)$$

where A_h is the Hamaker coefficient, d_v is the distance of the effective van der Waals plane out from the mass-average water–lipid interface (LeNeveu et al., 1977; Marra & Israelachvili, 1985; Evans & Needham, 1987)

$$P_{fl} = (-z/\lambda)\left(\sqrt{P_o}/\lambda\right) \exp(-d_w/2\lambda)$$

(Evans & Needham, 1987) in the limit where van der Waals attraction is negligible

$$z = \pi kT/16\sqrt{B}$$

and B is the bilayer bending modulus ($\sim 10^{-12}$ erg).

In the past we have estimated the hydration parameters λ and P_o from the best-fit line to the log P vs d_w curves in their linear region where hydration repulsion dominates. However, the parameters so derived are sensitive to the form and scatter of the relationship between d and c used to determine d_w, particularly for those lipids which cannot be dehydrated over a large range. We have both circumvented this difficulty and also reconciled these data with the independently measured compressibility moduli of these bilayers (Evans & Needham, 1987). As a result two methods, suggested to us by E. Evans (personal communication), have been used to measure the parameters that characterize the hydration force between these bilayers. In the first method the structural parameters d_l and d_w for the osmotic stress data have been determined by (a) choosing the bilayer thickness, d_l^* at log $P^* = 7$, where the water content is known accurately and the compressibility of the bilayer is in the linear range, and (b) using the compressibility moduli K (dyn/cm) measured by Evans and Needham (1987) to calculate varying bilayer thickness, d_l, and separation, $d_w = d - d_l$, for all the experimental points where log $P < 8$. Thus

$$d_l/d_l^* = [K + (P - P^*)d]/[K + (P - P^*)d_l^*]$$

The linear part of the log P vs d_w curves is then best fitted to $P = P_o \exp(-d_w)/\lambda)$. The compressibility moduli used are 145 dyn/cm for egg PC, 233 dyn/cm for POPE, and 200 dyn/cm for the other lipids (Evans & Needham, 1987). The derived parameters are fairly insensitive to the absolute value of K (derived decay lengths differ by about 10% over this range of K) and are independent of the chosen osmotic pressure for log $P^* < 7.5$.

In the second method in addition to the above adjustments and in order to take account of van der Waals attraction to the net repulsive pressure, particularly important near full hydration, a van der Waals contribution, $A_h/[6\pi(d_w - 2d_v)^3]$, has been subtracted from the net repulsive pressure of the experimental points, which are then best fitted to $P = P_o \exp(-d_w/\lambda)$. A_h and d_v used were 1.2×10^{-14} and 3 Å (Evans & Needham, 1987).

Differences in the hydration force decay length λ for the data adjusted both for compressibility alone and for compressibility and a van der Waals contribution were estimated by t tests between all pairs of lipids.

We then use the data adjusted for compressibility and a van der Waals contribution to determine the structural parameters of the lamellar phases at equilibrium in excess water by extrapolation to negligible pressures, i.e., log $P = 1$. These are then compared with the same parameters from the gravimetric data and are seen to be little sensitive to which method is used in their derivation.

The three methods of deriving decay lengths give different absolute values of λ. Therefore in order to compare lipids, consistent methods are required. Each method using the measured compressibility of bilayers, a requirement when X-ray data are used, shows that (a) differences in λ exist and (b) the statistical significance of these differences is the same for each of the methods of derivation. We emphasize therefore that the absolute value of λ is sensitive to the protocol of its derivation but the relative values are not. This makes problematic comparison of decay distances derived herein with those derived by other protocols, particularly those that do not use the independent compressibility measurements.

Results

POPE and SOPC. We first studied the structural dimensions of the lamellar phase formed by POPE and SOPC, in defined amounts of water and at 30 °C where both lipids have melted hydrocarbon chains. Figure 1 shows the variation of both the lamellar repeat spacing and the molecular area A with the concentration of lipid. At water contents lower than those shown, the hydrocarbon chains of these molecules become crystalline. Table III provides the structural dimensions of the lamellar phase at maximum hydration. POPE takes up water to a maximum level of 21–23 wt % where it occupies an area of 56 Å^2 per molecule. SOPC takes up water to a maximum of 37–38 wt % and shows a systematic increase in molecular area from 58 to 64–66 Å^2 with lipid hydration. Plotted also in Figure 1 are the model-dependent parameters of bilayer and water layer thicknesses as they vary with water content. It shows that interbilayer distances, at maximum hydration, are two times larger for SOPC than for POPE. Bilayer, and hydrocarbon, thicknesses on the other hand are relatively constant although a systematic bilayer thinning with hydration can be detected with SOPC.

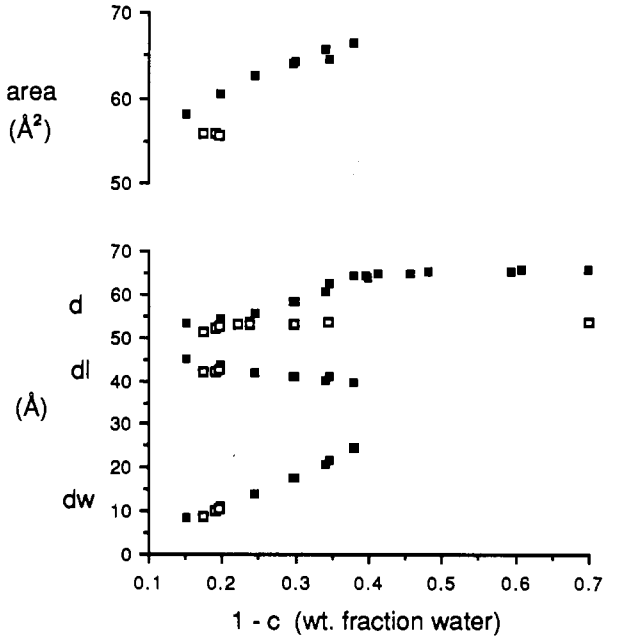

FIGURE 1: Structural parameters of the lamellar phase formed by POPE (open symbols) and SOPC (closed symbols) as they vary with water content. d = lamellar repeat spacing, d_l = thickness of the bilayer, d_w = bilayer separation, and area = molecular area A.

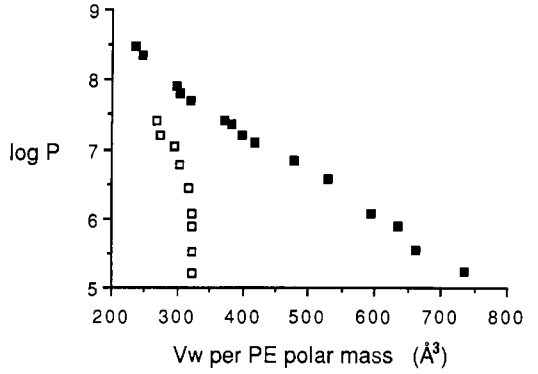

FIGURE 2: Net interbilayer pressure P (or the osmotic pressure of the lamellar phase) as it varies with the water content V_w, the volume of water associated with a polar group mass equal to that of PE. (■) SOPC; (□) POPE.

We then measured the energetics of dehydrating these POPE and SOPC lamellar phases. Figure 2 shows the relation between the volume of water *per PE polar head group mass* and lamellar phase osmotic pressure. V_w for SOPC is two to three times that of POPE near full hydration. Per polar group, V_w would be 20% more for SOPC than that plotted. In addition, the variation of pressure with V_w is very different for these two lipids. While SOPC can be dehydrated over a wide range, POPE undergoes a phase transition to a lamellar phase with frozen hydrocarbon chains when dehydrated to the limited extent described here.

Figure 3 translates these data into bilayer separation and net interbilayer pressure. Below equilibrium separations, the exponential pressure/distance relation can be characterized by hydration coefficients and decay lengths derived as described under Materials and Methods and shown in Table II. When fitted with measured bilayer compressibilities with and without estimated van der Waals attractive pressures, the decay length of POPE differs significantly from that of SOPC with a probability of >99.9%.

In sum, and consistent with previous observations on these two types of lipid (Jendrasiak & Mendible, 1976), there is a

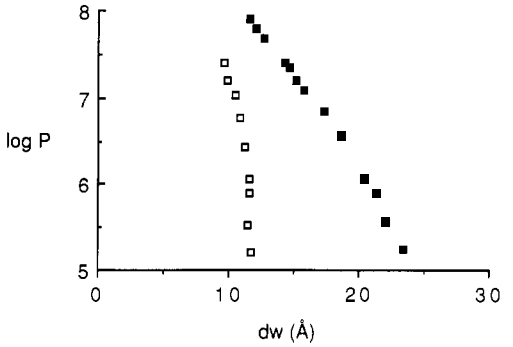

FIGURE 3: Net interbilayer pressure P as it varies with the distance between bilayers d_w. d_w was derived from constant compressibility as described under Materials and Methods. (■) SOPC; (□) POPE.

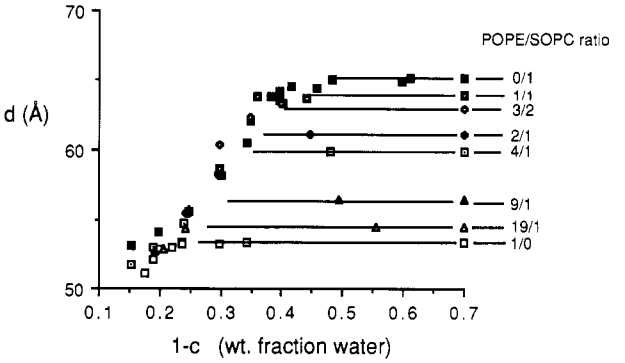

FIGURE 4: Lamellar repeat distance d as it varies with the weight fraction of water for the indicated POPE/SOPC mole ratios. The symbols on the right give the equilibrium spacings in excess water. Notable is that the same change in d spacing from that of the pure lipid takes place at 1/1 for POPE added to SOPC and at 19/1 for SOPC added to POPE.

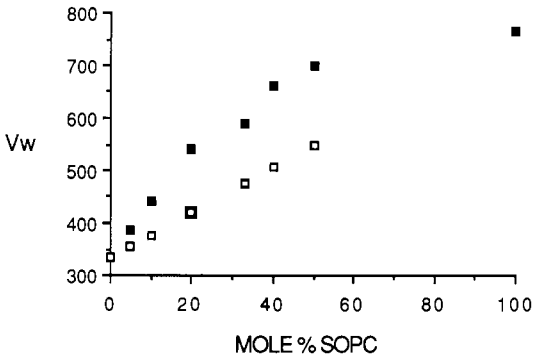

FIGURE 5: Volume of water per polar head group, V_w, in the maximally swelled lamellar phase formed by the indicated POPE/SOPC mixtures. (■) V_w observed; (□) V_w expected assuming that each lipid brings to the mixture an amount of water associated with it in its unmixed phase.

major difference in the amount of water these lipids imbibe from excess solution, POPE taking up less than half the amount of water per polar group mass than SOPC. In addition, the difference in decay lengths shows there is a large difference in the ease with which POPE and SOPC can be dehydrated, POPE requiring much larger changes in osmotic pressure to effect removal of equivalent amounts of water.

SOPC/POPE Mixtures. How do these two different lipids contribute to the hydration properties of bilayers made of their mixtures? Figure 4 shows the dependence of the lamellar repeat spacing, d, on the water content of the lipid for various mixtures of SOPC and POPE. At water contents below the maximum for any mixture, the spacings are similar for all mixtures. However, as the SOPC content increases the

Table II: Hydration Parameters, λ and P_o, and t Tests of the Differences in λ between Pairs of Lipid Species[a]

unadjusted hydration parameters

	λ	0.84	2.08	2.06	2.33	2.38	2.29	2.65
	log Po	12.5	12.3	12.5	10.3	10.4	10.5	10.6

compressibility adjusted (P<8)

	λ	0.82	1.08	1.32	1.76	1.83	1.98	2.07
	log Po	12.5	12.3	12.5	10.3	10.4	10.5	10.6

compressibility and vdw adjusted (P<8)

λ log Po		POPE	egg PE-t	egg PE	MMPE	DMPE	SOPC	egg PC
1.04 11.6	POPE		ns	p>98%	p>99%	p>99.9%	p>99.9%	p>99.9%
1.17 12.0	egg PE-t	ns		ns	p>99%	p>99.9%	p>99.9%	p>99.9%
1.36 12.3	egg PE	ns	ns		p>98%	p>99.9%	p>99.9%	p>99.9%
1.86 10.2	MMPE	p>99%	p>99.5	p>99.5		ns	ns	ns
1.96 10.2	DMPE	p>99.9%	p>99.9%	p>99.9%	ns		ns	ns
2.11 10.4	SOPC	p>99.9%	p>99.9%	p>99.9%	ns	ns		ns
2.16 10.5	egg PC	p>99.5%	p>99.9%	p>99.9%	ns	ns	ns	

[a] For the first horizontal row the hydration parameters were estimated with the unadjusted osmotic stress data. In the second row those data were adjusted for constant compressibility as measured by Evans and Needham (1987). For the vertical column the hydration parameters were estimated by further adjusting the osmotic stress data by an estimation of a van der Waals contribution to the osmotic stress (see Materials and Methods). p values indicate the probability that the decay lengths are different; ns indicates that $p < 98\%$.

Table III: Structural Parameters of Fully Hydrated Lamellar Phases Derived from Their Phase Diagrams[a]

	POPE	egg PE-T	egg PE	SOPC
d (Å)	53.2	52.0	52.9	64.6
c_o (wt %)	77 (79)	70 (72)	61 (64)	60 (63)
V_w (Å)	327	518	768	825
A (Å^2)	56 (57)	66 (65)	75 (72)	66 (64)
d_{hc} (Å)	36.2	32.5	28.7	32.0
d_{wp} (Å)	16.7	19.9	24.2	31.0
d_l (Å)	41.3 (41.8)	36.5 (37.4)	32.4 (33.8)	39 (40.6)
d_w (Å)	11.7 (11.4)	15.5 (14.6)	20.5 (19.1)	26 (24.0)
% poly	0	17	34	0
chain hetero	16:0, 18:1	16:0–18:2	16:0–22:6	18:0, 18:1

[a] The values in parentheses have been derived from the osmotic stress curves adjusted for both constant compressibility and a van der Waals contribution and then extrapolated to negligible stress (see Materials and Methods). % poly is the percentage of all chains that are at least doubly unsaturated. Chain hetero gives the range of chain types.

maximum degree of hydration and the final spacing increase disproportionately. Figure 5 shows the maximum volume of water taken up by the lamellar phase, per average polar group mass (solid squares). Indicated as well are the expected values of V_w assuming linear additive contributions from each lipid (open squares). It is clear that the addition of SOPC to POPE results in a disproportionate increased uptake of water in the resultant lamellar phase. For POPE/SOPC ratios from between 4/1 and 9/1 up to equimolar, the observed excess is approximately 30% more than would be expected if the mixed lipid surfaces hydrated proportional to the hydration of each of the pure components. These results suggest that beyond bringing their complement of water to these mixtures SOPC induces a structural change in POPE bilayers that results in further hydration, a structural change that is maximized at a POPE/SOPC ratio of about 6/1. Such a disproportionate effect of PC was seen for egg PC/egg PE mixtures (Jendrasiak & Mendible 1976).

Variation in the Hydration of Different Species of Phosphatidylethanolamine. We find large differences in the degree of maximum hydration among PE's. Figure 6 shows the relation between water content, lamellar repeat spacing, and molecular area for three phosphatidylethanolamines, (i) that extracted from egg, (ii) that transphosphatidylated onto egg PC, and (iii) POPE (30 °C). Each swells in excess solution to very similar values of repeat spacing but different values of water content and molecular area. A comparison of their structural dimensions at maximum hydration and of their chain composition is shown in Table III. At full hydration the amount of water varies by more than a factor of 2 and correlates with equilibrium areas. The major structural differences, i.e., molecular area and polar and hydrocarbon thicknesses, correlate well with the most obvious differences in chain composition, the degree of heterogeneity and pro-

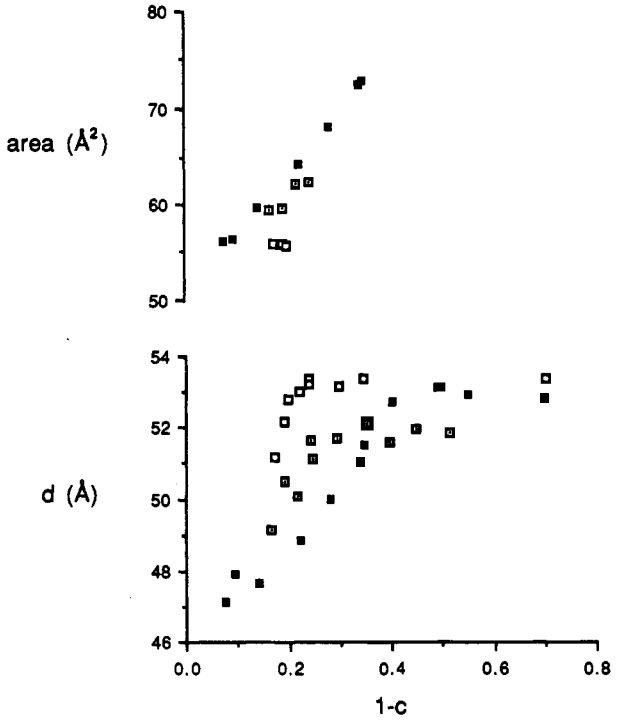

FIGURE 6: Lamellar repeat spacing, d, and molecular area for three differnt PE's as they vary with water content. (□) POPE; (⊡) egg PE-T; (■) egg PE.

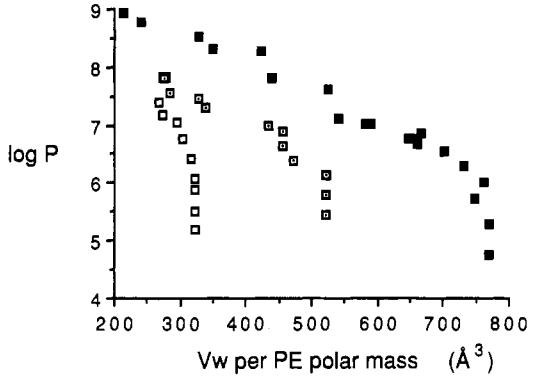

FIGURE 7: Net interbilayer repulsive pressure, P, as it varies with V_w, the volume of water per phospholipid molecule. (□) POPE; (⊡) egg PE-T; (■) egg PE.

portion of polyunsaturated species (Table III). This suggests that hydration is sensitive to chain heterogeneity and/or polyunsaturation in the polar group layers of PE.

Figure 7 shows the relation between the amount of water per molecule and the osmotic pressure exerted on the lamellar phases formed by these different PE's. Even when dehydrated by the same controlled osmotic stress, these three PE's maintain their differential degrees of hydration.

Figure 8 translates these data into interbilayer pressure as it varies with bilayer separation. Below equilibrium separations, the exponential pressure/distance relation can be characterized by hydration coefficients and decay lengths derived as described under Materials and Methods and shown in Table II. While there appears a progressive increase in decay distance with degree of hydration among these lipids, these differences are not statistically significant, with the possible exception of POPE and egg PE not corrected for van der Waals pressure. On the other hand, λ for all the PE's are significantly different from those of the PC's, MMPE, and DMPE with a probability greater than at least 98%.

What is striking among the PE's is the large difference in maximum hydration of lipids with the same head group,

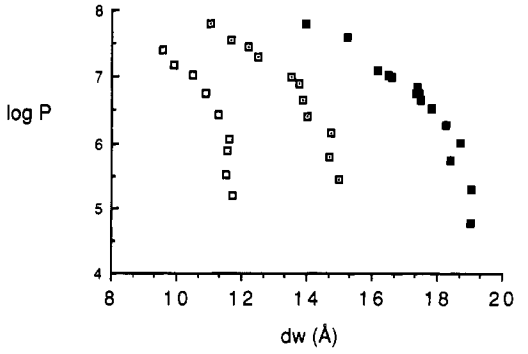

FIGURE 8: Interbilayer repulsive pressure, P, as it varies with d_w, the distance between bilayers. d_w was derived from constant compressibility as described under Materials and Methods. (□) POPE; (⊡) egg PE-T; (■) egg PE.

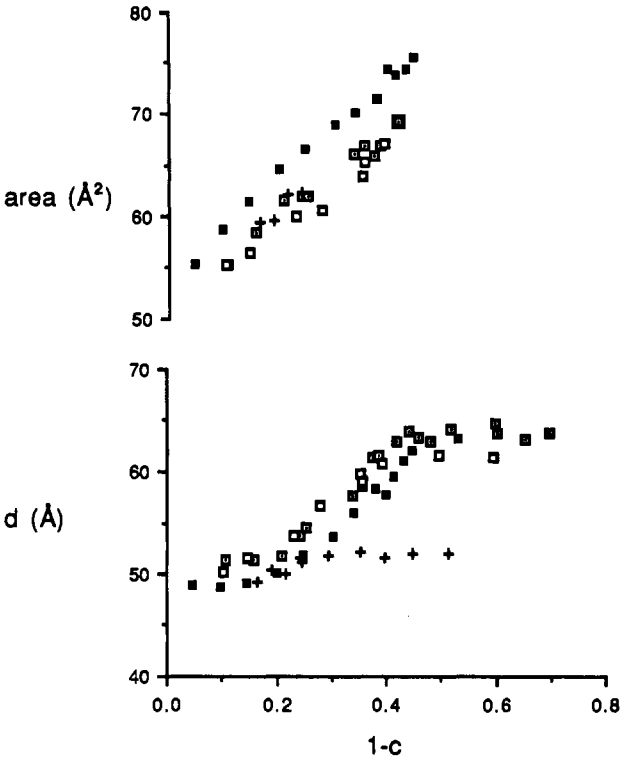

FIGURE 9: Lamellar repeat spacings, d, and molecular areas, as they vary with water content. (+) Egg PE-T; (□) MMPE; (⊡) DMPE; (■) egg PC.

showing a sensitive dependence of hydration on the composition of the hydrocarbon chains (Table III). The trend suggests that increased hydration results from the larger lateral disorder expected from more heterogeneous or more polyunsaturated chains.

Derivatives of Phosphatidylethanolamine. We have also measured the effects of successive methylation of phosphatidylethanolamine polar groups on these hydration properties. Figure 9 shows the relation between water content and either repeat spacing, d, or molecular area, A, for egg PE-T and its mono-, di-, and trimethylated derivatives. A comparison of model-independent and model-dependent structural parameters at full hydration is provided in Table IV. It is striking that for the fully hydrated lipid a single methylation results in the larger spacing and water content characteristic of the dimethylated species and egg PC. There appears only a very weak dependence of maximum spacing, molecular area, and maximum degree of hydration with subsequent successive methylation of the head group.

Table IV: Structural Parameters of Fully Hydrated Lamellar Phases of Egg PE-T and Its Methylated Derivatives[a]

	egg PE-T	MMPE	DMPE	egg PC
d (Å)	52.0	61.8	63.1	61.9
c_o (wt %)	70 (72)	60 (66)	58 (64)	57 (60)
V_w (Å^3)	518	827	938	991
A (Å^2)	66 (65)	67 (61)	70 (63)	74 (70)
d_{hc} (Å)	32.5	32.2	31	29.4
d_{wp} (Å)	19.9	29.5	32.1	32.4
d_l (Å)	36.5 (37.4)	37.1 (40.8)	36.3 (40.3)	35.1 (37.0)
d_w (Å)	15.5 (14.6)	24.7 (21.0)	26.8 (22.8)	26.8 (24.9)

[a] The values in parentheses have been derived from the osmotic stress curves adjusted for both consatnt compressibility and a van der Waals contribution and then extrapolated to negligible stress (see Materials and Method).

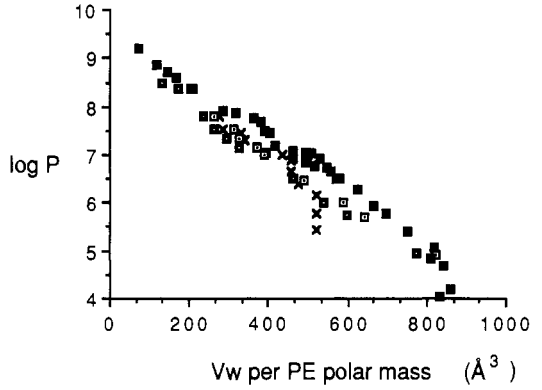

FIGURE 10: Net interbilayer pressure P (or the osmotic pressure of the lamellar phase) as it varies with the water content V_w, the volume of water associated with a polar group mass equal to that of PE. (×) Egg PE-T; (▣) MMPE; (⊡) DMPE; (■) egg PC.

Figure 10 shows the relation between the volume of water, *normalized to the mass of the PE head group*, and the osmotic pressure of the lamellar phase for these lipid species. The methylated species are remarkably similar. PE differs from them in the degree of hydration only near full hydration.

Figure 11 shows the interbilayer pressure as it varies with bilayer separation d_w. Below equilibrium separations, the exponential pressure/distance relation can be characterized by hydration coefficients and decay lengths derived as described under Materials and Methods and shown in Table II. When fitted with a constant bilayer compressibility, with and without van der Waals pressure, the decay distance for egg PE-T is significantly different from those of all its methylated derivatives, all of which are the same. A single methylation results in an increase of the decay distance to a value which is the same for all the methylated species.

Discussion

These results show that the extent of bilayer hydration and interbilayer forces are strikingly sensitive to seemingly small changes in their molecular constitution. Until recently, measured interactions between neutral bilayers, either adhesive (Evans & Metcalfe, 1984; Evans & Parsegian, 1983) or repulsive (Lis et al., 1981; Rand, 1981), have been accounted for by the sum of van der Waals attraction, hydration repulsion, and, more recently, steric repulsion, P_{fl}, due to mechanical fluctuations (Evans & Parsegian, 1986; Evans & Needham, 1987) (see eq 1).

The stronger adhesion and lower hydration of PE bilayers compared to PC bilayers has been attributed either to a weaker hydration coefficient (Cevc, 1985) or to a larger van der Waals Hamaker coefficient (Lis et al., 1982), although the latter has been revised downward with the inclusion of fluctuation-enhanced steric repulsion P_{fl} (Evans & Needham, 1986, 1987). The data of Figures 3, 8, and 11 can be fitted with these forces as described by eq 1, and to a large extent this theoretical correlation is adequate. However, there are a number of features that strain this interpretation and support the suggestions of Kolber and Haynes (1979), Parsegian et al. (1985), and McIntosh and Simon (1986b) that water bridges add an attraction between some surfaces.

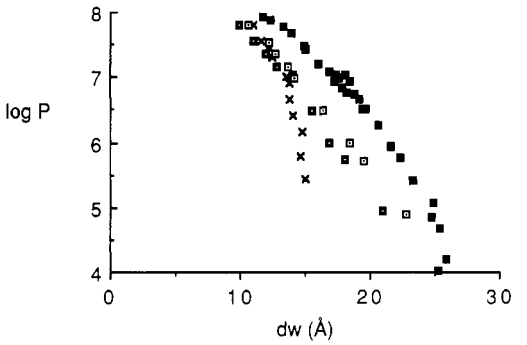

FIGURE 11: Net interbilayer pressure P as it varies with d_w, the distance between bilayers. d_w was derived from constant compressibility as described under Materials and Methods. (×) Egg PE-T; (▣) MMPE; (⊡) DMPE; (■) egg PC.

Why is the decay of the repulsive pressure, either with bilayer separation or with volume of intervening water, more rapid for POPE than for SOPC bilayers? Not only do these differences in decay distances emerge in this study, but measured adhesion energies between POPE-containing unilamellar vesicles also require such small decay distances even when enhanced van der Waals attraction and fluctuation-enhanced repulsion are included in those energies (Evans & Needham, 1986). Do the smaller measured decay distances of the PE's result from a truly lower intrinsic hydration decay length λ of eq 1, or more likely to us, do they emerge from additional forces contributing to the work of dehydrating the lamellar phase and not included in eq 1?

Why should PE's which differ only in the composition of their melted hydrocarbon chains swell to different extents? Although P_o and A_h in eq 1 can be adjusted to account for this, the adjustments are large given the chemical differences. In particular, egg PE and egg PE-T differ in the required Hamaker coefficient by a factor of 5. Further, POPE requires a hydration coefficient 2 orders of magnitude larger than those of the other lipids, or a redefined plane of van der Waals interaction distance to go with its extremely rapid decay length.

Why should the hydration of singly methylated PE bilayers act almost exactly like that PC (trimethylated PE), with little further hydration upon successive methylations?

Why is the swelling of PE/PC mixtures not proportional to the lipid ratios in the mixtures? Small amounts of PC added to PE multilayers effect disproportionate increases in swelling. Direct measurement of the adhesion energy of vesicles made of these POPE/SOPC mixtures (in preparation) can be fitted with a van der Waals power law attraction, refined to include separate polar group and hydrocarbon chain contributions. However even when fluctuation-enhanced repulsion is added to the force balance, both an unexpectedly high-power dependence of the separation distance is required and one again must accept the very different measured parameters, P_o and λ, that characterize the dehydration of POPE.

As an alternative to such refinements to the formalism of van der Waals attraction and hydration repulsion within the force balance of eq 1, we are tempted to suggest that hydration forces can include an attractive component based on the hydrogen-bonding or solubility properties of polar groups. We

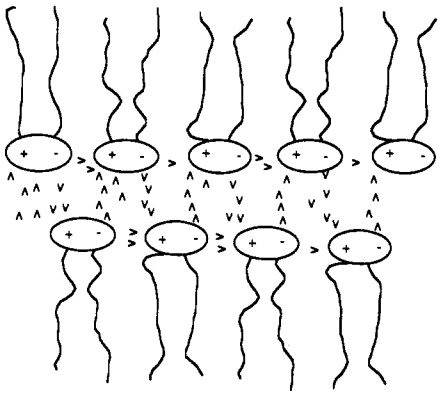

FIGURE 12: Schematic diagram showing spatial correlations of the polar heads of the phospholipid molecules, each of which has groups capable of polarizing water in opposite directions. Hydrogen-bonded water bridges can then span the space between complementary groups which may not themselves hydrogen bond because of constraints imposed by the packing of the other parts of the molecule.

examine this possibility in terms of the formalism introduced previously to explain net hydration repulsion and that can account for the strong attraction of DNA double helices modified by the binding of certain counterions (Rau et al., 1984).

Hydration Attraction. Crystal structures have provided direct observation of intermolecular ammonium–phosphate linkages having the dual character of both salt bridge and hydrogen bond in DLPE bilayers [for a review, see Hauser et al. (1981)]. A variety of water-mediated H-bonded linkages between the phosphate oxygens both within and between apposing DMPC bilayer planes has also been described in DMPC crystals (Hauser et al., 1981). One expects such arrangements when the number of water molecules per polar group is small enough that discrete polar and water layers are less likely. Kolber and Haynes (1979) and McIntosh and Simon (1986b) have suggested that such arrangements occur at the low hydration levels seen in PE bilayers. Water networks, with both attractive and repulsive orientations, exist in other crystalline structures (Savage, 1986). Although bilayers with melted hydrocarbon chains are molecularly more dynamic than the crystals, one would still expect such networks to exist between molecules whose mobility is less than that of water itself. Equilibrium hydration always represents a balance of all attractive and repulsive components. What we propose here is that the observed range of hydration results in part from changes in that balance resulting from attractive as well as repulsive orientations of water between bilayers.

More specifically, the attractive force may be thought of as resulting from a complementarity between phosphate and amine polar charges on facing surfaces, interacting by structurally perturbing the intervening water. Specifically, if simplistically, one expects that the amine and the phosphate regions on the PE head group polarize or otherwise perturb water in opposite directions. The nature of this perturbation and of its propagation into the aqueous space is thought of as a restructuring of solvent molecules as formulated earlier (Marcelja & Radic, 1976). There are two extremes in the relative orientations of the polar groups sitting on apposing surfaces: amine to amine and phosphate to phosphate, noncomplementary and repulsive; or amine to phosphate, complementary and expected to be attractive (see Figure 12). Totally random orientation, unless the strengths of amine and phosphate are closely matched, will lead to net repulsion. We suggest all orientations can exist between bilayers, their balance being determined by the correlations that are allowed under

Table V: Water Contents of the Saturated Solutions and Precipitates of the Isolated Polar Groups (Schumacher & Sandermann, 1976) and of the Maximally Hydrated Lamellar Phases

	no. of H_2O per polar group		
	PC	PE	PS
saturated solution	110	20	55
lamellar phase	25 (SOPC)	11 (POPE)	18 (DOPS)
precipitate	11	5	7

the constraints of molecular packing within the bilayers (Figure 12). Such obvious complementarity as amine and phosphate groups is not required to achieve such water bridges between groups. For example water-mediated H-bonded linkages between phospholipid oxygens are seen between apposing DMPC bilayer planes in DMPC crystals. We emphasize that any complementarity is likely to be dynamic in that the polar groups on both surfaces are in continual motion, although much slower than the intervening water molecules, while interacting with each other. In some ways the attraction thus resembles the mutual motion and perturbation that is the basis of very low frequency van der Waals forces (Mahanty & Ninham, 1976; Parsegian, 1975). These will be opposed by noncomplementing polar groups, the original hydration repulsion.

From another perspective, one that would connect solution chemistry and these measured hydration properties of bilayers, it is worth recalling the measurements of solubility of unattached PC, PE, and PS polar groups, phosphocholine, -ethanolamine, and -serine. Table V shows the number of waters per unattached polar group both at saturating concentrations and precipitated from that solution (Schumacher & Sandermann, 1976). It also shows that within the maximally hydrated lamellar phase these polar groups have intermediate numbers of water between the latter two extremes. They appear therefore to be restrained at supersaturating concentrations. So, built into the constraint of anchoring these polar species onto hydrocarbon chains and assembling them into bilayers is a restraint on their precipitation that would appear as an attractive force. The greater the restraint, which is reflected in the greater imposed area per molecule, the less able are the polar groups to collapse across the interbilayer space.

The formalism of Marcelja and Radic that originally accounted for the repulsive hydration forces leads also to hydration attractive forces. The interaction of two planar surfaces can be a combination of attractive

$$A/4 \cosh^2 (d/2\lambda)$$

and repulsive

$$R/4 \sinh^2 (d/2\lambda)$$

regions where λ is a characteristic decay constant. At large separations each of these forms goes over to an exponential decay. At short distances, the repulsive component must ultimately dominate. At intermediate separations when $A \sim R$ there will be an energy minimum where the two components exactly cancel and stable apposition is achieved.

We use this pair of interactions to estimate the depth of energy minimum reflected in the P vs d_w force curves and to see whether they are of the same magnitude as directly measured. For this we use d taken as equivalent to d_w as determined in the Luzzati tradition. Our strategy is to fit the pressure–distance curves under the constraint that A, R, and λ automatically balance at the equilibrium separation in excess water, d_o. We pick a λ which then gives a ratio A/R and then

Table VI: Calculated Values for POPE of Adhesion Energy, $G(d_o)$ (dyn/cm), and of Net Repulsion Derived Using either van der Waals (P_v) or Hydration (P_h) Attraction[a]

		$G(d_o)$						computed net pressures from either van der Waals (P_v) or hydration attraction (P_h)	
		d_o (Å) (λ = 2.4 Å)			λ (Å) (d_o = 11.8 Å)				
log P	d_w	11.6	11.8	12.0	2.4	2.6	2.8	log P_v	log P_h
7.4	9.6	0.10	0.08	0.06	0.08	0.10	0.13	7.21	7.55
7.19	9.8	0.08	0.06	0.05	0.06	0.08	0.10	7.17	7.50
7.04	10.5	0.14	0.11	0.08	0.11	0.13	0.16	6.72	7.07
6.77	10.8	0.13	0.09	0.07	0.09	0.12	0.14	6.53	6.89
6.43	11.2	0.18	0.10	0.06	0.10	0.12	0.14		
6.06	11.5	0.25	0.08	0.05	0.08	0.10	0.12		
5.89	11.5	0.16	0.05	0.03	0.05	0.07	0.08		
5.52	11.5	0.06	0.02	0.01	0.02	0.03	0.03		
5.2	11.5	0.04	0.01	0.01	0.01	0.01	0.02		

[a] P is measured net replusion.

compute A and R, or equivalently compute the energy minimum $G(d_o)$ per unit area.

Specifically

$$P(d) = \frac{R}{4\sinh^2(d/2\lambda)} - \frac{A}{4\cosh^2(d/2\lambda)}$$
$$= Re^{-d/\lambda}(1 + 2e^{-d/\lambda}) - Ae^{-d/\lambda}(1 - 2e^{-d/\lambda})$$
$$= (R - A)e^{-d/\lambda} + 2(R + A)e^{-2d/\lambda}$$

[It is worth recalling here that with rigid helical biopolymers (Rau et al., 1984; Rau & Parsegian, 1987) the measured decays clearly fall into two categories as expected, λ (2.8–3.4) and $\lambda/2$ (1.4–1.6).]

$$G(d_o) = -\int P(d)\,dd$$
$$= (R - A)\lambda e^{-d_o/\lambda} + (R + A)\lambda e^{-2d_o/\lambda}$$

But at $d = d_o$, $P = 0$ so that

$$R + A = -\frac{R - A}{2}e^{+d_o/\lambda}$$

to allow us to write

$$G(d_o) = \frac{R - A}{2}\lambda e^{-d_o/\lambda}$$

and

$$P(d) = (R - A)e^{-d/\lambda}[1 - e^{-(d-d_o)/\lambda}]$$

Then

$$P(d) = 2\frac{G(d_o)}{\lambda}e^{-(d-d_o)/\lambda}[1 - e^{-(d-d_o)/\lambda}]$$

or

$$G(d_o) = \frac{\lambda P(d)}{2}\frac{1}{e^{-(d-d_o)/\lambda}[1 - e^{-(d-d_o)/\lambda}]} \quad (2)$$

Equation 2 then would apply when hydration attraction dominates.

In a similar way a parameterization of eq 1 (suggested by E. Evans) leads to the alternative relationship where the dominant attraction is traditional van der Waals:

$$G(d_o) = \frac{\lambda_v P(d)(d_o/2\lambda_v - 1)}{d_o^3/d^3 - e^{-(d-d_o)/\lambda_v}} \quad (3)$$

The decay λ in eq 2 is, as originally postulated, taken as an intrinsic, constant value independent of lipid species and reflecting the properties of water. On the other hand, λ_v in eq 3 is taken to be variable and dependent on lipid species as indicated by the fitted values given in Table II. These last relations, eq 2 and 3, provide a prescription for using each data point, $P(d)$, to estimate the depth of the energy minimum $G(d_o)$ at the point where attractive and repulsive hydration forces balance. Alternatively, at fixed measured $G(d_o)$, $P(d)$ can be calculated and compared with measured values. However we emphasize that the use and comparison of these two relations assumes a priori the dominance of these particular interactions, hydration repulsion against *either* hydration attraction *or* van der Waals attraction. In particular, because of the rapid decay of hydration attraction, the cases where it might dominate also happen to be the cases of low hydration and where P vs d can be measured only over a small range. At large hydrations and distances the softer van der Waals attraction will dominate; importantly, one expects a mix of both attractions at intermediate separations. The plausibility of this picture at this stage can only be indicated by how reasonable are the computed energy minima (Evans & Needham, 1986, 1987) or net repulsions compared to measured values.

Data for POPE. For an intrinsic decay of 2.4 and with d_o = 11.6–12 Å and for decays of 2.4–2.8 with d_o = 11.8 Å we have tabulated estimates of adhesion energy $G(d_o)$ for each of our directly measured pressures and interbilayer separations (Table VI). Except near the limit of swelling, where the approaching zero in the denominator of eq 1 amplifies small experimental errors, this set of computations gives a remarkably consistent set of energy estimates, pleasingly close to the value of 0.15 erg/cm^2 reported by Evans and Needham for interacting POPE bilayers. It must be emphasized that this procedure extracts $G(d_o)$ values assuming only the form of the competing attractive and repulsive hydration forces. The dependence of these energies on the choice of particular decay constants or swelling limits can be seen in Table VI. Yet they give qualitatively correct magnitudes of energy minima. The numbers could have come out on the order of 1 or 0.01 rather than 0.1 since we have had no control by parameter fitting. Consequently, we suggest that hydration attraction, created from the same kind of structural perturbation of water on which hydration repulsion is based, be considered a possibility in these interactions.

So far the data do not allow a critical distinction between domination by hydration attraction and that by van der Waals attraction. The data of Table VI also compare, for POPE, measured $P(d)$ with those calculated with the relations of eq 2 and 3, and each correlate equally well. Similar comparisons for egg PE and egg PE-T cannot be made since the adhesion measurements have not been made, and it is likely that, be-

tween these lipid bilayers, neither attraction would dominate.

But hydration attraction does address the above-mentioned difficulties with the traditional view. One can retain the idea of a hydration force decay length that is constant and depends primarily on the properties of the medium. One need not assume radically different van der Waals attraction between bilayers. We suggest that the different chains with their different packing and resultant areas sensitively affect the degree of polar group correlations and thereby the contribution of hydration attraction to the interactions. PE's with crystalline acyl chains hardly imbibe water at all (Seddon et al., 1984; McIntosh & Simon, 1986b), as if precipitated; synthetics such as POPE will take up some ten water molecules each; natural PE's with large surface areas swell to two or three times this amount. The metastability observed for some synthetic PE's that dehydrate and crystallize with time (Mulukutla & Shipley, 1984; Seddon et al., 1984) may result from a slow relaxation of the constraints of chain packing, allowing the polar groups to attract and remove some intervening water. In that way of thinking then, it appears that PE bilayer attraction is a frustrated version of the forces driving precipitation of isolated head groups. The sudden effect of polar group methylation may be to spoil, through decreased ability of methylated ethanolamine to hydrogen bond with water, the strength of complementarity and the neat arrangements of polar groups required for spatial correlation.

In addition, several examples of enhanced hydration of mixed phospholipid bilayers suggest that the mixed polar groups act to spoil the head group ordering required for hydration. PC added to PE would not only add hydration repulsion through its phosphate group but might act as a kind of contaminant to spoil PE head group ordering and reduce hydration attraction. This would account for the disproportionate increase in polar group hydration that PC endows in the mixtures. Small and specific amounts of cholesterol or benzene cause sudden swelling of the gel phase of DPPC (Rand et al., 1980; McDaniel et al., 1982). At 10 mol % cholesterol the bilayers increase their separation from 20 to 35 Å with no detectable melting of the hydrocarbon chains. Diacylglycerol added to egg PC bilayers causes a small increase in bilayer separation (Das & Rand, 1986), as if a small residual attractive component is removed as the polar groups are spread further apart. Might this be caused by the spoiling effect described above where cholesterol or diacylglycerol disorders an interbilayer hydrogen-bonded structure produced by otherwise uncontaminated PC bilayers? For these mixed systems, however, without better knowledge of how polar groups affect the coefficients of hydration repulsion and van der Waals attraction, it is possible that the changes in the polar group region may be enough to modify the traditional forces and account for the results.

One should not be surprised to see a contribution of such attractive forces holding together bilayers that contain lipids capable of forming hydrogen bonds (Boggs, 1984), and for such forces to be modified by the constraints on these polar groups and by other factors that affect polar group solubility or precipitation. The low maximum hydration of glycolipids (Marra, 1985), of phosphatidylserine (Loosley-Millman et al., 1982), and of phosphatidylglycerol (Marra, 1986) in high salt and the large adhesion energies measured between digalactosyldiacylglycerol bilayer vesicles (Evans & Needham, 1987) all suggest their action. In fact what stands out is the exceptionally large hydration of methylated species compared to the rest, those described herein, and also dihexadecyldimethylammonium in high salt [Pashley et al. (1986) and our unpublished force measurements]. It is as if the role of methylation is to preclude the formation of hydrogen bond mediated attractive interactions between bilayers.

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