Water adsorption isotherms and hydration forces for lysolipids and diacyl phospholipids

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ABSTRACT The repulsive forces in a wide range of diacyl and monoacyl phospholipid systems have been obtained from the adsorption isotherms for water. From the exponential dependence of the repulsive pressure on the water content, information has been deduced regarding the hydration force. For diacyl phosphatidylcholines the strength of the hydration force and its characteristic decay length are in good agreement with values previously obtained by x-ray diffraction methods. For natural and synthetic diacyl phosphatidylcholines in the fluid lamellar phase, the hydration force extrapolated to zero layer separation (P_o) is in the range $4–5.10^8$ N.m^{-2} and the decay length is ~0.3 nm. The results for dimyristoyl, dipalmitoyl, and distearoyl phosphatidylcholines in the gel phase are very similar with $P_o \approx 2.5.10^8$ N.m^{-2} and decay length of ~0.2 nm. Egg monomethyl phosphatidylethanolamine is less strongly hydrated: $P_o = 2.3.10^9$ N.m^{-2}, with a decay length of 0.3 nm. Egg phosphatidylethanolamine and bovine phosphatidylserine hydrate even more weakly with $P_o \approx 1.3.10^8$ N.m^{-2} and decay length of ~0.15 nm. Mixtures with cholesterol or phosphatidylcholine increase both P_o and the decay length for phosphatidylethanolamine to values closer to those for phosphatidylcholine. The repulsive forces deduced for egg lysophosphatidylcholine at 40°C display a biphasic water dependence, with the low water phase being similar to lamellar egg phosphatidylcholine, and the phase at higher water content having a smaller value of $P_o = 2.10^8$ N.m^{-2} but a longer decay length of ~0.45 nm, corresponding to a nonlamellar configuration. Bovine lysophosphatidylserine similarly yields values of $P_o = 1.2.10^8$ N.m^{-2} and an effective decay length of 0.64 nm. The hydration behavior of the various diacyl phospholipids has been interpreted in terms of the mean-field molecular force theory of lipid hydration, and values deduced for the surface hydration potential of the various lipids. This analysis extends previous results on hydration forces, particularly to lysolipids and nonlamellar phases.

INTRODUCTION

The hydration of phospholipids can be usefully studied using the water adsorption isotherms (1–4, 24, 25). Analysis of the isotherms can, in principle, yield detailed information on the energetics of association of the water molecules with the lipid polar headgroups (see e.g., reference 5). An important consequence of this association is the creation of water-mediated repulsive forces between the hydrated lipid layers (5–8). These hydration forces arise from the structuring of the water molecules at the lipid polar surface and are found to decay approximately exponentially with separation between the lipid layers (5–7). At close distances of approach the hydration repulsion overcomes the van der Waals attraction and is responsible, at least in part, for the stability of biological membranes against spontaneous coalescence and fusion.

Water adsorption isotherms can be used to study the total repulsive forces, and hence the hydration forces, in the regions of close approach between the lipid layers. The isotherm specifies the activity of the adsorbed water, and hence the work required to transfer water from the bulk to the region between the lipid layers. From a knowledge of the characteristic dimensions and the partial molar volume of water in the lipid system, the effective force or pressure between the layers may then be calculated (7, 32). In the region of low hydration, it has recently been suggested that the repulsive forces are dominated by steric interactions between the lipid headgroups (9). However, the repulsive force was found to be a continuous function of the number of water molecules associated with the lipids, i.e., of the energetics of hydration, and therefore the adsorption isotherms present a true reflection of the hydration forces, when extrapolated to higher water contents.

In the present communication, the repulsive forces have been deduced from published adsorption isotherms (1–4, 24, 25), for a wide range of different phospholipids. Good agreement in the strength and range dependence of the extrapolated hydration force is found with the direct results for phosphatidylcholine (PC)[1] systems which have been determined by x-ray diffraction measurements (7,

[1]Abbreviations used in this paper are: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; MePE, monomethyl phosphatidylethanolamine; chol, cholesterol; C(18:2), linoleoyl; C(18:1), oleoyl; C(10:0), caproyl; C(14:0), myristoyl; C(16:0), palmitoyl; C(18:0), stearoyl.

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8). This gives confidence in using the adsorption isotherms to determine the hydration forces in systems that have not previously been studied (e.g., monomethyl phosphatidylethanolamine, and didecanoyl and dilinoleoyl phosphatidylcholines amongst the diacyl phospholipids). The data so obtained then allow a more detailed comparison with theoretical descriptions of the hydration forces (5, 14, 20–22). In particular, new results are presented for lysolipids, which give an opportunity to investigate hydration forces in nonlamellar phases. Currently, rather little information is available on the repulsive and hydration forces in nonlamellar phases (18), especially those of the "oil-in-water" (type I) configuration.

METHODS

The adsorption isotherm specifies the number of water molecules taken up by the lipid at equilibrium with water vapor of a given partial pressure, p. The relative vapor pressure in the system, p/p_o, can be identified with the activity of water, a_w, associated with the polar headgroups of the lipid layers. Thus the free energy change associated with hydration of the lipid layers is given by:

$$\Delta G_{hyd} = -RT \cdot \ln(p/p_o), \quad (1)$$

where p_0 is the saturated vapor pressure of water. The effective total pressure between the layers can therefore be defined by (6, 7):

$$P_{tot} = -(RT/V_w) \cdot \ln(p/p_o), \quad (2)$$

where V_w is the partial molar volume of water in the lipid polar group region. Using the adsorption isotherm, the total pressure, P_{tot}, can then be related to the average number of water molecules, n_w, associated with each lipid molecule, at a particular partial pressure (32).

In principle, the total repulsive pressure is made up of several contributions:

$$P_{tot} = P_s + P_{hyd} + P_{el} - P_{vdw}, \quad (3)$$

where the steric repulsion between the headgroups, P_s, and the hydration repulsion, P_{hyd}, are likely to be the dominant components at the relatively low hydration levels appropriate to the adsorption isotherms. The Van der Waals attraction, P_{vdw}, is considerably smaller than the latter contributions, at close distances of approach (6, 7). The electrostatic repulsion, P_{el}, applies only to charged lipids and is likely to make only small contributions to the net repulsion at low water contents for which the lipid charge will be effectively neutralized by the close proximity of the counterions (cf. reference 19). Fluctuation forces, arising from modulation of the interlayer forces by thermally induced undulations in the lipid layers, are also not expected to contribute appreciably to the interlayer interactions. This is because the undulations are effectively damped by the close apposition of the lipid layers at low water content (10). Recently it has been demonstrated that the steric repulsions dominate at very low levels of hydration (9). Nevertheless, it was found that the logarithm of the total repulsive pressure was a linear function of the number of water molecules associated with the lipid, over the entire hydration range. Therefore the characteristic parameters of the hydration force can be obtained by extrapolation to the regions of higher water content at which the hydration force dominates.

In the region where n_w is sufficiently large ($\geq$3 mol/mol), the thickness of the water space between the lipid layers can be approximated from the degree of hydration, n_w, by density considerations:

$$d_w = 2n_w \cdot V_w/(N_A \cdot A), \quad (4)$$

where N_A is Avogadro's number and A is the area per lipid molecule.[2] In this way the total pressure between the lipid layers can be related not only to the extent of hydration, n_w, but also to the separation of the layers, d_w. Eq. 4 applies to a lamellar geometry, and assumes that V_w is constant, which was found to hold for egg phosphatidylcholine (23). In the case of e.g., an inverted hexagonal phase, H_{II}, the diameter of the water cylinders is:

$$d_w = 4n_w \cdot V_w/(N_A \cdot A). \quad (5)$$

For the normal hexagonal phase, H_I, the minimum water layer thickness between the lipid cylinders is:

$$d_w = \{[8\pi(1 + n_w/Mv_l)/3\sqrt{3}]^{1/2} - 2\} \cdot 2Mv_l/(N_A \cdot A), \quad (6)$$

where M is the lipid molecular weight and v_l is the lipid partial specific volume.

For regions of low water content, such as the initial parts of the adsorption isotherm, it has been suggested that the partial molar volume of water could be smaller than the value $V_w = 18.10^{-6}$ m^3 found in free water (11, 12). However, recent direct measurements have demonstrated that the partial specific volume of water remains close to its bulk value throughout the full hydration range of egg phosphatidylcholine-water mixtures (23). If V_w were smaller in some systems, the effective total pressure given by Eq. 2 would be correspondingly greater. Even so, it has been shown in reference 9 that the logarithm of the effective pressure is a linear function of the number of water molecules associated with the lipid, over the entire hydration range. Therefore, as noted above, the results deduced from the hydration isotherms can be extrapolated to the region in which water attains the bulk value of its partial molar volume, and Eqs. 4–6 may then be used reliably.

RESULTS

The effective repulsive bilayer pressure is given as a function of water content for a number of different natural phospholipid systems in Fig. 1. The values of P_{tot} were deduced from the adsorption isotherms of reference 2, using the bulk value for the partial molar volume of water in Eq. 2. As seen from Fig. 1, the repulsive pressure depends exponentially on the water content, n_w. Because the three systems shown are almost certainly in a lamellar configuration, Eq. 4 implies that the hydration pressure decays exponentially with the bilayer separation, d_w, if the lipid molecular area does not change appreciably with hydration (cf. also footnote 2). X-ray diffraction measurements (7) have shown that the area/molecule for egg phosphatidylcholine does not change by more than 10% over the range of water contents $3 \leq n_w \leq 10$. (In fact, for egg phosphatidylcholine the point at high water content does deviate very significantly from the straight line, and

[2]This equation assumes that the lipid surface is defined in terms of a lipid layer whose thickness is determined by the partial specific volume of the lipid, $\bar{v}_l$, as is done in e.g., references 6–8.

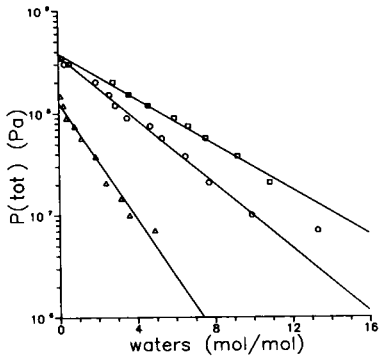

FIGURE 1 Bilayer repulsive pressure, P_{tot}, as a function of water to lipid mole ratio, n_w, deduced from the adsorption isotherms of reference 2. (O) egg phosphatidylcholine; (□) egg phosphatidylcholine/cholesterol (1:1 mol/mol); (△) bovine phosphatidylserine. T = 22°C. The ordinate is logarithmic, extending from 10^6 to 10^9 N.m^{-2}, and the lines are obtained by linear regression. A value of $V_w = 18.10^{-6}$ m^3 was assumed in calculating P_{tot}.

can be brought into agreement with the linear dependence by correction for the increase in area/molecule using the data of reference 7.) By taking the mean molecular area over the relevant hydration range, the uncertainty associated with the changes in lipid area is halved. This procedure is justified by the comparison with the results obtained from x-ray data given below.

The adsorption isotherms of several other different natural phospholipid systems have been analyzed in a similar way and, with the exception of egg phosphatidylethanolamine, yield exponential water dependences similar to those given in Fig. 1. The parameters of the repulsive pressure have been analyzed by linear regression according to the equation (cf. Fig. 1):

$$P_{tot} = P_o \cdot \exp(-n_w/n_\xi), \quad (7)$$

where n_ξ is the characteristic exponential decay constant, expressed in terms of water molecules/lipid molecule. The parameters have also been expressed in terms of a characteristic decay length, ξ:

$$P_{tot} = P_o \cdot \exp(-d_w/\xi), \quad (8)$$

where d_w is given by Eq. 4, calculated with a mean value of A. Data for $n_w \geq 10$ have been omitted from the linear regression because of the increase in area/lipid molecule with increasing hydration, as discussed above. The parameters obtained for the various systems are summarized in Table 1, together with the extrapolated values for the strength of the hydration potential, ψ_{ho}, deduced from P_o (see later Discussion). For egg phosphatidylethanolamine the isotherms indicate that very little hydration takes place at low water activity and for this reason these points have been omitted from the linear regression. At

TABLE 1 Parameters of the total repulsive forces (P_o, n_ξ) and hydration forces (ψ_{ho}, ξ) in natural phospholipid systems and mixtures with cholesterol, deduced from the adsorption isotherms for water*

Lipid	T	P_o	n_ξ	ψ_{ho}	ξ	Reference‡
	°C	$N \times m^{-2}$	mol/mol	V	nm	
Egg PC	40	5.37×10^8	2.80	1.16	0.28	1
	25	5.00×10^8	2.55	1.02	0.26	1
	22	3.62×10^8	2.77	0.94	0.28	2
Egg MePE	22	2.29×10^8	2.89	0.78	0.29	4
Egg PE	22	1.38×10^8	1.19	0.27	0.13	2
Bovine PS	22	1.26×10^8	1.53	0.33	0.17	2
Egg PE/egg PC (1:1 mol/mol)	22	3.56×10^8	2.54	0.86	0.25	4
Egg PC/chol (1:1 mol/mol)	22	3.79×10^8	3.92	1.36	0.39	2
Egg PE/chol (1:1 mol/mol)	22	2.56×10^8	2.49	0.71	0.25	4

*The parameters for the hydration force, ψ_{ho} and ξ, are those which would be observed in the region of higher water contents, at which the hydration force dominates (see text).

‡Adsorption isotherms are from the following references: (1) Elworthy (1967); (2) Jendrasiak and Hasty (1974); (4) Jendrasiak and Mendible (1976*b*).

higher activities the water dependence is biphasic and the linear regression parameters reported in Table 1 are restricted to the region $n_w < 4$. There are only two points for higher water activities, but the slope of the plot is then closer to that for the phosphatidylcholine systems (data not shown).

The results of Table 1 can be compared with those obtained for similar systems at higher water content, using the x-ray diffraction method. For egg phosphatidylcholine, values of $P_o = 5.75.10^8$ N.m^{-2} and $\xi = 0.26$ nm were obtained at 25°C (8). These values are similar to those shown in Table 1, particularly for the decay length, ξ.[3] This gives confidence in using the adsorption isotherms to obtain information about the hydration repulsion pressure in other systems. For egg phosphatidylcholine/cholesterol (1:1 mol/mol), values of $P_o = 3.98.10^{11}$ N.m^{-2} and $\xi = 0.14$ nm were obtained at 25°C (8). These values differ greatly from those given in Table 1, and from those obtained in the other lipid systems studied by x-ray diffraction. It could be that the behavior at high water content is different from that at low water content shown in Table 1. For egg phosphatidylethanolamine values of

[3]If the absorption isotherm from reference 2 is analyzed using the individual values of the area/lipid molecule derived from x-ray measurements (7), values of $P_o = 3.99.10^8$ N.m^{-2} and $\xi = 0.26$ nm are obtained. These results are similar to those given in Table 1, which were calculated using a fixed area/molecule, confirming that the error introduced by the mean area approximation is not too great.

$P_0 = 3.72.10^9$ N.m^{-2} and $\xi = 0.21$ nm were obtained at high water content and 25°C (8). From the biphasic response deduced from the adsorption isotherms it is clear that the behavior at low water content is very different in this case.

Representative results deduced from the adsorption isotherms of synthetic phosphatidylcholines are given in Fig. 2. Again the typical exponential dependence on degree of hydration is obtained for all except the highest water contents. The corresponding parameters of the repulsive pressure for various homogeneous phosphatidylcholine systems and for dipalmitoyl phosphatidylethanolamine are given in Table 2. For comparison, values of $P_0 = 3.98.10^8$ N.m^{-2} and $\xi = 0.29$ nm were obtained for dioleoyl phosphatidylcholine at 25°C and higher water contents (8), in good agreement with the results in Table 2. Correspondingly, values of $P_0 = 6.76.10^8$ ($1.35.10^8$) N.m^{-2} and $\xi = 0.20$ (0.36) nm were obtained for dipalmitoyl (distearoyl) phosphatidylcholine at 25°C (8), although a clear exponential separation dependence was not observed for distearoyl phosphatidylcholine. The results for dipalmitoyl phosphatidylcholine agree reasonably well with those in Table 2, especially with regard to the reduction in the decay length relative to unsaturated phosphatidylcholines. Values of $P_0 = 8.71.10^8$ N.m^{-2} with $\xi = 0.26$ nm for dimyristoyl phosphatidycholine at 27°C, and $P_0 = 9.77.10^9$ N.m^{-2} with $\xi = 0.22$ nm for dipalmitoyl phosphatidylcholine at 50°C, have been obtained at higher water content by x-ray diffraction (8). Although agreeing qualitatively with the results of Table 2, the differences in P_0 values do suggest a somewhat different behavior at high degrees of hydration in the fluid phase. For dipalmitoyl phosphatidylcholine in 1:1 mol/mol mixture with cholesterol values of $P_0 = 1.55.10^8$ N.m^{-2} and

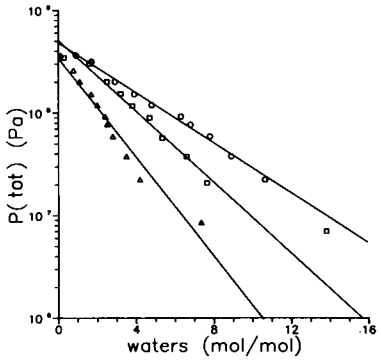

FIGURE 2 Bilayer repulsive pressure, P_{tot}, as a function of water to lipid mole ratio, n_w, deduced from the adsorption isotherms of references 2 and 3. (○) dilinoleoyl phosphatidylcholine; (□) dicaproyl phosphatidylcholine; (△) dipalmitoyl phosphatidylcholine. $T = 22$°C. The ordinate is logarithmic, extending from 10^6 to 10^9 N.m^{-2}, and the lines are obtained by linear regression. A value of $V_w = 18.10^{-6}$ m^3 was assumed in calculating P_{tot}.

TABLE 2 Parameters of the total repulsive forces (P_0, n_ξ) and hydration forces (ψ_{ho}, ξ) in synthetic phosphatidylcholine and phosphatidylethanolamine systems and mixtures with cholesterol, deduced from the adsorption isotherms for water*

Lipid	T	P_0	n_ξ	ψ_{ho}	ξ	Reference‡
	°C	$N \times m^{-2}$	mol/mol	V	nm	
diC(18:2)PC	22	4.80×10^8	3.56	1.33	0.34	2
diC(18:1)PC	22	4.06×10^8	3.23	1.11	0.31	2
diC(10:0)PC	22	5.04×10^8	2.52	1.10	0.27	3
diC(14:0)PC	22	3.69×10^8	1.74	0.76	0.22	3
	15	6.78×10^8	1.96	1.03	0.22	25
	20	5.77×10^8	2.05	1.11	0.26	25
	25	4.01×10^8	2.33	0.91	0.25	25
	35	3.99×10^8	2.28	0.89	0.25	25
diC(16:0)PC	22	3.40×10^8	1.80	0.75	0.23	2,3
	25	5.23×10^8	1.95	1.01	0.25	24
	40	5.36×10^8	2.11	1.04	0.25	24
diC(18:0)PC	22	3.40×10^8	1.84	0.75	0.23	3
diC(16:0)PE	25	2.53×10^8	0.57	0.24	0.08	24
	40	2.65×10^8	0.58	0.24	0.08	24
diC(16:0)PC/ chol (1:1)	22	5.00×10^8	1.88	0.75	0.19	3

*The parameters for the hydration force, ψ_{ho} and ξ, are those which would be observed in the region of higher water contents, at which the hydration force dominates (see text).

‡Adsorption isotherms are from the following references: (2) Jendrasiak and Hasty (1974); (3) Jendrasiak and Mendible (1976*a*); (24) Elworthy (1962); (25) Wilkinson et al. (1977).

$\xi = 0.32$ nm at 25°C were obtained by x-ray diffraction (8). These values are substantially different from those given in Table 2, and may again point to a difference in behavior between the states of low and high hydration.

Of most interest is the behavior of lyso phospholipids, because these are likely to have a considerably different phase behavior from diacyl phospholipids, and the repulsive and hydration forces have not previously been studied in these systems. The effective repulsive pressure derived from the desorption isotherm of egg lysophosphatidylcholine at 40°C, taken from reference 1, is given in Fig. 3. The dependence on water content is clearly biexponential with a break point at $n_w \approx 5$, suggestive of a phase change. Reference to the lipid-water phase diagrams of lysophosphatidylcholines (13) supports this interpretation. Parameters of the hydration force for both phases of egg lysophosphatidylcholine, together with accompanying data from the adsorption isotherm of bovine lysophosphatidylserine, are given in Table 3. For egg lysophosphatidylcholine at 25°C a single exponential water dependence is observed, corresponding to the low water region at 40°C. As for phosphatidylethanolamine, the adsorption isotherm of lysophosphatidylserine indicates that this

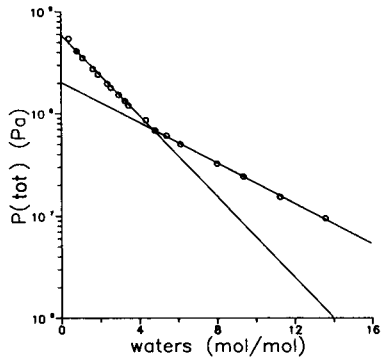

FIGURE 3 Interlayer repulsive pressure, P_{tot}, as a function of water to lipid mole ratio, n_w, deduced from the desorption isotherms of egg lysophosphatidylcholine at 40°C (reference 1). The ordinate is logarithmic, extending from 10^6 to 10^9 N.m^{-2}, and the lines are obtained by linear regression over the appropriate ranges. A value of $V_w = 18.10^{-6}$ m^3 was assumed in calculating P_{tot}.

lipid hydrates only very weakly at low water activity, and therefore these data points have been omitted from the linear regression.

Values for the area per phospholipid molecule, A, which were used with Eq. 4 for calculating the values of ξ in Tables 1–3, were taken from x-ray diffraction measurements. Wherever possible, measurements at high lipid concentrations were used. For egg phosphatidylcholine a mean value of $A = 0.5978$ nm^2 was taken from reference 7. For egg phosphatidylethanolamine, a value of $A = 0.55$ nm^2 was taken from reference 8. The same value was assumed for bovine phosphatidylserine at low water contents. A value of $A = 0.63$ nm^2 was taken from reference 8 for dioleoyl phosphatidylcholine, and the same value was also assumed for dilinoleoyl phosphatidylcholine. Values of $A = 0.475$ nm^2 were taken for saturated phosphatidylcholines in the intermediate gel phase (8, 26–28), and 0.505 nm^2 for dipalmitoyl phosphatidylcholine at 40°C (29). For dimyristoyl and distearoyl phosphatidylcholines in the low temperature gel phase, values of $A = 0.53$ and 0.484 nm^2 were taken from references 27, 28, and 26, respectively. A value of $A = 0.55$ nm^2 was taken for saturated phosphatidylcholines in the fluid phase (8). For dipalmitoyl phosphatidylethanolamine in the gel phase, $A = 0.415$ nm^2 was taken from corresponding data on the didodecyl derivative (30). A mean value of $A = 0.605$ nm^2 was obtained for egg lysophosphatidylcholine from reference 31. For all other phospholipids, a value of $A = 0.6$ nm^2, corresponding to the fluid phase of egg phosphatidylcholine, was assumed. The value for the area/molecule, A, enters linearly into the water layer thickness, d_w, in Eqs. 4–6. Therefore, to a first approximation, the fractional error in the decay length, ξ, (and in ln P_0) will be equal to that in the value assumed for the area/molecule (cf. also footnote 3).

TABLE 3 Parameters of the total repulsive forces (P_0, n_ξ) and hydration forces (ψ_{ho}, ξ) in lysophospholipid systems, deduced from the desorption or adsorption isotherms for water*

Lipid	T	P_0	n_ξ	ψ_{ho}	ξ	Reference‡
	°C	$N \times m^{-2}$	mol/mol	V	*nm*	
Egg lysoPC	40	5.91×10^8	2.19	0.96	0.22	1
		2.03×10^8	4.38	1.13	0.44	
	25	4.69×10^8	2.43	0.94	0.24	1
Bovine lysoPS	22	1.16×10^8	6.38	1.23	0.64	2

*The parameters for the hydration force, ψ_{ho} and ξ, are those which would be observed in the region of higher water contents, at which the hydration force dominates (see text).
‡De/adsorption isotherms are from the following references: (1) Elworthy (1967); (2) Jendrasiak and Hasty (1974).

DISCUSSION

The effective interlayer repulsive pressure has been deduced from the absorption isotherms by using Eq. 2 and assuming that the partial molar volume of water was equal to its bulk value. The exact molecular details of the headgroup-water interaction may be complex at low levels of hydration, the steric headgroup interactions being thought to dominate the interlamellar repulsion. However, the exponential dependence of the repulsive pressure on water content deduced from the absorption isotherms has been shown to remain continuous into the region of higher water content (9), where the hydration forces dominate and Eq. 4 can be used to predict the interlayer separation. Therefore, the parameters deduced from Eq. 8 for the low water region, represent closely the parameters of the hydration force that would be observed in the higher water region. The reason for this is that the adsorption isotherm determines the free energy of hydration according to Eq. 1, and hence is a direct measure of the hydration pressure between the layers, if the shape and size of the polar headgroups can be neglected. The experimental justification for this view comes from the good agreement between the parameters of the hydration forces for egg, dioleoyl and dipalmitoyl phosphatidylcholines deduced from the absorption isotherms, and those obtained directly from measurements with x-ray diffraction that were performed at considerably higher water contents. Fluctuation forces only come to be appreciable at yet higher water contents, close to the equilibrium separation (10). A complication arises in the choice of the positions of the lipid surfaces relative to which d_w is referred (35). This will primarily affect the values of P_0, the extrapolated hydration pressure at vanishing separation of the surfaces, but should have lesser effect on the

values deduced for the decay length, ξ, which depends only on the slope of the separation dependence. The convention for determining the position of the lipid surfaces (and for measuring d_w) chosen here corresponds mostly closely to that of references (6–8, 18, 32, 33), and for this reason comparison has been made with the data from those references (cf. also footnote 2).

The correspondence between the data deduced from the adsorption isotherms and from the measurements with x-ray diffraction is not always so good. In these latter cases, the differences most probably can be attributed to a different hydration and phase behavior at the low water contents. Complications may also arise from the extrapolation to higher water contents for these samples that are rather weakly hydrated. In support of this, the agreement between the different methods is found to be particularly good for egg and dioleoyl phosphatidylcholine, which are both more strongly hydrated and in a highly fluid state. It is also noteworthy that the agreement is best for those systems for which a large number of data points have been collected by the two methods.

Using the reasoning given at the beginning of this section, the parameters for the total repulsive force, deduced from the absorption isotherms and given in Tables 1–3, provide estimates for the values of the limiting hydration pressure at close approach, P_o, and its decay length, ξ, at the higher water contents for which the hydration force dominates. In connection with this extrapolation, which is based on the dependence with respect to the number of water molecules (Figs. 1–3; and Fig. 2 of reference 9), it should be noted that in several studies, the area per lipid molecule was found not to vary greatly over this higher range of either water content or interlayer separation (see [35] and references therein). Thus Eqs. 4–6, with an approximately constant value of A, should hold in the extrapolated region.

The data of Table 1 very clearly illustrate the differences in hydration behavior between the various phospholipid headgroup types. (At these rather low values of hydration, the counterions remain associated with the phosphatidylserine headgroups, and therefore the electrostatic repulsive pressure is relatively unimportant compared with the hydration forces). The strength of the hydration pressure can be related to the surface hydration potential, ψ_{ho}, using the mean-field molecular force theory of lipid hydration (5, 14):

$$P_{hyd} = -(2\chi\psi_{ho}^2/\xi^2) \cdot \exp(-d_w/\xi), \qquad (9)$$

where ξ is the decay length of the water polarization and $\chi = \epsilon_o\epsilon_\infty(\epsilon - \epsilon_\infty)/\epsilon$ is the water polarization susceptibility, ϵ_∞ being the high frequency dielectric constant, characteristic of water polarization in rapidly varying fields. Values for the hydration potential determined from the limiting hydration pressure, P_o $[=2\epsilon_o\epsilon_\infty)(\epsilon - \epsilon_\infty)\psi_{ho}^2/\epsilon\xi^2]$, with ϵ = 80 and ϵ_∞ = 1.8, are given for the various lipid systems in Tables 1–3. Clearly from Table 1, phosphatidylcholine is much more strongly hydrated than phosphatidylethanolamine and phosphatidylserine, and monomethyl phosphatidylethanolamine has an intermediate degree of hydration. Differences in hydration between phosphatidylcholines and phosphatidylethanolamines have already been pointed out by other workers (33, 34). The relative values of ψ_{ho} are in qualitative agreement with theoretical predictions based on the local excess surface charge density estimated from quantum mechanical calculations of electron density distributions (Table 3.2 of reference 5). For dipalmitoyl phosphatidylethanolamine the values of ψ_{ho} are even smaller than for the unsaturated natural analogue (cf. Tables 1 and 2). This could arise from the closer polar group packing in the saturated lipid, which would further reduce the accessibility to water. On the other hand, it is notable that mixture of phosphatidylethanolamine with either phosphatidylcholine or cholesterol increases ψ_{ho} to values more closely approaching that for phosphatidylcholine. Presumably the intermolecular hydrogen bonding of the phosphatidylethanolamine is disrupted, freeing additional parts of the polar group for interaction with water and hence increasing the hydration potential.

Table 1 also indicates rather small values for the decay length of the hydration forces in the natural phosphatidylethanolamine and phosphatidylserine systems. For saturated phosphatidylethanolamine the decay length is even shorter (see Table 2). One possible reason for these small values could lie in the phase behavior at low water contents, as discussed above. It is known that both of these lipid types can display metastability and reversion to crystalline phases, and the kinetics associated with the metastability can, in certain cases, be rather slow (15–17). Therefore it cannot be totally excluded that the samples in the adsorption experiments were not (partially) in a metastable state.

The results of Table 2 allow comparison of saturated and unsaturated phosphatidylcholines, of saturated phosphatidylcholines of different chainlengths, and of phosphatidylcholines in their fluid and gel phases. The parameters for dilinoleoyl, dioleoyl, and dicaproyl phosphatidylcholines are relatively similar and are characteristic of those for highly hydrated fluid bilayers (cf. reference 8). Egg phosphatidylcholine also exhibits rather similar values, with relatively little temperature dependence in the fluid phase (Table 1). At these levels of hydration dicaproyl phosphatidylcholine most probably is in a fluid lamellar phase, like the unsaturated phosphatidylcholines. The parameters for dimyristoyl, dipalmitoyl, and distearoyl phosphatidylcholines, deduced from the data of reference 3, are all very closely similar, because their adsorption isotherms are practi-

cally identical. Evidently at these low water contents, dimyristoyl phosphatidylcholine, in addition to dipalmitoyl and distearoyl phosphatidylcholine, is in a gel phase at 22°C. The hydration parameters for dipalmitoyl phosphatidylcholine deduced from the isotherms of reference 24 are in reasonable agreement with those found from the data of reference 3, although the values of ψ_{ho} from the former reference are slightly higher. The hydration surface potentials for the saturated phosphatidylcholines are, with one exception, smaller than those for the unsaturated phosphatidylcholines. In addition, the decay lengths are shorter for the saturated phosphatidylcholines than for the unsaturated phosphatidylcholines, paralleling the differences found with the less-hydrated phosphatidylethanolamine and phosphatidylserine in Table 1. A decrease in decay length on going from the fluid to the gel phase is obtained from the temperature dependence of the isotherms for dimyristoyl phosphatidylcholine of reference 25, when comparison is made with the data at 15°C. In this latter case, the hydration potentials increase slightly going into the gel phase. There are at least two mechanisms which may be operative in producing a difference in the hydration forces between saturated and unsaturated phospholipids and between fluid and gel phases. These arise from the differences in headgroup packing density. A closer packing will reduce the accessibility of the polar headgroups to water, but, on the other hand, will give rise to a stronger water-polarizing potential from the higher polar group density. These are opposing effects, and the data from the adsorption isotherms tend to suggest that the first of the two effects dominates for unsaturated phosphatidylcholines relative to saturated phosphatidylcholines.

Of special interest is the behavior of the lysophospholipids, because the hydration forces have not been investigated previously in these systems. Nor have hydration forces been measured previously in normal (type I), as opposed to inverted (type II), nonlamellar phospholipid phases. Because the lysolipids form small micelles in excess water, methods are required which concentrate on low water contents. The adsorption isotherms are therefore particularly appropriate here. Comparison with the water-composition phase diagrams of lysophosphatidylcholines (13) suggests that the low water region in Fig. 3 corresponds to egg lysophosphatidylcholine in a fluid lamellar phase at 40°C, and that the region of higher water content corresponds to a normal hexagonal phase, (or possibly a cubic phase). The position of the transition for egg lysophosphatidylcholine, at $n_w \approx 5$ in Fig. 3, corresponds quite well with that for the transition from the fluid lamellar to normal hexagonal phase at $n_w \approx 6$ for lysopalmitoyl phosphatidylcholine (13). The hydration potential and decay length in the low water phase of egg lysophosphatidylcholine are rather similar to those obtained for diacyl egg phosphatidylcholine (cf. Table 1), as might be expected. In the higher water phase, the hydration potential is similar, but the decay length is longer. Somewhat surprisingly, the desorption isotherm for egg lysophosphatidylcholine at 25°C gives no evidence for a phase change with increasing hydration. The lysolipid appears to remain in a fluid lamellar phase, over the range of water contents studied at this temperature. It is significant to note that lysophosphatidylserine displays a rather long decay length, similar to the higher water phase of egg lysophosphatidylcholine at 40°C (see Table 3). This most probably also corresponds to the hydration forces in a nonlamellar phase, because diacyl phosphatidylserine displays a much shorter decay length (cf. Table 1). As mentioned previously, electrostatic repulsions are expected to be relatively unimportant in this hydration regime. Added to which, hydration forces are expected, in any case, to dominate over electrostatic repulsions at small interlayer separations (19).

With respect to the hydration behavior of the lysolipids, it is interesting to note that an approximately exponential decay with a decay length of ~0.33 nm has been observed for a different nonlamellar phase, namely the inverted hexagonal phase of dioleoyl phosphatidylethanolamine (18). Reference to Eqs. 4–6 emphasizes that differences in effective decay length are to be expected because of the different geometries of the nonlamellar phases. For example, the effective decay length referred relative to the water cylinder diameter in the H_{II} phase (Eq. 5) is twice that referred to the equivalent water layer thickness in the lamellar phase (Eq. 4). An additional complication is that the hydration theory appropriate to the nonlamellar phases yields rather different expressions for the separation dependence than does that for the lamellar phase (5, 20). It is predicted that the dimensional dependence should be considerably weaker than that actually observed in the H_{II} phase (18). In this connection, the additional experimental information provided by the analysis of the hydration forces in other nonlamellar phases can be expected to be particularly valuable.

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REFERENCES

1. Elworthy, P. H. 1961. The absorption of water vapour by lecithin and lysolecithin, and the hydration of lysolecithin micelles. *J. Chem. Soc.* 5385–5389.
2. Jendrasiak, G. L., and J. H. Hasty. 1974. The hydration of phospholipids. *Biochim. Biophys. Acta.* 337:79–91.

3. Jendrasiak, G. L., and J. C. Mendible. 1976*a*. The effect of the phase transition on the hydration and electrical conductivity of phospholipids. *Biochim. Biophys. Acta.* 424:133–148.
4. Jendrasiak, G. L., and J. C. Mendible. 1976*b*. The phospholipid head-group orientation: effect on hydration and electrical conductivity. *Biochim. Biophys. Acta.* 424:149–158.
5. Cevc, G., and D. Marsh. 1987. Phospholipid Bilayers. Physical Principles and Models. John Wiley & Sons, Inc., New York. 442 pp.
6. Rand, R. P. 1981. Interacting phospholipid bilayers: measured forces and induced structural changes. *Annu. Rev. Biophys. Bioeng.* 10:277–314.
7. Parsegian, V. A., N. Fuller, and R. P. Rand. 1979. Measured work of deformation and repulsion of lecithin bilayers. *Proc. Natl. Acad. Sci. USA.* 76:2750–2754.
8. Lis, L. J., M. McAlister, N. Fuller, R. P. Rand, and V. A. Parsegian. 1982. Interactions between neutral phospholipid bilayers. *Biophys. J.* 37:657–666.
9. McIntosh, T. J., A. D. Magid, and S. A. Simon. 1987. Steric repulsion between phosphatidylcholine bilayers. *Biochemistry.* 26:7325–7332.
10. Evans, E. A., and V. A. Parsegian. 1986. Thermal-mechanical fluctuations enhance repulsion between bimolecular layers. *Proc. Natl. Acad. Sci. USA.* 83:7132–7136.
11. White, S. H., and G. I. King. 1985. Molecular packing and area compressibility of lipid bilayers. *Proc. Natl. Acad. Sci. USA.* 82:6532–6536.
12. Scherer, J. R. 1987. The partial molar volume of water in biological membranes. *Proc. Natl. Acad. Sci. USA.* 84:7938–7942.
13. Arvidson, G., I. Brentel, A. Khan, G. Lindblom, and K. Fontell. 1985. Phase equilibria in four lysophosphatidylcholine/water systems. Exceptional behaviour of 1-palmitoyl-glycerophosphocholine. *Eur. J. Biochem.* 152:753–759.
14. Cevc, G., and D. Marsh. 1985. Hydration of noncharged lipid bilayer membranes. Theory and experiments with phosphatidylethanolamines. *Biophys. J.* 47:21–31.
15. Seddon, J. M., K. Harlos, and D. Marsh. 1983. Metastability and polymorphism in the gel and fluid bilayer phases of dilauroylphosphatidylethanolamine. Two crystalline forms in excess water. *J. Biol. Chem.* 258:3850–3854.
16. Seddon, J. M., G. Cevc, R. D. Kaye, and D. Marsh. 1984. X-ray diffraction study of the polymorphism of hydrated diacyl- and dialkylphosphatidylethanolamines. *Biochemistry.* 23:2634–2644.
17. Cevc, G., J. M. Seddon, and D. Marsh. 1985. Thermodynamic and structural properties of phosphatidylserine bilayer membranes in the presence of lithium ions and protons. *Biochim. Biophys. Acta.* 814:141–150.
18. Gruner, S. M., V. A. Parsegian, and R. P. Rand. 1986. Directly measured deformation energy of phospholipid H_{II} hexagonal phases. *Faraday Discuss. Chem. Soc.* 81:29–37.
19. Cowley, A. C., N. Fuller, R. P. Rand, and V. A. Parsegian. 1978. Measurement of repulsion between charged phospholipid bilayers. *Biochemistry.* 17:3163–3168.
20. Kirk, G. L., S. M. Gruner, and D. L. Stein. 1984. A thermodynamic model of the lamellar to inverse hexagonal phase transition of lipid membrane-water systems. *Biochemistry.* 23:1093–1102.
21. Marcelja, S., and N. Radic. 1976. Repulsion of interfaces due to boundary water. *Chem. Phys. Lett.* 42:129–130.
22. Marcelja, S. 1977. Structural contribution to solute-solute interaction. *Croat. Chem. Acta.* 49:347–358.
23. White, S. H., R. E. Jacobs, and G. I. King. 1987. Partial specific volumes of lipid and water in mixtures of egg lecithin and water. *Biophys. J.* 52:663–665.
24. Elworthy, P. H. 1962. Sorption studies on phosphatides. Part II. Sorption of water vapour by a synthetic lecithin and cephalin. *J. Chem. Soc.* 4897–4900.
25. Wilkinson, D. A., H. J. Morowitz, and J. H. Prestegard. 1977. Hydration of phosphatidylcholine. Adsorption isotherm and proton nuclear magnetic resonance studies. *Biophys. J.* 20:169–179.
26. Tardieu, A., V. Luzzati, and F. C. Reman. 1973. Structure and polymorphism of the hydrocarbon chains of lipids: a study of lecithin-water phases. *J. Mol. Biol.* 75:711–733.
27. Janiak, M. J., D. M. Small, and G. G. Shipley. 1976. Nature of the thermal pretransition of synthetic phospholipids: dimyristoyl- and dipalmitoyllecithin. *Biochemistry.* 15:4575–4580.
28. Janiak, M. J., D. M. Small, and G. G. Shipley. 1979. Temperature and composition dependence of the structure of hydrated dimyristoyl lecithin. *J. Biol. Chem.* 254:6068–6078.
29. Inoko, Y., and T. Mitsui. 1978. Structural parameters of dipalmitoyl phosphatidylcholine lamellar phases and bilayer phase transitions. *J. Phys. Soc. Jpn.* 44:1918–1924.
30. Seddon, J. M., G. Cevc, R. D. Kaye, and D. Marsh. 1984. X-ray diffraction study of the polymorphism of hydrated diacyl- and dialkylphosphatidylethanolamines. *Biochemistry.* 23:2634–2644.
31. Reiss-Husson, F. 1967. Structure des phases liquides-cristallines de différents phospholipides, monoglycérides, sphingolipides, anhydres ou en présence d'eau. *J. Mol. Biol.* 25:363–382.
32. Le Neveu, D. M., R. P. Rand, V. A. Parsegian, and D. Gingell. 1977. Measurement and modification of forces between lecithin bilayers. *Biophys. J.* 18:209–230.
33. Rand, R. P., N. Fuller, V. A. Parsegian, and D. C. Rau. 1988. Variation in hydration forces between neutral phospholipid bilayers: evidence for hydration attraction. *Biochemistry.* 27:7711–7722.
34. McIntosh, T. J., and S. A. Simon. 1986. Area per molecule and distribution of water in fully hydrated dilauroylphosphatidylethanolamine bilayers. *Biochemistry.* 25:4948–4952.
35. McIntosh, T. J., and S. A. Simon. 1986. Hydration force and bilayer deformation: a reevaluation. *Biochemistry.* 25:4058–4066.