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Water Adsorption Isotherms of Lipids

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ABSTRACT Hydration of bilayer lipids is a fundamental property of biological membranes. The available database of lipid hydration isotherms is fitted over the entire range of water activities by using a statistical mechanical approach that is an extension of the common Brunauer-Emmett-Teller model, to include differential energies of association for water molecules beyond the first strongly bound layer. Three-parameter fits are obtained that can be used to represent the experimental isotherms to a good degree of accuracy over the complete range of water-binding activities. Fits are also made in terms of the hydration pressure and correlation length of water ordering, by using the polarization theory of lipid hydration. The relationship of the latter approach to measurements of hydration forces between lipid bilayers is discussed.

INTRODUCTION

The natural environment of biological membranes is water. Hydration of phospholipid bilayers controls the thermodynamic, dynamic, and structural properties of the membrane (see, e.g., Cevc and Marsh (1) and Marsh (2)). For instance, the chain-melting transition between gel and fluid lamellar states decreases strongly in temperature with increasing hydration (3), whereas the transition from the fluid lamellar to inverse hexagonal structure is favored by decreasing lipid hydration (4).

The most direct approach to lipid hydration is gravimetric determination of the water-binding isotherms as a function of the water vapor pressure, p, under which the lipid is equilibrated, relative to that of saturated water vapor (p_o) in equilibrium with free water. The water activity is given by the ratio $a_w = p/p_o$, and binding is characterized as the number of waters, n_w, associated with each lipid. Hydration isotherms have been determined for a variety of phospholipids at different temperatures (5–14). In certain cases, the isotherms have been characterized by the Brunauer-Emmett-Teller (BET) model (15), but this is found to apply only over a limited range of water activities (typically $a_w = 0.05-\sim0.5$). The purpose of this article is to fit the hydration isotherms that are currently available for the full range of water activities, so as to present the entire database in a more readily accessible form.

Here, I fit the various isotherms by using a statistical mechanical model that is a generalization of the BET approach, which allows a differential binding energy to be associated with the second and subsequent layers of bound water molecules. This introduces one further fitting parameter, relative to the standard BET model, but is then able to reproduce the binding isotherms with good accuracy, over the complete range of water activities.

A related issue is the description of water binding isotherms in terms of the water-polarization theory of lipid hydration (1,3). I do this here for the same experimental database of adsorption isotherms that was used with the statistical mechanical approach. Fits with water-polarization theory are inferior to those from the statistical mechanical approach, but nonetheless are capable of describing the essential features of the hydration isotherms, and they employ one less parameter. In principle, water-polarization theory yields values for the hydration pressure, $p_{hyd}(0)$, between apposing lipid bilayers and also the correlation length, λ_{hyd}, of the water ordering (16). This aspect is also considered here, in relation to experimental determinations of hydration forces from x-ray diffraction, which restrict themselves to an intermediate range of water activities so as to avoid contributions from steric headgroup interactions at low water contents and fluctuation and van der Waals forces at high water contents.

Thermodynamic background

Statistical mechanics of adsorption

In modeling adsorption isotherms, it is usually assumed that only the first layer binds strongly and that molecules in all subsequent adsorbed layers have the same statistical weight, $a_w q$, where $q \equiv q_2 = q_3 = \ldots. = q_{nt}$ and n_t is the total number of adsorbed layers (cf. Langmuir (17), case VI). The statistical weight of molecules in the first layer is $a_w q_1$ and the grand partition function is given by ((18), and see Appendix)

$$Z = \left(1 + q_1 a_w + q_1 q a_w^2 + \ldots + q_1 q^{n_t-1} a_w^{n_t}\right)^{n_{w1}}, \quad (1)$$

where a_w is the water activity, and n_{w1} is the number of water molecules that can be accommodated in the first layer and, by definition, in all following layers. Note that the definition of the second and subsequent "layers" is merely a mathematical convenience. It is likely that a physical layer

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covering the membrane surface will be composed of more than one of the model "layers". The significant feature is that all these subsequent layers have the same partition function q, i.e., the constituent water molecules are assumed to have the same thermodynamic properties.

The number of adsorbed water molecules is given by the following general relation for the grand partition function (cf. Appendix):

$$n_w = a_w \frac{\partial \ln Z}{\partial a_w}. \tag{2}$$

From Eqs. 1 and 2, the total number of adsorbed water molecules is therefore given by (1)

$$n_w(a_w) = n_{w1}\left(\frac{q_1 a_w}{1 - q a_w}\right) \times \left(\frac{1 - (n_t + 1)q^{n_t} a_w^{n_t} + n_t q^{n_t+1} a_w^{n_t+1}}{1 + (q_1 - q)a_w - q_1 q^{n_t} a_w^{n_t+1}}\right). \tag{3}$$

In the limit of a large number of adsorbed layers ($n_t \to \infty$, with $a_w < 1$), the adsorption isotherm then becomes

$$n_w(a_w) = n_{w1}\frac{q_1 a_w}{(1 - q a_w)(1 + (q_1 - q)a_w)}. \tag{4}$$

The adsorption isotherm that is given by Eq. 4 corresponds to Langmuir's original case VI (17). The well-known BET isotherm (15) corresponds to the further assumption that $q = 1$, i.e., adsorbed water layers beyond the first are indistinguishable from bulk water. The BET isotherm is normally restricted to fitting isotherms over a limited range of water activities ($a_w = 0.05-\sim 0.5$; see Elworthy (5,6) and Jendrasiak et al. (10)). It is less successful in fitting the adsorption isotherms of lipids over the full range of water activities than is the version in which q is treated as an additional parameter (see Fig. 1). In most cases, it is found below that the isotherm with a specific number of layers, n_t, does not perform better than that with an unrestricted number of layers. This may well be because, as noted, n_t is a model variable that is greater than the number of physical water layers.

Hydration polarization theory

In the polarization theory of lipid hydration, the free energy of hydration per lipid is given by (1,3)

$$\Delta G_{hyd}(n_w) = \Delta G_{hyd}(\infty)\tanh\left(\frac{n_w v_w}{A_l \lambda_{hyd}}\right), \tag{5}$$

where λ_{hyd} is the correlation length of the water polarization, A_l is the area per lipid molecule, and v_w is the volume of a water molecule. The contribution of lipid hydration to the chemical potential of water, $\Delta\mu_w = \partial \Delta G_{hyd}/\partial n_w$, is then given by

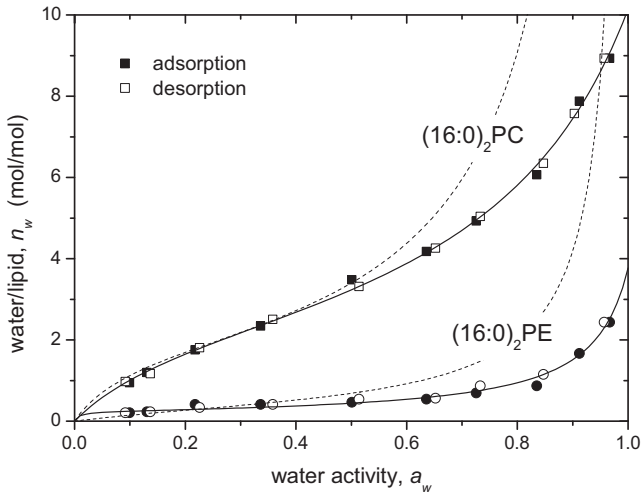

FIGURE 1 Water adsorption (*solid symbols*) and desorption (*open symbols*) isotherms for dipalmitoyl phosphatidylcholine, $(16:0)_2$PC (*squares*), and dipalmitoyl phosphatidylethanolamine, $(16:0)_2$PE (*circles*), at 25°C (6). (*Solid lines*) Nonlinear least-squares fits of Eq. 4 to the isotherms and (*dashed lines*) fits of the BET isotherm (Eq. 4 with $q = 1$) given in the original publication (6) for water activities: $a_w = 0.1-0.4$ for $(16:0)_2$PC and $a_w = 0.1-0.3$ for $(16:0)_2$PE.

$$\Delta\mu_w(n_w) = \Delta\mu_w(0)\,\mathrm{sech}^2\left(\frac{n_w v_w}{A_l \lambda_{hyd}}\right). \tag{6}$$

Defined in terms of water activity, the hydration contribution to the chemical potential also can be written as

$$\Delta\mu_w(n_w) \equiv \mu_w - \mu_w^o = k_B T \ln a_w, \tag{7}$$

where $\mu_w{}^o$ is the chemical potential in bulk water and k_B is Boltzmann's constant. Equating the right-hand sides of Eqs. 6 and 7, the water adsorption isotherm that is predicted from hydration theory is given by (1)

$$n_w = \frac{A_l \lambda_{hyd}}{v_w}\cosh^{-1}\left(\frac{\Delta\mu_w(0)}{k_B T \ln(a_w)}\right)^{1/2}, \tag{8}$$

where $\Delta\mu_w(0)$, the initial contribution of hydration to the chemical potential, is negative. Note that the latter is closely related to the hydration pressure between bilayers, $p_{hyd} = -\partial \Delta G_{hyd}/\partial(n_w v_w)$, by

$$\Delta\mu_w(0) = -p_{hyd}(0) v_w, \tag{9}$$

where $p_{hyd}(0)$ is the limiting hydration pressure between closely apposed bilayers.

Maximum water uptake

Unless especial precautions are taken to avoid temperature gradients (9), the maximum level of lipid hydration that is achieved on exposure to an atmosphere of saturated water vapor is less than that achieved by dispersing zwitterionic lipids directly in excess water. For instance, the gel-to-fluid

chain-melting temperature is not reduced to the minimum value that is achieved in excess water, and multibilayer stacks do not swell to the extent attained in direct contact with excess water. This phenomenon has been referred to as the vapor pressure paradox (19), which was finally resolved by diffraction measurements in a humidity chamber that was specially designed to eliminate sublimation of water from the hydrated lipid sample and condensation on cooler surfaces in the cell (20–22). It is the latter that causes lipids in an atmosphere of 100% relative humidity to be less than fully hydrated.

A consequence of the vapor pressure "paradox" is that most published adsorption isotherms for lipids do not reach maximum hydration at the maximum water activity, $a_w = 1$. Table 1 compares the number of waters per lipid, n_w^{sat}, adsorbed in the presence of saturated water vapor, given by the various hydration isotherms, with the maximum level of hydration, n_w^{max} per lipid, that is determined from x-ray diffraction. In a swelling experiment, the x-ray repeat distance, d_{100}, of a multibilayer stack increases linearly with number of waters per lipid, n_w, according to

$$d_{100} = d_l\left(1 + n_w\frac{v_w}{v_l}\right), \tag{10}$$

where d_l is the anhydrous thickness of the bilayer and v_l is the volume of a lipid molecule. The maximum hydration corresponds to that value of n_w at which d_{100} no longer increases further with added water. As can be seen in Table 1, for phosphatidylcholine (PC) and phosphatidylethanolamine (PE), the values of maximum hydration are always greater than those of n_w^{sat}, especially in the more strongly hydrated fluid membrane phase. Only in the case of palmitoyl-oleoyl phosphatidylcholine ((16:0/18:1cΔ^9)PC)—for which particular attention was paid to the effects of temperature gradients in determining the adsorption isotherm (9)—does the value of n_w^{sat} approach anywhere near to that of n_w^{max} for a phosphatidylcholine in the fluid L_α phase.

TABLE 1 Maximum level of hydration (waters per lipid) deduced from water adsorption isotherms in saturated water vapor, n_w^{sat}, and from x-ray diffraction of multibilayers in excess water, n_w^{max}

Lipid	T (°C)	n_w^{sat} (mol/mol)	Ref.	T (°C)	n_w^{max} (mol/mol)	Ref.
(10:0)$_2$PC	22	15.1	(8)	35	30	(29)
(14:0)$_2$PC	22	9.1	(8)	20	15.4	(30)
(16:0)$_2$PC	22	9.0	(7)	19	11.8	(31)
				20	13.6	(32,33)
				20	17.5	(34)
	25	10.52	(6)	24	12.6	(35)
				25	12.0	(36)
				25	14.3	(19,37)
	45	11.15	(6)	45	22.9	(38)
				45	27.2	(33)
(18:0)$_2$PC	22	9.0	(8)	19	11.7	(31)
				20	17.9	(32)
				25	17.9	(19,37)
				25	12.0	(36)
(18:1cΔ^9)$_2$PC	22	17.2	(7)	20	33.2 ± 0.8	(39)
					29.1	(40)
				25	33.2 ± 0.8	(39)
(16:0/18:1cΔ^9)PC	25	21.6	(9)	25	27.4	(9)
(16:0)$_2$PE	25	3.8	(6)	20	6.7	(41)
	40	3.8	(6)	25	4.9	(42)

Note that the effects of temperature gradients are particularly felt close to the saturated vapor pressure, where a small temperature difference results in a large change in water activity (23). They are unlikely to have a large effect on the initial parts of the adsorption isotherms that correspond to strong association of water with the lipid headgroups.

Fitting hydration isotherms

Fig. 1 gives the experimental water adsorption isotherms for a strongly hydrating lipid, dipalmitoyl phosphatidylcholine ((16:0)$_2$PC), and a weakly hydrating lipid dipalmitoyl phosphatidylethanolamine ((16:0)$_2$PE), at 23°C (6). The solid lines are nonlinear least-squares fits of Eq. 4, which is capable of describing both isotherms with a high degree of precision. In contrast, the dotted lines represent fits of the BET model, which is capable of describing the isotherm well over a limited range of activity (a_w = 0.1 to 0.3–0.4), but not outside this range.

Water adsorption isotherms for phosphatidylcholines

Fig. 2 shows the water adsorption isotherms for saturated phosphatidylcholines of different chain lengths. The solid lines represent nonlinear least-squares fits of Eq. 4 to the experimental isotherms. Except at very low water activities ($a_w < 0.19$), these are capable of describing the experimental data with reasonably high accuracy. The fitting parameters are given in Table 2. Note that in a few instances, when insufficient data points are present at low water activities, it is necessary to constrain the fits by limiting the total number of water layers, i.e., by using the isotherm of Eq. 3 instead of Eq. 4. Where this is done, it is indicated in the footnotes to the tables.

Values of q_1 are much larger than those of q (Table 2) because they correspond to direct association at the principal hydration sites on the headgroup of phosphatidylcholine. The values of q, which correspond to subsequent surface adsorption, are close to unity—indicating that these layers are much more liquidlike. Most of the values of q are somewhat less than unity. This reflects the fact that q is simply an effective parameter, which represents all layers subsequent to the first and also incorporates (in a single-particle fashion) the interactions between sites (see Appendix). (A similar result for a quite different system was found by Guggenheim (18), but parameterized by downscaling the activity.) The values of q_1 in Table 2 differ,

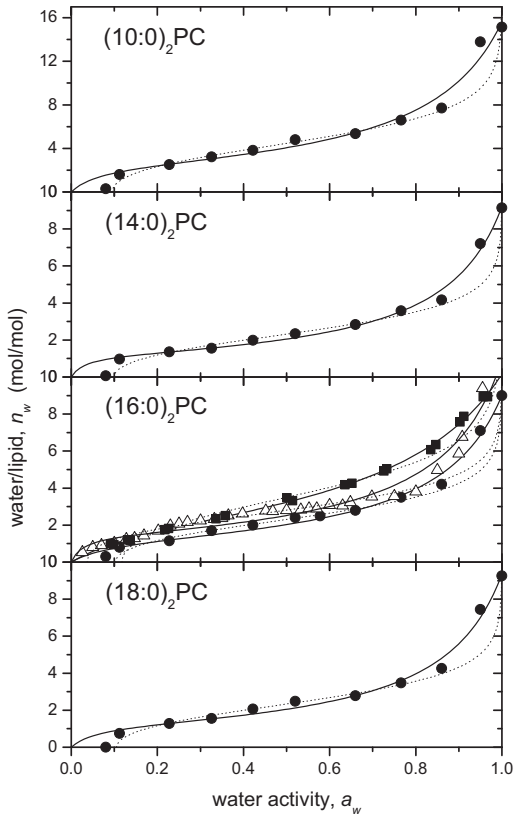

FIGURE 2 Water adsorption isotherms of saturated phosphatidylcholines. (*Top* to *bottom*) Dicapryl phosphatidylcholine, $(10:0)_2$PC, at 22°C (8); dimyristoyl phosphatidylcholine, $(14:0)_2$PC, at 22°C (8); dipalmitoyl phosphatidylcholine, $(16:0)_2$PC, at 22°C (*circles* (8)) and at 25°C (*squares* (6), *triangles* (14)); and distearoyl phosphatidylcholine, $(18:0)_2$PC, at 22°C (8). (*Solid lines*) Nonlinear least-squares fits of Eq. 4 (*dotted lines*) and of Eq. 8 from hydration theory.

even though they correspond to the same lipid polar group, because the experimental isotherms differ. This must be attributed to different preparative and equilibration procedures (see discussion in the previous section). The values of n_{w1} in Table 2 are in the range 1−3 corresponding to the limited number of strong hydration sites on the phosphocholine headgroup. Mostly, n_{w1} is larger in the fluid lipid state than in the gel state, which correlates with an increased headgroup accessibility that accompanies the lateral expansion on chain melting.

The dotted lines in Fig. 2 alternatively represent fits of Eq. 8 from polarization theory to the various experimental isotherms. Except at high water activities, polarization theory provides an adequate representation of the isotherms, with one less fitting parameter than the statistical thermodynamic treatment provided by Eq. 4. Fitting parameters for saturated phosphatidylcholines that are derived from hydration theory are given later in Table 4. In this table, the chemical potential parameter, $\Delta\mu_w(0)$ is expressed in terms of the hydration pressure parameter, $p_{hyd}(0)$ by using Eq. 9.

Water adsorption isotherms for phosphatidylethanolamines, sphingomyelins, and charged lipids

Fitting of Eq. 4 was also performed with the available adsorption or desorption isotherms for sphingomyelins (SM) and phosphatidylethanolamines (PE), as well as the charged lipids phosphatidylglycerol (PG), phosphatidylserine (PS), phosphatidic acid (PA), and diphosphatidylglycerol (i.e., cardiolipin, CL). The fitting parameters of Eq. 4 are listed for these lipids in Table 3. Isotherms for weakly hydrating lipids that do not adsorb water until a relatively high threshold water activity is achieved (corresponding to a phase change from a nonhydrated to a hydrated state) (14) are excluded from this analysis. The corresponding parameters from hydration theory that are required to fit Eq. 8 to this set of isotherms are included with the phosphatidylcholine values in Table 4.

Although the differences in q_1 between different lipid headgroups in Table 3 might be influenced by differences in experimental protocol, they reflect real differences in hydration potential ((1,3); and see also Table 4) between different polar groups, including also phosphocholine (Table 2). The values of n_{w1} in Table 3 also differ among the different lipid species, and from phosphatidylcholine in Table 2. The very low values for phosphatidylethanolamine, for instance, reflect the fact that the crystalline (L_c) state does not hydrate readily until heated above the chain-melting transition. For cardiolipin, n_{w1} is twice the value for egg phosphatidic acid because the tetraacyl lipid has two phosphate groups in the polar headgroup.

Bilayer-bilayer interactions

The parameters of hydration theory that are obtained by fitting the water adsorption isotherms with Eqs. 8 and 9 specify the hydration-force component of the interaction between bilayers. (Note that the controversy between hydration forces and molecular protrusion has been decided experimentally in favor of hydration forces—see, e.g., McIntosh and Simon (24).) In addition to the hydration forces, which are mediated by polarization of water at the lipid surface, there are also short-ranged steric forces between lipid headgroups at low hydrations (25). On the other hand, at large interbilayer separations (i.e., high hydration levels), the van der Waals forces between lipid layers come to dominate and, at intermediate hydrations, fluctuation forces arising from thermally excited elastic undulations of the lipid layers (26) make an important contribution to the repulsion between bilayers (27). Experimentally, in x-ray swelling experiments, hydration pressures are determined from the region of water activities beyond those at which steric forces dominate and before those at which fluctuation forces (and van der Waals attraction) contribute appreciably (25,27). Therefore, the

TABLE 2 Parameters for fitting Eq. 4 to the water adsorption isotherms of phosphatidylcholines

Lipid	T (°C)	n_{w1} (mol/mol)	q_1	q	Ref.
$(10:0)_2$PC	22	2.54 ± 0.30	15.1 ± 16.6	0.839 ± 0.020	(8)
$(14:0)_2$PC	15	2.61 ± 0.08	15.5 ± 7.0	0.743 ± 0.045	(43)
	20	2.50 ± 0.08	10.5 ± 2.0	0.50 ± 0.009	(44)
	22	1.20 ± 0.08	32.2 ± 37.5	0.870 ± 0.001	(8)
		2.05 ± 0.08	21.9 ± 29.4	0.900 ± 0.108*	(43)
	25	1.84 ± 0.16	15.6 ± 9.6	0.876 ± 0.025	(43)
	35	1.25 ± 0.17	25.3 ± 39.2	1.031 ± 0.022	(43)
$(16:0)_2$PC	20	2.50 ± 0.08	10.5 ± 2.0	0.50 ± 0.009	(44)
	22	1.28 ± 0.10	10.5 ± 6.1	0.860 ± 0.012	(7)
	25	2.40 ± 0.07	5.81 ± 0.63	0.772 ± 0.008	(6)
		1.47 ± 0.08	36.7 ± 23.8	0.865 ± 0.014	(14)
		1.94 ± 0.19	11.3 ± 5.4	0.916 ± 0.031†	(44)
	40	2.32 ± 0.12	7.77 ± 1.81	0.805 ± 0.013	(6)
$(18:0)_2$PC	20	2.50 ± 0.08	10.5 ± 2.0	0.50 ± 0.009	(44)
	22	1.24 ± 0.10	17.2 ± 16.2	0.869 ± 0.012	(8)
$(18:1c\Delta^9)_2$PC	22	3.03 ± 0.33	5.9 ± 3.3	0.830 ± 0.019	(7)
	25	3.06 ± 0.03	5.09 ± 0.19	0.765 ± 0.003	(12)
$(14:2c\Delta^{11,13})_2$PC		2.52 ± 0.08	4.90 ± 0.60	0.846 ± 0.006	(45)
$(18:2c\Delta^{9,12})_2$PC	22	3.63 ± 0.43	6.7 ± 4.3	0.838 ± 0.020	(7)
$(18:2t\Delta^{2,4})_2$PC‡	25	1.94 ± 0.04	6.28 ± 0.50	0.855 ± 0.005	(46)
$(16:0/18:1c\Delta^9)$PC	25	2.91 ± 0.14	2.91 ± 0.36	0.789 ± 0.011	(11)
		2.86 ± 0.06	8.55 ± 0.71	0.797 ± 0.005	(47)
		1.53 ± 0.18	14.3 ± 29.3	0.921 ± 0.010	(9)
		3.48 ± 0.29	3.26 ± 0.84	0.829 ± 0.017	(14)
$(18:0/22:6c\Delta^{4,7,10,13,16,19})$PC	25	3.12 ± 0.07	5.83 ± 0.44	0.802 ± 0.006	(47)
Egg PC§	22	2.87 ± 0.58	2.9 ± 2.0	0.807 ± 0.035	(7)
	25	2.09 ± 0.11	20.6 ± 13.1	0.922 ± 0.009	(5)
	40	2.54 ± 0.03	7.56 ± 0.45	0.876 ± 0.002	(5)
Egg lysoPC¶	25	2.40 ± 0.13	7.5 ± 1.8	0.819 ± 0.013	(5)
	40	2.62 ± 0.22	13.0 ± 7.7	0.882 ± 0.015	(5)
$(18:1c\Delta^9)_2$EtPC‖	22	0.92 ± 0.05	16.4 ± 9.3	0.843 ± 0.009	(10)

*Number of layers, $n_t = 11.1 \pm 1.4$ (see Eq. 3).
†Number of layers, $n_t = 19.7 \pm 2.3$ (see Eq. 3).
‡Desorption isotherm.
§Phosphatidylcholine from hen egg yolk.
¶1-acyl-2-lyso phosphatidylcholine from egg PC.
‖EtPC = 1,2-diacyl-*sn*-glycero-3-*O*-ethylphosphocholine.

hydration-force parameters that are obtained by fitting the entire hydration isotherm with Eqs. 8 and 9 are only effective values because they represent a global fit, including water activities at which the hydration force is not the major contribution to the interbilayer forces.

Empirically, the hydration-force contribution to the adsorption isotherm can be determined by using the same approach as in the x-ray swelling experiments. This is done by isolating the exponential part of the decrease in hydration pressure with increasing water content per lipid (19). From the definition of interbilayer pressure ($p_{int} = -\partial\Delta G_{int}/\partial V$), together with Eq. 7, the dependence of the hydration pressure on water activity is given by

$$p_{hyd} = -\frac{k_B T}{v_w} \ln a_w. \tag{11}$$

Fig. 3 shows the dependence of hydration pressure on water/lipid ratio that is obtained from the hydration isotherms for dioleoyl and palmitoyl-oleoyl phosphatidylcholine by using Eq. 11. The region of bilayer pressures over which the hydration forces dominate is identified from the linear section in the semilogarithmic plots. According to Eqs. 7 and 9, the dependence of the hydration pressure on water content is approximately exponential,

$$p_{hyd}(n_w) \cong p_{hyd}(0) \exp\left(\frac{-2n_w v_w}{A_l \lambda_{hyd}}\right), \tag{12}$$

for large bilayer separations, $n_w > A_l\lambda_{hyd}/(2v_w)$. Values of λ_{hyd} and $p_{hyd}(0)$ that are deduced in this way from the exponential parts of the water dependence are given in parentheses in Table 4. (Note that hydration forces deduced from x-ray measurements are invariably interpreted in terms of an exponential dependence on bilayer separation, d_w, as predicted by Eq. 12, where $d_w = 2n_w v_w/A_l$.) Generally, the values of $p_{hyd}(0)$ obtained for the hydration force from the exponential dependence are larger than the effective values that are obtained by fitting the entire isotherm with Eq. 8. Correspondingly, the values of the polarization correlation length for the exponential dependence are shorter than the

TABLE 3 Parameters for fitting Eq. 4 to the water adsorption isotherms of phosphatidylethanolamines, sphingomyelins, and charged phospholipids

Lipid	T (°C)	n_{w1} (mol/mol)	q_1	q	Ref.
$(16:0)_2$PE	25	0.24 ± 0.01	121 ± 378	0.937 ± 0.007	(6)
		2.27 ± 85.8	$6.9.10^{-6}$ ± $8.8.10^{-4}$	2.586 ± 33.4*	(14)
	40	0.23 ± 0.01	99 ± 325	0.958 ± 0.006	(6)
Egg PE†	22	0.19 ± 0.05	6.8 ± 25.7	1.090 ± 0.012‡	(7)
$(14:2c\Delta^{11,13})_2$PE		1.10 ± 0.08	1.15 ± 0.18	0.927 ± 0.007	(45)
$(18:1c\Delta^{9})_2$PE§	25	1.47 ± 0.29	0.069 ± 0.20	0.832 ± 0.023	(46)
Brain SM¶	22	1.19 ± 0.26	16.1 ± 58.9	0.880 ± 0.026	(13)
Milk SM‖	22	0.46 ± 0.08	96.0 ± 484	1.048 ± 0.024**	(13)
Egg SM††	22	0.52 ± 0.08	2.81 ± 2.0	0.894 ± 0.015	(13)
$(16:0)_2$PG	25	1.10 ± 0.05	3.30 ± 0.65	0.926 ± 0.006	(14)
Brain PS‡‡	22	1.29 ± 0.79	0.34 ± 0.30	0.860 ± 0.039	(7)
Brain lysoPS§§	22	4.98 ± 10.4	0.25 ± 0.62	0.915 ± 0.139	(7)
$(16:0)_2$PA	25	1.24 ± 0.10	5.29 ± 1.57	0.808 ± 0.021	(14)
$(16:0/18:1c\Delta^{9})$PA	25	1.38 ± 0.09	2.46 ± 0.70	0.972 ± 0.005	(14)
Egg PA¶¶	22	3.14 ± 0.17	5.6 ± 1.3	0.793 ± 0.011	(10)
Mitochondrial CL‖ ‖	22	6.27 ± 1.33	1.57 ± 0.87	0.841 ± 0.026	(10)

*Number of layers, $n_t = 9.91 \pm 18.1$ (see Eq. 3).
†Phosphatidylethanolamine from hen egg yolk.
‡Number of layers, $n_t = 59.6 \pm 10.5$ (see Eq. 3).
§Desorption isotherm. A transition from the inverted hexagonal (H_{II}) phase to a ribbon (P_α) phase takes place at $a_w \approx 0.5-0.4$ (46).
¶Sphingomyelin from porcine brain.
‖Sphingomyelin from bovine milk.
**Number of layers, $n_t = 35.1 \pm 3.0$ (see Eq. 3).
††Sphingomyelin from hen egg yolk.
‡‡Phosphatidylserine from bovine brain.
§§1-Acyl-2-lyso phosphatidylserine from bovine brain PS.
¶¶Phosphatidic acid produced from hen egg yolk phosphatidylcholine.
‖ ‖Cardiolipin from beef heart mitochondria.

effective values obtained from fitting the entire isotherm. The exception to this is the charged lipids, for which the contribution of electrostatic forces overlaps with that from hydration forces (and is of longer range).

CONCLUSIONS

The primary aim of this contribution is to present a readily accessible numerical representation of the water adsorption isotherms of phospholipids. This is done with Eq. 4 and the numerical parameters presented in Table 2. These data can be used, for instance, to investigate the strength of water binding by lipids (using the values of q_1), or the hydration forces between lipid bilayers by means of Eq. 11 (as was done for the data in parentheses in Table 4—albeit using the original experimental data).

For fully hydrated lipids that form fluid phases at the temperature of the isotherm, a lyotropic gel-to-fluid transition may occur at some water/lipid ratio within the range of the adsorption isotherm. Only in high-resolution measurements are small discontinuities that correspond to such lyotropic transitions discerned in the adsorption isotherms of phosphatidylcholines (11,12). The data presented here average over these small discontinuities for phosphatidylcholines. In certain other cases, for phosphatidylethanolamines or phosphatidylserines, water adsorption does not occur until rather high water activities are achieved (14). Not all of these isotherms can be analyzed satisfactorily with this method and, where desorption isotherms are not available, they are omitted from the study.

Lipid water-adsorption isotherms are intimately connected with the hydration forces between phospholipid bilayers. The parameters that are given in parentheses in Table 4 contain data from isotherms additional to those used in an earlier study (16). This, therefore, makes a further contribution to the study of bilayer-bilayer interactions.

APPENDIX: GRAND PARTITION FUNCTION AND ADSORPTION MODEL

The model used is one of adsorption of water molecules in layers at fixed, independent sites on the lipid surface. As regards the first layer, crystal structures of hydrated (or solvated) lipids indicate that there are fixed binding sites for water (or solvent) molecules at the lipid headgroups (see, e.g., Cevc and Marsh (1)). The assumption of independent sites does not imply that there is no interaction between sites, but simply that the interactions are represented by an effective value averaged over neighboring sites (see Guggenheim (18) for further discussion). Explicit allowance for interaction between sites would require introducing additional parameters into the model.

Adsorption to a single site that can accommodate just one water molecule in each layer is treated first (see main text for discussion of the significance of layers). After this, the extension to a finite number (n_{w1}) of sites is considered. Finally, various aspects of the particular hydration model that is used in fitting the isotherms are discussed.

TABLE 4 Parameters of polarization hydration theory obtained from fitting Eq. 8 to the water adsorption isotherms of different lipids

Lipid	T (°C)	$p_{hyd}(0)$ (MPa)	λ_{hyd} (nm)	Ref.
$(10:0)_2$PC	22	320 ± 19	0.183 ± 0.006	(8)
		(611 ± 40)	(0.231 ± 0.007)	
$(14:0)_2$PC	15	340 ± 45	0.188 ± 0.011	(43)
		(693 ± 87)	(0.234 ± 0.013)	
	20	262 ± 28	0.235 ± 0.016	(44)
		(559 ± 47)	(0.353 ± 0.017)	
		280 ± 29	0.199 ± 0.014	(43)
		(406 ± 38)	(0.250 ± 0.009)	
	22	314 ± 23	0.115 ± 0.005	(8)
		(589 ± 37)	(0.149 ± 0.005)	
	25	270 ± 44	0.154 ± 0.015	(43)
		(607 ± 66)	(0.173 ± 0.010)	
	35	287 ± 85	0.135 ± 0.029	(43)
		(556 ± 56)	(0.162 ± 0.012)	
$(16:0)_2$PC	20	262 ± 28	0.235 ± 0.016	(44)
		(406 ± 38)	(0.353 ± 0.017)	
	22	310 ± 19	0.115 ± 0.004	(7)
		(538 ± 27)	(0.154 ± 0.004)	
	25	294 ± 14	0.180 ± 0.005	(6)
		(448 ± 16)	(0.258 ± 0.004)	
		452 ± 30	0.119 ± 0.003	(14)
		(1800 ± 190)	(0.114 ± 0.004)	
		335 ± 26	0.174 ± 0.008	(44)
		(546 ± 17)	(0.240 ± 0.005)	
	40	305 ± 13	0.195 ± 0.005	(6)
		(492 ± 19)	(0.272 ± 0.005)	
$(18:0)_2$PC	20	262 ± 28	0.235 ± 0.016	(44)
		(406 ± 38)	(0.353 ± 0.017)	
	22	306 ± 20	0.117 ± 0.004	(8)
		(536 ± 33)	(0.156 ± 0.005)	
$(18:1c\Delta^9)_2$PC	22	266 ± 18	0.224 ± 0.010	(7)
		(422 ± 19)	(0.316 ± 0.009)	
	25	290 ± 11	0.183 ± 0.004	(12)
		(423 ± 4)	(0.272 ± 0.001)	
$(14:2c\Delta^{11,13})_2$PC		257 ± 18	0.183 ± 0.009	(45)
		(308 ± 7)	(0.307 ± 0.004)	
$(18:2c\Delta^{9,12})_2$PC	22	283 ± 18	0.260 ± 0.010	(7)
		(483 ± 31)	(0.347 ± 0.012)	
$(18:2t\Delta^{2,4})_2$PC*	25	267 ± 15	0.141 ± 0.005	(46)
		(249 ± 4)	(0.279 ± 0.002)	
(16:0/18:1cΔ^9)PC	25	251 ± 17	0.175 ± 0.008	(11)
		(306 ± 11)	(0.296 ± 0.006)	
		336 ± 21	0.179 ± 0.007	(47)
		(457 ± 9)	(0.274 ± 0.003)	
		208 ± 19	0.190 ± 0.013	(9)
		(247 ± 30)	(0.321 ± 0.012)	
		231 ± 11	0.246 ± 0.008	(14)
		(308 ± 10)	(0.392 ± 0.007)	
(18:0/22:6 c$\Delta^{4,7,10,13,16,19}$)PC	25	317 ± 23	0.190 ± 0.009	(47)
		(352 ± 7)	(0.332 ± 0.003)	
Egg PC	22	247 ± 0.21	0.186 ± 0.010	(7)
		(362 ± 22)	(0.275 ± 0.008)	
	25	295 ± 30	0.200 ± 0.013	(5)
		(459 ± 21)	(0.283 ± 0.007)	
	40	333 ± 26	0.205 ± 0.010	(5)
		(548 ± 17)	(0.282 ± 0.005)	
Egg lysoPC	25	303 ± 19	0.165 ± 0.007	(5)
		(446 ± 17)	(0.247 ± 0.004)	
	40	311 ± 28	0.183 ± 0.011	(5)
		(518 ± 20)	(0.249 ± 0.007)	
Milk SM	22	295 ± 39	0.071 ± 0.006	(13)
		(57 ± 8)	(0.49 ± 0.05)	
Egg SM	22	251 ± 12	0.127 ± 0.004	(13)
		(52 ± 18)	(0.21 ± 0.04)	
Brain SM	22	250 ± 26	0.046 ± 0.004	(13)
		(556 ± 44)	(0.152 ± 0.006)	
$(16:0)_2$PE	25	373 ± 53	0.030 ± 0.002	(6)
		(61 ± 8)	(0.159 ± 0.013)	
	40	307 ± 39	0.034 ± 0.003	(6)
		(58 ± 7)	(0.186 ± 0.015)	
$(14:2c\Delta^{11,13})_2$PE		207 ± 20	0.073 ± 0.004	(45)
		(83 ± 5)	(0.330 ± 0.013)	
$(18:1c\Delta^9)_2$PE†	25	187 ± 12	0.073 ± 0.004	(46)
		(136 ± 4)	(0.184 ± 0.003)	
$(16:0)_2$PG	25	237 ± 14	0.110 ± 0.005	(14)
		(290 ± 5)	(0.186 ± 0.002)	
$(16:0)_2$PA	25	278 ± 13	0.127 ± 0.004	(14)
		(577 ± 30)	(0.127 ± 0.003)	
(16:0/18:1cΔ^9)PA	25	252 ± 19	0.100 ± 0.006	(14)
		(98 ± 4)	(0.52 ± 0.02)	
Egg PA	22	298 ± 22	0.199 ± 0.009	(10)
		(485 ± 20)	(0.279 ± 0.006)	
Mitochondrial CL	22	217 ± 22	0.44 ± 0.04	(10)
		(271 ± 34)	(0.72 ± 0.04)	
$(18:1c\Delta^9)_2$EtPC	22	292 ± 31	0.080 ± 0.005	(10)
		(220 ± 18)	(0.167 ± 0.006)	

Note that 1 MPa $\equiv 10^6$ N.m$^{-2} = 10^7$ dyn.cm^{-2}. Values of the correlation length are calculated by assuming a constant area per lipid of $A_l = 0.6$ nm^2 in the fluid phase, and 0.5 nm^2 in the gel phase (0.4 nm^2 for phosphatidylethanolamine).

*Desorption isotherm.

†Desorption isotherm. A transition from the inverted hexagonal (H_{II}) phase to a ribbon (P_α) phase takes place at $a_w \approx 0.5-0.4$ (46).

For an open system, the statistical weight of a water molecule in the ith layer is $a_w q_i$, where q_i is the ordinary partition function for a water molecule at this position (see, e.g., Guggenheim (18)). For the ith layer to be occupied, all lower layers must be occupied at this site. The statistical weight of this state is therefore given by

$$w_i = a_w^i \prod_{j=1}^{i} q_j, \tag{A1}$$

where the product is over all layers from the first to the ith. The grand partition coefficient, z_s, corresponding to a single adsorption site is given by the summation of Eq. A1 over all layers at this site,

$$z_s = \sum_{i=0}^{n_t} w_i = 1 + \sum_{i=1}^{n_t} a_w^i \prod_{j=1}^{i} q_j, \tag{A2}$$

where n_t is the total number of layers, and $w_0 = 1$ is the statistical weight of the state with no water adsorbed.

Equation A2 applies to a single-site subsystem. In fact, there are n_{w1} sites per lipid in each layer. The number of ways of distributing n_i water molecules (in the ith layer) between n_{w1} sites, namely, $n_{w1}!/[n_i!(n_{w1}-n_i)!]$, is given by the successive terms in a binomial (or multinomial) expansion to the power n_{w1}. Thus, the grand partition function for the full system is given by

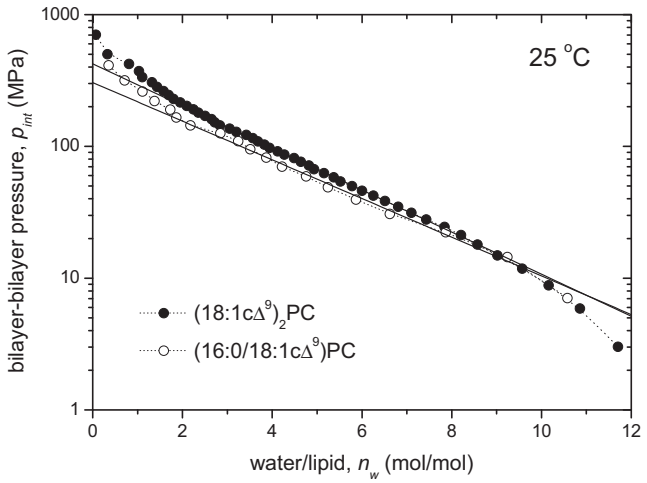

FIGURE 3 Dependence of the interbilayer repulsive pressure, p_{int}, on degree of hydration, n_w, deduced from the water adsorption isotherms for dioleoyl phosphatidylcholine, (18:1cΔ^9)$_2$PC (*solid circles*), and 1-palmitoyl-2-oleoyl phosphatidylcholine, (16:0/18:1cΔ^9)PC (*open circles*), at 25°C, according to Eq. 11. Data from Binder et al. (11,12). (*Straight solid lines*) Single exponential fits over the range of water contents: $2 \leq n_w \leq 9$.

$$Z = z_s^{n_{w1}} = \left(1 + \sum_{i=1}^{n_t} a_w^i \prod_{j=1}^{i} q_j\right)^{n_{w1}}. \qquad \text{(A3)}$$

With $q_i \equiv q$ for $i > 1$, Eq. A3 is identical to Eq. 1 of the main text.

From the expressions for the statistical weights, the mean number of water molecules adsorbed is given by

$$n_w = \frac{n_{w1}}{z_s} \sum_{i=1}^{n_t} i a_w^i \prod_{j=1}^{i} q_j, \qquad \text{(A4)}$$

which is a particular case of Eq. 2 in the main text.

Note that the division of the water adsorption into a first layer and subsequent "layers" ($q_i = q$ for $i > 1$) corresponds to primary binding to the lipid headgroups as the first layer, plus weaker adsorption in subsequent layers. (See main text for the definition of "layers" in the model.) This basic division into two main categories finds wide application to a variety of studies on lipid hydration (see, e.g., Binder (28) and references therein for a review). Binding in the first layer is described here by a Langmuir-type monolayer adsorption isotherm (case I in Langmuir (17)) that is characterized by q_1 and dominates the experimental isotherm at low water activities. Departures from this simple binding isotherm at higher water activities correspond to adsorption in the subsequent "layers" that are characterized by an effective partition coefficient (q) for all these layers. Whereas the driving force for formation of the first layer is binding to the polar headgroup, it is likely that adsorption of the subsequent layers may be driven by polarization of water by the surface (1,3,24). This disrupts the hydrogen-bonded structure of bulk water, consistent with the finding that sorption of the subsequent layers to phosphatidylcholines is endothermic, i.e., is driven by entropy (11,12). Ultimately, the total amount of water adsorbed by a multilayer system is determined by the water-layer thickness at which attractive and repulsive interbilayer forces exactly balance.

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