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Magnitude of the solvation pressure depends on dipole potential

(surfaces/bilayers/monolayers/structural forces/hydration pressure)

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ABSTRACT **As polar surfaces in solvent are brought together, they experience a large repulsive interaction, termed the solvation pressure. The solvation pressure between rough surfaces, such as lipid bilayers, has been shown previously to decay exponentially with distance between surfaces. In this paper, we compare measured values of the solvation pressure between bilayers and the dipole potential for monolayers in equilibrium with bilayers. For a variety of polar solvents and lipid phases, we find a correlation between the measured solvation pressures and dipole potentials. Analysis of the data indicates that the magnitude of the solvation pressure is proportional to the square of the dipole potential. Our experiments also show that the oriented dipoles in the lipid headgroup region, including those of both the lipid and solvent molecules, contribute to the dipole potential. We argue that (*i*) the field produced by these interfacial dipoles polarizes the interbilayer solvent molecules giving rise to the solvation pressure and (*ii*) both the solvation pressure and the dipole potential decay exponentially with distance from the bilayer surface, with a decay constant that depends on the packing density of the interbilayer solvent molecules (1–2 Å in water). These results may have importance in cell adhesion, adsorption of proteins to membranes, characteristics of channel permeability, and the interpretation of electrokinetic experiments.**

The close approach of adjacent biological or lipid bilayer membranes is resisted by several repulsive pressures, one being the solvation or hydration pressure (if the solvent is water). The solvation pressure is thought to be the dominant interbilayer repulsive pressure for bilayer separations of about 5–20 Å (1–6). For rough surfaces, such as lipid bilayers, it has been found that the solvation pressure, P_h, decays exponentially with increasing separations, such that $P_h = P_0\exp(-d_f/\lambda)$, where d_f is the distance between adjacent bilayers (1, 2, 4–6) and λ is the decay length. A goal of both experimentalists and theoreticians is to determine characteristics of the surface and solvents that give rise to specific values of P_0 and λ.

Numerous theoretical treatments (7–19) have been proposed to explain the range and magnitude of the solvation pressure. Most theories are general in that they do not consider the molecular structure of the solvated surface or the specific interactions of the surface with the solvent. However, it is generally agreed that solvation repulsion arises from the polarization and reorganization of solvent molecules near the membrane surface.

In their pioneering studies to explain the magnitude of the hydration pressure between specific lipid bilayers, Cevc and Marsh (13, 20) proposed that the electric field that polarizes solvent molecules arises from fixed charges and perpendicular components of the "multipole surface charge densities" in the polar headgroup of lipids. Using a theory based on polarization of water molecules by the lipid headgroups, they concluded that

$$P_0 = \frac{2\varepsilon_0(\varepsilon - 1)}{\varepsilon}\left(\frac{\psi(0)}{\lambda}\right)^2, \qquad [1]$$

where $\psi(0)$ is the hydration potential at $d_f = 0$, ε is the bulk dielectric constant of the solvent, and ε_0 is the permittivity of free space. Cevc and Marsh (13) calculated $\psi(0)$ for a variety of lipids by summing the different dipole moments of the polar components of the lipid headgroup. This approach implies that the "plane of origin" of P_h is positioned so that the entire headgroup region contributes to the hydration potential. Recently, the work of Marcelja and coworkers (8, 16) and Cevc and Marsh (13, 20) has been extended by Belaya *et al.* (17) and Dzhavakhidze *et al.* (18, 19) by using a nonlocal electrostatic approach to calculate P_h. They noted that P_h could arise "as a result of nonlocal polarization of water by permanent dipole moments at the surface—either due to oriented polar groups of the surface itself or due to chemisorbed water molecules" (18). They found that the magnitude of P_h depends on the perpendicular component of the dipolar polarization and on the extent that the solvent penetrates into the polar headgroup. Other treatments have been generally less specific than these in that P_0 was either taken as an experimental parameter or predicted to be dependent on the concentration, orientation, and type of defects in the solvent (2, 7, 10, 12, 15, 16).

We have taken an experimental approach to determine the factors responsible for both λ and P_0. For lecithin bilayers in water and nonaqueous polar solvents, McIntosh *et al.* (21) have shown experimentally that λ depends on the number of dipolar solvent molecules per unit volume in the interbilayer space. In considering P_0, we have used Eq. **1**, but have equated the hydration potential, $\psi(0)$, to ΔV_d, where ΔV_d is the Volta or dipole potential measured in monolayers in equilibrium with liposomes (22). Thus we predict that

$$P_0 = \frac{2\varepsilon_0(\varepsilon - 1)}{\varepsilon}\left(\frac{\Delta V_d}{\lambda}\right)^2. \qquad [2]$$

For uncharged bilayers, we have found that Eq. **2** gave good agreement with the measured values of P_0 when the plane of origin of P_h was taken as the physical edge of the bilayer (4–6, 23, 24). For uncharged lipids, such as the ones discussed in this paper, ΔV_d can arise only from the vector sum of perpendicular components of the dipole (and multipole) moments of the lipid *and the solvent* molecules (25–27).

In this paper, we develop further the hypothesis that the potential arising from the arrangement of dipoles of solvent and lipid molecules predominantly determines P_0, and we also justify the assumption that the plane of origin of the solvation pressure is near the physical edge of the bilayer.

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Abbreviations: E-PtdCho, egg phosphatidylcholine; PVP, polyvinylpyrrolidone; Pam_2-PtdCho, dipalmitoylphosphatidylcholine.

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Furthermore, based on the observed equivalence of $\psi(0)$ and ΔV_d, we propose that the dipole potential decays exponentially into the solvent phase with a decay length λ.

MATERIALS AND METHODS

The data used in this paper have been previously published (4, 6, 20, 23, 24) and hence the methods will be described briefly. The lipids used were egg phosphatidylcholine (E-PtdCho), E-PtdCho/cholesterol of various mole ratios, and dipalmitoylphosphatidylcholine (Pam_2-PtdCho). The bilayers were in the liquid-crystalline phase (L_α) or gel phase ($L_{\beta'}$). To obtain pressure–fluid space relationships, osmotic pressures in the range of 0 to 6×10^7 dynes/cm^2 (1 dyne = 10 μN) were applied to lipid multilayers by suspending the lipids in water, formamide, or 1,3-propanediol containing various concentrations of polyvinylpyrrolidone (PVP). Because the PVP molecules are too large to enter between the lipid multilayers, the PVP competes for solvent with the lipid and therefore compresses the lamellar lattice (1, 2). Osmotic pressure measurements of the PVP in water, formamide, and 1,3-propanediol have been described elsewhere (20). For each multilamellar suspension, x-ray diffraction patterns were recorded and electron density profiles were calculated (4–6, 20, 24). These profiles were used to estimate the width of the bilayer and the fluid space between bilayers for each applied osmotic pressure. Over the range of pressures considered in this paper, steric interactions are negligible, and the osmotic pressure approximately equals the solvation pressure (6, 24).

The dipole potential for lipids at the solvent/air interface was measured with a Ag/AgCl electrode in the subphase and a polonium electrode in air. Lipid monolayers were spread in excess over water, formamide, or 1,3-propanediol (all containing 0.01 M KCl) as described (22).

RESULTS AND DISCUSSION

Determination of the Magnitude of the Solvation Pressure. Fig. 1 shows plots of the logarithm of applied pressure versus the distance between bilayer surfaces for Pam_2-PtdCho in the gel phase and E-PtdCho in the liquid-crystalline phase. For the range of pressures shown, the dominant repulsive pressure resisting compression of the multilayers is P_h, so that $P \cong P_h$. For each data set, the experimental values fit quite well to a linear regression ($r^2 > 0.96$ in each case), implying that the hydration pressure decays exponentially with increasing distance between bilayer surfaces (1, 2, 4–6), so that $P_h = P_0 \exp(-d_f/\lambda)$. Values of P_0 and λ for several bilayer systems and three solvents are given in Table 1. Sources of experimental uncertainty of these parameters have been described elsewhere (4–6, 24). Note that P_0 (the value of P_h at zero bilayer separation) is larger in the gel phase than in the liquid-crystalline phase (Fig. 1).

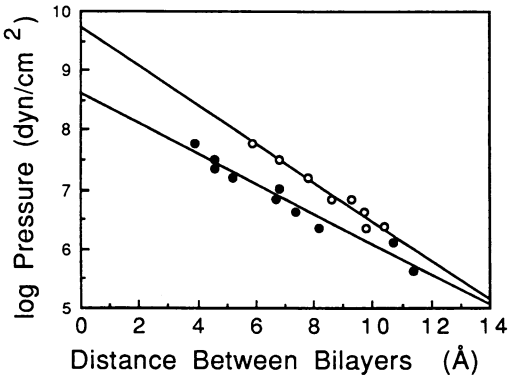

FIG. 1. Logarithm of applied osmotic pressure plotted versus fluid spacing, d_f, for gel phase bilayers of Pam_2-PtdCho (○) and for liquid-crystalline bilayers of E-PtdCho (●). The solid lines are linear regressions to the data sets. The values of the regressions at $d_f = 0$ correspond to P_0. Data are redrawn from McIntosh and Simon (4).

The choice of plane of origin (where $d_f = 0$) is critical in determining P_0 because of the exponential dependence between P_h and d_f. For phosphatidylcholine bilayers, we (4–6, 24) assigned the plane of origin at the physical edge of the bilayer (Fig. 2). This reference plane was chosen for three reasons. The first is that further reduction in fluid space results in steric repulsion between the polar headgroups, which is much larger than solvation pressure (6, 24). The second concerns the differences in organization of solvent molecules in two regions—in the fluid space between bilayers (shaded area in Fig. 2), where most solvent molecules are in contact with other solvent molecules, and in the headgroup region of the bilayer, where the solvent molecules are intercalated between the lipid polar headgroups. Previously we have shown (20) that, for $d_f > 0$, P_h decays with $\lambda \propto (1/n_s)^{1/3}$, where n_s is the number of solvent molecules per unit volume in the interbilayer space as calculated from bulk solvent values. However, for phosphatidylcholine bilayers, P_h cannot decay as $n_s^{-1/3}$ for $d_f < 0$, because solvent molecules intercalated into the headgroup region do not have the same number of nearest neighbors as do interbilayer solvent molecules. The third reason is that, as will be shown in this paper, solvent molecules in the polar headgroup region contribute to the electrical field that determines P_0. The location of $d_f = 0$ at the physical edge of the bilayer takes into account these solvent molecules. Of course the construction of a plane at $d_f = 0$ is a simplification that arises from assumptions that the dipoles can be treated as points and that the bilayer surface is molecularly smooth. However, the electric fields, E_0, giving rise to P_0 must be at least one molecular layer thick (see Fig. 2) and, as discussed previously (6, 24), the surface of a phospholipid bilayer is molecularly rough.

Dependence of Solvation Pressure on Dipole Potential. Table 1 and Fig. 3 show P_0 as obtained by x-ray diffraction experiments (4–6, 20, 23) and as calculated from Eq. 2 for a variety of bilayer systems. In these experiments, ΔV_d was varied experimentally in three ways: (*i*) by changing the area per lipid molecule, A, by the use of different lamellar phases (5), (*ii*) by using nonaqueous solvents in place of water (20), and (*iii*) by varying the concentration of cholesterol in the bilayer (6). It is seen from Fig. 3 that Eq. 2 yields quantitative agreement between P_0 obtained from x-ray diffraction and dipole potential measurements ($r^2 = 0.921$); that is, the regression to the experimental data (solid line) has a slope and intercept similar to a plot of Eq. 2 (dotted line). We consider the agreement between experiment and theory to be good, particularly since measurements of ΔV_d and λ are based on different physical principles and P_0 was obtained by extrapolation of an exponential to $d_f = 0$ (Fig. 1). Another potential problem is that our measured ΔV_d may not be equal to the hydration potential because of an additional potential of unknown magnitude arising from the air/hydrocarbon interface in monolayers, which is not present in bilayers. This could add a constant voltage of unknown magnitude and sign to our measured values of ΔV_d, which would result in a curvature to the plot in Fig. 3. We argue that this additive constant voltage should be relatively small, for two reasons. First, the fit to a straight line ($r^2 = 0.921$) indicates that there is relatively little curvature. Second, ΔV_d for monolayers of phosphatidylcholines with both hydrocarbon chains brominated at the 15, 16 position is 387 ± 4.5 mV ($n = 3$), which is quite similar to values obtained for nonbrominated phosphatidylcholines with similar acyl chain lengths (Table 1). Since the dipole moment of a C—H bond is different from that of a C—Br bond, this result suggests that the voltage drop

Table 1. Range and magnitude of solvation pressure for lecithin bilayers

Lipid	Solvent	λ, Å	ΔV_d, mV	P_0, (dynes/cm^2) × 10^{-8}	$2\varepsilon_0\left(\frac{\varepsilon - 1}{\varepsilon}\right)\left(\frac{\Delta V_d}{\lambda}\right)^2$, (dynes/cm^2) × 10^{-8}
E-PtdCho	Water	1.7	415	4.0	10.0
	Formamide	2.4	266	1.8	2.2
	1,3-Propanediol	2.6	223	1.1	1.3
Pam_2-PtdCho	Water	1.3	575	47.0	34.6
E-PtdCho/cholesterol, 4:1	Water	1.6	446	12.2	13.8
E-PtdCho/cholesterol, 2:1	Water	1.4	463	16.6	19.4
E-PtdCho/cholesterol, 1:1	Water	2.1	493	3.2	9.6
	Formamide	2.9	353	0.75	2.6
	1,3-Propanediol	3.1	292	0.59	1.5

Data in this table are taken from refs. 4–6, 20, and 28. All lipids are in the liquid-crystalline phase except for Pam_2-PtdCho, which is in the gel phase.

across the air/hydrocarbon interface is small compared to ΔV_d.

Dipole potentials can also be measured by conductance and binding experiments with bilayers (29, 30). For a variety of lipids, dipole potential measurements in bilayers are always about 100–150 mV (29, 30) smaller than those in monolayers. This difference is not well understood, but it should be noted that dipole potential measurements with bilayers depend on the use of a pair of large probes that are assumed to interact identically with water. In addition, these probes may perturb the interface (see figure 7 in ref. 31).

We have also found reasonable agreement between measured values of P_0 and values calculated from Eq. 2 for two lipids whose headgroups are not zwitterionic [namely, monoeladin and monocaprylin in water (23)]. These data are not plotted in Fig. 1 because the orientation of the small monoglyceride polar headgroups is not known, so that the plane of origin is difficult to define in a way similar to that used for phosphatidylcholine bilayers.

Contributions to the Dipole Potential. Since the magnitude of the hydration pressure depends on the dipole potential (Fig. 3), it is important to analyze the factors that determine ΔV_d. It would be expected that ΔV_d should depend on *all* the perpendicularly oriented dipoles at the bilayer/solvent interface—that is, dipoles in the lipid headgroup as well the dipoles of the solvent molecules that are intercalated into the headgroup region (5, 17–19).

First, let us consider the contributions to ΔV_d from the lipid headgroups. It has been suggested that both the zwitterionic phosphocholine moiety (29) as well as the carbonyl groups (28) contribute to ΔV_d. The effect of the lipid headgroup on ΔV_d can be shown in experiments where the area per lipid molecule (A) at the interface is varied. That is, for a single-component lipid, when A is increased, the number of headgroups per unit at the interface decreases, and ΔV_d decreases. In fact, the data of MacDonald and Simon (22) show that ΔV_d is inversely proportional to A for dimyristoylphosphatidylcholine monolayers. The inverse relationship between ΔV_d and A, which may reflect that increasing A increases the fraction of the surface occupied by hydrophobic residues, explains why P_0 is higher in gel than liquid-crystalline phases (4–5, 23).

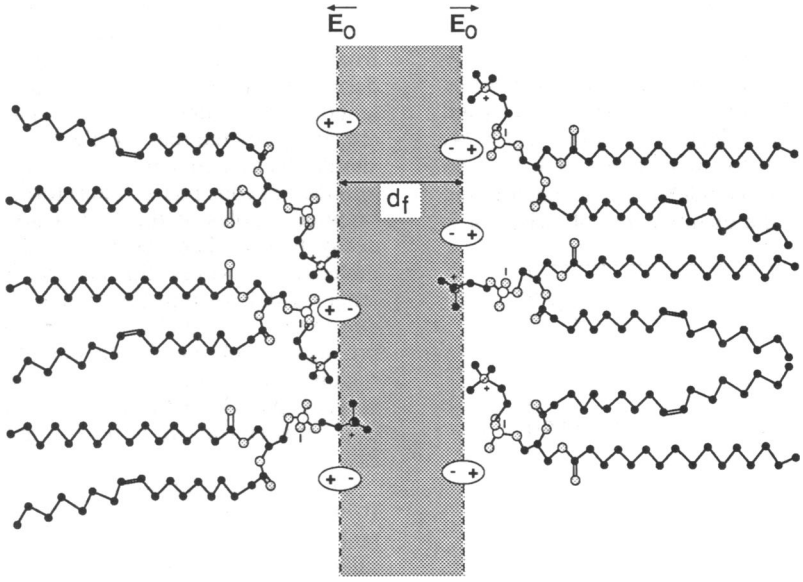

FIG. 2. Schematic diagram of the fluid space between apposing E-PtdCho bilayers. The plane of origin of the hydration pressure (where d_f = 0 in Fig. 1) is taken as the edge of the bilayer when the phosphocholine moiety is in its usual position parallel to the plane of the membrane. The headgroups rotate so that the trimethylammonium moiety can extend 2–3 Å beyond this plane into the interbilayer fluid space (shaded region). Two lipids having their headgroups in this extended conformation are shown. The positive and negative charges on the lipid molecules represent the location of the trimethylammonium and phosphate moieties, respectively. Solvent dipoles are schematically shown as ellipses at the plane of origin. They are oriented so that, on average, their positive charges point toward the hydrocarbon region of the bilayer. The net orientation of the lipid and solvent dipoles will give rise to an electric field ($\vec{E}_0$) at the plane of origin. This electric field will affect solvent molecules in the interbilayer space and decay rapidly in this region. This figure was redrawn from ref. 24.

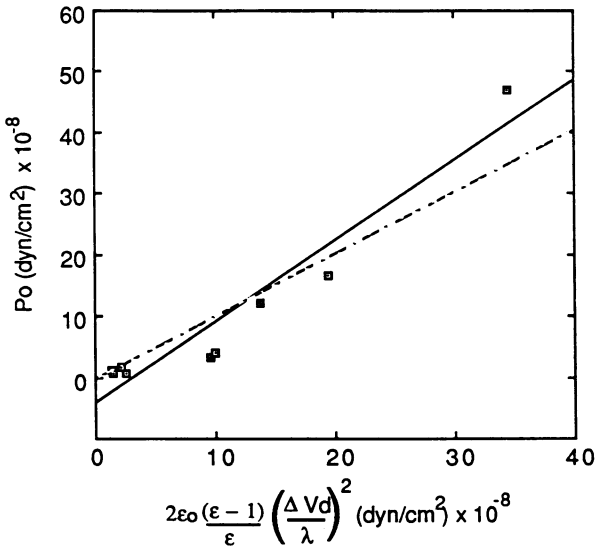

FIG. 3. Plot of P_0 obtained by x-ray analysis as indicated in Fig. 1 versus $2\varepsilon_0 \frac{(\varepsilon - 1)}{\varepsilon}\left(\frac{\Delta V_d}{\lambda}\right)^2$, where ΔV_d is the measured dipole potential of monolayers, λ is the decay length of the hydration pressure, ε is the dielectric constant of the solvent, and ε_0 is the permittivity of free space. The numerical values for these data are taken from refs. 4–6, 20, and 23 and are given in Table 1. The solid line is a least-squares fit to the data points. It has a regression coefficient $r^2 = 0.921$, a slope of 1.32, and an intercept of -4.20×10^8 dynes/cm^2. The dotted line is the relationship between P_0 and ΔV_d predicted by Eq. 2.

Evidence for the contribution of interfacial solvent to the dipole potential comes from several sources. First, the role of solvent molecules in determining ΔV_d has been previously noted in studies of monolayers and electrodes in water (25, 26, 32). Second, the dipole of lipid monolayers has been shown to depend strongly on the solvent in the subphase (Table 1; refs. 20, 33–35). Since the area of equimolar E-PtdCho/cholesterol bilayers is approximately the same in water, formamide, and 1,3-propanediol (20), the large (200-mV) differences in ΔV_d for these systems (Table 1) cannot be explained by changes in A. The relationship between ΔV_d and the properties of the solvent molecules is shown in Fig. 4, where ΔV_d, as measured (20) for monolayers of E-PtdCho over four solvents (water, formamide, 1,3-propanediol, and glycerol), is plotted against the quantity $n\mu/\varepsilon A$, where n is

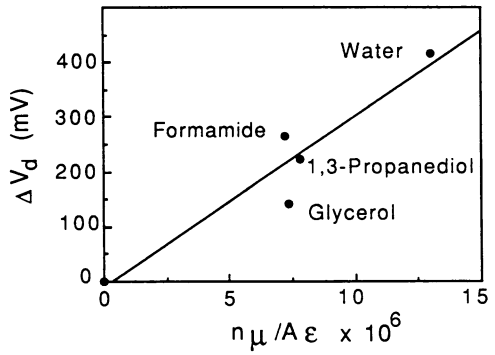

FIG. 4. Plot of the measured dipole potential ΔV_d of E-PtdCho monolayers (in equilibrium with bilayers) versus $n\mu/A\varepsilon$, where n is the number of solvent molecules per unit volume, μ is the dipole moment of the solvent in Debye units, A is the area per lipid molecule in Å^2 (Table 1), and ε is the bulk dielectric constant. The line is a least-squares fit ($r^2 = 0.91$) to the four data points plus a point at the origin.

the number of solvent molecules per unit volume in bulk solvent, μ is the dipole moment of the solvent, ε is the bulk dielectric constant of the solvent, and A is the area per lipid molecule at the interface. This plot indicates that the ratio $n\mu/\varepsilon A$ is proportional to ΔV_d, as would be expected from nonlocal electrostatic theory (17–19) or from treatments of the dipole layer as a parallel plate capacitor (25). A third indication that the solvent molecules, as well as the lipid headgroups, contribute to ΔV_d can be inferred from measurements of ΔV_d for myristic acid (33), stearic acid (34), 1,2-hydroxystearic acid methyl ester (35), hexadecanol (36), octadecyl methyl ester (37), and monoelaidin (23). All of these neutral lipids, with vastly different headgroups and approximately the same area per molecule (≈20 Å^2), have dipole potentials of about 400 mV when spread over aqueous subphases. Cholesterol, with a larger area per molecule (≈38 Å^2), also has ΔV_d of 400 mV (37). That lipid molecules having only a hydroxyl moiety for a polar headgroup, such as hexadecanol and cholesterol, can generate dipole potentials almost as large as lipids having zwitterionic headgroups, such as E-PtdCho, suggests that ΔV_d arises from both the covalently bonded dipoles of the lipids and the solvent molecules in contact with them. In fact, it has been noted by several authors (37–39) that only a few percent of the total interfacial solvent dipoles need be oriented to generate large dipole potentials.¶ Finally, based on deuterium NMR studies, Bechinger *et al.* (43) stated, "the structuring effect of polyhydroxyl compounds on water appears to create a more negative electric dipolar field in the vicinity of the phosphocholine head group." That is, one would expect that compounds such as glycerol would lower ΔV_d, as has been observed (20, 33, 34).

Propagation of the Dipole Field into Fluid Space. Now let us consider how the electric field arising from the oriented interfacial dipoles propagates into the surrounding solvent phase. Cevc and Marsh (13) extended the formalism of Gruen and Marcelja (8) and showed that the hydration potential (ψ) from a monolayer surface should decay as

$$\psi = \psi(0)\exp(-d_f/\lambda). \quad [3]$$

Therefore, since we find experimentally that $\psi(0)$ can be approximated by ΔV_d, we argue that the potential arising from oriented dipoles at the lipid–solvent interface should decay exponentially into the aqueous phase with a decay length on the order of 1–2 Å. Similarly, Dzhavakhidze *et al.* (18, 19) predicted that the electric field (and hence potential) should decay exponentially from the surface of the bilayer into the aqueous phase. Therefore, the potential arising from oriented dipoles at the E-PtdCho–solvent interface should decay to less than 100 mV over a distance of one water molecule. However, this electric field should propagate for longer distances into the hydrocarbon region of the bilayer due to the lower dielectric constant of this region. Both the propagation of the dipole potential into the hydrocarbon region and the decay of the hydration potential into the solvent space have been treated separately, but the connection between the two cases has not been recognized explicitly.

Our analysis indicates that $\psi(0) \cong \Delta V_d \cong 400$ mV for E-PtdCho bilayers at $d_f = 0$. However, electrophoretic mobility measurements of phosphatidylcholine vesicles in salts, made at the plane of shear, correspond to ζ potentials

¶Cholesterol, protonated fatty acids, and saturated phosphatidylethanolamines all have dipole potentials of about 400 mV but have very narrow fluid spaces between adjacent bilayers (40–42). These lipids may exclude water by forming H-bond bridges between apposing bilayers (41, 42). Therefore, bilayers or macromolecules with small equilibrium fluid spaces can have either small dipole potentials (and therefore small repulsive solvation pressures) or else form interbilayer bonds.

of a few millivolts (44, 45). This implies, for our location of the plane of origin of P_h to be correct, that ΔV_d must decay about 400 mV from the plane of origin to the plane of shear. The following analysis argues that this potential drop is consistent with the location of these two molecular "planes." If ΔV_d decays exponentially into the fluid phase (Eq. 3), then for a decay length of 1.5 Å (average of λ for Pam_2-PtdCho and E-PtdCho in water; Table 1), ΔV_d would decay from 400 mV to 2 mV over a distance of 8 Å or about three water molecules. The problem is to determine the physical location of the plane of shear with respect to the plane of origin of the hydration or dipole potential. In their studies of the adsorption of cations to phospholipids, Eisenberg *et al.* (46), using Gouy–Chapman–Stern analysis, concluded that the hydrodynamic plane of shear is about 2 Å from the "surface" of phosphatidylcholine/phosphatidylserine bilayers. However, for the following reasons, it is reasonable to conclude that our plane of origin and the plane of shear could be separated by 8 Å. First, the polar headgroups can extend a few angstroms from their in-plane orientation ($d_f = 0$) into the aqueous phase (ref. 24 and Fig. 2), especially in the presence of salts (47), implying that the effective bilayer surface may be 2–3 Å further out into the aqueous from our plane of origin. Second, lipids exhibit "breathing" modes or fluctuations in thickness, generated by thermal energy, which also diffuse the plane of origin. Third, in the calculations of ζ potential from the electrophoretic mobility measurements, the "no slip" boundary condition requires one to two immobile water layers [i.e., 3 to 6 Å (32)]. Fourth, the molecules giving rise to the polarizing electric field must be at least one molecule in width (i.e., 3 Å; Fig. 2). Thus our plane of origin might well be 8 Å from the plane of shear, and therefore the results of our measurements of P_h and electrophoretic mobility measurements appear to be consistent.

Physiological Significance. The large potential drop from the bilayer surface into the aqueous phase can have many important physiological consequences when molecules in water approach to distances within 8 Å of the membrane; all charged and dipolar molecules should be affected by this potential over this range. For instance, the binding of lipophilic ions to lipid bilayers (29, 38, 39) and the gating of ion channels (27, 29, 48) are affected by changes in ΔV_d. The organization of solvents near the mouth or vestibule of channels may also modify ion selectivity, the total electrochemical potential, and hence ion mobility within the channel (49). The extent of the perturbation of the electrochemical potential on electrolyte mobility will depend on vestibule geometry and the orientation of the protein and solvent dipoles (50). Finally, proteins in the fluid space can be affected by the dipole potential. As first mentioned by Flewelling and Hubbell (29) and later elaborated on by D. Cafiso (personal communication), the dipole potential should orient dipolar proteins so that they may partition into the membrane with the "correct" orientation (i.e., lowest energy).

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