The hydration pressure between lipid bilayers

Comparison of measurements using x-ray diffraction and calorimetry

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ABSTRACT The hydration pressure between dipalmitoyl phosphatidyl-*N*,*N*-dimethylethanolamine (DPPE-Me_2) bilayers has been analyzed by both x-ray diffraction measurements of osmotically stressed liposomes and by differential scanning calorimetry. By the x-ray method, we obtain a magnitude (P_o) and decay length (λ) for the hydration pressure which are both quite similar to those found for bilayers of other zwitterionic lipids, such as phosphatidylcholines. That is, x-ray analysis of DPPE-Me_2 in the gel phase gives $\lambda = 1.3$ Å, the same as that previously measured for the analogous gel phase lipid dipalmitoylphosphatidylcholine (DPPC), and $P_o = 3.9 \times 10^9$ dyn/cm^2, which is in excellent agreement with the value of 3.6×10^9 dyn/cm^2 calculated from the measured Volta potential of DPPE-Me_2 monolayers in equilibrium with liposomes. These results indicate that the removal of one methyl group to convert DPPC to DPPE-Me_2 does not markedly alter the range or magnitude of the hydration pressure. Calorimetry shows that the main gel to liquid-crystalline phase transition temperature of DPPE-Me_2 is approximately constant for water contents ranging from 80 to 10 water molecules per lipid molecule, but increases monotonically with decreasing water content below 10 waters per lipid. A theoretical fit to these temperature vs. water content data predicts $\lambda = 6.7$ Å. The difference in observed values of λ for x-ray and calorimetry measurements can be explained by effects on the thermograms of additional intra- and intermolecular interactions which occur at low water contents where apposing bilayers are in contact. We conclude that, although calorimetry provides important data on the energetics of bilayer hydration, it is difficult to obtain quantitative information on the hydration pressure from this technique.

INTRODUCTION

In many biological processes, such as cell adhesion, membrane fusion, and hormone (drug)-receptor interactions, interstitial water must be removed so that the apposing surfaces can come into physical contact. In several quite different systems, including lipid bilayers (LeNeveu et al., 1977; McIntosh and Simon, 1986*b*), DNA helices (Parsegian et al., 1985), and carbohydrate molecules (Rau and Parsegian, 1987), it has been shown that a major barrier to close approach of apposing surfaces is due to the hydration pressure, P_h, which arises from the polarization of water by the polar surfaces (Marcelja and Radic, 1976; Rand and Parsegian, 1989).

Although the hydration pressure has been observed directly for lipid bilayers with a surface force apparatus (Marra and Israelachvili, 1985), the magnitude and decay length of P_h have been quantitated with only one technique, x-ray diffraction analysis of osmotically stressed systems (Parsegian et al., 1979; McIntosh and Simon, 1986*b*). That is, for lipid bilayers subjected to osmotic stresses, x-ray diffraction data have shown that $P_h = P_o \cdot \exp(-d_f/\lambda)$, where P_o is the magnitude of P_h, d_f is the distance between bilayers, and λ is the decay length, which has been measured to be 1–2 Å for a variety of bilayers in water (McIntosh and Simon, 1986*b*; Rand and Parsegian, 1989). Based on these experimental data, several theoretical models have been forwarded with the purpose of revealing the physiochemical mechanisms underlying the hydration pressure. Since, for most lipids, measurements of P_o and λ have been generated using only the x-ray method, it would be useful if these important results could be confirmed with another method based on different physical principles.

Such an alternative experimental approach was taken by Cevc and Marsh (1985). In their pioneering study, they used differential scanning calorimetry (DSC) to analyze the hydration of a series of saturated phosphatidylethanolamines (PEs). Using a theoretical analysis of lipid hydration, Cevc and Marsh (1985) found that a decay length of $\lambda \sim 2.5$ Å was consistent with the observed changes as a function of water content of the temperature, enthalpy, and entropy of the gel to liquid-crystalline phase transition. They also analyzed x-ray diffraction data from dilauroyl-phosphatidylethanolamine (DLPE) at various water contents (corresponding to 5–9 waters/lipid) and calculated a decay length $\lambda = 2.5$ Å. Thus, there appeared to be general agreement between the x-ray diffraction and calorimetric methods of obtaining λ. However, later x-ray diffraction experi-

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ments of osmotically stressed natural and synthetic PEs gave considerably lower values for λ, ranging from 0.8 to 1.3 Å (Rand et al., 1988).

Phosphatidylethanolamines are not the best lipids to use for obtaining values of P_o and λ of the hydration pressure for several reasons. First and foremost, PEs imbibe relatively few water molecules compared with other phospholipids so that the fluid spacing between adjacent PE bilayers in excess water is only ~5 Å (McIntosh and Simon, 1986*a*). Second, PE bilayers crystallize into different phases at low water contents (Seddon et al., 1984; Xu et al., 1988). This places additional limits on the range of water contents that can be analyzed by x-ray diffraction. Therefore, osmotic stress experiments can only change the fluid spacing between PE bilayers by one or two Ångstroms, making it difficult to accurately determine λ with the x-ray method (McIntosh and Simon, 1986*a*). Thus, Cevc and Marsh could not directly measure the decay length of the hydration pressure and had to make several assumptions to calculate λ from their x-ray data. Third, PE bilayers likely contain an additional short range attractive interaction (McIntosh and Simon, 1986*a*; Boggs, 1987; Rand et al., 1988), which further complicates the analysis of force vs. distance relationships (McIntosh and Simon, 1986*a*). An additional potential problem with the calorimetric method is that λ may be different for gel and liquid-crystalline phases (McIntosh and Simon, 1986*b*; Simon et al., 1988; Rand and Parsegian, 1989).

Because of these problems with PE bilayers, we decided to reinvestigate the possibility of using calorimetry to obtain λ using a lipid where, for comparison, P_o and λ could be determined directly using x-ray diffraction. The optimal lipid for these studies should have the following characteristics. First, it should be chemically pure and have homogeneous acyl chain lengths so that sharp thermal transitions are obtained. Second, it should be unchanged or have a zwitterionic head group, so that electrostatic interactions are negligible. Third, it should imbibe relatively large quantities of water compared with PEs, so that a range of fluid spacings can be obtained with osmotic stress experiments. Fourth, it should exhibit a single gel to liquid-crystalline phase transition at experimentally accessible temperatures so that problems involving multiple transitions can be avoided. Fifth, it should not change its phase over a relatively wide range of water contents. One lipid that satisfies all these criteria is dipalmitoyl phosphatidyl-*N*,*N*-dimethylethanolamine (DPPE-Me_2). DPPE-Me_2 has been characterized previously by both x-ray diffraction (Mulukutla and Shipley, 1984) and calorimetry (Vaughn and Keough, 1974; Casal and Mantsch, 1983; Mio et al., 1984; Chowdhry and Dalziel, 1985; Gagne et al., 1985), and shown to meet the above criteria. Most importantly, it imbibes much more water than PE so that λ can be accurately determined by x-ray diffraction.

Another reason for measuring the hydration pressure for DPPE-Me_2 is that it provides another system to test the hypothesis (Simon and McIntosh, 1989) that there is a correlation between the magnitude of the hydration pressure and the Volta potential (V), which is equivalent to the dipole potential for uncharged bilayers. Cevc and Marsh (1985) derived the expression $P_o = 2\chi(\Psi/\lambda)^2$, where Ψ is the "hydration potential" at $d_f = 0$, χ is the susceptibility of the interlamellar water and is equal to $\epsilon_o(\epsilon - 1)/\epsilon$, where ϵ is the bulk dielectric constant of water and ϵ_o is the permittivity of free space. For a variety of phosphatidylcholine (PC) and PC/cholesterol bilayers, we (Simon and McIntosh, 1989) showed that there was a good correlation between the hydration potential (Ψ) and the Volta potential (V) measured for monolayers in equilibrium with liposomes by demonstrating agreement between P_o as measured by x-ray diffraction and the quantity $2\chi(V/\lambda)^2$. We also argued that V contains contributions from the dipoles (and multipoles) of the lipid as well as intercalated solvent molecules. However, in our experimental correlation between P_o and $2\chi(V/\lambda)^2$, most of the values of V were between 200 and 500 mV, and we had only one value of V of over 500 mV. Therefore, to further test this correlation, it would be advantageous to find a lipid with a large Volta potential. Preliminary results showed that V is over 600 mV for DPPE-Me_2, making it a useful lipid to further test our hypothesis that the magnitude of the hydration potential can be accurately predicted by the measured Volta potential.

MATERIALS AND METHODS

Materials

L-α-dipalmitoyl phosphatidyl-*N*,*N*-dimethylethanolamine (Lot 118F8458) was used as purchased from Sigma Chemical Co. (St. Louis, MO). Polyvinylpyrrolidone (PVP) was obtained from Sigma Chemical Co. Triply distilled water was used to make PVP-water solutions in the range of 0–50% wt/wt.

X-Ray diffraction

For x-ray diffraction experiments, osmotic pressures were applied to DPPE-Me_2 suspensions by an "osmotic stress" technique (LeNeveu et al., 1977). An excess amount (>80% by weight) of the appropriate PVP-water solution was added to dry DPPE-Me_2. These suspensions were incubated, with periodic vortexing, for several hours at 60°C, a temperature above the lipid gel to liquid-crystalline phase transition temperature. Because PVP is too large to enter between the lipid multilayers, it competes for water with the lipid and compresses the

lipid lattice (Parsegian et al., 1979). For these PVP solutions, we used values of osmotic pressure as calculated from the virial coefficients of Vink (1971). These values of osmotic pressure are in close agreement to measured values (Parsegian et al., 1986; McIntosh et al., 1989*b*). The lipid suspensions were sealed in quartz glass capillary tubes and mounted in a point collimation x-ray camera containing three or more sheets of Kodak DEF x-ray film in a flat plate film cassette. Specimen-to-film distance was 10 cm, and exposure times were on the order of 4 to 8 h. All diffraction patterns were recorded at 20 ± 2°C. X-Ray films were processed by standard techniques and densitometered with a Joyce-Loebl model MKIIIC microdensitometer. The background curve was subtracted and integrated intensities, $I(h)$, for each order h were obtained by measuring the area under each diffraction peak. For these unoriented suspensions the structure amplitude for each order h was set equal to $[h^2I(h)]^{1/2}$ (Blaurock and Worthington, 1966). Structure amplitudes were normalized according to the procedure of Blaurock (1971) and phase angles for each diffraction order were obtained as previously described (McIntosh and Holloway, 1987). In brief, continuous transforms were calculated for each possible phase angle combination by the sampling theorem. The correct phase combination was taken as the one whose continuous transform most closely matched the experimental structure factors for all data sets (see Fig. 2).

Volta potential measurements

Volta potentials, V, were measured as described previously (MacDonald and Simon, 1987). Briefly, monolayers of DPPE-Me_2 were formed by spreading 10 μL of a 25-mg/ml solution onto a subphase of 1 mM KCl in a trough having a surface area of ~30 cm^2 at room temperature. The KCl had been roasted at 650°C for 24 h to remove organic impurities. Under these conditions it has been shown that the packing of the lipid molecules in the monolayer is approximately the same as it is in a bilayer (MacDonald and Simon, 1987). The Volta potential was measured between an Ag/AgCl electrode in the subphase and a polonium electrode in air using a Keithley electrometer (model 602; Keithley Instruments Co., Cleveland, OH). The reported values of V represent the difference in potential of the surface in the presence and absence of the monolayer.

Differential scanning calorimetry

Thermograms were obtained using a Perkin-Elmer DSC7 calorimeter with the Perkin-Elmer Corp. (Norwalk, CT) data station. Before preparation of samples for calorimetry, the DPPE-Me_2 was desiccated for 40 h at 500 μm Hg to remove absorbed water. The lipid was then weighed into a stainless steel sample pan and the appropriate amount of deionized, doubly distilled water was added with a microsyringe. The sample was then sealed and reweighed. After completion of the calorimetry run, the sample was reweighed to insure that no water had evaporated. Samples were preheated at 80°C in the calorimeter for ~5–10 min. and were usually scanned at 5°C/min. The samples were heated and recooled at least two times or until reproducible thermograms were obtained on consecutive scans. The relatively short times spent below the transition temperature are insufficient for the gel phase to be converted into a low temperature "crystalline" phase (Mulukutla and Shipley, 1984). The protocol outlined above is similar to the one used by Cevc and Marsh (1985). For the main endothermic transition, transition temperatures (taken at the peak of the transition) are reproducible to 0.3°C. The transition enthalpies were calculated by integrating the area under the endothermic transition peaks using the data station software.

RESULTS

X-Ray diffraction

The diffraction pattern of DPPE-Me_2 in excess water contained five orders of a lamellar repeat period of 64.6 Å and a wide-angle reflection centered at 4.14 Å. The wide-angle reflection was broader than the sharp reflections obtained from gel phase lipids with untilted chains, as it extended from ~4.07 to 4.17 Å. It was difficult to determine if this wide-angle pattern consisted of two reflections, a sharp reflection centered at 4.14 Å superimposed on a broad band, or a single, somewhat broad reflection. For either case, the spacing and width of the broad component of this reflection (or reflections) indicate that the hydrocarbon chains in DPPE-Me_2 are tightly packed in a gel phase and tilted relative to the hydrocarbon water interface (see below). Using infrared spectroscopy, Casal and Mantsch (1984) had previously deduced that the acyl chains of DPPE-Me_2 are tilted.

For all PVP concentrations, the same wide angle pattern was recorded. However, the lamellar repeat period decreased monotonically with increasing PVP concentration. Fig. 1 shows a plot of the natural logarithm of applied osmotic pressure (ln P) vs. lamellar repeat period for these PVP experiments. A least squares analysis provides a good straight line fit ($r^2 = 0.99$).

Estimates for the width of the DPPE-Me_2 bilayer as a function of PVP concentration in the swelling solution can be obtained by a Fourier analysis of the lamellar diffraction data (McIntosh and Simon, 1986*a*, *b*; McIntosh et al., 1987; McIntosh et al., 1989*a*, *b*). Fig. 2 shows a plot of structure factors vs. reciprocal spacing for the diffraction data from DPPE-Me_2 in water and the

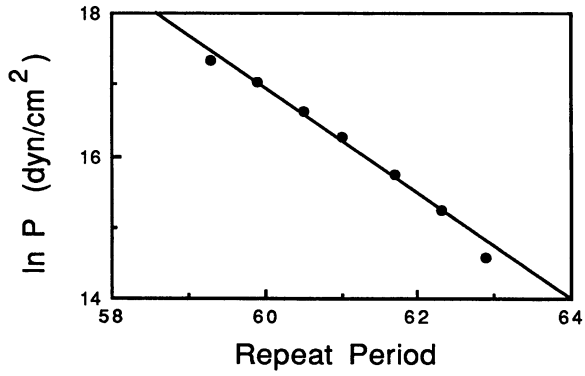

FIGURE 1 Natural logarithm of applied pressure (ln P) plotted vs. lamellar repeat period for DPPE-Me_2 suspensions. The straight line is a least squares fit to the data ($r^2 = 0.99$).

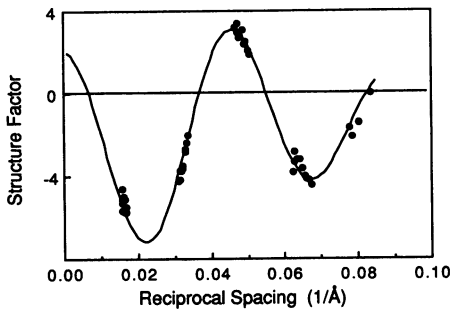

FIGURE 2 Structure factors for DPPE-Me_2 plotted vs. reciprocal space coordinate. The circles represent structure factors for osmotic pressure experiments in PVP solutions. The solid line is the continuous Fourier transform calculated using the sampling theorem for one data set.

various PVP concentrations. The solid curve is the continuous transform of one data set calculated using the sampling theorem. Note that all of the structure factors fall closely to this transform. This indicates that the bilayer thickness does not appreciably change over the range of applied osmotic stresses shown in Fig. 1 (McIntosh and Simon, 1986*b*; Simon et al., 1988). Fig. 3 shows two representative electron density profiles for DPPE-Me_2 at the minimum ($P = 0$, in excess water) and maximum ($P = 3.2 \times 10^7$ dyn/cm^2, in 50% PVP) applied pressures used in these experiments. In both profiles, the high density peaks centered at about ±22 Å correspond to the high density lipid head groups. The electron density trough in the geometric center of each profile represents the terminal methyl groups, and the medium density region between the head group peaks

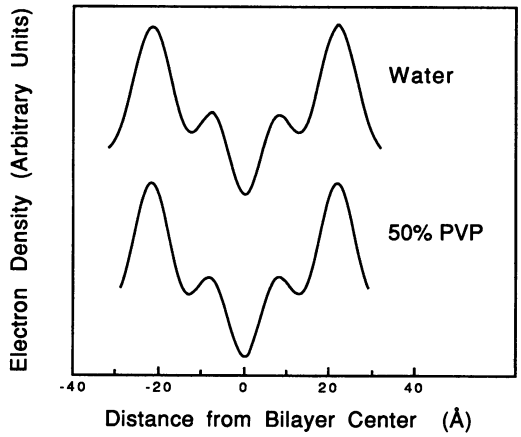

FIGURE 3 Electron density profiles for DPPE-Me_2 bilayers in water and in 50% PVP.

and the terminal methyl trough represents the methylene groups of the acyl chains. The medium density regions at the outer edges of each profile correspond to one-half of the fluid space between adjacent bilayers. Note that the distance between head group peaks across the bilayer is nearly the same in the two profiles. In fact, for all PVP concentrations used, the distance between head group peaks across the bilayer stayed nearly constant, as the mean head group separation was 43.7 Å ± 0.9 Å (mean ± standard deviation for 10 experiments). This implies that the bilayer thickness and area per lipid molecule remained approximately constant for this entire range of PVP concentrations. A similar result has been obtained for phosphatidylcholine and phosphatidylethanolamine bilayers subjected to similar osmotic stresses (McIntosh and Simon, 1986*b*). Since the bilayer width remains approximately constant for all PVP concentrations, the decrease in repeat period with increasing osmotic stress (Fig. 1) must be the consequence of a decrease in fluid thickness between bilayers.

The measured head group peak separation of 43.7 Å is consistent with the hydrocarbon chains in DPPE-Me_2 being tilted relative to the plane of the bilayer. That is, head group peak separations of 42 and 49 Å have been previously measured for dipalmitoylphosphatidylcholine (DPPC) bilayers with tilted and untilted chains, respectively (McIntosh, 1980). From these values, we calculate the chain tilt of DPPE-Me_2 to be ~27°.

To estimate the fluid thickness between apposing bilayers it is necessary to determine the bilayer width and subtract its value from the repeat period. The definition of bilayer width is somewhat arbitrary, as there is not a sharp boundary between water and lipid. That is, the lipid/water interface is not molecularly smooth and water penetrates into the head group region of the bilayer. Previously we (McIntosh et al., 1986*a*, *b*; McIntosh et al., 1987; McIntosh et al., 1989*a*, *b*; Simon and McIntosh, 1989) have operationally defined the bilayer width as the total geometric thickness of the lipid bilayer. We use that same procedure here to compare our previous results to those from DPPE-Me_2. At the resolution of the profiles in Fig. 3, the head group peaks correspond to the center of the lipid head group, between the phosphate moiety and the glycerol backbone. Single crystal data (Hauser et al., 1988) have previously been used to estimate the distance from the center of the head group to the physical edge of the bilayer. For phosphatidylethanolamine (PE) and phosphatidylcholine (PC) bilayers this distance has been estimated as 4 and 5 Å, respectively (McIntosh and Simon, 1986*a*, *b*). The DPPE-Me_2 head group is intermediate in size between that of PE and PC. Therefore, if we

assume that the orientation of the head group is the same in hydrated DPPE-Me_2 as it is in hydrated PE and PC bilayers (that is the phosphoro-dimethylethanolamine moiety lies parallel to the plane of the bilayer), then the distance between the head group peak in the electron density profile (Fig. 3) and the edge of the bilayer is between 4 and 5 Å. Several lines of evidence indicate that the head group orientation is similar for DPPE-Me_2, PC, and PE bilayers. First, in electron density profiles at comparable resolutions, the total widths at half height of the head group peaks are similar for gel phase bilayers of PE, DPPE-Me_2, and PC, being 7.6 ± 0.2 Å for DLPE (McIntosh and Simon, 1986*a*), 7.9 ± 0.5 Å for DPPE-Me_2, and 8.5 ± 0.7 Å for DPPC (McIntosh and Simon, 1986*b*). Second, the magnitude of the Volta potential is more positive for DPPE-Me_2 than for DPPC (see below). If the large dipole between the phosphate and dimethylamine were oriented perpendicular to the plane of the bilayer, then it would be expected that the Volta potential would be significantly lower for DPPE-Me_2 than for DPPC (Tocanne and Tessie, 1990). Third, Dorset and Zhang (1990) have recently shown that epitaxially grown crystals of DPPE-Me_2 have the same head group orientation as crystals of PC and PE and that the lipid thickness depends on the size of the head group. They found that the distance from the lipid phosphate group to the edge of the unit cell (equivalent to the edge of the bilayer) is ~0.2 Å smaller for DPPE-Me_2 than for PC. We use this result to estimate that for DPPE-Me_2 the distance from each peak in the electron density profile to the outer edge of the bilayer is 5 − 0.2 Å = 4.8 Å. Therefore we estimate the thickness of DPPE-Me_2 bilayers to be 43.7 Å + 9.6 Å = 53.3 Å.

Fig. 4 shows a plot of the natural logarithm of pressure (ln P) vs. the distance between bilayers, calculated assuming a constant bilayer thickness of 53.3 Å for DPPE-Me_2. The straight line is a least squares fit ($r^2 = 0.99$) to the data points. The decay length for this line is $\lambda = 1.3$ Å and extrapolation to zero fluid space gives $P_o = 3.6 \times 10^9$ dyn/cm^2. For comparison, data points for DPPC (McIntosh and Simon, 1986*b*) are also shown in Fig. 4. As can be seen, the pressure-distance relationships are quite similar for the two lipids.

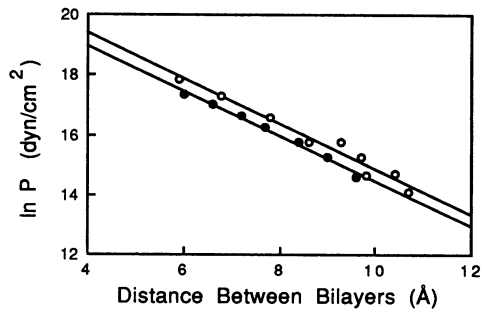

FIGURE 4 Natural logarithm of applied pressure (ln P) plotted vs. lamellar repeat period for DPPE-Me_2 (*solid circles*) and DPPC (*open circles*) suspensions. The DPPC data were taken from McIntosh and Simon (1986*b*). The straight lines are least squares fit to the two data sets ($r^2 = 0.99$ for DPPE-Me_2 and $r^2 = 0.97$ for DPPC). For both lipids $\lambda = 1.3$ Å.

Volta potential

The Volta potential, V, for DPPE-Me_2 monolayers in equilibrium with liposomes was measured to be 612 ± 2 mV ($N = 3$). Using the relationship $P_o = 2\chi(V/\lambda)^2$ (see Introduction) and the measured values of V and λ, we calculated a value of $P_o = 3.9 \times 10^9$ dyn/cm^2 for the magnitude of the hydration pressure. This value is in excellent agreement with the value of $P_o = 3.6 \times 10^9$ dyn/cm^2 obtained from x-ray diffraction measurements (see above). Fig. 5 shows values of P_o as obtained from x-ray diffraction and from the relation $P_o = 2\chi(V/\lambda)^2$ for DPPE-Me_2 as well as a variety of PC and PC/cholesterol bilayer systems in water and other solvents.

Differential scanning calorimetry

Fig. 6 shows the thermograms of DPPE-ME_2 vacuum dried, fully hydrated, and with 10 wt% water. In the absence of water, two endothermic transitions were observed. The temperatures and enthalpies of these two transitions were 66.4°C, 0.24 kcal/mol and 111.9°C, 16.5

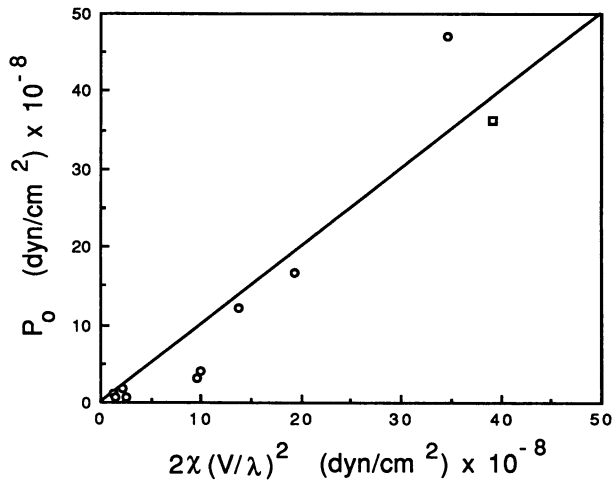

FIGURE 5 A plot of the magnitude of the hydration pressure (P_o) vs. the quantity $2\chi(V/\lambda)^2$. The open square represents data from DPPE-Me_2 and the open circles represent data for PC and PC/cholesterol bilayers taken from Simon and McIntosh (1989). The solid line is a plot of the theoretical prediction $P_o = 2\chi(V/\lambda)^2$.

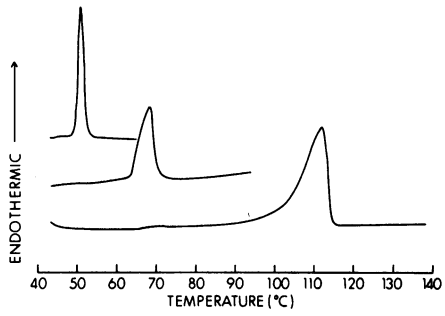

FIGURE 6 Differential calorimetric scans of DPPE-Me_2 at full hydration (*left trace*), at 10 wt% water (*middle trace*) and after vacuum drying (*right trace*). The weights of the three samples were different. The enthalpies are given in the text.

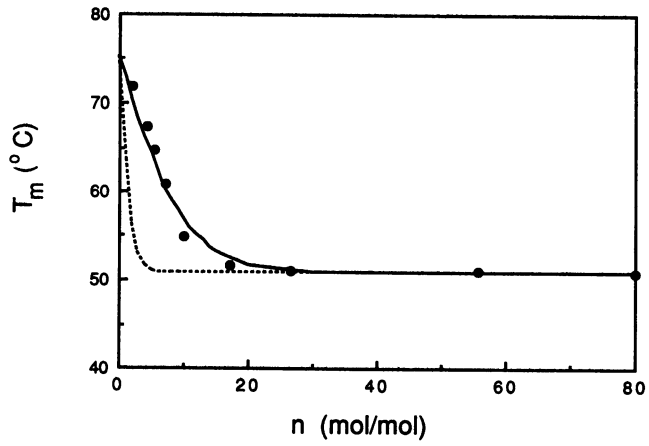

FIGURE 7 Transition temperature (T_m) vs. the number (n) of water molecules per DPPE-Me_2. The circles represent experimental values. The solid line is a fit to the data points using Eq. 1, which yields a value of $\lambda = 6.7$ Å. The dotted line represents a plot of Eq. 1 using $\lambda = 1.3$ Å, the value obtained from x-ray diffraction experiments. See text for details.

kcal/mol, respectively. The addition of 10 wt% water reduces T_m and ΔH_m to 67.3°C and 6.67 kcal/mol, respectively. These measurements show that the vacuum dried sample imbibed a little water. For fully hydrated DPPE-Me_2 one endothermic peak was observed over the range 10–80°C, with a transition temperature of $T_m = 50.8$°C and an enthalpy of $\Delta H_m = 9.6$ kcal/mol. The peak width at half height, $T_{1/2}$ was 1.4°C and the specific heat was C_p max = 10.1 cal/°K gm. The values of T_m and ΔH_m for fully hydrated DPPE-Me_2 are in good agreement with several previous calorimetric measurements (Vaughn and Keough, 1974; Casal and Mantsch, 1983; Mio et al., 1984; Gagne et al., 1985). However, Chowdhry and Dalziel (1985), using extremely pure samples and scanning at much slower rates, obtained smaller values of $T_{1/2}$ and larger values of C_p max then we or other investigators have measured.

The values of T_m for DPPE-Me_2 obtained at various states of hydration are presented in Fig. 7. We omit the point at zero water content, as the structure of DPPE-Me_2 may be different under these conditions (Seddon et al., 1984; Pascher and Sundell, 1986). The values of T_m of the main endothermic transition decrease with increasing water content. For the first several waters of hydration, T_m decreases abruptly with increasing water content. However, after ~10 water molecules per lipid are added, the decrease in T_m with water content becomes more gradual and finally asymptotically approaches the limiting value of 50.8°C.

DISCUSSION

Hydration pressure as obtained from x-ray diffraction data

Methylated phosphatidylethanolamines imbibe considerably more water than do phosphatidylethanolamines, making them more amenable for analysis of hydration pressures (Rand and Parsegian, 1989). As shown in Fig. 4, the total pressure between DPPE-Me_2 bilayers decays exponentially with increasing interbilayer separation. Over this range of applied pressures it has been previously shown that the major repulsive pressure is the hydration pressure (Parsegian et al., 1979). Therefore, P_h decays exponentially with increasing separation between bilayers, as it does for a variety of other lipids (LeNeveu et al., 1977; McIntosh et al., 1986*b*; Rand and Parsegian, 1989; McIntosh et al., 1989*a*, *c*). The measured decay length of 1.3 Å for DPPE-Me_2 is the same as the decay length for DPPC (see Fig. 4). This is somewhat smaller than the decay length of 1.8 Å obtained for liquid-crystalline phase egg PE-Me_2 bilayers (Rand et al., 1988). The reason for the observed difference in decay lengths between gel and liquid-crystalline dimethyl PEs is not known, although it is interesting to note that there is a similar difference in decay lengths between gel and liquid-crystalline phase PC bilayers (McIntosh and Simon, 1986*b*).

The equilibrium fluid spacing (the bilayer separation at zero applied pressure and full hydration) is 11.3 Å for DPPE-Me_2 bilayers, which is similar to the value of 11.7 Å obtained for DPPC bilayers (McIntosh and Simon, 1986*b*), but considerably larger than the equilibrium fluid spacing of 5 Å obtained for saturated PE bilayers (McIntosh and Simon, 1986*a*). Thus, in agreement with previous studies (Rand et al., 1988), we find that methylation of the PE head group increases the fluid spacing between apposing bilayers.

The data in Fig. 4 show that the hydration pressure is

very similar in both magnitude and decay length for DPPE-Me_2 and DPPC bilayers. This demonstrates that substitution of a methyl group in DPPC for a proton in DPPE-Me_2 has a very small effect on the magnitude and range of P_h.

As shown in Fig. 5, there is good quantitative agreement between P_o as obtained by x-ray diffraction and the quantity $2\chi(V/\lambda)^2$ calculated from measurements of Volta potential for both PC bilayers and for DPPE-Me_2 bilayers. It should be noted that, because of the exponential dependence of P_h on bilayer separation, the value of P_o obtained by x-ray diffraction depends critically on the choice of the plane of origin of the hydration pressure (Simon and McIntosh, 1989). For all of the bilayer systems represented in Fig. 5 we chose the plane of origin to be at the physical edge of the bilayer. As described in Simon and McIntosh (1989), this choice for the plane of origin was made for several reasons. One of the most important reasons is that all of the perpendicular components of the oriented dipoles, including those of both the lipid and water molecules in the head group region, contribute to the measured Volta potential. The location of the plane of origin at the physical edge of the bilayer takes into account the contributions of all of these dipoles. Our determination of the position of the edge of the bilayer makes the assumption that the DPPE-Me_2 head group is oriented parallel to the plane of the bilayer in hydrated liposomes. Although this assumption cannot be rigorously proven, we argue that the Volta potential data and the structural analysis of Dorset and Zhang (1990) make this the most probable head group orientation (see Results).

Analysis of calorimetry data

In their analysis of hydration-induced transition temperature shifts, Cevc and Marsh (1985) derived expressions for the hydration free energy, enthalpy, entropy, and transition temperature as a function of water content. They used each of these relations to calculate a value for the decay length, λ, of the hydration pressure, and obtained similar values of λ from each expression. In this paper, we consider only the observed change in transition temperature for several reasons. First, the transition temperature is the most accurately measured parameter from the calorimetry data. Second, the theoretical expression derived by Cevc and Marsh (1985) for the change in transition temperature with hydration contains only one unknown parameter, λ (see below), whereas the expressions for the change in free energy, enthalpy, and entropy contain other nonmeasurable parameters. Third, there are other potential problems encountered in analyzing the enthalpy of hydration, such as the presence of multiple peaks in the thermograms at low water contents. Although all of these peaks may contribute to the free energy of hydration, not all may be associated with the main gel to liquid-crystalline phase transition (Kodama et al., 1982) that was considered by Cevc and Marsh in their derivation of the expression relating enthalpy and water content. Moreover, some of these transitions, especially for PEs, may represent metastable or nonequilibrium states (Seddon et al., 1983; Xu et al., 1988).

Cevc and Marsh (1985) derived the following equation relating the decrease in transition temperature (ΔT_m) to the decay length, λ, of the hydration pressure:

$$\Delta T_m = T_m - T_{anhyd} = \Delta T_m(0) \cdot \tanh(nV_w/\lambda A), \qquad (1)$$

where n is the number of water molecules per lipid molecule, V_w is the molar volume of water (30 Å^3), A is the area per lipid molecule (which we assume is 45 Å^2), $\Delta T_m(0)$ is the difference in transition temperature at full hydration, and T_{anhyd} is the transition temperature for the anhydrous lipid. Because DPPE-Me_2 is in a different phase in the complete absence of water, we use an extrapolated value for T_{anhyd} = 75.3°C. As seen in Fig. 7, our observed transition temperatures as a function of water content can be fit quite closely with the expression given in Eq. 1. However, the best fit is obtained with a value of λ = 6.7 Å. Thus, the value of λ calculated using the calorimetry data and the formalism of Eq. 1 is considerably higher than the value of λ = 1.3 Å obtained from x-ray diffraction measurements. For comparison, the dotted line in Fig. 7 shows a plot of Eq. 1 using the value of λ = 1.3 Å obtained from the x-ray diffraction. For high water contents (n > 15), the two curves obtained with λ = 1.3 and 6.7 Å are quite similar. However, they are significantly different at lower water contents.

There are several possible reasons why λ obtained from x-ray diffraction and calorimetry are not in agreement. The most important factor is that most of the decrease in T_m shown in Fig. 7 occurs for low water contents. Approximately the first 10 water molecules added to the anhydrous bilayer partition into the polar head group region of the bilayer (McIntosh et al., 1987). The addition of this water increases the volume of the polar head group, wedging apart the acyl chains and thereby decreasing T_m. Because these first water molecules are located primarily in the head group region of the bilayer, and not between adjacent bilayers, the dominant interbilayer interaction for these low water contents is *not* the hydration pressure, but rather interactions that occur when polar groups from apposing bilayers are in contact, such as steric repulsion and attractive interactions between headgroups (McIntosh and Simon, 1986*a*; McIntosh et al., 1987; McIntosh et al., 1989*a*). In addition, there are several other impor-

tant intra- and intermolecular contributions to the transition enthalpy, such as rotational isomerism of the hydrocarbon chains, excluded volume interactions in the plane of the bilayer, and van der Waals interactions between hydrocarbon chains (Nagle and Wilkinson, 1978; Nagle, 1980). These in-plane interactions would be expected to be sensitive to the area per lipid molecule, and thus to the number of water molecules in the lipid head group region. Corrections for these additional pressures cannot easily be made when analyzing calorimetric thermograms. At higher water contents ($n > 10$), where the water partitions primarily between apposing head groups, the hydration pressure *is* the dominant repulsive interbilayer pressure. For $n > 10$, T_m would not be expected to change markedly with increasing water content, since both the area per lipid molecule (McIntosh et al., 1987) and ΔH_m remain approximately constant. Thus, fits to the transition temperature versus water content relationships (Fig. 7) using Eq. 1 are most sensitive to the low water content region where there is the greatest change in the packing of the acyl chains and where short range steric and attractive interactions dominate. In contrast, in the high water content regions, where hydration repulsion dominates, the wide-angle x-ray diffraction data and the electron density profiles (Fig. 3) show that there is no measureable change in acyl chain packing, and therefore very small measured changes in T_m (Fig. 7).

Therefore, although Eq. 1 correctly predicts the form of the T_m vs. n relation (Fig. 7) for DPPE-Me_2, it is difficult to use this expression to obtain the decay length of the hydration pressure.

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REFERENCES

Blaurock, A. E. 1971. Structure of the nerve myelin membrane: proof of the low-resolution profile. *J. Mol. Biol.* 56:35–52.

Blaurock, A. E., and C. R. Worthington. 1966. Treatment of low angle x-ray data from planar and concentric multilayered structures. *Biophys. J.* 6:305–312.

Boggs, J. M. 1987. Lipid intermolecular hydrogen bonding: influence on structural organization and membrane function. *Biochim. Biophys. Acta.* 906:353–404.

Casal, H. L., and H. H. Mantsch. 1983. The thermotropic phase behavior of *N*-methylated DPPEs. *Biochim. Biophys. Acta.* 735:387–396.

Casal, H. L., and H. H. Mantsch. 1984. Polymorphic phase behavior of phospholipid membranes studied by infrared spectroscopy. *Biochim. Biophys. Acta.* 779:381–401.

Cevc, G., and D. Marsh. 1985. Hydration of noncharged lipid bilayer membranes. Theory and experiments with phosphatidylethanolamine. *Biophys. J.* 47:21–32.

Chowdhry, B. Z., and A. W. Dalziel. 1985. Phase transition of 1,2- and 1,3-diacylphosphatidylethanolamines with modified head groups. *Biochemistry.* 24:4109–4117.

Dorset, D. L., and W. Zhang. 1990. Lamellar packing of a chiral *N,N*-dimethyl phosphatidylethanolamine: electron diffraction evidence for a lecithin-type headgroup conformation. *Biochim. Biophys. Acta.* In press.

Gagne, J., L. Stamatatos, T. Diacovo, S. W. Hui, P. L. Yeagle, and J. R. Silvius. 1985. Physical properties and surface interactions of bilayer membranes containing *N*-methylated phosphatidylethanolamines. *Biochemistry.* 24:4400–4408.

Hauser, H., I. Pascher, and S. Sundell. 1988. Preferred conformations and dynamics of the glycerol backbone in phospholipids. An NMR and x-ray single crystal analysis. *Biochemistry.* 27:9166–9174.

Kodama, M., M. Kuwabara, and S. Saki. 1982. Successive phase transition phenomena and phase diagram of the phosphatidylcholine-water system as revealed by differential scanning calorimetry. *Biochim. Biophys. Acta.* 689:567–570.

LeNeveu, 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.

MacDonald, R. C., and S. A. Simon. 1987. Lipid monolayer states and their relationship to bilayers. *Proc. Natl. Acad. Sci. USA.* 84:4089–4094.

Marcelja, S., and N. Radic. 1976. Repulsion of interfaces due to boundary water. *Chem. Phys. Lett.* 42:129–130.

Marra, J., and J. Israelachvili. 1985. Direct measurements of forces between PC and PE bilayers in aqueous electrolyte solutions. *Biochemistry.* 24:4608–4618.

McIntosh, T. J. 1980. Differences in hydrocarbon chain tilt between hydrated phosphatidylethanolamine and phosphatidylcholine bilayers: a molecular packing model. *Biophys. J* 29:237–246.

McIntosh, T. J., and S. A. Simon. 1986*a*. Area per molecule and distribution of water in fully hydrated dilauroylphosphatidylethanolamine bilayers. *Biochemistry.* 25:4948–4952.

McIntosh, T. J., and S. A. Simon. 1986*b*. The hydration force and bilayer deformation: a reevaluation. *Biochemistry.* 25:4058–4066.

McIntosh, T. J., and P. W. Holloway. 1987. Determination of the depth of bromine atoms in bilayers formed from bromolipid probes. *Biochemistry.* 26:1783–1788.

McIntosh, T. J., A. D. Magid, and S. A. Simon. 1987. Steric interaction between phosphocholine bilayers. *Biochemistry.* 26:7325–7332.

McIntosh, T. J., A. D. Magid, and S. A. Simon. 1989*a*. Cholesterol modifies the short-range repulsive interactions between phosphatidylcholine membranes. *Biochemistry.* 28:17–25.

McIntosh, T. J., A. D. Magid, and S. A. Simon. 1986*b*. Range of the solvation pressure between lipid membranes: dependence on the packing of solvent molecules. *Biochemistry.* 28:7904–7912.

McIntosh, T. J., A. D. Magid, and S. A. Simon. 1989*c*. Repulsive interactions between uncharged bilayers. Hydration and fluctuation pressures for monoglycerides. *Biophys. J.* 55:897–904.

Mio, M., M. Okamoto, M. Akagi, and K. Tasaka. 1984. Effect of *N*-methylation of phosphatidylethanolamine on the fluidity of phospholipid bilayers. *Biochem. Biophys. Res. Commun.* 120:989–995.

Mulukutla, S., and G. G. Shipley. 1984. Structure and thermotropic properties of phosphatidylethanolamine and its *N*-methyl derivatives. *Biochemistry.* 23:2514–2519.

Nagle, J. F. 1980. Theory of the main lipid bilayer phase transition. *Annu. Rev. Phys. Chem.* 31:157–195.

Nagle, J. F., and D. A. Wilkinson. 1978. Lecithin bilayers, density measurements and molecular interactions. *Biophys. J.* 23:159–175.

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.

Parsegian, V. A., R. P. Rand, N. L. Fuller, and R. C. Rau. 1986. Osmotic Stress for the direct measurement of intermolecular forces. *Methods Enzymol.* 127:400–416.

Parsegian, V. A., R. P. Rand, and D. C. Rau. 1985. Hydration forces: What next? *Chemica Scripta.* 25:28–31.

Pascher, I., and S. Sundell. 1986. Membrane lipids: preferred conformational states and their interplay. The crystal structure of dilauroylphosphatidyl, *N,N,*-dimethylethanoloamine. *Biochim. Biophys. Acta.* 855:68–78.

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.

Rand, R. P., and V. P. Parsegian. 1989. Hydration forces between phospholipid bilayers. *Biochim. Biophys. Acta.* 988:351–376.

Rau, D. C., and V. A. Parsegian. 1987. Measurement of forces between xanthan polysaccharides. *Biophys. J.* 51:503*a*. (Abstr.)

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.

Seddon, J. M., K. Harlos, and D. Marsh. 1983. Metastability and polymorphism in the gel and fluid phases of DLPE. *J. Biol. Chem.* 258:3850–3854.

Simon, S. A., and T. J. McIntosh. 1989. Magnitude of the solvation pressure depends on dipole potential. *Proc. Natl. Acad. Sci. USA.* 86:9263–9267.

Simon, S. A., T. J. McIntosh, and A. D. Magid. 1988. Magnitude and range of the hydration pressure between lecithin bilayers as a function of headgroup density. *J. Colloid Interface Sci.* 126(1):74–83.

Tocanne, J.-F., and J. Tessie. 1990. Ionization of phospholipids and phospholipid-supported interfacial lateral diffusion of protons in membrane model systems. *Biochem. Biophys. Acta.* 1031:111–142.

Vaughn, D. J., and K. M. Keough. 1974. Changes in phase transitions of phosphatidylethanolamine- and phosphatidylcholine-water dispersions induced by small modification in the headgroup and headgroup and backbone regions. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 47:158–161.

Vink, H. 1971. Precision measurements of osmotic pressure in concentrated polymer solutions. *Eur. Polym. J.* 7:1411–1419.

Xu, H., F. A. Stephenson, H.-n. Lin, and C.-h. Huang. 1988. Phase metastability and supercooled metastable state of diundecanoylphosphatidylethanolamine bilayers. *Biochim. Biophys. Acta.* 943:63–75.