Repulsive interactions between uncharged bilayers

Hydration and fluctuation pressures for monoglycerides

Thomas J. McIntosh,* Alan D. Magid,* and Sidney A. Simon‡
Departments of *Cell Biology, ‡Neurobiology, and ‡Anesthesiology, Duke University Medical Center, Durham, North Carolina 27710

ABSTRACT Pressure versus distance relations have been obtained for solid (gel) and neat (liquid-crystalline) phase uncharged lipid bilayers by the use of x-ray diffraction analysis of osmotically stressed monoglyceride aqueous dispersions and multilayers. For solid phase monoelaidin bilayers, the interbilayer repulsive pressure decays exponentially from a bilayer separation of ~7 Å at an applied pressure of 3×10^7 dyn/cm^2 to a separation of ~11 Å at zero applied pressure, where an excess water phase forms. The decay length is ~1.3 Å, which is similar to the value previously measured for gel phase phosphatidylcholine bilayers. This implies that the decay length of the hydration pressure does not depend critically on the presence of zwitterionic head groups in the bilayer surface. For liquid-crystalline monocaprylin, the repulsive pressure versus distance curve has two distinct regions. In the first region, for bilayer separations of ~3–8 Å and applied pressures of 3×10^8 to 4×10^6 dyn/cm^2, the pressure decays exponentially with a decay length of ~1.3 Å. In the second region, for bilayer separations of ~8–22 Å and applied pressures of 4×10^6 to 1×10^5 dyn/cm^2, the pressure decays much more gradually and is inversely proportional to the cube of the distance between bilayers. These data imply that two repulsive pressures operate between liquid-crystalline monocaprylin bilayers, the hydration pressure, which dominates at small (3–8 Å) bilayer separations, and the fluctuation pressure, which dominates at larger bilayer separations (>8 Å) and strongly influences the hydration properties of the liquid-crystalline bilayers. Thus, due primarily to thermally induced fluctuations, monocaprylin bilayers imbibe considerably more water than do monoelaidin bilayers. For both monoelaidin and monocaprylin, the measured magnitude of the hydration pressure is found to be proportional to the square of the dipole potential.

INTRODUCTION

Three distinct repulsive interactions are thought to exist between neutral phospholipid bilayers: (*a*) steric interactions between the bulky phospholipid head groups (1, 2), (*b*) solvation or hydration repulsion, due to the orientation of solvent molecules by the electric fields surrounding the lipid head groups (3–5), and (*c*) fluctuation pressure, due to thermally induced undulations or fluctuations in the bilayer surface (6–9). Total pressure versus distance relationships have been obtained for several phospholipid bilayer systems (3, 10–12). Although it is difficult to parcel the total pressure into its component pressures, we have argued in the case of phosphatidylcholine bilayers that steric hindrance between head groups is the dominant pressure at small (< ~5 Å) bilayer separations (1, 2), whereas hydration pressure, as enhanced by thermal fluctuations, provides the major repulsive barrier for fluid spacings >5 Å (1, 3, 4, 7). Thermally induced out-of-plane fluctuations have also recently been observed for the lamellar phase of the quaternary mixture of sodium dodecyl sulfate, water, pentanol, and dodecane (13).

To obtain additional information on the relative magnitudes of the hydration and fluctuation pressures, and on the dependence of these pressures on bilayer structure, we have investigated the repulsive interactions between bilayers composed of the monoglycerides monocaprylin (MC) and monoelaidin (ME). These monoglycerides were of interest for several reasons. First, their polar head group is smaller than the head groups of phospholipids such as phosphatidylcholine. Therefore, as compared with phospholipids, for monoglycerides the range of steric interactions should be smaller and hence the effective range over which hydration and fluctuation pressures can be studied should be larger. Second, monoglycerides are uncharged whereas phospholipids contain zwitterionic or charged head groups. It would be expected that the hydration pressure should be different for monoglycerides and phospholipids, considering the differences in hydration energy of dipoles and formal charges (14). Third, theoretical treatments of fluctuation pressures can be directly tested with MC and ME bilayers. That is, the analyses of Helfrich and colleagues (6, 8) predict that the magnitude of the fluctuation pressure is inversely proportional to the bending modulus of the bilayer. Because MC bilayers are fluid and significantly thinner than gel state ME bilayers, the bending modulus for MC should be orders of magnitude smaller than that of ME. This implies that the fluctuation pressure should be considerably larger for MC bilayers as compared with ME bilayers.

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Fourth, the glycerol-water head group region of the monoglycerides has a relatively high static dielectric constant, and therefore should not contribute appreciably to the attractive van der Waals pressure (15–18). This makes the analysis of van der Waals interactions more straightforward for monoglycerides as compared with phospholipids. Fifth, understanding the hydration properties of monoglycerides is significant in itself, because these surfactants are important in fat digestion in the intestine and as emulsifiers in the processing of various fats (19).

In this paper, by the use of x-ray diffraction analysis of bilayers brought together by applied osmotic pressure, we have measured pressure versus bilayer separation for liquid-crystalline phase MC and gel phase ME bilayers. For both MC and ME bilayers we find that the hydration pressure has a decay length of ~1.3 Å, which is very similar to that found for gel phase phosphatidylcholine bilayers (11). Moreover, for both MC and ME bilayers we find that the magnitude of the hydration pressure is approximately equal to $2\chi(V_d/\lambda)^2$, where χ is the dielectric susceptibility, V_d is the dipole potential, and λ is the measured decay length. Our data also indicate that the fluctuation pressure is much larger for MC bilayers than for ME bilayers, and demonstrate the importance of thermal undulations in the hydration properties of liquid-crystalline membranes.

MATERIALS AND METHODS

Poly(vinylpyrrolidone) (PVP), dextran, and the monoglycerides 1-monooctanoyl-rac-glycerol (monocaprylin) and 1-mono-[(*trans*)-9-octadecenoyl]-rac-glycerol (monoelaidin) were used as obtained from Sigma Chemical Co., St. Louis, MO. Triple-distilled water was used to make PVP-water solutions and dextran-water solutions in the range of 0–50% wt/wt. Osmotic pressures in the range of 1.1×10^5 to 3.2×10^7 dyn/cm^2 were applied to unoriented MC and ME suspensions by the "osmotic stress" procedures of Parsegian, Rand, and colleagues (3, 20, 21). In brief, an excess amount (usually 80% by weight) of the appropriate dextran or PVP solution was added to the dry lipid. The suspensions were covered with nitrogen and incubated for several hours with periodic vortexing at temperatures (20°C for MC and 40°C for ME) such that the lipid was in its liquid-crystalline or neat phase (19, 22, 23). Because dextran and PVP molecules are too large to enter between the lipid multilayers, they compete for water and thereby compress the lipid lattice (3, 4). Osmotic pressures for the dextran and PVP solutions were calculated from the virial coefficients obtained by Vink (24). These extrapolated pressures are in close agreement with values measured by Parsegian et al. (20) and by us (unpublished results). The lipid-polymer suspensions were sealed in quartz-glass x-ray capillary tubes and mounted in a point collimation x-ray camera.

Vapor pressures in the range of 2.8×10^7 to 2.2×10^9 dyn/cm^2 were applied to oriented MC and ME multilayers by published procedures (1, 4). The oriented specimen was formed by placing a small drop of monoglyceride-chloroform solution on a piece of aluminum foil and slowly evaporating the chloroform. The foil substrate was given a convex curvature and mounted in a controlled humidity chamber on a line-focused single-mirror x-ray camera, where the x-ray beam was oriented at a grazing angle relative to the multilayers. The humidity chamber consisted of a canister with two Mylar windows for passage of the x-ray beam. The vapor pressure was controlled by means of a cup of saturated salt solution in the chamber. To speed equilibration, a gentle stream of nitrogen gas was passed through a flask of the saturated salt solution and then through the chamber. The ratios of the vapor pressure (p) of the various saturated salt solutions to the vapor pressure of pure water (p_0) have been measured (25, 26). The following saturated salt solutions were used to obtain the relative vapor pressures (p/p_0) indicated in parentheses: $CuSO_4$ (0.98), Na_2SO_4 (0.93), KCl (0.87), NH_4Cl (0.80), $NaNO_2$ (0.66), $CaCl_2$ (0.32), and $KC_2H_3O_2$ (0.20). The applied pressure is given by

$$P = -(RT/V_w)\, ln\, (p/p_0), \tag{1}$$

where R is the molar gas constant, T is the temperature (293° K for all experiments), and V_w is the molar volume of water (which was taken as its value in bulk solution).

To obtain information on the partial thickness of the lipid layer (d_l) and the partial thickness of the fluid layer (d_f), lipid-water phase diagrams were obtained following the method of Luzzati (27). Carefully weighed lipid-water mixtures were allowed to incubate for several hours at 20 and 40°C for MC and ME, respectively. The specimens were then sealed in capillary tubes and mounted in a point collimation x-ray camera. The partial lipid and fluid thicknesses were calculated from

$$d_l = \{cv_l/[cv_l + (1 - c)v_f]\}d \tag{2}$$

and

$$d_f = d - d_l, \tag{3}$$

where c is the weight fraction of lipid, v_l and v_f are the partial specific volumes of lipid and water, respectively, and d is the lamellar repeat period. For these calculations v_f was set equal to 1.0 and values of v_l = 0.95 and 0.90 were used for MC and ME, respectively. The average area available per lipid molecule at the lipid/water interface was calculated from

$$A = 2Mv_l/d_lN, \tag{4}$$

where M is the molecular weight of the lipid and N is Avogadro's number.

For all specimens, oriented multilayers and unoriented lipid-polymer-water and lipid-water suspensions, x-ray diffraction patterns were recorded on Kodak DEF 5 x-ray film. X-Rays were obtained from a Jarrell-Ash Microfocus generator, and exposure times were between 2 and 8 h. All patterns were recorded at 20°C.

For dipole potential measurements, monolayers of ME or MC were formed by spreading 10–40 μl of a lipid-chloroform solution (25 mg/ml) onto 1 mM KCl in a Teflon trough with a surface area of ~30 cm^2 as described previously (28). To insure that the surface was free of surface active impurities, the KCl was roasted at 600°C and the subphase was vacuum aspirated immediately before the monolayer was spread. The trough was emptied and thoroughly cleaned between runs. The dipole potential was measured between a Ag/AgCl electrode in the suphase and a polonium electrode in the air which were connected to an electrometer (model 602, Keithley Instruments, Inc., Cleveland, OH). The reported values of dipole potential represent the difference in the potential of the subphase surface in the presence and absence of the monolayer.

RESULTS

For MC specimens over a broad range of applied pressures and water contents, the x-ray diffraction patterns consisted of two low-angle reflections, which indexed as orders of a lamellar repeat period, and a broad wide-angle band with a spacing of ~4.5 Å. These patterns are typical of bilayers in the liquid-crystalline (29) or neat (22) phase. For the MC-polymer-water suspensions, the lamellar repeat period (d) varied from 43.7 Å in 5.1% PVP to 29.0 Å in 50.5% dextran. For the oriented MC multilayers, the repeat period varied from 28.8 Å at $p/p_0 = 0.98$ to 25.9 Å at $p/p_0 = 0.66$. For lower values of relative vapor pressure discrete lamellar reflections were not visible, and the low-angle pattern consisted of a single broad band centered at ~23.5 Å. Thus, for this entire range of applied pressures the lamellar repeat period for MC changes by ~28 Å, which can be compared with the 10 Å change in d observed for egg phosphatidylcholine multilayers over a comparable pressure range (1). The natural logarithm of applied pressure ($ln\ P$) is plotted versus d in Fig. 1. This plot consists of two distinct regions, one in the range $11 < ln\ P < 15$ and the other in the range $15 < ln\ P < 21$. The rate of decrease in repeat period with increasing pressure is significantly smaller in the higher pressure region than in the lower pressure region.

For ME specimens for a range of applied pressures $12.9 < ln\ P < 17.1$, diffraction patterns consisted of four to six low-angle reflections, which indexed as orders of a lamellar repeat period, and a single, sharp, wide-angle reflection at 4.18 Å. For this pressure range, the lamellar repeat periods varied from 63.4 to 58.9 Å, whereas the spacing of the wide-angle reflection remained constant to within 0.02 Å. For applied pressures $ln\ P > 17.1$, a different lipid phase was present, as the diffraction pattern consisted of a lamellar repeat period of 50 Å, and multiple sharp wide-angle reflections, with the two most intense reflections at 4.52 and 3.94 Å (data not shown). This second lipid phase was also observed occasionally after long (2–3–d) incubation periods at applied pressures $ln\ P < 17.1$. A plot of $ln\ P$ versus d for ME is also shown in Fig. 1.

Plots of d, d_l, and d_f as a function of water content for MC-water and ME-water suspensions are shown in Fig. 2, *A* and *B*, respectively. Several of the data points in Fig. 2 *A* are from the work of Larsson (19). As can be seen, our values for d, d_l, and d_f are in close agreement with Larsson's (19). In the case of MC, the repeat period increased monotonically with increasing water content up to a limiting value of ~50% water (Fig. 2 *A*). For higher water contents sharp lamellar reflections were not observed. Larsson (19) has presented arguments which indicate that this high water content phase consists of concentric bilayers with alternating water layers. Presumably at these high water contents the water layers are large enough and of variable thickness so that discrete reflections are not observed. In the case of ME, the lamellar repeat period increased with water content only up to a limiting value of ~18% water, whereupon the

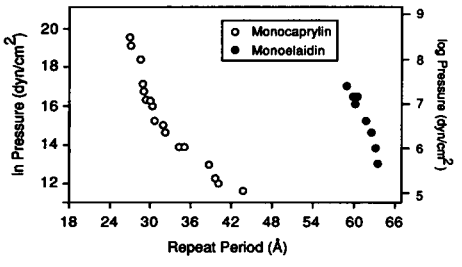

FIGURE 1 Natural logarithm (left scale) and common (base 10) logarithm (right scale) of applied pressure versus lamellar repeat period for MC bilayers (*open circles*) and ME bilayers (*solid circles*).

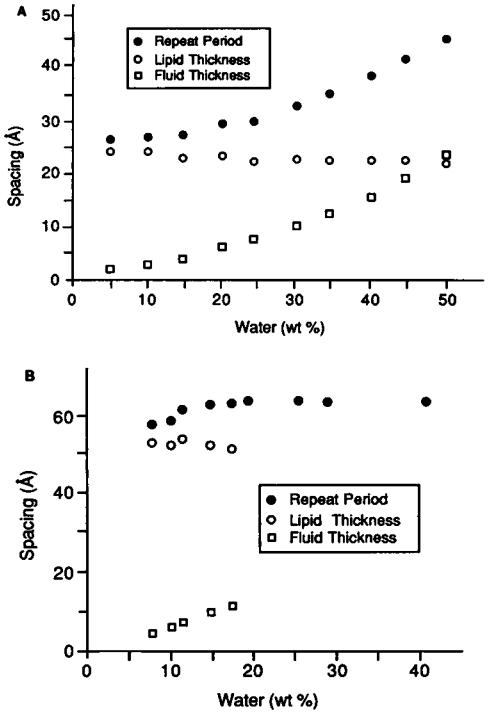

FIGURE 2 Plots of lamellar repeat period (*solid circles*), partial lipid thickness (*open circles*), and partial fluid thickness (*open squares*) versus water content for (*A*) MC bilayers and (*B*) ME bilayers. In *A* the values at 5, 10, 20, 30, 40, and 50% water are from Larsson (1967) and the other points are from the present study.

repeat period stayed nearly constant with further increases in water content (Fig. 2 *B*).

Following the procedure of LeNeveu et al. (3), we used the data of Fig. 2, *A* and *B*, to determine the partial fluid thickness for each value of d in Fig. 1. The resulting plots of $ln\ P$ versus d_f for MC and ME are shown in Fig. 3. For $ln\ P > 14$ the values of d_f for MC and ME are quite similar. However, for $ln\ P < 14$, the values of d_f are significantly larger for MC as compared with ME. For example, for ME bilayers the maximum value of d_f where an excess fluid phase forms is 10.9 Å, a width which could be spanned by about four water molecules. However, for MC bilayers d_f reached a value of about 22 Å before the discrete low-angle reflections could no longer be observed.

The area available per ME molecule at the hydrocarbon-water interface, as calculated from Eq. 4 and the d_1 values in Fig. 2 *B*, was found to be 20.3 ± 0.4 Å^2 (mean ± SD, five experiments). The area per lipid hydrocarbon chain was calculated from the wide-angle spacing to be (4.18 Å)$^2 \times 2/(3)^{1/2} = 20.2$ Å^2. Both the similarity of the calculated area per molecule and area per hydrocarbon chain, and the sharpness of the wide-angle reflection provide strong evidence (29) that the gel phase hydrocarbon chains for ME are oriented approximately perpendicular to the plane of the bilayer. For the case of MC, Larsson (19) has calculated, using his data shown in Fig. 2 *A*, that the area per MC molecule varies from 32.4 Å^2 at 50% water to 29.4 Å^2 at 5% water. Our values at intermediate water contents (Fig. 2 A) lie between these limiting values.

The dipole potential was measured to be 347 ± 2 mV (mean ± SE, $n = 2$ experiments) and 317 ± 13 mV ($n = 7$ experiments) for monolayers of ME and MC, respectively. These values are in close agreement with dipole potential measurements made with glycerol monooleate-decane monolayers (30).

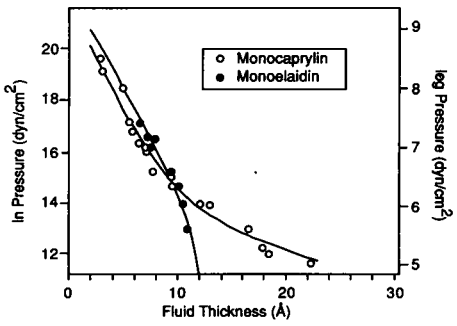

FIGURE 3 Natural logarithm (left scale) and common (base 10) logarithm (right scale) of applied pressure versus partial fluid thickness, d, for MC bilayers (*open circles*) and ME bilayers (*solid circles*). The solid lines represent the least squares fit to the data. For MC, $Pt = 1.8 \times 10^9 \exp(-d_f/1.3) + 2.4 \times 10^{-15}/d_f^3 - 1.6 \times 10^{-14}/6\pi d_f^3$ and for ME, $Pt = 5.1 \times 10^9 \exp(-d_f/1.3) - 1.5 \times 10^{-14}/6\pi d_f^3$.

DISCUSSION

The data in Fig. 3 represent the total pressure (P_t) as a function of fluid spacing for liquid-crystalline (MC) and gel phase (ME) monoglyceride bilayers. This total pressure is composed of several component pressures. Between the surfaces of electrically neutral bilayers, it has been demonstrated that there are three repulsive pressures—steric (P_s), hydration (P_h), and fluctuation (P_f)—as well as the attractive van der Waals pressure (P_v). Previous studies with phospholipid bilayers (1, 3, 4, 11) have found that P_s and P_h decay exponentially with increasing fluid spacings, whereas P_v has the functional form $P_v = -H/6\pi d_f^3$ (3), where H is the Hamaker constant. Recent theoretical analyses (6, 8) predict that P_f has the functional form $P_f = 2(kT)^2/Bd_{eff}^3$, where k is Boltzmann's constant, T is temperature, d_{eff} is an effective fluid spacing expected to be somewhat small than d_f (6), and B is the bilayer bending or curvature elastic modulus.[1] In these osmotic stress experiments, the total repulsive pressure is balanced by the sum of P_v and the applied pressure. The first task is to estimate the relative contributions of the three repulsive pressures for MC and ME bilayers for the range of fluid spacings shown in Fig. 3.

Let us first consider the case of ME bilayers. The plot of $ln\ P$ versus d_f (Fig. 3) is approximately linear for $14 < ln\ P < 17$, implying that a single exponential repulsive pressure dominates over this pressure range. Two lines of evidence indicate that, of the three possible repulsive pressures listed above, the dominant interaction is the hydration pressure. First, the relatively thick gel phase ME bilayers would be expected to have a very large bending modulus (7, 9, 31), implying that P_f should be negligible compared with P_h (9). Second, the following geometric considerations make it improbable that steric interactions can be an appreciable factor for most of the repeat period range shown in Fig. 1 for ME bilayers. An estimate for the total thickness of ME bilayers can obtained from the work of Kodali et al. (32), who measured repeat periods for two types of crystals for a series of saturated monoglycerides containing $n = 10$ to

[1] A different expression for P_f has been derived by Evans and Parsegian (7). However, that formalism is generally applicable for values of d_f less than the equilibrium separation (9), where P_h is the dominant interaction. In the case of MC bilayers, fluctuations occur at separations where P_v is comparable in magnitude to P_h. For this case, the formalism of Harbich and Helfrich (6) is more appropriate (9) and is used in our analysis.

22 carbons per hydrocarbon chain. One crystal form, the alpha form, has a single wide-angle reflection at a spacing similar to the value we found for hydrated ME. For this crystal form, Kodali et al. (32) found $d = 48.5$ Å for $n = 18$, with a chain tilt of 35 degrees relative to the bilayer normal. This implies that the maximum thickness for a bilayer with $n = 18$ with no chain tilt would be 48.5 Å/cos (35°) = 59.2 Å. Because the increment per CH_2 group is 1.25 Å and there are 18 carbons per chain, this implies that the total hydrocarbon thickness is $d_h = 45$ Å, and the thickness of two fully extended glycerol head groups is ~14.2 Å. (The *trans* double bond in ME would be expected to decrease the bilayer thickness by a fraction of an angstrom.) Comparing this value of 59.2 Å (for the maximum bilayer width) with the repeat periods considered in our analysis of 58.9 to 63.4 Å (Fig. 1), we conclude that steric interactions would be possible only for the two or three smallest values of d, and should not contribute appreciably for the larger values of d. We note that this estimate of 59.2 Å for the maximum bilayer width is considerably larger than the value of $d_l = 52.5$ Å obtained from Eq. 2 (Fig. 2 *B*). This occurs, at least partly, because the partial lipid thickness, d_l, will underestimate the total bilayer thickness if water penetrates into the lipid head group region (11).

From the above considerations, for ME bilayers the total pressure as a function of separation between bilayers may be written $P_t = P_h - P_v$. As noted by Evans and Parsegian (7), the planes of origin may differ for P_h and P_v, and there is no clear definition for any of these planes. Following their lead, we also assume that all pressures are measured relative to the same zero separation, and use the partial fluid thickness, d_f, for the distance between these planes of origin. (The assumption of common planes of origin is even easier to justify for monoglycerides than for phospholipids, due to the relatively small volume of the glycerol head group.) The energy of adhesion can then be calculated by integrating P_t and evaluating the energy at the equilibrium fluid separation, $d_f = 10.9$ Å (Fig. 3). The Hamaker constant H was estimated to be 1.5×10^{-14} erg by setting the calculated energy of adhesion equal to -0.05 erg/cm^2. This value of adhesion energy was chosen as it corresponds to the adhesion energy calculated for gel state phosphatidylcholine bilayers, based on adhesion experiments between gel and liquid-crystalline bilayers (David Needham, personal communication). From a least squares fit to the data ($r^2 = 0.970$) we find $P_h = 5.1 \times 10^9 \exp(-d_f/1.3)$. The total pressure is plotted in Fig. 3. The value of 1.3 Å for the decay length for P_h for ME bilayers is very similar to that found for gel phase phosphatidylcholine (PC) bilayers (11, 12). This similarity between ME and PC bilayers implies that the decay length is not dependent (or, at best, weakly dependent) on the type or surface density of zwitterions in a bilayer. This result is not in agreement with one theoretical treatment of the hydration pressure which predicts that the decay length should be strongly dependent on the zwitterionic density (33).

For applied pressures $>2.4 \times 10^7$ dyn/cm^2 ($ln\ P > 17$), the ME bilayers convert to a phase with crystalline hydrocarbon chains and a very small repeat period. This spontaneous dehydration could be due to hydrogen bonding between adjacent ME bilayers. A similar crystallization behavior has previously been noted for phosphatidylethanolamine bilayers (34).

The case of MC is somewhat more complicated to model, due to the break in the plot of $ln\ P$ versus d_f (Fig. 3). That is, for MC there are two distinct regions in the $ln\ P$ versus d_f graph, one for 3 Å $< d_f <$ 8 Å and the other for 8 Å $< d_f <$ 22 Å. This indicates that there are at least two repulsive pressures operating. For the first region, the data points for MC lie approximately parallel to those for ME. Thus, it appears likely that this region is dominated by the hydration pressure. To determine whether steric interactions could be a factor for the smaller measured values of d and d_f, it is necessary to estimate the total thickness for MC bilayers. The calculation of the total bilayer thickness for MC depends on additional assumptions, because there are no crystal data available for liquid-crystalline monoglycerides. To estimate the thickness per CH_2 group in MC bilayers, we multiply the incremental thickness in gel bilayers by the ratios of area per molecule obtained in ME and MC bilayers. This gives a value of 0.85 Å per CH_2 in MC bilayers, and a hydrocarbon thickness of $d_h = 13.6$ Å. By adding 14.2 Å for the width of two fully extended glycerol head groups, we obtain the maximum bilayer thickness for MC of 27.8 Å. Comparing this number to the range of d of 26.9 to 40.0 Å used in our analysis (Fig. 1), we conclude that steric interactions can only be appreciable for the lowest two or three values of d.

The fluctuation pressure is expected to be the dominant pressure in the second region (8 Å $< d_f <$ 22 Å) because P_f is expected to be longer ranged than P_h and would be expected to be large for a thin, fluid bilayer (6). That is, it has been predicted (6) that P_f is inversely proportional to the bilayer bending modulus, $B = (1/2)K(d_h)^2$, where K is the bilayer area compressibility modules. The area compressibility modulus is equal to $K = T(\Delta A/A)$, where T is the isotropic bilayer tension. The formalism of Parsegian et al. (4) relates T to the applied osmotic pressure, such that $T = -Pd_f$ for a bilayer. Therefore $K = Pd_f/\Delta A/A_o)$ and can be calculated using the pressure/fluid spacing data presented in Fig. 3 and the changes in area calculated from Eq. 4 for MC bilayers and determined from the wide-angle patterns for gel phase ME bilayers. For

the pressure range $13 < ln\ P < 20$, a plot of Pd_f versus $\Delta A/A_o$ gives $K = 147$ dyn/cm, a value similar to that measured for liquid-crystalline PC bilayers (31), and a value of $B = 1.4 \times 10^{-12}$ dyn cm, which is similar to the bending modulus of egg PC bilayers (35).[2] For the gel phase ME bilayers the change in area is much smaller. Based on the wide-angle diffraction data where the hydrocarbon spacing of 4.18 Å changes < 0.02 Å over the pressure range shown in Fig. 3, we estimate that $\Delta A/A_o$ is 2.3×10^{-5}. This gives $K > 7.7 \times 10^4$ dyn/cm and $B > 7.8 \times 10^{-9}$ dyn cm. Thus, B is over 3 orders of magnitude larger for ME than MC bilayers, so that based on the Helfrich analysis (6, 8) it is expected that the magnitude of P_f should be over 3 orders of magnitude larger for MC bilayers than for ME bilayers. Thus, the major difference in the data in Fig. 3 for ME and MC bilayers appears to be due to the presence of undulations in MC bilayers.

To model the MC data in terms of component pressures, we set $P_t = P_h + P_f - P_v = P_{ho} \exp(-d_f/\lambda) + 2(KT)^2/Bd_{eff}^3 - H/6\pi d_f^3$. Summing the component pressures in this manner makes explicit assumptions about the potential energy profiles and may not be strictly correct, but it should provide useful approximations for the relative magnitudes of the pressures. Another problem with this analysis is that the relationship between d_{eff} and d_f is not known, particularly at small values of d_f. In general, d_{eff} is smaller than d_f, but the difference between d_f and d_{eff} is thought to decrease as apposing bilayers are brought together (6). As a first approximation, we set $d_{eff} = d_f$ and vary the parameters P_{ho}, λ, and H to fit the experimental data shown in Fig. 3. The least squares ($r^2 = 0.968$) fit to the data gives $P_{ho} = 1.76 \times 10^9$ dyn/cm^2, $\lambda = 1.3$ Å, and $H = 1.6 \times 10^{-14}$ erg. This total pressure curve is plotted in Fig. 3. We note that this is a new method for determining the Hamaker constant, and does not depend on measurements of the energy of adhesion, but does depend on the value assumed for d_{eff}.

The primary difference between the curves for MC and ME bilayers (Fig. 3) is the presence of the long-range fluctuation pressure for the fluid MC bilayers. The presence of P_f is primarily responsible for the vast difference in hydration properties of MC and ME bilayers, with the MC bilayers taking up much more water at low applied pressures ($ln\ P < 14$). For instance, in excess water in the absence of any applied pressure, the fluid space for ME reaches a maximum value of ~11 Å, whereas the fluid space for MC bilayers is so large and variable that lamellar diffraction is not recorded.

It should also be noted that the effects of thermal undulations are larger for MC bilayers than for egg PC bilayers (7). The reasons for this difference are that, compared with egg PC bilayers, MC bilayers have a smaller attractive pressure (smaller Hamaker constant) and a larger fluctuation pressure due to a smaller bending modulus, B. Thus, over the same range of bilayer separations, P_f is larger than P_v for MC bilayers, whereas P_v is larger than P_f for egg PC.

The theoretical analysis of Cevc and Marsh (36) predicts that the magnitude of P_h should be proportional to the square of the bilayer hydration potential. We (2, 12) have argued that the dipole potential, as measured in a monolayer with the same area per molecule as the bilayer (28), provides a reasonable estimate for the hydration potential in the case of phospholipids. To test this hypothesis for monoglyceride bilayers, we write $P_h(d_f = 0) = 2\chi (V_d/\lambda)^2$, where χ is the dielectric susceptibility and V_d is the measured dipole potential (12, 20). Using our measured values for V_d and χ, we obtain values for $P_h(0)$ of 1.2×10^9 and 1.8×10^9 dyn/cm^2 for ME and MC bilayers, respectively. These values can be compared with our extrapolated values for $P_h(0)$ of 5.1×10^9 and 1.8×10^9 dyn/cm^2 for ME and MC bilayers, respectively (Fig. 3). The theoretical and experimental values are the same for MC bilayers and within a factor of 4 of each other for ME bilayers, which is in reasonable agreement considering the experimental uncertainty involved, particularly in defining the origin for the hydration pressure. For example, we note that if the origin of P_h had been chosen 0.9 Å farther from the bilayer center than given by the definition of the partial lipid thickness (Eq. 2), then exact agreement between the theoretical and experimental values of $P_h(0)$ would be obtained for ME. Therefore, for ME and MC bilayers the potential arising from the oriented dipoles of the lipid and its associated water can account for the magnitude of the hydration potential, and the magnitude of P_h.

The values of V_d for ME and MC are less than those measured for gel or liquid-crystalline phase phosphatidylcholines (28). Likewise, the values of $P_h(0)$ for ME and MC are less than those for phosphatidylcholines (3, 10, 21), when the partial fluid thickness (Eq. 3) is

[2]In their elegant x-ray analysis, Safinya et al. (13) obtained a value of $B = 2$–8×10^{-14} ergs for the quaternary microemulsion system of sodium dodecyl sulfate (SDS), water, pentanol, and dodecane. This value of B is 18–70 times smaller than our value for MC bilayers, and 30–115 times smaller than the value measured for egg PC bilayers (35). Because the hydrocarbon thickness of MC and SDS-water-pentanol bilayers are about the same, this implies that K for the latter system is in the range of 2–8 dyn/cm. This value is too low for a liquid-crystalline bilayer (28). In the presence of dodecane, where the SDS-pentanol-dodecane bilayer thickness swells to >100 Å (or more than twice the fully extended length of two SDS molecules), K would be <0.2 dyn/cm. For the quaternary microemulsion system, the small values of B, at least in the case of low dodecane concentrations, can be partially reconciled by use of the value of the bulk modulus $C = 7.3 \times 10^9$ dyn/cm^2, obtained by Liu and Kay (37), rather than the value of $C = 6 \times 10^7$ dyn/cm^2 used by Safinya et al.

used to define the interbilayer distance for both classes of lipids. For example, Rand et al. (21) determined $P_h(0) = 3.2 \times 10^{10}$ dyn/cm^2 for egg phosphatidylcholine bilayers. Thus, with regard to the hydration pressure, the major difference between surfaces composed of zwitterions and dipoles, such as phosphatidylcholine bilayers, and purely dipolar surfaces, such as monoglyceride bilayers, appears to be the magnitude of the electric field (proportional to V_d/λ) which orients the interbilayer water molecules.

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