Membrane dipole potentials, hydration forces, and the ordering of water at membrane surfaces

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ABSTRACT We have compared hydration forces, electrical dipole potentials, and structural parameters of dispersions of dipalmitoylphosphatidylcholine (DPPC) and dihexadecylphosphatidylcholine (DHPC) to evaluate the influence of fatty acid carbonyl groups on phospholipid bilayers. NMR and x-ray investigations performed over a wide range of water concentrations in the samples show, that in the liquid crystalline lamellar phase, the presence of carbonyl groups is not essential for lipid structure and hydration. Within experimental error, the two lipids have identical repulsive hydration forces between their bilayers. The higher transport rate of the negatively charged tetraphenylboron over the positively charged tetraphenylarsonium indicates that the dipole potential is positive inside the membranes of both lipids. However, the lack of fatty acid carbonyl groups in the ether lipid DHPC decreased the potential by (118 ± 15) mV. By considering the sign of the potential and the orientation of carbonyl groups and headgroups, we conclude that the first layer of water molecules at the lipid water interface makes a major contribution to the dipole potential.

INTRODUCTION

Ever since the identification and characterization of hydration forces as the dominant interaction between membranes or macromolecules approaching contact, there has been a serious need to identify those properties of the molecular surface that establish hydration. Even before, the organization of water around amphiphiles had been the subject of extensive spectroscopic and electrical study. Despite many efforts to investigate the structure of water in hydration shells, there is controversy over the structural order of water at surfaces. For example, if we compare the ^{2}H-NMR quadrupolar splitting of deuterated water at membrane surfaces with the expected quadrupolar splitting of perfectly ordered water, we end up with a tiny order parameter of $\sim$0.01–0.02 even for a first hydration shell. This is in apparent contradiction to the experimental observation that water molecules form hydrogen bonds with the more ordered lipid surfaces (Fookson and Wallach, 1978; Wong and Mantsch, 1988) or the theoretical prediction that water molecules near a polar surface are oriented by an electric field associated with the discrete surface charges (Kjellander and Marcelja, 1985a,b).

The question of water ordering at membrane surfaces is not only of interest from a spectroscopic viewpoint. Every water molecule has a dipole moment of $\sim$1.8 Debye (Smyth, 1955). Ordering or disturbance of the dipole moments of head groups or of the fatty acid carbonyls themselves, or of water rearranged around the lipid headgroups, all could contribute to electric potentials at the surface.

It is often noted that the electric fields bounding or traversing membranes can be comparable to those effecting dielectric breakdown of macroscopic materials. The clear implication is that one is dealing with fields capable of significant molecular rearrangement. Conversely though, it is insufficiently recognized that a relatively small degree of ordering of the water molecules could generate significant drops in electrostatic potential over relatively small distances.

In recent years water ordering has also been considered to be the source of hydration forces between membrane surfaces. One popular thought is that hydrated surfaces bear an effective polarizing or, better, ordering potential (Marcelja and Radic, 1976; Gruen et al., 1984; Cevc, 1987). Similarly, it has been suggested that the water near the surface is organized by the surface dipole potential (Simon and McIntosh, 1989). Good evidence has been given that there is a correlation between the coefficient of the exponentially decaying solvation force and the square of the dipole potential measured on monolayers of the same phospholipids (Simon et al., 1991). One still does not know whether water of hydration is oriented by electric fields from the surface or whether water of hydration is oriented by the constraints of hydrogen bonding.

The purpose of this paper is to evaluate the influence of lipid carbonyl groups on bilayer structure and hydration and to examine the relation between the strength of hydration repulsion between bilayers and the dipole potential. We have chosen two different phosphatidylcholines for these investigations, dipalmitoylphosphatidylcholine (DPPC) with ester bonds and dihexadecylphos-

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phatidylcholine (DHPC) with ether bonds between the glycerol and the fatty acids. The reason for that choice was that lipid carbonyl groups are thought to contribute heavily to the formation of a membrane dipole potential (Flewelling and Hubbell, 1986). There is strong evidence by x-ray diffraction (Pearson and Pascher, 1979) and IR-spectroscopy (Wong and Mantsch, 1988) that the carbonyl group of the *sn*-2 chain is directed towards the water phase. The carbonyl group is a strong electrical dipole with the positive charge located on the carbon atom. Lipid carbonyls are in a region of low polarizability, and can thus make large contributions to the membrane dipole potentials.

Differences in dipole potentials between ester and ether lipids of the order of 100 mV were reported in the literature from monolayer and bilayer measurements (Paltauf et al., 1971; Pickar and Benz, 1978). We have measured (*a*) dipole potentials using the trans-bilayer conductance of lipophilic ions, (*b*) hydration forces using x-ray diffraction and osmotic stress, (*c*) ^{31}P-NMR of phospholipids to compare lipid headgroup conformations and mobilities, and (*d*) ^{2}H-NMR of deuterated water to determine an order parameter of water O-^{2}H bonds as a function of sample water concentration and temperature.

If lipid carbonyl groups contribute to the hydration of lipid membranes to any significant extent, we expect a measurable difference in the repulsive hydration force between bilayers of the two lipids. Parallel NMR investigations on the same lipids under similar experimental conditions should indicate differences in lipid headgroup ordering and the water structure.

MATERIALS AND METHODS

L-α-dipalmitoylphosphatidylcholine (DPPC) was obtained from Avanti Polar Lipids, Inc. (Pelham, AL) and L-α-dihexadecylphosphatidylcholine (DHPC) from Fluka (Buchs, Switzerland). A part of the NMR measurements at higher water concentrations were performed with DL-α-dihexadecylphosphatidylcholine, which was a kind gift of Dr. M. V. Anikin (Institute of Fine Chemical Technology, Moscow).

We verified lipid purity by thin layer chromatography. All products were judged to be at least 98% pure.

NMR measurements

The ^{31}P- and ^{2}H-NMR experiments were performed on a Bruker MSL-300 spectrometer (Karlsruhe, Germany) using a high power probe with an 8-mm solenoidal sample coil which was doubly tuned for an X-nucleus and protons.

^{2}H-NMR spectra were observed at a resonance frequency of 46.073 MHZ using a quadrupolar echo sequence with phase cycling as described by Davis et al. (1976). An interpulse delay of 100 μs and a recycle delay of 250 ms or 1 s were chosen.

Gated broadband decoupled ^{31}P spectra were observed at a resonance frequency of 121.513 MHz with a phase cycled Hahn echo sequence, as described by Rance and Byrd (1983). A delay time between the 90° and 180° pulse of 100 μs was chosen. Typically 1,024 scans with a recycle delay time of 1 s were accumulated.

Sample temperatures were adjusted by a Bruker temperature control unit, and measured by a digital thermometer, the sensor of which was placed close to the solenoid coil.

Quadrupolar splittings, ^{31}P-NMR anisotropies of chemical shift, and relative intensities of overlapping resonance signals of different phases were determined by a lineshape fitting procedure using the sum of mean square deviations between measured and calculated intensities as an indicator for the quality of the fit. Most of the experimental spectra were analyzed using methods based on the analysis of a set of calculated spectra.

The lipids were dried over phosphorus pentoxide in an evacuated desiccator before sample preparation. Between 50 and 100 mg of the lipid were filled into short sample tubes of 8 mm diameter. The exact amount of lipid was determined with balances. To prevent the uptake of water by the dried lipid via the air humidity, sample preparation was performed in a glove bag (Instruments for Research and Industry, Cheltenham, PA) filled with dry nitrogen. The glass tubes were sealed with Parafilm and constricted above the lipid in a flame. Heavy water, 99.6% deuterated (Cambridge Isotope Laboratories, Woburn, MA), was added with a microsyringe through the constriction which was flame sealed afterwards. The amount of water added was checked by weight. The sample contents were homogenized by backward and forward centrifugation.

Before the measurements the samples were stored for several days at a temperature of 60°C.

X-ray measurements

Lamellar phase structural parameters

Water content was set by weighing dry lipid and 2 mM TES buffer into small weighing bottles. These bottles were then sealed and allowed to equilibrate for ~2 d at room temperature (~20°C). No water loss was detected before the next step: mounting the hydrated lipid into x-ray sample holders to be further equilibrated at the temperature of measurement. The lipid was combined with a little powdered teflon (for camera calibration), then sealed between mica windows 1 mm apart. We used x-ray diffraction to characterize the structures formed and to measure their lattice dimensions. The x-ray camera was of the Guinier type operating in vacuo, using the Cu(K_α) line (λ = 1.54 Å) isolated by a bent quartz crystal monochromator. Diffraction was recorded photographically. The temperature was 50°C maintained to ±0.5°C using thermoelectric controls. In this study, only lamellar phases were observed. Their diffraction lines corresponded to those of a single dimension, the repeat spacing d, which could be measured to an accuracy of ±0.1 Å.

Data are given in the form of the relation between the measured water content of the lamellar phase and the x-ray repeat spacing d. We have chosen the unit cell to contain one phospholipid molecule, of volume L, plus the measured volume of water, V_w, per lipid molecule in the lamellar phase. The quantity

$$\phi = 1/(1 + (1 - c)\, v_w/c \cdot v_l)$$

is the volume fraction of lipid, where c is the weight fraction lipid in the phase; v_l and v_w are the partial specific volumes of lipid and water, each taken as 1.0 cm^3/g, accurate to within 1.5% of the values measured over the entire hydration range for egg PC (White et al., 1987).

The repeat spacing can be converted to the thickness of the bilayer, d_l, taken as a layer that contains all the lipid and only the lipid in the sample, and the distance d_w equal to the thickness of a layer that contains all the water. This division of the repeat spacing follows the

Luzzati tradition (Luzzati and Husson, 1962; Luzzati, 1968) of using the mass average thicknesses based on measured sample composition. Then

$$d_l = \phi\, d \quad \text{and} \quad d_w = (1 - \phi)\, d.$$

Interbilayer forces

Forces between bilayers in the multilayer array were directly measured using the osmotic stress technique as described elsewhere (Parsegian et al., 1986). The multilayers were equilibrated against polyethylene glycol (PEG, MW 15,000–20,000; Sigma Chemical Co., St. Louis, MO) solutions of known osmotic pressure. PEG solutions were dialyzed extensively against distilled water to remove any residual salts and osmotic pressures were measured directly on a membrane osmometer. The resulting x-ray repeat spacing at each PEG osmotic pressure was determined by x-ray diffraction.

Bilayer thickness, d_l, and bilayer separation, d_w, for the osmotic stress data make use of the independently measured compressibility of bilayers and have been derived as previously described (Rand and Parsegian, 1989). Briefly, the single bilayer thickness d_l^* at $\log P^* = 7$ is calculated from the corresponding d spacing using the Luzzati definition as described above. The compressibility modulus, K dyn/cm, measured by Evans and Needham, (1987) is then used to calculate bilayer thickness, d_l, and separation, $d_w = d - d_l$, for all the osmotic stress experimental points where $\log P < 8$. We have assumed K, measured as 145 dyn/cm for DMPC in the liquid crystalline phase, is the same for DPPC and DHPC in the liquid crystalline phase. The bilayer thicknesses and separations are then derived as follows. K is the fractional change in area for a change in bilayer tension T, and is equal to $\Delta T/\Delta A/A_0$. For osmotic stress, changes from P^* to P cause changes in lateral tension, $\Delta T = (P - P^*)d_w$. The fractional change in area $\Delta A/A_0 = -\Delta d_l/d_l^* = (d_l^* - d_l)/d_l^*$ for constant lipid molecular volume.

Hence,

$$d_l/d_l^* = 1 + (P - P^*/K)d_w,$$

and since $d = d_l + d_w$, then

$$d_l/d_l^* = (K + (P - P^*)d)/(K + (P - P^*)d_l^*),$$

from which can be derived for each d and P, the d_l and d_w.

Dipole potential measurements

Planar phospholipid bilayers were prepared on a 1.5-mm hole in a teflon partition separating two Lucite chambers by the Mueller-Rudin technique (Mueller et al., 1963). The membrane-forming solution was a 2% solution of either DPPC or DHPC in *n*-decane. Good solubility was achieved by gentle heating of the solvent. Membrane area was measured by microscopic observation. 3 ml of 0.1 M NaCl were added to each chamber, and the membrane-forming solution was applied with a sable-hair brush. After membrane thinning, the membrane conductance, g, was determined through stepwise changes in applied voltage, ΔV, and the resultant step in current, ΔI. Current is measured as previously described (Zimmerberg and Parsegian, 1986). Temperature was controlled by irradiating the entire Faraday cage in which the bilayer was situated with an infrared light source. The temperature was measured with a small temperature sensor in one of the chambers. After the measurement of the unmodified bilayer conductance, small amounts of either an aqueous solution of tetraphenylarsonium or a methanolic solution of tetraphenylboron was added symmetrically to both aqueous solutions bathing the bilayer. Conductance was then measured again with various steps in voltage. For tetraphenylarsonium, the steady-state currents at different manually applied voltages were measured using a chart recorder. For tetraphenylboron, the current relaxation curve in response to an electronically controlled step in voltage was measured using either an oscilloscope or by digitalization of the signal using a computer interface (Stimers et al., 1987). Both the time constant of the relaxation and the amplitude of the current after the capacitative transient were measured, and the initial current was obtained by exponential extrapolation (Andersen and Fuchs, 1975; Andersen et al., 1978). This initial current was used for the conductance calculation.

As expected, addition of either tetraphenylboron or tetraphenylarsonium to either DPPC or DHPC planar bilayers resulted in increased membrane conductance. Since these lipids are in the gel state at room temperature, experiments were performed well above the transition temperature, between 47 and 54°C. The variation of conductance was measured as a function of temperature to determine the effect of temperature control on experimental uncertainty of the final results. These temperatures also led to a high rate of evaporation. To correct for the effect of evaporation on solution ion concentration, the volume of the aqueous solutions was determined by weighing the chamber.

RESULTS

NMR

Samples containing between 1 and 40 water molecules per DHPC were prepared as described in Materials and Methods and investigated in the temperature interval from 20 to 80°C. In good agreement with results reported earlier for DPPC, the ^{31}P-NMR anisotropy of chemical shift of the phosphate groups is typically ~ -46 ppm in the liquid crystalline phase and decreases to values of ~ -53 ppm during the phase transition into the gel phase (see Fig. 1). In excess water DHPC enters the liquid crystalline lamellar phase at a temperature of $\sim$43°C. The phase transition temperature is higher at lower water concentrations.

The measured quadrupolar splitting of deuterated water in DHPC water dispersions is phase-state dependent too. The quadrupolar splittings of water in the liquid crystalline phase are bigger than the splittings in the crystalline phase at the same water concentration with a sudden jump between both values at the point of phase transition. Within the phases, the measured values were nearly temperature independent for temperature intervals of $\sim \pm 20$°C. That is in remarkable contrast to results of quadrupolar splitting of water in DPPC lamellar phases. With DPPC bilayers the quadrupolar splitting goes through a minimum at the phase transition between crystalline and liquid crystalline phases (Salsbury et al., 1972). Details of the temperature dependence and water concentration dependence of the NMR spectra will be presented in a separate publication.

In Fig. 2. the quadrupolar splittings of deuterated water measured as a function of water concentration in the liquid crystalline phase of DPPC and DHPC are compared. Also, the values measured in DPPC disper-

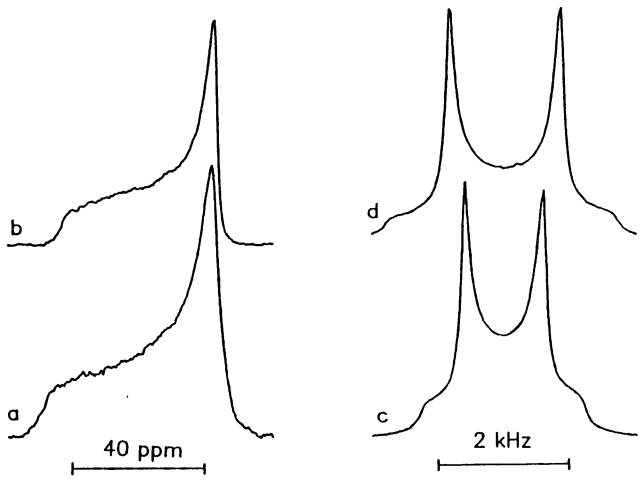

FIGURE 1 ^{31}P- and ^{2}H-NMR spectra of DHPC dispersed in heavy water. The water concentration is 10.2 water molecules per lipid. The absolute value of the ^{31}P-NMR anisotropy of chemical shift of DHPC phosphates is increased in the gel phase, while the quadrupolar splitting of deuterated water is decreased. (*a*) ^{31}P-NMR spectrum, liquid-crystalline phase (58°C), $\Delta\sigma = -47$ ppm. (*b*) ^{31}P-NMR spectrum, gel phase (38°C), $\Delta\sigma = -52$ ppm. (*c*)^{2}H-NMR spectrum, liquid-crystalline phase, $\Delta\nu = 1.7$ kHz. (*d*)^{2}H-NMR spectrum, gel phase, $\Delta\nu = 1.3$ kHz.

sions are smaller than those measured in DHPC dispersions, one has to keep in mind that both splittings are close to each other if compared with the value of completely immobilized water molecules.

Hydration forces

Fig. 3 shows the relation between the total repeat spacing and weight fraction of water of the multilamellar

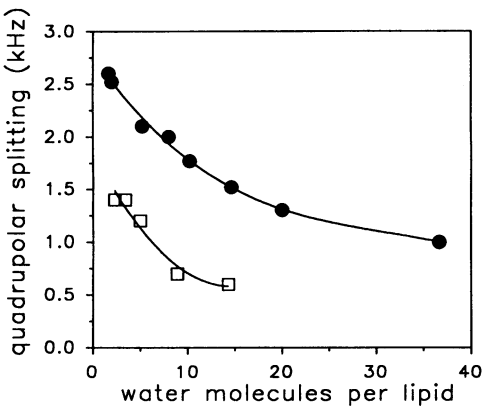

FIGURE 2 Quadrupolar splitting of deuterated water in DHPC/water dispersions (●) and DPPC/water dispersions (□) measured 10°C above the main chain melting temperature as a function of water content (DPPC data from Gawrisch et al., 1978).

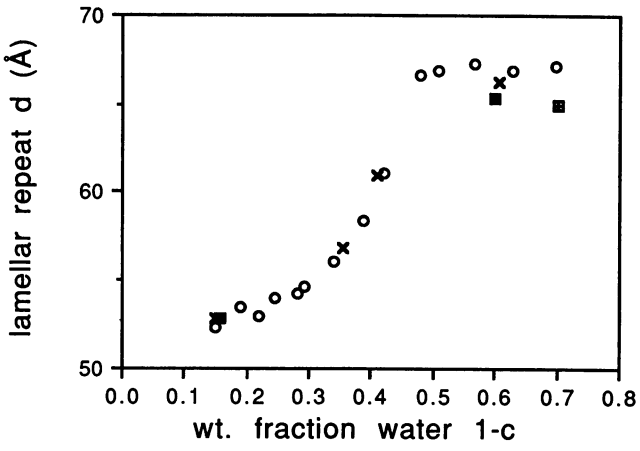

FIGURE 3 X-ray repeat spacing, *d*, as it varies with water content of the multilamellar phase of DPPC (■, ○) and DHPC (x) for 50°C. DPPC data is either previously published (Lis et al. 1982 (○)) or with new samples (■) as a confirmation. The symbol (□) represents a sample of both DPPC and DHPC comounted in excess solution and separated by a dialysis membrane. This shows that no detectable difference could be found in the lamellar repeat of these two lipids at full hydration.

phases formed by DPPC and DHPC in 2 mM TES buffer (pH 7.4) at 50°C. In order to determine whether the small differing seen between the two PCs result from sample-to-sample experimental errors we mounted DHPC and DPPC together, separated by a dialysis membrane in the same sample holder and in excess solution. The repeat spacings of DHPC and DPPC, measured under these conditions were indistinguishable.

Fig. 4 shows the relation between the osmotic pressure with which the lipid was equilibrated and the resultant x-ray repeat spacing. To avoid small differ-

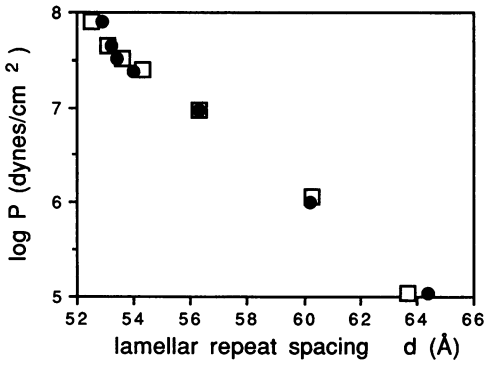

FIGURE 4 Interbilayer pressure, *P*, as it varies with bilayer separation, *d*, for DPPC (□) and DHPC (●) at 50°C.

ences of osmotic pressures DHPC and DPPC were often mounted together in the same sample holder.

In Fig. 5 the thickness of the water layer between DPPC and DHPC bilayers as a function of osmotic stress is given, where the water layer thickness is determined by the compressibility method described above. The data can be fitted by the equation

$$P = P_o \exp(d_w/\lambda),$$

where $\log P_o = 10.67 \pm 0.25$ (P in dyn/cm) and $\lambda = (2.20 \pm 0.28)$ Å for DPPC, and $\log P_o = 10.62 \pm 0.20$ and $\lambda = (2.24 \pm 0.18)$ Å for DHPC. Errors are the 95% confidence limits. We have had to assume equal compressibility moduli K for DPPC and DHPC even though they have not been directly measured. If there were a significant difference in lateral compressibilities, this ought to effect a detectable difference in coefficients P_o. However, such a difference is hard to reconcile with the stoichiometric data of Fig. 3, showing that all structural parameters are the same for both lipids.

Dipole potentials

To measure the dipole potentials of these two lipids, the transport of two hydrophobic and oppositely charged ions, tetraphenylarsonium and tetraphenylboron, were measured in response to steps in electrical field across unmodified lipid membranes. We measured the membrane current using a voltage-clamp technique. In each case, the resulting conductance was determined as a function of ion concentration (e.g., Fig. 6), temperature (Fig. 7), and voltage (Fig. 8). In order to use molar conductances as a measure of the concentrations of ions within the bilayer in the absence of both field effects and ion–ion interactions, we have to extrapolate the conductance to zero applied voltage and zero ion concentration. In practice, this was achieved by using the constant conductance in the linear region of the voltage/current relationship (Fig. 8) and the initial linear region of the ion concentration/conductance curve (Fig. 6). In the case of DHPC and tetraphenylboron, it was necessary to extrapolate the molar conductance linearly to zero concentration, because noise precluded measurements

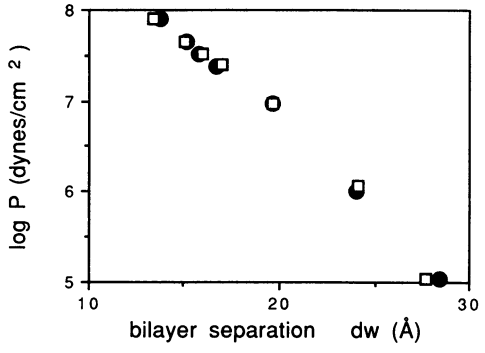

FIGURE 5 Interbilayer pressure, P, as it varies with bilayer separation, d_w, for DPPC (□) and DHPC (●) at 50°C. Bilayer separation was derived from the repeat spacing d and bilayer compressibility as described in Methods. The data are fitted by the line $P = 10^{10.67\pm0.25} \cdot \exp(-d_w/\lambda)$, $\lambda = (2.20 \pm 0.28)$ Å for DPPC, and $P = 10^{10.62\pm0.20} \cdot \exp(-d_w/\lambda)$. $\lambda = (2.24 \pm 0.18)$ Å for DHPC.

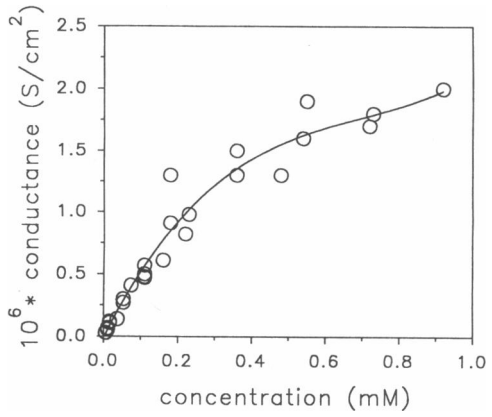

FIGURE 6 Specific conductance of a DHPC – BLM in 0.1 M NaCl as a function of $TPhAs^+$ concentration at 50°C. The membrane was formed from a n-decane solution of DHPC. A stock solution of 10 or 100 mM $TPhAs^+$ in ethanol was added in equal amounts to both sides of the BLM. Pulses of ±10 mV were applied via Calomel electrodes (Beckman) and the resultant currents were measured. The surface area of the bilayer was determined by use of an ocular retical in a microscope.

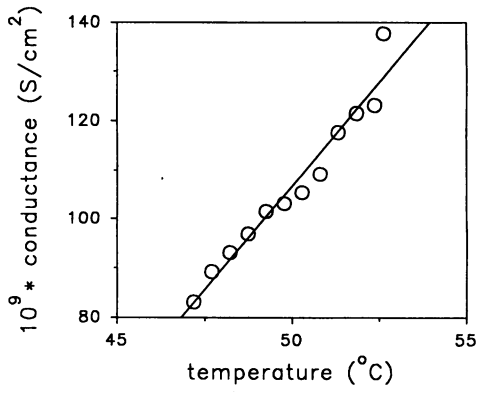

FIGURE 7 Specific conductance of DHPC BLM as a function of temperature in the presence of 16 μM $TPhAs^+$. Experimental conditions were identical to experiments in Fig. 6. Curves like this, prepared for each lipid-probe pair, were used to correct conductances to 50°C. It was assumed that the change in conductance with temperature given in a percentage relative to the conductance measured 50°C is the same for all concentrations.

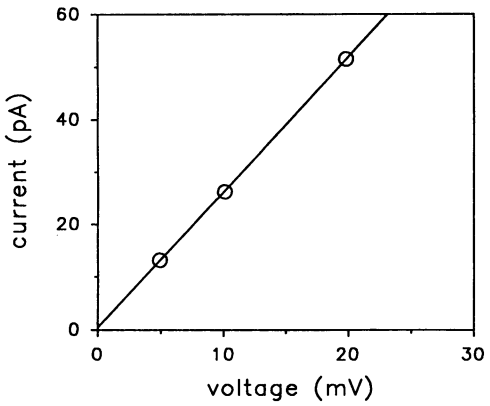

FIGURE 8 Current through BLM formed of DHPC in 0.1 M NaCl as a function of voltage at 50°C. The $TPhAs^+$ concentration is 36.4 μM. All other experimental conditions were identical to experiments in Fig. 6. The linear relations indicates that conductance is ohmic.

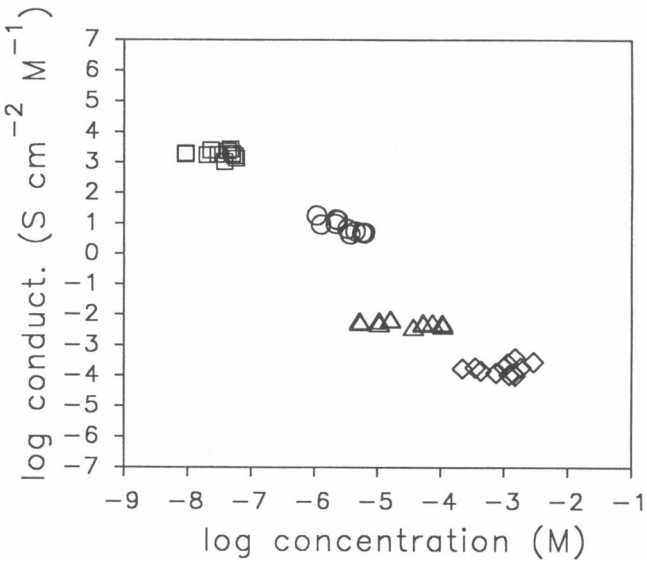

FIGURE 9 Conductance of DPPC and DHPC bilayers as a function of $TPhAs^+$ and $TPhB^-$ concentrations at 50°C. Assuming that the differences in membrane conductance are caused by different membrane concentrations of hydrophobic ions, the dipole potentials given in Table 1 can be calculated. It was supposed that the concentration of hydrophobic ions in the membrane depends solely on the dipole potential and the net charge of the hydrophobic ion. (□) DPPC + $TPhB^-$, (◇) DPPC + $TPhAs^+$, (○) DHPC + $TPhB^-$, (△) DHPC + $TPhAs^+$.

at lower concentrations of tetraphenylboron. To normalize for temperature, conductance was measured as a function of temperature at one concentration (Fig. 7), and subsequent measurements at particular concentrations and temperatures were adjusted to 50°C. For that purpose the temperature dependence of the conductance was renormalized, setting the conductance at 50°C to 100%. It was assumed that the slope of the curve does not depend on the concentration of the hydrophobic ion.

The linear data for all four lipid/ion pairs clearly show their molar conductances to be discretely separated over seven orders of magnitude (Fig. 9). For both lipids, transport of negatively charged tetraphenylboron was higher than that of positively charged tetraphenylarsonium. Assuming that the only difference between the two ions to be the charge, and the mobility of the ions in the bilayer to be the same, the interior of the bilayer must be positive with respect to the aqueous solutions (Andersen et al., 1976; Pickar and Benz, 1978; Ross et al., 1986). Using a Boltzmann distribution of concentrations to determine this internal potential,

$$\psi_d = \tfrac{1}{2}\frac{kT}{e}\ln\left(g_{TPhB^-}/g_{TPhAs^+}\right).$$

The magnitude of this internal, or dipole potential, is 227 mV for DPPC and 109 mV for DHPC bilayers drawn from decane/PC mixtures. g_{TPhB^-} and g_{TPhAs^+} are the specific conductances of the bilayer in the presence of the appropriate hydrophobic ion, extrapolated to low ion concentrations, and ψ_d is the dipole potential. By this measure, the difference in dipole potential between DHPC and DPPC is 227 − 109 = 118 mV.

It should be mentioned that the absolute values of the dipole potentials given above may contain contributions from differences in hydration energies of the positively and negatively charged ions and differences in potential energies inside the bilayer caused by other than electrostatic interactions. Differences in hydration energies might be caused by the slightly smaller covalent radius of the Boron atom (0.88 Å) in comparison with the Arsonium atom (1.18 Å) (see Pauling, 1960). Further, if quadrupolar terms of the electrical interaction between water molecules and ions are taken into consideration, even ions with identical covalent radii but different signs of charges have slightly different hydration energies (Bockris and Reddy, 1973). Coetzee and Sharpe (1971) observed slight differences in the nuclear magnetic resonance shift of solvent molecules in the presence of positively and negatively charged hydrophobic ions, which might be connected with a different arrangement of water molecules around these ions. Additional energies would show up as an additive constant in the dipole potential. For the calculation of differences of dipole potentials such an unknown constant would cancel. Therefore the differences should be considered as more accurate than the absolute values.

However, there is general agreement that the solvation of hydrophobic ions is determined mainly by the presence of the four phenyl groups and not by the

central charge. The assumption of the identity of hydration energies of positively and negatively charged ions is widely used in physical chemistry without causing any contradictions (see e.g., Krishan and Fridman, 1976; Flewelling and Hubbell, 1986). It has been pointed out by Cafiso and co-workers (Ellena et al., 1987) that at low concentration the two kinds of lipophilic ion sit essentially at the same position in the bilayer and will sense the same dipole potential.

DISCUSSION

For the lamellar liquid crystalline phases of ester-linked DPPC or ether-linked DHPC we have observed that there is: (*a*) no detectable difference in the bilayer structural parameters nor in hydration forces between bilayers (Figs. 3, 4 and 5) as measured by x-ray diffraction and osmotic stress; (*b*) a difference of 118 mV in the dipole potential inferred from bilayer transport measurements, 227 mV for DPPC but 109 mV for the ether-linked DHPC (Table 1); (*c*) identical ^{31}P-NMR anisotropies of the chemical shifts of the phosphate groups of both lipids (Fig. 1 and Arnold et al., 1981; Hauser, 1981a; (*d*) ^{2}H-NMR quadrupolar splittings of the order of 1 to 3 kHz for deuterated water at DPPC and DHPC surfaces (Figs. 1 and 2).

Water contribution to the dipole potential

The fact that the replacement of ester bonds by ether bonds yields no measurable influence on the hydration force in the liquid crystalline lamellar phase compels one to see that the influence of carbonyls on lipid hydration is minor in comparison with the influence of the rest of the parts of the polar group region. In contrast, for example, large changes in hydration are caused by the addition of one methyl group to the phosphatidylethanolamine headgroup (Gruner et al., 1988; Rand et al., 1988; Rand and Parsegian, 1989).

The similarity of structural data of DPPC and DHPC dispersions in the liquid-crystalline lamellar phase was observed in earlier experiments. Lohner et al. (1987) measured a slight decrease of d_w in DHPC bilayers in comparison with DPPC in the presence of excess water. Kim et al., (1987) observed an almost negligible compositional dependence of the repeat spacing in DHPC/DPPC mixtures in excess water with a tendency for DHPC having a slightly higher value (1.4 Å). Bigger differences between DHPC and DPPC dispersions were measured for lipid gel phases. In the gel phase the hydrocarbon chains are packed in a crystalline lattice and the difference in the linkage of chains to the glycerol becomes important. DHPC forms a chain-interdigitated gel phase at water concentrations higher than 30 wt% which is not observed in pure DPPC (Ruocco et al., 1985a; Haas et al., 1990). Smaby et al. (1983) observed packing differences between ester and ether phospholipids in monolayers. However, in the liquid crystalline bilayer phase the packing and conformation of ether and ester lipids are virtually identical (Hauser et al., 1981a,b; Ruocco et al., 1985b).

Both the dipole potential measurements on bilayers and monolayers (Paltauf et al., 1971) agree that the potential is positive inside the hydrocarbon region of membranes for DPPC and DHPC. Despite the fact that the absolute value of the potential is still not known with sufficient accuracy, the contribution of water molecules to the potential can be estimated.

The membrane dipole potential is a manifestation of a nonrandom orientation of the electric dipoles in lipid headgroups, fatty acid carbonyl groups and water (Fig. 10). According to x-ray data on crystalline PC (Pearson and Pascher, 1979) the carbonyl group of the *sn*-1 acyl chain is nearly in the plane of the bilayer, while the carbonyl group of the *sn*-2 chain is directed towards the water phase, with the positive charge inside the membrane. The strongest dipole in the lipids, 18.5 to 25 Debye for the PC headgroup, is oriented more parallel to the membrane surface (Seelig, 1978). Thus, the normal component of that dipole moment is reduced to a value of 3.0–9.5 Debye (Frischleder and Peinel, 1982) with the positive charge oriented towards the water phase. The anisotropies of chemical shifts of the phosphate groups of DPPC and DHPC are nearly identical at

TABLE 1 Results of dipole potential measurements

	$TPHB^-$	$TPhAs^+$	
DPPC	$g = (1.91 \pm 0.50)*10^3$ S cm^{-2} M^{-1}	$g = (1.59 \pm 0.51)*10^{-4}$ S cm^{-4} M^{-1} → $\psi_d = (227 \pm 9)$ mV	
DHPC	$g = (1.37 \pm 0.52)*10^1$ S cm^{-2} M^{-1}	$g = (5.44 \pm 0.10)*10^{-3}$ S cm^{-2} M^{-1} → $\psi_d = (109 \pm 6)$ mV	
	↓	↓	
	$\Delta\psi_{(DPPC\text{-}DHPC)} = (137 \pm 18)$ mV	$\Delta\psi_{(DPPC\text{-}DHPC)} = (98 \pm 10)$ mV	$\Delta\psi_{(DPPC\text{-}DHPC)} = (118 \pm 15)$ mV (average of all measurements)

(*g*) Bilayer conductivity. $\psi_d = kT/2e \ln (g_{TPhAs^+}/g_{TPhB^-})$. $\Delta\psi_{(DPPC\text{-}DHPC)} = \pm kT/e \ln (g_{DHPC}/g_{DPPC})$.

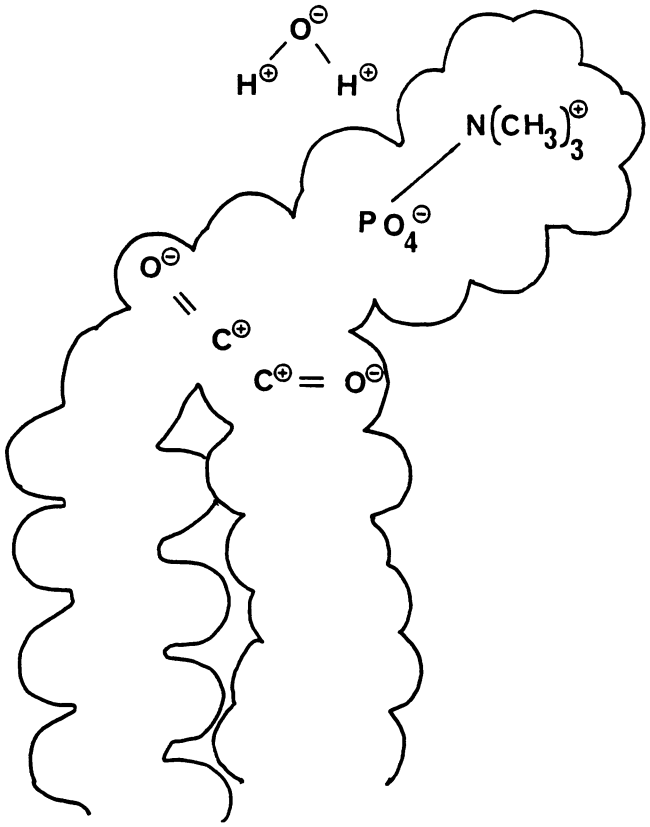

FIGURE 10 Orientation of electric dipoles of DPPC molecules and adjacent water molecules in lipid bilayers. In the case of DHPC, which lacks the carbonyl groups, the dipoles of water molecules have to overcompensate the normal component of phosphatidylcholine headgroups to result in a positive potential inside the membrane.

all water concentrations in the liquid crystalline lamellar phase. It has been shown (Akutsu and Seelig, 1981) that the anisotropy of chemical shift is sensitive to changes in headgroup orientation. Earlier NMR studies performed in excess water by Hauser et al. (1981a,b) and Ruocco et al. (1985b) did not reveal any significant structural difference between the headgroup regions of DPPC and DHPC either. That indicates that the presence of carbonyls had no measurable influence on the orientation of the lipid headgroups. It is reasonable to assume that the normal component of the headgroup dipole moment is essentially the same for both lipids. The larger dipole potential of DPPC vs. DHPC may be caused by the carbonyl dipoles of DPPC.

In the case of DHPC lacking the carbonyls, a positive potential inside the membrane requires that the normal component of the headgroup dipole be overcompensated by the sum of the dipole moments of water molecules surrounding the headgroup. That indicates that the sum of normal components of all dipole moments of the hydration shell per lipid molecule is of the order of 5–10 Debye. However, this quantitative estimation depends heavily on the knowledge of the normal components of the headgroup dipole and the value of the dipole potential. Water molecules were always considered to contribute to membrane dipole potentials (see e.g., Simon and McIntosh, 1989). To obtain a potential that is positive inside the DHPC membranes, the contribution caused by the orientation of water molecules has to be the determining one.

Water at membrane surfaces

If the dipole potential contains a major contribution from the existence of an ordered water layer, then by NMR spectroscopy we should see significant water ordering at the membrane surface. Such water ordering at membrane surfaces is reflected by a quadrupolar splitting of deuterated water. The splitting is caused by an interaction between the internal electric field gradients of the O–^{2}H bond of the water molecule and the electric quadrupolar moment of the deuterium nucleus. For immobile water molecules the splitting would be 166 kHz (Halle and Wennerström, 1981). Measured quadrupolar splittings are on the order of 1 or 2% of the value for completely immobile water only. This corresponds to a very low order parameter of the O–^{2}H bonds of water with respect to the magnetic field, apparently not high enough to justify a significant contribution of water to the dipole potential. One way to solve this contradiction is to assume that the water molecules perform rapid motions around their symmetry axis. The quadrupolar splitting can be given as

$$\Delta\nu = (3e^2Qq/4h)(3\cos^2\theta - 1),$$

where eQ is the quadrupolar moment of the deuterium nucleus, eq the internal electric field gradient of the water molecule, and θ the angle between the field gradient and the outer magnetic field. If the water molecules perform fast motions, around an axis that is a bisection of the bond angle of water (109°), the field gradient is transferred into a new coordinate system by the same Legendre polynomial of second order as given above. The O–^{2}H bonds of water would form an angle of 54.5° with the z-axis of this coordinate system which is almost the so-called Magic angle. Then a fast reorientation of water molecules around this bisection would reduce the measured quadrupolar splitting nearly to zero but it would not cause a similar reduction of the normal component of the electrical dipole moment.

The residual quadrupolar splitting would be determined primarily by small variations in the water bond angle. Additional exchange processes are responsible for further reductions. The measured values are a time

average over an observation time of at least 10^{-5} s. Thus, the motions of the lipid matrix, the fast diffusion of the water molecules within the aqueous space, and even the diffusion of water molecules through bilayers may cause a further reduction of the measured splittings. It has been shown that radii of curvature of bilayers up to 1 μm have an influence on the measured splittings, making ^{2}H-NMR of deuterated water sensitive also to changes in the superstructure of the lipid dispersions (Finer and Darke, 1974; Gawrisch et al., 1985).

We suggest that the fastest reorientation of water molecules at the membrane surface are flipping motions around an axis that is a bisection of the bond angle of water, the symmetry axis of the water molecule. As shown above, this flipping would reduce the measured quadrupolar splittings nearly to zero, but it would have no influence on the water contribution to the membrane dipole potential

Hydration forces and dipole potentials

Is there then any significant correlation between dipole potentials and the hydration force?

We consider a force with the empirical form

$$P_h = P_o \exp(-d_w/\lambda),$$

where d_w is the bilayer separation and λ a decay distance. We consider further the correlation between the coefficient P_o and the square of the dipole potential (Simon and McIntosh, 1989; Simon et al., 1991). For two systems with the same decay distance λ, a change in P_o will create a horizontal shift in the P_h vs. d_w curve of an amount

$$\lambda \ln(P_{o1}/P_{o2}) = \lambda \ln(\psi_1/\psi_2)^2$$

for different dipole potentials ψ.

The expected shift, then, for a decay distance λ = 2.2 Å and dipole potentials 227 and 109 mV is

$$2.2(\text{Å}) \times 2 \times \ln(227/109) = 3.2 \text{ Å}.$$

Such a shift, well within experimental accuracy, is not seen (Fig. 5). In fact, if there is any disparity in bilayer separations, DHPC with its lower bilayer potential seems to swell slightly more than DPPC. To this extent there does not seem to be good correlation between dipole potential as measured from ion transport and the hydration force.

Simon and McIntosh (1988) used dipole potentials derived from monolayer films spread at air/water interfaces to test for a correlation between dipole potentials and hydration forces. These potentials are some 200 mV greater than those seen directly on bilayers but differences between monolayer potentials are very close to those between corresponding bilayer potentials (Pickar and Benz, 1978). Were one then to add 200 mV to each of our bilayer-measured dipole potentials, one would expect a shift in the hydration force curve of only

$$2.2(\text{Å}) \times 2 \times \ln(427/309) = 1.4 \text{ Å},$$

which is just within experimental error of the measurements.

Both the monolayer- and bilayer-derived potentials suffer from unknown additive constants to the dipole potential. Potentials measured on monlayers will also reflect any polarization at the hydrocarbon air interface. The terminal CH_3 group can make a contribution with the reported value of 0.4 Debye, for the C^+–H^- bond (Bernett et al., 1964). Further, the "zero" of the monolayer–dipole potential is the pure water/air interface. There is a lot of controversy over the potential at a water/air interface (cf. Wilson et al., 1988).

The measurement of absolute dipole potentials with hydrophobic ion transport as a measure of ion partitioning into the bilayer is based upon assumptions too. First, that the ionic mobility within the bilayer is the same for both positively and negatively charges ions. Second, that the hydration energies of the two ions are identical. A critical discussion of the validity of this assumption was given in the Experimental Results section.

Despite the questions about the absolute values of dipole potentials, there is a rather good agreement about the influence of carbonyl groups on the dipole potential. The measured contribution of the two carbonyl groups is equivalent to an increase of the dipole potential of ~100 mV. If we follow the qualitative considerations in the paper of Flewelling and Hubbell (1986), a normal component of the dipole moment of carbonyls of ~1 Debye would be necessary to explain that increase.

At present, we tentatively conclude that there is no direct correlation between the dipole potential measured by ion transport and the hydration force, at least for the two lipid systems investigated here. The same conclusion can not be as easily reached if one uses the larger dipole potentials from spread monolayers. Recently, McIntosh and Simon obtained further experimental evidence for a correlation between dipole potentials measured on monolayers and solvation forces (T. McIntosh, personal communication). Grounds for agreement with our experiments might be found by recognizing that a significant contribution to the dipole potential is caused by the solvating molecules themselves. It is very likely that this contribution to the dipole potential and the hydration force parameters correlate with each other. Comparison between the two approaches must recognize the use of different methods to measure

dipole potentials and different conventions for defining the bilayer thickness. In the approach of Simon and McIntosh, a certain number of water molecules, which is roughly equivalent with a first hydration shell around the phospholipid headgroup, belongs to the lipid phase (Simon and McIntosh, 1989).

Rather than seeing the dipole potential necessarily as a cause of hydration forces, we suggest that the water contribution to the dipole potential and the hydration forces have the same molecular origin, the water structure of the hydration layer around lipid headgroups. Hydrogen bonding between lipid headgroups and water might be the ordering potential for this hydration layer.

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