Molecular origin of the internal dipole potential in lipid bilayers: calculation of the electrostatic potential

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ABSTRACT The finite difference linearized Poisson-Boltzmann equation was solved for a segment of bilayer for two lipids (phosphatidylcholine dihydrate and phosphatidylethanolamine-acetic acid) in order to obtain the transbilayer electrostatic potential. Atomic coordinates derived from the crystal structures of these lipids were used, and partial charges were assigned to all atoms in the polar parts of the molecules. These calculations confirmed that a dipole potential exists in the uncharged hydrophobic interior of a bilayer. The phosphocholine and phosphoethanolamine groups make negative contributions to the internal potential, and the glycerol acyl esters make positive contributions, but the sum of these terms is negative. The water of hydration in phosphatidylcholine, and the acetic acid which is present in the phosphatidylethanolamine crystal structure, make positive contributions to the internal potential. It is concluded that the water of hydration in fully hydrated lipid bilayers is mainly responsible for the experimentally inferred positive sign of the internal potential.

INTRODUCTION

The objective of this work is to determine the molecular origin of the internal dipole potential in lipid bilayers. This is a problem which has eluded solution by experimental means but can now be studied by using a computational approach.

It has been known for several years that the passive permeability of lipid bilayers is considerably greater for anions than for cations (Lieberman and Topaly, 1969; LeBlanc, 1970; Flewelling and Hubbell, 1986*a*). The bilayer translocation rate and partition coefficient are both several orders of magnitude larger for the anion tetraphenylboron (TPB^-) than they are for the structurally similar tetraphenylphosphonium (TPP^+) cation. Lieberman and Topaly (1969), as well as LeBlanc (1970), proposed that the observed differential permeability to anions and cations could be explained by an electrostatic potential in the interior of the bilayer which is positive relative to the aqueous medium. This proposal has since been generally accepted (Haydon and Hladky, 1972; McLaughlin, 1977; Flewelling and Hubbell, 1986*a*, *b*; Honig et al., 1986). LeBlanc (1970) estimated that an internal potential of 100 to 150 mV would account for his observations, while Flewelling and Hubbell (1986*a*) obtained a value of 240 mV from their measurements on fully hydrated phosphatidylcholine vesicles and planar bilayers.

The internal potential is a dipole potential since it is found in bilayers of zwitterionic lipids which bear no net charge. Its molecular origin has been a subject of some speculation, however. Haydon and Hladky (1972) suggested that oriented water molecules associated with the bilayer surface could produce a positive internal potential. McLaughlin (1977) listed three possible sources of an internal dipole potential: oriented water molecules, the lipid polar head groups, and the glycerol acyl esters; but he also remarked that "the origin of the dipole potential remains obscure". Honig et al. (1986) repeated the list of possible sources given by McLaughlin, and offered the opinion that the principal source is "probably the lipid carbonyls", while at the same time mentioning that the water and the polar head groups may also contribute. The importance of the water of hydration has been emphasized by Simon and McIntosh (1989).

The potential energy barrier for an ionic substance crossing a lipid bilayer can be described as a sum of several components. Ketterer et al. (1971) gave an expression consisting of two electrical terms (the Born and image energies) and an additional term to account for all other interactions. Flewelling and Hubbell (1986*b*) added an internal dipole term to the expression of Ketterer et al. (1971) thus obtaining the following equation:

$$W_{tot}(z) = W_B(z) + W_I(z) + W_N(z) + W_D(z). \quad (1)$$

$W_{tot}(z)$ is the mean potential energy of an ion as a function of its position along the normal to the bilayer plane. $W_B(z)$ and $W_I(z)$ are the Born and image (or polarization) energies, respectively; these terms account for the change in electrostatic energy which results when a charge is moved across an interface between regions of differing permittivity, but give no discrimination between positive and negative charges since they depend only the square of the charge. The term $W_N(z)$ accounts for other effects such as hydration, nonbonded energy, and hydrophobic interactions. $W_D(z)$ is the internal dipole potential which is proposed to exist in the nonpolar hydrophobic interior of the bilayer. $W_D(z)$ is the only term in Eq. 1 which is sensitive to the sign of the ionic charge; if the internal potential is positive, cations will tend to be excluded from the hydrophobic region as compared to anions, thereby causing a relative decrease in both the membrane/water partition coefficient and the bilayer translocation rate for cations.

An important assumption which is made in the application of Eq. 1 is that $W_N(z)$ is the same for the homologous molecules TPP^+ and TPB^-, so that differences in the total potential for the two ions can be ascribed to

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0006-3495/92/10/935/07 $2.00

their charge differences alone. It is unlikely that this assumption is rigorously correct, but it is probably a good approximation, and the body of available evidence strongly supports the qualitative interpretation that the sign of the charge of membrane permeant species has a significant effect on their rate of transport.

We have calculated the internal dipole potential of phosphatidylcholine and phosphatidylethanolamine bilayers by solving the finite difference linearized Poisson-Boltzmann equation (Davis and McCammon, 1989, 1990). This method permits the use of different values of the dielectric constant in different regions of space, and it takes account of the Born, image, and dipole terms included in the potential function given in Eq. 1. The calculations were carried out using atomic coordinates derived from the single crystal x-ray diffraction analyses of these lipids (Hitchcock et al., 1974; Elder et al., 1977; Pearson and Pascher, 1979). The structures in the crystals correspond to gel state lipid bilayers with little or no water present. For the present calculations, a finite segment of bilayer was generated by the application of symmetry operations to the atomic coordinates; the bilayer was bounded on either side by regions of high dielectric constant representing bulk water. Only the four water molecules in the asymmetric unit of phosphatidylcholine, and the acetic acid molecule in the phosphatidylethanolamine structure, were explicitly included in the calculations (i.e., in addition to the lipid molecules) since their coordinates were available from the x-ray analyses. The membrane transport data used to derive the experimental internal dipole potentials were obtained using liquid crystalline lipids in the fully hydrated state, however. Under those conditions, the number of hydrated water molecules is considerably larger than the number of discrete water molecules included in the computations; the surface area per molecule is also larger, and the molecular conformations may differ to some extent. On the other hand, the fundamental bilayer topology is the same in the computational model as in the experimental system, and the major features of the electrostatic potential should also be similar.

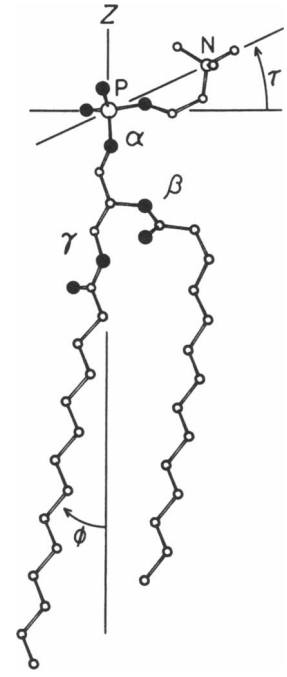

FIGURE 1 Diagram of DMPC showing the various quantities referred to in the text. The z axis is normal to the bilayer plane. τ is the angle between the P–N vector and the bilayer plane, and the chain tilt angle ϕ is measured between the bilayer normal and the mean acyl chain orientation. α, β, and γ denote the three ester groups on the glycerol backbone, being the phosphocholine head group and the two fatty acyl esters, respectively. Hydrogen atoms were omitted for clarity, and the oxygen atoms are filled.

METHODS

Atomic coordinates and partial charges

The multibilayer crystal structures of 1,2-dilauroyl-DL-phosphatidylethanolamine:acetic acid (DLPE:HAc) and of 1,2-dimyristoyl-sn-glycero-3-phosphocholine dihydrate (DMPC:2H_2O) have been determined by x-ray diffraction (Hitchcock et al., 1974; Elder et al., 1977; Pearson and Pascher, 1979; Hauser et al., 1981). The x-ray coordinates for these structures have recently been refined by energy minimization procedures (Vanderkooi 1990*a*, *b*; 1991), and the bilayer atomic coordinates obtained by the energy refinement were employed for the present electrostatic calculations. The overlap-normalized CNDO/2 atomic charges used for energy minimization were also used for the potential calculations; partial charges were assigned to all atoms in the polar head group, the glycerol acyl ester region, and the associated water or acetic acid molecules, but the atoms beyond C4 in the acyl chains were uncharged. Fig. 1 shows the structure of DMPC and the meaning of the various terms referred to in the text.

Translational symmetry operations were used to generate the coordinates of a bilayer segment consisting of a 5 × 5 block of unit cells, with both halves of the bilayer included. For DLPE:HAc each unit cell contained four symmetry related DLPE molecules and four HAc molecules. In the case of DMPC:2H_2O, the crystallographic asymmetric unit includes two nonequivalent DMPC molecules and four water molecules; there are two asymmetric units in the unit cell related by a twofold screw axis.

Electrostatic potential calculations

The linearized Poisson-Boltzmann equation was solved using a finite difference algorithm (Davis and McCammon, 1989, 1990). In this method, the molecular system of interest is placed within a three-dimensional rectangular grid, thereby dividing the molecular system into a large number of small cubic cells. The charges within each grid cell are assigned to the verticies of the grid in a prescribed manner. The equally spaced array of point charges which results gives rise to a sparse set of coupled linear equations (see Davis and McCammon, 1989). Grid cells containing atoms of lipid, explicit water molecules, or acetic acid are assigned a dielectric constant of 2, while empty grid cells outside of the bilayer are assigned a dielectric constant of 78, representing bulk water. The contribution of bound or partially immobilized water to the potential is underestimated by this approach since the lipid hydration number is considerably larger in the fully-hydrated state than it is in the crystalline state (see Discussion). The grid size employed in this work was 0.8 × 0.8 × 0.8 Å, with 86 grid cells in each direction. The zero of potential is taken as that in bulk water far from the surface of the bilayer.

The solution of the set of coupled differential equations yields the values of the electrostatic potential at each grid point. We are interested in the mean transbilayer potential calculated as a function of the z coordinate, which is normal to the bilayer plane. This value was obtained by averaging the potential over the xy grid points for a bilayer area corresponding to that of one crystallographic unit cell at the center of the bilayer fragment employed.

It is recognized that the results obtained using a finite fragment of a bilayer will not be equivalent to carrying out a calculation on an infinite array. The results obtained should give the essential features of the infinite array potential, however, since the unit cell from which the potentials are taken is surrounded on all sides by 15–20 Å of bilayer.

Calculations were carried out on a Silicon Graphics workstation and on the Cray Y/MP at the National Center for Supercomputing Applications at Urbana, Illinois.

RESULTS

Electrostatic potential calculations on DMPC:2H_2O

The two nonequivalent DMPC molecules in the DMPC:2H_2O crystal structure have significantly different polar group and glycerol ester conformations, and are offset one relative to the other with respect to the bilayer normal. This offset facilitates the packing of their rather bulky polar groups in the bilayer plane. The four nonidentical water molecules in the asymmetric unit are hydrogen bonded to the DMPC phosphate oxygens and to each other. In the x-ray structure, one of the water molecules makes a hydrogen bonded bridge between the head groups of adjacent bilayers (Pearson and Pascher, 1979). Minimization of the crystal energy of the multibilayer structure, starting from the x-ray structure, gave two minimized structures (I and II) corresponding to different local energy minima (Vanderkooi, 1991). Both minimized structures differed from the x-ray structure in that the acyl chain disorder found in the x-ray structure was removed, and structure II also differed in that no interbilayer water bridging was present. Structure II closely corresponded to the energy minimum for an isolated single bilayer. For these reasons the coordinates of structure II were used in the calculations reported here.

The mean transbilayer potential for DMPC:2H_2O is given as the solid line in the upper part of Fig. 2. A section of bilayer is shown at the bottom of the figure, drawn to the same scale as the graphs, from which the origins of the potential peaks and troughs in the polar regions may be inferred. The positive and negative peaks correspond to regions in which there is a preponderance of positive or negative charge, respectively. The number of peaks is larger than one might expect since the head groups of the two DMPC molecules in the asymmetric unit are at different levels in the bilayer. The apparent asymmetry between the potential profiles of the two halves of the bilayer is a spurious result arising from the fact that the potential was averaged over grid points for an area approximately equal to that of one crystallographic unit cell, but the crystallographic long axis runs at an angle to the bilayer normal whereas the potential

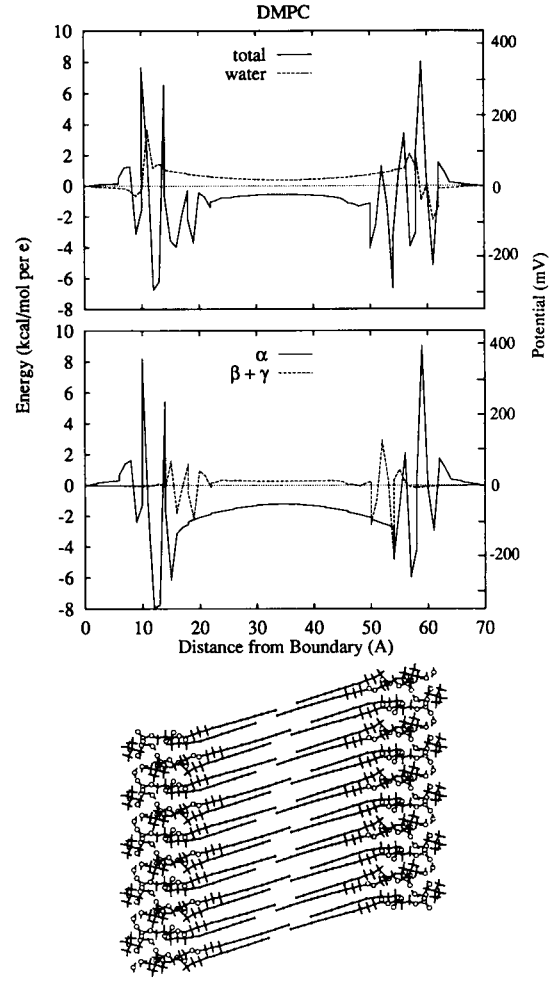

FIGURE 2 Electrostatic potential for DMPC:2H_2O. The total potential and the component contribution due to water are shown in the upper panel, and the contributions due to the α chain (phosphocholine) and the β and γ chains (glycerol acyl esters) are given in the lower panel. The bilayer projection at the bottom of the figure is drawn to the same scale as the graphs. The energy scale (*left ordinate*) gives the energy change for bringing a positive charge from a point far removed from the bilayer to a point within the bilayer. The right ordinate gives the mean potential in millivolts. The potentials are plotted as a function of the distance from the edge of the cubic array for which the calculations were performed.

averaging was over a segment taken at right angles to the bilayer plane; thus, a somewhat different assemblage of atoms was included in the averaging for the two sides of the bilayer.

The potential is nonzero across the entire central hydrophobic region of the bilayer, and is negative (Fig. 2). By contrast, in the space external to the bilayer surface, the potential is slightly positive but decays quickly to zero. The potential in both of these regions is of dipolar origin, arising from field effects of the partial charge distribution in the polar head group and bound water domain.

Separate calculations were carried out to determine the contributions of the various molecular segments to

TABLE 1 **Components of the electrostatic potential at the bilayer midplane**

	kcal/mol	mV
DMPC:2H_2O		
phosphocholine	−1.21	−52.7
glycerol acyl esters	0.26	11.5
water	0.40	17.5
Total	−0.55	−23.7
DLPE:HAc		
phosphoethanolamine	−1.07	−46.5
glycerol acyl esters	0.34	14.9
acetic acid	0.24	10.4
Total	−0.49	−21.2

the total potential. These calculations were made by setting the partial charges equal to zero on all atoms except those of the group in question. The upper part of Fig. 2 includes the potential due to the water molecules, and the lower graph shows the contributions of the α chain (phosphocholine group) and the β and γ chains (glycerol acyl esters). The sum of the potentials for these three groups equal the total potential. The internal potential component due to the phosphocholine group is negative, whereas that from the water of hydration and the acyl esters is positive. The values of the potential contributions at the bilayer midplane are given in Table 1.

Electrostatic potential calculations on DLPE:HAc

The calculations on DLPE:HAc were carried out for a single bilayer having the same structure as obtained by energy refinement of the multibilayer crystal structure (Vanderkooi 1990*a*, *b*). No water molecules were present in this crystal structure, and none were included explicitly in the calculations. Fig. 3 gives the total potential and the contribution of the cocrystallized acetic acid in the upper part, and the contributions of the α chain (phosphoethanolamine group) and the β and γ chains (glycerol acyl esters) in the lower part. The qualitative features of the DLPE potential are similar to those of DMPC; the phosphoethanolamine head group makes a negative contribution to the internal potential, and the glycerol acyl esters and the acetic acid make positive contributions, but the total potential remains negative in the central part of the bilayer. The values of the component potentials at the bilayer midplane are included in Table 1.

DISCUSSION

The computational results confirm that there is a nonzero dipole potential across the hydrophobic interior of a bilayer of zwitterionic lipids as a result of the partial charge distribution in the polar regions. Exterior to the bilayer surface, the potential falls to zero within less than 10 Å of the outermost bilayer atoms. This observation is in agreement with the conclusion drawn by Simon and McIntosh (1989) on the basis of hydration pressure measurements that the potential outside a bilayer drops quickly to zero.

Our calculations show that the positive contribution of the acyl ester groups to the internal potential is of insufficient magnitude to overcome the negative contribution due to the phosphocholine or phosphoethanolamine head group. Thus, the positive sign of the internal potential cannot be accounted for by the lipids alone. It seems evident that the water of hydration must be responsible for the positive sign of the internal potential; this result was not actually realized in the present calculations since the only water molecules explicitly included in the calculations were those for which coordinates were available from the crystal structure analysis.

Polar head group

The *P–N* vector in the polar head group does not lie parallel to the bilayer plane as has often been claimed, but makes an angle of 15° with the bilayer plane in

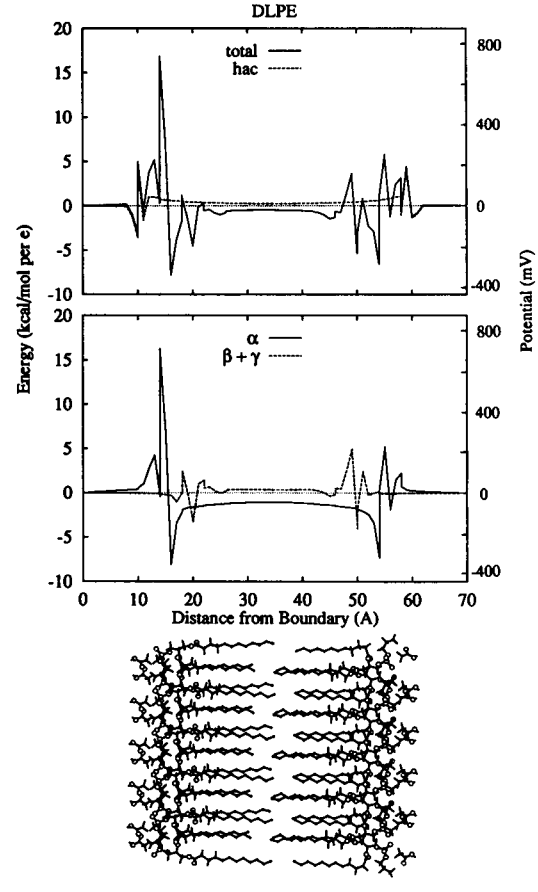

FIGURE 3 Electrostatic potential of DLPE:HAc. The total potential and the component potential due to the acetic acid are given in the upper part of the figure, and the component potentials for the α chain (phosphoethanolamine) and the β and γ chains (glycerol acyl esters) are given in the lower panel. See the legend to Fig. 2 for further details.

DLPE (τ in Fig. 1), and 9° and 25°, respectively, in the two independent DMPC molecules. This gives the result that the z component of the head group dipole is nonzero with the negative end of the dipole pointing toward the bilayer, thereby causing the head group contribution to the internal potential to be negative. The acyl esters make a positive contribution to the internal potential, but the magnitude of this contribution is only one quarter to one third that of the polar head group, as can be seen from Table 1. This result is obtained in spite of the fact that the acyl esters are closer to the hydrophobic core and are less shielded by the high dielectric constant external region than is the head group. The results obtained with DMPC and DLPE are essentially the same, and it seems clear that if one considers only the internal potential arising from the partial charge distribution on the lipid molecules alone, a negative value will be obtained.

Water

The cocrystallized water molecules in DMPC:2H_2O act as hydrogen bond donors to the phosphate groups and to other water molecules which are in turn hydrogen bonded to the phosphates, since there are no hydrogen bond donor groups on DMPC. The result is that the average orientation of the hydrated water molecules is with the H atoms pointing toward the bilayer, and this in turn is responsible for the positive dipole contribution of the water molecules to the internal potential. In fully hydrated DMPC the number of water molecules which are associated with and partially immobilized by the bilayer is considerably greater than that found in the crystalline dihydrate; by most estimates, 8–10 water molecules are associated with the DMPC head group under such conditions (Small et al., 1967; Kodama et al., 1982; McIntosh and Simon, 1986; McIntosh et al., 1987; Simon et al., 1991). The additional water molecules will not be bound as tightly as the two which are present in the crystal structure, but they will necessarily be oriented, on the average, with the positive ends of the O—H bonds pointing toward the bilayer, since DMPC can only act as a hydrogen bond acceptor. Thus, in the fully hydrated state the positive contribution of the interfacial water to the internal potential may be expected to be considerably greater than that calculated for the dihydrate. For example, if eight water molecules were to bind and make the same average contribution per water molecule to the internal potential as calculated for the dihydrate, the internal potential would become +29 mV, based on the data given in Table 1. We conclude that water of hydration holds the key to understanding the experimentally derived positive sign of the internal dipole potential. Simon and McIntosh (1989) have also argued on the basis of a variety of experimental observations that the surface potential of monolayers is dominated by contributions from the interfacial solvent.

The experimentally derived positive sign of the internal potential can be accounted for if allowance is made for the water in the fully hydrated state, but the magnitude of the potential estimated in this manner is still considerably less than the value of 100–240 mV which has been derived from transport data (LeBlanc, 1970; Flewelling and Hubbell, 1986*a*). We do not have an explanation for this, but may suggest that either the water contribution to the internal potential is larger than calculated, or else that the experimental values are overestimates of the true value. Other factors which may be expected to affect the internal potential are the molecular conformation, molecular orientation, and the packing density of the lipid molecules. Beitinger et al. (1989) consider that the methyl group dipoles are important for understanding the surface potential measurements of monolayers, but Simon and McIntosh (1989) label this contribution as small relative to the other terms involved. The dipole moment of the methyl group is certainly small as compared to that of the more polar parts of the molecule, and in the liquid crystalline state the degree of disorder experienced by the acyl chains near the bilayer midplane will cause the orientations of these terminal methyl dipoles to be fairly randomly distributed. Thus, we do not expect that the methyl groups will make a significant contribution to the internal dipole potential, but this possibility should be further investigated.

Effects of other molecules

The calculations on DLPE:HAc show that molecules other than water may have an appreciable effect on the internal potential. The HAc molecules in the DLPE:HAc structure are almost completely surrounded by bulk water (represented as a continuum of high dielectric constant) but still make a significant contribution to the internal potential.

Experimental observations on the effects of small molecules on bilayer permeability have been summarized by McLaughlin (1977). Salicylamide and phloretin cause the cation conductance of bilayers to increase and the anion conductance to decrease by the same proportion; salicylamide causes conductance changes by a factor of 20, and phloretin by a factor of 1,000 (Andersen et al., 1976). These molecules are weak acids, but they were shown to affect bilayer permeability in their electrically neutral states. The experimental results were interpreted to mean that the binding of these small molecules to the bilayer caused the internal potential to become less positive. Recent NMR studies by Bechinger and Seelig (1991) showed that the binding of phloretin to phosphatidylcholine vesicles resulted in conformational changes of the phosphocholine polar group and a displacement of bound water. The phloretin-induced change in the internal dipole potential implied by the ion transport measurements is therefore evidently the net result of the lipid structural effects noted by NMR, together with the

direct contribution to the internal potential of the large dipole moment of phloretin itself.

An extensive literature exists on the surface potentials of monolayers, and considerable progress has been made in extracting molecular dipole moments from these data (e.g., Beitinger et al., 1989), as well as in relating monolayer states to corresponding bilayer states (MacDonald and Simon, 1987). It will be a subject for future work to calculate the dipole moments of monolayers and to compare the results obtained with the monolayer literature.

In summary, we have shown that each of the three lipid bilayer components mentioned by McLaughlin (1977) makes a contribution to the internal potential, but the water of hydration must be largely responsible for the positive sign of the internal potential implied by the experimental observations, since the acyl ester contribution is too weak to overcome the much stronger negative head group internal potential.

ADDENDUM

Following the completion of this work, a copy of the paper by Gawrisch et al. (1992) was received. These authors used ion transport measurements to compare the internal potential of L-α-dipalmitoyl phosphatidylcholine (DPPC) with that of L-α-dihexadecyl phosphatidylcholine (DHPC). These lipids are structurally similar except that DHPC lacks the acyl ester groups which are present in DPPC. They found that the internal potentials of bilayers formed from both of these lipids are positive. The potential of DHPC was somewhat less positive than that of DPPC, indicating that the ester groups in DPPC do indeed make a positive contribution to the total potential, but the fact that the potential in DHPC is still net positive shows that there must be another positive contribution in addition to that of the ester groups. They conclude that the water of hydration makes an important contribution to the internal potential, and hence are in agreement with the conclusion arrived at in this paper.

This research was supported in part by a National Science Foundation Presidential Young Investigator Award to C. Zheng; by a computing time grant from the National Center for Supercomputing Applications at the University of Illinois at Urbana-Champaign; and by the Northern Illinois University Graduate School.

Received for publication 25 November 1991 and in final form 15 June 1992.

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