Eur Biophys J (2009) 39:103–110
DOI 10.1007/s00249-008-0392-y

ORIGINAL PAPER

Effect of headgroup on the dipole potential of phospholipid vesicles

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Received: 22 October 2008 / Revised: 1 December 2008 / Accepted: 8 December 2008 / Published online: 9 January 2009

Abstract The dipole potentials, ψ_d, of phospholipid vesicles composed of pure dimyristoylphosphatidylcholine (DMPC) or vesicles in which 50 mol% of the DMPC was substituted by dimyristoylphosphatidylserine (DMPS), dimyristoylphosphatidylglycerol (DMPG), dimyristoylethanolamine (DMPE), dimyristoylphosphatidic acid (DMPA) or monomyristoylphosphatidylcholine (MMPC) were measured via a fluorescent ratiometric method utilizing the probe di-8-ANEPPS. The PS and PG headgroups were found to cause only minor changes in ψ_d. PE caused an increase in ψ_d of 51 mV. This could be explained by a decrease in the dielectric constant of the glycerol backbone region as well as a movement of the P^-–N^+ dipole of the less bulky PE headgroup to a position more parallel to the membrane surface than in PC. The negatively charged PA headgroup increases ψ_d by 215 mV relative to PC alone. This indicates that the positive pole of the dipole predominantly responsible for the dipole potential is located at a position closer to the interior of the membrane than the phosphate group. The increase in the charge of the negative pole of the dipole by the phosphate group of PA increases the electrical potential drop across the lipid headgroup region. The incorporation of the single chain lipid MMPC into the membrane causes a decrease in ψ_d of 142 mV. This can be explained by a decrease in packing density within the membrane of carbonyl dipoles from the *sn*-2 chain of DMPC. The results presented should contribute to a better understanding of the electrical effect of lipid headgroups on the functioning of membrane proteins.

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Keywords Voltage-sensitive styryl dye · Fluorescence · Phosphatidylcholine · Phosphatidylethanolamine · Phosphatidic acid · Lysophosphatidylcholine

Introduction

The dipole potential, ψ_d, of a phospholipid membrane is an electrical potential that exists between the polar exterior of a membrane and its hydrocarbon interior (Liberman and Topaly 1969; Haydon and Myers 1973; Hladky and Haydon 1973; Brockman 1994; Clarke 2001). This potential difference arises from the orientation of polar lipid residues and water dipoles near the surface of the membrane. For saturated phosphatidylcholine (PC) membranes reported values of the dipole potential range from approximately 300 to 400 mV, which, because of the short distances over which it drops, results in electric field strengths in the range of 10^8–10^9 V/m (Clarke 2001; Schamberger and Clarke 2002). In these membranes, the hydrocarbon-like interior is at a positive potential relative to the surface. For comparison, a typical membrane potential of 100 mV across the entire membrane of 4 nm thickness results in an electric field strength of 2.5×10^7 V/m (Clarke 2001; Schamberger and Clarke 2002). Since it is known that field strengths of this magnitude can alter the conformation and orientation of membrane proteins, it is reasonable to expect that the much higher field strength caused by the dipole potential could also significantly influence the conformation and orientation, and hence the activity, of membrane proteins. Changes in the dipole potential, then, could induce changes in protein activity and thus serve regulatory purposes.

Several studies suggest that the dipole potential does in fact influence membrane protein function. Work in this

laboratory showed an increase in the molecular activity of the Na^+, K^+-ATPase (Starke-Peterkovic et al. 2005). Cladera and O'Shea (1998) studied the effects of the dipole potential on the insertion and folding of amphiphilic peptides in membranes and Maggio (1999) reported on the modulation of phospholipase A_2 by the membrane dipole potential. In addition to effects on membrane proteins, the dipole potential has also been shown to affect several other membrane processes such as the kinetics of redox reactions at membrane surfaces (Alakoskela and Kinnunen 2001), skin permeability (Cladera et al. 2003), membrane partitioning of anaesthetics (Alakoskela et al. 2004), and membrane fusion (Cladera et al. 1999).

Because of the difficulties of both experimentally measuring the dipole potential and of theoretically calculating it, its exact origin is still not entirely clear. Nevertheless, based on experimental and theoretical approaches it seems most likely that for PC membranes oriented water dipoles are the major contributor (Gawrisch et al. 1992; Zheng and Vanderkooi 1992; Shinoda et al. 1998; Mashl et al. 2001). It also seems clear that the carbonyl groups of the ester linkages between the headgroup and the hydrocarbon chains play an important role, because ether lipids lacking the carbonyl group have a much lower dipole potential (Gawrisch et al. 1992; Clarke 1997). The carbonyl groups may in fact contribute to the polarisation of the water dipoles by hydrogen bonding. In the case of PC it seems unlikely that the P^-–N^+ dipole of the lipid headgroup could make a positive contribution to the magnitude of the dipole potential, because, based on the chemical structure of the PC molecule (see Fig. 1), the negatively charged phosphate group would be expected to be closer to the membrane interior than the positively charged choline. Hence, the P^-–N^+ dipole would tend to make the dipole potential negative within the membrane interior, whereas in fact it is positive. Molecular dynamics simulations (Shinoda et al. 1998) also support a negative contribution to the dipole potential from the P^-–N^+ dipole, which according to their calculations is overcompensated for by a more positive contribution from oriented water dipoles. However, NMR studies (Semchyschyn and Macdonald 2004) have shown that the angle of the P^-–N^+ dipole moment relative to the membrane surface can vary depending on the surface charge of the membrane. Therefore, although the dipole of the PC headgroup alone cannot explain the positive polarity of the dipole potential, any changes in the angle of the P^-–N^+ dipole relative to the membrane surface would be likely to modulate its magnitude.

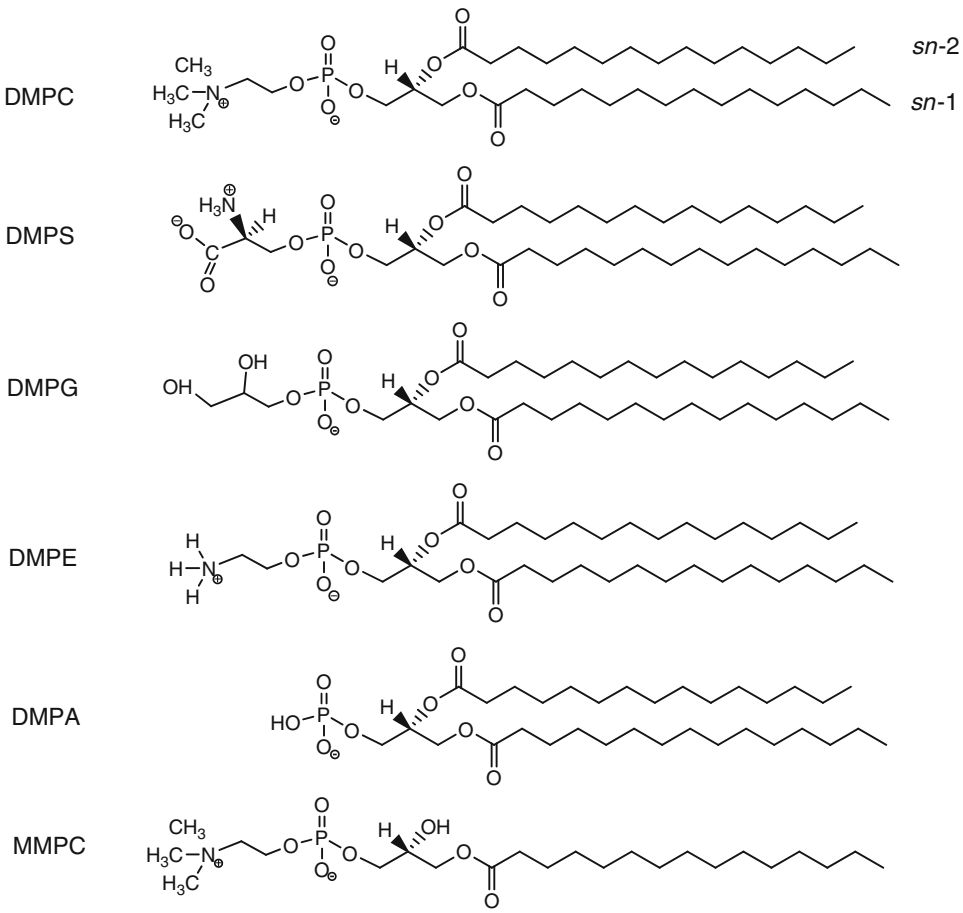

Fig. 1 Chemical structures of dimyristoylphosphatidylcholine (DMPC), dimyristoylphosphatidylserine (DMPS), dimyristoylphosphatidylglycerol (DMPG), dimyristoylphosphatidylethanolamine (DMPE), dimyristoylphosphatidic acid (DMPA) and monomyristoylphosphatidylcholine (MMPC)

The purpose of the work described here is to determine experimentally the effect that the chemical structure of the lipid headgroup has on the magnitude of the dipole potential. This will provide useful data for comparison with molecular dynamics simulations of phospholipid membrane bilayers (Shinoda et al. 1998; Mashl et al. 2001) and, thus, hopefully lead to a more accurate description of the membrane and its properties at an atomic level. Up to now most experimental work on the dipole potential has concentrated on derivatives of PC (Pickar and Benz 1978; Gawrisch et al. 1992; Clarke 1997). Gross et al. (1994) did investigate the effect of phosphatidylserine (PS) (see Fig. 1) on the dipole potential using the voltage-sensitive fluorescent probe di-8-ANEPPS, but found that exchanging the PC headgroup for PS had no effect, even though the PS headgroup is negatively charged in contrast to the zwitterionic PC. Here we apply the same fluorescent probe to investigate the effect of a range of lipid headgroups on the dipole potential. Although some, such as PS do not cause any major changes relative to PC, we show here that this is not generally the case. The lipid headgroup is, thus, an important factor in determining the magnitude of the dipole potential.

Materials and methods

All phospholipids used in this study were purchased from Avanti Polar Lipids (Alabaster, AL, USA) and were used as received. Ethylenediamine tetraacetic acid disodium salt (approximately 99%), and tris[hydroxymethyl]aminomethane (Tris, minimum 99.9%) were purchased from Sigma (Castle Hill, NSW, Australia) and used as received. Sodium chloride was from Merck (Darmstadt, Germany), 4-(2-(6-(dioctylamino)-2-naphthalenyl)ethyl-1-(3-sulphopropyl)-pyridinium inner salt (di-8-ANEPPS) was from Molecular Probes (Eugene, OR, USA), and concentrated hydrochloric acid was from Univar (Seven Hills, NSW, Australia). All were used as received. Purified water (18.2 MΩ) was obtained from a Millipore Direct-Q system.

Vesicle preparation: extrusion method

Large unilamellar lipid vesicles were prepared via the extrusion method. In this method, 0.024 g total of dimyristoylphosphatidylcholine (DMPC) plus other phospholipids were dissolved in 1 mL of chloroform. The chloroform was then removed via at least 2 h rotoevaporation at a slow rotation rate to form a thin lipid film on the walls of a roundbottom flask. Throughout the rotoevaporation the thermal bath was kept at a temperature of approximately 30°C, i.e. above the main phase transition temperature of DMPC of 23°C (Cevc 1993). The material was resuspended in 10 mL of buffer (preheated to 30°C) by allowing the material to rotate at high rotation rate on the rotary evaporator (without vacuum) for 1 h. The buffer was 30 mM Tris, 150 mM NaCl, 1 mM EDTA, and adjusted to pH 7.2 using hydrochloric acid. The suspension was vortexed for 30–60 s prior to extrusion in the next step. The suspension was then extruded through a 100 nm Nucleopore polycarbonate membrane a minimum of 11 times using an Avanti Mini-Extruder (Alabaster, AL, USA). Throughout the resuspension/hydration and extrusion process the temperature was maintained at 30°C. After extrusion the final phosphatidylcholine concentrations were usually in the range 2–4 mM. These were determined using a Phospholipid C test kit from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Dye solutions

A solution of dye was prepared by dissolving approximately 5 mg of di-8-ANEPPS in 9 mL of ethanol, which theoretically yields a concentration of 0.937 mM. The measured concentration was found to be 0.862 ± 0.09 mM using an estimate of the molar absorptivity (37,000 ± 4,000 M^{-1} cm^{-1} at 498 nm in methanol) provided by Molecular Probes on the certificate of analysis. For fluorescence measurements, 30 μL of this ethanolic dye solution were added to 4 mL of vesicle suspension. The ratio of the lipid to dye concentrations in these suspensions is approximately 450–500. The suspensions were vortexed for a few seconds and then allowed to remain at 30°C overnight for dye aggregates to dissociate and for dye monomers to insert themselves into the lipid membrane. No significant changes in the maximum wavelength of fluorescence emission are observed after 3 h. The effects of small volumes of ethanol added to these suspensions on the emission and excitation spectra were previously checked (Clarke and Kane 1997; Vitha and Clarke 2007) and found to be negligible in both cases. Furthermore, essentially tripling the total dye concentration in the final suspensions by adding 15 μL instead of 5 μL to a 1 mL aliquot of vesicle suspension was found to have no effect on the measured emission and excitation spectra, verifying that the dye is dilute enough that dye–dye interactions within the membrane are insignificant.

Fluorescence measurements of the dipole potential

Fluorescence measurements were performed on a Shimadzu RF-5301 PC spectrofluorophotometer. Quartz semi-micro cuvettes were used for all measurements. The temperature was maintained at 30°C using a circulating water bath connected to the sample holder. The emission

wavelength was set at 670 nm for all excitation spectra with an RG645 cutoff filter (Schott, Mainz, Germany) in the emission path. Excitation spectra were corrected for the wavelength dependence of the excitation monochromator's transmission efficiency using rhodamine B as a quantum counter. The excitation ratios, R_{ex}, are defined here as the ratio of the emission intensity at 670 nm caused by excitation with 420 and 520 nm light. The wavelengths were carefully chosen to avoid any effects of changes in membrane fluidity on the measured excitation ratios (Clarke and Kane 1997).

In all cases R_{ex} was obtained as an average of a minimum of five spectra recorded in succession. Based on a comparison of measured R_{ex} values with literature values of the dipole potential, we have previously established that an approximately linear correlation exists between R_{ex} and ψ_d (Starke-Peterkovic et al. 2005). This correlation is described by the following equation:

$$\psi_d = \frac{R_{ex} + 0.3}{4.3 \times 10^{-3}} \quad (1)$$

Based on least squares regression analysis of the correlation, the parameter values in this equation together with their associated errors are 4.3 ($\pm$1.2) $\times$ 10^{-3} mV^{-1} and 0.3 ($\pm$0.4). Using this equation, therefore, allows the magnitude of the dipole potential, ψ_d, to be estimated from experimental measurements of R_{ex}.

An assumption implicit in the use of Eq. 1 and the comparison of dipole potential values thus derived for different lipid environments is that the location of the probe is the same in all membranes. This would seem to be justified based on the probe's structure. Its hydrophilic sulphonate group and its hydrocarbon chains would act as anchors in the membrane-aqueous solution interface and the membrane hydrocarbon interior, respectively, which should limit translational movement into or out of the bilayer. However, rotational motion (within a cone with its axis perpendicular to the membrane surface) may still be possible. This could lead to differences in the R_{ex} value, because the molecular groups which give rise to the dipole potential (i.e. the head group and the esters of the glycerol linker region) are known to fluctuate over a finite width (Wiener and White 1992; Klauda et al. 2006). Time-resolved fluorescence anisotropy studies (Visser et al. 1995) have already been carried out on the related probe RH421 in DMPC vesicles, which would support such an idea. The rotational diffusion of the probe was described by a wobbling in a cone model (Kinosita et al. 1984) with a cone angle of 48–50° relative to the membrane normal. Whether there are any significant differences in the degree of allowed rotational motion of di-8-ANEPPS in the lipid membranes studied here is unknown at this stage.

Results and discussion

To determine the effect of different headgroups on the dipole potential of phospholipid membrane, vesicles were prepared as described under "Materials and methods" containing 50 mol% of DMPC and 50 mol% of either dimyristoylphosphatidylserine (DMPS), dimyristoylphosphatidylglycerol (DMPG), dimyristoylphosphatidylethanolamine (DMPE), dimyristoylphosphatidic acid (DMPA) or monomyristoylphosphatidylcholine (MMPC). The last lipid is also referred to as myristoyllysophosphatidylcholine. All of the lipids used, therefore, have an identical hydrocarbon chain length and they are all fully saturated. Therefore, any differences in dipole potential observed cannot be attributed to the chain length or degree of saturation. In an earlier study (Clarke 1997) the degree of saturation was shown to have a significant effect on the dipole potential via a change in the density of lipid packing and possibly the extent of water penetration. Comparisons between the dipole potential of DMPC and vesicles composed of 50% mixtures of DMPC and DMPS, DMPG, DMPE, or DMPA allow the effect of the lipid head group on the dipole potential to be deduced. The lipid MMPC has a single hydrocarbon chain, in comparison to the two chains of DMPC. Therefore, the results obtained with MMPC allow one to identify the effect of decreasing the number of chains per lipid. The structures of all the lipids used are shown in Fig. 1.

The fluorescence ratios obtained and their corresponding values of ψ_d are given in Table 1. There it can be seen that the headgroups PS and phosphatidylglycerol (PG) only cause relatively minor decreases in the dipole potential relative to the value obtained for pure PC. The negative charge on the serine residue of PS or the dipoles of the hydroxyl groups of the PG headgroup might have been expected to significantly modify ψ_d, but this is not the case. For PS this result is consistent with previous findings by Gross et al. (1994). Presumably the negative charge of the serine and the hydroxyl groups of the glycerol are far enough out towards the adjacent aqueous solution that they do not significantly affect the dipole potential. The aqueous solution contains a high salt concentration of 150 mM NaCl, which could effectively screen the negative charge of the serine, and the dielectric constant of the medium around the serine and the hydroxyl groups is likely to be high (close to the value of 80 of water), which would significantly reduce the electric field strength that their charge or dipoles would produce.

In Table 1 it can also be seen that the dipole potential of vesicles containing 50% of phosphatidylethanolamine (PE) is higher than that of vesicles composed purely of PC. This is in contrast to surface potential measurements of monolayers of PC and PE, which do not appear to

Table 1 Fluorescence ratios, R_{ex}, and dipole potential values, ψ_d, of dimyristoylphosphatidylcholine (DMPC) alone and combined at a 50 mol% ratio with dimyristoylphosphatidylserine (DMPS), dimyristoylphosphatidylglycerol (DMPG), diphosphatidylethanolamine (DMPE), dimyristoylphosphatidylethanolamine (DMPA) and monomyristoylphosphatidylcholine (MMPC)

Phospholipid	R_{ex}	ψ_d/mV[a]
DMPC	1.463 (±0.010)[b]	410 (±145)**
DMPC + DMPS	1.408 (±0.006)	397 (±143)
DMPC + DMPG	1.341 (±0.008)	382 (±138)
DMPC + DMPE	1.684 (±0.017)	461 (±157)
DMPC + DMPA	2.387 (±0.010)	625 (±194)
DMPC + MMPC	0.851 (±0.003)	268 (±119)

[a] The ψ_d values were calculated from R_{ex} using Eq. 1

[b] The error estimates in the R_{ex} values represent the standard deviation of 5 individual measurements

** The error estimates in the *absolute* values of ψ_d include significant contributions from the standard errors in the slope and intercept of the calibration curve (Starke-Peterkovic et al. 2005) on which Eq. 1 is based. However, the accuracy of the R_{ex} values are all within approximately ±1%, which allows comparisons of differences in ψ_d between different lipids to be made, even if the absolute magnitudes of ψ_d are not precisely known

show any significant difference between the two different lipids (Smaby and Brockman 1990). One possible explanation for the difference observed here is that it could be related to the smaller size of the PE headgroup relative to PC, because the only difference between the two is that PC has methyl groups attached to the nitrogen whereas PE has hydrogen atoms. The less bulky PE headgroup might allow the lipids to pack together more tightly within the bilayer, thus producing a higher density of the dipoles responsible for the dipole potential and possibly reducing water penetration and hence a higher dipole potential. Such an effect has been suggested to explain the decrease in dipole potential due to increased hydrocarbon chain unsaturation due to the increased space requirements of kinked chains produced by *cis* double bonds (Clarke 1997). However, if PE lipids were packed more tightly than PC lipids then one would also expect that the rotational dynamics of the chains of PE should be restricted. Hunter and Squier (1998), however, have found that this is not the case. Using fluorescently labelled phospholipids and studying chain motion via time-resolved fluorescence anisotropy, they found that incorporation of PE into PC lipid vesicles up to 50 mol% caused no significant change in the rate of rotational motion of the hydrocarbon chains. This is consistent with structural measurements showing only a slightly lower cross-sectional area per headgroup of PE versus PC (Rand and Parsegian 1989; Lewis and Engelman 1983). For example, DOPE and DOPC have been reported to have cross-sectional head group areas of 65 and 70 Å^2, respectively (Rand and Parsegian 1989).

However, what Hunter and Squier (1998) did find was that PE causes a significant increase in the fluorescence lifetime of the probe TMA-DPH, which they attributed to a reduction in the polarity of the surroundings of the chromophore by PE. They suggested that the reduction in polarity could come about via a structural change in either the conformation of the glycerol backbone or in the hydration of the PE and PC headgroups. The latter is supported by measurements of water uptake by lipid multilayers reported by Rand and Parsegian (1989), who found that successive methylations of the PE headgroup of egg PE to yield egg PE-Me, PE-Me$_2$ and finally egg PC resulted in stepwise increases in hydration of 28, 7 and 16%, respectively. If the dipoles of the additional waters of hydration of PC over PE polarise themselves so as to oppose the existing dipole potential, the dipole potential would be expected to decrease. Therefore, both the results of Hunter and Squier (1998) and those of Rand and Parsegian (1989) are consistent with the higher dipole potential caused by PE found here.

If the polarity of the membrane is decreased by PE relative to PC, i.e. the dielectric constant is decreased, one would expect the dipole potential to increase (as observed here), because of the increase in electric field strength that dipolar groups in the membrane would cause. Mathematically, the dipole potential is related to the dielectric constant of the surroundings, ε, by the Helmholtz equation:

$$\psi_d = \frac{\mu_\perp}{A\varepsilon_o\varepsilon} \quad (2)$$

where $\mu_\perp$ is the average component of the lipid dipole moment (including membrane-associated water molecules) perpendicular to the plane of the membrane, ε_o is the permittivity of free space, and A is the area occupied per lipid molecule in the surface of the membrane. The application of this equation to a microscopic system such as a membrane is slightly confusing, because the dielectric constant, ε, is the macroscopic manifestation of the density of dipoles (if one ignores electronic polarisation). Therefore, one needs to make clear which dipoles are contributing to $\mu_\perp$ and which are contributing to ε. The dipoles contributing to $\mu_\perp$ can be considered as those (whatever they might be) causing the dipole potential to be positive within the membrane. The dipoles contributing to ε can be considered as all other dipoles which are free to undergo orientation polarisation so that they oppose the polarity of the dipole potential and, therefore, reduce its value. Without any more precise knowledge of the origin of the dipole potential, this is the clearest distinction that can be made between $\mu_\perp$ and ε at present.

Hunter and Squier (1998) furthermore proposed that, since the phospholipid acyl chain dynamics are sensitive to the cross-sectional area within the membrane, *A*, the fact that the rotational dynamics of both lipids are the same implies compensatory changes in the average orientation of the PE and PC headgroups, so that PC and PE in spite of their different headgroup sizes both maintain optimal contact interactions between the phospholipid acyl chains. Any change in the tilt angle of the P^-–N^+ dipole relative to the membrane surface could also potentially significantly change the magnitude of the dipole potential. Thus, if the P^-–N^+ dipole lies flatter to the membrane surface in PE relative to PC this could reduce the negative contribution to the dipole potential, hence resulting overall in a more positive value. Changes in the orientation of the P^-–N^+ dipole are supported by measurements of the quadrupole splitting of NMR spectra of deuterated phospholipids (Seelig et al. 1987; Semchyschyn and Macdonald 2004). According to Seelig et al. (1987), the P^-–N^+ dipole of PC can move through an angle of 40° due to changes in the surface charge of the membrane.

The result obtained with phosphatidic acid (PA) is very interesting (see Table 1). The PA headgroup causes an increase in the dipole potential of over 200 mV. This must be due to the negative charge of the phosphate group or alternatively the lack of any positive charge in the headgroup. This result is consistent with previous experiments (Clarke and Lüpfert 1999), in which we studied the effect of cation binding to the phosphate of PC vesicles. There it was found that partial neutralisation of the negative charge of the phosphate via cation binding caused a decrease in magnitude of the dipole potential (see Fig. 6 of Clarke and Lüpfert 1999). In an analogous fashion here the lack of any neutralisation of the phosphate group by a positively charged headgroup causes the dipole potential to increase (see Fig. 2). Therefore, this result, as well as that of Clarke and Lüpfert (1999), strongly suggest that the positive charge responsible for the positive polarity of the dipole potential lies in a position within the membrane which is deeper than that of the phosphate group, i.e. closer towards the membrane interior. If the positive charge responsible for the positive polarity of the dipole potential were at the same membrane depth as the phosphate group, then its neutralisation should result in a net increase in positive charge within the membrane and hence an increase in the magnitude of the dipole potential, i.e. opposite to the observed experimental behaviour (Clarke and Lüpfert 1999).

Finally, the result obtained with MMPC is particularly interesting because it provides information on the molecular origin of the dipole potential. The result shown in Table 1 indicates that the inclusion of 50 mol% of MMPC in the membrane causes a drop in the dipole potential of

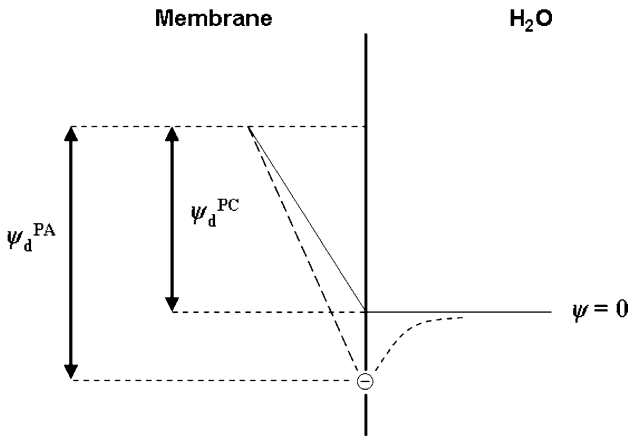

Fig. 2 Proposed mechanism for the increase in dipole potential, ψ_d, by the incorporation of dimyristoylphosphatidic acid (DMPA) into a membrane of dimyristoylphosphatidylcholine (DMPC). The *solid lines* represent the profile of the electrical potential, ψ, for DMPC alone. The *dotted lines* represent the profile of ψ for a DMPC membrane incorporating DMPA. The electrical potential is defined to be zero in the aqueous solution far from the membrane surface. ψ_d, as measured by the dye di-8-ANEPPS, is defined as the difference in the electrical potential between the membrane interior and the membrane surface (not the bulk aqueous phase), i.e. where the dye is located. ψ_d^{PC} and ψ_d^{PA} represent the dipole potentials of a pure DMPC membrane and one in which 50 mol% DMPA has been incorporated, respectively. DMPC, because it has a zwitterionic headgroup, is assumed to produce no surface potential, whereas DMPA with its negatively charged phosphate group produces a negative surface potential

142 mV. This drop cannot be due to any headgroup difference because the headgroups of DMPC and MMPC are both PC. Therefore, it must be related to the different number of chains. DMPC has two acyl chains, both linked to the headgroup via ester linkages, whereas MMPC has only a single acyl chain and a single ester linkage. However, the two acyl chains of DMPC are not identical. The *sn*-1 acyl chain of DMPC extends straight down from the phosphate group towards the centre of the membrane. The *sn*-2 chain forms a kink from the phosphate group before extending down into the membrane (see Fig. 1). Because of the kink the *sn*-2 chain does not extend quite as far as the *sn*-1 chain into the membrane interior. According to X-ray crystal structural data (Pearson and Pascher 1979), the carbonyl group of the *sn*-1 chain is expected to be approximately in the plane of the bilayer, whereas the oxygen of the carbonyl group of the *sn*-2 chain is directed more towards the membrane–water interface. Therefore, as pointed out by Seelig et al. (1987) and Gawrisch et al. (1992), the carbonyl dipole of the *sn*-2 chain would be expected to make a far greater contribution to the dipole potential than that of the *sn*-1 chain. Since MMPC has only a single acyl chain directed straight down into the membrane interior, it is, therefore, most likely that its carbonyl dipole has a similar orientation within the membrane to the

sn-1 chain of DMPC, i.e. in the plane of the bilayer. Therefore, the large drop in dipole potential on incorporating MMPC into the membrane observed here can be explained by the approximately 50% decrease in density of *sn*-2 chain carbonyl dipoles within the membrane.

It is hoped that the results presented here will contribute to a better understanding of how variation in lipid headgroup may electrically modulate the function of membrane proteins. In addition, we expect that the experimental data given here will provide a valuable resource for the testing of the accuracy of molecular dynamics simulations of lipid membranes and, thus, lead to a more refined description of phospholipid membrane structure and function.

Acknowledgments R. J. Clarke acknowledges with gratitude financial support from the Australian Research Council (Discovery Grant DP-0208282) and from the Australian Research Council/National Health and Medical Research Council funded Research Network "Fluorescence Applications in Biotechnology and the Life Sciences" (RN0460002).

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