Response of the Phosphatidylcholine Headgroup to Membrane Surface Charge in Ternary Mixtures of Neutral, Cationic, and Anionic Lipids: A Deuterium NMR Study†

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ABSTRACT: Deuterium nuclear magnetic resonance (^{2}H NMR) spectroscopy was used to investigate the response of the phosphatidylcholine headgroup of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) to changes in surface electrostatic charge in membranes consisting of ternary mixtures of lipids. DMPC was deuterated at the choline α- and β-methylene segments. The membrane surface charge was manipulated by the simultaneous addition of cationic didodecyldimethylammonium bromide (DDAB) and anionic 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol (DMPG) to neutral DMPC. Addition of increasing amounts of DDAB caused a progressive decrease (increase) in the ^{2}H NMR quadrupole splitting from DMPC-α-d_2 (DMPC-β-d_2). Addition of increasing amounts of DMPG caused a progressive increase (decrease) in the quadrupole splitting from DMPC-α-d_2 (DMPC-β-d_2). Qualitatively, the ^{2}H NMR quadrupole splitting charge response exhibited the same main features for ternary mixtures of DDAB/DMPG/DMPC and binary mixtures of DDAB/DMPC or DMPG/DMPC. Quantitatively, however, the ^{2}H NMR quadrupole splittings obtained from ternary mixtures did not coincide with those obtained from binary mixtures of nominally identical surface charge densities. Hence, the quadrupole splitting did not respond directly to the net membrane surface charge. Instead, the quadrupole splitting measured for a given ternary lipid composition could be reproduced by summing the individual effects of the charged lipids in binary mixtures, weighted according to their appropriate mole fractions.

^{2}H and ^{31}P NMR[1] studies of phospholipid bilayers have demonstrated unambiguously that the presence of charged species at the surface of a membrane induces a conformational change in the headgroup of the phospholipids phosphatidylcholine (Sixl & Watts, 1983; Seelig et al., 1987; Scherer & Seelig, 1989), phosphatidylserine (Roux et al., 1989; de Kroon et al., 1991), and phosphatidylglycerol (Marassi & Macdonald, 1991). The origin of the surface charges may be extrinsic to the membrane, as is the case for the binding of surface ligands like divalent cations or peptides, or intrinsic to the membrane, as is the case when mixing in charged phospholipids or amphiphiles. For phosphatidylcholine, the conformational change is believed to involve an alignment of the P^-–N^+ dipole of the phosphocholine headgroup under the influence of the electrostatic field emanating from the membrane surface (Akutsu & Seelig, 1981; Roux et al., 1989; Scherer & Seelig, 1989; Macdonald et al., 1991). Thus, a negative surface charge attracts the positively charged quaternary nitrogen toward the membrane surface, while positive surface charge has the opposite effect. This model reproduces all of the essential features of the dependence of the ^{2}H NMR quadrupole splitting on both positive and negative surface charge in binary mixed membranes. Because phosphatidylcholine responds to and, via ^{2}H NMR, reports on membrane surface charge, it is described as behaving like a *molecular voltmeter*.

Recently, a new aspect of the *molecular voltmeter* has become apparent. We have noted that, for membranes consisting of a ternary mixture of neutral phosphatidylcholine plus both a cationic and an anionic amphiphile, the ^{2}H NMR quadrupole splittings do not correspond to those measured from binary mixtures having nominally identical surface charge densities (Marassi & Macdonald, 1991). This observation suggests that in such ternary mixtures the charge response of phosphatidylcholine cannot be interpreted simply as an interaction of the phosphatidylcholine headgroup with the net uniform surface electrostatic field expected from a quantitative addition of all the charges from all the membrane components.

Such an effect has been observed previously in ternary mixtures of DMPC/DMPS/melittin (Dempsey et al., 1989), in ternary mixtures of POPC/POPG/melittin (Beschiaschvili & Seelig, 1990), and in ternary mixtures containing POPC, POPG, and Ca^{2+} ions (Macdonald & Seelig, 1987). In all these cases the positively charged species were extrinsic to the bilayer proper. This introduces ambiguities regarding variables such as the amount of charged species bound, the location of binding, and, in some cases, the effective charge once bound. Hence, one is uncertain whether the discrepancies in the values of the quadrupole splitting for binary versus ternary mixed membranes of nominally identical surface charge are real or merely the result of some uncertainty in the determination of the aforementioned variables.

In the present work, we investigate the response of phosphatidylcholine to surface charge in ternary mixed membranes. To this end, we have mixed zwitterionic 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) simultaneously with anionic 1,2-dimyristoyl-*sn*-glycero-3-phospho-

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[1] Abbreviations: NMR, nuclear magnetic resonance; ^{2}H, deuterium; ^{1}H, proton; ^{31}P, phosphorus; $\Delta\nu$, quadrupole splitting; $\Delta\sigma$, chemical shift anisotropy; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; DMPG, 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol; DMPS, 1,2-dimyristoyl-*sn*-glycero-3-phosphoserine; DMPA, 1,2-dimyristoyl-*sn*-glycero-3-phosphatidic acid; DDAB, didodecyldimethylammonium bromide; HEPES, 4-(2-hydroxyethyl)-piperazine-1-ethanesulfonic acid; TPS, 2,3,5-triisopropylbenzenesulfonyl chloride; TLC, thin-layer chromatography.

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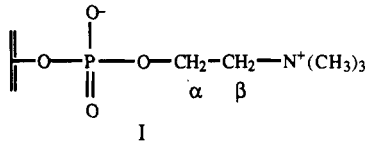
glycerol (DMPG) and cationic didodecyldimethylammonium bromide (DDAB). Since all the lipids are bilayer-forming, the membrane composition is determined unambiguously by the lipid mixing ratio. In addition, the charged groups of such amphiphiles occupy similar locations at the membrane surface. Furthermore, the effective charge in both DDAB and DMPG is simply equal to the formal valence carried by these lipids. These factors combine in our model system to reduce to a minimum uncertainties regarding the charge at the membrane surface. We compare the ^{2}H NMR charge response of choline-deuterated DMPC in such ternary mixtures to the charge response in binary mixtures composed of DMPC plus either DMPG or DDAB.

MATERIALS AND METHODS

Synthesis of Headgroup-Deuterated DMPC. The structures of the phosphatidylcholine (I) and phosphatidylglycerol (II) headgroups and of DDAB (III) are shown below. The



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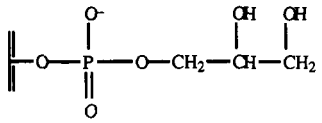

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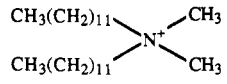

III

α and β nomenclature employed for the deuterium-labeled segments of phosphatidylcholine is also indicated. Nondeuterated lipids were purchased from Avanti (Alabaster, AL). Choline was selectively deuterated at the α- and β-methylene segments by a combination of the methods described by Harbisson and Griffin (1984) and Aloy and Rabaut (1913). DMPC-α-d_2 and DMPC-β-d_2 were prepared by coupling 1,2-dimyristoyl-*sn*-glycero-3-phosphatidic acid (DMPA) with choline tetraphenylboron salt, selectively deuterated at the α or β segments, using 2,3,5-triisopropylbenzenesulfonyl chloride (TPS) as the condensing agent (Aneja et al., 1970). The choline-deuterated DMPC was purified by chromatography on silica gel and CM-52 as described by Comfurius and Zwaal (1977), and its purity was checked by TLC, ^{1}H NMR, and differential scanning calorimetry.

Sample Preparation. Samples for solid state NMR studies were prepared as follows. Deuterated DMPC (5–10 mg) plus the desired amount of DMPG and/or DDAB, all in chloroform solution, were mixed by vortexing. The solvent was removed under a stream of nitrogen, followed by high vacuum, and the dry lipids were dispersed in excess deuterium-depleted aqueous buffer (150 mM NaCl, 10 mM HEPES, pH 7) by vortexing and warming to 35 °C in order to ensure homogeneous mixing. The lipid dispersions were then centrifuged at 13000*g*, and the pellets were tranferred to 5-mm sample tubes for NMR measurement.

NMR Measurements. ^{2}H NMR spectra were recorded at 35 °C on a Chemagnetics CMX300 NMR spectrometer, operating at 45.98 MHz, equipped with a temperature control unit. The quadrupole echo technique (Davis et al., 1976) was employed, using quadrature detection with complete phase cycling of the pulse pairs (Griffin, 1981) and a 90° pulse length of 2.0 μs, an interpulse delay of 40 μs, a recycle delay of 250 ms, a spectral width of 100 KHz, and a data size of 2K. ^{31}P spectra were recorded at 121.25 MHz using a Hahn echo sequence (Rance & Byrd, 1983) with proton decoupling. The following experimental conditions were employed: a 90° pulse length of 4.25 μs, an echo spacing of 40 μs, a recycle delay of 1 s, a spectral width of 100 KHz, and a data size of 2K. The ^{31}P spectra were acquired at 35 °C. Prior to the NMR experiments, the samples were equilibrated at 35 °C inside the NMR probe for about 20 min. At 35 °C, for all mole fractions of DMPC, DMPG, and DDAB, the bilayers are completely liquid-crystalline, as judged by differential scanning calorimetry (Marassi & Macdonald, 1991). The temperature was controlled to within 0.1 °C.

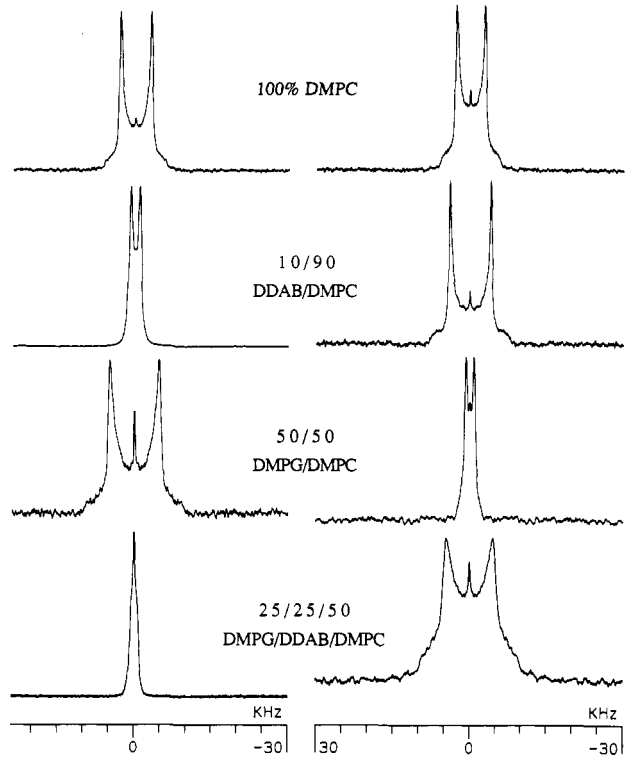

FIGURE 1: ^{2}H NMR spectra at 35 °C from DMPC-α-d_2 (left) and DMPC-β-d_2 (right) in membranes containing lipid mixtures with the molar compositions, from top to bottom, of 100% DMPC, 10/90 DDAB/DMPC, 50/50 DMPG/DMPC, and 25/25/50 DDAB/DMPG/DMPC. All spectra have been symmetrized.

RESULTS

The ^{2}H NMR spectra at 35 °C from DMPC-α-d_2 (left) and DMPC-β-d_2 (right) in binary mixtures with either anionic DMPG or cationic DDAB, and in ternary mixture with both DDAB and DMPG are shown in Figure 1. The spectra are consistent with a bilayer arrangement of liquid-crystalline lipids (Seelig, 1977; Davis, 1983). Furthermore, they each consist of a single Pake pattern, indicating that DMPC senses and reports on the globally averaged lipid environment. Addition of cationic DDAB causes the ^{2}H NMR quadrupole splitting, $\Delta\nu$, measured as the separation in Hertz between the maxima of the spectrum, to decrease in the case of DMPC-α-d_2 and to increase in the case of DMPC-β-d_2. Conversely, addition of anionic DMPG causes the $\Delta\nu$ from DMPC-α-d_2 to increase and that from DMPC-β-d_2 to decrease. Qualitatively and quantitatively, this is the typical response of headgroup-deuterated phosphatidylcholine to positive and negative membrane surface charge (Seelig et al., 1987). The counterdirectional change in the ^{2}H NMR quadrupole splitting from DMPC-α-d_2 versus DMPC-β-d_2 is characteristic and

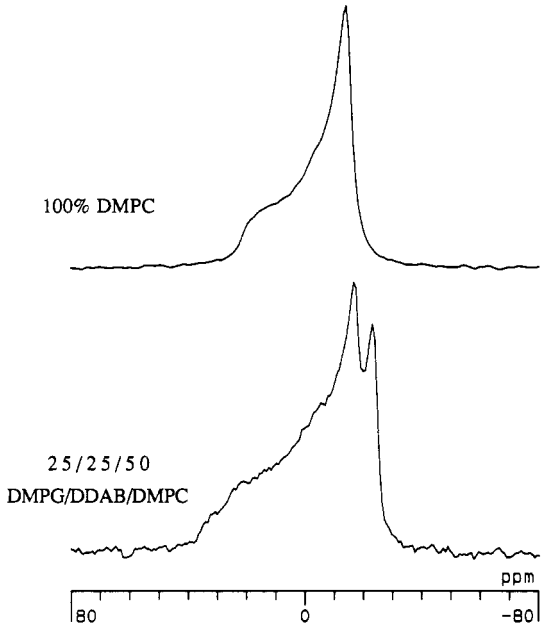

FIGURE 2: ^{31}P NMR spectra at 35 °C from membranes containing 100% DMPC (upper spectrum) and a ternary mixture of molar composition 25/25/50 DDAB/DMPG/DMPC (lower spectrum).

points to a concerted conformational change of the phosphocholine headgroup. Furthermore, the measured quadrupole splittings are in good agreement with previous reports on the effects of charged amphiphiles on the ^{2}H NMR spectrum of phosphatidylcholine (Scherer & Seelig, 1989; Macdonald et al., 1991). Thus, at 35 °C DMPC experiences the full effect of the surface charge density expected after addition of either cationic or anionic amphiphile. The central, sharp, isotropic peak visible in the ^{2}H NMR spectra of membranes containing charged amphiphiles is probably the result of a minor population of small lipid vesicles. Normally these are removed by centrifugation of the lipid suspensions. However, with charged amphiphiles the bilayers swell due to electrostatic repulsions and centrifugation becomes difficult.

Membranes containing 100% DMPC and 25/25/50 DMPG/DDAB/DMPC will have identical surface charge densities, since the negative and positive charges should cancel. However, the ^{2}H NMR quadrupole splittings measured from these two membranes are clearly different, in the case of both DMPC-α-d_2 and DMPC-β-d_2. Specifically, the quadrupole splitting of DMPC-α-d_2 in the mixture containing DMPG/DDAB/DMPC in the molar ratio 25/25/50 is smaller relative to that in 100% DMPC-α-d_2, while the opposite is true for the quadrupole splitting of DMPC-β-d_2.

The corresponding ^{31}P NMR spectra of membranes containing 100% DMPC and 25/25/50 DMPG/DDAB/DMPC are shown in Figure 2. The spectrum for 100% DMPC (top) is typical of liquid-crystalline lipids in a bilayer arrangement. The chemical shift anisotropy, $\Delta\sigma$, of 100% DMPC was measured to be –47 ppm, in good agreement with previously reported ^{31}P NMR data for the phosphate group of POPC (Tamm & Seelig, 1983). In an earlier report (Marassi & Macdonald, 1991), we have shown that the addition of anionic DMPG to DMPC decreases the magnitude of the chemical shift anisotropy ($|\Delta\sigma|$) for DMPC. By contrast, increasing the positive surface charge density through admixture of cationic DDAB has the effect of increasing $|\Delta\sigma|$. The simultaneous addition of 25 mol % DMPG and 25 mol % DDAB to 50 mol % DMPC yields a ^{31}P NMR spectrum (bottom) consisting of two superimposed, axially symmetric powder patterns having distinct $\Delta\sigma$'s. As discussed previously, the spectral component with the larger $|\Delta\sigma|$ is attributed to DMPC while the smaller $|\Delta\sigma|$ is attributed to DMPG. However, note that, although the nominal surface charge of the 25/25/50 mixture is neutral, as expected upon charge cancellation, the $\Delta\sigma$ measured from DMPC in this mixture is –58 ppm, which is 11 ppm larger in magnitude than the $|\Delta\sigma|$ for a membrane containing 100% DMPC. This difference indicates that the conformations of the phosphate group of DMPC in the 25/25/50 mixture and in 100% DMPC are different from one another, even though these membranes both bear a nominally identical surface charge. Thus, both the ^{2}H and ^{31}P NMR results suggest that in the DDAB/DMPG/DMPC ternary mixtures the effect of net surface charge density alone is not sufficient to explain the observed changes in the $\Delta\nu$ and $\Delta\sigma$ values measured from phosphatidylcholine.

Figure 3 summarizes the changes in the quadrupole splittings from DMPC-α-d_2 (a) and DMPC-β-d_2 (b) as a function of the net mole fraction of charge ($X_+ - X_-$) at 35 °C. The data for the binary and ternary mixtures are both shown. The binary mixtures containing DMPC/DDAB are characterized by molar concentrations X_0 and X_+ of neutral and cationic amphiphile. The binary mixtures containing DMPC/DMPG are characterized by molar concentrations X_0 and X_- of neutral and anionic amphiphile. The ternary mixtures containing DMPC/DDAB/DMPG are characterized by molar concentrations X_0, X_+, and X_- of neutral, cationic, and anionic amphiphiles. Plotting the quadrupole splitting as a function of ($X_+ - X_-$) allows a direct comparison of the ^{2}H NMR charge response in binary or ternary mixed membranes of identical surface charge densities.

In the binary mixtures, the quadrupole splitting from DMPC-α-d_2 increases with increasing mole fraction of anionic DMPG. Conversely, with increasing mole fraction of cationic DDAB, the quadrupole splitting decreases, collapsing to zero and reversing its sign at about 15 mol % DDAB. On the other hand, the quadrupole splitting from DMPC-β-d_2 decreases with increasing mole fraction of anionic DMPG and increases with increasing mole fraction of cationic DDAB. Note that the assignment of a positive or negative quadrupole splitting is arbitrary since ^{2}H NMR reports only the size of the quadrupole splitting and not its sign. The curvilinear progression of $\Delta\nu$, and its approach toward a limiting value with increasing amounts of either negative or positive surface charge, parallel the behavior observed in other investigations of the effects of surface charge on the quadrupole splittings from deuterated phosphatidylcholine (Seelig et al., 1987; Scherer & Seelig, 1989; Macdonald et al., 1991).

In the case of the ternary mixtures containing DMPG/DDAB/DMPC, the dependence of the quadrupole splitting on the net mole fraction of charge retains the essential features observed with the binary mixtures. The change in $\Delta\nu$ is once again counterdirectional for DMPC-α-d_2 versus DMPC-β-d_2 and for positive versus negative charge. Once again, the progression of $\Delta\nu$ is curvilinear, and $\Delta\nu$ appears to approach limiting values at higher mole fractions of charged lipid. However, the quadrupole splittings measured from deuterated DMPC present in binary and ternary mixtures of nominally identical surface charge densities are clearly different from one another. In the ternary mixtures, the presence of cationic DDAB reverses to some extent the effect of anionic DMPG on the quadrupole splitting of DMPC. However, the resulting quadrupole splitting is not that expected from the net cancellation of the negative charge by the added amount of

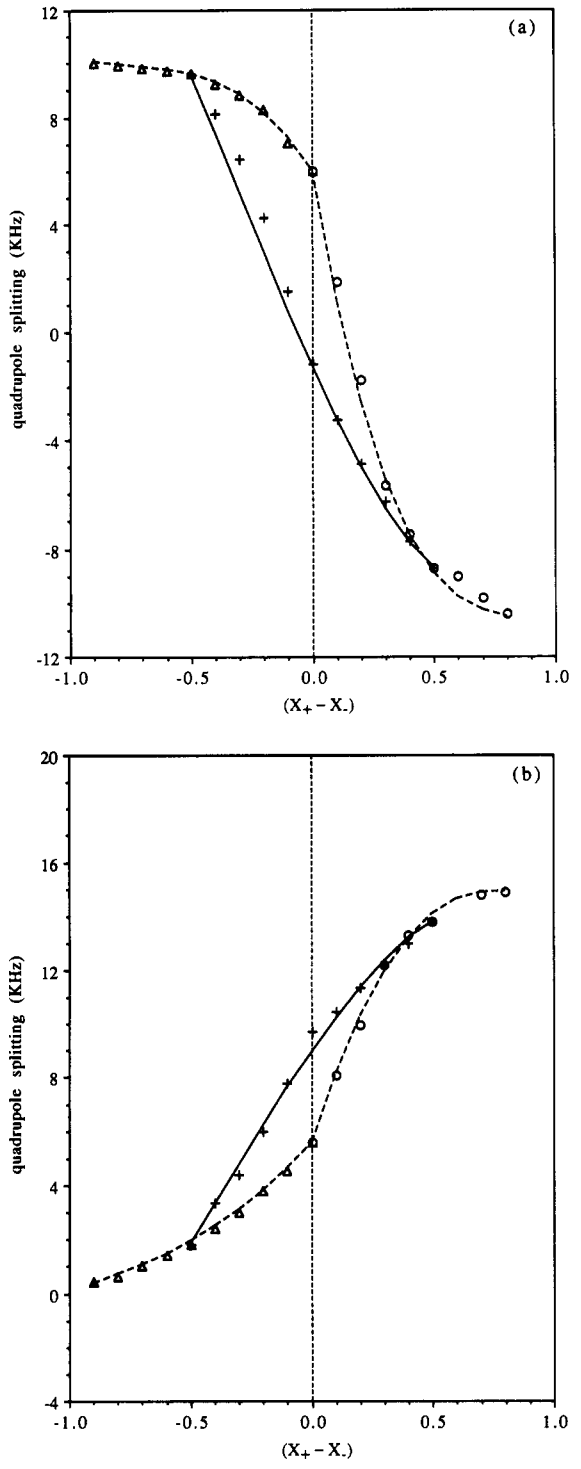

FIGURE 3: Dependence of the ^{2}H NMR quadrupole splitting at 35 °C from DMPC-α-d_2 (a) and DMPC-β-d_2 (b) on the net mole fraction of charge, $(X_+ - X_-)$. X_+ and X_- are the mole fractions of DDAB and of DMPG, respectively. (○) DDAB/DMPC binary mixtures. (△) DMPG/DMPC binary mixtures. (+) DDAB/DMPG/50 mol% DMPC ternary mixtures. The solid lines are the fits to the ternary mixtures data calculated using eq 2. The vertical dotted line indicates nominal charge neutrality (i.e., $X_+ = X_-$).

DDAB. Note, for example, that for DMPC-α-d_2 the quadrupole splitting is 6.01 kHz for a 100% DMPC membrane and −1.17 kHz in the ternary mixture containing 25/25/50 DMPG/DDAB/DMPC, even though both membranes bear a nominally neutral surface charge. Likewise, the quadrupole splitting from DMPC-β-d_2 is 5.57 kHz for a 100% DMPC membrane but 9.70 kHz in the ternary mixture containing 25/25/50 DMPG/DDAB/DMPC. For DMPC-α-d_2 the quadrupole splittings measured from ternary mixtures are consistently smaller than those measured from binary mixtures of nominally equal surface charge density. On the other hand, for DMPC-β-d_2 the opposite is true, and the quadrupole splittings measured from ternary mixtures are consistently larger than those measured from binary mixtures of nominally equal surface charge density. Thus, the ^{2}H NMR data demonstrate that for ternary mixtures the quadrupole splitting is not responding directly to the net membrane surface charge.

DISCUSSION

Under Results, we have shown that in both the binary and ternary mixed membranes the quadrupole splitting from headgroup-deuterated phosphatidylcholine behaves as expected upon the deposition of charge at the membrane surface. Nevertheless, in ternary mixtures containing DMPC plus both cationic and anionic lipids in a molar ratio leading to membrane neutrality, the quadrupole splittings measured from either the α- or β-choline segments are not equal to those obtained from 100% DMPC neutral membranes. Moreover, it is possible to obtain a quadrupole splitting characteristic of neutral membranes with a nominally negative membrane containing DDAB/DMPG/DMPC. This difference between the charge response of phosphatidylcholine in binary versus ternary mixtures of charged amphiphiles indicates that the headgroup conformation is not directly a function of the net membrane surface charge. Indeed, if this were the case, then the charge response profile of the ternary mixtures should be identical to that observed for the binary mixtures. In the following section we demonstrate that for the ternary mixtures the relationship between the quadrupole splitting and the net mole fraction of charged lipid can be predicted, simply and rather accurately, if the individual responses of phosphatidylcholine to positive and negative charge in binary mixed membranes are known. This treatment is consistent with a scenario wherein the quadrupole splitting in ternary mixed membranes is the result of pairwise, electrostatic interactions between DMPC and individual charged lipids.

Analysis of the ^{2}H NMR Data for Ternary Mixtures. We hypothesize that for a ternary mixture of molar concentrations X_n of amphiphiles of charge n = +, −, and 0, where $X_+ + X_- + X_0 = 1$, the headgroup quadrupole splitting, $\Delta\nu_t$, is perturbed relative to the value $\Delta\nu_o$ for a neutral membrane containing 100% DMPC (i.e., $X_0 = 1$) by an amount which can be written as the sum of the perturbations of the quadrupole splitting, $\Delta\nu_b$, for the individual binary mixtures of corresponding compositions, relative to $\Delta\nu_o$. Accordingly, one may write this additivity hypothesis as

$$[\Delta\nu_t(X_+,X_-) - \Delta\nu_o] = [\Delta\nu_b(X_+) - \Delta\nu_o] + [\Delta\nu_b(X_-) - \Delta\nu_o] \quad (1)$$

where $\Delta\nu_t(X_+,X_-)$ represents the quadrupole splitting for a ternary mixture of composition (X_+,X_-), while $\Delta\nu_b(X_+)$ and $\Delta\nu_b(X_-)$ represent the quadrupole splittings for binary mixtures of corresponding mole fractions of cationic or anionic amphiphiles. Rearranging eq 1 yields an expression which allows one to predict values for $\Delta\nu_t(X_+,X_-)$ using measured values of $\Delta\nu_b(X_+)$,$\Delta\nu_b(X_-)$, and $\Delta\nu_o$:

$$\Delta\nu_t(X_+,X_-) = \Delta\nu_b(X_+) + \Delta\nu_b(X_-) - \Delta\nu_o \quad (2)$$

The values of $\Delta\nu_t(X_+,X_-)$ thus calculated define the solid lines shown in Figure 3, panels a and b. They are found to be in excellent agreement with the quadrupole splittings measured experimentally from DMPC-α-d_2 and DMPC-β-d_2 in the ternary mixtures. The fits to the ^{2}H NMR data obtained

Table I: Experimental Slopes and Intercepts for the Relationship between the ^{2}H NMR Quadrupole Splitting and $(X_+ - X_-)$ in the Range of $0.40 \leq (X_+ - X_-) \leq 0.40$[a]

membrane components	slope (kHz)		intercept (kHz)	
	$(m)_\alpha$	$(m)_\beta$	$(\Delta\nu_0)_\alpha$	$(\Delta\nu_0)_\beta$
DDAB/DMPC	−38.67 (0.999)	21.74 (0.997)	6.01	5.57
DMPG/DMPC	9.62 (0.979)	−8.42 (0.995)	6.01	5.57
DDAB/DMPG/ 50% DMPC	−22.15 (0.990)	15.29 (0.993)	−1.17	9.70

[a] Note that for the ternary mixtures the intercepts, $\Delta\nu_0$, correspond to the quadrupole splittings measured from nominally neutral membranes (i.e., $X_+ = X_-$). The subscripts α and β indicate the respective ^{2}H-labeled positions. The linear regression coefficients are listed in brackets.

from eq 2 indicate that the phosphatidylcholine ^{2}H NMR charge response in the ternary mixed membranes of DDAB/DMPG/DMPC can be predicted completely and accurately if the individual ^{2}H NMR charge responses in the binary mixed membranes, DDAB/DMPC and DMPG/DMPC, are known.

Linear Approximations. In Figure 3 the relationships between the quadrupole splitting and the mole fraction of charge are linear, to a good approximation, in the range of $-0.4 \leq (X_+ - X_-) \leq 0.4$. Linear regression analysis of the binary mixtures data yields

$$\Delta\nu_b(X_+) = m_+X_+ + \Delta\nu_0$$

$$\Delta\nu_b(X_-) = m_-X_- + \Delta\nu_0 \quad (3)$$

whose slopes, m, and intercepts, $\Delta\nu_0$, are listed in Table I. Rearranging eq 3 yields the terms $[\Delta\nu_b(X_+) - \Delta\nu_0]$ and $[\Delta\nu_b(X_-) - \Delta\nu_0]$ which can be substituted into eq 1 to obtain the expression

$$[\Delta\nu_t(X_+,X_-) - \Delta\nu_0] = m_+X_+ + m_-X_- \quad (4)$$

In the ternary mixed membranes $1 - X_0 = X_+ + X_-$; therefore, substituting for X_+ and X_- in eq 4 and rearranging yields

$$[\Delta\nu_t(X_+,X_-) - \Delta\nu_0] = (m_+ + m_-)(1 - X_0) - m_+X_- - m_-X_+ \quad (5)$$

Two limiting cases illustrate the predicted behavior of the quadrupole splittings in the ternary mixed membranes. In the case where $X_+ = X_-$, corresponding to an overall neutral membrane surface charge density, eq 5 reduces to

$$[\Delta\nu_t(X_+,X_-) - \Delta\nu_0] = (m_+ + m_-)(1 - X_0)/2 \quad (6)$$

Equation 6 predicts that the quadrupole splitting for a ternary mixture of nominally neutral surface charge will deviate from that of a 100% DMPC membrane in proportion to the difference between the absolute values of the slopes m_+ versus m_-. For example, using the slopes in Table I, eq 6 predicts quadrupole splittings equal to −1.25 and 9.34 kHz for DMPC-α-d_2 and DMPC-β-d_2, respectively, in ternary mixtures of 25/25/50 mol % DDAB/DMPG/DMPC. These are remarkably close to the measured values of −1.17 and 9.70 kHz listed as intercepts for these membranes in Table I. Furthermore, eq 6 indicates that the deviation of $\Delta\nu_t$ from $\Delta\nu_0$ will scale according to $(1 - X_0)$. To confirm the validity of this prediction, we measured the quadrupole splitting for the ternary mixture DDAB/DMPG/DMPC in the nominally neutral molar ratio 12.5/12.5/75. The value of 7.23 kHz predicted by eq 6 agrees well with the measured value of 7.00 kHz.

A second limiting case is that where $|m_+| = |m_-|$. In this instance $m_+ = -m_-$, and equation 6 reduces to

$$[\Delta\nu_t(X_+,X_-) - \Delta\nu_0] = m_+(X_+ - X_-)$$

or

$$[\Delta\nu_t(X_+,X_-) - \Delta\nu_0] = m_-(X_- - X_+) \quad (7)$$

Equation 7 indicates that the quadrupole splitting in such a ternary mixture depends directly on the net surface charge as represented by the difference $(X_+ - X_-)$. In this case, the ^{2}H NMR charge response in the ternary mixed membranes would appear identical to the charge response in the binary mixed membranes, and, for nominally neutral membranes, $\Delta\nu_t$ would be equal to $\Delta\nu_0$. In all cases investigated to date, the slopes m_+ and m_- are not equal in magnitude, leading to the observed differences between the charge response of binary versus ternary mixed membranes.

CONCLUSIONS

In the present study we have shown that, in membranes composed of ternary mixtures of cationic and anionic lipids plus neutral phosphatidylcholine, both the positive and negative lipids induce a charge response in the phosphatidylcholine headgroup, measurable through the ^{2}H NMR quadrupole splitting. However, in such ternary mixtures the observed quadrupole splitting is not simply a function of the uniform, surface electrostatic field expected upon the net cancellation of charges. Instead, our analysis indicates that the individual effects of positive and negative charge on the headgroup quadrupole splitting are additive and that their superposition gives the perturbation of the headgroup quadrupole splitting in the ternary mixtures. Using this hypothesis, we have shown that if the individual responses of phosphatidylcholine to positive and negative charge in binary mixed membranes are known, then it is possible to predict the charge response of phosphatidylcholine in ternary mixed membranes simply and accurately.

Our results require that the current model for the origin of the surface charge response of the ^{2}H NMR quadrupole splitting be modified. We hypothesize that individual pairwise interactions between DMPC and the charged lipids result in distinct conformations of the phosphocholine headgroup. These are assumed to arise from interactions between the phosphocholine P^-–N^+ dipole and the charged group of the particular species being considered. Thus, the *choline tilt* model, in which the entire phoshocholine headgroup tilts with respect to the plane of the membrane surface, need not be abandoned. However, the driving force for the conformational change is not the net membrane surface electrostatic field, but rather the intermolecular interaction between neighboring lipids. Hence, the measured quadrupole splitting in the ternary mixtures, which reflects the conformation of the phosphocholine headgroup, becomes a weighted average of the quadrupole splittings measured in the individual binary mixtures. This finding implies that, in cases where there is complete miscibility of the membrane components, the molecular voltmeter is sensitive to local intermolecular interactions and may have important implications for the study of protein–lipid interactions where proteins carrying net positive charges are believed to form long-lived complexes with negatively charged lipids.

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