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Surface charge response of the phosphatidylcholine head group in bilayered micelles from phosphorus and deuterium nuclear magnetic resonance

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Abstract

Solid-state phosphorus (^{31}P) and deuterium (^{2}H) nuclear magnetic resonance (NMR) spectroscopy over the temperature range of 25–50°C were used to investigate bilayered micelles (bicelles) composed of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC) in the presence of either the anionic lipid 1,2-dimyristoyl-*sn*-3-phosphoglycerol (DMPG) or the cationic lipid 1,2-dimyristoyl-3-trimethylammonium-propane (DMTAP). The ^{31}P-NMR spectra demonstrate that bicellar structures form with DMPG/DMPC ratios ranging from 0 to 50/50 and with DMTAP/DMPC ratios from 0 to 40/60, while the overall concentration of DHPC remains constant. The formation of bicelles containing charged amphiphiles is contingent upon the presence of NaCl, with 50 mM NaCl being sufficient for bicelle formation at all concentrations of charged amphiphile investigated, while 150 mM NaCl affords better resolution of the various ^{31}P-NMR resonance signals. The ^{2}H-NMR spectra demonstrate that the quadrupolar splittings ($\Delta\nu$) of head group-deuterated DMPC change inversely as a function of the amount of negative versus positive charge present, and that the changes for deuterons on the α-carbon are opposite in sense to those for deuterons on the β-carbon. This indicates that head group-deuterated phosphatidylcholine functions as a molecular voltmeter in bicelles in much the same fashion as it does in spherical vesicles.

Keywords: Surface charge; Bicelle; Deuterium NMR; Phosphorus NMR

1. Introduction

Phospholipid vesicles have long been utilised in the study of membranes via solid-state ^{31}P- and ^{2}H-NMR spectroscopy, including both unilamellar [1–3] and multilamellar vesicles (MLVs) [4–13]. Recently a new type of phospholipid system, the bilayered micelle or 'bicelle', has come into use [14–17]. Unlike large spherical unilamellar vesicles and MLVs, bicelles are disc-shaped with no aqueous compartment in their interior. These structures form upon mixing long- and short-chain lipid molecules, such as 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC). In bicelles, the long-chain lipid molecules form a bilayer and the short-chain lipid molecules cover the edges of the bilayer. A particular attraction of bicelles is their tendency to spontaneously align in a magnetic field. This alignment is a result of the natural magnetic susceptibility of

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phospholipid bilayers and occurs such that the average normal to the bilayer lies perpendicular to the applied magnetic field.

Once aligned, these structures allow the acquisition of high-resolution solid-state NMR spectra of comparable quality to those obtained with samples that have been mechanically oriented on glass or polymer slides. The use of bicelles to obtain oriented samples is attractive for a multitude of reasons ([15,17] and references cited therein), but in particular because a wide variety of peptides and proteins can be reconstituted into bicelles [18–20], where their biological activity is retained [21].

Alignment of bicelles in a magnetic field can be demonstrated using ^{31}P- and ^{2}H-NMR [14–26]. Here, the powder pattern line shapes characteristic of giant unilamellar vesicles (GUVs) and MLVs are replaced by relatively narrow, orientation-dependent resonance lines. The advantages of ^{31}P-NMR are that no isotopic labelling is required, and that one may quantify the proportions of the phosphorus-containing lipids. ^{2}H-NMR requires isotopic labelling, but provides a means of determining the order parameters along the lipid acyl chains, or of exploring the dynamics of the phosphocholine head group.

What has not been examined in detail as yet are the consequences of altering bicellar surface charge through the inclusion of charged lipid molecules. There are many good reasons for including charged lipid molecules in bicelles, one being that they promote binding of oppositely charged peptides. To include charged lipid molecules in GUVs or MLVs is a trivial matter, and their effects on surface charge can be monitored using solid state ^{31}P- and ^{2}H-NMR spectroscopy [10–13,27–32] of the phosphocholine head group.

To date, there have been few reports on the effects of including charged lipids in bicelles, and only one specifically involving head group-deuterated phosphatidylcholine [17]. In that study, a small amount of the anionic phospholipid 1,2-dimyristoyl-*sn*-glycero-3-phosphatidic acid (DMPA) was incorporated into DHPC/DMPC bicelles in the presence of Lanthanides, and the bicelles were shown to align in a magnetic field. Additionally, the anionic phospholipid 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylserine (DMPS) was successfully introduced into DMPC/DHPC bicelles.

In this report, we describe solid-state ^{31}P- and ^{2}H-NMR studies of the effect of adding the anionic phospholipid 1,2-dimyristoyl-*sn*-3-phosphoglycerol (DMPG) and the cationic amphiphile 1,2-dimyristoyl-3-trimethylammonium-propane (DMTAP) to DHPC/DMPC bicelles. The ^{31}P-NMR spectra are consistent with alignment of the bicelles in the magnetic field and show resolution of all phospholipid species present as a function of both charged amphiphile content and temperature. Additionally, ^{2}H-NMR spectra demonstrate that the well-documented molecular voltmeter response of the phosphocholine head group [27,28,31–33] is manifest in this new model membrane system.

2. Materials and methods

2.1. Materials

1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine-1,1,2,2-d_4 (DMPC-α,β-d_4), 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC), 1,2-dimyristoyl-*sn*-3-phosphoglycerol (DMPG) and 1,2-dimyristoyl-3-trimethylammonium-propane (DMTAP) were purchased from Avanti Polar Lipids (Alabaster, AL). All other materials were purchased from Aldrich (Milwaukee, WI).

2.2. Preparation of bilayered micelles (bicelles)

The details of bicelle preparation have been reported elsewhere [17]. Briefly, bicelles used in this report were prepared such that q, the ratio of long-chain to short-chain lipid molecules, was 4.5. The ratio q is strictly speaking a ratio of the total cross-sectional surface area occupied by the head group of the long-chain lipid molecules to that occupied by the head group of the short-chain lipid molecules. In the case that the surface areas per molecule are effectively the same, as it is when considering the phosphocholine and phosphoglycerol head groups, q reduces to a concentration ratio. In our samples, the proportion of DHPC is kept constant, relative to the sum of the number of long-chain lipid molecules (DMPG+DMPC or DMTAP+DMPC). As for the selection of the value of 4.5 for q, it is known that

when $q \leq 2.5$, the bicelles are effectively isotropic and do not spontaneously align in a magnetic field, and that for $q \geq 3$ the bicellar diameter increases linearly with q [16]. Accordingly, a value of $q = 4.5$ was selected to ensure that the bicelles were large enough to spontaneously align.

Since DHPC is extremely hygroscopic, an aqueous DHPC solution was prepared immediately upon opening the container for the first time, from which the necessary amount was added to dry DMPC plus DMPG or DMTAP. NaCl was then added from an aqueous stock solution to produce a final NaCl concentration of either 50 or 150 mM. Typically, samples were composed of 25% w/v of lipid in aqueous solution, with the total amount of lipid being 40–50 mg. Once all the components were present, the samples were gently mixed and centrifuged, followed by several cycles of heating to 40°C and cooling to 4°C. Samples were stored at 4°C prior to use. Bicelle formation was indicated by the presence of an optically clear solution.

2.3. NMR measurements

^{31}P-NMR spectra were recorded on a Chemagnetics CMX300 NMR spectrometer operating at 121.25 MHz, using a Chemagnetics double-resonance magic angle spinning (MAS) probe, but without sample spinning. The Hahn echo sequence with complete phase cycling of the pulses and proton decoupling during acquisition was employed as described by Rance and Byrd [34]. The 90° pulse length was 6 μs, the echo spacing 30 μs, the recycle delay 2.5 s, the spectral width 100 kHz, and the data size 2K. Spectra were typically processed with zero filling and exponential broadening of 5–25 Hz. Chemical shifts were referenced to external 85% H_3PO_4.

^{2}H-NMR spectra were recorded on the same spectrometer operating at 45.98 MHz, using a Chemagnetics wideline deuterium probe. The quadrupolar echo sequence [35] was employed using quadrature detection with complete phase cycling of the pulse pairs, a 90° pulse length of 2.0 μs, an interpulse delay of 40 μs, a recycle delay of 100 ms, a spectral width of 100 kHz, and a 4K data size. Spectra were typically processed with zero filling and exponential broadening of 25–75 Hz.

Sample temperature was regulated during acquisition of the NMR spectra using a thermocouple linked to a feed-back loop controlling the operation of a resistive heater through which the temperature-regulating air stream passed prior to reaching the NMR sample. The sample was always held at the desired temperature for 30 min prior to signal acquisition. All samples were equilibrated in the magnetic field for a minimum of 30 min to ensure maximum possible alignment.

3. Results and discussion

3.1. ^{31}P-NMR evidence for formation and alignment of DMPG- and DMTAP-containing bicelles in the presence of NaCl

In attempting to verify bicelle formation and their subsequent alignment in a magnetic field one may use either ^{31}P- or ^{2}H-NMR spectroscopy. As phospholipids require no specific labelling to enable acquisition of ^{31}P-NMR spectra, one has available a simple and effective probe of bicelle formation and alignment. Once conditions of bicelle formation are established, deuterated samples can be used to probe either acyl chain order or head group dynamics, depending on the location of the deuterons. In this report, the particular interest is whether or not the deuterated phosphocholine head group functions as a molecular voltmeter in the same fashion as it does in spherical vesicles.

In attempting to assemble bicelles containing a particular charged amphiphile, the first task is to prepare a sample that macroscopically appears to be bicellar. This is generally indicated by the presence of an optically clear solution. Initial attempts at incorporating DMPG or DMTAP into DMPC/DHPC bicelles without using NaCl were unsuccessful. Our previous experience with manipulating membrane surface charge in GUVs and MLVs suggested that the formation of charged bicelles might be favoured by including salt and/or buffer, their effect being to screen inter-bicellar repulsion. When samples with DMPG/DMPC = 10/90 and NaCl concentrations of either 50 or 150 mM were prepared, an optically clear solution resulted, indicating probable bicelle formation. Forming DMTAP-containing bicelles proved to be more difficult, even in the pres-

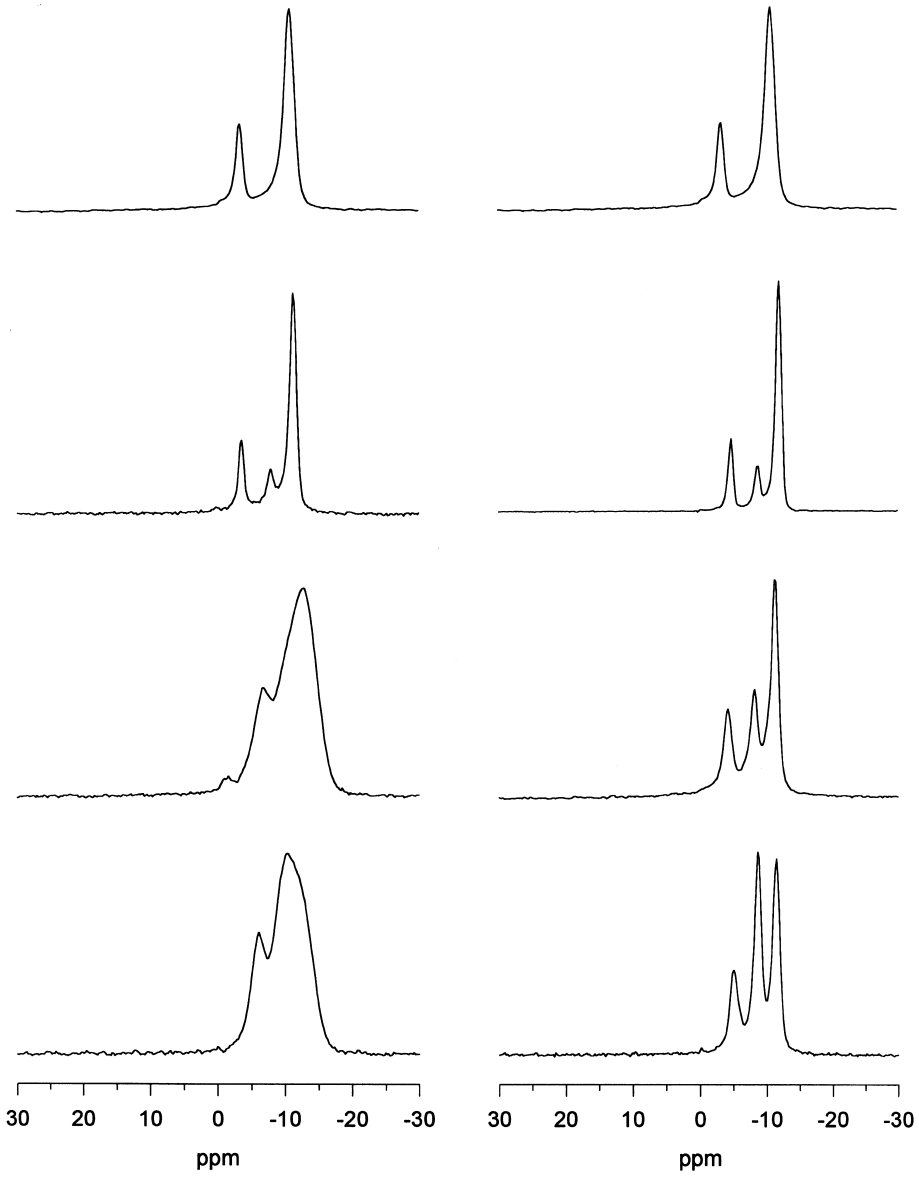

Fig. 1. Representative ^{31}P-NMR spectra at 30°C of DMPC/DHPC bicelles ($q = 4.5$) as a function of added DMPG. Ratios of DMPG/DMPC are, from top to bottom, 0, 10/90, 30/70 and 50/50. Spectra on the left are for samples prepared in 50 mM NaCl, while those on the right are for samples prepared in 150 mM NaCl.

ence of NaCl. Following lipid hydration, the mixing of the contents had to be carried out very gently, or else the results were inconsistent.

Preliminary ^{31}P-NMR results showed that DMPG-containing bicelles (DMPG/DMPC = 10/90, where all ratio are mole ratios) at both 50 and 150 mM NaCl aligned in a magnetic field. Consequently, a series of samples was prepared with various DMPG/DMPC ratios between 0 and 50/50, in the presence of either 50 or 150 mM NaCl. All displayed the optical clarity associated with bicelles. Their ^{31}P-NMR spectra are presented in Fig. 1. All spectra were acquired at 30°C after first heating the samples to 50°C in the magnet. The spectra on the left represent samples prepared in 50 mM NaCl, while those on the right represent samples prepared in 150 mM NaCl. From top to bottom the DMPG/DMPC ratios are 0, 10/90, 30/70, and 50/50, respectively.

There are several important features of these spec-

tra worthy of comment. First, one can unambiguously assign the observed resonances. For the samples where no DMPG has been added (top spectra), the downfield, low-intensity resonance belongs to the phosphorus of DHPC, while the upfield, high-intensity resonance belongs to the phosphorus of DMPC. This assignment is consistent with previous reports [23]. Integration of these spectra yields a DMPC/DHPC ratio of approximately 4, which is close to the expected value of 4.5. This modest difference can be attributed to the extremely hygroscopic nature of DHPC, which makes preparation of an accurate stock solution difficult.

Assignment of the resonance belonging to the phosphorus of DMPG is facilitated by observing a series of increasing DMPG concentrations in DHPC plus DMPC mixtures where all three resonances are resolved, and noting which one increases in direct proportion to the amount of DMPG added. Hence, the DMPG phosphorus resonance is seen to have a chemical shift intermediate to those of DHPC and DMPC. This result is consistent with ^{31}P-NMR spectra of multilamellar POPG/POPC vesicles obtained under magic-angle spinning (MAS) conditions where the POPG signal is downfield of the POPC signal [36].

Another noteworthy feature of the spectra in Fig. 1 is the difference between the spectral line shapes for the 50- and 150-mM NaCl samples, in the case of DMPG/DMPC = 30/70 and 50/50. Despite the similarities in their macroscopic appearance, the samples prepared in 50 mM NaCl yield poorly resolved ^{31}P-NMR resonances compared to the samples prepared in 150 mM NaCl. From the appearance of these spectra, it is not obvious if the poor resolution results from poor orientation in the magnet, or from some distribution of composition or bicelle size. It is possible that the structures formed in the case of 50 mM NaCl and high DMPG content are not bicelles, although their optical clarity suggests that some sort of bicelle population is present. Regardless, it is a condition that is alleviated by an increase in the NaCl concentration, since all samples prepared in 150 mM NaCl exhibit well-resolved signals indicative of bicelle alignment in the magnetic field.

A further feature of these spectra is that the line width of the DMPC signal is noticeably broader (by approximately a factor of 2) when DMPG (or DMTAP) is absent. As line width is generally a reflection of T_2 relaxation, this would indicate that spin–spin relaxation is longer in the presence of charged amphiphiles. Further experiments are necessary before the origin of this effect can be ascertained.

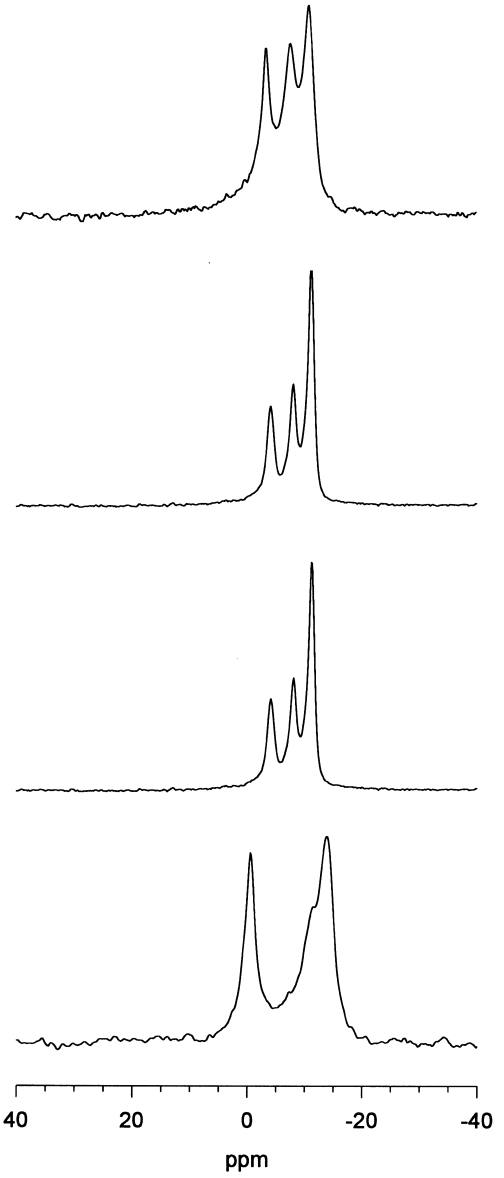

Fig. 2. Representative ^{31}P-NMR spectra of DMPC/DHPC bicelles (q = 4.5), where DMPG/DMPC = 30/70, as a function of temperature. The temperatures are, from top to bottom, 25, 30, 40 and 50°C.

3.2. Effect of temperature on the ^{31}P-NMR spectra of bicelles

Temperature is a critical determinant of the ability of bicelles to form and to align in a magnetic field. For instance, below 23°C, the gel-to-liquid-crystalline phase transition of DMPC, the mixtures investigated here yielded broad, featureless ^{31}P-NMR spectra. Alignment is in fact achieved only at temperatures above the gel-to-liquid-crystalline phase transition. Fig. 2 illustrates the temperature dependence of the ^{31}P-NMR spectra of (DMPC/DMPG)/DHPC bicelles, with $q = 4.5$, for the case of a DMPG/DMPC ratio of 30/70. The temperatures investigated were, from top to bottom, 25, 30, 40 and 50°C. Essentially identical results were obtained with bicelles containing DMPG/DMPC ratios of 0, 10/90, and 50/50, and for a DMTAP/DMPC ratio of 10/90. Examination of the spectra over this temperature range demonstrates several important points. First, at 25°C, a temperature very close to the gel-to-liquid-crystal transition temperature, it is possible to resolve resonances from all three phosphorus-containing species. However, the spectra still retain some of the characteristics of a powder pattern and therefore are not fully aligned, although the chemical shift anisotropy (CSA) is clearly reduced relative to that of non-oriented DMPC-containing MLVs, i.e. −47 ppm [29]. This alone is not an indication of bicelle formation since a reduction in the CSA can occur when MLVs distort from a spherical geometry under the influence of a magnetic field.

The spectra acquired at 30 and 40°C show three narrow, well-resolved resonances, with intensities proportional to the expected ratios of DHPC/DMPG/DMPC. Since the chemical shifts of the DMPC and DMPG resonances fall in the region that one expects for the 90° shoulder of the corresponding powder pattern line shapes in non-oriented MLVs, we conclude that at these temperatures we have achieved alignment of the bicelles in the magnetic field and that the normal to the plane of the bicelle is oriented at 90° to the direction of the magnetic field, as expected.

The same cannot be said for the spectrum at 50°C, where the three expected components are no longer resolved. Moreover, there is a large isotropic signal having an integrated intensity, relative to that of the broad upfield signal, much larger than would be expected if it corresponded to DHPC. We believe that this spectrum reflects a change in the fundamental organisation of the bicelles at this elevated temperature, rather than any chemical degradation of the component lipids. First, previous researchers have noted a conversion from a nematic phase to a mixture of smectic and isotropic phases at elevated temperatures in DMPC/DHPC bicelles oriented at 0° relative to the magnetic field [17]. Second, when the DHPC/DMPG/DMPC mixture is cooled from 50°C back to 40°C, the spectrum recovers to give the three well-resolved resonances observed prior to heating to 50°C. In fact, there is even some improvement in the quality of the spectrum in that the resonances appear even narrower than before heating.

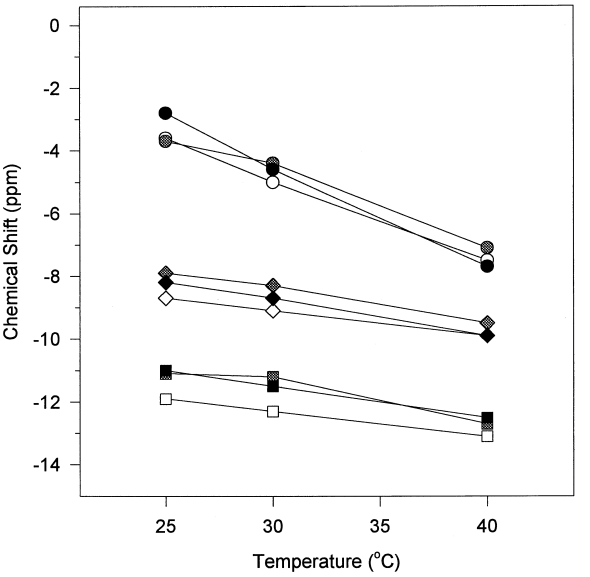

Fig. 3. Changes in the ^{31}P-NMR chemical shifts (relative to 85% H_3PO_4) from DHPC/DMPG/DMPC bicelles as a function of temperature. Circles (●), DHPC; diamonds (◆), DMPG; and squares (■), DMPC. Open symbols, DMPG/DMPC = 10/90; grey symbols, DMPG/DMPC = 30/70; closed symbols, DMPG/DMPC = 50/50.

A final point concerns the effects of temperature on the ^{31}P-NMR chemical shifts of the various resonances, as summarised in Fig. 3. All three species exhibit a similar decrease in chemical shift with increasing temperature, with the DHPC resonance changing the most over the temperature range observed. Further, the data indicate that a lower DMPG content correlates with a more negative chemical shift of the DMPC resonance. For example,

at 40°C the bicelles with DMPG/DMPC = 10/90 exhibit a DMPC ^{31}P resonance at −13.1 ppm, compared to −12.3 ppm for the bicelles with DMPG/DMPC = 50/50. The direction of this change in chemical shift with added DMPG is consistent with observations made on MLVs, where it was found that the CSA of DMPC MLVs decreased by approximately 8 ppm upon the addition of DMPG [29]. Note that data for the chemical shifts measured at 50°C are not included in Fig. 3 due to the ambiguity of resonance assignments at this temperature.

As for DMTAP-containing bicelles, ^{31}P-NMR spectra were acquired only for the case of a DMTAP/DMPC ratio of 10/90. These spectra (not shown) confirmed that conditions for bicelle formation and alignment already established for DMPG-containing bicelles were equally suitable for DMTAP-containing bicelles. Verification of the formation and alignment of these cationic bicelles is in fact demonstrable via ^{2}H-NMR spectra, as discussed below.

3.3. *^{2}H-NMR evidence of a molecular voltmeter response of the phosphocholine head group in bicelles*

A primary goal of the investigations undertaken here is to determine whether or not the phosphocholine head group of phosphatidylcholine responds to surface charge in bicelles in a manner analogous to that known to occur in MLVs and GUVs. The results described above establish conditions for successfully incorporating cationic or anionic amphiphiles into DMPC/DHPC bicelles which align in a magnetic field. It is now possible, therefore, to proceed to employ ^{2}H-NMR spectroscopy of choline-deuterated DMPC in order to address the question of the effects of surface charge on the conformation of the phosphocholine group.

DMPC-α,β-d_4 was incorporated into bicelles containing various proportions of DMPG or DMTAP, all prepared in 150 mM NaCl. Control experiments indicated that the optimal temperature for ^{2}H-NMR was 40°C, in agreement with the ^{31}P-NMR results. Fig. 4 shows a series of such ^{2}H-NMR spectra for bicelles containing the following proportions of long-chain amphiphiles, from top to bottom: DMPG/DMPC = 50/50, DMPG/DMPC = 20/80, DMPC = 100, DMTAP/DMPC = 10/90, and DMTAP/DMPC = 30/70.

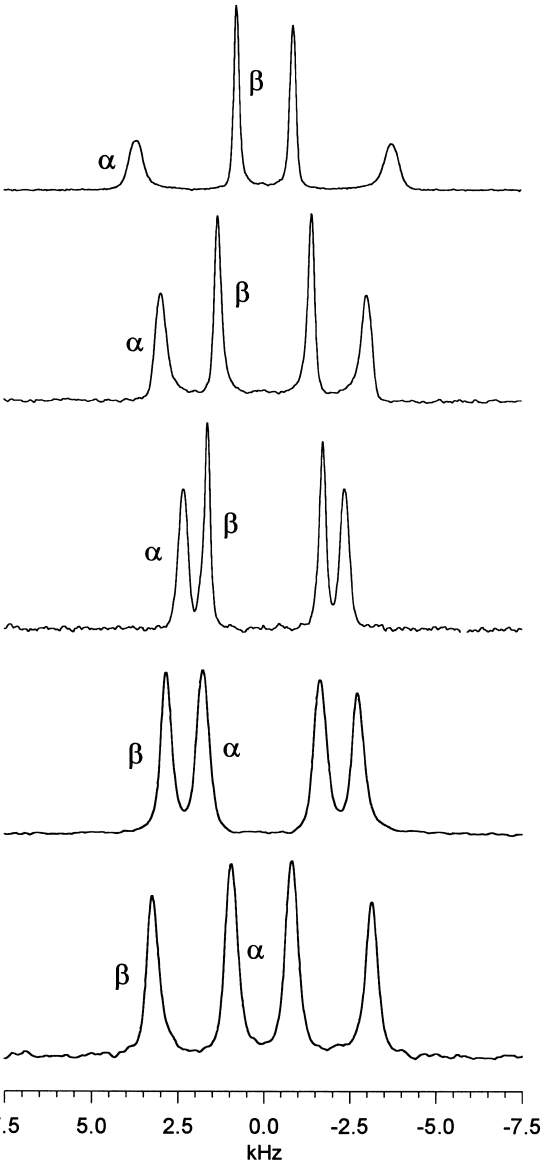

Fig. 4. Representative ^{2}H-NMR spectra at 40°C of DMPC/DHPC bicelles (q = 4.5) as a function of the DMPG/DMPC or DMTAP/DMPC ratio. The compositions are, from top to bottom, DMPG/DMPC = 50/50, DMPG/DMPC = 20/80, DMPC = 100, DMTAP/DMPC = 10/90, and DMTAP/DMPC = 30/70. The doublets assigned as originating from the α- and β-deuterons are indicated.

At 40°C, the ^{2}H-NMR spectra in every case consist of a pair of well-resolved narrow doublets, one for the α-deuterons and one for the β-deuterons of the phosphocholine head group of DMPC. The fact that the resonances are so narrow indicates a high

degree of alignment of the bicelles in the magnetic field. The values of the actual quadrupolar splitting measured for the case of no added charged amphiphiles (4.7 kHz for the α-deuterons and 3.3 kHz for the β-deuterons) is consistent with an alignment in which the normal to the plane of the bicelles is oriented at 90° to the direction of the magnetic field, again as expected.

These spectra demonstrate that the quadrupolar splittings respond to the introduction of surface charges into the bicellar membrane in a fashion virtually identical to that known to occur in MLV membranes. Specifically, the quadrupolar splitting for the α-deuterons increase as progressively more DMPG is added to the bicelles, while that for the β-deuterons decreases. Furthermore, when DMTAP is introduced into the bicelles, the response of the α- and β-deuterons is opposite to that observed in the presence of DMPG.

We would note at this point that it was difficult to obtain such high quality ^{2}H-NMR spectra for mixtures containing DMTAP/DMPC ratios of 40/60 and 50/50. For the former, a large isotropic resonance was present in the spectrum in addition to a pair of well-resolved doublets. For the latter, only the isotropic resonance was present in the spectrum. It is possible that at higher proportions DMTAP has difficulty mixing with the other lipids present in the bicelle preparations, thereby producing an artificially high level of DHPC which in turn produces small isotropically tumbling structures.

Fig. 5 shows a calibration curve relating the perturbation from control values of the quadrupolar splitting for the α- and β-deuterons of DMPC-α,β-d_4 as a function of the amount of charged amphiphile added to the bicelles. Here, $\Delta\nu_i$ represents the quadrupolar splitting at a particular DMPG or DMTAP content, while $\Delta\nu_o$ represents the quadrupolar splitting in the absence of charged amphiphile. Clearly, there is a progressive change in the quadrupolar splittings at the two deuterated positions as the amount of charge is increased, and the direction of this change is opposite in sense for α- versus β-deuterons and for cationic versus anionic charge. This fact indicates that the phosphocholine head group in bilayered micelles does indeed function as a molecular voltmeter as originally detailed by Seelig et al. [27] for spherical multilamellar phospholipid vesicles.

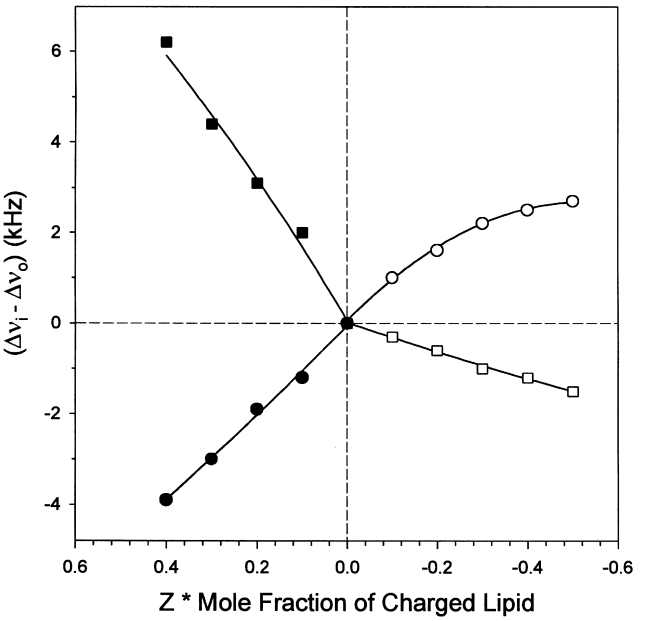

Fig. 5. Calibration curves relating the perturbation of the quadrupolar splitting for a given level of added charged amphiphile relative to control values in the absence of added charged amphiphile ($\Delta\nu_i - \Delta\nu_o$) as a function of the amount of added DMPG or DMTAP. Squares, α-deuterons; circles, β-deuterons. Open symbols, DMPG fraction; closed symbols, DMTAP fraction. Z represents the valence charge on the DMTAP (+) or DMPG (−).

It is interesting to note the values of these quadrupolar splittings and how they compare to those measured in other head group-deuterated phosphocholine lipid assemblies. For example, in multilamellar dispersions of DMPC above the gel-to-liquid-crystal transition temperature, the quadrupolar splittings for DMPC-α-d_2 and DMPC-β-d_2 deuterons are approximately 6.2 and 5.8 kHz, respectively [34]. These are much higher than the values of 4.7 and 3.3 kHz measured here in DMPC/DHPC bicelles.

^{2}H-NMR quadrupolar splittings reflect both conformation and orientational order at the position of the deuterons [4–6,37–39]. It is possible that this difference in quadrupolar splittings in bicelles versus MLVs might be due to a greater orientational disorder of the phosphocholine head group in bicelles. Evidence supporting this notion comes from a comparison of the order parameters for the first two positions of the acyl chains of DMPC in MLVs at 37°C, where $S_f = 0.22$ [6], and DMPC in bicelles at 35°C, where $S_f = 0.18$ [17]. This approximately 20%

decrease in orientational order of the acyl chains in bicelles versus MLVs is sufficient to account for the decreased quadrupolar splitting of the α-deuterons in bicelles versus MLVs. It may be that the gradient of orientational order is steeper in bicelles than in MLVs, and that this accounts for the remaining differences in the quadrupolar splittings of the β-deuterons in these two systems. In the absence of more experimental data, further speculation regarding this point is unwarranted.

An additional difference with respect to bicelles versus MLVs is the sensitivity of the quadrupolar splittings from the α- and β-deuterons to a given level of charged amphiphile. In MLVs, at a temperature of 35°C, the quadrupolar splitting for α-deuterons increases by approximately 3.3 kHz for a DMPG/DMPC ratio of 50/50, while for β-deuterons, the quadrupolar splitting decreases by approximately 3.6 kHz [33]. For the bicelles investigated here, the comparable values are 2.7 and 1.4 kHz, respectively. For DMTAP/DMPC MLVs, there are no data available regarding quadrupolar splittings of head group-deuterated DMPC, so a direct comparison with bicelles is not possible. However, our laboratory regularly uses DOTAP/POPC MLVs, and these display a response to cationic surfaces charge which is much more sensitive to changes in surface charge than the values observed here for bicelles. The reason for this apparent reduction in sensitivity in bicelles to either cationic or anionic charge (especially for the β-deuterons) is not certain. However, a lower orientational order parameter in bicelles would dampen the change in quadrupolar splitting expected for a given change in conformation.

Apart from the immediate questions raised by this report, there are numerous aspects of the surface electrostatic charge in bilayered micelles that deserve further investigation. These include studies of the effects of including other charged lipid molecules, or adding simple ions or polyelectrolytes. Since bicelles are used to reconstitute peptides, it would be interesting to have information regarding the details of the bicelle's surface charge during peptide/bicelle association. Finally, since bicelle alignment can be changed from the spontaneous case, where the bilayer normal is perpendicular to the static magnetic field, to one where it is parallel, by the addition of certain Lanthanide ions, it would be prudent to explore the consequences for the bicelle's surface charge of the interactions of these ions with the bicelle's surface. The sensitivity of the head group of DMPC to changes in membrane surface charge, as demonstrated in this report, indicates that ^{2}H-NMR of phosphocholine-deuterated DMPC is an ideal route to investigating such questions.

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