Magnetically Aligned Membrane Model Systems with Positive Order Parameter: Switching the Sign of S_{zz} with Paramagnetic Ions

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NMR studies of biological membrane constituents have traditionally been carried out on samples of multilamellar phospholipid assemblies,[1−4] suspensions of unilamellar vesicles,[5,6] or detergent micelles.[7] The use of multilamellar bilayer vesicles is hampered by the usual solid-state NMR problems: very broad spectra and concomitant poor signal-to-noise and the need for magic angle spinning techniques or specific isotopic labeling. Unilamellar vesicles can be used for high-resolution studies,[6] but short T_2's limit their utility, and the use of detergent micelles inevitably raises the question about matrix curvature and the biological relevance of detergents.

Macroscopic alignment of phospholipid bilayers greatly facilitates structural and dynamical NMR studies of membrane constituents. Samples are generally prepared in one of two forms: multilamellar dispersions aligned mechanically by shear forces on thin glass plates,[8−10] or magnetically aligned, oblate, bilayered micelles,[11] appropriately named "bicelles" by Sanders and Landis.[12] The former approach has the advantage that the samples can be mounted on a goniometer and rotated in the magnetic field, thereby yielding information similar to that obtained from single crystals. On the other hand, the preparation of multiplate samples[10] is cumbersome and sample deterioration is a problem. The use of magnetically aligned phospholipid bilayer samples has many advantages: lower viscosity and increased mobility result in longer T_2's and higher signal-to-noise; proper hydration is straightforward to maintain; and large interbilayer spacings facilities attachment of membrane-associated peptides and proteins.[13] Unfortunately, the sign of the magnetic susceptibility anisotropy of phospholipid bilayers dictates that the bicelles align themselves with the bilayer normal perpendicular to the magnetic field. This orientation ($\beta_{nl} = 90°$) is defined by an order parameter $S_{zz} \equiv \langle {}^1/_2(3\cos^2\beta_{nl} - 1)\rangle = -{}^1/_2$. For bilayer constituents undergoing rapid axially symmetric reorientation with respect to the normal (small drug molecules, peptides, hormones, and the phospholipid molecules themselves), the only disadvantage is that the anisotropic shifts and splittings will be scaled by a factor of $-{}^1/_2$. However, when bound to a bicelle, large, slowly reorienting intrinsic proteins will exhibit cylindrical powder patterns rather than sharp single lines or multiplets. Consequently, the advantage of working with macroscopically oriented systems is lost, and for a long time the hope has been to design membrane model systems with $S_{zz} > 0$. Using deuterium NMR spectroscopy, we have discovered that the addition of moderate amounts of paramagnetic ions has the effect of flipping phospholipid bicelles from $S_{zz} < 0$ to $S_{zz} > 0$. For 22% (w/v) lecithin samples, well-aligned bicelles with $S_{zz} > 0$ were observed to form when the ion:lipid ratio was equal to or greater than 0.09.

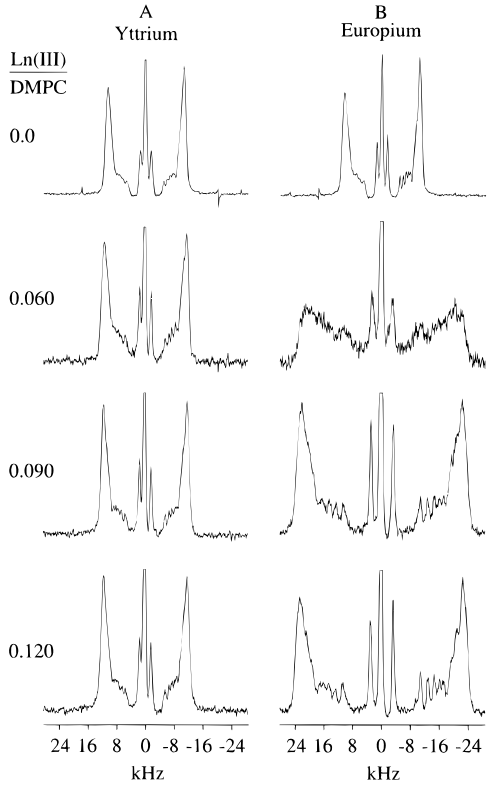

Figure 1. Deuterium quadrupole echo NMR spectra of DMPC-d_{54} in phospholipid bicellar solutions containing varied amounts of Y^{3+} and Eu^{3+} ions. The spectra were obtained at 55.3 MHz and 37 °C on a spectrometer consisting of a General Electric GN console equipped with an ENI LPI-10 rf amplifier, an 8.5 T Oxford Instruments wide-bore magnet, and a homebuilt probe delivering 4.2 μs 90° pulses to a 10 mm o.d. sample. Five hundred or 1000 transients were accumulated with a 2 s repetition time, and the free induction decays were processed with a 100 Hz exponential line broadening. Small differences in resolution (see, for example, the two top control spectra) are due to the use of different aligning times in the magnetic field. The isotropic peak observed in the center of all the spectra may reflect the fact that we are working near the upper limit (1:3.5) for achieving magnetic alignment of DMPC/DHPC mixtures.[12] The intensity of the isotropic peak increases upon addition of lanthanide cations, possibly indicating slow phospholipid exchange between bicelles and solution. The slight asymmetry in spectral resolution is most likely due to combined effects of temperature and field gradients across the sample.

Nematically ordered bicellar samples of a 1:3.4 mixture of 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC) and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), both from Avanti Polar Lipids, in deuterium-depleted water were prepared as described by Sanders and Landis.[12] To monitor bicellar orientation we used a mixture of regular and chain-perdeuterated DMPC (also from Avanti Polar Lipids) where DMPC:DMPC-d_{54} = 28:1. Deuterium quadrupole echo NMR spectra of oriented chain-perdeuterated DMPC obtained at 55.3 MHz and 37 °C are shown at the top of Figure 1. The two samples were prepared from the same batch of DMPC/DHPC solution and then titrated with small amounts of 0.55 M solutions of yttrium(III) chloride (99.999%) and europium(III) chloride (99.99%)

(1) Seelig, J. *Q. Rev. Biophys.* **1977**, *10*, 353.
(2) Griffin, R. G. *Methods Enzymol.* **1981**, *72*, 108.
(3) Smith, R. L.; Oldfield, E. *Science* **1984**, *255*, 280.
(4) Cross, T. A.; Opella, S. J. *Curr. Opin. Struct. Biol.* **1994**, *4*, 574−581.
(5) Brown, L. R.; Wüthrich, K. *Biochim. Biophys. Acta* **1977**, *468*, 389−410.
(6) Ulrich, E. L.; Girvin, M. E.; Cramer, W. A.; Markley, J. L. *Biochemistry* **1985**, *24*, 2501.
(7) Brown, L. R.; Braun, W.; Kumar, A.; Wüthrich, K. *Biophys. J.* **1982**, *37*, 319−328.
(8) Seelig, J.; Gally, H.-U. *Biochemistry* **1976**, *15*, 5199.
(9) Bechinger, B.; Opella, S. J. *J. Magn. Reson.* **1991**, *95*, 585.
(10) Prosser, R. S.; Hunt, S. A.; Vold, R. R. *J. Magn. Reson.* **1995**, *B109*, 109.
(11) Sanders, C. R.; Hare, B. J.; Howard, K. P.; Prestegard, J. H. *Prog. Nucl. Magn. Reson. Spectrosc.* **1994**, *26*, 421−444.
(12) Sanders, C. R., II; Landis, G. C. *Biochemistry* **1995**, *43*, 4030−4040.
(13) Sanders, C. R.; Landis, G. C. *J. Am. Chem. Soc.* **1994**, *116*, 6470−6471.

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(both from Aldrich Chemicals). To ensure complete mixing the samples were thoroughly stirred, alternatively left at 4 and 45 °C for 30 min each, and annealed in the magnetic field for a few hours before data acquisition. The appearance of well-resolved deuterium doublets (except for one spectrum in Figure 1B) following the addition of lanthanide solution attests to sample homogeneity.

The effect of adding diamagnetic YCl_3 to magnetically oriented DMPC/DHPC bicelles is illustrated in Figure 1A. An 8% increase of the deuterium quadrupolar splitting, Δ_Q, was observed for both the outermost (methylene "plateau region") and innermost (terminal CD_3) doublets, the result of a change in the order parameter $S_Q \equiv \langle {}^1/_2(3\cos^2\beta_{nm} - 1)\rangle$ of the phospholipid molecules relative to the bilayer normal. Addition of di- and trivalent cations has been observed to affect both the residual ^{31}P shielding anisotropy[14] and deuterium quadrupolar splittings[15] and has been shown to be the result of a change in the orientation of the phosphocholine headgroup relative to the bilayer.

The effect of adding $EuCl_3$ to the bicellar solutions is much more dramatic. As shown in Figure 1B, addition of a small amount of paramagnetic ion (Eu^{3+}:DMPC = 0.060) results in a very broad, noisy, intermediate spectrum, where the original plateau region doublet can barely be detected at $\Delta_Q = 20.8$ kHz. Additional $EuCl_3$ causes a doubling of all quadrupolar splittings and a sharpening of all resonances. We ascribe this to a switch in the ordering of the bilayer director from $S_{zz} \approx -{}^1/_2$ to $S_{zz} \approx 1$, induced by the binding of paramagnetic Eu^{3+} ions to the phospholipid headgroups. Apparently, the anisotropy of the bilayer susceptibility changes sign when a sufficiently anisotropic Eu^{3+} **g** tensor is created upon complex formation.

The quadrupolar splittings do not double exactly when enough Eu^{3+} is added. Rather, Δ_Q increases by a factor of 2.14 for both the plateau methylene and the terminal methyl deuterons. This is not surprising since one expects an increase of 5−10% simply from the addition of trivalent cations.[15] A comparison of the splittings observed in the presence of high equivalent concentrations of Eu^{3+} and Y^{3+} is more meaningful, and the ratio $\Delta_Q(Eu)/\Delta_Q(Y)$ was found to be 1.99 ± 0.02.

Eu^{3+}-induced doubling of Δ_Q is not confined to the alkyl chain. While NMR spectra are not shown here, choline headgroup deuterons, $-POCD_2-$, $-CD_2N-$, and $-N(CD_3)-$, are affected in a similar manner. Reflecting the proximity of the paramagnetic ions, these headgroup deuterons also exhibit upfield pseudocontact shifts in a manner reminiscent of observations made by Akutsu and Seelig[15] for headgroup deuterons in multilamellar DPPC samples. While the significance was unrecognized at the time, Akutsu and Seelig[15] reported that ^2H spectra of choline headgroup deuterated multilamellar suspensions of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) differed markedly from those obtained following addition of several diamagnetic di- and trivalent cations (La^{3+}, Ca^{2+}, Mg^{2+}, and Cd^{+2}). Figure 3 of ref 15 illustrates the Eu^{3+} effects on bilayer susceptibility for multilamellar bilayer samples with slow exchange between differently ordered regions of the sample.

Having the option of working with magnetically oriented bilayers with positive order parameters improves opportunities for spectroscopic studies of membrane-bound proteins, peptides, and assorted other molecules. We are currently seeking to elucidate the effects of several physicochemical parameters, such as the concentration of negatively charged phospholipids, ionic strength, and temperature, as well as the utility of other paramagnetic cations. (See Note Added in Proof).

We finally note that for molecules bound to the membrane surface, rather than buried in the interior of the membrane, the presence of a paramagnetic "shift reagent" may complicate assignment and interpretation of NMR spectra. However, for deuterium the paramagnetic effects are confined to the chemical shifts, while the quadrupolar splittings still yield valuable information about the organization and dynamics of membrane-associated species. Furthermore, it is likely that most of the observed shifts will be free of large contact shift contributions, and we predict that the shifts may provide important clues about the location of solute spins in phospholipid bilayers.

Note Added in Proof: Additional experiments have confirmed that only those lanthanide ions[16]—Eu^{3+}, Er^{3+}, Tm^{3+}, and Yb^{3+}—with magnetic anisotropy $\Delta\chi = \chi_{\parallel} - \chi_{\perp} > 0$, where $\chi_{\parallel}$ is along the bicelle normal, will "flip" the bicelles.

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(14) Brown, M. F.; Seelig, J. *Nature* **1977**, *269*, 721.
(15) Akutsu, H.; Seelig, J. *Biochemistry* **1981**, *20*, 7366.
(16) Horrocks, W. D. In *NMR of Paramagnetic Molecules*; La Mar, G. N., Horrocks, W. DeW., Holm, R. H., Eds.; Academic Press: New York, 1973; p 489.