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Novel Chelate-Induced Magnetic Alignment of Biological Membranes

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ABSTRACT A phospholipid chelate complexed with ytterbium (DMPE-DTPA:Yb^{3+}) is shown to be readily incorporated into a model membrane system, which may then be aligned in a magnetic field such that the average bilayer normal lies along the field. This so-called positively ordered smectic phase, whose lipids consist of less than 1% DMPE-DTPA:Yb^{3+}, is ideally suited to structural studies of membrane proteins by solid-state NMR, low-angle diffraction, and spectroscopic techniques that require oriented samples. The chelate, 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine diethylenetriaminepentaacetic acid, which strongly binds the lanthanide ions and serves to orient the membrane in a magnetic field, prevents direct lanthanide-protein interactions and significantly reduces paramagnetic shifts and line broadening. Similar low-spin lanthanide chelates may have applications in field-ordered solution NMR studies of water-soluble proteins and in the design of new magnetically aligned liquid crystalline phases.

INTRODUCTION

Although membrane proteins are estimated to constitute one-third of all proteins, disproportionately few three-dimensional structures are known (Fraser et al., 1995; Goffeau, 1995). This reflects the difficulties in applying conventional protein structure techniques (i.e., x-ray diffraction and high-resolution solution NMR) to membrane proteins. X-ray diffraction studies have been limited by the difficulty of cocrystallizing membrane proteins with detergents and/or phospholipids (the photosynthetic reaction center complexes, annexin, and cytochrome *c* oxidase represent outstanding exceptions to this rule). Three-dimensional structures of membrane proteins have been successfully obtained in several cases by electron diffraction of 2D crystals of membrane-protein complexes, although 2D diffractable crystals are also difficult to obtain. High-resolution solution NMR spectroscopy is a highly effective technique for the determination of the structure of membrane peptides and proteins, whose combined masses (i.e., detergent micelle plus protein) may soon routinely exceed 40 kDa (Pervushin et al., 1997). One concern with solution NMR spectroscopy of membrane peptides in detergents is that the limited size and high curvature strain of the micelle result in a conformation that may not be representative of the native state (Vinogradova et al., 1997). Solid-state NMR is perhaps the least restrictive of the above-mentioned techniques in terms of sample preparation. Well-resolved resonances and consequent high-resolution structures may be obtained from macroscopically aligned samples (a feat that is relatively easy to perform in membranes) or, in the case of rapid magic angle sample spinning, from unoriented powder samples. Macroscopic ordering of detergent-free membranes (and resident membrane proteins) is commonly achieved by either shear forces achieved with glass plates, or by spontaneous alignment in a magnetic field. This paper demonstrates the use of a lanthanide-phospholipid chelate complex (DMPE-DTPA:Yb^{3+}), which when combined with a model membrane system in the presence of a magnetic field, will spontaneously align such that the average bilayer normal is parallel to this field, as illustrated in Fig. 1. This orientation is critical to the successful study of large (nearly immobile) membrane proteins. The chelate complex not only possesses an enormous molecular magnetic susceptibility anisotropy and can thus be used in small concentrations to affect the desired orientation of the membrane, but it will be shown that the undesirable side effects of the paramagnetic ions (line broadening and shifts), in addition to direct interactions between the lanthanides and the membrane protein, can be virtually eliminated.

Magnetic alignment arises from the anisotropy of the diamagnetic susceptibility tensor, $\Delta\chi = \chi_{\parallel} - \chi_{\perp}$, where $\chi_{\parallel}$ and $\chi_{\perp}$ represent volume magnetic susceptibilities parallel and perpendicular to the long molecular axis. The associated orientation-dependent term for the Helmholtz free energy density is expressed as (Scholz et al., 1984)

$$F(\beta_{ln}) = -\frac{1}{2} N\Delta\chi(\vec{n}\cdot\vec{H})^2, \qquad (1)$$

where β_{ln} represents the angle between the magnetic field, $\vec{H}$, and the molecular director, $\vec{n}$, and N is the number of molecules per unit volume. A particularly fashionable way of aligning phospholipid membranes is to prepare a 15–25% (w/w) aqueous dispersion of long-chain phospholipid such as dimyristoylphosphatidylcholine (DMPC) and short-chain phospholipid such as dihexanoylphosphatidylcholine (DHPC) in which the mole ratio DMPC/DHPC = 2.75. This is thought to result in the formation of disk-shaped aggregates consisting of a planar DMPC-rich bilayer, whose hydrophobic edges are coated with DHPC. These bilayered micelles or "bicelles" have several virtues that make them suitable for in-depth structural and dynamical NMR studies

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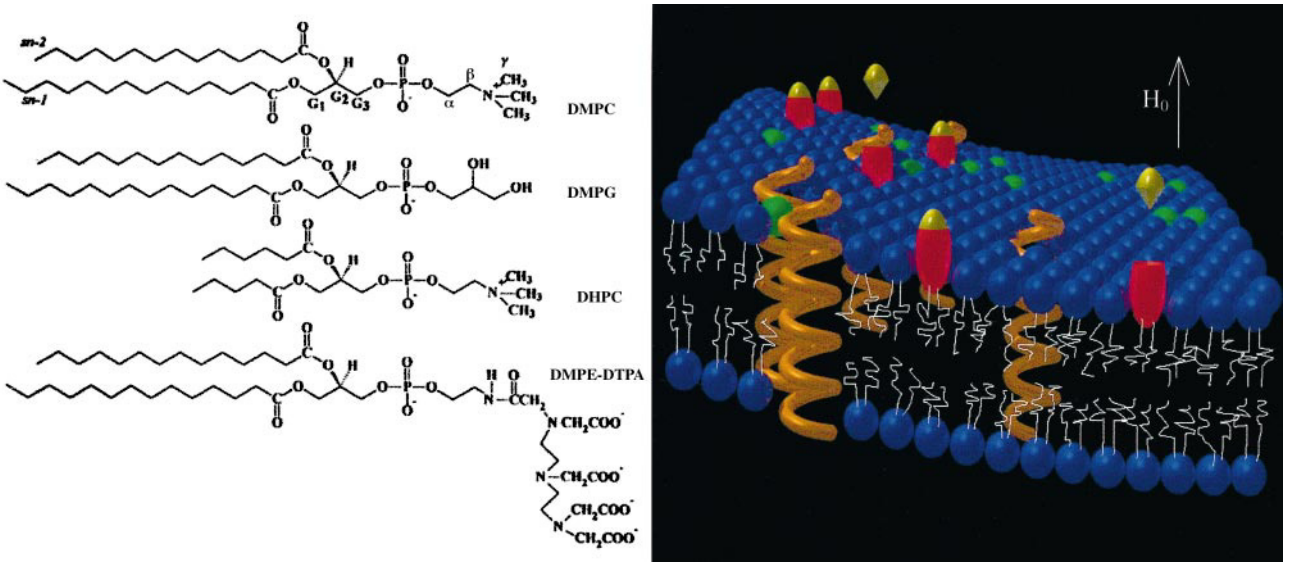

FIGURE 1 Structural formulae of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1,2-dimyristoyl-*sn*-glycero-3-[phospho-rac-(1-glycerol)] (DMPG), 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC), and 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolaminediethylenetriaminepentaacetate (DMPE-DTPA). The relative concentrations of the long-chain phospholipids and their bilayer organization are shown on the right for a sample containing the integral membrane peptide, gramicidin A′, discussed in the text. Blue, green, and red stylized molecules represent DMPC, DMPG, and DMPE-DTPA, respectively, and yellow represents the Yb^{3+} ion. DHPC, which is believed to be sequestered in curvature defect regions, is not shown in this figure. All of the above lipids were obtained commercially from Avanti Polar Lipids (Alabaster, Al).

of membrane polypeptides (Sanders and Schwonek, 1992; Sanders and Landis, 1994; Sanders et al., 1994). Bicelles are readily prepared, spontaneously align in the magnetic field, and are stable over a range of temperature, water concentration, pH, and salt concentration. Their lipid composition (for example, charged lipid content and fraction of unsaturated chains) can be adjusted to mimic different membranes. Furthermore, the "detergent-free" environment seems to be suitable for preserving membrane protein function. Finally, the bilayer order may be continuously scaled to the isotropic limit by the addition of short-chain lipid, DHPC. Thus the chemical shift resonances in the oriented system may be assigned by connecting them with their assigned isotropic values.

One disadvantage of bicelles in the study of membrane proteins is that they are, in fact, negatively aligned; $\Delta\chi$ of the constituent long-chain phospholipids is negative, and the bicelles are most stably arranged with the bilayer normals perpendicular to the magnetic field (i.e., the resulting positive free energy term in Eq. 1 is minimized if $\vec{n} \cdot \vec{H} = 0$). The order parameter used to describe the orientation, $\beta_{l\vec{n}}$, of the average bilayer normal with respect to the magnetic field is therefore negative: $S_{l\vec{n}} = \langle 3\cos^2\beta_{l\vec{n}} - 1\rangle/2 = -1/2$. In this case, inhomogeneous broadening of NMR lines can only be avoided if the membrane protein undergoes fast, axially symmetrical motion. In other words, their application is restricted to smaller membrane peptides. Recently, it was shown that the addition of certain trivalent paramagnetic ions (i.e., Eu^{3+}, Er^{3+}, Tm^{3+}, or Yb^{3+}) to a bicellar system creates a stable liquid crystalline phase with the average bilayer normal aligned along the magnetic field (Prosser et al., 1996, 1998a). These ions, which are known to bind the phospholipid headgroups, confer a large positive $\Delta\chi$ on the membrane, which arises from the crystal field coupling terms. In the case of Tm^{3+}, whose $\Delta\chi$ is largest among the above lanthanides, alignment can be obtained with as little as one Tm^{3+} ion per 155 DMPC lipids (Prosser et al., 1998b). These so-called positively aligned (i.e., $S_{l\vec{n}} = +1$) membranes were recognized to have potential for the study of large membrane proteins (Howard and Opella, 1996), although the problems associated with the paramagnetics have limited their implementation. Lanthanides are known to both shift and broaden NMR lines because of dipolar and contact effects, thus complicating the interpretation of chemical shift resonances. Moreover, lanthanides might bind to sites of negative (or partial negative) charge on the protein of interest, thus altering its conformation (Prosser et al., manuscript submitted for publication).

One way of overcoming the limitations of the paramagnetics in the positively aligned lanthanide-doped membranes is to incorporate a phospholipid molecule that strongly binds or chelates the lanthanides. Clearly, such a molecule would have the advantage that the remaining phospholipids and proteins would not directly coordinate the lanthanide ions. The first priority would be that the chelate must preserve the positive magnetic susceptibility anisotropy. This is related to the molecular order parameter associated with the chelate and the relative orientation of the ligand fields that coordinate the lanthanide ion. Ideally, the chelate should resemble a phospholipid and should not phase separate or partition when mixed with the regular phospholipids. The chelate should also exist in relatively low concentration, so that the membrane consists primarily of the phospholipid(s) of choice. These issues are addressed

in the following sections through a variety of ^2H, ^{31}P, and ^{13}C NMR experiments on the lanthanide-doped and lanthanide-chelated model membrane system. The phase properties and some of the hazards, such as precipitation, associated with the chelate will then be discussed, as will the measures we have taken to avoid such hazards. Finally, results of NMR measurements of three membrane peptides oriented in the above lanthanide-chelated model membrane system are presented, and future directions are discussed.

MATERIALS AND METHODS

Sample preparation

The phospholipids, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1,2-dimyristoyl-*sn*-glycero-3-[phospho-rac-(1-glycerol)] (DMPG), 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC), and 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolaminediethylenetriaminepentaacetate (DMPE-DTPA), were obtained from Avanti Polar Lipids (Alabaster, AL). DMPE-DTPA, originally synthesized by Grant et al. (1989), was custom synthesized and purified by Avanti by a novel proprietary method. Dry mixtures of DMPC, DMPG, and DMPE-DTPA were combined with stock solutions of DHPC and lanthanide chloride hexahydrate (99.99–99.999%; Aldrich Chemicals, Milwaukee, WI). HEPES (100 mM, pH 7.0; Sigma Chemicals, St. Louis, MO) buffer in D_2O (99.999%; Cambridge Isotopes, Cambridge, MA) was then added, and a transparent solution was obtained in less than 1 h by stirring, vortexing, heating to 40°C, cooling to 0°C, and centrifuging (2000 rpm). The sample could easily be transferred to an NMR tube by first cooling the sample to 0°C. Gramicidin A′, which was obtained from Sigma Chemicals, was first exchange labeled at room temperature in deuterated methanol for 1 week; combined with DMPC, DHPC, and DMPG and lyophilized; and then rehydrated in 100 mM HEPES D_2O buffer (pH 7.0). The membrane-associated peptide CP-11C, whose sequence is ILKKWPWWPWRRK, was generously provided by Prof. R. Hancock (University of British Columbia). The unmyristoylated version of the N-terminal fragment of the human ADP-ribosylation factor 1 (ARF1), whose sequence is GNIFANLFKGLFGKK, was generously provided by Prof. J. Prestegard (Complex Carbohydrate Research Center, Athens, GA). CP-11C and the N-terminal ARF1 model peptide were simply codissolved with the previously prepared lanthanide-chelated liquid crystal.

The reader may be skeptical of any multicomponent model membrane system consisting of no less than four phospholipids (DMPC, DHPC, DMPG, and DMPE-DTPA), lanthanides, water, and a peptide. Our experience has been that the model system is straightforward to prepare and align, and amenable to the addition of peptide. Surface-associated peptides could be loaded into the system such that peptide/(DMPC + DMPG) ratios were as high as 1:40, whereas the maximum peptide/(DMPC + DMPG) ratio of gramicidin was found to be ~1:50. Thus mechanically aligned model membrane samples have the advantage that higher concentrations of integral membrane peptide may be used at the expense of sample volume losses resulting from glass plates (Moll and Cross, 1990). Depending on the peptide or protein added, a larger concentration of lanthanide than that prescribed in this paper may be required to achieve alignment. Our suggestion is therefore to begin with a Yb/DMPC ratio of ~1:90 or ~1:100 (or in the case of diffraction studies in which paramagnetic broadening is not an issue, Tm/DMPC = 1:140) and simply titrate the lanthanide until alignment is observed. Alignment can be easily monitored by ^{31}P NMR or ^2H NMR of ^2H_2O.

Apparatus and NMR measurements

^{31}P, ^{13}C, and ^2H NMR spectra were acquired at 161.98 MHz, 100.61 MHz, and 61.42 MHz, respectively, using a Bruker 400 DMX wide-bore NMR spectrometer. ^{31}P NMR experiments were carried out using a 10-mm Bruker HX probe and a one-pulse detect sequence with MLEV-16 ^1H decoupling, 16–512 transients, a 40-ms acquisition time, a 4-s repetition time, and ^{31}P and ^1H $\pi/2$ pulse lengths of 15 μs and 35 μs, respectively. NOE-enhanced ^{13}C NMR spectra were typically acquired using a standard 5-mm Bruker HNC probe and 4000 transients on a Bruker 400 DMX wide-bore NMR spectrometer. The one-pulse detect sequence with MLEV-16 ^1H decoupling typically featured a 70-ms acquisition time, a 1.5-s repetition time, and ^{13}C and ^1H $\pi/2$ pulse lengths of 15 μs and 8 μs, respectively. ^{13}C spectra were processed using 3-Hz line broadening. ^2H spectra were acquired with a Bruker 7 mm HX MAS probe. The pulse sequence consisted of a soft (π = 90 μs) water inversion pulse, followed by a 77-ms delay and a quadrupolar echo sequence ($\pi/2$ = 4.0 μs), which employed Levitt-Suter-Ernst composite 90° pulses, a 35-μs interpulse spacing, and a 160-ms repetition time.

RESULTS AND DISCUSSION

The ^{31}P NMR and ^{13}C NMR spectra in Fig. 2 illustrate the effect of the paramagnetic lanthanide on the positively aligned ($S_{l\bar{n}} = +1$) bilayer phase. ^{31}P NMR is an ideal probe of both the phase of the membrane and the degree of paramagnetic broadening, because the lanthanides are known to coordinate with the lipid phosphodiester group in the absence of chelate. The ^{31}P NMR spectrum in Fig. 2 *A* (*left*) is the result of a near-minimal concentration of Tm^{3+} (DMPC/Tm^{3+} = 150) necessary for positive alignment. The resonance for DHPC, which is believed to undergo additional motional averaging through lateral diffusion over micellar-like curvature defects, is observed to the right (upfield) of the DMPC line. Addition of Tm^{3+} to A results in significant upfield shifts and severe line broadening, as shown in Fig. 2 *B* (*left*), where the two ^{31}P lines can no longer be distinguished. This effect is paralleled in the glycerol region of the ^{13}C NMR spectrum (Fig. 2 *B, right*), in which none of the glycerol peaks can be resolved (the sharp peaks result from DHPC, which is partly expelled from the bilayer upon the addition of sufficient amounts of lanthanide). Addition of the DMPE-DTPA chelate such that DMPE-DTPA and Tm^{3+} ions are equimolar renders both the DMPC and DHPC ^{31}P NMR chemical shifts clearly resolvable (Fig. 2 *C, left*). Moreover, the shifts and the linewidths are comparable to those observed for the most dilutely doped, positively aligned membranes (i.e., DMPC/Tm^{3+} = 150). The ^{13}C NMR spectra show a similar effect; upon the addition of chelate, the upfield paramagnetic induced Cγ shift is reduced to the value observed for the most dilute lanthanide concentration, and the oriented glycerol peaks are recovered and the isotropic components are not observed (Fig. 2 *C, right*). Deuterium spectra of chain-perdeuterated DMPC (not shown) further reveal that the addition of chelate, such that the lanthanide and chelate concentrations are equimolar, does not alter the ordering of the bilayer. As discussed by Prosser et al. (1998a), the chain plateau orientational order parameter is estimated to be 0.20 at 40°C.

We can estimate the magnetic susceptibility anisotropy, $\Delta\chi$, of the chelate based on the following arguments. The magnetic anisotropy of DMPC has been measured to be -0.58×10^{-8} erg cm^{-3} G^{-2} (Scholz et al., 1984) and is

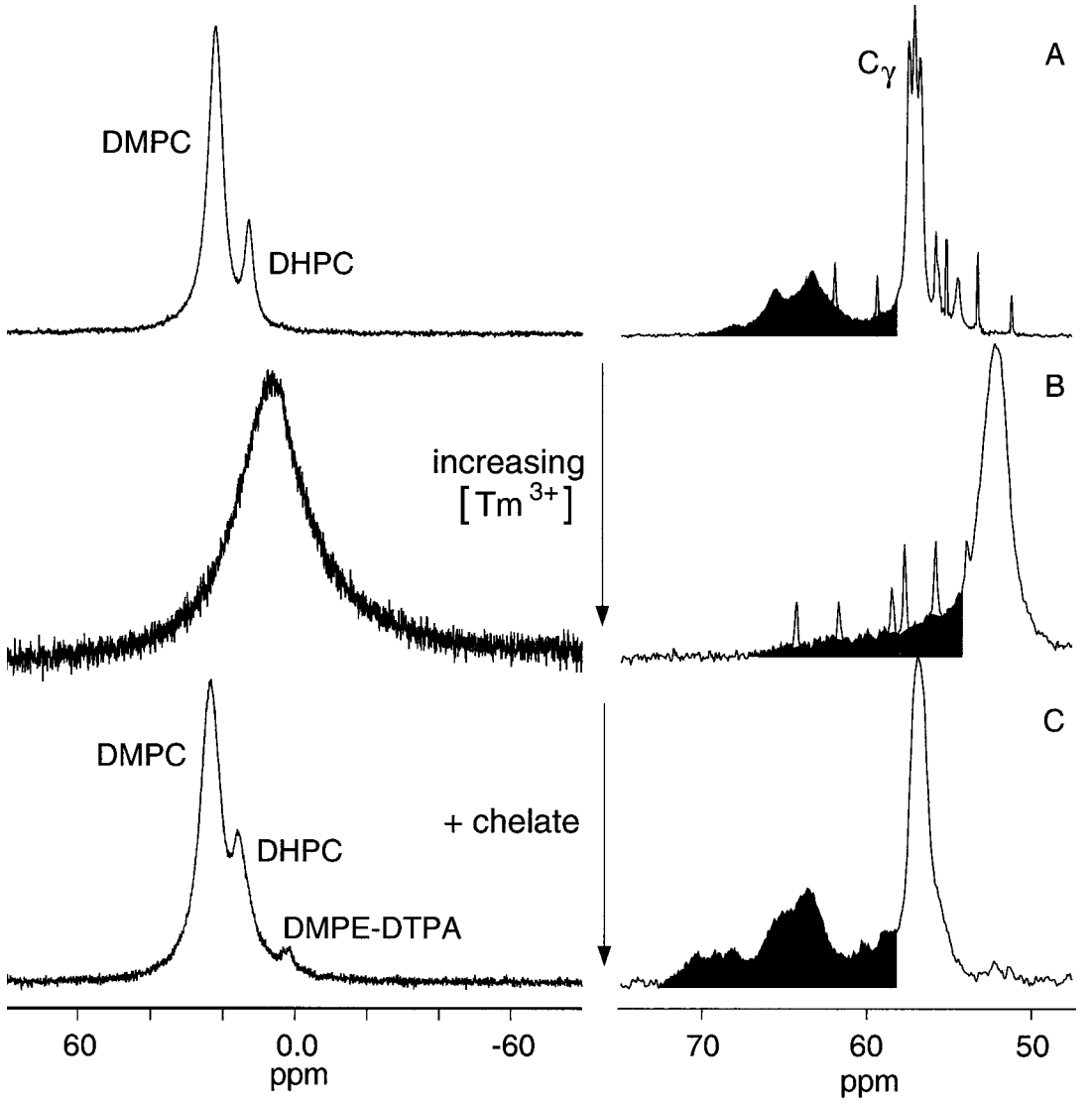

FIGURE 2 ^{31}P NMR spectra (*left column*) and the headgroup region from ^{13}C NMR spectra (*right column*) of (*A*) 40% (w/w) dispersion (in 100 mM pH 7.0 HEPES buffer) DMPC/DHPC = 6.0 Tm^{3+}-doped membrane (DMPC/Tm^{3+} = 150) at 35°C. (*B*) As in *A,* except DMPC/Tm^{3+} = 40. (*C*) As in *B,* with added DMPE-DTPA chelate such that DMPE-DTPA/Tm^{3+} = 1.0. The glycerol region of the ^{13}C spectrum is shaded for clarity.

sufficient to yield negatively aligned bicelles, as discussed by Sanders et al. (1994). The addition of Tm^{3+} or Yb^{3+} such that the Tm/DMPC or Yb/DMPC ratio is at least 0.0067 or 0.0100, respectively, is sufficient to flip the bilayers from negative to positive alignment. In the presence of equimolar DMPE-DTPA chelate, the minimal lanthanide/DMPC ratios are observed to be 0.0054 and 0.0080, for Tm^{3+}- and Yb^{3+}-doped systems, respectively. Thus, barring morphological changes in the liquid crystal introduced by the addition of a small amount of cation, the effective (motionally averaged) $\Delta\chi$ of the Tm^{3+}-doped and Yb^{3+}-doped chelate should be at least 185× and 125× that of DMPC (i.e., $+90 \times 10^{-8}$ erg cm^{-3} G^{-2} and $+60 \times 10^{-8}$ erg cm^{-3} G^{-2}, respectively). By comparison, the magnetic anisotropy of a benzene ring is ~10× that of DMPC (Sanders et al., 1993).

To demonstrate the application of the lanthanide-chelated model membrane to membrane protein and peptide studies, we investigated an integral membrane peptide, gramicidin A′, and a surface-associated peptide, whose sequence is GNIFANLFKGLFGKK. Gramicidin is known to bind a variety of cations and admit sodium ions through a channel it forms in the bilayer and is expected to strongly interact with the lanthanide ions at the water interface (Maruyama and Takeuchi, 1997; Hao et al., 1997), making it a good candidate for testing the efficacy of the chelate in the positively aligned lanthanide-chelated model membrane system. This peptide, whose structure is very sensitive to environment (Bouchard and Auger, 1993; Bouchard et al., 1995), was previously shown not to align in a bicellar phase (Sanders and Landis, 1995). Fig. 3 contrasts the ^{2}H NMR spectra of ^{2}H exchange-labeled gramicidin A′ in a posi-

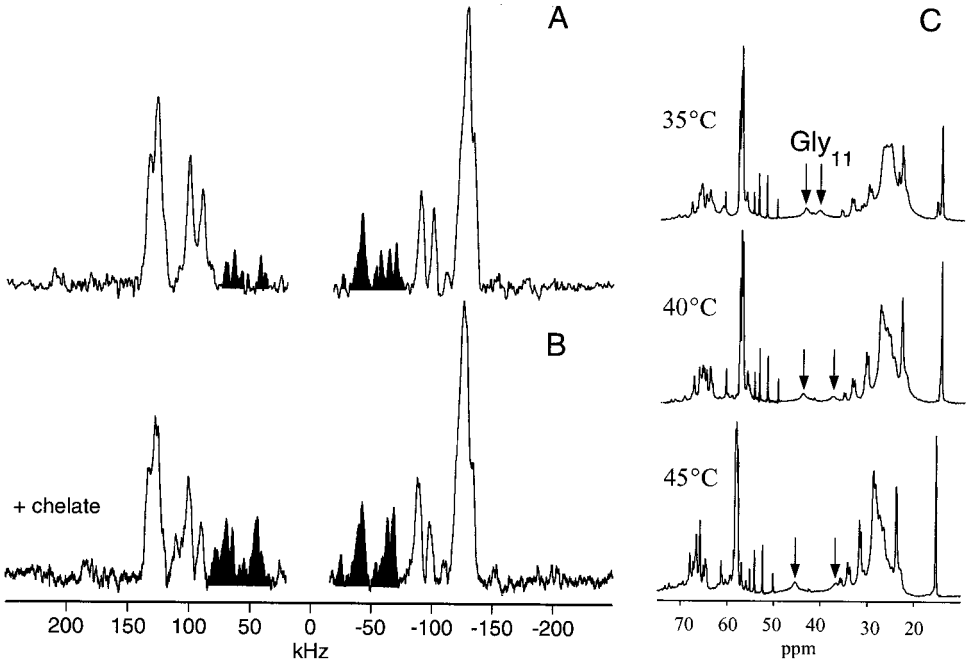

FIGURE 3 (*A*) ^{2}H NMR spectra of ^{2}H exchange-labeled gramicidin A′ in a 40% (w/w) DMPC/DHPC = 6.0, Yb^{3+}-doped membrane with DMPC/Yb^{3+} = 60, DMPC/DMPG = 20, and DMPC/gramicidin = 50 at 40°C. (*B*) As in *A,* with added chelate such that DMPE-DTPA/Yb^{3+} = 1.0. Both spectra were the result of 600,000 transients and a 1000-Hz line broadening. The shaded region is assigned to the tryptophan indole groups, and the central region of the spectrum is not shown, because this region consisted of residual water signal, which was further removed by a polynomial subtraction algorithm. (*C*) ^{13}C NMR spectrum showing the phospholipid aliphatic and glycerol region in addition to the carbonyl-alpha carbon splitting ($J + D$) arising from Gly^{11} in the unmyristoylated N-terminal fragment of the human ADP-ribosylation factor 1 (ARF1), whose sequence is GNIFANLFKGLFGKK. (Losonczi and Prestegard, 1998). This sample consisted of a 40% (w/w) solution of DMPC/DMPG/DHPC/chelate/peptide in a 6/0.3/1.0/0.06/0.12 molar ratio, and 100 mM HEPES pH 7.0 buffer. The spectra were acquired with 40,000 scans and MLEV-16 ^{1}H decoupling, using an 8-μs ^{1}H $\pi/2$ pulse.

tively aligned Yb^{3+}-doped membrane with and without equimolar DMPE-DTPA chelate. The quadrupolar splittings reflect the average orientation of the ^{2}H-N bond with respect to the peptide helix axis, and in the absence of dipolar dephasing, paramagnetic shifts, or pulse imperfections, a symmetrical spectrum would be expected. The spectra, which may be compared with published spectra of oriented exchange-labeled gramicidin (Prosser et al., 1994), are both consistent with the channel structure model recently proposed by Ketchem et al. (1997). There are two noteworthy effects of the chelate observable in the gramicidin spectra. The innermost doublets, attributed to the side-chain tryptophan indoles, appear to be more symmetrical. The total intensity of the upfield shaded resonances divided by the total intensity of the downfield indole resonances is observed to change from 2.9 ± 0.1 to 1.1 ± 0.1 upon the addition of chelate. (The signal-to-noise ratio is the same in the two gramicidin spectra after accounting for the fact that there was ~10% less sample in the chelate sample. The upfield intensity of the backbone resonances (*unshaded*) divided by that of the downfield backbone resonances changes by 36%.) Furthermore, the inner resonances amount to 14% of the total spectral area in the spectrum in Fig. 3 *A,* whereas they represent 28% of the total spectral intensity in Fig. 3 *B.* These indole resonances are primarily associated with sites that lie close to the membrane-water interface and therefore might be susceptible to paramagnetic interactions in the absence of chelate. Therefore, the dramatically improved symmetry in the indole doublets and the increased intensity are interpreted to be a result of the fact that the chelate sequesters the lanthanides away from the peptide-binding site. The combination of chelate and negative lipid also makes this oriented phase much more robust; in the absence of chelate the gramicidin sample was observed to precipitate above 40°C, and upon the addition of chelate, an oriented phase could be observed up to 70°C. Recently, we have tested the use of the DMPE-DTPA chelate in positively aligned Yb^{3+}-doped bilayers for two membrane-associated peptides: CP-11C (an antibacterial peptide whose primary sequence is ILKKWPWWPWRRK) (Falla and Hancock, 1997) and the unmyristoylated version of the N-terminal fragment of the human ADP-ribosylation factor 1 (ARF1), whose sequence is GNIFANLFKGLFGKK (Losonczi and Prestegard, 1998). In both cases, the spectra appeared to be well aligned and stable. ^{13}C spectra of the labeled N-terminal ARF 1 model peptide, in an aligned ($S_{\bar{1}\bar{1}} = +1$) Yb^{3+}-chelated phase, are shown in Fig. 3 *C.* Note that the splitting associated with the coupling between the carbonyl and alpha-carbon of Gly^{10} increases with increasing temperature. Because this splitting is the result of a scalar interaction, *J*, which is essentially independent of temperature and orientation, and a dipolar interaction, *D*, whose magnitude decreases with increasing temperature, the sign of the dipole coupling can be easily

determined in this instance (Sanders et al., 1994). The spectra in Fig. 3 *C* also show very well-resolved glycerol peaks (60–75 ppm), which result from the use of minimal concentrations of Yb^{3+} and chelate.

It should be noted that paramagnetic broadening is determined by $\Delta\chi$, the concentration, electronic relaxation time, and the spin quantum number associated with the lanthanide (Bleaney, 1972; Horrocks, 1973; Lenkinski, 1984). Although Tm^{3+} concentrations necessary for positive alignment were significantly lower than corresponding minimal Yb^{3+} concentrations, ^{31}P and ^{13}C spectra from Yb^{3+}-doped bilayers exhibited the best resolution among Eu^{3+}, Er^{3+}, Tm^{3+}, or Yb^{3+} ions (Prosser et al., 1998b) and are thus recommended for solid-state NMR purposes. Our experience has been that the combination of Yb^{3+} and chelate at minimal lanthanide concentrations results in ^{13}C lipid spectra whose resolution is slightly better than that of published spectra of lanthanide-free bicelles (Sanders and Schwonek, 1992). (Dipolar and quadrupolar splittings and CSA offsets in the ($S_{l\bar{n}} = +1$) system are at least twice those measured in bicelles, whereas line widths are similar, rendering the lines easier to resolve.) Therefore, under these optimal conditions the two sources of paramagnetic line broadening, through bond contact interactions and dipolar broadening (Bleaney, 1972), are expected to be negligible. Certainly, contact effects can be discounted, because the lanthanides are sequestered by the chelate. However, linewidths from ^{31}P NMR spectra of Yb^{3+}-chelated model systems at minimal lanthanide concentrations are larger than those observed in bicelles. This discrepancy can be explained by recognizing that the bilayer undergoes collective fluctuations of the director or surface undulations, which partly determine transverse relaxation times and linewidths (Althoff et al., 1996). This relaxation mechanism will of course depend on the magnitude and nature of the spin-spin interaction and on the orientation of the sample with respect to the magnetic field. Typically, linewidths are observed to be narrowest for $S_{l\bar{n}} = -½$. This effect is observed in the above ($S_{l\bar{n}} = +1$) ^{2}H gramicidin spectra, where linewidths are several times greater than those reported in a similar mechanically aligned system (Prosser et al., 1994) for which $S_{l\bar{n}} = -½$. However, if we compare spectra at the same orientation ($S_{l\bar{n}} = +1$) and reduced temperature, the resolution demonstrated in the above gramicidin spectrum (Fig. 3) was in fact slightly better than that observed in the mechanically aligned sample (Prosser et al., 1994). (Reduced temperature is defined as the difference between the measured temperature and the lipid main phase transition temperature, divided by the transition temperature.)

In summary, the model membrane consisting of the phospholipids DMPC and DHPC, in combination with the negatively charged lipid, DMPG, and a minimal concentration of DMPE-DTPA chelate, complexed with equimolar Yb^{3+}, results in a stable positively aligned bilayer phase suitable for membrane protein studies. We typically use either a 25% (w/w) dispersion of DMPC/DHPC/DMPG/DMPE-DTPA:Yb^{3+} in a 2.75/1.00/0.1375/0.0306 mole ratio or a 40% (w/w) dispersion of DMPC/DHPC/DMPG/DMPE-DTPA:Yb^{3+} in a 2.75/0.458/0.1375/0.0306 mole ratio; the 25% mixture often results in the best linewidths, whereas the latter combination yields larger order parameters and chemical shift offsets. The addition of peptide often necessitates higher lanthanide concentrations to maintain alignment. In such cases, the chelate is expected to be essential in sequestering the lanthanides away from the protein or molecule of interest and minimizing paramagnetic broadening. Both ^{31}P and ^{13}C NMR spectra reveal that the DMPE-DTPA chelate, in combination with minimal concentrations of Yb^{3+}, significantly reduces paramagnetic shifts and broadening in both lipid and peptide spectra. The minimal Yb^{3+} concentration for positive alignment in the presence of equimolar chelate is observed to be ~25% smaller (i.e., Yb^{3+}/DMPC = 0.008 with chelate versus Yb^{3+}/DMPC = 0.010 without chelate). Furthermore, the use of negatively charged lipid and chelate stabilizes the liquid crystalline phase as a function of temperature and seems to prevent long-term sample degradation. The use of negatively charged lipid in combination with the chelate is essential. The samples used to obtain the spectra in Fig. 2 did not contain negatively charged lipid, simply for purposes of illustrating the function of the chelate. The greatest advantage of the positively aligned lanthanide-chelate membranes lies in their application to the study of large immobile membrane proteins, which, as discussed in the introduction, are not amenable to bicelles. Low-angle neutron diffraction studies have demonstrated that well-resolved Bragg peaks can be obtained from magnetically aligned lanthanide-doped bilayers (Katsaras et al., 1997); the DMPE-DTPA chelate, which clearly sequesters the lanthanide ions, now makes such low-angle diffraction experiments feasible for membrane proteins. In addition, the positively aligned lanthanide-chelated bilayers, which are transparent, should be useful for membrane peptide studies by a variety of optical techniques that require oriented transition dipole moments (e.g., linear dichroism, ATR-FTIR, fluorescence anisotropy). Finally, the combination of lanthanide chelates with huge magnetic susceptibility anisotropies and low-spin paramagnetic species such as Ce^{3+} should have many applications beyond membranes. Lanthanide chelates with large $\Delta\chi$ might facilitate the design of new metallomesogens and liquid crystal polymers (Galyametdinov et al., 1996). Similar chelates could easily be introduced into water-soluble proteins and macromolecules for high-field NMR studies such as those proposed by Prestegard, Tolman (Tolman et al., 1995), and others.

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