Orientational behavior of phosphatidylcholine bilayers in the presence of aromatic amphiphiles and a magnetic field

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ABSTRACT A number of aromatic-containing additives which can influence the orientation of fragments of lipid bilayer membranes by a magnetic field have been investigated. Two properties of these additives prove important: (1) sufficient detergency to facilitate reorganization of bilayer components and (2), sufficient anisotropy in magnetic susceptibility to alter the preferred direction of fragment orientation. Triton X-100 is identified as effective in terms of facilitating magnetic field ordering of bilayer fragments but does not alter the preferred direction of orientation. A combination of the detergent CHAPSO (3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate) and the aromatic alcohol 1-naphthol facilitates both ordering and alters the preferred direction of bilayer orientation. As mixtures of dimyristoylphosphatidylcholine (DMPC) and CHAPSO, which orient with bilayer normals perpendicular to the magnetic field, were titrated with 1-naphthol, the assemblies underwent transitions, first to random orientation, and then to an orientation with bilayer normals parallel to the field. Based on temperature-induced phase transitions and the extent of motional averaging of the ^{31}P shielding tensor of the DMPC headgroup, the DMPC in these oriented samples appears to maintain a bilayer morphology during transitions. The insight provided in this study regarding factors which influence fragment stability and orientation lays the groundwork for the design of improved field-oriented media for spectroscopic investigation of membrane components.

INTRODUCTION

Under certain conditions it is possible to magnetically orient phospholipid bilayers such that their normals are perpendicular to the direction of the magnetic field (1–5). Observations of this phenomenon are not surprising, since it is known that the presence of a magnetic field can produce nematic order in various detergent-based bilayered lyotropic mesophases (6, 7). It is also known that such detergent-based nematic bilayer systems can be divided into two classes: those which orient with their bilayer normals parallel to the field, and those which orient with their bilayer normals perpendicular to the field (6, 7). However, reports of phospholipid-based systems which orient with normals perpendicular to the field have been rare. In the cases which have been reported, the systems contain significant levels of non-lipid components or involve whole native membranes (8–11).

In this communication, we investigate possible strategies for deliberately producing simple phospholipid-based aggregates which orient with their bilayer normals parallel to the field direction. The rationale for attempting this feat lies in the advantages such media offer for spectroscopic (NMR) observation of membrane constituents. The parallel orientation produces maximal dipolar and quadrupolar splittings in cases where rapid axial rotation of molecules about the bilayer normal occurs. Parallel orientation of bilayer normals also produces chemical shift offsets due to chemical shift anisotropies that are twice as large and in the opposite direction as those observed with perpendicular orientation. In addition, unlike other orientations, parallel orientations for axially symmetric systems give rise to spectra free of powder patterns regardless of the rates of molecular motion.

All molecules have a natural tendency to adopt a preferred orientation in the presence of a magnetic field. When placed in a field, changes in electron motion modify the applied field in proportion to the susceptibility of the molecule, χ. The additional field contributions can be viewed as arising from induced magnetic dipoles centered in the molecule of interest. For diamagnetic materials, these dipoles oppose the field (H) and hence give rise to an unfavorable energy of interaction with the field ($-\chi H^2$). Because electronic structure is not isotropic, χ is actually a tensor, and one orientation of the molecule (the preferred orientation) will have a lower energy of interaction ($-\Delta\chi H^2$, where $\Delta\chi = \chi\| - \chi\perp$ for an axially symmetric system). The energy differences for single molecules are normally very small compared to thermal energy (k_BT). So, departures from isotropic behavior are seldom observed in simple solutions (see 12). However, in cooperative systems, such as found in phospholipid based bilayers or nematic arrays of bilayer fragments, energy differences can become large, and one might expect spontaneous adoption of a preferred orientation (6, 7).

For phospholipids, the principal axis of the susceptibility tensor is believed to lie along the average direction of the long hydrocarbon chains of the fatty acids (13, 14). This becomes the direction least likely to lie along the magnetic field due to the negative anisotropy ($\Delta\chi$). Hence, simple bilayer fragments should orient with their

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normals perpendicular to the field. Altering this behavior requires altering the effective susceptibility tensor of the bilayer. One approach involves adding amphiphiles which have their principal susceptibility axis in the plane of the bilayer. Amphiphiles based on aromatic ring systems provide one such option. A single phenyl ring has an anisotropic susceptibility approximately 10 times that of a rigid phosphatidylcholine molecule (12, 14). Amphiphiles containing these moieties should prove to be powerful agents for modifying orientational behavior of bilayer fragments. If such molecules are inserted into bilayers with a very high degree of order, it should be possible to overcome the inherent susceptibility of phosphatidylcholine and reorient the membrane fragments at mole ratios of approximately 1:10.

Having a proper magnitude and orientation of an effective susceptibility tensor is not the only requirement for producing media with desired field-induced orientational properties. There are, in fact, many bilayer systems which have large and anisotropic susceptibility tensors which do not exhibit field-induced orientation. It has previously been postulated (5) that the primary barrier to the magnetic orientation in these cases is kinetic, associated with the formation of very extended multilamellar structures which cannot easily reorient independent of one another. This barrier can be overcome by adding certain detergent-like molecules to break up the extended lamellae into detergent-stabilized bilayered discoidal particles which easily rotate in the presence of a magnetic field. The most effective detergents are believed to preferentially segregate to the edges of bilayers providing stabilization of smaller discoidal fragments, as discussed in (5). (Sanders, C. R., and J. P. Schwonek. 1992. *Biochemistry.* 31:8898–8905). It is therefore desirable to try to combine moieties with highly anisotropic magnetic susceptibilities with amphipathic properties that promote bilayer disk formation. We will, therefore, focus on simple aromatic ring based amphiphiles in this study. While not ideal in terms of the relationship to natural membranes, an understanding of the behavior of these systems may lay the groundwork for the design of field-ordered membrane mimetic media which can be of use in spectroscopic studies of membrane-associated molecules.

METHODS

Aromatic chemicals were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). Triton X-100 and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) were purchased from Sigma Chemical Co. (St. Louis, MO). All compounds were used without further purification. Sample preparation was analogous to that described in a previous characterization of orientable mixtures of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine(DMPC)and3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate (CHAPSO, 5). Titrations of DMPC with Triton were executed by adding aliquots of 25% Triton X-100 stock solutions directly to DMPC dispersions in 5 mm NMR tubes. 1-Naphthol was added directly to CHAPSO-DMPC mixtures followed by extended cycles of heating, cooling, and mixing until the mixtures were homogeneous (this was not attained at the highest levels of naphthol). Other amphipathic aromatics were mixed with DMPC and hydrated with 0.1 M KCl, followed by extended heating, cooling, and mixing. In some cases, the samples remained visibly inhomogeneous.

^{31}P nuclear magnetic resonance (NMR) spectra were acquired at 202 MHz with 5 Watts broadband ^{1}H decoupling using a Bruker AM-500 spectrometer. Samples were not spun and spectra were acquired under unlocked conditions. Typically 200 scans were acquired using a 12 μs 90° pulse and a repetition rate of 2 s. Spectra were processed with 5–75 Hz exponential linebroadening to improve signal-to-noise ratios in spectra displayed.

RESULTS

"Amphipathic" aromatic-DMPC mixtures

It is apparent that any strategy for homogeneously aligning PC bilayers with normals *parallel* to the field direction must take two things into consideration: the need to produce discrete bilayer fragments, and the need to overcome the intrinsic $\Delta\chi$ of saturated PC bilayers. Ideally one might find a molecule to mix with DMPC which is capable of stabilizing the edges of bilayers *and* which possesses a $\Delta\chi$ of the correct sign and orientation to overcome the inherent $\Delta\chi$ of PC. Our first attempts will be centered around this strategy.

Amphipathic molecules possessing asymmetric distributions of polar groups such that one side is hydrophobic and the other is hydrophilic can, in principal, stabilize the edges of bilayers. Aromatic molecules in which one side of the ring system was functionalized with polar moieties while the other is either not functionalized, or functionalized only with apolar groups might fit this description. We envision these molecules as potentially intercalating with the acyl chains, such that the polar functionalized edge would face the solution and the aromatic ring system normals would always be in the bilayer plane. Since the $\Delta\chi$ of aromatic rings is negative and lies along the aromatic ring system normal, this arrangement would result in a parallel-orienting force on the bilayers in the presence of a magnetic field. A number of amphipathic aromatics were, therefore, purchased and mixed with hydrated DMPC in the presence of excess water.

^{31}P NMR resonances from the phosphate containing headgroups of phopholipids provide a convenient means of monitoring bilayer orientation in these mixtures. For a phosphodiester, the anisotropy of the chemical shift tensor is approximately 250 ppm (15). For randomly oriented immobile bilayer fragments, resonance intensity will be spread over this entire region, giving a classical powder pattern. Even for random distributions, powder patterns are not featureless because certain orientations of the principal axis of a shift tensor are more probable than others. Vectors pointing at the equator of a globe (90°) are, for example, more probable than vectors pointing at the poles (0°). Hence, powder patterns for axially symmetric shift tensors have a sharp, high

amplitude 90° edge, and an abrupt low amplitude termination at the 0° edge.

Because of averaging in typical bilayer-based liquid crystal media, the span of a typical powder pattern is not 250 ppm, but is closer to 45 ppm, with the 90° edge normally appearing upfield (16). As a sample becomes oriented, this powder pattern will disappear, and will be replaced with a single Lorentzian line. If a motional director axis is oriented perpendicular to the field and none of the internal molecular motions have changed, the line will lie at the 90° edge of the original powder pattern (approximately +15 ppm relative to an isotropic system). If the director is aligned parallel to the field and none of the internal molecular motions have changed, the line will appear at the 0° edge (approximately −30 ppm relative to an isotropic system).

The contents of DMPC–aromatic amphiphile mixtures and the resulting ^{31}P spectra at 40° are illustrated in Fig. 1. In a number of cases, it was not possible to achieve a homogeneous mixture of the lipid and the added aromatic compound. Compositions therefore correspond to total amounts in the samples and not concentrations of any one phase. In the absence of added amphiphile, a classical axially symmetric powder pattern is observed. The shape is indicative of a random orientation of bilayer fragments. In a few cases, addition of the "amphipathic" aromatic led to a reduction in the intensity in the downfield region of the powder pattern in favor of increased intensity at the upfield edge. This is indicative of preferred orientation with the bilayer normal perpendicular to the field. Thus, some of these compounds promote field-induced orientation, but only at 90° (e.g., dithranol). In no case did a distinct 0° spectral component appear in the 20–40 PPM region. It is difficult to ascertain exactly why this approach failed. However, the limited solubility and tendency of bilayer fragments to remain randomly dispersed suggest that most do not have detergency properties adequate to break extended bilayers into easily reorientable fragments. In the cases where reorientation did occur, but not to the desired 0° orientation, the problem could lie in either insufficient solubilization of the amphiphile or improper aromatic placement and orientation within the bilayer assembly.

Triton X-100–DMPC mixtures

In an attempt to improve the detergency properties we explored the use of Triton X-100. The interaction of Triton X-100 and related detergents with phosphatidylcholine (PC) has been subjected to extensive study (c.f. references 17 and 18). Although much remains to be learned about specific structures formed, the ability of Triton to solubilize membrane structures is well known, and it does contain a phenyl group.

Fig. 2 shows that the addition of even small amounts of Triton X-100 (1:7.7 mole ratio relative to DMPC) results in collapse of the usual ^{31}P powder pattern into a single sharp peak located at the resonance position of the 90° component of the powder pattern. The appearance of this dominant, nearly symmetric peak is most likely the result of orientation of the DMPC bilayers, such that their bilayer normals are perpendicular to the direction of the magnetic field. The detergent properties of Triton provide an effective mechanism for reorientation of fragments. On further addition of moderate amounts of Triton, the only changes observed are slight shifts toward the isotropic position of the ^{31}P resonance. These shifts are expected even if no perturbation of average DMPC structure occurs because tendencies of detergent to produce smaller bilayer fragments (disks) allow greater wobble about an average orientation and some reduction in orientation dependent shift. In fact, such shifts are observed for the CHAPSO–DMPC system at comparable levels of CHAPSO (5), but the Triton-induced shifts are much less, suggesting a somewhat larger fragment size for the DMPC–Triton system. Recent measurements of anisotropic diffusion in the CHAPSO–DMPC system have led to an estimate of disk diameter of 207 Å for a 3:1 DMPC:CHAPSO ratio (Chung, N. N., and J. H. Prestegard, manuscript submitted for publication). Data on the Triton–DMPC system at low Triton levels confirm a dramatic difference in size or geometry of the oriented arrays, but did not allow accurate estimates of particle size. The retention of a very large orientation dependent shift also suggests that while DMPC promotes bilayer orientation, it does not significantly perturb the overall motions of the bilayers or the individual DMPC molecules within the bilayers.

At a detergent:DMPC ratio of 1:1.7 a second resonance near the isotropic shift of DMPC appears. This is consistent with the appearance of an apparent isotropic lipid phase in equilibrium with the oriented phase. Micellar aggregates in equilibria with extended bilayers have been postulated in previous studies of Triton-phospholipid systems (18). At no point is a 0° orientation component seen. This could be the result of inadequate immobilization of the phenyl ring in the bilayer matrix. Triton differs from the more idealized aromatic systems discussed above by having rather flexible linkages to hydrophilic and hydrophobic portions of the molecule.

The Triton system is nevertheless interesting because of its ability to induce orientation at low levels with seemingly minor perturbations of native bilayer structure. For this reason, some additional investigations were undertaken. A 1:3.5 Triton:DMPC mixture was examined at 40° at concentrations of 5, 20 (see Fig. 3), and 45% total amphiphile:salt solution (wt/vol). The 5 and 45% samples yielded spectra very similar to the 40° spectrum depicted in Fig. 3. This result suggests that oriented phases are formed over a wide range of water contents. Since larger bilayer fragments can sterically interact to form cooperative domains at lower overall lipid contents than small discoidal fragments, the observation also supports the existence of rather extended bilayer structures.

The temperature dependence of orientation in Triton-

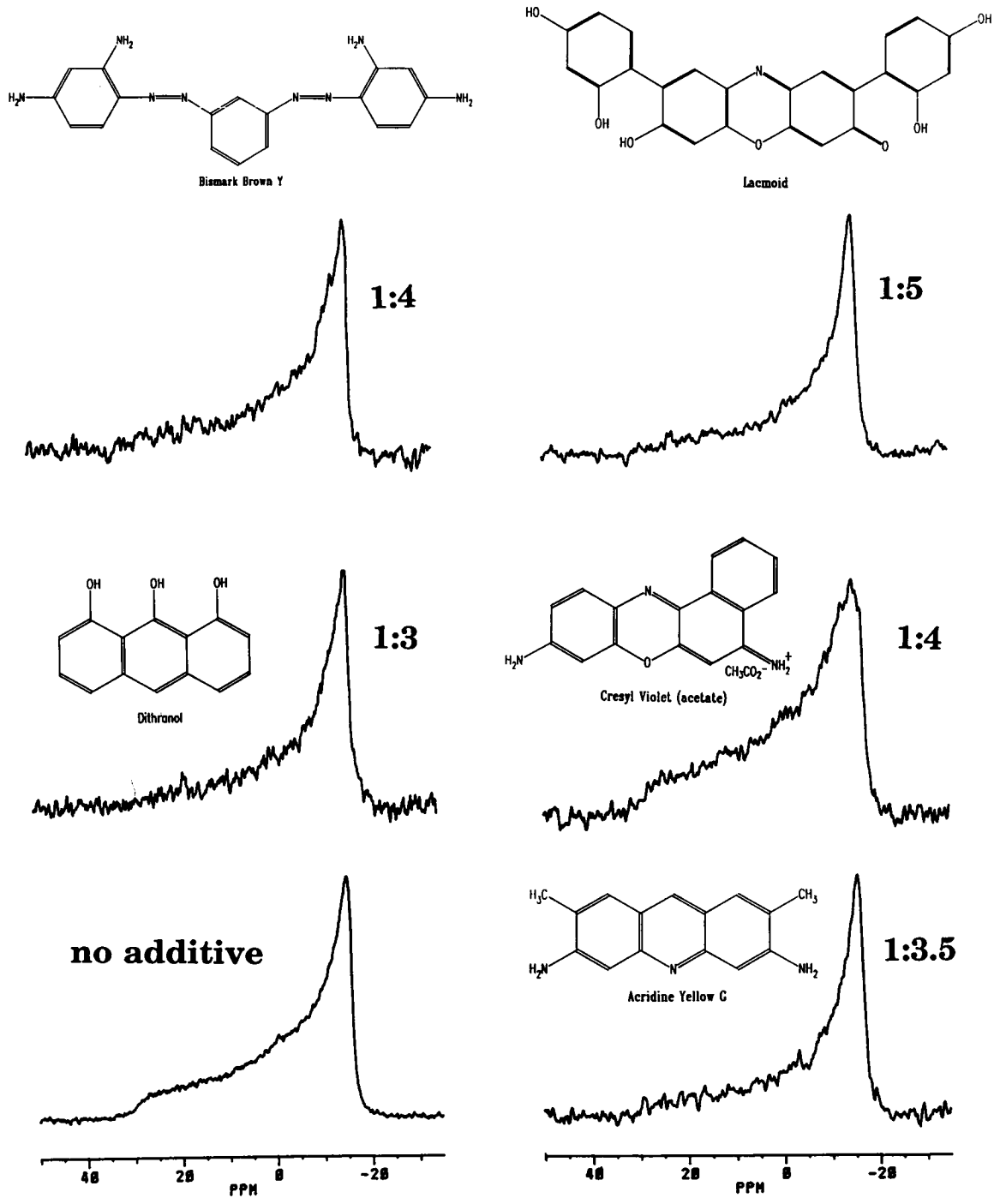

FIGURE 1 40° ^{31}P (^{1}H-decoupled) spectra of mixtures of DMPC with various "amphipathic" aromatic molecules. The spectra are labeled both with the aromatic additive present and the additive: DMPC ratio. These samples contained 30% (wt/vol) total "amphiphile" (aromatic + DMPC) in 0.1 M KCl/D_2O.

DMPC mixtures is shown in Fig. 3. It can be observed that between 23 and 20° orientation is apparently lost and a very broad powder pattern appears. This spectral transition is expected for L_α-like bilayers when the temperature is lowered below the L_α–L_β transition temperature, T_m. This transition was observed to occur in the Triton–DMPC mixture at a temperature within a few degrees of the T_m for pure DMPC (24°), providing additional support for an L_α-like bilayer morphology in Triton–DMPC mixtures at ratios of at least 1:3.5.

We also acquired a ^{2}H NMR spectrum from a 25% 1:5 Triton:DMPC sample containing a few mgs of acyl-perdeuterated DMPC. This spectrum is shown in both minimally apodized and Gaussian resolution-enhanced forms in Fig. 4. The powder pattern for unoriented d_{54}-DMPC at 40° has been reported (5) and it is possible to

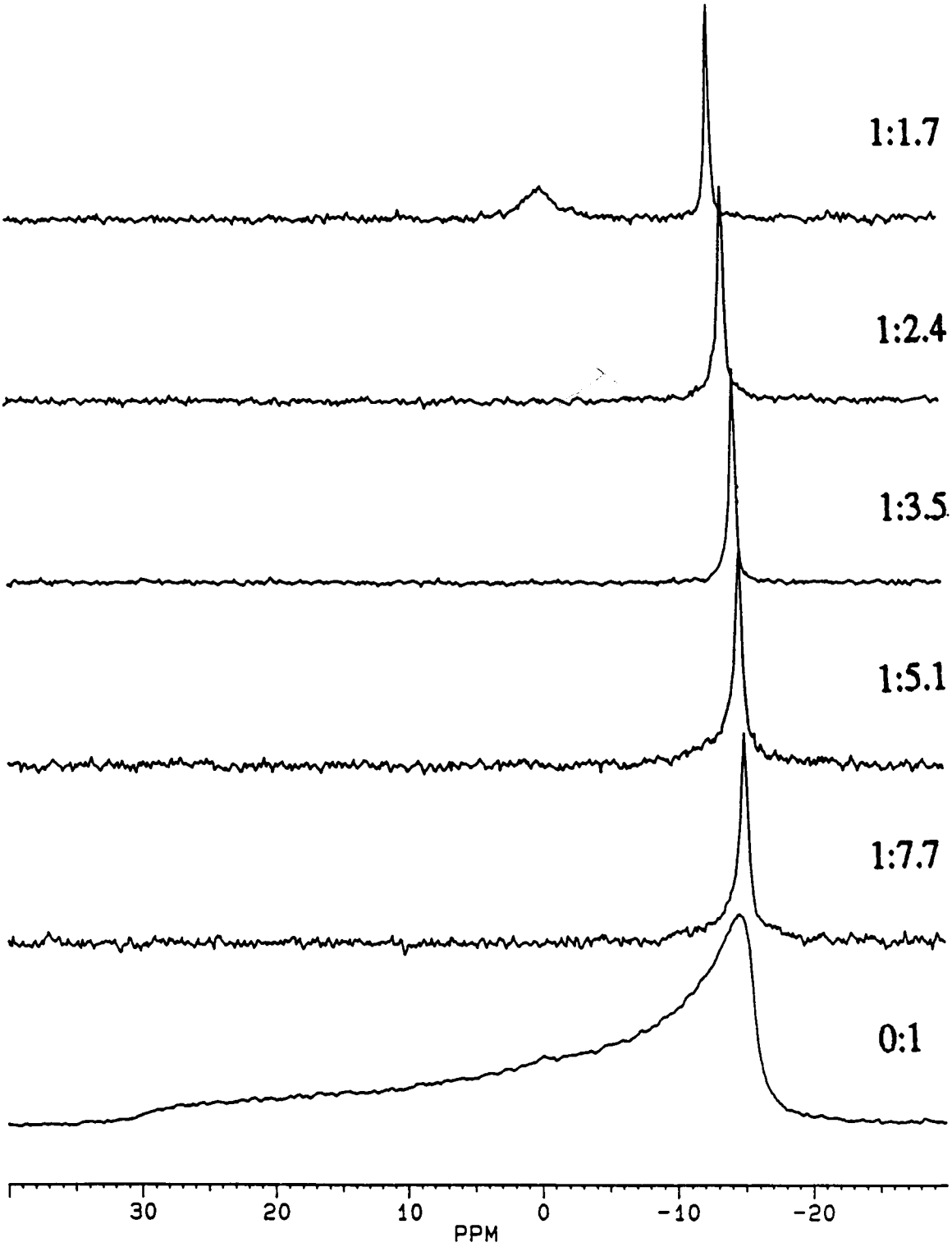

FIGURE 2 ^{31}P spectra (^{1}H-decoupled) of DMPC during titration by Triton X-100 at 40°C. The mixtures contained 20% (wt/vol) total amphiphile in 0.1 M KCl/D_2O. Spectra are labeled with the Triton:DMPC molar ratios and are referenced to external H_3PO_4.

observe that the components in the high order (large splittings) portion of the spectrum in Fig. 4 are scaled down by factors which are roughly 0.94, while the terminal methyl splitting is scaled down by a factor of 0.82. The scaling observed for the "high order" splittings roughly corresponds to the small shift towards isotropic position observed in the ^{31}P spectrum, suggesting that this scaling reflects a small increase in global bilayer disorder, while the additional scaling observed for the terminal methyls reflects a Triton-induced disordering of the

interior of the bilayers. The latter effect appears to be small. The ^2H spectrum is, thus, fully consistent with maintenance of an liquid crystalline (L_α)-like bilayered state in the highly oriented samples.

1-Naphthol–CHAPSO–DMPC mixtures

In a final attempt to achieve our objective of altering the orientational preference of bilayer fragments, we decided to separate the requirements for stabilization of bilayer fragments and alteration of susceptibility. Much of our previous work has been done using the nonionic bile salt detergent, CHAPSO (5). It should be noted that while the $\Delta\chi$ of CHAPSO and other bile salts is unknown, it is unlikely to play a major role in determining the preferred orientation of DMPC assemblies. There are no areas of extensive conjugation and the mole fraction of CHAPSO in DMPC can be kept relatively low.

In terms of choosing a suitable aromatic we might consider either a molecule totally bereft of polar groups or one which contains only a single polar moiety. Fully non-polar aromatics would be predicted to partition to the interior of the membrane where, due, to the acyl chains, they might be expected to remain oriented such that their ring system normals are in a plane coplanar with the plane of the bilayer (and thereby promote parallel orientation). Those with a single polar "headgroup" would be predicted to partition at the interface in order to allow the polar moiety to be hydrated (19). Representatives from both classes of molecules were investigated.

^{31}P spectra at 40° were taken of 30% 1:4.5 CHAPSO–DMPC mixtures containing anthracene and naphthalene at ratios to DMPC of 1:2.25 and 1:3, respectively. No appearance of a parallel component in either case was observed (data not shown). It is quite possible that the interior of L_α-like bilayers is insufficiently ordered to constrain aromatic molecules via intercalation with the acyl chains.

As a representative of aromatic molecules possessing a polar "headgroup" we chose 1-naphthol. The ^{31}P powder pattern for a random dispersion of fully hydrated pure DMPC above its L_β–L_α phase transition is illustrated at the bottom of Fig. 5. Addition of CHAPSO to this sample (1:4.2 CHAPSO:DMPC mole ratio) results in elimination of the powder pattern as a result of magnetically-induced orientation. The Lorentzian resonance is near −15 ppm, indicating orientation with normals perpendicular to the magnetic field. There is a small downfield shift (several ppm) that is consistent with a shift of overall bilayer order towards isotropic behavior.

The 1:4.2 CHAPSO:DMPC sample was then titrated with 1-naphthol. Initial addition of naphthol (1:5.4 naphthol:DMPC) does not significantly affect the ^{31}P spectrum of DMPC (Fig. 5) suggesting little perturbation of the orientation, structure, or order of the CHAPSO–DMPC aggregates by the aromatic compound. At 1:3.6, an isotropic-like spectral component near 0 PPM appears. In addition, the main upfield spectral component shifts upfield by a couple of PPM to −13 PPM and becomes distinctly asymmetric. The upfield shift of this peak may result from a perturbation in overall bilayer order, a perturbation of the static tensor components of the phosphorus, or from a small change in averaged tensor orientation due to the presence of the bicyclic additive. Asymmetry in this peak may reflect residual sample heterogeneity.

Addition of 1-naphthol to 1:2.7 resulted in the appearance of a third spectral component near 26 PPM, a reduction in the 90° oriented component, and another increase in intensity of the isotropic-like component. The +26 PPM peak is asymmetric. At both 1:2.2 and 1.8:1 naphthol:DMPC the 90° oriented component is almost completely eliminated and the (now symmetric) +26 PPM component dominates the spectra. This component does not appear to represent a hexagonal phase (where the cylinder axes would be parallel with the field) because the ^{31}P signal from such a phase would show up around +13 PPM due to axial averaging about *both* long molecular axes and the cylindrical phase axes (16). Instead, it can be observed that the +26 PPM resonance appears at exactly −2 times the chemical shift of the 90° oriented component. This observation strongly suggests that bilayers are being oriented with normals parallel to the field as a result of the addition of 1-naphthol.

It is believed that when 1-naphthol and other aromatic monoalcohols bind to a membrane surface the hydroxyl moiety insures that their average position in the bilayer is at the interface in order to maintain hydration of the -OH moiety (19, 20). Because the interface is sterically crowded, the aromatic ring system will insert into the bilayer. This would place the normal to the ring system in the bilayer plane. The molar $\Delta\chi$ has been determined for 1-naphthol (21) and it is well over an order of magnitude greater than that of DMPC in an L_α state (13). Thus, as DMPC bilayers are titrated with 1-naphthol it would be expected that the $\Delta\chi$ of the DMPC would be canceled out, first to produce assemblies with no net diamagnetic susceptibility (and hence isotropic behavior, if they possess enough motional freedom), and finally, at higher naphthol concentrations, parallel oriented bilayers.

There are a few problems that should be considered before concluding that these observations are totally in accord with expectations. First, the ^{31}P spectra indicate coexistence of multiple orientational states in several samples. Second, the amount of naphthol needed to tip the orientation in favor of the parallel state was much higher than would be predicted simply by comparison of the $\Delta\chi$s for DMPC and naphthol.

The slow-exchange coexistence of phases of differing orientational states as revealed in the spectra of Fig. 5 likely arises from sample inhomogeneity. It was observed that at naphthol levels above 1:3.6 it was very difficult to produce visually homogeneous samples. Sev-

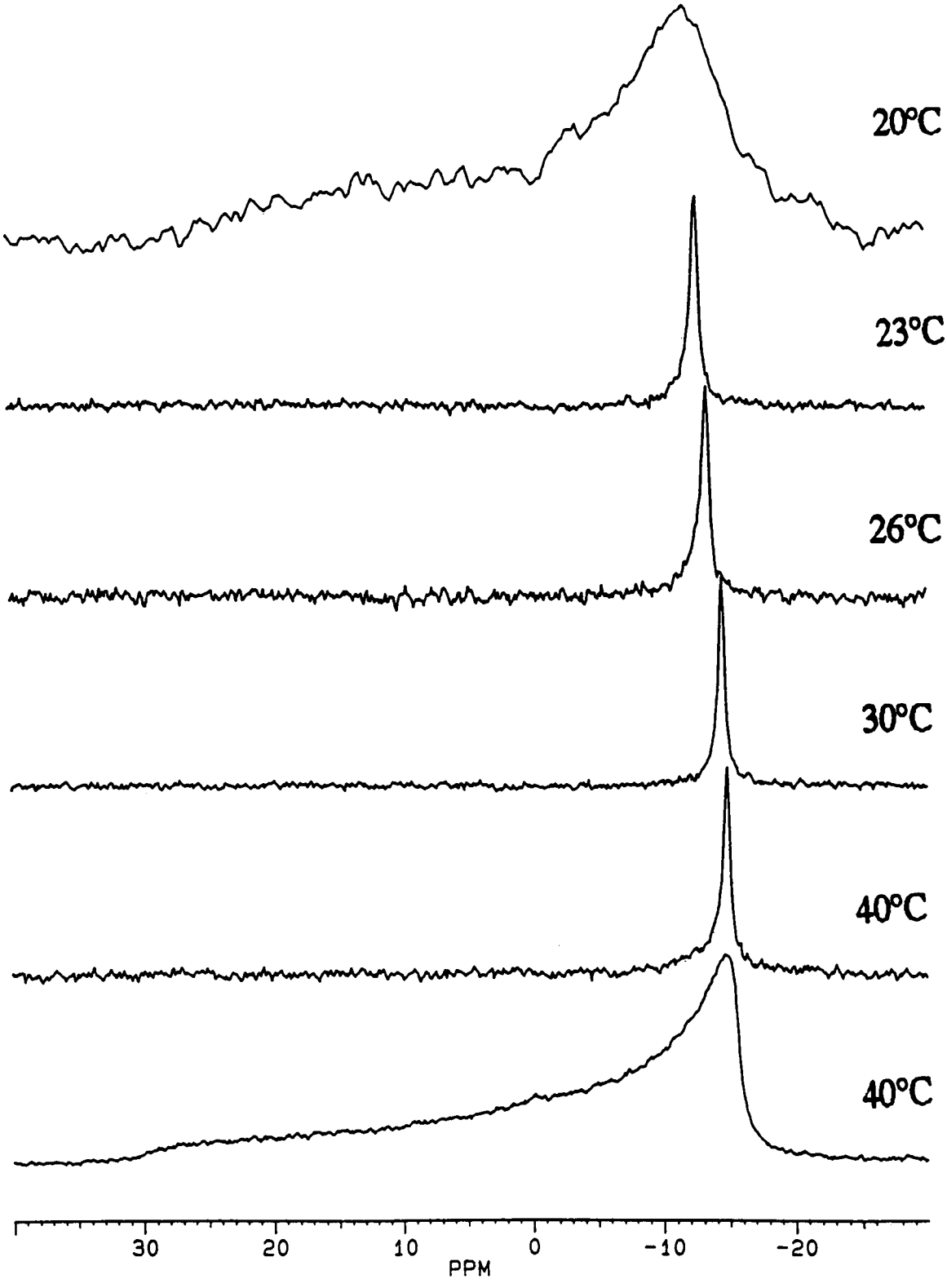

FIGURE 3 ^{31}P spectra (^{1}H-decoupled) of a 1:3.5 Triton X-100:DMPC sample at various temperatures (°C). The sample contained 20% wt/vol total amphiphile in 0.1 M KCl/D_2O. The bottom spectrum is a powder pattern from hydrated DMPC with no Triton present.

eral attempts were made to address this problem, including preparation of additional samples and repeated mixing and incubation between spectral acquisitions. While exact ratios of the three spectral components from the 1:2.7–1:1.8 samples varied somewhat during these attempts, the appearance remained qualitatively the same,

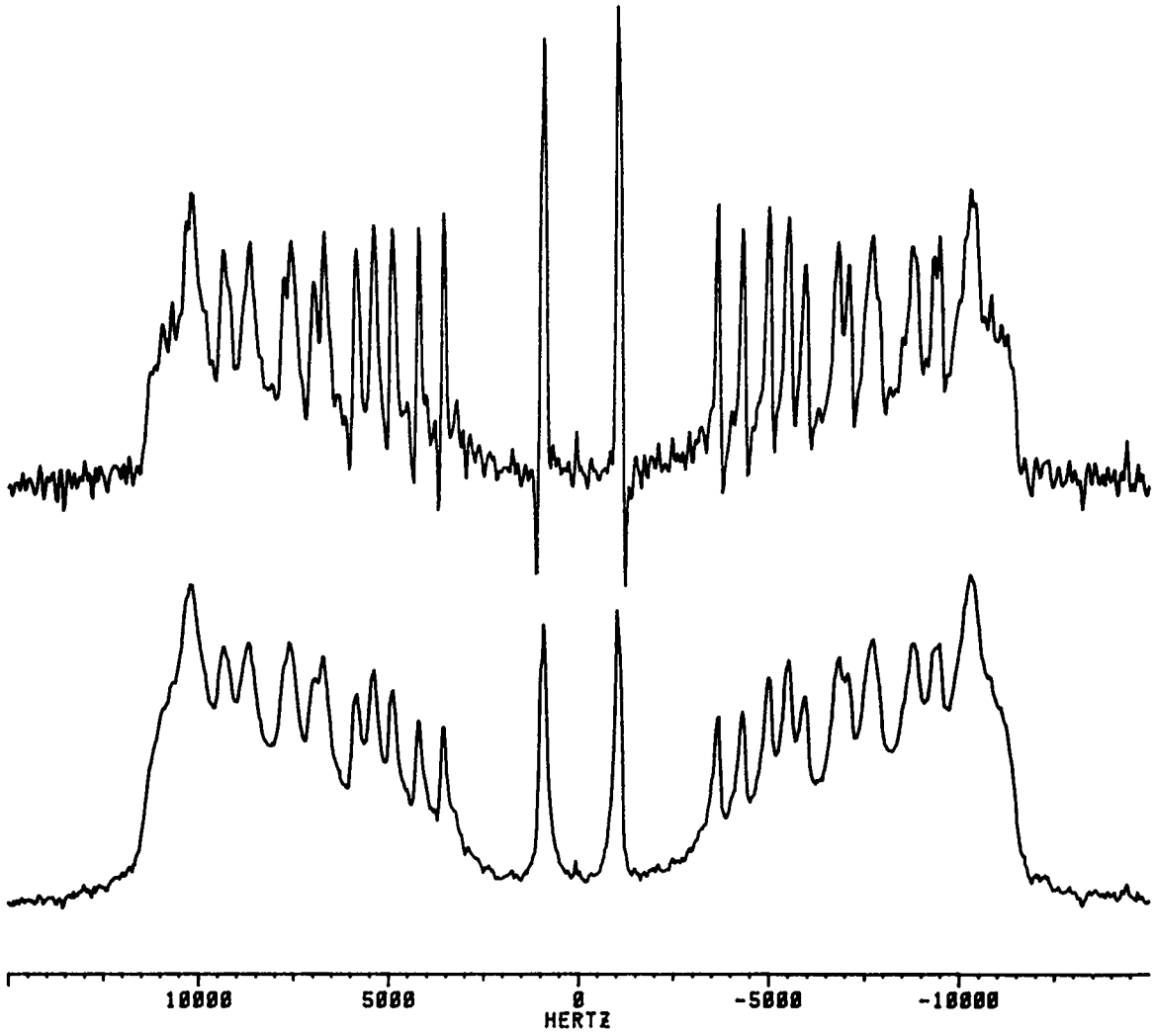

FIGURE 4 Solids echo ^2H NMR spectra of 1:5 Triton X-100:DMPC, 25% total amphiphile in 0.1 M KCl/^2H-depleted H_2O at 40°. The DMPC in this sample has been doped with acyl-perdeuterated DMPC. The top spectrum was produced following Gaussian multiplication of free induction decay, while the bottom spectrum was produced following exponential multiplication (40 Hz linebroadening). The spectra represent 2,048 data points (prior to zero-filling) and 80,000 scans.

and it was not possible to produce a homogeneous sample with only parallel orientation. It is possible that some phase equilibrium involving a limiting amount of CHAPSO dictates this behavior.

The fact that a large amount of naphthol was required to bring about the parallel orientation is very likely a consequence of the fact that the above model for the mechanism of the orientational process is overly idealized. The degree to which naphthol is ordered at the surface is unknown and may in fact be less than the average alkyl chain. Also, the model fails to take into account the possibility of naphthol partitioning along the CHAPSO-stabilized edges of the disks. In any case, the essential components of the model are qualitatively consistent with the experimental data.

Support for a bilayer-dominant morphology in the °0 oriented assemblies is two-fold. First, a change in morphology might be expected to be accompanied by either a perturbation in the average orientation of the phosphorus tensor with respect to the (new) axis of axial motional averaging and/or a perturbation in the eigenvalues/vectors of the static tensor. The fact that the 0° oriented assemblies resonate at exactly twice the shift of the 90° oriented assemblies (which are believed to be bilayers, see reference 5) suggests that no fundamental morphological change has occurred. Other support for a bilayer-like morphology can be found in examining the temperature-dependence of a 1:2.2 naphthol:DMPC sample. Fig. 6 demonstrates that between 15 and 20°, the 0° oriented sample clearly undergoes a dramatic spectral transition to what appears to be a phase characterized by an axially symmetric powder pattern. We have previously noted a similar spectral transition near T_m (24°) for DMPC-detergent mixtures which orient with bilayer normals perpendicular to the field. While the nature of this phase transition cannot be unequivo-

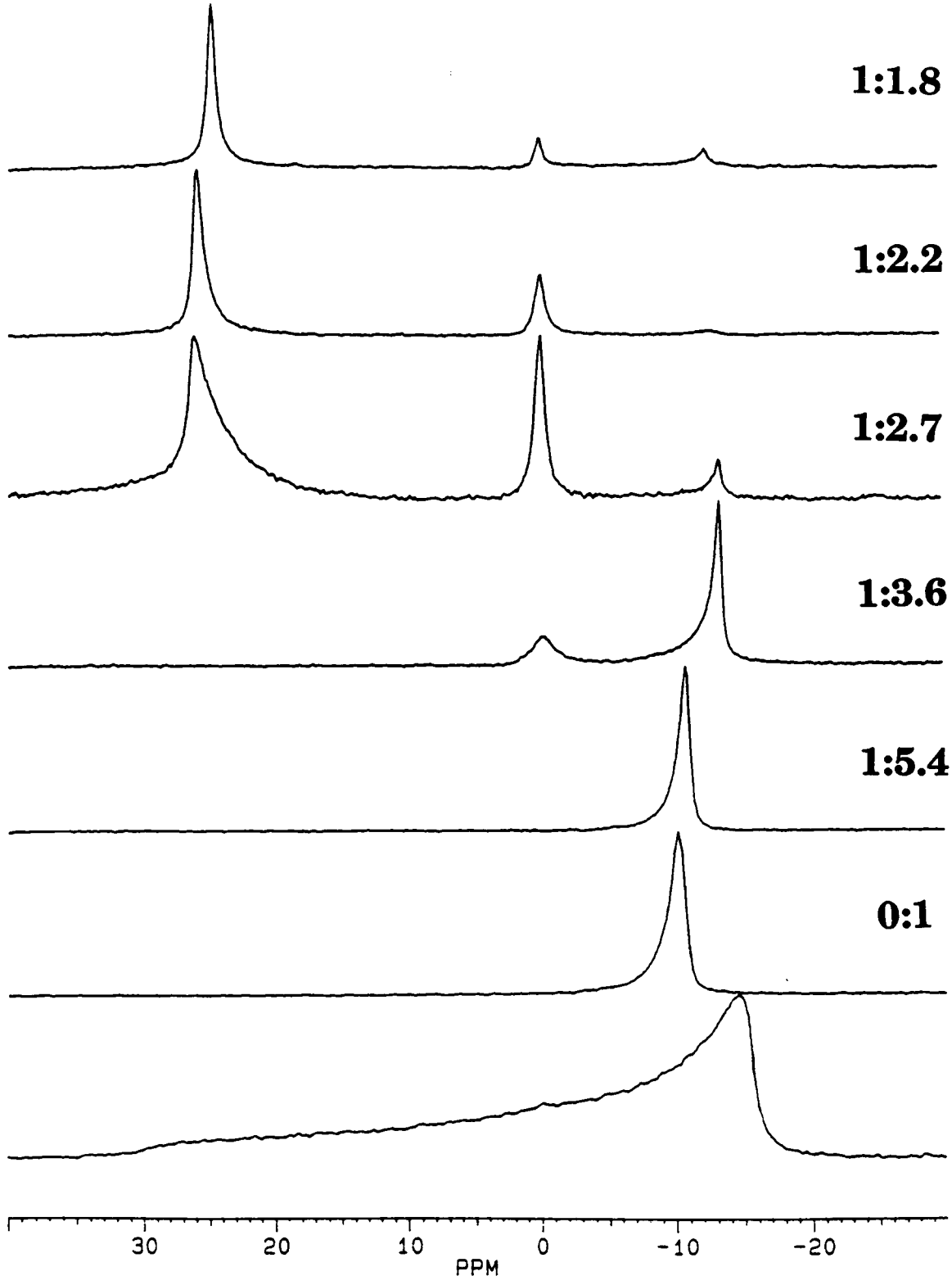

FIGURE 5 ^{31}P (^{1}H-decoupled) spectra from titration of 1:4.2 (mol:mol) CHAPSO:DMPC with 1-naphthol at 40°C. The total "amphiphile" (naphthol + CHAPSO + DMPC) wt/vol in 0.1 M KCl/D_2O was maintained at 31% throughout the titrations. The spectra are labeled with the 1-naphthol:DMPC mol:mol ratio. The bottom spectrum is a 40° powder pattern from hydrated DMPC with no CHAPSO present.

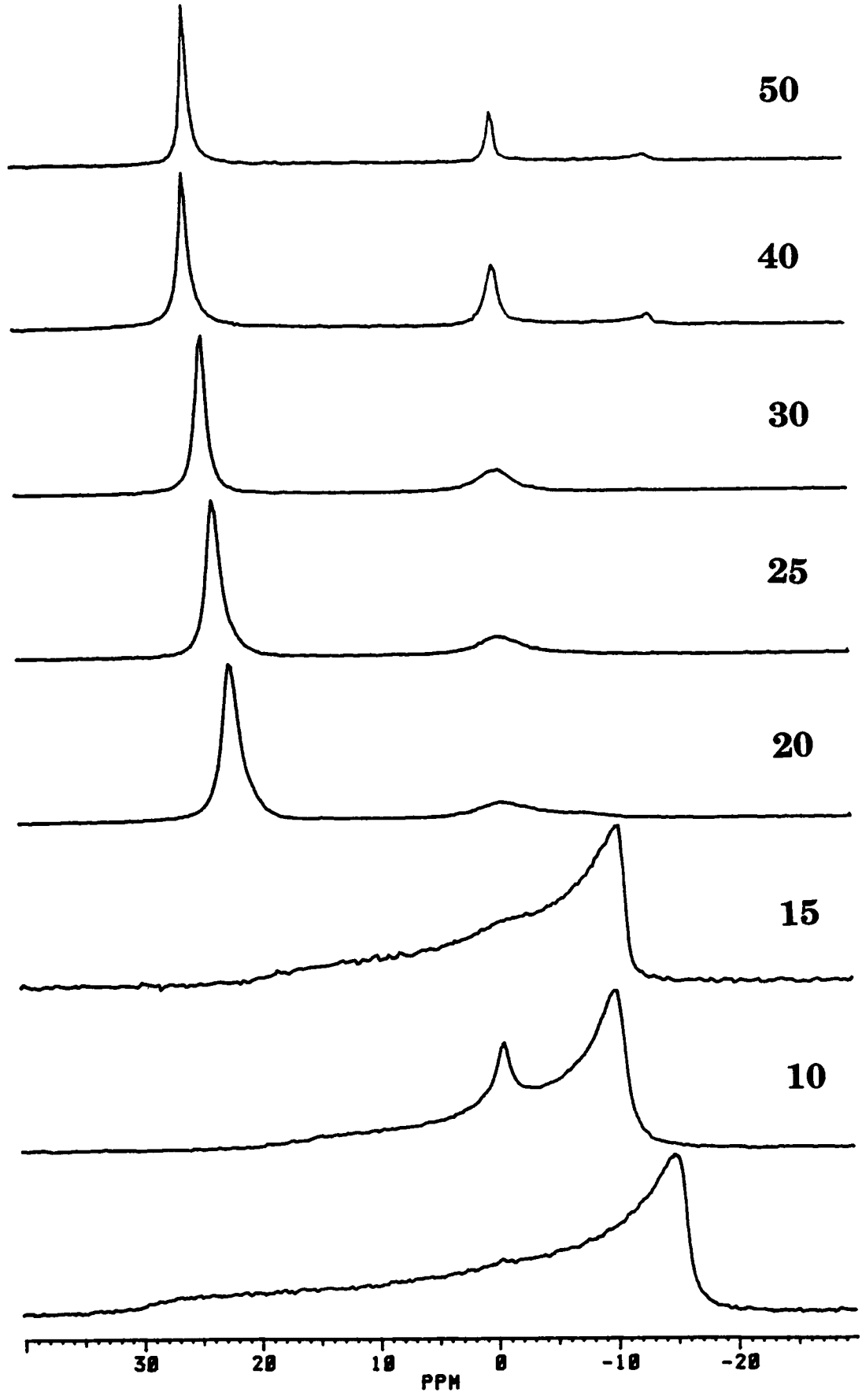

FIGURE 6 ^{31}P spectra taken of a 1.0:0.47:2.2 1-naphthol:CHAPSO:DMPC mixture (31% wt/vol in 0.1 M KCl/D_2O at different temperatures (°C). The bottom spectrum is from a 40° DMPC dispersion with no CHAPSO or naphthol being present.

cally determined from the data, it is consistent with a phase transition between analogs of the commonly observed L_β and L_α lamellar states.

DISCUSSION

The above studies lead to two useful observations. First, a new agent has been identified for facilitating orientation of lipid bilayer systems (Triton) which appears to act at lower concentrations and maintain larger, more nearly ideal bilayer membrane fragments. Second, we have been able to demonstrate, in principle, the ability to redefine the preferred direction of orientation for bilayer fragments.

While Triton X-100 evidently facilitates the magnetic orientation of phospholipid bilayers, the mechanism by which it does so may be different from that of the bile salt analog CHAPSO (5) or short-chain PC (Sanders, C. R. and J. P. Schwonek. 1992. *Biochemistry.* 31:8898–8905). Titrations of DMPC by these latter detergents are accompanied both by bilayer orientation and by a gradual scaling down of overall bilayer order until final convergence to the isotropic limit. This phenomena has been attributed to the ability of the bile salts and short chain PC to break down PC lamellae into successively smaller discrete fragments which are edge-stabilized by the detergent-like component. The fact that Triton is effective at lower concentrations and with much less scaling down of bilayer order suggests that Triton's ability to facilitate orientation may not stem from such "edge activity," but rather from some other property that maintains larger bilayer fragments.

The results using the CHAPSO–naphthol system demonstrate that it is possible to alter the preferred direction of orientation for a magnetically oriented phospholipid system. Based on previous characterization of the CHAPSO–DMPC mixtures and the results of this work, it appears likely that the morphology of the oriented aggregates is predominately that of discoidal patches of DMPC bilayers whose edges are stabilized by interaction with CHAPSO, and that these discoids orient with bilayer normals parallel to the field. The agent used to achieve this change, 1-naphthol, is not an ideal substance to use in media intended to model membrane environments. However, these observations contribute to our understanding of principles which dictate orientational behavior in magnetic field and should form the basis for the rational design of other magnetically orientable systems which may play useful roles in spectroscopic studies of membrane systems.

Not all molecules which might prove effective in the design of field oriented systems need to be based on aromatic moieties. One example documented in the literature is based on amphipathic α-helical peptides. It is known that such peptides exhibit a relatively large positive $\Delta\chi$ of which the principal component lies along the helical axis (22). Thus, if these peptides were to bind to the edge of the bilayer, such that the helices were parallel to the bilayer normal, a parallel orientation would be induced by a magnetic field. Indeed, it has been noted that presence of the hydrophobic peptide gramicidin in PC bilayers will promote a certain amount of parallel bilayer orientation, even without the co-presence of a reorientation-facilitating molecule (8). It is also likely that the presence of significant amounts of well-ordered proteins is responsible for the parallel orientation observed for several natural membranes in the presence of a magnetic field (9–11). Previous studies of the venom peptide melittin have indicated that while this peptide can indeed stabilize the edges of bilayers, only 90° orientation is observed in a magnetic field (23). This probably results either from the peptide binding to the edges in an incorrect orientation to promote parallel orientation (see Fig. 10 in Reference 24) or because the total $\Delta\chi$ of the bound melittin is insufficient to induce a change in preferred orientation. We have also investigated a synthetic, idealized amphipathic peptide known as 18A (25, 26) and have found, using ^{31}P as a probe, that this peptide promotes bilayer disc formation and magnetic orientation, but results only in orthogonally oriented bilayers (Prestegard, J., and Y. Kim, unpublished observation). Understanding and optimizing the behavior of these peptide systems is an obvious target for the future.

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