CROSS POLARIZATION P-31 NUCLEAR MAGNETIC RESONANCE OF PHOSPHOLIPIDS

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ABSTRACT P-31 single-pulse and cross-polarization (CP) nuclear magnetic resonance spectra were obtained of aqueous dispersions of pure phospholipids. Dimyristoyl phosphatidylcholine, dipalmitoylphosphatidylcholine, 1-palmitoyl-2-oleoyl phosphatidylcholine, egg phosphatidylcholine, bovine brain sphingomyelin, and transphosphatidylated (from egg phosphatidylcholine) phosphatidylethanolamine were studied. The spectra from all the phospholipids, taken in the usual single-pulse mode, showed the pseudo-axially symmetric powder pattern typical of phospholipids in a hydrated lamellar form. P-31 CP spectra of all the phosphatidylcholines and phosphatidylethanolamine revealed a decrease in intensity in the vicinity of the isotropic chemical shift as long as the lipid was above the gel-to-liquid crystalline phase transition temperature. This intensity pattern has been observed previously for C-13 CP spectra of molecules rotating rapidly about a single well-defined axis (e.g., solid benzene) (Pines, A., M.G. Gibby, and J.S. Waugh, 1973, *J. Chem. Phys.*, 59:569–590). Pure lipid dispersions below their gel-to-liquid crystalline phase transition temperature, including dipalmitoylphosphatidylcholine and sphingomyelin, do not exhibit a local minimum in the CP spectrum at the position of the isotropic chemical shift. Thus, below the phase transition temperature, there is not the same rapid rotation of the headgroup about a well-defined axis. A dramatic change in the rate of headgroup rotation is shown to take place at the pretransition of dipalmitoylphosphatidylcholine. P-31 CP spectra were also obtained for bovine rod outer segment disk membranes, rabbit muscle sarcoplasmic reticulum membranes, a total lipid extract of the latter, and a recombined membrane containing human erythrocyte glycophorin. The CP spectra were similar to the single-pulse spectra, indicating a substantial difference in behavior from pure phospholipid dispersions. This is interpreted in terms of a slower headgroup rotation.

Phospholipids in membranes inhabit a liquid-crystalline environment, as opposed to a solid or an isotropic liquid. Most P-31 nuclear magnetic resonance (NMR) studies of phospholipid bilayers have used essentially liquid-state P-31 NMR techniques to study the phospholipid headgroups (1, 2). However, because the liquid-crystalline nature of the membrane imparts extreme anisotropy to the molecular motion, solid-state NMR techniques can provide interesting additional information on membrane structure. P-31 NMR is a particularly advantageous approach. The P-31 nucleus is 100% naturally abundant, so that no probes need to be added and no chemical modifications need to be made. Here we report a systematic study with cross-polarization (CP) P-31 NMR of phospholipids in model and biological membranes. These studies present a new picture of phospholipid headgroup rotation and of the behavior of the director characterizing that rotation.

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MATERIALS AND METHODS

Egg phosphatidylcholine, 1-palmitoyl,2-oleoyl phosphatidylcholine (16:0, 18:1 PC), dimyristoyl phosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), bovine brain sphingomyelin, and transphosphatidylated (from egg PC) phosphatidylethanolamine (PE) were obtained from Avanti Polar Lipids, Inc. (Birmingham, AL). Octylglucoside was purchased from Calbiochem-Behring Corp. (La Jolla, CA). Multilamellar liposomes were formed by adding buffer (10 mM histidine, pH 7) to lyophilized phospholipids, followed by gentle shaking. Large unilamellar vesicles were formed by solubilization of the phospholipid with octylglucoside and subsequent dialysis, following procedures described previously (3). Vesicles 1,500–2,500 Å in diameter are formed, and are unilamellar as shown by electron microscopy (4).

Rabbit muscle sarcoplasmic reticulum was isolated from the white hind leg muscle of New Zealand White rabbits (5). ATPase activity was assayed according to the method of Warren et al. (6) and calcium pumping activity was measured using Arsenazo III (7). ATPase activity was typically 1.0–2.0 μmol (mg protein)$^{-1}$ min^{-1} and calcium pumping, in the presence of oxalate, produced calcium transported/ATP hydrolyzed ratios of 1.2–1.6. Bovine rod outer segment disks were isolated from dark adapted retinas according to published procedures (8). Absorbance ratios (500 nm/280 nm) were typically 2.2. These preparations will activate phosphodiesterase in response to light. Glycophorin was isolated (9) from human erythrocytes. Glycophorin was recombined with 16:0, 18:1 PC according to previously published procedures (10). Ordinary single-pulse and CP P-31 NMR spectra were obtained on a Nicolet NT-150 NMR spectrometer (Nicolet Instruments, Santa Barbara, CA) using a home-built CP probe. During all measurements (except where

noted), the sample was bathed in a stream of nitrogen gas at 25°C. All spectra were obtained with a H-1 decoupling field of 50 kHz during data acquisition. The samples were aqueous except for one lyophilized powder. Chemical shifts are in parts per minute from external 85% phosphoric acid. Normally excited spectra were also obtained on a JEOL FX-270 (JEOL USA, Analytical Instruments Div., Cranford, NJ) at 109 MHz using a fully phase cycled spin echo sequence (11) at 22°C.

RESULTS

The CP P-31 NMR spectrum of lyophilized egg PC produces an asymmetric powder pattern. It extends over the full range of the chemical shift tensor, showing the three principle elements of the chemical shift tensor, as reported previously (11). The normally excited spectrum of hydrated egg PC produces a pseudo-axially symmetric powder pattern (Fig. 1), as usually seen from phospholipids in a lamellar configuration (1). The reduced expression of the chemical shift tensor in this powder pattern has been explained by a rapid rotation of the phospholipid headgroup about a single axis (12), with a correlation time of about a nanosecond (13). The same resonance shape is seen for DMPC, DPPC, and 16:0, 18:1 PC when observed in the single-pulse mode.

Fig. 2 *A* presents the CP spectrum of DMPC at 25°C. Although the width of the resonance is the same as seen in the normally excited spectrum, a striking difference is the intensity minimum near the isotropic chemical shift of 2 ppm. This pattern repeated for 16:0, 18:1, PC, egg PC, and DPPC (the latter at 42°C). This shape is the same as that observed in C-13 CP spectrum of solid benzene (14). Fig. 2 *B* also shows a simulation of an axially symmetric powder pattern with intensity scaled by the geometric term in the P-H dipolar second moment (14) for comparison.

The region of the intensity minimum represents an orientation of the axis of rotation or director characterized by weak H-P dipolar interaction and consequently poor CP efficiency. Diffusion from orientations of effective CP to orientations with ineffective CP, arising from lateral diffusion of the phospholipid or tumbling of the liposomes, clearly does not occur in this system on the (approximately) millisecond time scale of the CP experiment. The simulation in Fig. 2 *B* is the resonance shape predicted from headgroup rotation, taking into account the averaging of the dipolar interactions affected by the axial rotation. The agreement with the observed data lends strong support to this interpretation. A similar conclusion was reached based on similar lineshapes in another system (14).

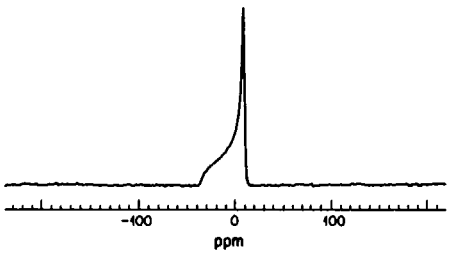

FIGURE 1 Normally excited P-31 NMR spectra at 109 MHz, obtained as described in the text with a phase cycled spin echo, for hydrated egg phosphatidylcholine, at a concentration of 100 mg/ml in 10 mM histidine, pH 7. The spectrum was obtained with 1,024 data points and 50 Hz linebroadening.

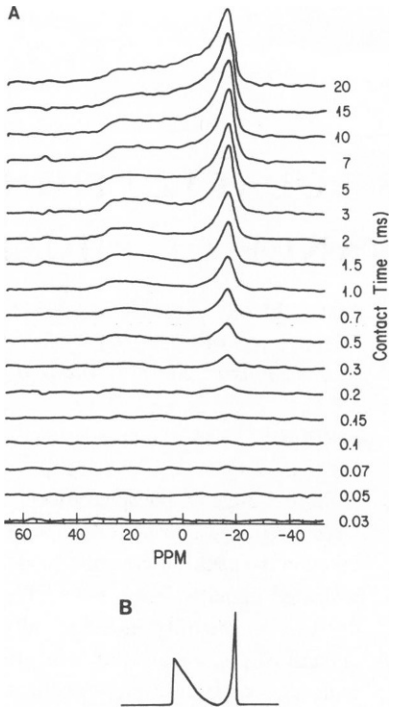

FIGURE 2 (*A*) CP P-31 NMR spectra, obtained as described in the text, for DMPC as a function of contact time in 10 mM histidine, pH 7. 500 scans were obtained with 1,024 data points, 100 Hz linebroadening, and a recycle time of 1 s. The sample contained 100 mg of phospholipid. (*B*) Spectral simulation of the lineshape containing the local minimum at the position of the isotropic chemical shift. The equations of Seelig (1) were used, as described elsewhere (17, 18), except that the resulting powder pattern was scaled by the geometric term in the P-H dipolar second moment (15).

The shape of the resonance was then examined as a function of contact time. Fig. 2 *A* shows the results for DMPC. At long contact times, the minimum becomes less obvious and a resonance similar to that seen in the single-pulse spectrum is observed. This effect of long contact time was also observed for solid benzene (14). The initial growth of P-31 magnetization is governed by an exponential time constant T_{PH}, reflecting the efficiency of the CP (14). The subsequent decline in intensity is governed, in part, by the time constant $T_{1\rho}$ (H), the decay of proton magnetization in the rotating frame (14).

The second class of common zwitterionic phospholipids to be examined was phosphatidylethanolamine. Fig. 3 shows the results of a CP study for a hydrated, unsaturated PE in the liquid crystal state. As observed for DMPC in the liquid crystal state, the CP spectra for PE show a prominent local minimum at short contact times. In contrast to DMPC, however, CP spectra of PE at long contact times still show the local minimum. Otherwise the T_{PH} and $T_{1\rho}$ (^{1}H) for PE are similar to DMPC.

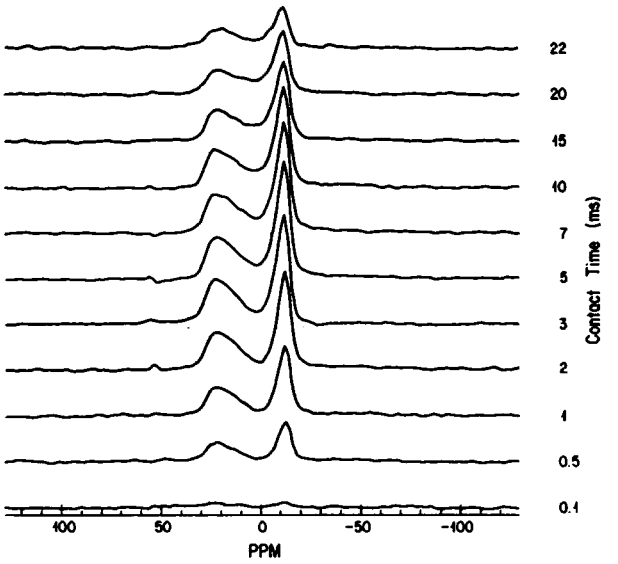

FIGURE 3 CP P-31 NMR spectra of PE as a function of contact time. 500 scans were obtained using 1,024 data points, 100 Hz linebroadening, and a recycle time of 1 s. The sample contained 100 mg of phospholipid.

The last common phosphate-containing zwitterionic lipid, sphingomyelin, was then examined at 25°C. (At higher temperatures the morphology of this material becomes complex, introducing additional motional averaging, and thus this material is not suitable for these studies at higher temperatures [15]). It shows a distinctly different behavior. The single-pulse spectrum closely resembles that observed for the other phospholipids (15), but the CP spectrum also shows a normal lamellar resonance (Fig. 4). This suggests significant differences in the molecular motion of this lipid compared with PC and PE. T_{PH} at δl and δl is similar to PC and PE.

At the temperature of the latter experiment, sphingomyelin was below the temperature of its gel-to-liquid crystalline phase transition. All the phospholipids described before sphingomyelin were measured above their analogous phase transition temperature. Therefore it was important to measure the properties of a phospholipid at various temperatures surrounding the phase transition temperature. DPPC was chosen for this purpose because it is a well-characterized system with a gel-to-liquid crystalline phase transition temperature at 42°C. Fig. 5 shows the results between 30° and 40°C. Two distinct changes are

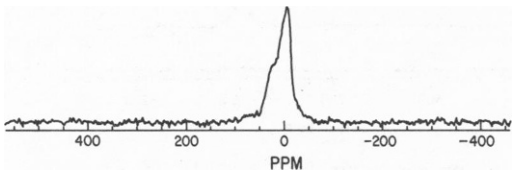

FIGURE 4 CP P-31 NMR spectrum of hydrated sphingomyelin at a 3-ms contact time at room temperature. 3,000 scans were obtained using 1,024 data points, 300 Hz linebroadening, and a recycle time of 1 s. The sample contained 50 mg of phospholipid.

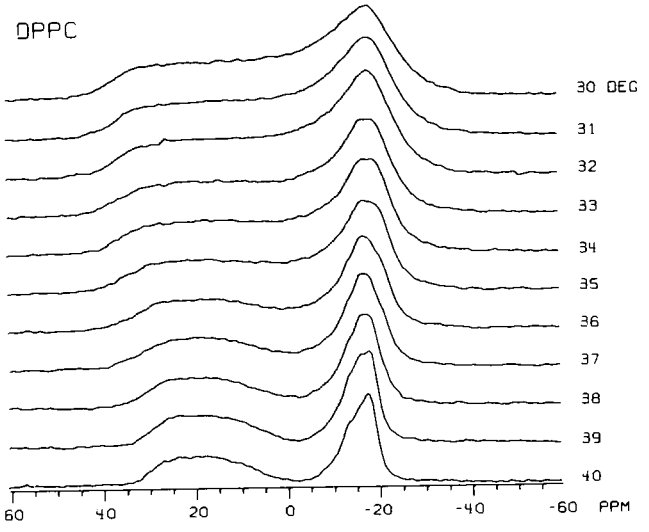

FIGURE 5 CP P-31 NMR spectrum of DPPC as a function of temperature at a 5-ms contact time. Other conditions were as in Fig. 2.

observed in the spectra in this region. The spectra narrow as the temperature increases from 30° to 40°C. This phenomenon is a well-known consequence of changes in the motional parameters of phospholipids as they leave the gel state and enter the liquid crystal state.

The second notable change seen in these spectra concerns the intensity minimum noted above in liquid crystalline phospholipids. At 30°C, the CP spectra are the same as the single-pulse spectra. The spectra up through 34°C also exhibit normal lineshapes with no evidence for orientation-dependent CP efficiency. However, at 35°C a minimum forms, and at 39°C and above, the spectra are identical with those obtained for the other phospholipids in the liquid crystalline state.

This observation is quantified in Fig. 6. Here the ratio of the intensity at 2 ppm to the intensity at the more shielded maximum of the powder pattern is plotted as a function of temperature. The onset of a transition at 35°C is apparent. In this same system the hydrocarbon chains melt at 42°C in a much narrower temperature range.

Having looked at the three most commonly found zwitterionic phospholipids in pure form (PC, PE, and sphingomyelin), it is now appropriate to look at mixtures of

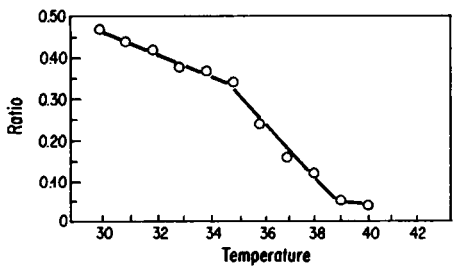

FIGURE 6 Plot of the ratio of the intensity of the local minimum at the isotropic chemical shift position to the intensity of the right-hand maximum for the data in Fig. 5.

lipids and at membranes that contain protein. The first example in this category is a mixture of natural phospholipids extracted from a biological membrane, the rabbit muscle sarcoplasmic reticulum, which consists largely of phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine and phosphatidylinositol (16). These are unsaturated phospholipids and this mixture is in the liquid-crystal state in these experiments.

The single-pulse spectrum shows the expected partially motionally averaged powder pattern from phospholipids in a bilayer (17). At moderate contact times, the CP spectra do not show the intensity minimum that is observed for pure PC and pure PE, even though those two phospholipids constitute the majority of the phospholipids in the extract and the pure and the mixed lipid systems are all in the liquid crystal state. The spectrum looks like that for sphingomyelin in Fig. 4.

Another case was examined in which a single pure membrane protein, human erythrocyte glycophorin, was introduced into a pure 1-palmitoyl-2 oleoyl phosphatidylcholine bilayer. The single-pulse spectrum is similar to the spectrum expected from a phospholipid bilayer (10). The CP spectrum appears in Fig. 7. At short contact times a local minimum is observed near the isotropic chemical shift, but it disappears at moderate contact times. Thus the T_{PH} associated with the position of the isotropic chemical shift is longer than the T_{PH} of the upfield maximum, but shorter than the T_{PH} associated with the isotropic chemical shift of the pure phospholipids. Apparently, the presence of a single membrane protein is sufficient to cause a change in the behavior of the phospholipid headgroups. This must be a long range effect or else it must result from a rapid exchange of phospholipids among sites, since all the phospholipid headgroups contributing to the observed resonance are affected, even though only a minority of them can interact directly with the protein surface at any given instant.

The single-pulse spectra from two biological membranes, the bovine rod outer segment disk membrane, and the rabbit muscle sarcoplasmic reticulum membrane, both show as the dominant spectral feature a partially motionally averaged powder pattern characteristic of a phospholipid bilayer. These spectra are more complex than is found for a pure phospholipid bilayer and are the subject of separate studies (17, 18). The CP spectra for one of these biological membranes is shown in Fig. 8. An intensity minimum is observed at the isotropic chemical shift, but much less pronounced than that seen in the pure phospholipid samples.

All the pure phospholipid dispersions for which data were presented above existed in a multilamellar form. In contrast, all the membranes containing protein, whether biological membranes or reconstituted membranes, consist of unilamellar vesicles (electron micrograph data not shown). In addition the unilamellar vesicles are considerably smaller than the multilamellar liposomes. Therefore it was important to determine whether the smaller size or the unilamellar nature of the vesicles containing protein contributed to the differences in lineshape from the pure phospholipid multilamellar dispersions. To do this, unilamellar vesicles of egg phosphatidylcholine were prepared that were of the same size as the vesicles containing protein. The CP spectra of such a system exhibit resonance shapes similar to those of the pure phospholipid multilamellar dispersions, and different from the protein-containing systems, so the size of the vesicle is not important to the CP dynamics in these samples.

We have collected data on T_{PH} for these systems, but must be cautious in their interpretation. In highly mobile systems the H-1–H-1 dipolar interaction is reduced from values typical of a rigid solid, and a consequence is that the value of T_{PH} is very sensitive to mismatch in the Hartmann-Hahn condition (14, 19, 20). For the present systems the signal intensity was observed to decrease twofold with a 0.2-dB deviation from the Hartmann-Hahn condition. The instability of the transmitter power levels over the several hours required for a variable contact time study precludes an accurate determination of T_{PH} at present.

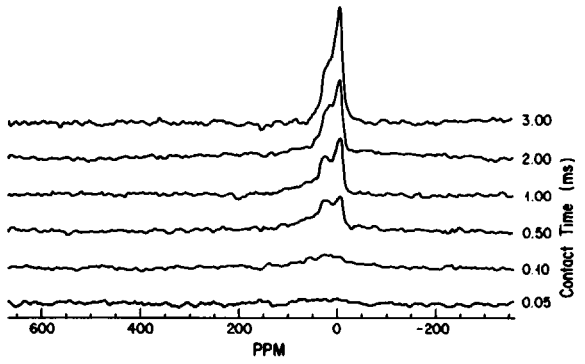

FIGURE 7 CP P-31 NMR spectra of glycophorin recombined with 16:0, 18:1 PC as a function of contact time. 5,000 scans were obtained for each spectrum using 1,024 data points, 300 Hz linebroadening, and a recycle time of 1 s.

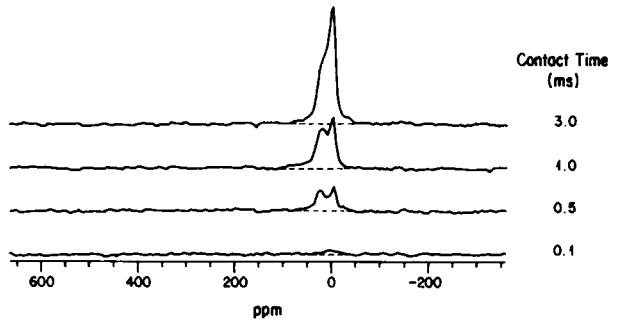

FIGURE 8 CP P-31 NMR spectra of bovine rod outer segment disk membranes as a function of contact time in 10 mM histidine, 1 mM EDTA, pH7. 20,000 scans were obtained for each spectrum, using 2,048 data points, 300 Hz linebroadening, and a recycle time of 1 s. The sample contained ~10 mg of membrane material.

Preliminary estimates indicate that T_{PH} is ~10 ms at the isotropic shift and ~2 ms at the extrema for the pure phospholipids studies.

DISCUSSION

The local minimum at the position of the isotropic chemical shift in the cross polarization spectra of, for example, DPPC, is indicative of weak dipolar interactions for some orientations of the director for rotation of the phospholipid phosphate chemical shift tensor in the membrane. The dipolar interactions are scaled by the factor $(3 \cos^2 \theta - 1)$ when the director is well defined. Therefore the dipolar interactions and thus the cross polarization efficiency are minimized at 54.7°. θ is the angle between the director and the external magnetic field. This effect is due to a well-defined axis of rotation as has been observed previously in another system using CP C-13 NMR (14).

To understand the ramifications of this observation for bilayer structure requires knowledge of the orientation of the P-31 chemical shift tensor for the phospholipid in the membrane. In a study of uniformly oriented membranes, the relationship between the orientation of the normal to the membrane surface (with respect to the external magnetic field) and chemical shift position in the observed P-31 powder pattern was experimentally determined (21). Using that relationship, we can assign the position of the local intensity minimum observed in the CP P-31 NMR spectra of the membrane studied here (at short contact times). This position corresponds to orienting the normal to the membrane surface (of the phospholipid bilayer) at an angle at 54.7° with respect to the external magnetic field. The director for the rotation of the headgroup is perpendicular to the membrane surface (21). Since the scaling factor described above requires a θ of 54.7°, the director for headgroup rotation and the rotation axis responsible for scaling the dipolar interactions (and consequently the cross polarization efficiency) are colinear. Therefore the molecular rotation detected here, leading to the observation of a local minimum in the CP P-31 NMR spectra of phospholipid bilayers, is consistent with previous studies of bilayer structure. Furthermore, this indicates that the CP P-31 NMR studies are sensitive to the behavior of the director for phospholipid headgroup rotation and to the rotational rate of the headgroups. The simulation in Fig. 2 shows that this explanation is adequate to describe the observed data.

PE represents a more extreme result, but it is explainable by the same model. The local minimum persists even at long contact times when $T_{1\rho}$ (H-1) is causing a loss of signal intensity. This may suggest a more ordered director for PE headgroup rotation than for PC headgroup rotation.

A careful consideration of the possibilities leads to the following as the best model for describing the CP P-31 NMR spectra in the temperature range of a phospholipid phase transition from the gel-to-liquid crystal state. At 34°C, calorimetry data indicate that the DPPC molecules are in the gel state (22). The pseudo-axially symmetric powder pattern observed here shows that axial rotation of the phospholipid headgroup is occurring. The absence of an orientation-dependent cross polarization efficiency indicates that the headgroup rotation rate is insufficient to motionally average the dipolar interactions according to the scaling mechanism described above. The rate of rotation is therefore $<10^6$ s^{-1}, because a rotation rate faster than that would achieve the motional averaging of the dipolar interactions that causes the local minimum observed at the isotropic chemical shift in the liquid crystal state. The rate of rotation is faster than 10^4 s^{-1}, because rotation slower than that would lead to a significant increase in the expression of the P-31 chemical shift anisotropy in the observed powder pattern. Thus we can put fairly close limits on the rotation rate for the phospholipid headgroup at this temperature. This result is in good quantitative agreement with a previously published report in which a total lineshape analysis of the P-31 resonance was performed (23). In that study, a rotational rate of 5×10^5 s^{-1} was obtained just below the pretransition temperature (23).

It is interesting to note the slope of the plot in Fig. 6, from 34° to 30°C. The ratio plotted is increasing as one goes to lower temperatures. These data provide a quantitative expression of the resonance shape change, interpreted previously as a monotonic decrease in headgroup rotation rate as the system goes deeper into the gel state (23).

At 40°C, the phospholipid hydrocarbon chains are in the gel state, but the headgroups have melted to the liquid crystal state. The former is well known from calorimetry experiments (22). The latter is obvious from the P-31 NMR spectra. The development of the local minimum in the CP P-31 NMR spectra shows that the rate of headgroup rotation has increased to that experienced in the liquid crystal state, since now the rotation is effective at averaging the dipolar interactions. In the liquid crystal state the headgroup rotation occurs with a correlation time of 10^{-9} s (24).

This model is adequate to explain the results obtained for sphingomyelin. At the temperature represented in Fig. 4, sphingomyelin is in the gel state. Therefore its spectral shape is similar to that observed for DPPC in the gel state.

At this point we wish to see whether this model is of any assistance in understanding the results obtained with mixed lipid systems or systems containing protein. In all these more complex systems, the observation of a local minimum is less prominent than in the pure PC or pure PE systems, particularly at intermediate or long contact times. However, there are some similarities. The overall powder pattern is similar, consistent with axial rotation of the headgroups in each case. With respect to the position corresponding to the isotropic chemical shift, the T_{PH} time constant is shorter in these more complex systems than in the liquid crystal state of the pure lipids, but much longer

than that observed for the gel state phospholipid. Therefore, the model described earlier suggests that the rate of headgroup rotation is somewhat slower in these systems, but not as slow as in gel state phospholipid.

During the review process of this paper, a CP P-31 NMR study of DPPC appeared that gave a temperature profile for the CP intensities across the powder pattern similar to that reported here (25). Thus the two studies agree on this point. However, the authors chose to plot the integrated intensity over the entire powder pattern rather than the intensity ratio plotted here, and they also inadvertently included the Hartmann-Hahn mismatch dependence on temperature (noted in the present report) together with the effects of the phase transition on the CP efficiency. These problems unnecessarily obscure the importance of the pretransition of the P-31 signal.

Thus the well known pretransition in the calorimetry of DPPC bilayers (22) appears to correspond to a differential melting of the phospholipid headgroups, before the melting of the hydrocarbon chains. The melting of the headgroups is characterized by a dramatic increase in the rate of rotation of the headgroups. This conclusion agrees with that of others (23).

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REFERENCES

1. Seelig, J. 1978. P-31 NMR and the headgroup structure of phospholipids in membranes. *Biochim. Biophys. Acta.* 515:105–134.
2. Yeagle, P. L. 1978. Phospholipid headgroup behavior in biological assemblies. *Accounts Chem. Res.* 11:321–326.
3. Mimms, L. T., G. Zampighi, Y. Nozaki, C. Tanford, and J. A. Reynolds. 1981. Phospholipid vesicle formation and transmembrane protein incorporation using octyl glucoside. *Biochemistry.* 20:833–840.
4. Yeagle, P. L., and J. Bensen. 1982. Molecular packing of cholesterol in phospholipid vesicles. *Biochim. Biophys. Acta.* 692:139–146.
5. Eletr, S., and G. Inesi. 1972. Phospholipid orientation in sarcoplasmic reticulum membrane: spin label ESR and proton NMR studies. *Biochim. Biophys. Acta.* 282:174–179.
6. Warren, G. B., P. A. Toon, N. J. M. Birdsall, A. G. Lee, and J. C. Metcalfe. 1974. Reconstitution of a calcium pump using defined membrane components. *Proc. Natl. Acad. Sci. USA.* 71:622–626.
7. Herbette, L., J. Marquardt, A. Scarpa, and J. K. Blasie. 1977. A direct analysis of lamellar X-ray diffraction from hydrated, oriented multilayers of fully functional sarcoplasmic reticulum. *Biophys. J.* 20:245–272.
8. Smith, H. G., Jr., G. W. Stubbs, and B. J. Litman. 1975. The isolation and purification of osmotically intact discs from retinal rod outer segments. *Exp. Eye Res.* 20:211–217.
9. Marchesi, V. T., and E. P. Andrews. 1971. Glycophorin isolation from cell membranes with lithium diiodosalicylate. *Science (Wash. DC).* 174:1247–1248.
10. Yeagle, P. L., and A. Y. Romans. 1981. Glycophorin-phospholipid interface in recombined systems: a P-31 NMR study. *Biophys. J.* 33:243–252.
11. Rance, M., and A. Byrd. 1983. Obtaining high fidelity spin 1/2 powder specta in anisotropic media: phase cycled Hahn echo spectroscopy. *J. Mag. Res.* 52:221–240.
12. Griffin, R. G. 1976. Observation of the effect of water on the P-31 nuclear magnetic resonance spectra of dipalmitoyllecithin. *J. Am. Chem. Soc.* 98:851–853.
13. Kohler, S. J., and M. P. Klein. 1977. Orientation and dynamics of phospholipid headgroups in bilayers and membranes determined from P-31 NMR chemical shielding tensors. *Biochemistry.* 16:519–526.
14. Pines, A., M. G. Gibby, and J. S. Waugh. 1973. Proton-enhanced NMR of dilute spins in solids. *J. Chem. Phys.* 59:569–590.
15. Hui, S. W., T. P. Stewart, and P. L. Yeagle. 1980. Temperature dependent morphological and phase behavior of sphingomyelin. *Biochim. Biophys. Acta.* 601:271–281.
16. Albert, A. D., M. Lund, and P. L. Yeagle. 1981. Evidence for the influence of the protein-phospholipid interface on sarcoplasmic reticulum Ca^{++} Mg^{++} ATPase activity. *Biophys. J.* 36:393–407.
17. Selinsky, B. S., and P. L. Yeagle. 1984. Two populations of phospholipids exist in sarcoplasmic reticulum and in recombined membranes containing Ca-ATPase. *Biochemistry.* 23:2281–2288.
18. Albert A. D., and P. L. Yeagle. 1983. Phospholipid domains in retinal rod outer segment disk membranes. *Proc. Natl. Acad. Sci. USA.* 80:7188–7191.
19. Mehring, M. 1983. Principles of High Resolution NMR in Solids. 2nd ed. Springer-Verlag, Berlin 1–39.
20. Demco, S., J. Tegenfeldt, and J. S. Waugh. 1975. Dynamics of cross polarization in nuclear magnetic resonance. *Phys. Rev.* B11:4133–4151.
21. Seelig, J., and H. Gally. 1976. Investigation of phosphatidylethanolamine bilayers by deuterium and phosphorous-31 nuclear magnetic resonance. *Biochemistry.* 15:5199–5204.
22. Estep, T. N., D. B. Mountcastle, R. L. Biltonen, and T. E. Thompson. 1978. Studies on the anomalous thermotropic behavior of aqueous dispersions of dipalmitoylphosphatidylcholine-cholesterol mixtures. *Biochemistry.* 17:1984–1989.
23. Campbell, R. F., E. Meirovitch, and J. H. Freed. 1979. Slow-motional NMR line shapes for very anisotropic rotational diffusion. Phosphorous-31 NMR of phospholipids. *J. Phys. Chem.* 83:525–533.
24. Yeagle, P. L., W. C. Hutton, C. Huang, and R. B. Martin. 1975. Headgroup conformation and lipid-cholesterol association in phosphatidylcholine vesicles: a P-31 (H-1) NOE study. *Proc. Natl. Acad. Sci USA.* 72:3477–3482.
25. Akutsu, Hideo, and Y. Kyogoku. 1984. Phase transitions of phosphatidylcholine bilayers as revealed by cross-polarization efficiency of P-31 NMR. *Biochim. Biophys. Acta.* 774:293–295.