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THE INTERPRETATION OF PROTON MAGNETIC RESONANCE LINEWIDTHS FOR LECITHIN DISPERSIONS

EFFECT OF PARTICLE SIZE AND CHAIN PACKING

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SUMMARY

Two previously reported theoretical treatments of the effect of sonication on the PMR spectrum of phospholipid bilayer membranes have led to divergent conclusions regarding the effects of sonication on the structure of the bilayer membrane. In this report these two theoretical treatments will be critically reviewed, and it will be shown that only the theory of Seiter and Chan (Seiter, C. H. A. and Chan, S. I. (1973) J. Am. Chem. Soc. 95, 7541–7553) yields predictions which are in agreement with experiment. Analysis of available and newly acquired NMR results for sonicated bilayer vesicles of different sizes, both above and below the thermal phase transition, indicates that sonication does disrupt the regular molecular packing of the phospholipid molecules in these systems.

INTRODUCTION

The proton magnetic resonance (PMR) spectra of the liquid-crystalline phase of unsonicated phospholipid dispersions are characterized by the conspicuous lack of high resolution features [1–6]. This result is to be expected for a liquid crystal, and indeed it is possible to account for the observed NMR lineshapes as well as the magnetic relaxation behavior in terms of the restricted and anisotropic motion expected for these systems [7].

It is well established that sonication of a phospholipid dispersion in its multilamellar state transforms it to a system of single-walled bilayer vesicles [8–14], and

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concurrent with this the PMR spectrum develops high resolution characteristics [14–19]. Two mathematical treatments have recently been developed to account for this dramatic behavior. First, on the basis of the stochastic linewidth theory of Anderson [20], extended properly to take into account both the restricted motion of the fatty acid chains as well as the overall Brownian tumbling of the bilayer vesicles, Seiter and Chan [7] concluded that the effect of sonication could only be accounted for by a disruption of the regular molecular packing of the hydrocarbon chains, presumably as a consequence of the high surface curvature inherent in vesicles 300 Å in diameter [14]. On the other hand, using the method of second moments introduced by Gutowsky and Pake [21] and adapting it for the present situation, Finer [22] concluded that the restriction of linewidth caused by sonication results merely from particle tumbling. This paper represents an attempt to resolve these divergent views regarding the effects of sonication on the PMR spectra of phospholipid dispersions.

THE TREATMENT BY FINER

The starting point of Finer's [22] treatment is the second moment result of Gutowsky and Pake [21], who argued that the NMR linewidth of a solid containing nuclei which undergo a "specialized" motion can be described by an equation of the form

$$W_i^2 = B^2 + (W_0^2 - B^2)\, t\,(W_i, \tau_c)$$

In this expression W_i is the linewidth observed for the NMR spectrum of the solid, W_0 is the rigid lattice linewidth, and B^2 is related to that part of the second moment which is not affected by the specialized motion. $t\,(W_i, \tau_c)$ is the narrowing function which describes mathematically how the second moment is reduced by the "specialized" motion and is usually represented by an inverse tangent function involving the correlation time associated with this "specialized" motion as well as the reduced second moment. It should be pointed out that inherent in the Gutowsky-Pake treatment is the assumption that the "specialized" motion must involve complete rotation about one axis. Only under this circumstance does the lineshape for the solid remain Gaussian after the onset of the "specialized" motion, and this feature of the lineshape is essential if the linewidth is to be related to the second moment in a simple way.

Finer [22] extended the above Gutowsky-Pake formula to consider the effects of sonication on the NMR spectrum of phospholipid dispersions. The multilamellar dispersion was treated as a solid with the fatty acid chains undergoing a "specialized" motion characterized by a correlation time τ_c. This "specialized" motion is assumed to reduce the linewidth of the spectrum of the polymethylene chains from its rigid lattice value of W_0 to W_i. The effect of sonication was thought to merely introduce rotational Brownian motion of the bilayer units and this effect of rotational tumbling of the bilayer vesicles was mathematically accounted for by introducing an additional narrowing function which further reduces the second moment, including that part of the second moment which was previously not affected by the "specialized" motion. The following ad hoc expression was introduced to obtain the linewidth W_m for a bilayer unit which undergoes tumbling motion on a time scale of τ_v,

$$W_m^2 = B^2\, t\,(W_m, \tau_v) + (W_0^2 - B^2)\, t\,(W_m, \tau_c)$$

Using this approach, Finer predicted linewidths for sonicated bilayer vesicles which were similar to those observed experimentally. On this basis he concluded that the reduced linewidths observed for sonicated bilayer dispersions can be explained solely in terms of the overall Brownian tumbling of the bilayer vesicles.

We believe that the procedure which Finer has used to treat the effects of Brownian motion on the NMR spectrum of the bilayer units is arbitrary and in fact incorrect. The Gutowsky-Pake expression really pertains only to inhomogeneously broadened (by static dipolar interactions) lines and hence cannot be applied to describe the homogeneous broadening produced by isotropic rotational tumbling of a bilayer vesicle*. Apart from this fundamental objection to Finer's ad hoc use of the Gutowsky-Pake relationship, we believe that Finer also chose the wrong value of W_i. The choice of a proper value of W_i is, of cource, important. The value of 250 Hz used by Finer seemed rather small and is based on what we consider an experimental

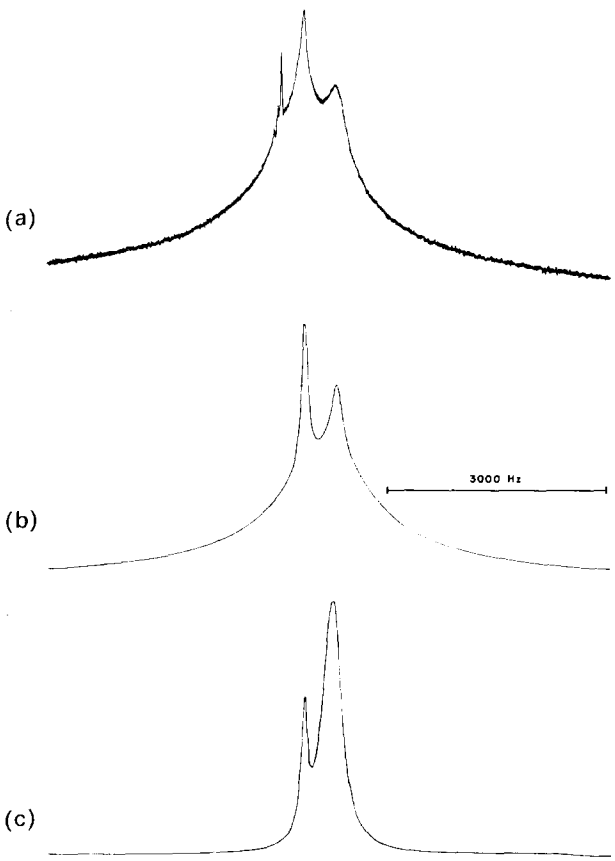

Fig. 1. (a) PMR spectrum of dimyristoyl lecithin multilayers at 220 MHz and 35 °C obtained using a 10 kHz sweepwidth. (b) and (c) Computer simulations of the PMR spectrum of lecithin multilayers (see ref. 28 of text for details of calculations). Spectra (b) and (c) were calculated using half-widths of 3000 and 250 Hz respectively for the methylene protons of the hydrocarbon chains.

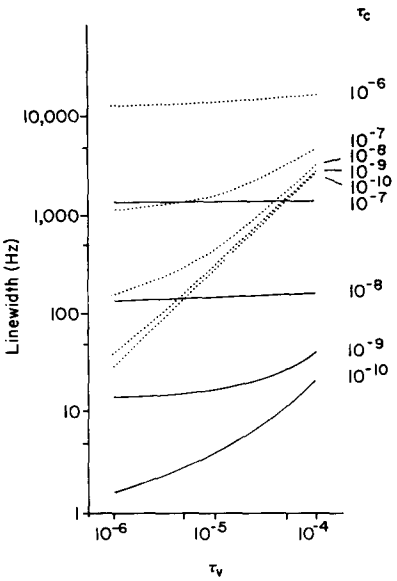

Fig. 2. Calculated linewidths of the chain methylene protons in bilayer vesicles as a function of the overall tumbling rate (τ_v^{-1}) and time scale of the local chain motion (τ_c) based on the results of Finer and obtained using two different values for W_i, 250 Hz (—) and 3000 Hz (· · ·).

artifact caused by instrumental limitations. The actual linewidth of the paraffinic protons of multilayers should be approximately 3000 Hz. This value is suggested by the apparent T_2 values observed for lecithin multilayers [23] as well as a number of other bilayer systems [24]. Moreover, a series of computer simulations of the PMR spectrum of lecithin bilayers indicate that the observed spectrum is reproduced only when a linewidth of approximately 3000 Hz is used for the protons of the polymethylene chain (cf. Fig. 1a and 1b). By contrast, a computer simulation using a value of 250 Hz for the linewidth of the methylene protons yielded a spectrum which shows little resemblance to the actual spectrum (cf. Fig. 1a and 1c). Recent order parameters based on the deuteron quadrupole coupling constant measured by Seelig [25] and others [26, 27] for ^{2}H-labeled bilayer systems also predict a proton NMR linewidth of several thousand hertz for the paraffinic protons in these systems.

Finally, using the value of 250 Hz for W_i, Finer concluded that the correlation time for the chain motion (τ_c) in vesicles is approximately 10^{-9} s, and that for this τ_c there is no strong dependence of the linewidth (approx. 15 Hz) upon the rate of overall tumbling of the bilayer vesicles (Fig. 2). Using what we consider to be the correct W_i value and Finer's value of τ_c, however, we find that the Finer expression predicts a linewidth close to the experimental value only when the overall tumbling rate of the vesicle is much faster than $2 \cdot 10^5$ Hz (Fig. 2), the maximum value which is expected for 300 Å diameter vesicles on the basis of the Stokes-Einstein relation-

* Actually the methylene signal from sonicated bilayer vesicles is also in part inhomogeneously broadened due to other sources, e.g. dispersion in chemical shifts, and there will be a range of T_2 values if a mobility gradient exists for the polymethylene chains. Our treatment here only pertains to the homogeneous linewidth.

ship at 50 °C in aqueous solution. In addition, we find that the linewidth then becomes strongly dependent upon the rate of tumbling (Fig. 2). This result indeed contradicts Finer's own viscosity measurements as well as those performed in this [14] and other laboratories [18]. It should be noted that at a slower τ_c (say 10^{-7} s), the linewidth is predicted not to depend upon τ_v, but much broader lines are then predicted.

THE SEITER-CHAN TREATMENT

In a series of recent papers [6, 7, 23, 28] Seiter, Feigenson and Chan showed that it was necessary to invoke motional restriction as well as anisotropic motion of the hydrocarbon chains in order to account for the NMR lineshape and magnetic relaxation behavior observed for phospholipid bilayers in their multilamellar state. These workers used Monte Carlo methods [29] to simulate the restricted motion for a methylene geminal pair as well as a rapidly spinning rotor and they carried out NMR lineshape calculations based on the stochastic linewidth theory of Anderson [20]. The most important conclusion to emerge from this work is that the hydrocarbon chains in these systems do possess an important degree of order and the NMR lineshape associated with the hydrocarbon chains is inhomogeneously broadened because of incomplete spatial averaging of magnetic dipolar interactions. In more quantitative terms Seiter and Chan show that the NMR lineshape and magnetic relaxation behaviour observed for lecithin multilayers above the thermal transition temperature could be explained if the interproton vector of geminal methylene pairs undergoes restricted motions of amplitude $\Delta\beta \approx 60°$ at a time scale of $\tau_\perp \approx 10^{-7}$ s while seemingly "reorienting" about the average chain axis at a time scale $\tau_\parallel \ll \tau_\perp$.

Seiter and Chan [7] have also extended Anderson's theory to account for the possible effects of sonication by superimposing overall rotational tumbling of the bilayer unit on the restricted and anisotropic local chain motion described above. The effect of rotational tumbling of the bilayer unit of course is to replace the inhomogeneous broadening present for multilayers by homogenous broadening, provided that the rate of tumbling of the bilayer unit is sufficiently fast. In this calculation, a third correlation time τ_v associated with the isotropic rotational tumbling of the bilayer unit was introduced in addition to $\tau_\parallel$ and $\tau_\perp$ and the important feature of motional restriction for the segmental motion was preserved.

The following result was obtained for the homogeneous T_2 resulting from the complex local motion and overall rotational tumbling of the bilayer unit.

$$\frac{1}{T_2} = \tfrac{1}{5}\alpha^2\{\bar{d}^2\tau_v + 2\bar{d}(\overline{d'^2})^{\frac{1}{2}}\sqrt{\tau_v\tau_*} + \overline{d'^2}\tau_*\}$$

where $\alpha^2 = 9.7 \cdot 10^9\ \mathrm{s}^{-2}$ for a germinal pair of protons,

$$\frac{1}{\tau_*} = \frac{1}{\tau_v} + \frac{1}{\tau_\perp}$$

and

$$\bar{d} = \overline{(1-3\cos^2\beta)}$$

$$\overline{d'^2} = \overline{(\bar{d}-(1-3\cos^2\beta))^2}.$$

Note that this particular relation is subject to the limitations $\tau_v > \tau_\perp \gg \tau_\parallel$ and in addition the two motions given by τ_v and $\tau_\perp$ must occur on sufficiently different times scales, so that it is possible to average over β independently of the averaging by the rotational diffusion of the bilayer unit. Also, for the resonance to be totally homogeneously broadened $\tau_v < 1/\alpha\bar{d}$, and thus for large vesicles with $\tau_v > 10^{-4}$ s the relation is not necessarily valid.

Fig. 3 depicts the dependence of the aliphatic proton linewidths on the rate of overall tumbling predicted by the above expression. These results are given for different amplitudes of angular excursion ($\Delta\beta$) as well as a range of time scales for the "out-of-axis" motion ($\tau_\perp$). These calculations indicate that the rate of overall rotational tumbling should have a large effect on the linewidth only when the motion is highly restricted, and that this dependence disappears when the motion becomes unrestricted. Moreover, for small $\Delta\beta$'s, this τ_v dependence of the linewidth is independent of $\tau_\perp$, the time scale of the off-axis motion for the interproton vectors. Only when $\Delta\beta$ approaches 90° does the τ_v dependence of the linewidth become more strongly dependent on $\tau_\perp$, but then the dependence of the linewidth on τ_v is small.

The above predictions are clearly at variance with the results of Finer's theory which predicts a stronger linewidth dependence on τ_v the shorter τ_c becomes; that is to say, the faster the rate of the specialized local motion, the more effective will the line narrowing be upon tumbling of the bilayer unit. This results we find difficult to understand in physical terms, and we believe it to be an artifact of the Finer treatment. However, the seemingly complicated results of the Seiter-Chan theory can be rationalized if we recall that, because of motional restriction, the NMR linewidth of a bilayer unit which is not undergoing rotational tumbling consists of both a homogeneous and an inhomogeneous component, and the relative importance of the two parts depends on the degree of motional restriction. Now for $\tau_v \gg \tau_\perp$,

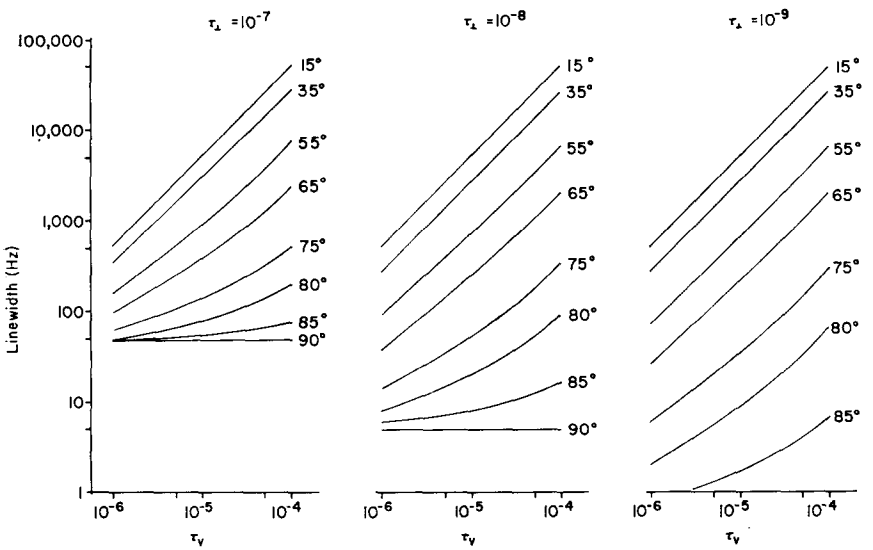

Fig. 3. Calculated linewidths of the chain methylene protons in bilayer vesicles as a function of the overall tumbling rate (τ_v^{-1}) and the degree of motional restriction ($\Delta\beta$) for three time scales of the local chain motion ($\tau_\perp$) based on the treatment of Seiter and Chan.

the effect of the overall tumbling is primarily to reduce the inhomogeneous broadening to homogeneous broadening as well, without greatly affecting that contribution to the linewidth which is already homogeneous in the absence of overall tumbling motion. Since this contribution to the homogeneous linewidth dominates when the local motion is highly restricted, we expect the linewidth to exhibit a strong dependence on the rate of tumbling under these conditions. Moreover this dependence should be essentially independent of $\tau_\perp$, since the τ_v dependence can only manifest itself through that part of the linewidth which is inhomogeneous in the absence of the overall tumbling motion. On the other hand, as the motion becomes less restricted, the two contributions will become more comparable. The linewidth would then be expected to exhibit a rather complex $\tau_\perp$ and τ_v dependence, although its dependence on the rate of tumbling should be limited only to the extent to which the inhomogeneous broadening dominates in the case of the stationary bilayer unit.

These theoretical predictions are best illustrated in Fig. 4, wherein we have considered the effect of a 10-fold increase in $\tau_\perp$ ($10^{-9} \rightarrow 10^{-8}$) on the PMR linewidth of sonicated bilayer vesicles tumbling at time scales of 10^{-4}, 10^{-5}, 10^{-6} s for various degrees of motional restriction for the local segmental motion. Similarly the effect of increasing the tumbling rate from 10^{+5} Hz to 10^{+6} Hz is also considered for various time scales of the local segmental motion (10^{-7}, 10^{-8}, 10^{-9} s). On the basis of these results, it is clear that for very restricted motions ($\Delta\beta < 50°$), a 10-fold narrowing is expected to accompany a tenfold increase in the overall tumbling rate, whereas a 10-fold increase in the rate of the off-axis local segmental motion has very little effect on the linewidth. As the motion gets less restricted, however, the effect of decreasing $\tau_\perp$ by a factor of 10 gradually leads to line narrowing, until in the limit of unrestricted motion ($\Delta\beta = 90°$) the linewidth has in fact reduced 10-fold. By

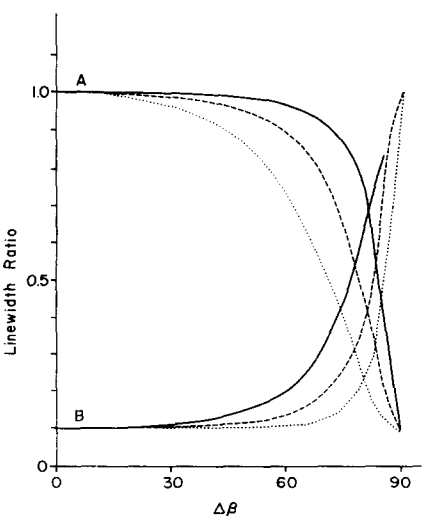

Fig. 4. The effect of a 10-fold increase in the rate of local motion (A) and the rate of overall tumbling (B) on the linewidth of the chain methylene protons in bilayer vesicles. In A the ratio of the linewidths corresponding to a decrease in $\tau_\perp$ from 10^{-8} to 10^{-9} s is given at $\tau_v = 10^{-6}$ s (· · ·), 10^{-5} s (- - -) and 10^{-4} s (—). In B the ratio of the linewidths associated with a decrease in τ_v from 10^{-5} s to 10^{-6} s is given at $\tau_\perp = 10^{-9}$ s (—), 10^{-8} s (- - -) and 10^{-7} s (· · ·).

contrast, a factor of 10 in the tumbling rate is reflected in the linewidth only for highly restricted motion, and this τ_v dependence of the linewidth is gradually suppressed as the motion gets less restricted, until in the limit of unrestricted motion there is no τ_v dependence of the linewidth irrespective of the time scale of the local segmental motion.

On the basis of Seiter and Chan calculations, it is evident that a linewidth similar to a value observed experimentally above the thermal phase transition for 300 Å diameter vesicles is only possible when the segmental motion is almost unrestricted ($\Delta\beta = 85$–$90°$) and $\tau_\perp$ is approximately 10^{-8} s. For such a motion the calculations predict only a minor if not negligible dependence of the linewidth of small (300 Å) sonicated bilayer vesicles on the rate of overall tumbling, in agreement with experiment. For a slower and more restricted motion, however, such as that which is necessary to account for the NMR lineshape and magnetic relaxation behaviour observed for phospholipid dispersions in their multilamellar state above the thermal phase-transition temperature, a homogeneous linewidth of the order of 500 Hz is predicted by the theory. Moreover, this linewidth should depend quite strongly on the overall tumbling rate, a result which is not borne out experimentally. It is on the basis of these compelling contradictions that Seiter and Chan [7] concluded that unsonicated and sonicated bilayers are characterized by quite different motional states. They attributed these differences to subtle differences in the molecular packing of the phospholipid molecules between these two systems. Sheetz and Chan [14] have previously argued that the small vesicle bilayer is significantly disordered at the molecular level because of the high surface curvature associated with these small particles.

THE MOTIONAL STATE OF SONICATED VESICLES

We present here further experimental results in support of our claim that surface curvature imposes structural constraints on the packing of the phospholipid molecules in lipid bilayers.

We first describe the PMR spectrum of small sonicated vesicles below the Chapman transition and analyze these spectral results in terms of the Seiter-Chan theory. This analysis will permit a comparison of the motional state of the hydrocarbon chains for these sonicated vesicles above and below the thermal phase transition as well as with their unsonicated counterparts.

Next, we shall consider the analysis of the PMR spectrum of large vesicles approximately 1000 Å in diameter. Because of the increase in particle size, these large bilayer vesicles tumble at a significantly slower rate. However, in terms of surface curvature, large vesicles 1000 Å in diameter are also intermediate between small sonicated vesicles and multilamellar dispersions. These latter experiments should therefore permit a delineation of the relative importance of the two types of motion, overall rotational tumbling vs local segmental motion, in determining the PMR spectral behavior of bilayer vesicles.

Small vesicles below the chapman transition

It is well established that multilayers in the crystalline state (below the thermal phase transition T_c) exhibit no high resolution features in the PMR spectrum [1–6].

The corresponding spectrum for small sonicated vesicles is however readily detectable [14]. The high resolution PMR spectrum of small dipalmitoyl lecithin vesicles at 18 °C, for example (Fig. 5a), is characterized by a relatively sharp choline signal (approx. 80 Hz in width and accounting for all the expected intensity) superimposed on a much broader aliphatic proton resonance (approx. 1000 Hz wide). It is not possible to account for these spectral differences between multilayers and small sonicated vesicles below the Chapman transition by the overall tumbling of the bilayer units alone. Although the rate of this tumbling for 300-Å vesicles is not readily ascertained below the thermal phase transition due to coagulation of the bilayer units under these conditions (Petersen, N. O., unpublished), this rate cannot be faster than 10^{+5} Hz for 300 Å vesicles at 18 °C. Even at this rate of tumbling, however, the aliphatic proton resonance is expected to have a width of approximately 5000 Hz. This assumes, of course, that the extent of the local segmental motion thought to prevail for multilayers in the crystalline state is carried over to the sonicated vesicles. Our present PMR results indicate that this cannot be the case and thus there must be disruption of the hexagonal regular close packing of the lipid molecules caused by sonication below the Chapman transition, in agreement with earlier PMR and dilatometry measurements [14].

According to the calculations to the calculations of Seiter and Chan, a line as narrow as approximately 1000 Hz is only possible if the interproton vectors of the hydrocarbon chains are allowed to traverse an angular range of 55–65 ° during segmental motion of the chain. For this $\Delta\beta$, the linewidth does not depend strongly on the rate of off-axis motion ($\tau_\perp$), although we do expect this motion to be slower than the $\tau_\perp$ value of approximately 10^{-8} s determined for above the thermal phase transition.

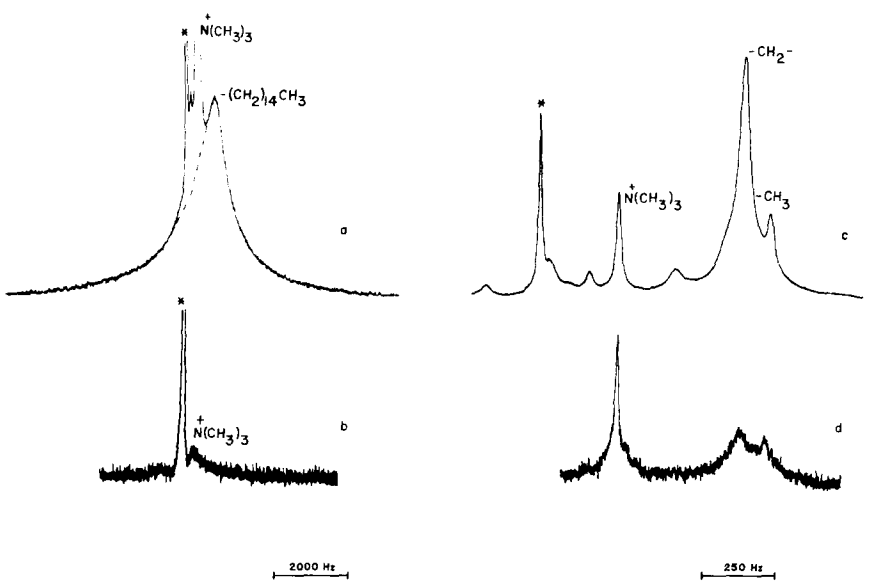

Fig. 5. 220 MHz PMR spectrum of sonicated dipalmitoyl lecithin dispersions. (a) Vesicles of approx. 300 Å at 18 °C, (b) vesicles of approx. 1000 Å at 18 °C, (c) vesicles of approx. 300 Å at 62 °C and (d) vesicles of approx. 1000 Å at 62 °C. *Residual H^2HO in sample.

The Seiter-Chan theory, however, does predict a fairly strong dependence of the linewidth on the rate of tumbling for these pseudo-crystalline bilayer vesicles. Experimentally we observed that both the choline and aliphatic resonances sharpen to approximately 80 % of their original linewidth upon a 10-fold dilution of a 27 % suspension of 300 Å vesicles at 23 °C. Although this dilution corresponds to about a factor of 3 reduction in the solution viscosity at this temperature, the exact decrease in τ_v is actually not known due to uncertainties resulting from coagulation of the vesicles under these conditions. Nevertheless, a τ_v dependence is indeed observed. By contrast we observe no similar change in the linewidth of the resonances of dipalmitoyl lecithin above the thermal phase transition for a similar 10-fold dilution of a 27 % suspension of lecithin vesicles at 50 °C, where solution viscosity decreases by a factor of 6.

Fig. 6 illustrates the temperature dependence of the linewidth of the bulk aliphatic resonance. This temperature dependence can be seen to be much more pronounced below than above the Chapman transition. Above T_c one would expect the linewidth to depend primarily on $\tau_\perp$, since $\Delta\beta$ is close to 90 ° as we have previously discussed (see earlier footnote). Below the phase transition, however, since $\Delta\beta \approx 60°$ at 18 °C, the linewidth should exhibit only a minor $\tau_\perp$ dependence. The large temperature dependence observed below T_c, we feel, reflects in part the effect of temperature on the bilayer tumbling rate, and in part increases in the amplitude of the local chain motion ($\Delta\beta$) accompanying the thermal expansion of the pseudo-crystalline lattice. Dilatometry measurements of Sheetz and Chan [14] have shown that the apparent partial molal volume of the lecithin is strongly temperature dependent below the Chapman transition. It should be pointed out that the linewidth of about 500 Hz

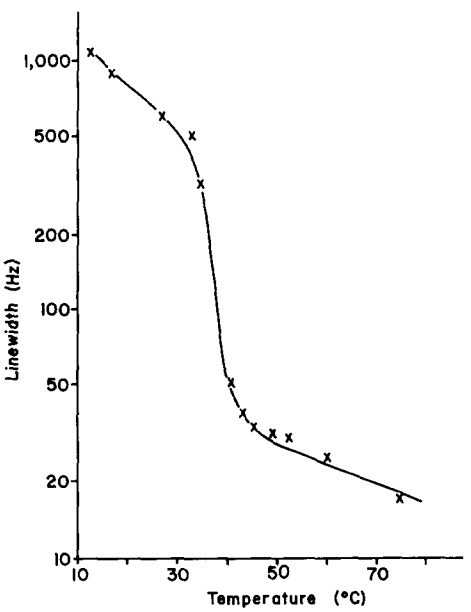

Fig. 6. Observed PMR linewitdhs of the chain protons (at 220 MHz) in small sonicated dipalmitoyl lecithin vesicles as a function of the temperature. The measurements below the thermal phase transitions were obtained with a 20-kHz sweepwidth, and those above with a 2.5-kHz sweepwidth.

observed for the paraffinic signal at 30° (below T_c) is also in part inhomogeneously broadened by the unresolved chemical shift difference between the terminal methyl resonances and the bulk methylene signal. Thus, in fact the aliphatic methylene signal is even narrower than what is observed at this temperature, further evidence of the pronounced packing changes accompanying the sonication process.

Large vesicles

For large vesicles approximately 1000 Å in diameter, only the choline head group is narrow enough (approx. 1000 Hz) to be detected below T_c, while the resonance due to the aliphatic protons is too broad to monitor even when a 20 000 Hz spectral width is used in the experiments. In terms of overall tumbling rates, these vesicles are now tumbling at a rate more than ten times slower than the smaller ones, if coagulation of the vesicles can be assumed to have no major effect on the tumbling rates of the individual bilayer units. In any case, the linewidth observed for the choline head groups indicates that there is some local motion of the head groups below the Chapman transition. This width could not be accounted for by the slower rate of overall tumbling of the bilayer unit compared with that for the smaller sonicated vesicles. The very narrow width of the choline head group signal in small sonicated vesicles under the same conditions indicates that the segmental motion of the head group is only slightly restricted ($\Delta\beta \approx 80°$ or more) and hence there should be at most a slight dependence of the linewidth upon the overall tumbling rate, if this almost unrestricted motion is preserved with this increase in the particle size. We therefore conclude that these spectral differences reflect the more regular molecular packing of the phospholipid molecules associated with the curvature decrease.

Above T_c, the lineshape of the paraffinic resonances is neither Lorentzian nor Gaussian and there is a conspicuous absence of intensities (Fig. 5d). It should be pointed out that insofar as intensities are concerned, the measured intensities are nothing but a reflection of lineshapes and linewidths. Since the only resonances narrow enough to be detected are usually monitored, the measured intensities might be a function of sweepwidth employed, an important point which has been neglected until recent works [5, 6, 14]. In the PMR spectra of large vesicles, we noted that the aliphatic resonance accounts for only about 50 % of the expected intensity at 50 °C, and even at 95 °C, only about 75 % of the paraffinic protons are detected in the spectrum. These observations may be compared with the value of 70 % of the fatty acid chain signals detected in the spectrum of small vesicles at 42 °C (above T_c), and the observation of the total intensity at about 62°. These differences in the spectral behavior between small and large vesicles, we feel, reflect differences in the flexibility gradient of the hydrocarbon chains between the two bilayer systems as a result of packing variations introduced by the change in surface curvature.

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NOTE ADDED IN PROOF

The rotational tumbling of bilayer units provides one of several mechanisms whereby the magnetic dipolar interactions can be averaged. As was pointed out in one of our earlier papers [14], in bilayer vesicles, lateral diffusion of the phospholipid molecules can also contribute to this averaging process. If this lateral diffusion is sufficiently rapid to be of significance, its contribution can be taken into account by replacing τ_v above in the Seiter-Chan treatment by τ_{eff}, where

$$\tau_{eff}^{-1} = \tau_v^{-1} + \tau_{ld}^{-1}$$

τ_{ld} corresponds to the correlation time associated with two-dimensional diffusion of the lipid molecules on the surface of the spherical bilayer unit. The lateral diffusion coefficient of phospholipid molecules in lecithin bilayers has been reported to be 10^{-7}–10^{-8} cm^2/s (Edidin, M. (1974) Annu. Rev. Biophys. Bioeng. 3, 179). Using this range of values, it is readily shown that $\tau_v \leq \tau_{ld}$ for a 300 Å diameter vesicle, and the general conclusions presented in this paper are valid. However, for a 1000 Å diameter bilayer unit, that part of the magnetic dipolar interactions which is not averaged out by the local segmental motion can only be effectively averaged by the lateral diffusion process.

REFERENCES

1 Finer, E. G., Flook, A. G. and Hauser, H. (1972) Biochim. Biophys. Acta 260, 59–69
2 Veksli, Z., Salsbury, N. J. and Chapman, D. (1969) Biochim. Biophys. Acta 183, 434–446
3 Penkett, S. A., Flook, A. G. and Chapman, D. (1968) Chem. Phys. Lipids 2, 273–290
4 Keough, K. M., Oldfield, E., Chapman, D. and Beynon, P. (1973) Chem. Phys. Lipids 10, 37–50
5 Seiter, C. H. A., Feigenson, G. W., Chan, S. I. and Hsu, M.-C. (1972) J. Am. Chem. Soc. 94, 2535–2537
6 Chan, S. I., Seiter, C. H. A. and Feigenson, G. W. (1972) Biochem. Biophys. Res. Commun. 46, 1488–1492
7 Seiter, C. H. A. and Chan, S. I. (1973) J. Am. Chem. Soc. 95, 7541–7553
8 Finer, E. G., Flook, A. G. and Hauser, H. (1972) Biochim. Biophys. Acta 260, 49–58
9 Huang, C.-H. (1969) Biochemistry 8, 344–352
10 Kornberg, R. D. and McConnell, H. M. (1971) Biochemistry 10, 1111–1120
11 Lee, A. G., Birdsall, N. J. M., Levine, Y. K. and Metcalfe, J. C. (1972) Biochim. Biophys. Acta 255, 43–56
12 Bangham, A. D. (1972) Chem. Phys. Lipids 8, 386–392
13 Papahadjopoulos, D. and Watkins, J. C. (1967) Biochim. Biophys. Acta 135, 639–652
14 Sheetz, M. P. and Chan, S. I. (1972) Biochemistry 11, 4573–4581
15 Sheard, B. (1969) Nature 223, 1057–1059
16 Levine, Y. K., Birdsall, N. J. M., Lee, A. G. and Metcalfe, J. C. (1972) Biochemistry 11, 1416–1421
17 Horwitz, A. F., Horsley, W. J. and Klein, M. P. (1972) Proc. Natl. Acad. Sci. U.S. 69, 590–593
18 Horwitz, A. F., Michaelson, D. and Klein, M. P. (1973) Biochim. Biophys. Acta 298, 1–7
19 Kohler, S. J., Horwitz, A. F. and Klein, M. P. (1972) Biochem. Biophys. Res. Commun. 49, 1414–1421
20 Anderson, P. W. (1954) J. Phys. Soc. Jap. 9, 316–339
21 Gutowsky, H. S. and Pake, G. E. (1950) J. Chem. Phys. 18, 162–170
22 Finer, E. G. (1974) J. Magn. Resonance 13, 76–86
23 Chan, S. I., Feigenson, G. W. and Seiter, C. H. A. (1971) Nature 231, 110–112
24 Charvolin, J. and Rigny, P. (1971) J. Magn. Resonance 4, 40–46
25 Seelig, J. and Niederberg, W. (1974) Biochemistry 13, 1585–1588, and references cited therein
26 Charvolin, J., Manneville, P. and Deloche, B. (1973) Chem. Phys. Lett. 23, 345–348
27 Oldfield, E., Chapman, D. and Derbyshire, W. (1971) FEBS Lett. 16, 102–104
28 Feigenson, G. W. and Chan, S. I. (1974) J. Am. Chem. Soc. 96, 1312–1319
29 Saunders, M. and Johnson, Jr, C. S. (1968) J. Chem. Phys. 48, 534–536