Biochimica et Biophysica Acta, 298 (1973) 1–7

BBA 76205

MAGNETIC RESONANCE STUDIES ON MEMBRANE AND MODEL MEMBRANE SYSTEMS

III. FATTY ACID MOTIONS IN AQUEOUS LECITHIN DISPERSIONS

ALAN F. HORWITZ, DANIEL MICHAELSON and MELVIN P. KLEIN

Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory University of California, Berkeley, Calif. 94720 (U.S.A.)

(Received August 21st, 1972)

SUMMARY

Magnetic resonance spectra and relaxation rates of sonicated and unsonicated vesicles of egg yolk lecithin are reviewed and compared. The NMR relaxation rates differ by about two orders of magnitude while the ESR order parameters show no such variation. The apparent contradiction may be removed by proposing that the ESR data reflect the order of segments of the fatty acids while the NMR relaxation rates reflect positional fluctuations. Macroscopic vesicular tumbling contributes insignificantly to the relaxation rates. Resonance and non-resonance data converge on a dynamic model in which the fatty acid molecules are configurationally mobile yet relatively ordered.

The NMR spectra of sonicated and unsonicated egg yolk lecithin dispersions are markedly different[1–3]. It is pertinent to ascertain if this difference has a simple origin, such as particle tumbling, or if it indeed reflects a structural difference between the two types of bilayers[1,4–6]. Publications from this laboratory have presented proton and phosphorus magnetic resonance spectra and relaxation rates of sonicated aqueous lecithin dispersions together with some plausible structures of the fatty acid chains[1,2,6]. We concluded from the T_2 data (or inverse linewidths) and temperature dependence of the T_1 data that the relaxation rates reflect the microscopic motions of the chains themselves rather than the macroscopic tumbling of the vesicles[1,6].

This conclusion was recently questioned by Finer *et al.*[4]. We present here four arguments that demonstrate the minor role of vesicle tumbling and suggest that these two types of bilayers have similar but different time dependent conformations.

Proton and phosphorus relaxation rates of sonicated egg yolk lecithin are not determined by vesicle tumbling

(A) Theoretical arguments do not support the contention that particle tumbling is important.

NMR relaxation rates are determined by the rates of nuclear motion. One effect of sonication is to disrupt the multilamellar vesicles of unsonicated lecithin

dispersions into smaller vesicles which undergo more rapid tumbling[1,7]. It is thus necessary to consider the contribution of vesicle tumbling to the nuclear relaxation rates. For molecules undergoing isotropic motion, Eqn 1 (ref. 8)

$$\Delta\omega \simeq \overline{\Delta\omega_0^2}\,\tau_c \tag{1}$$

can be used to estimate the motionally narrowed linewidth, $\Delta\omega$, where $\overline{\Delta\omega_0^2}$ is the rigid lattice second moment and τ_c is the rotational correlation time.

When the rigid lattice value of the second moment is used in Eqn 1 together with a value of τ_c appropriate to vesicle tumbling, the predicted and observed linewidths are vastly disparate[1,6]. Finer *et al.*[4] used a second moment estimated from the value of the methylene proton line-width of unsonicated lecithin rather than that of the rigid lattice. With that value of $\overline{\Delta\omega_0^2}$, tumbling times appropriate to sonicated vesicle radii, and a more sophisticated expression which becomes equivalent to Eqn 1 in the limit of rapid motion, they calculated linewidths agreeing reasonably with those displayed by the vesicles. The use of such a second moment was not justified and, in general, it is improper to do so[8].

In the special case of axial motion, however, the value of the rigid lattice ecsond moment used in Eqn 1 can be replaced by a new reduced second moment, $\overline{\Delta\omega'_0}^2$, which can often be estimated using Eqn 2,

$$\overline{\Delta\omega_0'^2} = \overline{\Delta\omega_0^2}\left(\frac{3\cos^2\theta - 1}{2}\right)^2 \tag{2}$$

where θ is the angle between the axis of rotation and the interproton vector[8,9]. [It follows that rapid motion about a second axis, different from the first, can reduce further the value of the second moment used in Eqn 1 (ref. 10).*] It is apparent from Eqn 2 that only rapid motion about an axis making an angle very near 54 ° 44′ with respect to the interproton vector will reduce the linewidth from the rigid lattice value of $\approx 7\cdot 10^4$ Hz to the value of $\approx 10^3$ Hz observed in unsonicated lecithin and used by Finer *et al.*[4] in Eqn 1. We do not view this as a physically plausible axis: The long axis of the fatty acid chain would appear more feasible for rapid axial motion[1]; were such motion to occur, the second moment would be reduced by a factor of 4.

---

* For rapid motion about more than one axis Eqn 2 must be modified to Eqn 2a

$$\Delta\omega'^2 = \overline{\Delta\omega_0^2}\left(\frac{3\cos^2\theta - 1}{2}\right)^2 \prod_{i=1}^{k}\left(\frac{3\cos^2\alpha_i - 1}{2}\right)^2 \tag{2a}$$

where θ is the angle between the interproton vector and the first axis of rotation, and where α_1 is the angle between the first axis of rotation and the second, *etc.* For methylene protons θ is 90° and α_i is the tetrahedral angle, 109° 28′. (θ is also the tetrahedral angle in ^{13}C studies. Application of this expression predicts an additional reduction of ^{13}C *vs* ^{1}H linewidths by a factor of 2.2). For methylene protons Eqn 2a becomes

$$\Delta\omega_0'^2 = \frac{\overline{\Delta\omega_0^2}}{4}(0.113)^n \tag{2b}$$

where $n+1$ is the number of bonds about which rapid reorientation occurs.

(B) There is no unique relaxation rate for the protons in sonicated egg yolk lecithin.

When the motion is complex and involves several correlation times (omitting the rapid axial case discussed in (A)), the net correlation time is given by Eqn 3,

$$\frac{1}{\tau_c} = \sum_i \frac{1}{\tau_{c_i}} \tag{3}$$

where $1/\tau_{c_i}$ are the correlation times for each motional component, *e.g.* vesicle tumbling and fatty acid chain motions*. If the narrow resonances observed in sonicated lecithin resulted primarily from vesicle tumbling (or from lateral diffusion of phospholipid molecules), a single value of τ_c and thus a single value of T_2 for all of the methylene resonances would be predicted, a prediction contrary to observation. The variety of T_2 and linewidth values observed for the resolved protons and the distribution of T_2 values for the methylene resonances themselves demonstrate clearly that fatty acid chain motion is at least as important as vesicle tumbling (or lateral diffusion)[6].

(C) Studies on membranes do not support this contention.

Proton magnetic spectra of rabbit sciatic nerve[11] and of rabbit sarcoplasmic reticular membrane preparations[12] have been reported and show relatively narrow resonances, qualitatively similar to those of sonicated lecithin, for the methylene and methyl protons. A size distribution of the sacroplasmic reticular membranes was not reported, but it is unlikely that the components of the sciatic nerve giving rise to the high resolution spectrum are similar in gross structure to sonicated egg yolk lecithin.

(D) The linewidths of sonicated egg yolk lecithin are independent of viscosity

The correlation time for particle tumbling is linear in viscosity (assuming the Stokes–Einstein relation). The data in Fig. 1 show that the proton and phosphorus linewidths are independent of glycerol concentration over a 5-fold range in viscosity. These observations are in accord with others made independently[13]; they provide clear evidence that particle tumbling does not affect significantly sonicated lecithin linewidths.

A comparison between sonicated and unsonicated egg yolk lecithin

The foregoing discussion suggests that the relatively long transverse relaxation rates (relatively narrow NMR lines) observed in sonicated lecithin reflect the dynamic structures of the fatty acids in these vesicles. It is obvious that the rotational correlation times will be substantially longer for the larger unsonicated vesicles than for their sonicated progeny. Since the tumbling of the smaller vesicles contributes little, if any, to the nuclear relaxation, these contributions in the unsonicated

---

* Eqn 3 is valid for isotropic motions, but for anisotropics motions Eqn 3a must be used (*c.f.* Woesner, D. E. (1962) *J. Chem. Phys.* 36, 1).

$$1/\tau_c = \sum_i C_i 1/\tau_{c_i} \tag{3a}$$

Using Eqn 2b it is easy to show that simultaneous, rapid axial motions about two bonds will reduce the linewidth by about an order of magnitude. This is similar to the total decrease in $1/T_2$ (or linewidth) observed as one proceeds along the entire methylene chain[14,15]. Thus each proton pair does not derive its linewidth solely from rapid axial motions.

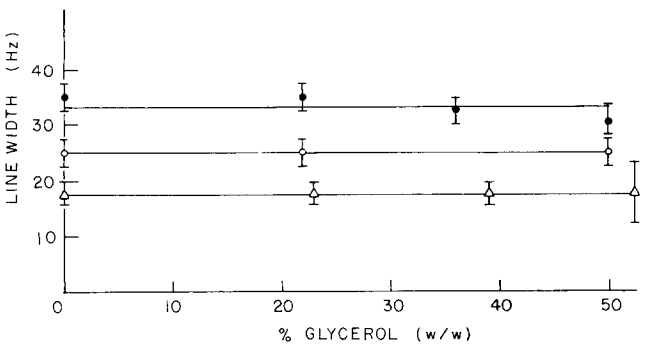

Fig. 1. The effect of glycerol on the NMR linewidths of sonicated egg yolk lecithin: ●, fatty acid methylene protons; ○, fatty acid methyl protons; △, phosphorus. Egg yolk lecithin was prepared according to the method of Singleton *et al.*[23] For proton experiments the lecithin was sonicated in 50 mM phosphated buffer containing 0.15 M KCl and 10^{-5} M EDTA, p^{2}H 7.5, and for phosphorus experiments it was sonicated in 50 mM tris buffer containing 0.15 M KCl and 10^{-5} M EDTA, p^{2}H 7.5. The details of the sample preparation have been described previously[2]. Glycerol was added to the samples after sonication, and they were allowed to stand for at least 30 min. Proton spectra were recorded at 20 °C on a Varian HR-220 NMR spectrometer, and the phosphorus spectra were recorded at 33 °C on the Fourier transform spectrometer described previously[2].

vesicles must be inconsequential. Thus the motional parameters underlying the nuclear relaxation are different in the two vesicular types. The methylene proton T_2 values are $\approx 10^{-4}$ s and $\approx 10^{-2}$ s for the unsonicated[14] and sonicated vesicles[6], respectively, implying a 100-fold difference in their correlation times.

The detailed differences between these two vesicles are unknown, although there are some similarities. While still uninterpreted, the differences between these two types of vesicles are evident in differential scanning calorimetry[24]. The evidence summarized below for both types of bilayers leads to the conclusion that there is an abrupt increase in motion very near the methyl terminus and that there is about an order of magnitude increase in a component of motion proceeding from the polar end to the methyl terminus of the fatty acid chains.

Chan *et al.*[14,16] have interpreted proton T_2 data for unsonicated lecithin as reflecting an order of magnitude decrease in a component of τ_c proceeding toward the center of the bilayer, and an abrupt increase in motion very near the terminal methyl.

Our proton T_1 and T_2 data for sonicated egg yolk and dimyristoyl lecithin were interpreted as revealing at least two components of motion: one fast which is roughly uniform over much of the fatty acid chain, and one slower which increases by a factor of ≈ 3 on progressing from the glycerol toward the methyl end of the fatty acids and then increases abruptly by another factor of 3–4 near this end[1,6]. The roughly uniform component of motion and the increase in motion near the methyl end is supported by a recent evaluation of ^{13}C T_1 data[17], in agreement with our previous interpretation of these data[6]. The temperature dependence of our T_1 values gave activation energies that agree well with those for internal rotations (trans to gauche isomerisations) in *n*-alkanes, suggesting that spin-lattice relaxation arises from frequent trans to gauche isomerisations[1,6].

Structured ESR spectra of vesicles containing nitroxide labeled phospholipid analogues have been interpreted in terms of an order parameter[18-20] while the individual NMR lines are analyzed in the framework of relaxation theory. Although it may not be useful, one can calculate order-parameters for the structureless NMR lines, from the ratio of their observed widths to the rigid lattice widths, and deduce that they are several orders of magnitude smaller than those determined by ESR. Also, one can calculate correlation times from the ESR spectra, by using Eqn 1, which agree reasonably well with those appropriate to NMR relaxation rates[6]. The spin-label order-parameters (by definition) are a measure of the time-average ordering at their locale on the fatty acid chains while the NMR relaxation rates reflect localized positional fluctuations. (Recall the different time scales for the two types of measurements.) That both the order-parameters and the relaxation rates decrease by about an order of magnitude from the polar to apolar ends of the molecule and show an abrupt increase in motion near the methyl end of the molecule, would suggest that the two methods reflect similar structural dynamics[1]. However, all NMR data reported for these lecithins show markedly broader lines in unsonicated than in sonicated vesicles; the former vesicles are reported to show a similar positional dependence of the relaxation rates as discussed above. By contrast, the ESR data do not exhibit a comparable difference[18,19]. The conformational constraints imposed by the nitroxides near their locale render it likely that these probes reflect the order along a finite length of the chain while the nuclear relaxation rates reflect motion at their locale. Such an interpretation envisions configurationally mobile yet relatively ordered chains. Sonication might then change the NMR correlation times without significantly modifying the ordering.

Evidence from several non-resonance techniques are in accord with the foregoing conclusions. X-ray scattering data show decreasing electron density along the methylene chain with an abrupt decrease near the methyl[21], laser Raman spectra show bands from individual fatty acids containing several gauche conformations[22]; finally, based on yet other types of experiments, Träuble has independently proposed "kinked" fatty acid conformations[22].

Our present ignorance precludes a discussion of any detailed structural differences between the two types of bilayers (which may involve the radii of curvature[15]).

A dynamic model of the fluid fatty acids

From an analysis of our proton magnetic resonance results we previously presented a dynamic model for the motion of the fatty acid chains in sonicated dimyristoyl and egg yolk lecithins[1,6]. This model is consistent with all of the data presented above, and therefore we recapitulate its salient features.

To account for the NMR T_1 data we suggested that fatty acid methylene groups are rapidly interconverting ($1/\tau_c \approx 10^{10}$ s^{-1}) between trans and gauche forms, and that this rate of interconversion is roughly uniform over much of the fatty acid chain. Fig. 2A shows a lecithin molecule containing a single gauche configuration. These laterally extended conformations affect all of the protons on methylene carbon atoms situated between the gauche bond and the methyl end of the molecule; due to interactions with neighboring chains the relative probability of the laterally extended conformations will probably increase as the position of the gauche bond progresses toward the methyl end. Therefore laterally extended

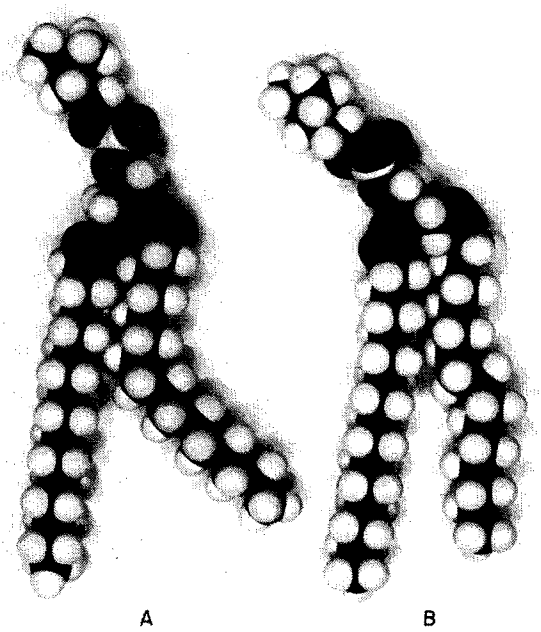

Fig. 2. A lecithin molecule containing: (A) a single gauche configuration, and (B) a β-coupled configuration.

conformations would not give rise to a roughly uniform value of T_1. On the other hand, conformations of the type shown in Fig. 2B result from a second gauche rotation of the opposite sense about a bond β to the first; these β-coupled configurations which result in straight conformations are likely to be of uniform or slightly increasing probability over much of the chain and therefore can lead to be observed T_1 data. Very near the methyl terminus laterally extended conformations will not be sterically unfavorable, the chains can isomerize without the aforementioned restrictions, and the relaxation times increase.

Aside from the β-coupled configurations are the less probable conformations which results from displacements of large segments of the fatty acids. These include coupled configurations in which the gauche rotations are separated by several rather than two bonds, or those shown in Fig. 2A. For the reasons discussed above, the probability that a particular carbon atom will find itself on such a segment will increase from the polar to the methyl end and thus account for the T_2 data.

In summary, the fatty acid chains in sonicated dimyristoyl and egg yolk lecithin are conformationally mobile yet relatively ordered. Such dynamic structures are consistent with the concept of mobile areas of "free volume" as a mechanism for small molecule transport, as discussed by Träuble and Haynes[23] and they are consistent with the concept of statistically bent fatty acids proposed by McFarland and McConnell[20]. Since T_1 data for unsonicated lecithin that are comparable to those for sonicated lecithin are not yet available, we do not know how the fatty

acid chain motions differ, nor do we know if the above model is qualitatively satisfactory for the unsonicated vesicles (see ref. 27 for a more complete discussion).

After this manuscript was written two papers were published that support the above model[25,26]. The first of these papers is a detailed analysis of laser-Raman spectra from sonicated lecithin. Laser-Raman spectra are sensitive to gauche and trans configurations in hydrocarbon chains, and they show that in sonicated lecithin the methylene groups along the fatty acid chain are in random configurations[25]. The second paper gives direct evidence for the tendency of gauche bonds to occur at a position β to a *cis*-unsaturate[26]. This reinforces the concept of β-coupled transitions in maintaining relatively straight chains.

ACKNOWLEDGMENT

This work was supported, in part, by funds from the U.S. Atomic Energy Commission

A. F. H. was a postdoctoral fellow of the National Heart and Lung Institute of the N.I.H., 1970-72.

REFERENCES

1 Horwitz, A. (1972) in *Membrane Molecular Biology* (Fox, C. F. and Keith, A. D., eds), pp. 164–191, Sinauer Associates, Stamford
2 Horwitz, A. and Klein, M. P. (1972) *J. Supramol. Struct.* 1, 19–28
3 Metcalfe, J. C., Birdsall, N. J. M., J., Lee, A. G., Levine, Y. K. and Partington, P. (1971) *Nature* 233, 199–201
4 Finer, E. G., Flook, A. G. and Hauser, H. (1972) *Biochim. Biophys. Acta* 260, 59–69
5 Sheard, B. (1969) *Nature* 223, 1057–1059
6 Horwitz, A. F., Horsley, W. J. and Klein, M. P. (1972) *Proc. Natl. Acad. Sci. U.S.* 69, 590–593
7 Attwood, D. and Saunders, L. (1965) *Biochim. Biophys. Acta* 98, 344–350
8 Abragam, A. (1961) *The Principles of Nuclear Magnetism*, Chapter 10, Oxford University Press, Clarendon, London
9 Andrew, E. R. and Eades, R. G. (1953) *Proc. R. Soc. A*, 218, 537–549
10 Wallach, D. (1967) *J. Chem. Phys.* 47, 5258–5268
11 Dea, P., Chan, S. I. and Dea, F. J. (1972) *Science* 175, 206–209
12 Davis, D. G. and Inesi, G. (1971) *Biochim. Biophys. Acta* 241, 1–8
13 Kornberg, R. D. and McConnell, H. M. (1971) *Proc. Natl. Acad. Sci. U.S.* 68, 2564–2568
14 Chan, S. I., Seiter, C. H. A. and Feigenson, G. W. (1972) *Biochem. Biophys. Res. Commun.* 46, 1488–1492
15 Steim, J. M. (1968) *Adv. Chem. Ser.* 84, 259–291
16 Chan, S. I., Feigenson, G. W. and Seiter, C. H. A. (1971) *Nature* 231, 110–112
17 Levine, Y. K., Partington, P., Roberts, G. C. K., Birdsall, N. M. J., Lee, A. G. and Metcalfe, J. C. (1972) *FEBS Lett.* 23, 203–207
18 Seelig, J. (1970) *J. Am. Chem. Soc.* 92, 3881–3887
19 Hubbell, W. L. and McConnell, H. M. (1971) *J. Am. Chem. Soc.* 93, 314–326
20 McFarland, B. G. and McConnell, H. M. (1971) *Proc. Natl. Acad. Sci. U.S.* 68, 1274–1278
21 Blaisie, J. K. (1972) in *ICN–UCLA Symposium in Molecular Biology* (Fox, C. F., ed.) ,Vol. 1, *Membrane Research*, pp. 3–14, Academic Press, New York
22 Lippert, J. L. and Peticolas, W. L. (1971) *Proc. Natl. Acad. Sci. U.S.* 68, 1572–1576
23 Träuble, H. and Haynes, D. H. (1971) *Chem. Phys. Lipids* 7, 324–335
24 Singleton, W. S., Gray, M. S., Brown, M. L. and White, J. L. (1965) *J. Am. Oil Chem. Soc.* 42, 53–56
25 Lippert, J. L. and Peticolas, W. L. (1972) *Biochim. Biophys. Acta* 282, 8–17
26 Batchelor, J. G., Prestegard, J. H., Cushley, R. J. and Lipsky, S. R. (1972) *Biochem. Biophys. Res. Commun.* 48, 70–75
27 Horwitz, A. F., Klein, M. P., Michaelson, O. M., Kohler, S. J. (1973) *Ann. N.Y. Acad. Sci.*, in the press