

Lawrence Berkeley National Laboratory

Recent Work

Title

MAGNETIC RESONANCE STUDIES ON MEMBRANE AND MODEL MEMBRANE SYSTEMS: III. A COMPARISON BETWEEN SONICATED AND UNSONICATED EGG YOLK LECITHIN

Permalink

<https://escholarship.org/uc/item/1sk8f1v5>

Author

Horwitz, Alan F.

Publication Date

1972-08-15

MAGNETIC RESONANCE STUDIES ON
MEMBRANE AND MODEL MEMBRANE SYSTEMS:
III. A COMPARISON BETWEEN SONICATED AND UNSONICATED
EGG YOLK LECITHIN

Alan F. Horwitz, Daniel M. Michaelson
and Melvin P. Klein

RECEIVED
LAWRENCE
BERNARD LABORATORY

August 15, 1972

LIBRARY AND
DOCUMENTS SECTION

AEC Contract No. W-7405-eng-48

For Reference

Not to be taken from this room



DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

Magnetic Resonance Studies on Membrane and Model Membrane Systems:

III. A comparison between sonicated and unsonicated egg yolk lecithin

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

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

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 of the fatty acid molecules containing several gauche conformations.

*A postdoctoral fellow of the National Heart and Lung Institute of the NIH, 1970-72.

The NMR spectra of sonicated and unsonicated egg yolk lecithin (EYL) dispersions are markedly different¹⁻³. 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 EYL 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, Eq. (1)⁸

$$\Delta\omega \approx \overline{\Delta\omega_0}^2 \tau_c \quad (1)$$

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 Eq. (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 linewidth of unsonicated EYL 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 Eq. (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 second moment used in Eq. (1) can be replaced by a new reduced second moment, $\Delta\omega_0^{\dagger 2}$, which can often be estimated using Eq. (2),

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

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 Eq. (1)¹⁰.) It is apparent from Eq. (2) that only rapid motion about an axis making an angle very near $54^\circ 44'$ with respect to the interproton vector will reduce the linewidth from the rigid lattice value of $\sim 7 \times 10^4$ Hz to the value of $\sim 10^3$ Hz observed in unsonicated EYL and used by Finer et al.⁴ in Eq. (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 motion¹; were such motion to occur, the second moment would be reduced by a factor of 4.

B) There is no unique relaxation rate for the protons in sonicated EYL.

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 Eq. (3),

$$\frac{1}{\tau_c} = \sum_i \frac{1}{\tau_{c_i}} \quad (3)$$

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 EYL 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 nerve¹¹ and of rabbit sarcoplasmic reticular membrane preparations¹² have been reported and show relatively narrow resonances, qualitatively similar to those of sonicated EYL, for the methylene and methyl protons. A size distribution of the sarcoplasmic 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 EYL.

D) The linewidths of sonicated EYL are independent of viscosity.

The correlation time for particle tumbling is linear in viscosity (assuming the Stokes-Einstein relation). The data in Table 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 independently¹³; they provide clear evidence that particle tumbling does not affect significantly sonicated EYL linewidths.

A Comparison between Sonicated and Unsonicated EYL.

The foregoing discussion suggests that the relatively long transverse relaxation rates (relatively narrow NMR lines) observed in sonicated EYL 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 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 $\sim 10^{-4}$ sec and $\sim 10^{-2}$ sec for the unsonicated¹⁴ and sonicated vesicles⁶, 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 calorimetry²⁴. 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.^{14,15} have interpreted proton T_2 data for unsonicated EYL 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 EYL and dimyristoyl lecithin were interpreted as revealing a roughly exponential decrease of a factor of 2-3 in a component of motion upon progressing from the glycerol end toward the methyl end of the fatty acids and an abrupt increase of another factor of about 3-4 near this end^{1,6}. The interpretation of an abrupt increase is supported by a recent evaluation of C^{13} T_1 data¹⁶, in agreement with our previous interpretation of these data⁶. There is no intention of implying that the polar ends of the fatty acids are highly immobilized; rather, they are only one order of magnitude less mobile than the methyl end, viz., $\tau_c = 10^{-8}$ sec vs $\tau_c = 10^{-9}$ sec. We suggested that the mobility of the bulk of the chain results principally from coupled trans-gauche isomerizations^{1,6}.

Structured ESR spectra of vesicles containing nitroxide labeled phospholipid analogues have been interpreted in terms of an order parameter¹⁷⁻¹⁹ 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 Eq. (1), which agree reasonably well with those appropriate to our NMR relaxation rates⁶. 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.) The facts 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, suggest that the two methods reflect similar structural dynamics¹.

The preceding discussion would lead to the conclusion that the ESR and NMR experiments report similar structural dynamics. All NMR data reported thus far show markedly broader lines in unsonicated than in sonicated vesicles; the former vesicles also show a parallel positional dependence of the relaxation rates as discussed above. By contrast, the ESR data do not exhibit a comparable difference^{17,18}. The conformational constraints imposed by the nitroxides near their locale render it likely that they reflect the order along a finite length of the chain while the nuclear relaxation rates reflect motion at their locale. Such an interpretation envisions fluid 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 methyl²⁰. Laser raman spectra show bands from individual fatty

acids containing several gauche conformations²¹. Finally, based on yet other types of experiments, Trüuble has independently proposed "kinked" fatty acid conformations²².

Our present state of ignorance precludes a discussion of any detailed structural differences between the two types of bilayers.

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

REFERENCES

1. A. Horwitz, in C. F. Fox and A. D. Keith, Membrane Molecular Biology Sinauer Associates, Stamford, 1972, p. 164.
2. A. Horwitz and M. P. Klein, J. Supra. Str., 1 (1972) 19.
3. J. C. Metcalfe, N. J. M. Birdsall, J. Feeny, A. G. Lee, Y. K. Levine and P. Partington, Nature, 233 (1971) 199.
4. E. G. Finer, A. G. Flook and H. Hauser, Biochim. Biophys. Acta, 260 (1972) 59.
5. B. Sheard, Nature, 223 (1969) 1057.
6. A. F. Horwitz, W. J. Horsley and M. P. Klein, Proc. Nat. Acad. Sci. U.S.A., 69 (1972) 590.
7. D. Attwood and L. Saunders, Biochim. Biophys. Acta, 98 (1965) 344.
8. A. Abragam, The Principles of Nuclear Magnetism (Oxford Univ. Press, Clarendon, London), Chap. 10, 1961.
9. E. R. Andrew and R. G. Eades, Proc. Roy. Soc. A, 218 (1953) 537.
10. D. Wallach, J. Chem. Phys., 47 (1967) 5258.
11. P. Dea, S. I. Chan and F. J. Dea, Science, 175 (1972) 209.

12. D. G. Davis and G. Inesi, Biochim. Biophys. Acta, 241 (1971) 1.
13. R. D. Kornberg and H. M. McConnell, Proc. Nat. Acad. Sci. U.S.A., 68 (1971) 2564.
14. S. I. Chan, C. H. A. Seiter and G. W. Feigenson, Biochem. Biophys. Res. Commun., 46 (1972) 1488.
15. S. I. Chan, G. W. Feigenson and C. H. A. Seiter, Nature, 231 (1971) 110.
16. Y. K. Levine, P. Partington, G. C. K. Roberts, N. J. M. Birdsall, A. G. Lee and J. C. Metcalfe, FEBS Letters, 23 (1972) 203.
17. J. Seelig, J. Amer. Chem. Soc., 92 (1970) 3881.
18. W. L. Hubbell and H. M. McConnell, J. Amer. Chem. Soc., 93 (1971) 314.
19. B. G. McFarland and H. M. McConnell, Proc. Nat. Acad. Sci. U.S.A., 68 (1971) 1274.
20. J. K. Blaisie, in C. F. Fox, ed., ICN-UCLA Symposium in Molecular Biology, Vol. 1, Membrane Research, Academic Press, 1972, in press.
21. J. L. Lippert and W. L. Peticolas, Proc. Nat. Acad. Sci. U.S.A., 68 (1971) 1572.
22. H. Trüuble and D. H. Haynes, Chem. Phys. Lipids, 7 (1971) 324.
23. W. S. Singleton, M. S. Gray, M. L. Brown and J. L. White, J. Amer. Oil Chem. Soc., 42 (1965) 53.
24. J. M. Steim, Adv. in Chem. Series, 84 (1968) 259.

Footnotes

† For rapid motion about more than one axis eq. (2) must be modified to eq. (2a)

$$\Delta\omega_o'^2 = \overline{\Delta\omega_o}^2 \left(\frac{3 \cos^2 \theta - 1}{2} \right)^2 \prod_{i=1}^n \left(\frac{3 \cos^2 \alpha_i - 1}{2} \right)^2 \quad (2a)$$

where θ is the angle between the interproton vector and the first axis of rotation, and where α_i 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, $109^\circ 28'$. (θ is also the tetrahedral angle in C^{13} studies. Application of this expression predicts an additional reduction of C^{13} vs H^1 linewidths by a factor of 2.2.) For methylene protons eq. (2a) becomes

$$\Delta\omega_o'^2 = \overline{\Delta\omega_o}^2 (0.113)^n \quad (2b)$$

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

† Equation (3) is valid for isotropic motions, but for anisotropic motions eq. (3a) must be used (c.f., D. E. Woessner, J. Chem. Phys., 36 (1962) 1).

$$1/\tau_c = \sum_i C_i 1/\tau_{ci} \quad (3a)$$

Using eq. (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.

FIGURE CAPTION

Figure 1. The effect of glycerol on the NMR linewidths of sonicated egg yolk lecithin:

- fatty acid methylene protons
- fatty acid methyl protons
- Δ 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, pD = 7.5, and for phosphorus experiments it was sonicated in 50 mM tris buffer containing 0.15 M KCl and 10^{-5} M EDTA, pD = 7.5. The details of the sample preparation have been described previously². Glycerol was added to the samples after sonication, and they were allowed to stand for at least 30 minutes. 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 previously².

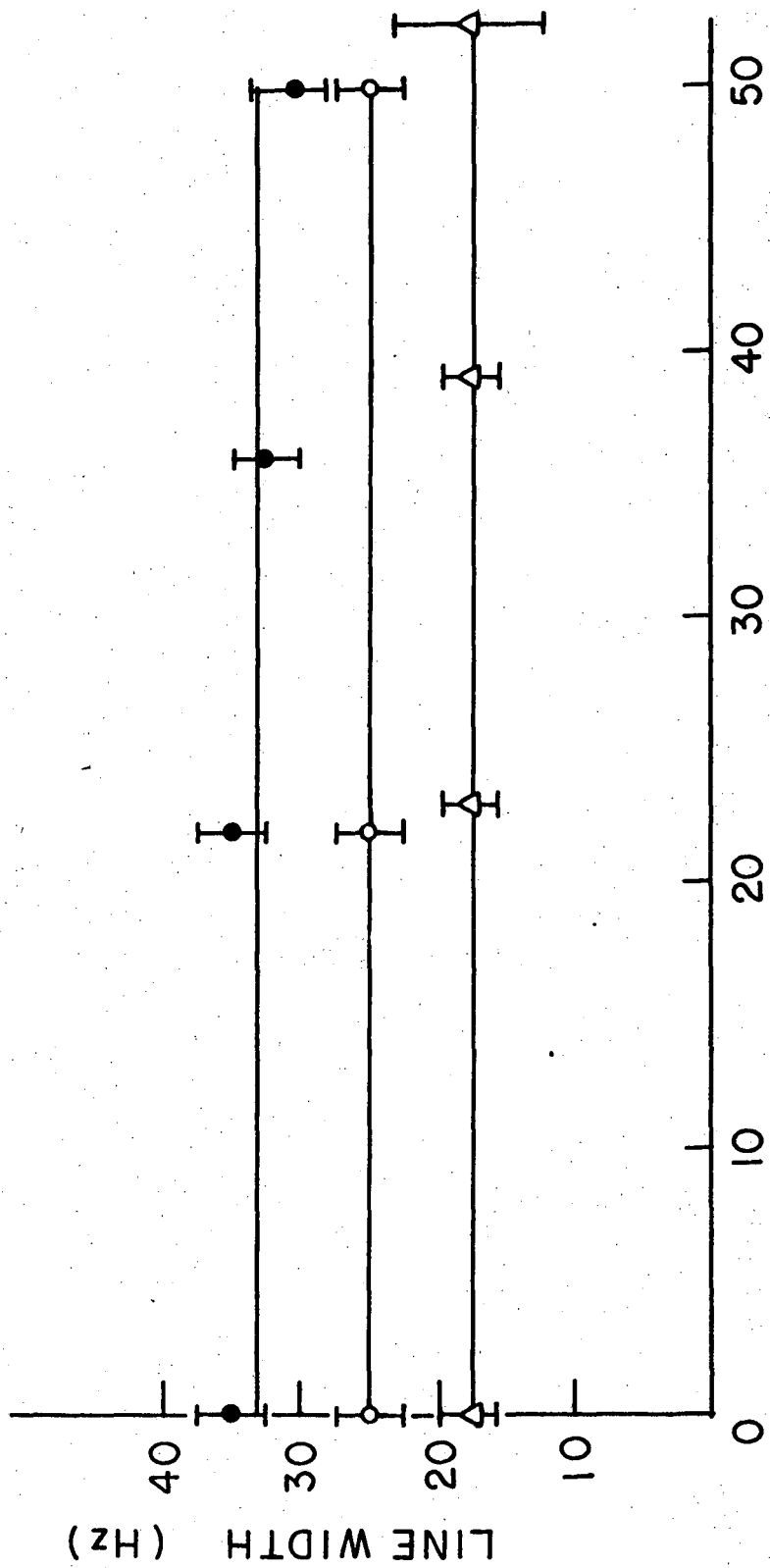


Fig. 1

XBL727-4698

LEGAL NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

TECHNICAL INFORMATION DIVISION
LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720