(27) The appearance of 0.62% Cl^- in I probably reflects encapsulated NaCl or KCl.
(28) The accuracy of elemental analysis determinations are as follows: Rh, ±0.4; P, ±0.3; B, ±0.3; Cl, ±0.3. Establishment of accurate ratios are difficult as the errors are cumulative.
(29) F. A. Cotton and G. Wilkinson, "Advanced Inorganic Chemistry," 3rd ed, Wiley, New York, N.Y., 1972, pp 884, 885 and 1023, 1024.
(30) ESCA spectra were recorded on a GCA/McPherson ESCA 36 spectrometer utilizing a magnesium (1253.6 eV) source. Samples were attached with sticky tape to an aluminum metal backing. We wish to thank Mr. Barry Matrana for the ESCA data, and The Aerospace Corporation, El Segundo, Calif., for the use of their spectrometer.
(31) W. L. Jolly, *Coord. Chem. Rev.*, **13**, 47 (1974).
(32) W. Bresmer, *Fortschr. Chem. Forsch.*, **36**, 1 (1973).
(33) E. L. Hoel, M. Talebinasab-Savari, and M. F. Hawthorne, *J. Am. Chem. Soc.*, **99**, 4356 (1977).
(34) A kinetic study of the hydrogenation of olefins using [3,3-$(Ph_3P)_2$-3-H-3,1,2-$RhC_2B_9H_{11}$] is in preparation: J. J. Wilczynski and M. F. Hawthorne.
(35) M. F. Hawthorne, D. C. Young, P. M. Garrett, D. A. Owen, S. G. Schwerin, F. N. Tebbe, and P. A. Wegner, *J. Am. Chem. Soc.*, **90**, 862 (1968).
(36) R. N. Grimes, "Carboranes", Academic Press, New York, N.Y., 1970, Chapter 6 and references contained therein.
(37) Contribution No. 3825.

B. A. Sosinsky, W. C. Kalb, R. A. Grey
V. A. Uski, M. F. Hawthorne*
Department of Chemistry, University of California
Los Angeles, California 90024[37]
Received May 2, 1977

Effect of Lectin-Induced Agglutination on Carbon-13 Nuclear Magnetic Resonance Line Width in Sonicated Phospholipid/Glycolipid Vesicles

Sir:

Sonication of aqueous dispersions of phosphatidylcholine results in the formation of small spherical unilamellar vesicles ~250 Å in diameter.[1] Structural similarities and differences between these sonicated vesicles and unsonicated multilamellar liposomes are of biological interest because these systems can serve as models of natural membranes with widely different radii of curvature.[2] Sonication of phosphatidylcholine dispersions causes narrowing of NMR resonance peaks assigned to the protons and carbons of phosphatidylcholine,[3-12] and the reasons for this have been debated. Some have argued that the line narrowing is due to increased disorder in the packing of the phospholipid molecules in the sonicated vesicles relative to that in unsonicated dispersions.[3-6] Others believe that the observed decrease in line width is not related to any structural difference between phospholipids in sonicated vesicles and in unsonicated dispersions, but is related to motional narrowing resulting from the faster tumbling rate of the small sonicated vesicles.[7-9] Chan et al.[4] and Horwitz et al.[6] attempted to eliminate tumbling as an agent of motional narrowing by showing that the ^1H NMR line widths of the phospholipid protons in sonicated vesicles are independent of solution viscosity. This interpretation has been questioned, however, because of the possibility that hydration layers at the vesicle surface could render vesicle tumbling insensitive to bulk solution viscosity.[9]

Since a variety of other physical techniques such as dilatometry,[3] differential scanning calorimetry,[13,14] electron spin resonance,[15] and fluorescence depolarization[13] have shown differences in the physical properties of sonicated and unsonicated phospholipid dispersions, it is of interest to determine whether the NMR spectral differences between sonicated and unsonicated dispersions are due, in fact, to a difference in tumbling rate or to real differences in phospholipid motion and packing. To answer this question, we have prepared phospholipid/glycolipid vesicles which can be quantitatively agglutinated by a plant lectin (carbohydrate-binding protein). These vesicle-lectin aggregates are large visible precipitates whose tumbling rate is similar to that of unsonicated dispersions. Using tri[*N-methyl*-^{13}C]choline labeled phosphatidylcholine (^{13}C-PC),[16,17] we studied the effect of lectin-induced agglutination on the line width of the [*N-methyl*-^{13}C]choline resonance of the sonicated vesicles.

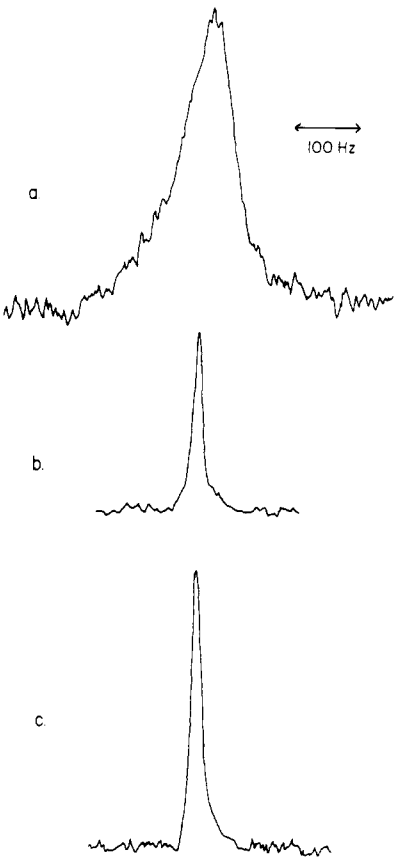

Figure 1. ^{13}C NMR spectra of ^{13}C-PC/LC mixtures obtained at 27 °C using a Bruker HX-270 Fourier transform spectrometer operating at 67.9 MHz with 3 W of decoupling power: (a) unsonicated ^{13}C-PC/LC (10:1, m/m) dispersion (7000 accumulations); (b) sonicated ^{13}C-PC/LC (10:1, m/m) vesicles (1000 accumulations); (c) lectin-agglutinated sonicated ^{13}C-PC/LC (10:1 m/m) vesicles (1500 accumulations). 8 K data points and a recycle time of 0.8 s were used to obtain the spectra.

An unsonicated dispersion of ^{13}C-PC/LC (10:1, mol/mol) exhibits the ^{13}C NMR spectrum shown in Figure 1a at 27 °C. The single *N*-methyl carbon resonance is broad ($\Delta\nu_{1/2}$ = 86 Hz) and asymmetric, indicative of restricted anisotropic motion of the phosphatidylcholine polar head group. This spectrum has the same line width and spectral appearance as that of unsonicated ^{13}C-PC alone. Sonicated vesicles of ^{13}C-PC/LC (10:1, mol/mol), on the other hand, exhibit a single sharp resonance ($\Delta\nu_{1/2}$ = 14 Hz), as shown in Figure 1b. This line width is slightly larger than that observed for ^{13}C-PC vesicles ($\Delta\nu_{1/2}$ = 12 Hz).

Addition of 400 μl of a solution of *R. communis* lectin[22] (4.88 mg/mL) to 1.2 mL of the clear sonicated ^{13}C-PC/LC (10:1, m/m) vesicle solution (3 μmol of ^{13}C-PC/mL) results in the formation of a flocculent white precipitate. The ^{13}C NMR spectrum of the redispersed lectin-vesicle aggregate, shown in Figure 1c, is similar to that of the unagglutinated ^{13}C-PC/LC vesicles (Figure 1b) although the line width for the aggregate is slightly larger ($\Delta\nu_{1/2}$ = 16 Hz). To ensure that the ^{13}C-PC/LC vesicles were quantitatively agglutinated by the quantity of lectin added, the agglutination was repeated under exactly the same conditions, using sonicated ^{14}C-PC/LC (10:1, mol/mol). After addition of lectin, the suspension was centrifuged at 12 000 × *g* for 20 min at 4 °C, resulting in sedimentation of >97% of the radioactive ^{14}C-PC, and thus

verifying quantitative agglutination. Addition of 4 μmol of lactose, which competes with the vesicles for the lectin, resulted in complete dissolution of the lectin-vesicle precipitate, giving rise to the resuspension of single unilamellar vesicles. Electron microscopy of the deagglutinated vesicle suspension showed only small particles, verifying that extensive vesicle fusion had not occurred.

The observation that the line width of the *N*-methyl carbon resonance is insensitive to lectin-induced vesicle agglutination is strong evidence that the tumbling of phospholipid vesicles is not the major mechanism for the line narrowing observed upon sonication of phosholipid dispersions. Preliminary experiments with the ^{13}C label in the acyl chain of phospholipid give similar results. Having eliminated tumbling as a significant factor, we conclude that the line narrowing observed in NMR spectra of sonicated vesicles is most likely due to increased structural disorder in the packing of the phospholipids, relative to unsonicated dispersions. The increased disorder in the packing of the phospholipid should also be reflected in an increased lateral diffusion rate in the plane of the bilayer. Recent work has suggested that such an increase in lateral diffusion rate may occur in sonicated vesicles.[25]

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References and Notes

(1) C. Huang, *Biochemistry*, **8**, 344 (1969).
(2) T. E. Thompson, C. Huang, and B. J. Litman in "The Cell Surface in Development", A. A. Moscona, Ed., New York, N.Y., 1974, p. 1.
(3) M. P. Sheetz and S. I. Chan, *Biochemistry*, **11**, 4573 (1972).
(4) S. I. Chan, M. P. Sheetz, C. H. Seiter, G. W. Feingenson, M. Hsu, A. Lau, and A. Yau, *Ann. N.Y. Acad. Sci.*, **222**, 499 (1972).
(5) C. H. Seiter and S. I. Chan, *J. Am. Chem. Soc.*, **95**, 7541 (1973).
(6) A. F. Horwitz, D. Michelson, and M. P. Klein, *Biochim. Biophys. Acta*, **298**, 1 (1973).
(7) E. G. Finer, A. G. Flook, and H. Hauser, *Biochim. Biophys. Acta*, **260**, 59 (1972).
(8) M. Bloom, E. E. Burnell, M. I. Valic, and G. Weeks, *Chem. Phys. Lipids*, **14**, 107 (1975).
(9) G. W. Stockton, C. F. Polnaszek, A. P. Tullock, F. Hasan, and I. C. P. Smith, *Biochemistry*, **15**, 954 (1976).
(10) B. Sears, W. C. Hutton, and T. E. Thompson, *Biochem. Biophys. Res. Commun.*, **60**, 1141 (1975).
(11) B. Sears, *J. Membr. Biol.*, **20**, 59 (1975).
(12) M. P. Gent and J. H. Prestegard, *Biochem. Biophys. Res. Commun.*, **58**, 549 (1974).
(13) J. Suurkuusk, B. R. Lentz, Y. Barenholz, R. L. Biltonen, and T. E. Thompson, *Biochemistry*, **15**, 1393 (1976).
(14) J. M. Sturtevant, *Ann. Rev. Biophys. Bioeng.*, **3**, 35 (1974).
(15) D. Marsh, A. D. Phillip, A. Watts, and P. F. Knowles, *Biochem. Biophys. Res. Commun.*, **49**, 641 (1972).
(16) Abbreviations: PC, phosphatidylcholine; ^{13}C-PC, tri[*N*-methyl-^{13}C]choline-labeled PC; ^{14}C-PC, [*N*-methyl-^{14}C]choline-labeled PC; LC, lactosyl ceramide.
(17) Egg yolk phosphatidylcholine was extracted and purified according to Litman.[18] Egg yolk phosphatidic acid was prepared by the digestion of egg yolk PC by phospholipase C according to Dawson and Hemington.[19] Tri[*N*-methyl-^{13}C]choline was synthesized by reaction of $^{13}CH_3I$ (Merck) and ethanolamine[20] and purified by ion-exchange chromatography. The ^{13}C-labeled choline was covalently attached to egg yolk phosphatidic acid as previously described.[21] [*N*-methyl-^{14}C]Choline-labeled egg PC was synthesized similarly using [*N*-methyl-^{14}C]choline from New England Nuclear, Boston, Mass. Lactosyl ceramide was purchased from Miles Laboratories, Elkhart, Ind. (*N*-palmitoyl dihydrolactocerebroside, lot no. 3). Preparation of unsonicated dispersions (multilamellar liposomes) was as follows. Solutions of LC and ^{13}C-PC or ^{14}C-PC in 2:1 chloroform–methanol were mixed in appropriate proportions in a round-bottom flask, and the solvent was removed in a rotary evaporator. After desiccation overnight against a vacuum, 4 mL of a buffer composed of 0.01 M Na_2HPO_4 and 0.2 M NaCl (pH 7.2) was added, and the lipid suspended by swirling the flask. To prepare sonicated vesicles, this dispersion was sonicated for 20 min at 4 °C under a N_2 atmosphere, followed by centrifugation at 12 000 × *g* for 20 min at 4 °C to remove titanium fragments and multilamellar liposomes.
(18) B. J. Litman, *Biochemistry*, **12**, 2545 (1973).
(19) R. M. Dawson and N. Hemington, *Biochem. J.*, **102**, 76 (1967).
(20) P. Brulet and H. M. McConnell, *J. Am. Chem. Soc.*, **98**, 1314 (1976).
(21) B. Sears, W. C. Hutton, and T. E. Thompson, *Biochemistry*, **15**, 1635 (1976).
(22) *R. communis* lectin was purified according to Nicolson and Blaustein[23] as modified by Podder et al.[24] The lectin was stored in 0.02 M Na_2HPO_4 plus 0.2 M NaCl (pH 7.2).
(23) G. L. Nicolson and J. Blaustein, *Biochim. Biophys. Acta*, **266**, 543 (1972).
(24) S. K. Podder, A. Surolia, and B. K. Backhawat, *J. Biochem.*, **44**, 151 (1974).
(25) P. Brulet and H. M. McConnell, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 1451 (1975).

W. Curatolo, G. G. Shipley, D. M. Small, B. Sears*
Biophysics Division, Department of Medicine
Boston University School of Medicine
Boston, Massachusetts 02118

L. J. Neuringer
Francis Bitter National Magnet Laboratory
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

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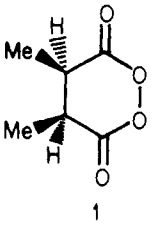
Six-Membered Cyclic Diacyl Peroxide Fragmentations. Thermal Decomposition of *meso*- and *dl*-2,3-Dimethylsuccinoyl Peroxides

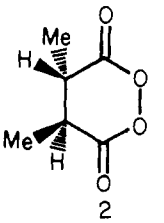
Sir:

Predictive criteria for those thermal reactions of high energy species which provide electronically excited states of product molecules are still not completely understood. The thermal decompositions of 1,2-dioxetanes which afford electronically excited n,π* states of carbonyl products are well known.[1] Efforts to study the thermal generation of electronically excited π,π* states of aromatics and simple alkenes have been hampered owing to the lack of suitable high energy precursors. Recent important work by Schuster[2] suggests that the thermal decomposition of a presumed six-membered cyclic diacyl peroxide intermediate is an efficient chemiluminescent process and provides a method for the direct chemical formation of aromatic hydrocarbon π,π* electronically excited states. This raises the question whether succinoyl peroxides on thermal decomposition would afford electronically excited states of simple alkenes. The mechanism of the decomposition of six-membered cyclic diacyl peroxides is not known and only a few examples of any six-membered cyclic diacyl peroxides exist in the literature.[3]

We report the syntheses and thermal decomposition of *meso*- and *dl*-2,3-dimethylsuccinoyl peroxides (**1** and **2**, respectively). These initial findings provide the first stereochemical study of six-membered cyclic diacyl peroxide thermal fragmentations. The data presented here are consistent with a common intermediate(s) but do not require the generation of electronically excited states of the 2-butene products.



1



2

Successive treatment of 98% isomerically pure *meso*-2,3-dimethylsuccinic acid (**3**),[4,5] mp 209–210 °C, with phosphorus pentachloride and sodium peroxide,[6] afforded a white crystalline solid whose NMR and IR spectra ($\nu_{C=O}$ 1812 (m), 1783 (s) cm^{-1}) were consistent with structure **1**. The isomeric purity of **1** was determined by treatment of the peroxide with