Title

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MAGNETIC RESONANCE STUDIES ON MEMBRANES AND MODEL MEMBRANE SYSTEMS

IV. A COMPARISON OF YEAST AND EGG LECITHIN DISPERSIONS

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Summary: Nuclear magnetic resonance (NMR) linewidths and relaxation rates for both protonated and deuterated yeast lecithins are smaller than those exhibited by egg lecithin. Relative to egg lecithin, the yeast lecithins contain a much higher percentage of unsaturated fatty acids, and a higher proportion of the saturated fatty acids are short chain. The relaxation times observed for deuterated yeast lecithin are 2-3 fold greater than those in the protonated analogs, thus identifying dipolar interactions as a major contributor to transverse relaxation. The possibility of using partially deuterated organisms for NMR studies of membranes is suggested.

From recent studies on unsonicated and sonicated aqueous dispersions of hen egg lecithin (EL) as well as dispersions of synthetic dipalmitoyl and dimyristoyl lecithins, a rough picture of the dynamics of the fluid fatty acid chains in phospholipid bilayers has emerged.[1-3] Proper biological activity is dependent on the fatty acid composition of the phospholipids[4] which in turn is believed to determine the membrane fluidity.[5,6] However, no explicit attempt to use NMR to assess the importance of fatty acid composi ion on bilayer fluidity has yet been reported.

We report differences between the fatty acid compositions of yeast and egg lecithins, compare the NMR parameters from dispersions of these lecithins, and relate the observed differences to fatty acid mobility in the bilayer. Additionally, the NMR data from dispersions of lecithin obtained from deuterium enriched yeast permit a partial evaluation of the contribution of the dipolar interactions to the proton relaxation rates.

Experimental

The yeast *R. pilimanae* was grown as previously described[7] with the addition of 0.1 mg Fe^{3+}/liter (as $FeNH_4(SO_4)_2$). The yeast was grown in 98.9% D_2O

for the production of deuterated yeast lecithin (DYL) and in H_2O for the production of normal (or "protonated") yeast lecithin (PYL). The cells were harvested after 10 days and stored frozen. To prepare for lecithin extraction, the cells were thawed, lyophilized, and sonicated for 15 minutes continuously in $CHCl_3$/MeOH, 2/1 (v/v). The samples were maintained in a dry ice-acetone bath under argon during the sonication with a Branson 185E sonicator fitted with a 1/2 inch tip.

The lipids were removed from the yeast by the method of Folch.[8] Lecithin was isolated from the crude extract by alumina chromatography,[9] and further purified by silica chromatography.[10] The final lecithin was determined to be pure by thin layer chromatography on silica (Adsorbosil-5, Applied Science Labs) using a solvent system of $CHCl_3$/MeOH/H_2O, 65/25/4. The overall lecithin yield was 15 mg/100 g yeast.

Lecithin dispersions in borate buffer (0.075 M H_3BO_3, 5 x 10^{-3} M $Na_2B_4O_7 \cdot 10\ H_2O$, 0.077 M NaCl, 10^{-4} M EDTA; pD = 8.5) were prepared in concentrations of 14-20 mg/ml as previously described.[11] Part of each dispersion was used directly for NMR; the remainder was diluted 1:1 with borate buffer and sonicated for 15 minutes. These samples provided the sonicated dispersions.

Proton magnetic resonance (PMR) measurements were taken in both the continuous wave and Fourier modes on a modified Varian HR-220 spectrometer. Samples in non-spinning capillaries were used to measure the spin-lattice relaxation times, T_1,[12] and the transverse relaxation times, T_2.[2] Linewidths were measured on the continuous wave spectra, and peak areas were determined from Fourier transformed spectra using caffeine as an internal standard.[13] Phosphorous magnetic resonance measurements were made in the Fourier mode at 24.3 MHz as described elsewhere,[11] and linewidths were measured from exponentially filtered Fourier transformed spectra.

After completion of the NMR experiments, portions of the lecithin dispersions were dialyzed against distilled water, stained with phosphotungstic acid, and examined at 30,000x by electron microscopy.[14] The remaining lecithin was extracted by the Bligh and Dyer method as described by Ames,[15] and was shown to be pure by thin layer chromatography. The fatty acid methyl esters[16] derived from the lecithin were analyzed by GLC on a column containing 10% DEGS on Chromsorb W. The deuterium content was measured by the method of DiMari.[17]

Results and Discussion

Representative continuous wave spectra for unsonicated and sonicated dispersions of PYL, DYL, and EL are shown in Figure 1. The PYL and DYL spectra closely resemble each other and spectra of sonicated EL, while those of unsonicated EL are quite different. The proton and phosphorous linewidth values are given in Table 1; it can be seen that the PYL and DYL linewidths change very little, while the EL linewidths decrease substantially when the dispersions are sonicated. Area measurements indicate that in all cases the observed signals represent a majority of the protons. The unsonicated yeast lecithin (YL) and EL dispersions are similar in that they both contain particles large enough to make the samples appear milky. The presence of large particles in both YL and EL unsonicated dispersions was affirmed by electron microscopy.

Values for the YL proton T_2 and T_1 relaxation times at various temperatures are given in Table 1. The T_1 values increase with increasing temperature and increase further when the samples have been sonicated. The methyl protons have longer T_1's than the methylene protons, and the T_1's for protons in DYL are longer than their counterparts in PYL. The methylene T_2's of both unsonicated and sonicated YL resemble those reported for sonicated EL in that there are at least two components for the transverse relaxation.[2] The fast components (less than 10 msec) were not measured in these experiments, and only the values

for the slow components are reported. In all cases the YL T_2 values are much smaller than the corresponding T_1's, and the measured T_2's are longer than T_2^*, where $(T_2^*)^{-1} = \pi\Delta\nu$ and $\Delta\nu$ is the linewidth. These results are similar to those reported for sonicated EL, and support a similar model of molecular motion.[1,2] In addition, the T_2 values for the N-methyl protons in the polar region of the molecule change more upon sonication than do the T_2 values for the protons in the methyl and methylene regions.

Results from other work[5,18] suggest that bilayers with a high percentage of unsaturated and/or short chain fatty acids would be more fluid and yield narrower NMR lines than their more saturated counterparts. The fatty acid composition of the yeast and egg lecithins are presented in Table 2, and it is evident that the PYL and DYL are highly unsaturated relative to EL. The NMR data reported above are indeed compatible with the proposal that the fatty acid chains in these unsonicated dispersions of YL are more mobile than those in EL.†

The importance of dipolar relaxation mechanisms may be estimated by comparing the relaxation times of PYL and DYL dispersions, since the dipolar interactions of a particular proton embedded in a hydrogenated matrix would be reduced by a factor of 0.024 were it to be placed in a totally deuterated matrix. The partially deuterated YL used in this work was found to be 70% enriched on the basis of deuterium analysis and integrated PMR spectra. The residual protons cannot be treated as isolated, and quantitative predictions are not feasible because the proton-proton distance distribution function is not known. Qualitatively, however, the effect of deuteration should be to decrease the dipolar contributions to the relaxation rates.

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†Since the majority of fatty acids in EL and YL are saturated or monounsaturated and the differences between these two lecithins are confined largely to these fatty acids, we can draw this conclusion only for these classes. Dispersions high in polyunsaturates may not be more fluid.

The proton spin-lattice relaxation times are dominated by dipolar interactions, and the 2-3 fold increase in the T_1 values observed in the DYL compared with those of the PYL samples may be taken as an empirical measure of the effect of partial deuteration on the dipolar interactions. Since a similar increase is seen in the T_2 of the DYL methylene protons, dipolar interactions are a major source of transverse relaxation in the yeast lecithin dispersions.

These YL results contradict the proposal[19,20] that decreased particle size and the consequent increased particle tumbling rate account for the sharpening observed in the spectra of sonicated EL. The particle tumbling argument would predict that the unsonicated to sonicated linewidth ratio of YL should be the same as that of EL; such is not the case. The spectra of the various sonicated dispersions are quite similar, while the spectra of the unsonicated dispersions reflect differences in the lecithins used. Since it appears that the unsonicated dispersions as observed by NMR are more sensitive to composition than the sonicated dispersions, such dispersions could be fruitfully exploited to study compositional differences. Finally, the resolution-enhancing effect of deuterium substitution may be of value in studying both model and biological membranes where NMR work is currently hampered by broad, poorly resolved peaks.

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References

1. A. F. Horwitz, in "Membrane Molecular Biology," Sinauer Associates, Stamford, Conn., 1972, Chapter 7.
2. A. F. Horwitz, W. J. Horsley, and M. P. Klein, Proc. Nat. Acad. Sci. U.S.A., 69, 590 (1972).
3. A. F. Horwitz, D. M. Michaelson, and M. P. Klein, Biochim. Biophys. Acta, in press.
4. C. F. Fox, in "Membrane Molecular Biology," Sinauer Associates, Stamford, Conn., 1972, Chapter 12.
5. J. M. Steim, M. E. Tourtellotte, J. C. Reinert, R. N. McElhaney, and R. L. Rader, Proc. Nat. Acad. Sci. U.S.A., 63, 104 (1969).
6. S. J. Singer and G. L. Nicholson, Science, 175, 720 (1972).
7. H. A. Akers, M. Llinas, and J. B. Neilands, Biochemistry, 11, 2283 (1972).
8. F. Folch, M. Lees, and G. H. Sloane-Stanley, J. Biol. Chem., 226, 497 (1957).
9. W. S. Singleton, M. S. Gray, M. L. Brown, and J. L. White, J. Am. Oil Soc., 42, 53 (1965).
10. C. C. Sweeley, in "Methods in Enzymology," vol. XIV, Academic Press, 1969.
11. A. F. Horwitz and M. P. Klein, J. Supramol. Struct., 1, 19 (1972).
12. R. L. Vold, J. S. Waugh, M. P. Klein, and D. E. Phelps, J. Chem. Phys., 48, 3831 (1968).
13. B. Sheard, Nature, 223, 1057 (1969).
14. D. Chapman, D. J. Fluck, S. A. Penkett, and G. G. Shipley, Biochim. Biophys. Acta, 163, 255 (1968).
15. G. Ames, J. Bacteriol., 95, 833 (1968).
16. C. J. Scandella and A. Kornberg, J. Bacteriol., 98, 82 (1969).
17. S. J. DiMari, C. D. Snyder, and H. Rapoport, Biochemistry, 7, 2301 (1968).
18. M. C. Phillips, R. M. Williams, and D. Chapman, Chem. Phys. Lipids, 3, 234 (1969).
19. E. G. Finer, A. G. Flook, and H. Hauser, Biochim. Biophys. Acta, 260, 59 (1972).
20. A. Darke, E. G. Finer, A. G. Flook, and M. C. Phillips, J. Mol. Biol., 63, 265 (1972).
21. D. Chapman and A. Morrison, J. Biol. Chem., 241, 5044 (1966).
22. P. Dea, S. I. Chan, and F. J. Dea, Science, 175, 206 (1972).
23. R. Kornberg and H. McConnell, Biochemistry, 10, 1111 (1971).

Table 1

Relaxation Times and Linewidths for Resonances of Yeast Lecithin Dispersions

	Unsonicated Dispersion					Sonicated Dispersion				
	$\Delta\nu$[†] in Hz	T_2[‡] in sec		T_1[‡] in sec		$\Delta\nu$ in Hz	T_2 in sec		T_1 in sec	
	20°	20°	20°	40°	60°	20°	20°	20°	40°	60°
Protonated Yeast Lecithin (PYL)										
N-methyl	32	0.02	0.21	0.30	0.41	15	0.09	0.27	0.38	0.53
methylene	70	0.02	0.33	0.44	0.58	40	0.02	0.38	0.45	0.83
methyl	30	0.02	0.4	0.6	0.8	20	0.02	0.6	0.8	0.6
phosphorous	56					21				
Deuterated Yeast Lecithin (DYL)										
N-methyl	40			0.97	1.2	20	0.10	0.83	0.96	1.6
methylene	58			1.0	1.3	37	0.08	0.98	1.1	1.3
methyl				1.1	1.5	26				2.0

[†]All values are ±5 Hz. The phosphorous spectra were taken at 31°, and the resonance of the unsonicated sample appears to have both broad and narrow components.

[‡]All values are ±0.01 sec and were obtained by least squares fit to one exponential. As explained in the text, only the slow components of the T_2's for the methylene protons are reported.

[‡]The N-methyl and methylene values are ±10% and were obtained by least squares fit to one exponential. The methyl values are ±25% and were obtained by least squares fit to two exponentials since the peaks occurred as shoulders on the methylene peaks.

Table 2

Fatty Acid Composition of Yeast Lecithins and Hen Egg Lecithin

	Composition in mole %						
	14:0	16:0	16:1	18:0	18:1	18:2	others
Protonated Yeast Lecithin (PYL)	1.0	11.1	4.1	0.2	65.3	16.0	2.6
Deuterated Yeast Lecithin (DYL)	1.5	18.4	3.2	1.0	53.9	18.6	3.4
Hen Egg Lecithin (EL)		28.5 26.5	0.5 1.8	11.5 13.3	25.2 32.3	12.5 12.9	 11.5

The egg lecithin composition is that of the two samples reported by Kornberg and McConnell.[23] The other fatty acids reported for egg lecithin are 20:4, 22:4, and 22:6. In the case of yeast lecithin, the other fatty acids are unidentified acids containing more than 18 carbons.

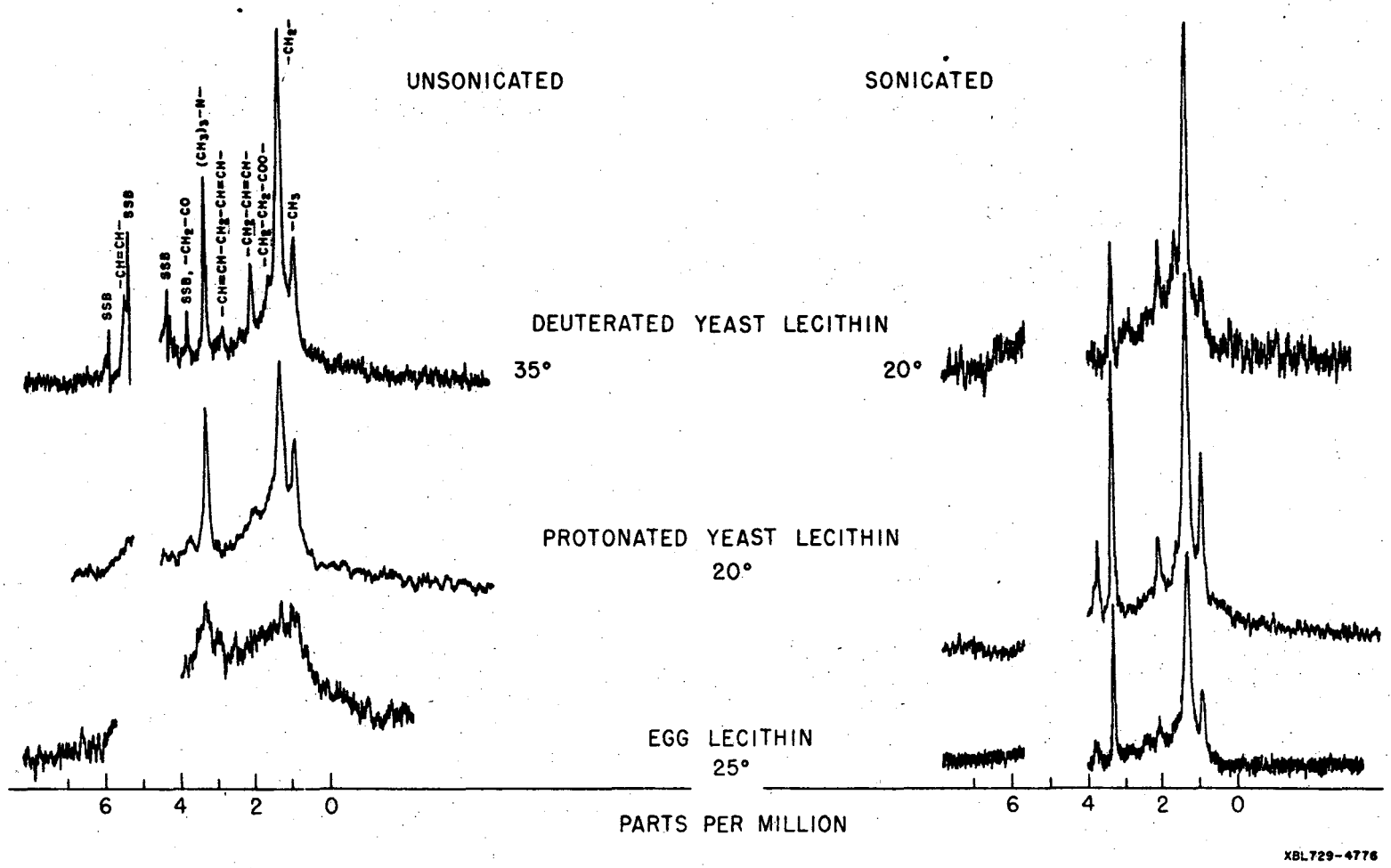

Figure 1. 220 MHz PMR spectra of yeast and egg lecithin dispersions. The spectrum of unsonicated DYL was recorded using a larger sample than was used for the other spectra. The changes in relative peak intensities between the PYL and DYL samples reflect variations in the amount of deuterium incorporated in the different positions of DYL. With the instrument settings used, the HOD peaks were off-scale and therefore were not scanned. The chemical shifts are relative to TMS and the resonances are assigned according to Chapman[21] and Dea.[22] SSB = spinning sideband of the HOD.

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