Carbon-13 Pulse Fourier Transform N.M.R. of Lecithins.

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Summary

Natural abundance carbon-13 proton noise decoupled spectra ($^{13}C-\{^{1}H\}$) of lecithins in organic solvent and in the smectic liquid crystalline phase, have been obtained. The preliminary results indicate that this technique may be useful in obtaining information about the mobility and organisation of lipids in liquid crystals and membranes.

Introduction

Both time and frequency domain n.m.r. spectroscopy have previously been applied to a study of the smectic liquid crystalline phase.[1,2] In this phase, broad lines with little structure are observed. It has been shown that in the smectic phases of potassium laurate/D_2O and egg lecithin/D_2O, that the proton linewidths appear to be field dependent,[1] so the observed linewidths may not be entirely due to homogeneous dipolar broadening.

Because of the small magnetic moment of ^{13}C, and hence less susceptibility to dipolar broadening, lack of appreciable $^{13}C-^{13}C$ scalar coupling, very large chemical shifts and the possibility of removing large 'solvent' absorptions in regions of interest by using a hetero(^{19}F or ^{2}H) lock signal for field-frequency stabilisation, we are investigating the possible uses of $^{13}C-\{^{1}H\}$ pulse Fourier transform n.m.r.[3] in studying the smectic liquid crystalline phase of egg lecithin, in the hope that this will provide a basis for a study of biological membranes.

Experimental

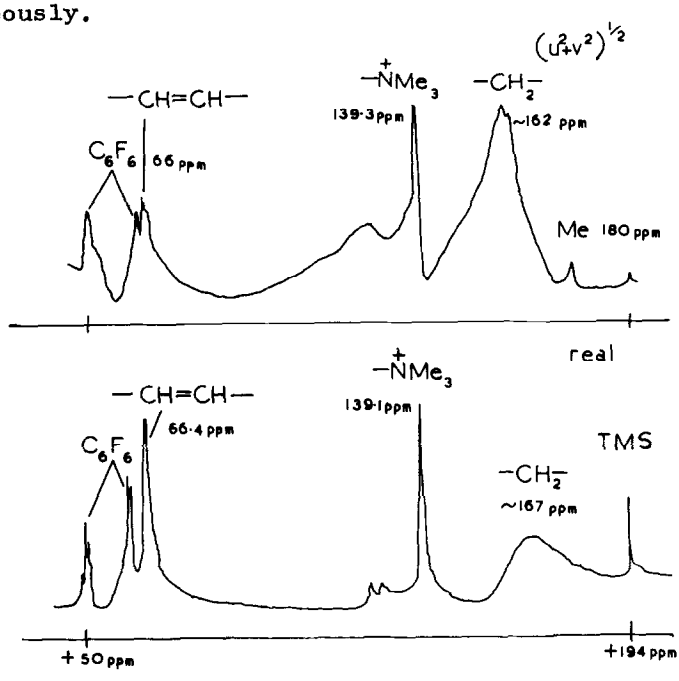
1,2-Dipalmitoyl-Lα-lecithin was purchased from Fluka, Buchs, and purified on SilicAR CC-7 (100-200 mesh) eluting with $CHCl_3$ -MeOH 2:1. Egg lecithin was obtained from Gallus domesticus eggs and purified on Woelm alumina (activity V) and SilicAR, basically according to Dawson[4]. Phosphatidyl serine Na^+ salt was obtained from Lipid Products, Epsom, England. 1,2-Dipalmitoyl diglyceride, choline chloride and methyl palmitate were from Fluka. The egg lecithin was dried in high vacuum prior to use.

Spectra were run on a Bruker spectrometer operating at 21.14 kG., using the widest broad-band noise decoupling ($\sim$1 KHz) available for ^1H. The ^{19}F resonance of C_6F_6 was used for field-frequency stabilisation, and T.M.S. was used as reference. Samples were spun in 11.6mm Wilmad tubes, or in 11.6mm tubes with a coaxial 5mm insert containing C_6F_6 and T.M.S., for aqueous samples. Typically 4-5,000 pulse responses were averaged for the solutions, and 20-30,000 for the smectic phase. Lecithin-water samples were kept at 25°C by a continuous nitrogen flow.

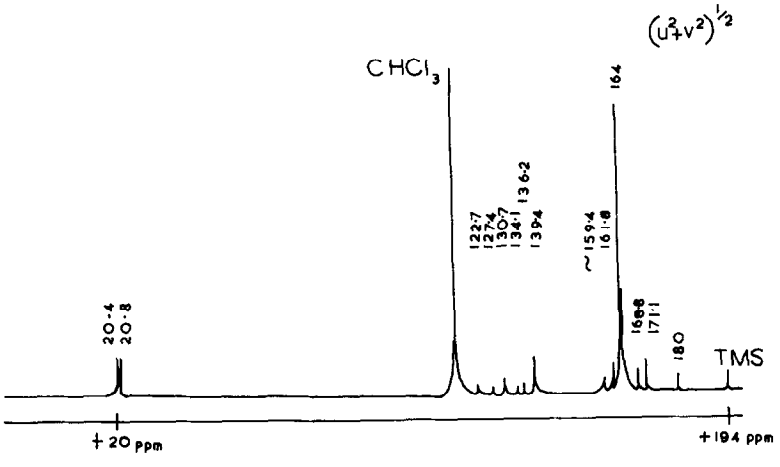
Results

A coarse dispersion of the smectic liquid crystalline phase of lecithin in water was prepared by mild hand agitation, and the natural abundance $^{13}C-\{^1H\}$ spectra of two samples are shown in Fig 1a. Fig 1b shows the spectrum of dipalmitoyl lecithin in $CHCl_3$. The spectra of the smectic phase show slight differences, partly due to whether real (absorption) or the square root of the power spectrum $(u^2+v^2)^{\frac{1}{2}}$, presentations are chosen. Pulse lengths[4] and sample homogeneity also affect

the appearance of the spectra. It proved to be difficult to optimise pulse lengths for all carbons in this system, simultaneously.



1a



1b

Figure 1a. $^{13}C-\{^1H\}$ spectra of two samples of egg yolk lecithin. Top spectrum is distorted by the superposition of a spurious sine-wave component. Lower spectrum was recorded at higher ^1H r.f. decoupling power.

1b. $^{13}C-\{^1H\}$ spectrum of dipalmitoyl lecithin dissolved in $CHCl_3$.

The $^{13}C-\{^{1}H\}$ spectrum in the liquid crystalline state of lecithin shows absorption maxima at 180, 162-7, 139.2 and 66.4 ppm, 'upfield' from CS_2. The peak at 139.2 ppm is quite prominent, and is assigned to the $-\overset{+}{N}Me_3$ carbons on the following basis. a) A corresponding peak is apparent in the dipalmitoyl lecithin $-CHCl_3$ spectrum, at 139.4 ppm. This peak is approximately 3 times the magnitude of the glyceryl phosphoryl choline moiety peaks adjacent, as might be expected since all these peaks are nonquaternary, and will thus have an equal overall Overhauser enhancement of three[5], assuming dipole-dipole coupling to be the dominant relaxation mechanism. b) $Me_4\overset{+}{N}\ Cl^-$ absorbs at 138 ppm and c) the $-\overset{+}{N}Me_3$ carbons of choline chloride absorb at 135.8 ppm ($J_{N-C}\sim4.3$Hz), this resonance is similarly three times the intensity of the $-CH_2\overset{+}{N}$-carbon (122.3 ppm, $J_{N-C}\sim1.8$Hz) and $-CH_2OH$ carbon (134.1 ppm) resonances. d) This peak is absent in the spectra of dipalmitoyl diglyceride and phosphatidyl serine, in $CHCl_3$.

The absorption at 66.4 ppm (Fig 1a) appears to arise from the olefinic carbons of the oleyl residue of egg lecithin; a similar absorption is seen in phosphatidyl serine in $CHCl_3$ at 64 ppm.

In the region of the $-CH_2-$ resonances, close similarity is seen between dipalmitoyl diglyceride, dipalmitoyl lecithin and methyl palmitate, in $CHCl_3$. However, the CO and αCH_2 groups in the lecithin and diglyceride 1 and 2 acyl chains have different chemical shifts (CO at 20.4 and 20.1 ppm in the diglyceride and 20.8 and 20.4 ppm in lecithin; αCH_2s at 159.5 and 159.7 ppm in the diglyceride and 159.2 and 159.5 ppm in lecithin).

In the liquid crystalline phase of lecithin, the individual

CH_2 resonances are not resolved at all. This could indicate restricted motion giving shorter T_2s, resulting in an 'envelope', or may be due to multiplet formation due to incomplete noise decoupling of the relatively wide ($\nu_{\frac{1}{2}} \sim 600Hz$) ^1H resonance, this latter factor will lead to a decreased signal intensity due to a reduced Overhauser enhancement, as will any deviations from the 'extreme narrowing condition'[5].

Conclusion

The observation of a narrow chemically shifted $-\overset{+}{N}Me_3$ carbon absorption in the egg lecithin liposome indicates that this group is relatively mobile. From the observation of other chemically shifted resonances, further studies, especially ^{13}C-T_1 measurements[6], possibly on specifically isotopically enriched groups, in similar liquid crystals and biological membranes, may give new information on the structures of these systems.

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References

1. Penkett, S.A., Flook, A.G. and Chapman, D. Chem. Phys. Lipids 2 273 (1968).
2. Hansen, J.R. and Lawson, K.D. Nature, 225 542 (1970)
3. Ernst, R.R. and Anderson, W.A., Rev. Sci. Instr. 37 93 (1966).
4. Dawson, R.M.C., Biochem. J. 88 414 (1963).
5. Kuhlmann, K.F., Grant D.M. and Harris R.K., J. Chem. Phys. 52 3439 (1970)
6. Freeman, R. and Hill, H.D.W., J. Chem. Phys. 53 4103 (1970).