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PROTON NMR BANDSHAPE STUDIES OF LAMELLAR LIQUID CRYSTALS AND GEL PHASES CONTAINING LECITHINS AND CHOLESTEROL

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SUMMARY

Proton NMR spectra for gel and liquid crystalline samples, composed of dimyristoyl and/or dipalmitoyl lecithin, cholesterol and water, can be consistently interpreted in terms of mesophase symmetry and molecular diffusion according to a model proposed by Wennerström (Wennerström, H. (1973) Chem. Phys. Lett. 18, 41–44).

It is shown by computer simulation that the characteristic "super-lorentzian" bandshape of the lamellar mesophase can be described by the superposition of three gaussian curves.

The NMR signal of the gel phase can be simulated by the superposition of two gaussian curves with widths at half height of 2.5 kHz and 19 kHz.

An upper limit of the lateral diffusion coefficient of the lecithin molecules in the gel phase is calculated to be about $5 \cdot 10^{-15}$ m^2/s. It is therefore concluded that the static intermolecular dipolar couplings average to zero in the lamellar mesophase.

An estimation of the order parameter of the liquid crystalline phase is made from experimental data and a calculated "rigid lattice" linewidth.

A two phase system is shown to exist in the temperature range 28–34 °C for a mesophase of a mixture of dimyristoyl and dipalmitoyl lecithin.

The presence of cholesterol results in enhanced lateral diffusion of the lecithin molecules at temperatures below the Chapman transition point.

INTRODUCTION

Most biological membranes contain a large amount of lipids, mainly different phospholipids such as lecithins, phosphatidylethanolamines, etc. It is generally agreed that the major part of the phospholipids form a bilayer matrix in the membrane [1–4]. The physical properties of the phospholipids in a bilayer have been studied by several experimental techniques [5]. One promising method is the use of nuclear magnetic resonance, which has the great advantage of not introducing any perturbations in the system studied.

In 1969 Chapman and coworkers [6] reported NMR investigations of the difference between the gel phase and the liquid crystalline phase of several phospholipids. They observed that there was a marked decrease in the proton NMR linewidth when the temperature was raised above the phase transition gel → liquid crystal. They concluded that the motion of the phospholipid molecules was considerably greater in the liquid crystalline phase than in the gel phase. However, no precise description of the nature of the increased molecular motion was made.

Recently, Chan and associates [7–9] made a thorough NMR investigation of the lamellar liquid crystalline phase composed of lecithin and water. It was shown that the NMR bandshape is dominated by contributions from static dipole-dipole interaction and a calculation of the bandshape was attempted.

It has been observed that the ^1H NMR spectrum of the long hydrocarbon chains in most lamellar liquid crystalline samples exhibits a characteristic "super-lorentzian" bandshape [10]. This feature was rationalized by Wennerström [11], who showed that the cylindrical symmetry of the lamellae, on the NMR time scale, should give rise to a typical "super-lorentzian" bandshape. A critical point in the derivation made was that the lateral diffusion has to be fast enough so that the intermolecular static dipole-dipole interactions average to zero.

We have investigated the proton NMR spectra of some lecithin water systems to attempt to give a consistent interpretation of the difference in the spectra obtained for the gel and liquid crystalline phases using the results of ref. 11.

MATERIAL AND METHODS

Dimyristoyl lecithin and dipalmitoyl lecithin were synthesised according to Cubero Robles and van den Berg [12]. The product was purified by silic acid column chromatography and yielded only one spot on thin-layer chromatography. In order to minimize the amount of water bound to the lecithin in the "dry" state, the lipid was precipitated in acetone and further dried in vacuo. Cholesterol was purchased from Merck AG and was purified by recrystallisation from ethanol. The lipid mixtures were prepared by dissolving appropriate amounts of the components in chloroform, which was then completely removed by evaporation in vacuo. This procedure was adopted in order to ensure complete mixing of the lipid molecules. The samples were prepared by weighing appropriate amounts of lecithin or lecithin/cholesterol mixtures, in a 12 mm NMR tube. Heavy water (99.8 % isotopic enrichment, purchased from Norsk Hydro) was then added and the tube then sealed. The samples were left to equilibrate for at least 24 h at a temperature above the transition point, gel → liquid crystal, often called the Chapman transition. The ^1H NMR spectra were recorded with a Varian XL-100 NMR spectrometer, operating in an unlocked mode. The magnetic field was swept using the manual controls. The spectra were calibrated by sweeping over both the centre band and side band. The sample temperature was controlled by a variable temperature control unit and was determined with a thermometer before and after running a spectrum. The sample was thermally equilibrated for at least 30 min before the NMR spectrum was traced. On all the samples studied the temperature dependence was investigated by both a heating and a cooling procedure.

LECITHIN/WATER LAMELLAR SYSTEMS

We investigated the temperature dependence of the proton magnetic resonance spectrum of two samples of pure lecithin and water (dimyristoyl lecithin or dipalmitoyl lecithin). The samples are denoted I and II in Table I. The spectra of the two samples show qualitatively the same temperature dependence. A broad ^1H NMR signal, as is shown in Fig. 1, is observed below the Chapman transition temperature, i.e. in the gel phase. The shape of this signal can approximately be described by a superposition of two gaussian curves with widths at half height of 2.5 kHz and 19 kHz, where the narrow component contains 20 % of the total intensity. It seems clear that this narrow component is chiefly due to the choline methyl protons, while the broad component mainly comes from the methylene protons in the carbon chains.

TABLE I

LIPID COMPOSITION OF THE SAMPLES STUDIED, WHICH ALL CONTAINED 20 % (V/W) OF ^2H_2O

Sample number	Lipid (w/w)
I	Dimyristoyl lecithin
II	Dipalmitoyl lecithin
III	Dimyristoyl lecithin/dipalmitoyl lecithin 1 : 1
IV	Dimyristoyl lecithin/cholesterol 4 : 1
V	Dipalmitoyl lecithin/cholesterol 4 : 1
VI	Dipalmitoyl lecithin/dimyristoyl lecithin/cholesterol 2 : 2 : 1

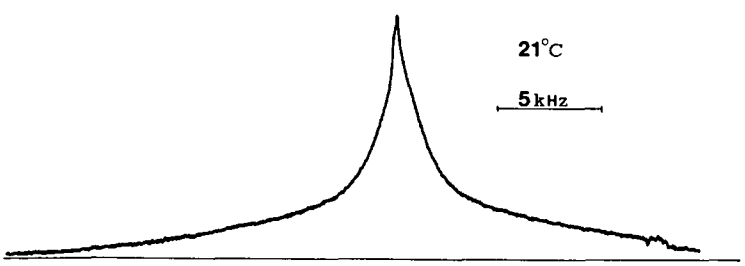

Fig. 1. Proton NMR spectrum from a gel phase of a dimyristoyl lecithin (80 %)/water sample. Temperature 21 °C.

There is a drastic change in the NMR bandshape when the temperature is raised above the Chapman transition point and a typical super-lorentzian curve is observed as is shown in Fig. 2a. It proved possible to simulate this super-lorentzian bandshape by the method described by Wennerström [11]. We assumed that the NMR spectrum for an oriented sample with a fixed direction of the lamellae, consisted of a superposition of three gaussian curves, namely one for the methylene protons plus the protons of the glycerol residue, one for the terminal methyl protons, and one for the choline methyl protons. The relative intensities of the curves were determined by the proportions of these three different groups of protons. The results of this

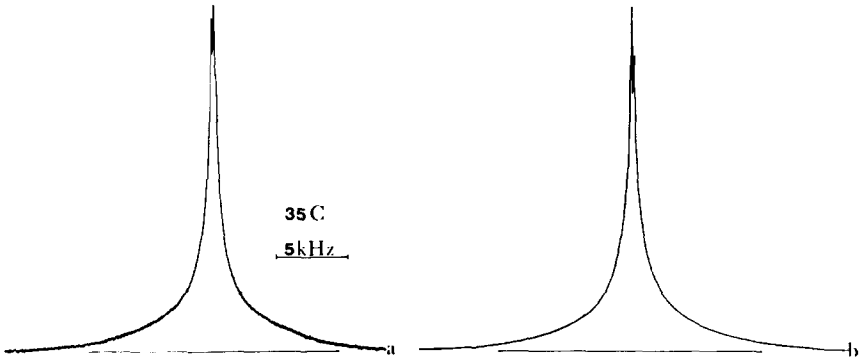

Fig. 2. Proton NMR spectrum from a lamellar liquid crystalline phase of a dimyristoyl lecithin (80 %)/water sample at 35 °C (a) and a comparison of the experimentally observed spectrum with a computer simulated spectrum (b). The simulated spectrum is composed of the following gaussian curves: (i) a curve 14 kHz wide containing 79 % of the intensity, centered at 0.0 ppm, corresponding to the methylene protons and the protons of the glycerol residue, (ii) a curve 4 kHz wide containing 9 % of the intensity, centered at +0.4 ppm, corresponding to the terminal methyl protons and (iii) a curve 2 kHz wide containing 12 % of the intensity, centered at −2.0 ppm, corresponding to the choline methyl protons. The extra peak (to the left) in a is due to residual water protons.

TABLE II

LINEWIDTH OF THE METHYLENE PROTON SIGNAL OF THE DIMYRISTOYL LECITHIN SAMPLE I IN LAMELLAE WITH THE DIRECTOR PARALLEL WITH THE MAGNETIC FIELD AND ORDER PARAMETERS OF THE METHYLENE PROTONS OF DIMYRISTOYL LECITHIN SAMPLE I

Temperature (°C)	Linewidth (kHz)	Order parameter, S
31	32±3	0.50±0.05
35	28±3	0.44±0.05
43	22±3	0.34±0.05

analysis for the dimyristoyl lecithin sample I at various temperatures is given in Table II and a calculated best fit curve to the spectrum in Fig. 2a is shown in Fig. 2b. An estimate of the average order parameter, S, can be obtained by assuming that the width of the gaussian curve representing the protons of the long carbon chains is determined by the interactions between two geminal methylene protons. This interaction gives a splitting of about 64 kHz when the proton-proton vector is parallel to the applied magnetic field. Estimated values of the order parameters at different temperatures are given in Table II. As may be seen from this table, the S values obtained are quite large ranging between 0.50 and 0.34 for the temperatures studied. It is interesting to note that in a recent paper by Seelig and Seelig [13] it was found from deuteron NMR studies of selectively deuterated dipalmitoyl lecithin in lamellar mesophase that the order parameter was approximately the same as ours. If the rotation of the methylene groups about the long axis of the hydrocarbon chains is taken into account in the calculation of the linewidth, we get a value of 32 kHz, i.e. the major part of the reduction in the order parameter when going from a rigid lattice to the liquid crystalline state, is caused by a rotation around the long symmetry axis of the carbon chains.

Seiter and Chan [9] concluded from their studies that the carbon chains move isotropically up to an angle of about 60° with respect to the normal to the lamellae and that there was a rotation about the long axis of the carbon chain. This corresponds to an order parameter of about 0.2, which is considerably smaller than the value found in this work.

It can be noted that it is a straightforward procedure to calculate the free induction decay of the NMR signal instead of the continuous wave bandshape. One can in this way obtain a better estimate of the width of the broad component of the NMR signal.

The calculated second moment of the NMR signal is somewhat, but not drastically, less for the liquid crystalline than for the gel phase. The marked difference in NMR bandshape for the two phases in question is caused by the static intermolecular dipolar couplings, which contribute to the NMR signal in the gel phase but not in the lamellar mesophase. These intermolecular couplings are probably small in magnitude (approximately 1–3 kHz) but it is crucial that they average to zero for the appearance of a super-lorentzian bandshape in the NMR spectrum.

In two-dimensional diffusion, a molecule travels a root mean square distance x in a time t where

$$x^2 = 4\,Dt$$

D is the diffusion coefficient. Using this formula with the assumption that the static intermolecular dipole-dipole interactions are 3 kHz and that the distance between nearest neighbours is 7.5 Å, one gets an upper limit of about $5 \cdot 10^{-15}$ m^2/s for the lateral diffusion coefficient in the gel phase. From recently measured lateral diffusion coefficients* for lamellar phospholipid mesophases [14, 15] one can then conclude that the intermolecular dipolar couplings should average to zero in the liquid crystalline phase.

MIXED LECITHINS AND LECITHIN CHOLESTEROL SYSTEMS

Shimshick and McConnell have suggested that there is a lateral phase separation in mixed lecithin/water systems [16]. We investigated this phenomenon using ^{1}H NMR for a 1 : 1 mixture of dimyristoyl lecithin and dipalmitoyl lecithin (sample III) at a lower water content than that used by Shimshick and McConnell. A typical gel phase spectrum is obtained for this sample up to 28 °C. In the temperature range 28–34 °C, there is a gradual change from a typical gel phase to a typical liquid crystalline phase spectrum, i.e. we have a two phase system in this temperature region. This is in excellent agreement with the findings of Shimshick and McConnell [16] in spite of the fact that the water contents were markedly different in the two investigations.

There has been a great interest in the literature on the question of the effect of cholesterol on lecithin-water systems [17–20]. The samples IV–VI were found to deviate markedly from the corresponding samples (I–III) in their temperature

* For a dipalmitoyl lecithin sample, the diffusion coefficient in a cubic mesophase has been determined by pulsed NMR technique to be about 10^{-12} m^2/s (Bull, T. E. and Lindblom, G., unpublished measurements).

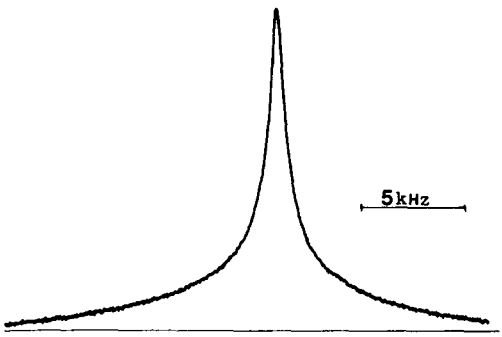

Fig. 3. Proton NMR spectrum from a dimyristoyl lecithin/cholesterol (4 : 1)/water mixture at 2 °C.

dependence. The cholesterol-containing samples all showed a typical super-lorentzian bandshape down to about 2 °C (cf. Fig. 3), which was the lowest temperature investigated. It thus seems clear that cholesterol has the effect of enhancing the lateral diffusion of the lecithin molecules at temperatures below the Chapman transition point for the pure lecithin/water samples. It should also be noted that our data are not consistent with a model proposed by Phillips and Finer [20], where large discrete regions of 1 : 1 complex between lecithin and cholesterol separate out in the bilayers.

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