BBA 75318

PHYSICAL STUDIES OF PHOSPHOLIPIDS

XII. NUCLEAR MAGNETIC RESONANCE STUDIES OF MOLECULAR MOTION IN SOME PURE LECITHIN–WATER SYSTEMS

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(Received April 8th, 1969)

SUMMARY

Proton magnetic resonance spectra of 1,2-dipalmitoyl-L-phosphatidylcholine and its perdeuterated analogue have been obtained at temperatures between 24 and 150° at various hydrations. The use of a perdeuterated lecithin enables the various lineshapes either to be assigned to the dipolar interactions of group protons, or to the long chain protons in the gel and liquid-crystalline phases.

In the gel phase the motion of the head group protons and hydrocarbon chain protons is dominated by the first 4–5 moles of water (^2H_2O) per mole of lecithin. The range n = 4–10 moles may indicate a weaker secondary hydration shell. The linewidths are not further reduced for n > 10. The decreasing linewidth corresponding to the CH_2 protons of the fatty acid residues parallels the reduction of the temperature (T_c) for the gel to liquid-crystalline phase transition.

Above the transition temperature a broad line (1.5 gauss) remains. Thus, for all hydrations, some residual anisotropy exists in the lamellar liquid crystalline phase for the head group protons, and this may be connected with some degree of order at the polar/apolar interface, that is, quase-orientation of the glycerol residue. A narrow component (approx. 10^{-2} gauss) is also observed and corresponds to the unresolved lineshapes due to the anisotropic but rapid molecular motion of the long chain protons and of the $N^+(CH_3)_3$ protons of the head group.

INTRODUCTION

The phospholipid family is one of the principal components of cell membranes. The organisation and the interaction of phospholipids with protein and water is considered to be associated with the structure and function of the membrane[1]. We are presently examining one class of interactions, that of a binary model system, phospholipid–water. In previous papers some physical characteristics of phospholipid–water system[2–4], the molecular motion of pure lipids[5,6] and of their aqueous dispersions[7,8] have been described. In this study we shall restrict our investigations to the phase diagram of 1,2-dipalmitoyl-o-phosphatidylcholine (lecithin) and water. Proton magnet-

Abbreviations: PMR, proton magnetic resonance; NMR, nuclear magnetic resonance.

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ic resonance (PMR) has been applied to this system in order to study the molecular reorientation of different groups of protons of the phospholipid, using heavy water, ^2H_2O, in place of ordinary water.

The observed linewidths and second moments of this system, and of the perdeuterated lecithin, will be discussed in terms of (a) the nature of the molecular and segmental motions of lecithin in the crystalline gel, the liquid-crystalline lamellar, and the viscous isotropic phases, and (b) the modifications of the motion of the hydrocarbon chains and of the head group protons, as a result of increasing temperature and degrees of hydration.

These studies are important as they lead up to the subsequent analysis of the spectra of cell membranes.

EXPERIMENTAL

Sample preparation

1,2-Dipalmitoyl-L-phosphatidylcholine (*i.e.* dipalmitoyl lecithin) and the corresponding lecithin with deuterated fatty acid chains were used. The former sample was obtained from Fluka, Basle, and was subsequently purified. The purity was checked by thin-layer chromatography. The perdeuterated dipalmitoyl lecithin and 1,2-dimyristoyl lecithin were synthesised by Dr. A. P. Davies and Mr. J. S. Chadha of this laboratory. Deuteration of the fatty acid chain protons was effective up to 95% (by high-resolution nuclear magnetic resonance (NMR) and mass spectrometry). In order to obtain a completely anhydrous lecithin, the samples were dried to constant weight under high vacuum. Using the dry material, the desired lecithin–water systems were prepared by weighing the components into a 5-mm NMR Pyrex sample tube with a central constriction. After sealing the tube the homogenization was achieved using the centrifugation technique previously described[4]. The samples were homogenized above the corresponding transition temperature.

Lecithin monohydrate samples were prepared on the vacuum line by equilibration of the anhydrous sample under ^2H_2O vapour at the ambient temperature. The relative amounts of the components in a given mixture are expressed by number (n) of moles ^2H_2O per mole of lecithin. The presence of ^2H_2O in the samples provides the possibility of studying the molecular motions of the lecithin molecule in the presence of water, but avoids a strong overlapping signal from protons of H_2O.

The transition temperatures were checked by differential thermal analysis and the polarised light microscope against published data[4].

PMR measurements

A 60-MHz broad-line NMR spectrometer (Varian Associates), with a 5-mm sample thermostat was used in this study, and has been described previously[5].

During the temperature studies the sample was held in the V-4331 R.F. probe at a given temperature for about 1 h and for 1–2 h near the transition temperature, before spectral measurements commenced. In order to demonstrate the existence of a broad line in the liquid-crystalline phase, one sample (n = 24 moles ^2H_2O) was equilibrated for several days at a temperature a few degrees above its transition temperature (T_c) of 41°.

Derivative absorption spectra were recorded using a multitude of magnetic

field sweeps (500–25000 mgauss) to resolve individual components of the composite spectra. In order to preserve the true shapes for individual components, sufficiently small magnitudes of 20 Hz modulation (0.03–1.3 gauss) were used, depending upon the observed linewidth. Similarly, the amplitude of the R.F. field was changed for the measurement of different components and in all cases maintained at sufficiently low level to avoid saturation. The linewidths and the second moments were obtained from at least four derivative spectra at a given temperature. First and second moments were calculated for the broad components observed below and above the transition by the method described by WILSON AND PAKE[9].

Due to the overlapping linewidths observed in this study, and to the low amplitudes of modulation utilized, the signal to noise ratio was, in general, rather low. In particular, the accuracy of linewidth measurements for some of the broader lines below the transition may be as much as ± 0.7 gauss. For a similar volume of the partly deuterated sample the number of protons is reduced to 26% of the number in the protonated sample, and the signal to noise ratio correspondingly decreased. The maximum error of linewidth measurement for the broad linewidths may then be ± 1.5 gauss.

RESULTS

Protonated dipalmitoyl lecithin

Below the transition temperature. At room temperature all the spectra show a similar composite line shape with three measurable linewidths which reduce as the water content increases. (a) A line of width (ΔH) 2.7–0.4 gauss, (b) a broad line of width 9–4 gauss, ΔH approx. 10^{-2} gauss, and (c) a narrow and weak absorption line of width ΔH 10^{-2} gauss.

Examples are shown in Fig. 1a; the intermediate linewidths (a) were obtained using more expanded field sweeps and smaller amplitudes of field modulation.

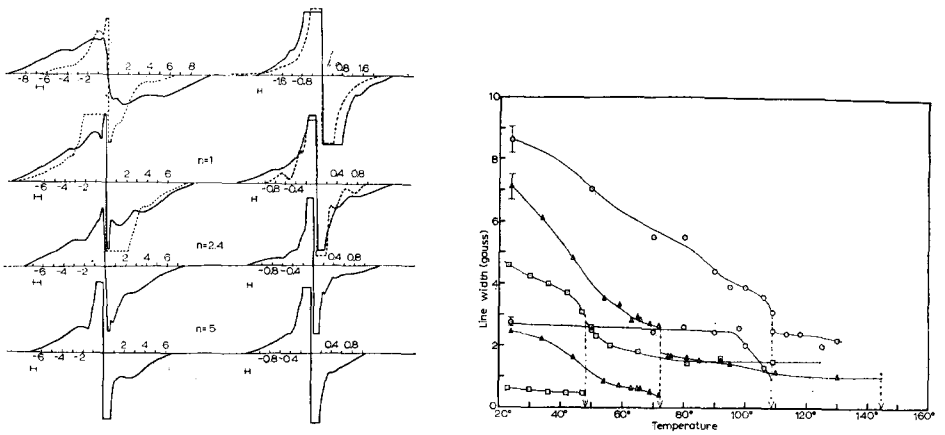

Fig. 1. PMR derivative absorption spectra of 1,2-dipalmitoyl-L-phosphatidylcholine (—) and the perdeuterated analogue (---) with various water contents at (a) 24° and (b) above the transition temperature (T_c). (1) n = 0, (2) n = 1, (3) n = 2.4 and (4) n = 5. The amplitude of modulation is indicated and the abscissae are calibrated in gauss.

Fig. 2. Linewidths as a function of temperature for 1,2-dipalmitoyl-L-phosphatidylcholine with various water contents. ⊙, anhydrous lecithin; △, lecithin + 1 ^2H_2O; and ⊡, lecithin + 5 ^2H_2O.

The variation of the broad and intermediate linewidths as functions of temperature for examples of different water contents is shown in Fig. 2. Both linewidths decrease gradually with increasing temperature. An abrupt narrowing of the broad and intermediate linewidths occurs at a temperature T_c consistent with the formation of the mesomorphic lamellar phase, as judged by the light microscope and differential thermal analysis measurements. Narrowing of the intermediate component is noticed in all systems a few degrees below this temperature. Increasing amounts of water up to 10 moles per mole of lecithin are seen to reduce the temperature at which the abrupt narrowing of the broad component is observed.

For a short range (10°) of temperature below the transition point, the intensity of the broad component decreases as the intensity of the narrow component increases. The effect was noticed in all systems, even with the anhydrous sample. This increase of intensity is particularly marked just below the transition temperature, and resolution of the derivative maxima and minima of the intermediate absorption line shape is obscured.

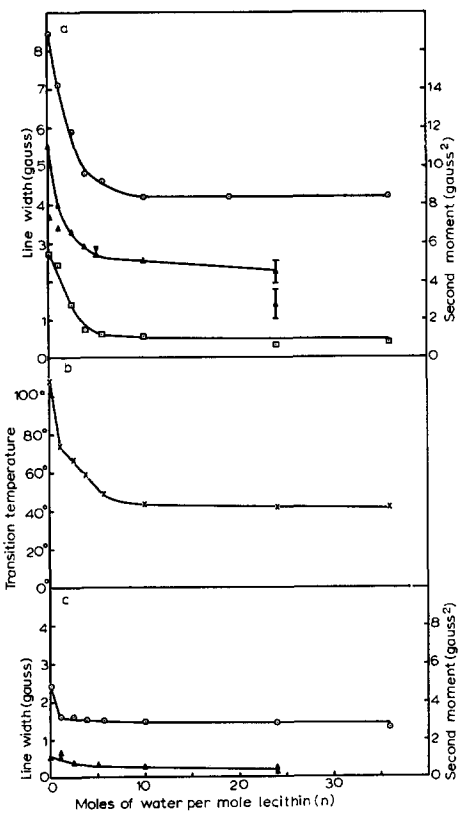

Fig. 3. Variation of linewidth and second moment for 1,2-dipalmitoyl-L-phosphatidylcholine as a function of water content; n moles ^2H_2O per mole of 1,2-dipalmitoyl-L-phosphatidylcholine; (a) Linewidth of broad (⊙) and intermediate (⊡) components and the second moment (△), in the gel phase at 24°. (b) The transition temperature T_c (×). (c) The linewidth (⊙) and second moment (△) of the broad component in the mesomorphic phase ($T > T_1$). The corresponding parameters for the perdeuterated analogue are shown by ▲. The symbol ▽ indicates the use of H_2O.

The linewidths of the broad and intermediate components depend strongly upon the extent of hydration of the lecithin up to a definite amount of water. This is illustrated in Fig. 3a. Both linewidths and the second moment decrease rapidly until 4 water molecules per lecithin molecule are introduced. A smaller linewidth and second moment reduction occurs from 4–10 water molecules. When more than 10 water molecules per lecithin molecule are introduced, the linewidths and second moments remain constant within the experimental error. The linewidth of the central narrow component (approx. 10^{-2} gauss) decreases slightly with increasing water content up to 10 water molecules. This line is assigned to the proton signal from ^{2}HHO arising from the residual protons in ^2H_2O. The integrated intensity of the narrow component at room temperature for the samples with the lower water concentrations is under 1% of the total intensity.

The temperature, at which the abrupt reduction of linewidth and second moment occurs, is shown in Fig. 3b as a function of water content. A limiting transition temperature of 41° is found for $n \geqslant 10$ moles ^2H_2O.

Above the transition temperature. In the mesomorphic lamellar phase, two lines, in general, are resolved as shown in Fig. 1b. These lines consist of: (a) an intense narrow component of width 10^{-2} gauss, having rather strong "wings", and (b) a previously unresolved[6] line of width 2.5–1.5 gauss, depending on the extent of hydration. A satellite of double the width of this line is also observed at low hydrations; at higher hydrations extended wings are seen. The breadth of this "broad" component is greater than that of the intermediate line observed below the transition point. Only a little narrowing of the broad component in the mesomorphic phase occurs with increasing temperature or hydration, as shown by Figs. 2 and 3c. The linewidth for the anhydrous sample is, however, somewhat greater than for the hydrated samples. An additional line of width ΔH of approx. 0.90 gauss for the anhydrous sample and for samples with $n \leqslant 2.4$ is also found. This line is not resolved for greater water contents.

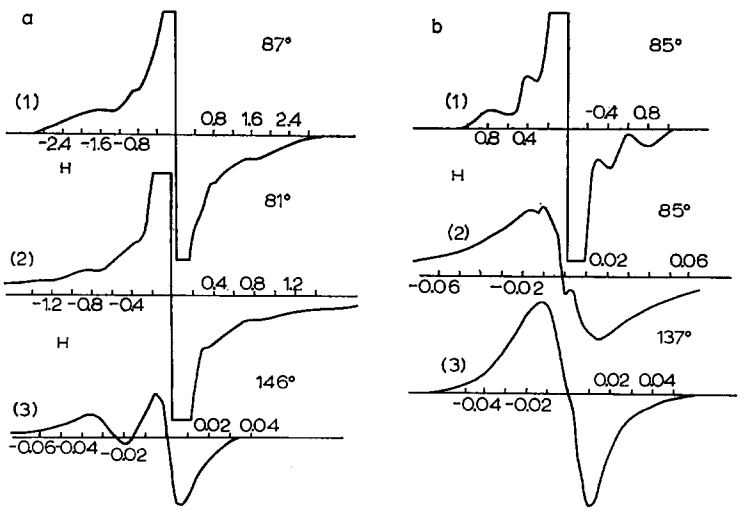

Fig. 4. PMR derivative absorption spectra of 1,2-dipalmitoyl-L-phosphatidylcholine in liquid-crystalline phases. (a) Lecithin + 1 ^2H_2O, (1) and (2) lamellar phase, (3) cubic phase. (b) Perdeutereated lecithin + 1 ^2H_2O, (1) and (2) lamellar phase, (3) cubic phase.

At higher temperatures the lecithin–water samples containing from 1 to 2.4 water molecules exhibit another phase transition[4]. Above about 145°, no broad component is obtained with these samples. The strong narrow line is resolved with minimum modulation and an expanded field sweep into two lines separated by approx. 2.0 ppm as shown in Fig. 4a for n = 1.

Perdeuterated dipalmitoyl lecithin

Below the transition temperature. The NMR spectra of the perdeuterated lecithin are shown in Fig. 1a, and are compared with the dipalmitoyl lecithin spectra recorded under similar experimental conditions. As shown in Fig. 3a, the second moment at low hydrations and for n = 24 is less than of the corresponding lecithin–^2H_2O sample. The intermediate and broad linewidths show a similar variation with temperature of the deuterated and protonated lecithin when n = 24.

The main difference between the two lecithin–water systems rests in the intensity ratio of the intermediate and broad components. This difference is illustrated for the anhydrous samples at 24° in Table I, where R is the ratio of the first moments of the intermediate and total broad ($\Delta H > 0.1$ gauss) derivative components.

TABLE I

INTENSITY RATIO (R) AND LINEWIDTHS OF THE INTERMEDIATE AND BROAD COMPONENTS OF ANHYDROUS 1,2 DIPALMITOYL-PHOSPHATIDYLCHOLINE

$n = 0$	*Protonated lecithin*	*Perdeuterated lecithin*
R, experimental ratio	14 ± 1.7%	47 ± 3.0%
R, theoretical ratio	11%	43%
ΔH (intermediate)	2.7 gauss	2.6 gauss
ΔH (broad)	8.5 gauss	8.0 gauss
$(\Delta H)^2$ broad	11.1 gauss^2	7.3 gauss^2

Assuming that the magnetic dipolar interactions in each case are the same, the intensity figures in Table I are approximately those expected if the $N^+(CH_3)_3$ group protons of the head group contribute only to the intermediate line — the fatty acid methylene groups.

Above the transition temperature. In comparison with the fully protonated lecithin the intensity of the strong narrow line is reduced. The second moments of the broad components are comparable, as shown in Fig. 3c. Comparison of linewidths and second moments of the "broad" component for the lecithin–water systems containing 24 moles of water at 75° is given in Table II. Close correspondence of the broad lines in the mesomorphic phase occurs.

The monohydrate (n = 1) gives rise to two well resolved "broad" components: the additional linewidth of approx. 0.90 gauss, and the outer linewidth of 1.5 gauss. The outer line does not contain the long wings characteristic of the protonated sample. With increasing temperature, or water content, the 0.90-gauss line is obscured by the narrow component. At higher temperature (approx. 140°) the monohydrate sample exhibits a phase transition analogous with the behaviour of the protonated sample.

"High resolution" conditions for the operation of the wide line spectrometer revealed a very narrow and asymmetric derivative spectrum, with an overall width of approx. 23 mgauss, as shown in Fig. 4b. To avoid the decomposition of the lecithin, the samples were not measured at temperatures higher than 150°.

TABLE II

$n = 24$ moles ^2H_2O	*Protonated lecithin*	*Perdeuterated lecithin*
ΔH broad	1.4 gauss	1.3 gauss
$(\Delta H)^2$ broad	0.4 gauss^2	0.2 gauss^2

Addition of ordinary water. In the previous experiments ^2H_2O was added to the phospholipids. Additional experiments were carried out corresponding to the addition of 5 molecules of ordinary water. No change in the characteristics of the spectra either above or below the transition temperature occur for equivalent hydrations of either 5 H_2O or 5^2H_2O. However, the narrow component shows a slight broadening (15 mgauss) in comparison with the linewidth (10 mgauss) of a similar total volume of bulk water.

1,2-Dimyristoylphosphatidylcholine

The behaviour of the linewidths in the transition region was investigated with this sample, having a hydration of n = 24 moles ^2H_2O. The transition temperature of 26° is preceded by analogous line narrowing of the broad component as was found for the dipalmitoyl lecithin. However, the intermediate component is reduced from 0.5 gauss at 10° to 0.2 gauss at 18°. A small endothermic peak is observed at this temperature by thermal analysis[4]. The narrow line intensity increases sharply at 26°.

DISCUSSION

SALSBURY AND CHAPMAN[5,6] have described the molecular reorientations of disaturated phosphatidylethanolamine and phosphatidylcholine[6] in the absence of water from —200 to 160°. In particular the reorientational processes responsible for the line narrowing at the transition temperature were analysed by the study of PMR second moments. The motion of the protons of the long chains in the mesomorphic phase were described as rapid ($\nu_c \geqslant 10^7$ Hz) with a distribution of correlation frequencies along the chain. A form of anisotropic rotation was proposed to account for the narrow lines observed. No broad PMR component was resolved for 1,2-distearoyl-L-phosphatidylcholine above its transition temperature.

In this discussion we shall be concerned primarily with the modifications to these motions as a result of the introduction of water between the polar head groups.

The gel phase

Assignments

The derivative line shapes were assigned by the comparison of the protonated and perdeuterated lecithin spectra and by the intensity ratio of the individual components.

(*a*) *The intermediate line* (*ΔH approx 2.7–0.4 gauss*). This line is assigned to residual dipolar interactions of the protons of the C–$N^+(CH_3)_3$ group. These nine protons are expected to undergo rapid reorientation about the C_3 axis of the methyl groups and/or about the C_3 axis of the fragment at low temperatures as shown for trimethylamine[10]. The small moment of inertia of the CH_3 rotator leads to a large reduction of linewidth and second moment near 150° for distearoyl lecithin[6], which is not observed for phosphatidylethanolamine containing N^+H_3 groups. For trimethylamine, one narrow and a broad component of 4.1 gauss are measured for the rotating phase[10]. It is therefore reasonable to assign the intermediate linewidth (2.7 gauss at 27°) to the protons of the $N^+(CH_3)_3$ head group in which the methyl groups are undergoing restricted rotation.

If all the CH_2 groups of the hydrocarbon chains of the glycerol residue and the $(CH_2)_2$ head group contribute to the broad component at 24°, then the relative intensity of the intermediate and broad lines shows good agreement with the expected intensity from the numbers of protons assigned to various line shapes as shown in Table I. The slightly higher percentage for the experimentally measured intermediate component is possibly due to imperfect decomposition of the composite spectrum or to different dipolar interactions in the head group of the deuterated lecithin.

(*b*) *The broad component* (*ΔH approx. 9–4 gauss*). This line is assigned to contributions from the protons of the CH_2 groups of the lipid chains in agreement with the study of distearoylphosphatidylcholine[8]. At 24° the CH_2 groups are undergoing restricted rotational and oscillatory modes of reorientation and the linewidth is considerably reduced from the rigid lattice value. The protons of the glycerol residue ($CH_2 \cdot CH \cdot CH_2$) and of the $(CH_2)_2N^+$ fragment of the head group also make a contribution to this signal (see Table I). These protons must be largely responsible for the 8.0-gauss linewidth of perdeuterated lecithin.

The second moment of 7.3 gauss[2] of the perdeuterated lecithin indicates that the methylene protons of the head group are undergoing restricted oscillation. However, these nine protons can contribute only about 10 % to the total second moment of the protonated dipalmitoyl lecithin at 24°. Thus the linewidth and the second moment of the broad component in protonated dipalmitoyl lecithin are dominated by the dipolar interactions of the protons of the lipid chains.

(*c*) *The narrow line* (*approx. 10^{-2} gauss*). The narrow line is assigned to the proton signal from ^2HHO. In the pre-transitional region this narrow component appears to grow at the expense of the broad component.

The assignment of the individual components contributing to the overall line shape gives us the possibility of following the molecular motion in different parts of the lecithin molecules when water is introduced and as the temperature is raised.

The effect of water

The introduction of water to the phosphatidylcholine system leads to the formation of water layers between the lipid lamellae in which the polar groups are in contact with the water[3,4].

In the anhydrous sample, coulombic interactions in the zwitterion and between neighbouring polar groups may prevent rapid reorientations of the protons of the head group. The lamellar organisation of the lipid molecules permits stronger interaction

between the head groups and thereby gives rise to close packing of the apolar chains in the crystalline phase. Thus the amplitude and frequency of molecular motion or segmental reorientations are likely to be increased by the introduction of water. This behaviour is shown in Fig. 3a.

The addition of up to n = 5 moles of water results in a narrowing of the broad linewidths by 50%; 5 additional moles of water account for the balance of the reduction observed for n ⩾ 10 moles ^2H_2O. The first 5 water molecules reduce the intermediate linewidth to 22% of that found for the anhydrous sample. The importance of the first 5 water molecules in the organization of the gel phase is therefore emphasised. Further, the dominant effect is on the linewidth assigned to the head group protons, as might be expected from the diminished strength of the coulombic interactions in the head groups. In addition, the Van der Waals forces between the hydrocarbon chains become less effective, and molecular reorientation of the head groups and hydrocarbon chains increases as water penetrates the zwitterionic lattice. X-ray studies have been interpreted to suggest that, upon hydration, some change of orientation of head groups occurs.

The first water molecule incorporates into the phosphatidylcholine head group, *i.e.* PO_4^- and $N^+(CH_3)_3$ as H^+ and OH^-, thereby weakening the ionic linkage. Thus the phosphorylcholine residue may be allowed to adopt a more extended arrangement. The second water molecule is likely to contribute to the hydrogen bonding structure originating from the polar groups. The formation of hydrogen bonding is confirmed by infrared studies[4]. The only difference of the infrared spectra between the anhydrous and hydrated lecithins is the shift of the 1252 cm^{-1} band to 1238 cm^{-1}. This band is assigned to vibrational modes of the phosphate ion[4].

Additional water molecules participate in further hydrogen bond formation which results in the weakening of inter-head group forces as shown by the influence of water upon the intermediate linewidth. The importance of 4–5 moles of water per mole of lecithin indicates a "primary" hydration layer which also modifies the motion of the long chains in addition to that of the head group protons.

The corresponding linewidth reductions parallel the reduction of the transition temperature (T_c) on increasing amount of water up to n = 10 moles ^2H_2O. The secondary class of 4–10 moles of water is no doubt a weaker hydration shell with a slight effect on the molecular motion. The role of these 10 water molecules has already been ascribed to the formation of a hydrate structure in the polar group region[4]. The additional water (n > 10) produces no observable effect on the motion of either head group protons or the long chain protons and forms a free water layer between the polar groups [4,12].

The PMR continuous wave experiments do not appear to be a viable means of studying the "bound" water in these systems. The narrow line observed from the use of ordinary water is only slightly but significantly broader than that of free water. The lipid-associated water must be undergoing sufficiently rapid motion that averages the dipolar broadening nearly to zero. Thus the directions and magnitudes of the aqueous interproton vectors must be varying rapidly ($\nu_c > 10^5$ Hz). No contribution of the water protons to the broad or intermediate component was found. However, deuteron magnetic resonance experiments[13] have shown that anisotropic reorientation of the ^2H_2O molecules occurs for up to n = 21 moles of ^2H_2O.

The liquid-crystalline phase

(a) *The narrow component*

The intense narrow line observed in the lamellar mesomorphic phase must be dominated by the contribution from the rapidly reorienting protons of the fatty acid residues of the phospholipid. This line grows at the expense of the broad component in the gel phase just below the transition temperature. Its width of 10^{-2} gauss above T_c corresponds with the narrow linewidth of distearoylphosphatidylcholine described by SALSBURY AND CHAPMAN[6].

However these studies reveal that the intermediate line of the gel phase is narrowed just below the transition temperature. This behaviour is observed more clearly for the dimyristoylphosphatidylcholine sample. The $N^+(CH_3)_3$ group must therefore be undergoing rapid reorientation with a correlation frequency $\nu_c > 10^5$ Hz in the liquid-crystalline phase with a linewidth less than 0.2 gauss. The motionally narrowed absorption must lie unresolved in this narrow component. Signals corresponding to these two groups of protons, $(CH_2)_n$ and $N^+(CH_3)_3$, are just resolved in the PMR spectrum of egg-yolk lecithin taken on a high resolution spectrometer[8].

In the lamellar phase a discrete long X-ray spacing is found[4], and the phospholipid molecules are aligned in two-dimensional sheets. The anisotropy of the molecular lamellar organisation does not allow complete isotropy of reorientation of the fatty acid residues, since even in the presence of water, a non-zero and finite second moment of the narrow component must be appropriate.

(b) *The broad component*

As shown in Table II and Fig. 3, the broad component of the liquid-crystalline phase of dipalmitoyl lecithin–water systems corresponds within the errors of measurement with that of the perdeuterated lecithin. Thus the line of width 1.4 gauss must correspond with residual dipolar interactions within the phospholipid head group. Since the $N^+(CH_3)_3$ line shape is considerably narrower, even below the transition point, the contributing groups must be the protons of the glycerol residue $CH_2 \cdot CH \cdot CH_2$, and the methylene groups $PO \cdot (CH_2)_2N^+$ of the phosphorylcholine residue. The broad line due to these protons suggests that the lamellar organization requires the maintenance of an anisotropic arrangement of the head groups at the lipid–water interface. The anisotropic organization is maintained inside the separate particles of the dispersion whilst the particles themselves are randomly oriented; the small linewidth and second moment of this component must, however, indicate the reduction of dipolar interactions by means of segmental motions. The residual linewidth indicates, therefore, slow and anisotropic motion ($\nu_c < 10^5$ Hz) of the glycerol and $(CH_2)_2N^+$ protons. The rates of reorientation are not sufficiently rapid to minimize dipole–dipole interaction. Thus the measured linewidths and second moments in the lamellar phase are a function of head group anisotropy. This anisotropy remains almost constant with increasing temperature.

In a soap–water system where only the alkyl chains can give rise to the NMR signal, one narrow line with very wide and strong "skirts" is observed. An absence of the well resolved "broad" component supports the former conclusion about the origin of this line.

The effect of water

A large change in the linewidth occurs when the first water molecule is introduced. This coincides with the observed modification of the head group conformation[4].

After the preferred conformation of the head groups is obtained, the further introduction of water ($n > 1$) does not change the linewidth. The existence of the broad component is therefore the responsibility of the symmetry of the organisation of the glycerol residues and the polar groups in the lamellar phase, without regard to the structure of the aqueous layer or the increasing temperature.

Sonication of lipid–water systems

We have shown that linewidths of less than 0.2 gauss and of about 10^{-2} gauss are expected for the $N^+(CH_3)_3$ and $(CH_2)_n$ protons of lecithin in a mesomorphic dispersion in water. Rapid rotation of lecithin aggregates at a frequency, ν_c, greater than these linewidths in frequency units should be a suitable mechanism for further line narrowing[8]. A reduction of particle radius to 100–400 Å, which can be obtained by ultrasonication of these dispersions, should be sufficient to do this[8]. Narrowing of the broad component (6 kHz) may, however, be a marginal effect. High resolution spectra of sonicated lipid dispersions showing chemically shifted lines have been reported[7]. A similar averaging process may explain the observation that sonication of erythrocyte ghosts also shows high resolution chemically shifted lines[15].

The cubic phase

The high temperature region of the lecithin water phase diagram includes a viscous isotropic phase at low water concentrations. The X-ray data is consistent with a cubic lattice. The detailed model is uncertain, but may be aqueous spheres ($r = 12$Å) surrounded by phospholipid[4], or of the rod-like class[15].

For values of $n \leqslant 3$ moles of ^2H_2O per mole of lecithin above 140° no broad component is observed. This new phase is characterized by narrow chemically shifted absorptions of the $N^+(CH_3)_3$ and $(CH_2)_n$ groups, separated by approx 2.0 ppm, in agreement with other authors[7]. The rather broad linewidths (approx. 50 Hz) and the long wings suggest that the molecules are not completely free in terms of molecular motions. Nevertheless, the presence of fine structure indicates a very effective averaging process.

The absence of the broad component, in spite of the high viscosity of this phase, must be related to an efficient averaging to almost zero of inter- and intra-molecular dipole–dipole interactions. PENKETT *et al.*[8] indicate that the spin-lattice relaxation time (T_1) shows no abrupt change at the lamellar–cubic phase boundary. The T_1 data may, however, refer to the $(CH_2)_n$ protons, for which $\nu_c > 10^7$ Hz.

However, the broad components of the lamellar phase are characterised by spin–spin relaxation times $T_2 \leqslant 1$ msec. Although the frequency of motion of the lipid segments may not be modified with respect to the lamellar phase, the local symmetry certainly is modified. The new symmetry of the cubic phase will create either rapid isotropic rotary motion[16], or molecular diffusion within a given sphere (or rod), as described by PENKETT *et al.*[8]. Such motions must occur at a frequency $\nu_c > 10^5$ Hz in order to narrow the "broad" component of the lamellar phase. The isotropic symmetry will further permit more degrees of freedom of reorientation than in the lamellar phase.

In a rod-like structure the polar head groups will retain a continuous arrangement resembling, in a given rod, the arrangement in the lamellar phase. If this is the correct structure, the phospholipid molecules must be capable of rapid and random molecular motion and translation to be able to give rise to the observed spectrum.

As the proposed structures are uncertain, it is difficult to determine the definite mechanism for the linewidth narrowing. Detailed relaxation measurements with a fast

recovery pulsed NMR spectrometer are being commenced at this laboratory. An insight into the modification of molecular motions at the phase boundary may then be gained.

Erythrocyte membranes

A typical wideline PMR spectrum of haemoglobin-free human erythrocyte ghost suspended in buffered ^2H_2O (pH 7.4) is shown in Fig. 5. At 24° broad components of 2, 3, 3.4 and 5.8 gauss are resolved. A strong narrow line, due principally to aqueous protons, dominates the observed spectrum. (A somewhat similar spectrum has been observed previously with erythrocyte ghosts at lower water content[17].)

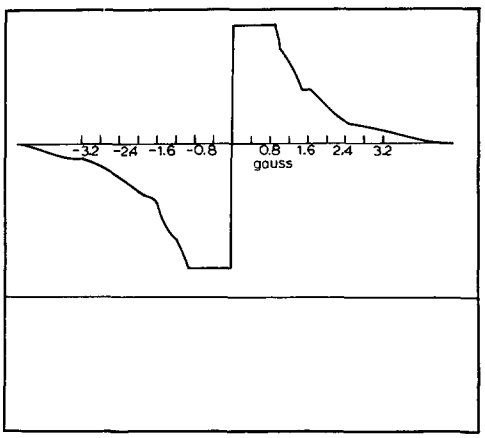

Fig. 5. A wideline derivative absorption spectrum of intact human erythrocyte ghosts in phosphate buffer (pH 7.4) in ^2H_2O at 24°. The abscissa is calibrated in gauss.

Although quantitative discussion is not yet possible for such a complex system, the magnitudes of these linewidths indicate the presence of different proton groups undergoing rather slow (approx. 10^5 Hz) reorientation.

The line of 5.8 gauss may arise from the protein moiety. (Lines of this width at similar temperatures have been observed with bovine serum albumin–water systems[18].) The remaining lines are broader than those observed with our lecithin–water systems and may originate from modified dipolar interactions involving lipid–cholesterol or lipid–protein interactions. High-resolution NMR studies of erythrocyte membranes have been interpreted to indicate that interactions of this type are occurring[14,19]. Future NMR studies will examine model lipid–protein systems to contrast their properties with natural membrane systems.

ACKNOWLEDGEMENTS

We wish to thank Mr. A. Darke for technical assistance, and to express our thanks to our colleagues at this laboratory, particularly Dr. A. Davies, Mr. J. S. Chadha, Mr. B. D. Ladbrooke and Dr. V. B. Kamat for the provision of, and auxiliary characterisation of, the samples utilised in this study.

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