LECITHIN TRANSLATIONAL DIFFUSION STUDIED BY PULSED NUCLEAR MAGNETIC RESONANCE

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ABSTRACT The translational diffusion coefficient of egg yolk and dilauroyl lecithin in optically isotropic phases containing sodium cholate has been measured using the pulsed NMR magnetic field gradient method. After a correction for geometrical factors the measured diffusion coefficient is found to agree well with previous determinations in phospholipid systems. The experimental data imply that the cubic mesophase of the lecithin-sodium cholate-water system contains continuous lipid aggregates. A possible model of the arrangement of the different amphiphile molecules in the cubic phase is discussed.

INTRODUCTION

In recent years several studies on the lateral diffusion of lecithin molecules in a bilayer have been reported (1–4). All these studies have been made with probe molecules, using spin labels in an electron spin resonance (ESR) experiment (1–3) or a chromophore probe in flash photolysis (4). Since the relevance of the ESR experiments have been questioned (5, 6) regarding the perturbation of the bilayer system made by the spin label, the need for a nonperturbatory method is obvious. An alternative method is provided by pulsed nuclear magnetic resonance (NMR), where no such perturbation is introduced. However, measurements of the diffusion coefficient of lecithin in a lamellar mesophase are not simple and straightforward. This is due to the static proton dipole-dipole interactions occurring in an anisotropic liquid crystalline phase (7). On the other hand, if the mesophase is isotropic on a time scale which is short compared with the inverse of the dipole interactions, the molecular motion will average the dipolar interactions to zero, and a conventional 90°-τ-180° pulsed NMR experiment using pulsed magnetic field gradients can be used for measurements of diffusion coefficients. This applies to cubic mesophases and some pulsed NMR studies of amphiphile diffusion for different amphiphilic cubic mesophases have been reported (8–10). Unfortunately, cubic phases of lecithin-water systems exist only at very low water contents and at high temperatures (11), so that diffusion coefficients determined for such phases may be of less value when considering a biomembrane bilayer system. (We have

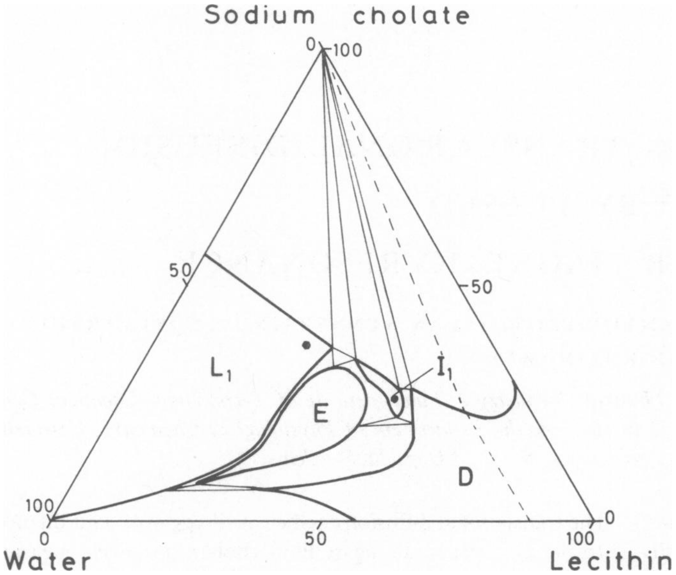

FIGURE 1 Phase diagram (from ref. 27) of the ternary system egg yolk lecithin-sodium-cholate-water (expressed as weight percent). Phase notations: L_1 is the isotropic micellar solution, D and E are the anisotropic lamellar and hexagonal mesophases, and I_1 is the isotropic cubic mesophase. Samples investigated are indicated by dots.

determined the diffusion coefficient of dipalmitoyl lecithin in a cubic phase [10] to be about 10^{-8} cm^2/s at 130°C.) However, if sodium cholate is added to egg yolk (or synthesized saturated) lecithin a cubic phase can be obtained (12) at room temperature having a water content varying between about 22 and 27 w-% (see Fig. 1). In this communication we present a determination of the translational diffusion coefficient of lecithin in cubic and micellar phases composed of egg yolk or dilauroyl lecithin, sodium cholate, and heavy water. From the data obtained an estimate of the lateral diffusion of lecithin in a bilayer is also made.

EXPERIMENTAL METHODS

The determinations of the diffusion coefficients were performed with the pulsed magnetic field gradient method described by Stejskal and Tanner (13) at 60 MHz with a Bruker 322s pulsed NMR spectrometer (Bruker Physik AG, Karlsruhe, W. Germany) equipped with a Bruker pulsed magnetic field gradient unit. The molecular diffusion will attenuate the spin echo amplitude E at 2τ according to the equation (13)

$$\ln \frac{E_g}{E_o} = -(\gamma g \delta)^2 D \left(\Delta - \frac{\delta}{3}\right) \tag{1}$$

where E_g/E_o is the echo attenuation. The gradient magnitude g and spacing Δ were kept constant at $\sim 2\,T/m$ and 60 ms, respectively, while the gradient width δ was varied between 1.2 and 10 ms. The lower limit for δ was chosen to ensure nearly ideal rectangular shapes of the gradient pulses. The magnitudes of δ and Δ were measured visually on an

TABLE I

MEASURED DIFFUSION COEFFICIENTS AT THREE TEMPERATURES FOR MICELLAR AND CUBIC PHASES COMPOSED OF LECITHIN, SODIUM CHOLATE, AND HEAVY WATER

All measurements were made relative to the diffusion of glycerol (14), $D = 1.9 \cdot 10^{-8}$ cm^2/s at 24.2°C.

Sample	Phase	$D \cdot 10^8$ cm^2/s		
		24.2°C	40.5°C	50.5°C
I	Micelle	0.7	0.8	1.2
II	Cubic	0.7	1.5	2.3
III	Cubic	0.7	–	2.4

oscilloscope. Three to eight echoes were accumulated at least three times on a signal averaging unit. The largest accumulated echo was used in the determination of the diffusion coefficient since all instabilities in the spectrometer tend to decrease the echo height. The diffusion coefficient, D, was calculated from a least square fit of the echo height against $\delta^2(\Delta - \delta/3)$ for both the sample and a glycerol reference. The ratios between the slopes and the known diffusion coefficient of glycerol (14) then gave the value of D for the sample. Every determination of D was repeated to establish the reproducibility. The random error in the diffusion coefficients we estimate to ±20%, where the smallest diffusion coefficients have the largest errors. In addition to this there is a systematic error mainly due to the uncertainty in the diffusion coefficient of the reference.

Egg yolk lecithin was prepared according to Singleton et al. (15). Dilauroyl lecithin was prepared as described previously (16). Sodium cholate and heavy water were purchased from British Drug Houses Ltd., Poole, Dorset, England and Norsk Hydro, Norway, respectively.

RESULTS

The diffusion coefficient was measured at three temperatures for three systems: a micellar solution containing 30% egg yolk lecithin, 36% sodium cholate, and 34% heavy water (I), and two cubic mesophase samples composed of 50% egg yolk lecithin, 26% sodium cholate, 24% heavy water (II), and 50% dilauroyl lecithin, 26% sodium cholate, 24% heavy water (III), respectively. The compositions are given in weight percentages. The diffusion coefficients obtained are summarized in Table I. All the coefficients measured are of the order of 10^{-8} cm^2/s.

DISCUSSION

General Comments

The systems studied contain two proton-rich compounds, lecithin and cholate, and the observed spin echoes could thus contain contributions from both of these. However, the echoes were observed after a long time, 90 ms, and it is then only signals with a comparatively long effective T_2 that contribute to the echo. From the high resolution ^{1}H NMR spectrum of a typical sample shown in Fig. 2, it can be seen that it is the lecithin methylenes that give rise to the most intense and sharp peak. We can

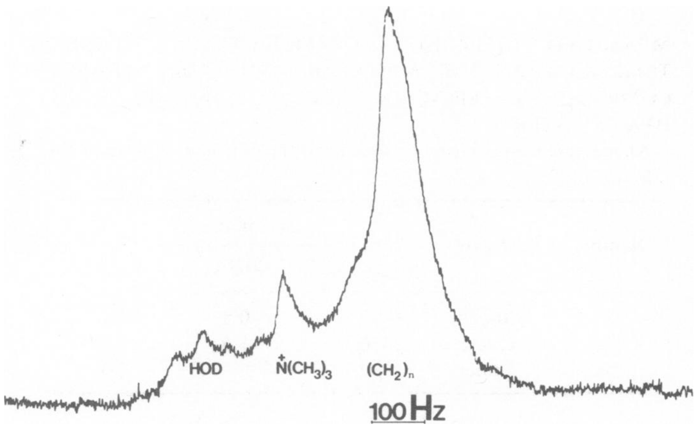

FIGURE 2 Proton NMR spectrum at 100 MHz for a cubic mesophase composed of 50% egg lecithin, 26% sodium cholate, and 24% D_2O. Note the sharp peaks due to choline and alkyl chains of the egg lecithin.

thus conclude that the echo at 90 ms is dominated by magnetization from lecithin protons. It thus seems reasonable to refer the measured diffusion coefficients to lecithin diffusion.

Micelles

At low amphiphile concentration it can be assumed that the micelles are sufficiently small so that the measured diffusion coefficient corresponds to the diffusivity of the micelles themselves. Then, assuming that the micelles are spherical, the radius of the aggregates can be calculated from the Stokes formula for translational diffusion (17). However, as the amphiphile concentration is increased the micelles begin to interact with each other and for some systems it has been observed that the micellar shape changes from a globular-like aggregate to a rod-shaped at higher concentrations (18–21). The rod-like micelles may be very long (22, 23).

In the interpretation of the experimental diffusion coefficients it should be noted that the NMR pulsed gradient method measures the diffusion that takes place between the two magnetic field gradient pulses. In our experiments these pulses were spaced by 60 ms and with a diffusion coefficient of 10^{-8} cm^2/s the formula $<x^2> = 2Dt$ shows that it is a translational diffusion over a distance of 300 nm that contributes to the attenuation of the echoes. For small ($r = 3.5$ nm) micellar aggregates it is thus only the diffusion of the micelle itself that contributes to the measured diffusion. However, for rod-shaped micelles with a length $l > 300$ nm the diffusion of lecithin within the micelles can contribute. There seems to be no thorough investigation of the shape of the lecithin-cholate micelles reported in the literature but the proton NMR spectrum, showing typical high resolution features, suggests a rotational correlation time of 10^{-5} or less and thus aggregates of limited extension. Consequently the main contribution to the measured diffusion coefficient is from the motion of the aggregates themselves.

Cubic Mesophases

The structure of the cubic phase is still not known, but essentially two different structures have been proposed (24). In one of these the phase is built up from globular micelles packed in a face-centered cubic lattice and the other one consists of a network of rod-shaped aggregates, *cf.* Fig. 3. Recently it was shown (9) that from a comparison of measured diffusion coefficients (using pulsed NMR) in the micellar solution and the cubic mesophases in the amphiphile system of dodecyltrimethylammonium chloride and water, it is possible to distinguish between the two structures proposed for cubic phases. This was feasible because the restricted diffusion within a small region like a globular micelle gives rise to a much smaller effective diffusion coefficient in the pulsed

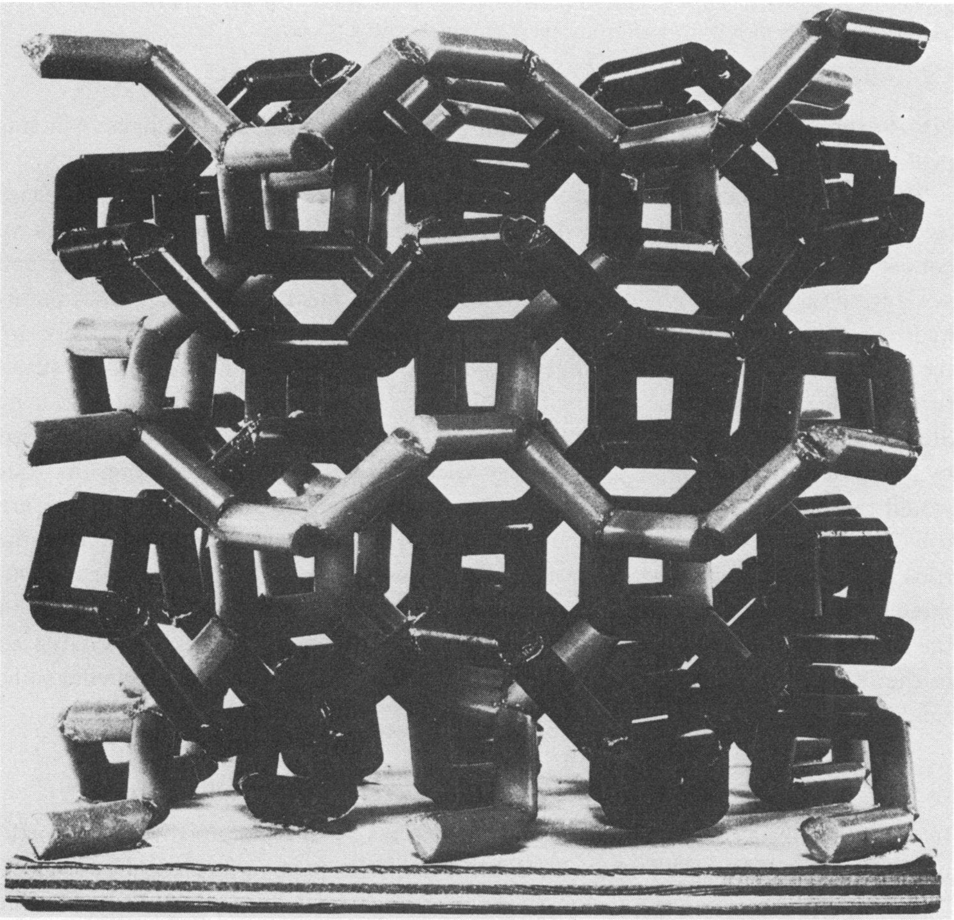

FIGURE 3 A picture of a model of the cubic mesophase made by K. Fontell (*J. Colloid Interface Sci.* 1973. **43**:156). The model illustrates the two interwoven but otherwise independent networks of the rod structure. The rods in the picture represent only the axis of the rod aggregates and the length and the diameter of the aggregates are almost equal. (By kind permission of K. Fontell.)

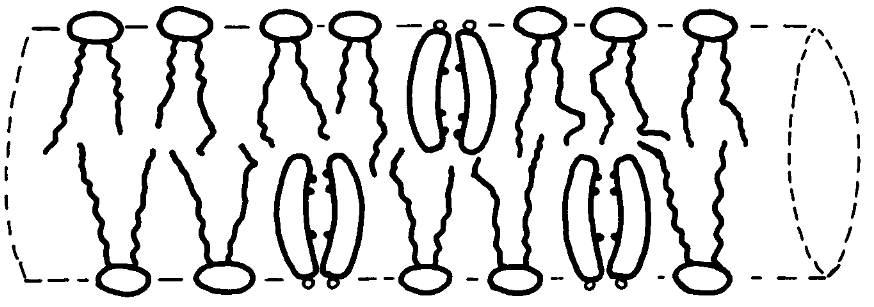

FIGURE 4 Schematic diagram of a possible structure of the rod-shaped aggregates in the cubic phase (not to scale). The lecithin molecules are proposed to have their polar head groups at the surface of the rods and the alkyl chains are pointing inwards towards the long axis of the cylinder. Cholate is solubilized in an aggregated form (e.g. dimers as shown in the picture) in order to account for the hydrophilic and hydrophobic parts of the cholate molecule.

NMR experiment than if the diffusion can occur over macroscopic distances which is possible in the network structure.

The diffusion coefficients in the cubic phases are larger than in the micellar phase (see Table I). Since interaggregate interactions should be larger in the cubic phases we conclude that the measured diffusion in these phases is due to molecular motion and not aggregate diffusion (*cf.* ref. 9). The cubic phase must then contain continuous hydrophobic regions as for example in the network structure in Fig. 3. Furthermore the lecithin should have the possibility of diffusing along the rods in the network structure. These rods can therefore not have the same structure as the one proposed by Small (25) for the rods in the hexagonal phase, *E*. In this model the rods are formed by piling discs of a bilayer type on top of each other. The diffusion along the rods would then have to include flip-flop motions of the lecithin molecules. These are usually very slow (26). An alternative model of the molecular arrangement in the rods is presented in Fig. 4. Note that the cholate molecule is hydrophilic (due to three hydroxylic groups) on one side of the steroid ring system and hydrophobic on the other side. Therefore it is suggested that the cholate molecules are solubilized in the lecithin rods as dimers or perhaps larger aggregates, having the hydrophilic sides "face-to-face."

Comparison with Other Studies of Diffusion in Lecithin Bilayer Systems

In order to make a comparison between diffusion coefficients obtained by other methods it is necessary to consider how the diffusion experiment is performed. For a lamellar mesophase the lateral diffusion coefficient, D_L, is usually measured (1–4). The diffusional motion in all directions *in* the bilayer is the same and the molecules in the lamellae do not "flip-flop." The diffusion tensor in the principal axis coordinate system can then be written

$$\mathsf{D} = \begin{pmatrix} D_L & 0 & 0 \\ 0 & D_L & 0 \\ 0 & 0 & 0 \end{pmatrix}$$

Let us now assume that the local conditions (as experienced by the lecithin molecules) in the lamellar and the cubic mesophases are the same. It is then convenient to define one diffusion coefficient $D_\parallel$ for the motion along the directions of the rods in the cubic phase, and another one, $D_\perp$, for the diffusion around the rods. The diffusion tensor is then given by

$$\mathsf{D} = \begin{pmatrix} D_\parallel & 0 & 0 \\ 0 & D_\perp & 0 \\ 0 & 0 & 0 \end{pmatrix}$$

neglecting translation along the radius. If the only difference between the lamellar and the cubic phases is geometric it is clear that $D_\parallel = D_\perp = D_L$. However, since in the pulsed NMR experiment, molecules diffusing over short distances will not influence the measured diffusion coefficient, the molecules traveling around the rods (having a radius of about 3.5 nm = the length of a lecithin molecule) will have an effective diffusion coefficient $D_{\perp \text{eff}} = 0$.

For an isotropic system as a cubic mesophase one measures an average value of the diffusion coefficient in all directions, which is equal to $\frac{1}{3}$ of the trace of the diffusion tensor, i.e. $\frac{1}{3}\, Tr\mathsf{D} = \frac{1}{3}\, D_\parallel \simeq \frac{1}{3}\, D_L$. Thus the lateral diffusion coefficient, D_L, obtained from other methods should be compared with a value which is three times the measured diffusion coefficient, D_{cub}, for the cubic phase. In Table II diffusion coefficients for some lecithin lamellar mesophases and biological membranes obtained in other studies are summarized. As may be inferred from this table, there is a reasonable agreement between our corrected value $3 \cdot D_{\text{cub}}$ and previous determinations.

TABLE II

LECITHIN LATERAL DIFFUSION COEFFICIENTS FOR SOME LIPID MODEL AND BIOLOGICAL MEMBRANES

The lateral diffusion coefficient for lecithin estimated in this work is equal to $3 \cdot D_{\text{cub}} = 2.1 \cdot 10^{-8}\ \text{cm}^2/\text{s}$ at 24.2°C.

System*	Method	$D_L \cdot 10^8\ \text{cm}^2/\text{s}$
Egg lecithin at 25°C [1]	ESR	1.8
Egg lecithin and cholesterol (4:1) at 25°C [3]	ESR	7
Sarcoplasmic reticulum at 37°C [2]	ESR	6
Dipalmitoyl lecithin at 5°C [4]	Flash photolysis	1.6

*Reference numbers in brackets.

CONCLUSION

In this work the diffusion coefficient of lecithin molecules in a cubic phase containing cholate has been determined. It should be noted that the diffusion of lecithin may be influenced by the presence of cholate, so that the translational motion of lecithin in our systems is different from lecithin in pure lecithin-water systems. In what way the diffusion coefficient will be affected by cholate is difficult to foresee and will need further experimental investigation. Unfortunately, the cubic mesophase exists over a rather narrow region of concentration (see Fig. 1) so that there are small possibilities of changing the lecithin-cholate ratio. Therefore studies on pure lecithin-water systems will be needed to clarify this point.

We are grateful to Jan Ulmius for recording the high resolution NMR spectra.

This work was supported by the Swedish National Research Council (project no. K 3500-003).

Received for publication 17 November 1975 and in revised form 27 April 1976.

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