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ZWITTERIONIC DIPOLES AS A DIELECTRIC PROBE FOR INVESTIGATING HEAD GROUP MOBILITY IN PHOSPHOLIPID MEMBRANES

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Summary

For phospholipid membranes with zwitterionic head groups, the dipole can be considered as a specific label for tracing the changes in the dynamic behaviour of this region of the bilayer in its various phases. Measurements of the dielectric properties of fully hydrated 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine bilayers in the frequency range 1—50 MHz show a dispersion which is attributed to the motion of the phosphocholine dipoles in the plane of the bilayers. When the temperature is varied, both the permittivity and loss factor increase sharply at the pretransition (35° C) and the main transition (42° C). The relaxation time and amplitude were also determined for this dispersion and these further reflect the structural changes occurring with temperature. The relaxation times varied between 4 ns at 30° C and 2.3 ns at 50° C. Due to steric hindrances a restriction in the angle of head group rotation occurs at lower temperatures but is greatly reduced above the main transition.

Introduction

Pure lipid bilayer systems have been investigated by various physical methods to determine their static and dynamic properties. Many of the lipids have a zwitterionic head group with a consequent large dipole moment and the present dielectric measurements take advantage of this fact that is evidently important for the overall behaviour of the system. Significant work to characterize the static properties of bilayers has been carried out by X-ray [1—3] and neutron diffraction [4,5], deuterium magnetic resonance [6], calorimetric methods [7—9], fluorescence, light scattering and absorption [10]. Dynamic properties have been mainly determined by NMR and ESR (for

Abbreviation: DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine.

references see refs. 6 and 11) and optical spectroscopy [12,13]. Some of these methods use labels at various parts of the lipid molecules with the consequent disadvantage of perturbing the system. In this respect the electric dipole moment of the head group provides naturally a sensitive probe to detect overall changes in the bilayer system as well as motions in the head group region.

Recently dielectric investigations in the GHz range (0.05—60 GHz) have been made on lipid systems in water [14]. The results have been interpreted in terms of two main dispersions, namely those of free and bound water. In the case of lysolecithin (from egg lecithin) solution, the high frequency end of a dispersion attributed to these zwitterions could also be detected at 50 MHz. Our measurements now cover the region below 50 MHz and, in contrast to the samples used in the above work, the present system does not contain excess water. Thus the co-existence of different phases, such as the multi-lamellar phase and vesicles in the excess water is avoided. An additional advantage is that our samples have very small conductivity, which facilitates the measurements. These studies of pure multi-lamellar systems will show sharp phase transitions reflecting strong cooperativity, as previously observed in calorimetric [9], spin label [15] and Raman [16] measurements. If small vesicles are present, their size limits the cooperativity of the transitions [15].

The 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC)-water bilayers chosen for investigation have the advantage of being well defined by previous work [2,5,6,17]. In the present studies the following three different phases of this system occur (nomenclature of Luzzati [1] and Tardieu et al. [2]): the lamellar phase $L_{\beta'}$ below 35° C (the so-called pretransition), the $P_{\beta'}$ phase between 35 and 42° C [2,17] with a two-dimensional lattice characterized by a ripple structure, and the lamellar phase L_{α} above 42° C (chain melting transition).

Materials and Methods

The DPPC was purchased from Fluka and its purity was checked by means of thin-layer chromatography. The bilayers were prepared in a similar manner to that followed by Janiak et al. [17]. According to these authors the maximum hydration of DPPC is 25 wt.% water at 20 and 39° C and 40 wt.% at 50° C. In the present case, in order to avoid the presence of bulk water at every temperature, 25 wt.% was chosen. The DPPC was added to the appropriate amount of water in a glass tube having two parts separated by a narrow constriction. After sealing the tube, the material was centrifuged eight times at a temperature above the phase transition to and fro through the constriction, thus ensuring a homogeneous mixture.

The measuring technique and the construction of the parallel plated stainless steel cell with a Perspex spacer fitting directly on to the terminals of a Boonton 33A bridge have been previously described [18]. In the present experiments the condenser plates were 0.15 cm apart and thus with a sample area of 0.5 cm^2 the amount of lipid required was about 60 mg. The cell was packed as full as possible with the bilayer material and the liquid filling holes sealed. Water and ethanol were used as standardising liquids of known permittivity. Since the conductivity at low frequency of the bilayers used was very small,

any errors due to electrode polarization in the frequency range used were negligible. Also no appreciable correction for inductive effects up to 50 MHz was found to be required. In the conventional manner, however, a small correction $2\sigma/f$ has been subtracted from the measured loss factor at each frequency f to allow for the effect of the ionic conductivity σ (as obtained by extrapolating the kHz values to very low frequency). Estimated relative experimental errors in the permittivity ϵ' and loss factor ϵ'' were about 1.5 and 3%, respectively. Absolute errors may be somewhat more due to incomplete filling of the cell.

Results

In a first experiment, the permittivity ϵ' and conductivity σ of the DPPC/H_2O mixture (75 wt.% lipid, 25 wt.% H_2O) were measured at 50 MHz as the temperature is slowly raised and lowered, waiting long enough at each temperature for stability to be reached. The experimental results, given in Figs. 1 and 2 show that the pre-transition at 35° C and the main transition at 42° C are clearly reflected by the dielectric behaviour. Both ϵ' and σ have sharp increases at the transition temperatures and the overall changes are relatively large, for example ϵ' is seen to almost double in value between 15 and 60° C. It is also interesting to note the hysteresis seen for ϵ' and σ in the pre-transition and the slight hysteresis in σ for the main transition.

These effects were now further investigated by experiments over a range of frequencies. The underlying dispersion which develops at 5 MHz or more as temperature is raised is illustrated in Fig. 3. To make a first approximate analysis, two Debye dispersions, one at low frequency and the other in the MHz range, are assumed. Thus

$$\epsilon' = \frac{A_1}{1 + (f/f_1)^2} + \frac{A_2}{1 + (f/f_2)^2} + \epsilon_\infty \tag{1}$$

and

$$\epsilon'' = \frac{A_1(f/f_1)}{1 + (f/f_1)^2} + \frac{A_2(f/f_2)}{1 + (f/f_2)^2} \tag{2}$$

A least-squares fit to the measured ϵ' and ϵ'' values then gives the relaxation frequency f_2 and the amplitude A_2 of the high frequency dispersion at each temperature, as shown in Figs. 4 and 5 (measured as the temperature is increased). ϵ_∞ is the permittivity of the system at frequencies much higher than the relaxation frequencies f_1 and f_2. For such a mixture of lipids and bound water it has been estimated to be approx. 4 and clamped at this value. The transition profiles of f_2 and A_2 in these graphs define the overall behaviour of the zwitterionic dispersion with temperature, whereas the form of the transition profiles measured at one particular frequency, as shown in Figs. 1 and 2, is somewhat dependent on the position of the frequency in the dispersion region. The small scatter of the individual points in Figs. 4 and 5 derived from the least squares fit shows that this dispersion can be well determined above 30° C in the frequency range investigated and that the overlap from the low frequency dispersion is adequately approximated by the first Debye term in Eqn. 1.

Measurements were also made with 12.5 wt.% H_2O and 12.5 wt.% ethylene

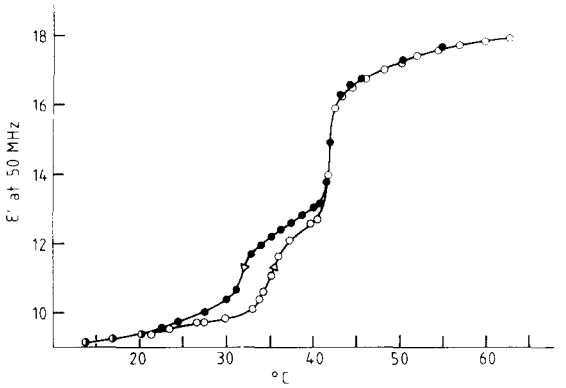

Fig. 1. Variation of permittivity ϵ' at 50 MHz for DPPC/25 wt.% H_2O. ○, increasing temperature; ●, decreasing temperature.

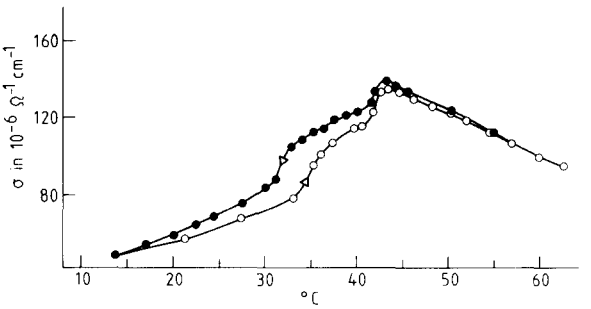

Fig. 2. Changes in conductivity σ at 50 MHz for DPPC/25 wt.% H_2O. ○, increasing temperature; ●, decreasing temperature.

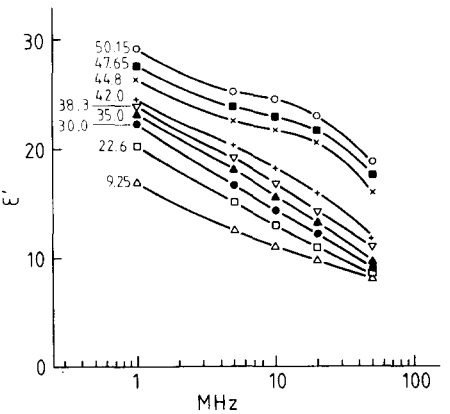

Fig. 3. Dispersion of DPPC/25 wt.% H_2O in MHz range as temperature is raised.

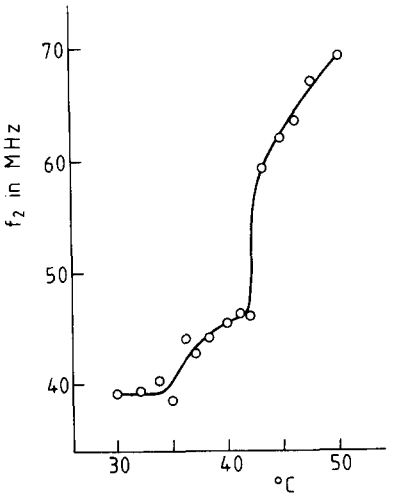

Fig. 4. Increase of relaxation frequency f_2 with rising temperature for the MHz dispersion.

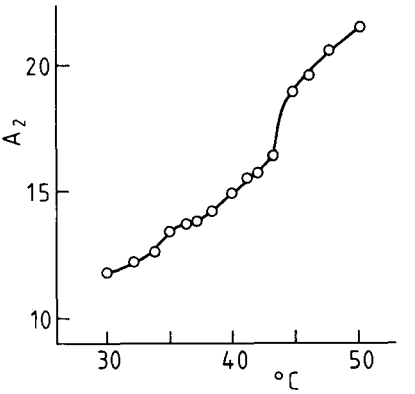

Fig. 5. Change with rising temperature of amplitude A_2 for the MHz dispersion.

glycol added to 75 wt.% DPPC in order to increase the viscosity of the fluid layers. In this case the relaxation frequencies associated with the dipolar head dispersion were found to be lower than those given with H_2O (e.g. at 30 and 50° C by factors of 2.1 and 1.3, respectively).

Discussion

Neutron scattering experiments in the gel phases ($L_{\beta'}$, $P_{\beta'}$) as well as in the liquid crystalline phase (L_α) showed the average orientation of the phosphocholine group of DPPC to be nearly parallel to the bilayer surface [5]. In addition, ^2H and ^{31}P NMR data in the L_α phase suggest that the orientation as well as the rotational motion of this group is predominantly parallel to the bilayer plane [19,20]. Our dielectric results also support these conclusions for when additional measurements were performed with a smaller sample thickness (0.5 mm instead of 1.5 mm) the permittivity values measures were found to be reduced (e.g. 27% reduction at 1 MHz and 30° C). This is considered to be due to the increased orientation of the individual multilayer stacks parallel to the condenser plates and consequent reduced polarization in the direction of the applied field (see the discussion on the orientation factor B in Appendix).

To obtain a better picture of the possibilities for rotation of the phosphocholine head groups in the plane of the bilayer, molecular models were constructed with the lipids arranged in a hexagonal lattice. For the $L_{\beta'}$ phase the area per molecule was taken to be 48.6 Å^2 [2] and for the L_α phase 57.0 Å^2. (This latter value was deduced from the variation in bilayer thickness between these phases as determined by neutron diffraction [5] but taking into account the small volume change of 3.9% [21].) The C(1)-C(2) bonds of the glycerol backbone (C(1) next to the phosphate group) were arranged perpendicular to the plane of the bilayer (which at least is very probable for the L_α phase

[19]). Very close packing and a large extent of steric hindrance are seen in the model for the $L_{\beta'}$ phase (Fig. 6a) and somewhat less for the L_α phase (Fig. 6b).

In view of these considerations, the present dispersion in the MHz region will be evaluated as a first approximation making the following simplifying assumptions: (a) that the zwitterionic dipole rotates as a unit in the plane of the bilayer; (b) that for the short time intervals in which the applied field alternates each dipole's motion is limited by steric hindrances to an angle 2ϕ in the bilayer surface. Many considerations have been overlooked in this simple treatment, for example: the fact that the head group is not a rigid unit but that rotation about all its bonds is possible [19]; the extent to which the movements of the head and tail groups of the lipids may be coupled (cf. Budo's theory for molecules with groups having relative rotation [22]); and also any detailed considerations of inter-molecular interactions. With these assumptions, the mean dipole $\bar{\mu}$ induced in the direction of the internal directing field F can be shown to be given by

$$\bar{\mu} = B\left(1 - \frac{\sin^2\phi}{\phi^2}\right)\left(\frac{\mu^2 F}{2\,kT}\right) = B\gamma\left(\frac{\mu^2 F}{2\,kT}\right) \tag{3}$$

where μ is the dipole of the head group, k Boltzmann's constant, T the absolute temperature and B an orientation factor for the multilayer stacks relative to the condenser plates (see Appendix). By means of X-ray diffraction, B has been estimated to be 0.52 for the sample thickness of 1.5 mm (for fully random orientation $B = 2/3$). No variation of B with temperature was observed. With regard to the rotational limitation, if a distribution of angles ϕ is assumed as seems likely, then the angle ϕ now discussed will correspond to the mean value of the restriction factor γ (see Appendix).

To relate the amplitude A_2 to the dipole μ, Kirkwood's equation for solutions of highly polar molecules [23] can (from Eqn. 3) be modified to the form

$$g^{1/2}\mu = 10^{18}(1000\,kT/2\pi N_A)^{1/2}(A_2/c)^{1/2}\left(\frac{2}{3B\gamma}\right)^{1/2} \text{ Debye} \tag{4}$$

where g is Kirkwood's correlation factor, N_A Avogadro's number, c the molar concentration of lipids and kT is inserted in ergs. In the case of zwitterionic amino acids in aqueous solution, reasonable values of dipole moment can be deduced from the unmodified equation by taking $g = 1$ [23] and this value will be assumed to be applicable here. Using the known specific volume for DPPC [2], its molarity c in the mixture can be deduced as 1.06 at 30°C and, allowing for the small volume changes [21] going from the $L_{\beta'}$ to the L_α phase, as 1.03 at 45°C. Taking the head group conformation suggested by Seelig et al. [19], the distance between the zwitterionic charges was estimated to be 3.9 Å, giving a dipole moment of 18.7 D. Now inserting these values in Eqn. 4, the mean restriction factor γ and the corresponding angle ϕ can be calculated from the amplitude A_2 at temperature T. At 30, 45 and 50°C, the angular range of rotation 2ϕ is thus estimated to be 150°, 230° and 270°, respectively. With regard to experimental errors, allowing a possible uncertainty in A_2 of ± 10%, we deduce the corresponding errors in 2ϕ to range up to a maximum value of 30° at 50°C.

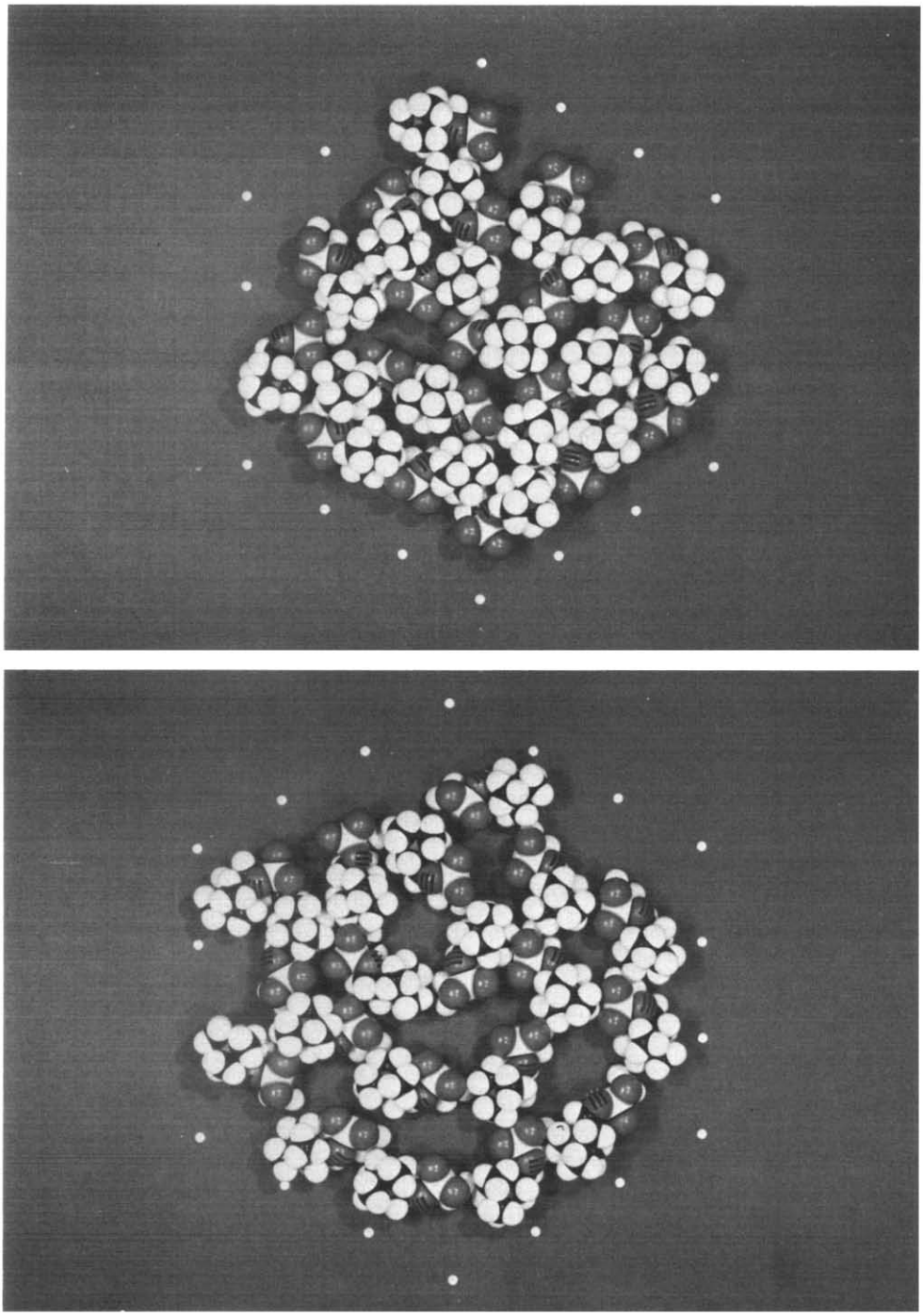

Fig. 6. Models for the arrangement of the phosphocholine head groups on a hexagonal lattice. The dipoles are oriented almost parallel to the bilayer surface with the C(1)-C(2) bonds of the glycerol backbone at right angles to this plane. The outer hexagon of white spots give the positions of the C(1)-C(2) bond axes for the neighbouring lipids. (a) $L_{\beta'}$ phase, area per head group, 48.3 $Å^2$. (b) L_α phase, area per head group, 57.0 $Å^2$.

The values, however, seem sufficiently accurate to indicate that a small degree of restriction still remains at 50° C, which would only disappear at still higher temperatures. Considering the close packing illustrated in Fig. 6 both below and above the phase transitions, the high degrees of freedom now found seem only possible if there is a considerable amount of conformational disorder in the head groups, and if the rotation is coupled with lateral diffusion of defects and molecules. Defects could, for example, be formed by a few head groups changing conformation so as to protrude out of the plane of the bilayer, or by variations in the lateral hexagonal packing.

The form of the amplitude versus temperature profile in Fig. 5 (contrasting with the decrease of amplitude with temperature for the dispersion of polar molecules in solution) can thus be interpreted in terms of an increase in the angular range of rotation 2ϕ corresponding to the increase in area per lipid molecule during the phase transitions. This area increase will inversely reflect the sharp bilayer thickness decrease [17] at the main phase transition, since the total volume increase of a lipid-water system at this phase transition is relatively small [21]. In addition, as more area becomes available, the rotational frictional coefficient becomes less, due to decreased interactions with neighbouring molecules, and the relaxation time shows a sharp decrease. This is reflected in Fig. 4, where the relaxation time decreases from about 4 ns at 30° C to 2.3 ns at 50° C ($1/\tau = 2\pi f$). The time of 2.3 ns is in good agreement with a correlation time of 1.4 ns observed in the nuclear magnetic resonance experiments on ^{31}P in the head groups of egg phosphatidylcholine vesicles above the main phase transition [24]. The present τ value reflects the motion of the whole phosphocholine group and is two orders of magnitude greater than the correlation time found by deuterium magnetic resonance for the more rapid rotational motion of the choline methyl groups [25]. The molecular weight of the phosphocholine head group is 182.2 and it is interesting to compare the present relaxation times with those of a zwitterionic molecule such as triglycine of similar molecular weight (189) in aqueous solution, 0.2 and 0.17 ns at 30 and 40° C, respectively [26]. It is evident that the restriction to motion in one plane (see Appendix), intermolecular interactions, and possible coupling between head and tail groups of the lipid have increased the relaxation times by roughly one order of magnitude.

Some remaining information can be extracted by regarding the dielectric relaxation as a rate process and assuming that the Eyring equation

$$\tau = \frac{h}{kT} \exp(\Delta G^*/RT) \tag{5}$$

$$= \frac{h}{kT} \exp(-\Delta S^*/R) \exp(\Delta H^*/RT)$$

is applicable. Normally ΔS^* and ΔH^* can be taken to be independent of temperature. A linear plot of ln $(T\tau)$ versus $1/T$ is then obtained and ΔH^* can be evaluated from the slope. In the present case, however, we obtain two distinct lines below and above the main phase transition at 42° C (Fig. 7). At the pre-transition (35° C) no clear break can be seen, owing to higher experi-

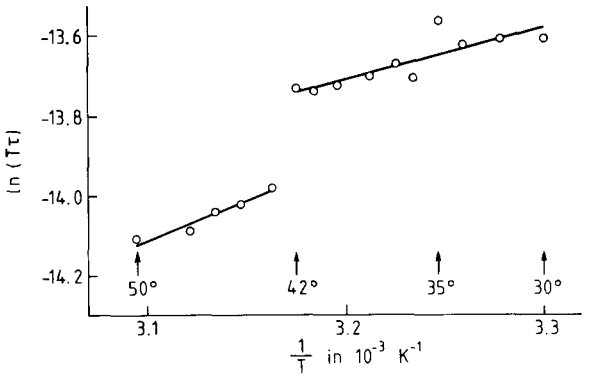

Fig. 7. Plot of ln ($T\tau$) against $1/T$, where τ in S is the relaxation time corresponding to the f_2 values in Fig. 4 ($1/\tau = 2\pi f_2$) and T the absolute temperature in K.

mental errors in this region. Least squares fits to these lines give ΔH^* values of 2.6 and 4.0 kcal/mol (10.7 and 16.6 J/mol) below and above 42° C, respectively. These are of an order of magnitude found for small molecules in water, but the significance of the difference between these values cannot as yet be seen. An interesting comparison can also be made with the value of 3.7 kcal/mol found for the above-mentioned motion of the choline methyl groups.

Further experiments on bilayers of other lipids with varying lengths of hydrocarbon chains and different head groups are now in progress. Thus, for example, fully hydrated 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine bilayers have been investigated and the pre- and main transitions seen clearly at 50 MHz to occur at 11 and 23° C. The method has also advantages for studies of lipid head group mobility in other circumstances, for instance in the presence of cholesterol or proteins in the membrane, or of ions in the neighbouring water.

Appendix

We consider all the bilayer surfaces such as S_θ with normals making an angle θ with the internal directing field F (Fig. 8a), and suppose that there are $m\delta x$ dipoles μ in these planes centred at angles between x and $x + \delta x$ to the field component F' ($= F\sin\theta$) in the plane and limited by steric hindrances to orientation within an angle ϕ on each side of OC (Fig. 8b). For a much lower frequency of directing field, it may well be that the dipoles have time to assume any orientation in the plane, so removing this angular limitation and producing additional polarization and a corresponding dispersion. This effect, however, need not be considered for the MHz field applied, and hence it can be taken that m is independent of angle x. Thus

$$n_\theta = 2\pi m \tag{A1}$$

where n_θ is the total number of dipoles in the planes S_θ. Assuming a Boltzman distribution for the $m\delta x$ dipoles in the 2 ϕ sector

$$m\delta x = \int_{x-\phi}^{x+\phi} A \exp(\alpha \cos \psi)\, d\psi \tag{A2}$$

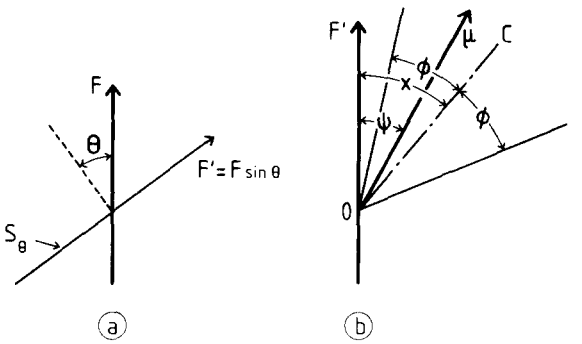

Fig. 8. (a) Side view of bilayer surface S_θ relative to directing field F. (b) Diagram drawn in the plane S_θ showing the angles x, ϕ and ψ.

where $\alpha = \mu F'/kT$ and A is a normalizing factor. Integrating Eqn. A2, for the small fields applied where $\mu F << kT$ ($\alpha \approx 10^{-6}$ or less), it is found that

$$A = \frac{m\delta x}{2(\phi + \alpha \cos x \sin \phi)} \tag{A3}$$

The mean dipole moment $\bar{\mu}_\theta$ in the direction of the field F' of all the dipoles in the planes S_θ is now given by

$$\bar{\mu}_\theta = \frac{1}{n_\theta} \int_0^{2\pi} \int_{x-\phi}^{x+\phi} \left\{ \frac{m \exp(\alpha \cos \psi)\, \mu \cos \psi}{2(\phi + \alpha \cos x \sin \phi)} \right\} d\psi\, dx \tag{A4}$$

By integration we obtain (again neglecting second-order terms in α)

$$\bar{\mu}_\theta = \frac{\mu\alpha}{2}\left(1 - \frac{\sin^2\phi}{\phi^2}\right) = \left(\frac{\mu^2 F \sin\theta}{2\,kT}\right)\gamma \tag{A5}$$

where

$$\gamma = 1 - \frac{\sin^2\phi}{\phi^2} \tag{A6}$$

Now let us suppose that n_θ is given by $\beta(\theta)\delta\Omega$ or $\beta(\theta)\,(2\pi \sin\theta\delta\theta)$, where $\beta(\theta)$ is a distribution function depending on the degree of orientation of the multilayer stacks parallel to the condenser plates. Then the total mean dipole in the field direction F may be expressed as

$$\bar{\mu} = \frac{\int \beta(\theta)\, \bar{\mu}_\theta \sin\theta\, d\Omega}{\int \beta(\theta)\, d\Omega} = \left(\frac{\mu^2 F}{2\,kT}\right)\gamma \frac{\int_0^{\pi/2} \beta(\theta) \sin^3\theta\, d\theta}{\int_0^{\pi/2} \beta(\theta) \sin\theta\, d\theta} = \left(\frac{\mu^2 F}{2\,kT}\right)\gamma B \tag{A7}$$

For a distribution of angles ϕ amongst the molecules, if n_ϕ is the number having

angles between ϕ and $\phi + \delta\phi$

$$\bar{\gamma} = \frac{\int_0^{\pi} n_\phi \gamma \mathrm{d}\phi}{\int_0^{\pi} n_\phi \, \mathrm{d}\phi} \tag{A8}$$

To avoid introducing additional parameters, no particular distribution will be here assumed. The ϕ value deduced will correspond to this mean restriction factor $\bar{\gamma}$. The second factor B depends only on the relative orientations of the bilayers to the condenser plates. For random orientations of the bilayers, B is seen by integration of Eqn. A7 to be 2/3, and with no angular restriction in the bilayer planes $\phi = \pi$ and $\gamma = 1$. In this case $\bar{\mu}$ reduces to $\mu^2 F/3kT$ as for dipoles with unrestricted orientation in solution. As the distance between the condenser plates decreases, the mean orientation of the multilayer stacks parallel to the plates increases (i.e. B decreases). For the present experiments with a separation of 1.5 mm, $\beta(\theta)$ was evaluated by means of X-ray diffraction in each of the three phases and B then deduced by numerical integration to be 0.52 independent of temperature.

In respect of the relaxation time for the MHz dispersion, the motion of the head groups can be considered on the lines of Budo's theory for the relaxation in solution of molecules containing rotating polar groups [22]. With the present approximations, if the head group dipole rotates in the plane of the bilayer with frictional coefficient ρ, and if the whole molecule were able to rotate about an axis XY parallel to the plane of the bilayer with frictional coefficient ρ_1, then the dielectric relaxation time τ can be shown to be given by

$$\frac{1}{\tau} = kT\left(\frac{1}{\rho} + \frac{1}{\rho_1}\right) \tag{A9}$$

In a bilayer, however, the motion about the axis XY is practically totally hindered. Hence ρ is very large and

$$\frac{1}{\tau} = \frac{kT}{\rho_1} \tag{A10}$$

Thus the relaxation time will tend to be larger than the value to be expected if motion about XY were possible. Also, as already pointed out in the discussion, other factors will lengthen τ as compared to the value to be expected from a head group freely rotating in water. Moreover, a coupling potential function dependent on the head-to-tail angular position about the C(1)-C(2) bond might give rise to several relaxation times [22]. The present data, however, did not justify the use of more parameters in the least squares fit.

Acknowledgements

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