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Brillouin light scattering measurement of the elastic properties of aligned multilamella lipid samples

(dipalmitoyl phosphatidylcholine/cholesterol/sound velocities)

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ABSTRACT Brillouin measurements of the elastic properties are presented for aligned multilamella samples of both pure dipalmitoyl phosphatidylcholine at various temperatures and water concentrations and for the same compound containing 33 mol % cholesterol at various temperatures and two water concentrations. For pure dipalmitoyl phosphatidylcholine the elastic moduli change significantly at the gel transition and the modulus for area compression of individual bilayers is inferred to be an order of magnitude larger below the gel transformation than above. The presence of cholesterol is shown to influence the elastic behavior of dipalmitoyl phosphatidylcholine.

Recent development of a technique for producing large aligned samples (0.1 mm × 1 cm × 1 cm) of the smectic phase of dipalmitoyl phosphatidylcholine (1, 2) (DPPC) facilitates physical measurements that have previously only been possible on simpler systems of no biological relevance, i.e., inorganic crystals, simple fluids, or thermotropic liquid crystals. We report here measurements of bulk elastic properties of pure DPPC at various water concentrations and temperatures and also a mixture of 33 mol % cholesterol in DPPC at two water concentrations and various temperatures. Although it is well known that upon cooling most lipids will undergo a phase transition, between 100° and ~0°, in which they become considerably more rigid, quantitative measurements of this change have not been made (3). Because the temperature dependence of some membrane-associated processes in living systems can be associated with phase transitions in the lipid fraction of these membranes, these measurements are important to their interpretation (3).

The Brillouin scattering technique employed in these measurements has been widely applied to the study of elastic properties of diverse systems (4), including a smectic liquid crystal unrelated to lipids (5). The beam from a single mode argon ion laser is scattered by a thermally excited sound wave into a detection apparatus that defines a scattering angle θ . Laser radiation with free space wavelength λ_0 in a medium with index of refraction n will be scattered by a sound wave with wavelength $\lambda_s = \lambda[2 \sin(\theta/2)]^{-1}$. The frequency shift $\pm\nu_s$ of the scattered light relative to the laser frequency is measured by a four-pass Fabry-Perot interferometer. This defines the frequency of the sound wave. The product $\lambda_s\nu_s$ is equal to the phase velocity of the sound wave. Experimental details, including index of refraction effects and data analysis, will be described in a separate publication. Typically, values of ν_s of the order of 10^{10} Hz are measured to $\pm 10^8$ Hz; however, the smallest value of ν_s that can be measured conveniently is approximately 10^9 Hz. The scattering geometry and sample orientation can be varied to measure the sound speed as a function

of both scattering angle θ and the angle ψ that the sound velocity makes with the normal to the lipid bilayers.

A general phenomenological theory for sound propagation in smectic liquid crystals (6, 7) of the DPPC type (8) predicts two sound waves with speeds that we designate as v_1 and v_3 , for historical reasons, and three elastic constants C_{11} , C_{33} , and C_{13}

$$v_1^2 + v_3^2 = \rho^{-1}[C_{11} + (C_{33} - C_{11}) \cos^2\psi]$$

$$v_1^2 v_3^2 = \rho^{-2}[C_{11}C_{33} - C_{13}^2] \sin^2\psi \cos^2\psi \quad [1]$$

in which ρ is the macroscopic density of the system. The physical significance of these three elastic constants can be understood in terms of the deformations that the lamella phase can be subjected to. The equilibrium state of a lamella phase can be characterized by the density ρ and the lamella repeat distance d that gives rise to the low-angle Bragg reflections observed by x-ray diffraction (3). At a fixed temperature both of these are functions of the force per unit area normal to the lamella, P_N , and an orthogonal force per unit area P that would tend to change the area per lipid molecule A . For an unstressed system $P_N = P =$ atmospheric pressure; however, because of the lamella structure it is possible to stress the system such that $\Phi \equiv P - P_N$ is not zero. The phenomenological theory also predicts that sound propagation occurs at constant entropy and constant water concentration (6, 8). Thus, of the three variables ρ , d , and A mentioned above only two can be taken to be independent $\delta\rho/\rho + \delta d/d + \delta A/A = 0$. Thermodynamics obtains $C_{11}, C_{33} > 0, C_{11}C_{33} - C_{13}^2 > 0$ in which

$$C_{33} = \rho(\partial P_N / \partial \rho)_A = -d(\partial P_N / \partial d)_A \quad [3a]$$

$$C_{11} = \rho(\partial P / \partial \rho)_d = -A(\partial P / \partial A)_d \quad [3b]$$

This can also be manipulated to obtain

$$C_{33} - C_{11} = A(\partial / \partial A)_\rho (P + P_N) \quad [3c]$$

Also

$$C_{11}C_{33} - C_{13}^2 = [\rho(\partial P_N / \partial \rho)_A][A(\partial \Phi / \partial A)_{P_N}] \quad [4a]$$

$$= [\rho(\partial P / \partial \rho)_d][d(\partial \Phi / \partial d)_P] \quad [4b]$$

Identities such as $d(\partial P / \partial d)_\rho = \rho(\partial \Phi / \partial \rho)_d$ can be employed to prove that there are only three independent thermodynamic derivatives (6). It is implicit that these are taken at constant water concentration and entropy. If the lamella structure did not influence the elasticity, P_N would equal P ($\Phi = 0$), the elastic properties would be the same as for an isotropic fluid ($C_{11} =$

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Abbreviation: DPPC, dipalmitoyl phosphatidylcholine.

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$C_{33} = C_{13}$), and the only elastic constant would be the bulk modulus. According to Eq. 1 the product $v_1^2 v_3^2$ would then be zero, indicating only one sound wave whose speed $v_3^2 = \rho^{-1} C_{11}$ would be isotropic (i.e., independent of ψ). In contrast to this, a positive value of $C_{33} - C_{11}$ indicates that the multilamella are more easily compressed laterally than along the normal and the effect of this is that $v_3^2(\psi = 0) > v_3^2(\psi = 90^\circ)$. If $|C_{33} - C_{11}| \ll C_{11}$, $v_1^2 \simeq \rho^{-1}(C_{11}C_{33} - C_{13}^2)C_{33}^{-1} \sin^2\psi \cos^2\psi$ and

$$v_3^2 \simeq \rho^{-1}[C_{11} + (C_{33} - C_{11}) \cos^2\psi] - \rho^{-1}[C_{11}C_{33} - C_{13}^2]C_{33}^{-1} \sin^2\psi \cos^2\psi. \quad [5]$$

If the lateral force per unit area is normalized to the lateral force per bilayer, then $dA(\partial\Phi/\partial A)_{P_N}$ might be compared with either the elasticity of an individual bilayer or two times the surface elastic modulus of monolayers at comparable surface densities (9).

$$-A(\partial\pi/\partial A) \sim d[C_{11}C_{33} - C_{13}^2]C_{33}^{-1}. \quad [6]$$

Finite values of this quantity will be observed as either finite values for v_1^2 or a dip in v_3^2 near $\psi = 45^\circ$. Light scattering from the v_1^2 wave is difficult to detect and for most of our data the best measures of $C_{11}C_{33} - C_{13}^2$ come from observing the dip in v_3^2 .

Because observable elastic constants are generally frequency dependent, it is important to note that these measurements obtain values appropriate to time scales of the order of 10^{-10} sec. Relaxation processes that are slow compared to 10^{-10} sec can cause elastic constants measured at lower frequencies to be smaller than those measured here (10, 11). On the other hand, 10^{-10} sec is not unreasonably fast for many molecular processes and the high-frequency elastic properties of lipids may be relevant to understanding the effects of lipid phase transformations on rapid conformational changes in membrane-associated proteins.

MATERIALS

Cholesterol and synthetic DPPC were obtained from Calbiochem and used without further purification. Techniques for making aligned samples, evaluating sample purity, and determining water content are identical to those described by Powers and Clark (1) for pure DPPC monohydrate and Powers and Pershan (2) for the other samples.

RESULTS

Fig. 1A illustrates the measured sound speeds as a function of ψ in the example where we have the best evidence for elastic anisotropy. With pure DPPC at 2.5 ± 1 wt % water both sound waves could be observed. The solid line represents the best fit to the data and allows the determination $\rho^{-1}C_{11} = 6.05 \pm 0.2$, $\rho^{-1}(C_{33} - C_{11}) = -3.4 \pm 0.1$ in units of 10^{10} cm²/sec² and $\rho^{-2}(C_{11}C_{33} - C_{13}^2) = 10 \pm 2$ in corresponding units. Fig. 1B, on the other hand, illustrates an example for which the evidence is barely sufficient to conclude the existence of anisotropy: 33 mol % cholesterol in DPPC with 25 wt % water at 28°. The bulk modulus is accurately determined $\rho^{-1}C_{11} = 3.15 \pm 0.13$; however, the relative errors are larger for $\rho^{-1}(C_{33} - C_{11}) \simeq 0.04 \pm 0.1$ and $\rho^{-2}(C_{11}C_{33} - C_{13}^2) \simeq 4 \pm 3$. If we take $\rho \sim 1$ g cm⁻³ and $d \sim 50$ Å, Eq. 6 obtains $-A(\partial\pi/\partial A) \sim (6.5 \pm 5) \times 10^3$ dyne cm⁻¹. (1 dyne = 10^{-5} N). The uncertainties assigned to the above measurements indicate the maximum range of values that could reasonably fit the data. The most probable value for $-A(\partial\pi/\partial A) \sim 6.5 \times 10^3$ dyne cm⁻¹ is more than an order of magnitude larger than generally accepted values of

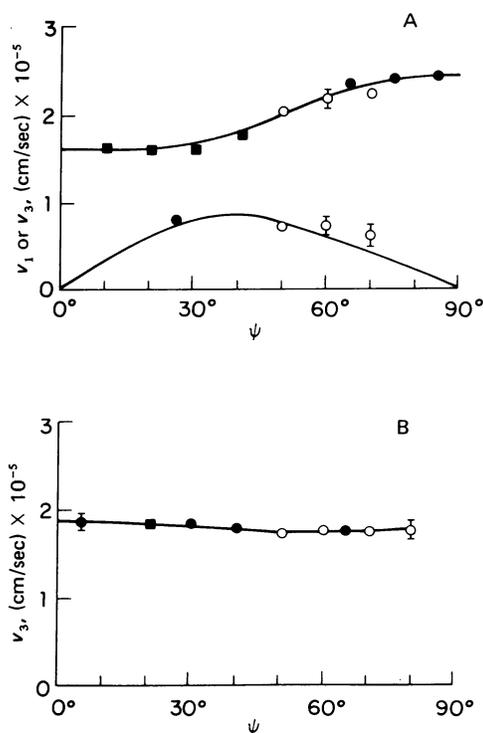


FIG. 1. Examples of sound speeds as a function of the angle ψ between the propagation direction and the lamella normal. Typical error bars are shown for representative points. (A) DPPC with 2.5 wt % water at 23°; (B) DPPC + 33 mol % cholesterol with 25 wt % water at 28°. Scattering angles θ : 60°, ○; 90°, ●; 120°, ■.

the area compressibility modulus for lipid bilayers (9, 12, 13). Even the lowest possible value of 1.5×10^3 dyne cm⁻¹, which is very unlikely, is more than 4 times larger than what is expected for fluid bilayers. We will comment on this comparison later; however, for now we simply point out that the smallest values of $C_{11}C_{33} - C_{13}^2$ that we can measure are so much greater than what is expected for fluid bilayers that a nonzero determination, even with large relative errors, has physical significance.

Fig. 2 illustrates the results of our measurements for $\rho^{-1}C_{11}$ as a function of temperature. For pure DPPC above the gel transition (3) the bulk modulus at 13% water is identical to the higher water values. Other data not shown here are consistent with the conclusion that the bulk modulus is independent of water in this region of the phase diagram (2, 3). Below the gel transition and at low water the system is significantly more rigid. A clear break in the temperature dependence is observed to coincide with the gel transition temperature ($\sim 41^\circ$) at high water (3). On the other hand, the presence of 33 mol % cholesterol suppresses any evidence of this transition and, as expected, the cholesterol stiffens the system above the gel and softens it below (14–16). The effects of lowering the water content are comparable with or without the cholesterol.

The elastic anisotropy specified by the difference $C_{33} - C_{11}$ is illustrated in Fig. 3 for both 33 mol % cholesterol in DPPC and for pure DPPC as a function of temperature and for different water contents. For pure DPPC at high water ($\geq 20\%$) and above the gel temperature ($\sim 41^\circ$) this anisotropy is too small to measure by our experiment, $|C_{33} - C_{11}| \lesssim 10^9$ dyne cm⁻². Below the gel at high water the system is measurably stiffer against compression of the lamella spacing at constant area than against area compression at fixed spacing. If $C_{33} - C_{11}$ is normalized to obtain an elastic anisotropy per lipid bi-

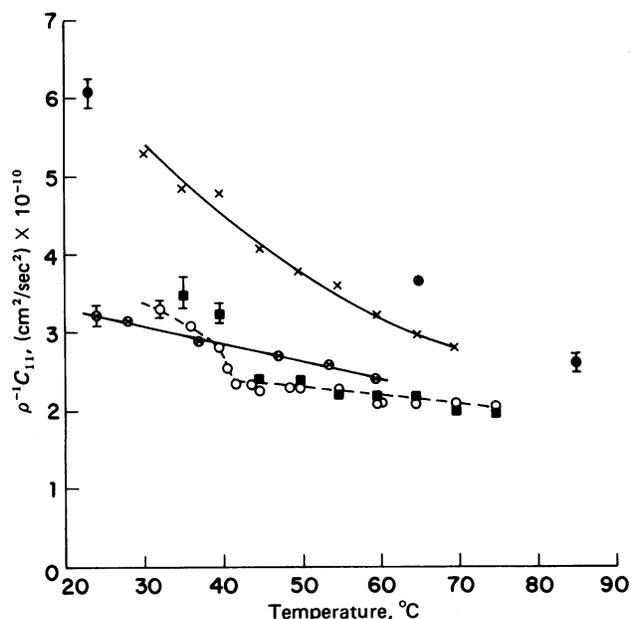


FIG. 2. Values of $\rho^{-1}C_{11}$ versus temperature for 33 mol % cholesterol in DPPC at 5 wt % water (X) and 25 wt % water (O). Also given are values for pure DPPC at 2.5 wt % water (●), 13 wt % water (■), and 25–30 wt % water (○). Typical errors are indicated for some representative points. The solid lines drawn through the cholesterol data and the broken line through the high water data for pure DPPC are simply guides for the eye.

layer, typical values below the gel transition are of the order of $1000 \text{ dyne cm}^{-1}$ and above the gel they are less than 500 dyne cm^{-1} . In the cholesterol sample there is no clear evidence for a gel transition, and even for high water the anisotropy is measurably different from zero above 41° . The effect of cholesterol is to stiffen the system, but the most interesting aspect of these data is that the effect of lower water on the pure DPPC is strikingly opposite to the effect on the cholesterol sample. The original data for low-water DPPC at 23° were shown in Fig. 1A, and we assert that the sign of this anisotropy is unambiguous, $C_{33} - C_{11} < 0$. This effect is just what would be expected from a model in which at low water the polar head groups of pure DPPC form a rigid two-dimensional network that stiffens the system against compression of the area per polar head group

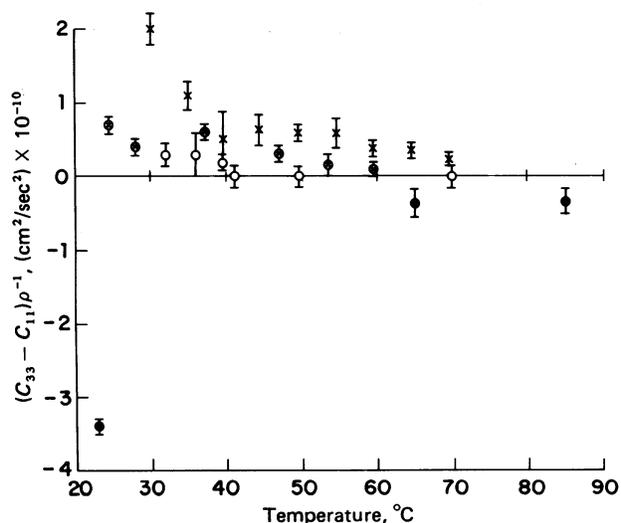


FIG. 3. Values of $(C_{33} - C_{11})\rho^{-1}$ versus temperature for the same samples as in Fig. 2. The symbols are the same as for Fig. 2.

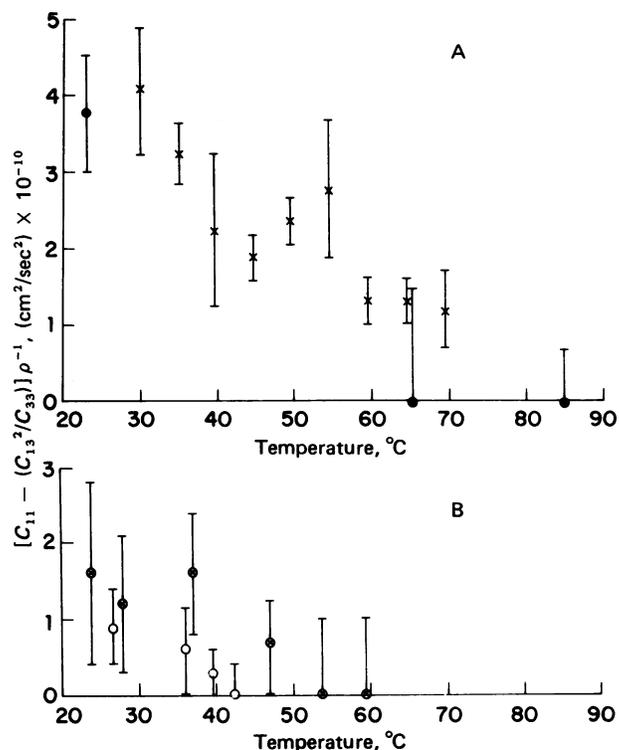


FIG. 4. Values of $[(C_{11} - C_{13}^2/C_{33})\rho^{-1}]$ as a function of temperature for (A) pure DPPC with 2.5 wt % water (●) and 33 mol % cholesterol in DPPC with 5 wt % water (X), and (B) pure DPPC (O) and 33 mol % cholesterol containing approximately 25 wt % water (⊙).

(9, 17–20). It is reasonable to speculate that although 33 mol % cholesterol can stiffen the DPPC system through its effect on the packing of the hydrocarbon region, it will disrupt the two-dimensional head group network sufficiently to suppress the large negative value of $C_{33} - C_{11}$ in low-water pure DPPC.

Actually, this type of argument must be used with some caution. From Eq. 3c one sees that negative values only indicate that the area per lipid molecule will decrease in response to an average pressure increase if the density is held fixed. From $C_{33} - C_{11}$ alone we do not know what anisotropic forces must be applied to maintain constant density. A better criterion for evaluating the modulus opposing area changes is the quantity shown in Fig. 4, $[C_{11} - (C_{13}^2/C_{33})]\rho^{-1}$, Eq. 4 or Eq. 6.

In pure DPPC at high water and for temperatures $\geq 41^\circ$, $[C_{11} - (C_{13}^2/C_{33})]$ is unmeasurable or less than $5 \times 10^9 \text{ dyne cm}^{-2}$. Below 41° the data indicate values that are greater than this with reasonable probability. For 33 mol % cholesterol in DPPC at high water and temperatures $\leq 50^\circ$, the case for finite values of $[C_{11} - (C_{13}^2/C_{33})]\rho^{-1}$ is even stronger. These might be compared with a typical value for the modulus of area compressibility of membranes taken from the work of Evans *et al.* (13), $\sim 300 \text{ dyne cm}^{-1}$. Experiments on lipid monolayers also yield results that can be used to obtain estimates of similar magnitude for bilayers (9, 12). If we consider a bilayer, together with its water layer, to be approximately 50 \AA thick, this translates into a bulk modulus of the order of $6 \times 10^8 \text{ dyne cm}^{-1}$. We suggest this is an appropriate value for $C_{11} - (C_{13}^2/C_{33})$ in pure DPPC above the gel transition, where we were unable to measure a nonzero value. Below the gel transition, measurements of the area compressibility for either membranes or monolayers are less reliable. Horn and Gershfeld (21) maintain that lipid monolayers are at best metastable for

surface pressures in excess of 0.1 dyne cm^{-1} , and we do not believe meaningful data exist for lipid bilayers below the gel transition. The data shown in Fig. 4 indicate that, below the gel transition, moduli of area compressibility are at least an order of magnitude stiffer than values appropriate to the more highly fluid systems above the gel.

Interestingly, the removal of water considerably stiffens the modulus of area compressibility in both pure DPPC and 33 mol % cholesterol. Fig. 4A indicates that near room temperature $C_{11} - (C_{13}^2/C_{33})$ has approximately the same value in both materials. Although Fig. 2 indicates that they also have approximately equal values for C_{11} , from Fig. 3 we see they have very different values for C_{33} . The implication is that both pure DPPC and the cholesterol sample have comparable values of elastic modulus opposing area changes regardless of whether the change is at fixed normal pressure or fixed density. If, however, the change is at fixed density, the normal force P_N that must be exerted to maintain constant density is considerably larger for the cholesterol sample than for the pure DPPC sample. This is easily seen from Eq. 3c. In the absence of elastic coupling between area and density, $A(\partial P/\partial A)_\rho$ would be negative and $A(\partial P_N/\partial A)_\rho$ would be zero, yielding a negative value for $C_{33} - C_{11}$. Because pure DPPC has a large negative value for this quantity, we can infer the area-density coupling is weaker in pure DPPC than in the cholesterol sample. This is the proper argument in support of a two-dimensional network for the polar groups of pure DPPC.

DISCUSSION

In this paper we have described physical measurements on aligned multilamella samples of DPPC and DPPC plus cholesterol at various water concentrations. It is reasonable to ask whether the physical properties of lipid bilayers in these samples are sufficiently similar to the physical properties of membrane lipids for the study on the multilamella to be relevant to membrane problems. This must ultimately be answered by experiment, and one purpose of this paper has been to demonstrate that experimental techniques previously applied by physicists to crystals and liquid crystals can be applied to lipid systems. The principal new physical result reported is a quantitative measure of the elastic rigidity of lipid systems below the gel transition and at lower water concentration. If we can assume that at 25% water the macroscopic elastic anisotropy in the multilamella system derives from the microscopic elasticity of individual bilayers, the data in Fig. 4 indicate that below the gel transition the bilayer modulus of area compressibility is probably an order of magnitude larger than the values obtained above the gel transition. The elastic anisotropy also supports a model in which the polar head groups form hydrogen-bonded two-dimensional networks at low water in pure DPPC. The effect of cholesterol appears to be to disrupt this network.

The principle shortcomings in the present measurements are:

(i) For pure DPPC more measurements need to be taken to map

out the very gross changes in elasticity that occur between 23° and 65° for water concentrations between 2.5% and 13%. (ii) Concentrations of cholesterol other than 33% should be investigated. A variety of different approaches to studying the cholesterol-DPPC-water phase diagram have been taken, and it is possible that elastic measurements will supplement other data (14-16, 22, 23). (iii) The Brillouin technique is limited to high-frequency sound waves and corresponding low resolution of elastic constants. Techniques should be developed to measure lower frequency elasticity to higher precision (11).

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