brief communication

Viscoelastic relaxation of bilayer lipid membranes

II. Temperature dependence of relaxation time

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ABSTRACT Recent studies have demonstrated the high frequency exponential relaxation of a viscoelastic modulus of lipid membranes. It is shown that the temperature dependence of the relaxation time can be extracted from published data on the relevant modulus for solvent-free bilayer lipid membranes of glycerol monoöleate. The relaxation is fastest close to the membrane transition temperature, where the time scale is ~20 μs. Possible causes of the transitional minimum of this time scale are discussed.

INTRODUCTION

Quasi-elastic light scattering from thermally excited capillary waves upon lipid membranes has recently been used to study the physical properties of such membranes (1, 2, 3). The capillary waves are governed by a membrane viscoelastic modulus, γ (comprising elastic and viscous portions), relating to shear stress normal to the membrane plane (the elastic part is equivalent to the membrane tension). In these studies, the viscoelastic relaxation of this stress has been observed (2). It is shown here that, despite previous doubts (2), the temperature dependence of the single exponential relaxation time can be extracted from observations of waves of a single wave-number. The relaxation is found to be fastest at the membrane transition.

For both bilayer lipid membranes (BLM) and fully compressed monolayers of glycerol monoöleate (GMO) the frequency dependence of the elastic and viscous portions of γ has been observed explicitly. In solvent-free bilayers at room temperature the time scale for relaxation is ~37 μs (2). Fully compressed monolayers of GMO at the air-water interface similarly show a single exponential relaxation process, of time scale ~9 μs (3). In the monolayer case the viscoelastic relaxation speeded up exponentially as the film was expanded from the fully compressed state (3); at all molecular areas only one relaxation process was apparent.

A recent study of the transitions of solvent-free BLM of GMO (1) has provided much of the information required to determine the temperature dependence of this relaxation. This experiment involved observation of fluctuations of a single fixed wave-number (q), yielding the frequency of these fluctuations as well as the appropriate values of the membrane tension and viscosity as functions of temperature. Provided that one process dominates the relaxation phenomena the relaxation time can be extracted from observations of fluctuations of a single frequency (3). For this analysis it is necessary to know the increase in the elastic part of the modulus over its low frequency, equilibrium value and the membrane viscosity. The data from Reference (1), covering a range 7 < T < 27°C ($T_t \sim 16.6$°C), are here used to infer the temperature variation of the time scale of the viscoelastic relaxation of γ for the case of solvent-free monolein BLM.

THEORETICAL BACKGROUND

In contrast to conventional rheological studies (4), which involve oscillatory stress of fixed frequency, these light scattering experiments involved (1) capillary waves of a selected q, their temporal evolution being observed by photon correlation. From the measured correlation functions the complex frequency ($\omega = \omega_0 + i\Gamma$) of the waves was estimated. This frequency is related to the wavenumber q via the appropriate dispersion equation (5), involving the physical properties of the system. For an interface such as a BLM these properties comprise the density and viscosity of the ambient fluid and one membrane viscoelastic modulus γ ($= \gamma_0 + i\omega\gamma'$). In particular the wave damping arises from dissipation in both the aqueous medium (due to its viscosity) and the BLM itself (due to the membrane viscosity γ'). Assuming that the ambient fluid properties have their accepted values, the dispersion equation can be solved, using the experimental values of ω_0 and Γ, to yield the only two unknowns: the BLM tension, γ_0, and the transverse shear membrane viscosity, γ'. The values thus determined are those appropriate to fluctuations of frequency ω_0 (2). It is worth reiterating that these BLM properties are hydrodynamically well defined quantities, referring to a specific membrane stress. In particular the viscosity involved is not the usual membrane viscosity, which relates to shear stress in the plane of the membrane.

Relaxation processes may affect the membrane mod-

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ulus γ, causing both the elastic and viscous portions to be frequency dependent. Following a usual convention from linear rheology (4) the oscillatory stress $T(t) = T^* e^{i\omega t}$ can be related to the strain $u(t) = u^* e^{i\omega t}$ as

$$T^* e^{i\omega t} = G^*(\omega) u^* e^{i\omega t}, \tag{1}$$

where the dynamic modulus can be expressed in terms of a storage modulus $G'(\omega)$ and a loss modulus $G''(\omega)$:

$$G^*(\omega) = G'(\omega) + iG''(\omega). \tag{2}$$

There is as yet no theoretical understanding of such viscoelastic relaxation in membranes, but it has been found, for both BLM and fully compressed monolayers of GMO, that the observed frequency dependences of γ_0 (identified with G') and γ' (identified with G''/ω) are adequately described by a particularly simple model, the Maxwell linear viscoelastic fluid. This derives from a simple exponential damping of the stresses within the system (6), leading to

$$G'(\omega) = G_e + G \frac{\omega^2\tau^2}{1 + \omega^2\tau^2} \tag{3}$$

$$G''(\omega) = G \frac{\omega\tau}{1 + \omega^2\tau^2}, \tag{4}$$

where G_e is the $\omega \longrightarrow 0$ value of the storage modulus, here the equilibrium membrane tension. Thus for the Maxwell fluid the viscosity is maximum for low shear rates ($= G\tau$) and falls when the frequency (ω) of the oscillatory stress $\gtrsim 1/\tau$, being converted into an elastic modulus (which is increased from its low frequency value by G as $\omega \longrightarrow \infty$). In previous studies (2, 3) this frequency dependence was explicitly demonstrated, τ being estimated by fitting γ, measured at different ω, with the forms of Eqs. 3 and 4. However these equations suggest that τ can be estimated from data for a single frequency. Rearranging and combining Eqs. 3 and 4 yields

$$\tau = \frac{G'(\omega) - G_e}{\omega G''(\omega)} = \frac{\Delta\gamma}{\omega_0^2 \gamma'}, \tag{5}$$

where $\Delta\gamma$ ($\equiv \gamma_0(\omega) - \gamma_0(\omega \longrightarrow 0)$) is the difference between the high frequency and the equilibrium values of the tension, and the frequency occurring in the viscoelastic model is taken to be that of the capillary waves observed. Substitution of the τ value thus found into Eqs. 3 or 4 yields an estimate of G.

This method of determining τ and G has been applied to data for monolayers of GMO at the air-water interface (3). Data for several different q values yielded consistent results, demonstrating the validity of this approach. We emphasize the basic assumption: only one exponential relaxation process is involved in the data being analyzed.

RESULTS

Our experimental approach, including data analysis methods, has been described elsewhere (1). The results presented here (from Reference [1]) derive from a single solvent-free BLM, formed from a dispersion of GMO in squalane. The BLM was formed at 25°C, subsequently being cooled quasi-statically to 7°C while light scattering observations were carried out. The analysis outlined above requires measurable values of $\Delta\gamma$ and γ': the first tends to zero for low q (i.e., low frequency), whilst the second decreases at high q (although the precision of determination of γ' increases as q is raised). The present light scattering data are for a single wavenumber, $q = 1{,}275 \text{ cm}^{-1}$, at which intermediate value both $\Delta\gamma$ and γ' are adequately determined (cf. [2]).

The values of ω_0 and Γ as functions of temperature were interpreted in terms of γ_0 and γ' (1). These data were somewhat scattered, and so their averages over 0.5°C intervals are used here (Fig. 1). Values of ω_0, similarly averaged, are shown in Fig. 2. The errors on the average values of γ_0 and ω_0 were much smaller than those upon γ' ($<1\%$ for ω_0 and $\sim 1.5\%$ for γ_0), and are omitted from the relevant figures. These data provide all but one of the pieces of information required to evaluate τ using Eq. 5: only the variation of the equilibrium ($\omega \longrightarrow 0$) BLM tension is missing.

No published data seem to exist for the temperature variation of the equilibrium tension of a bimolecular lipid membrane. Analysis of the frequency dependence of light scattering γ data for a different solvent-free BLM of GMO yielded a value of G_e (the equilibrium tension) of 3.07 mN/m at 24.7°C for GMO (2), agreeing with expectation (7). In the absence of direct evidence on the T variation of the equilibrium tension, we turn to other information (see Discussion). The bilayer tension is related to the tension of an oil-water interface supporting a lipid monolayer. The equilibrium interfacial tension of a solution of GMO in *n*-decane against 0.1 M NaCl increases at 0.034 mN/m/°C between 15 and 30°C (S. H. White, personal communication). No reliable data for GMO dispersed in squalane appear to exist, so we use these GMO/*n*-decane results. Assuming that these data extrapolate to somewhat lower temperatures, they suggest the variation of equilibrium bilayer tension shown in Fig. 1 *a*, the 24.7°C value given above being used to normalize the trend. The extrapolation to temperatures lower than 15°C requires some justification. Published data (8) for GMO in similar solutions tend to show some

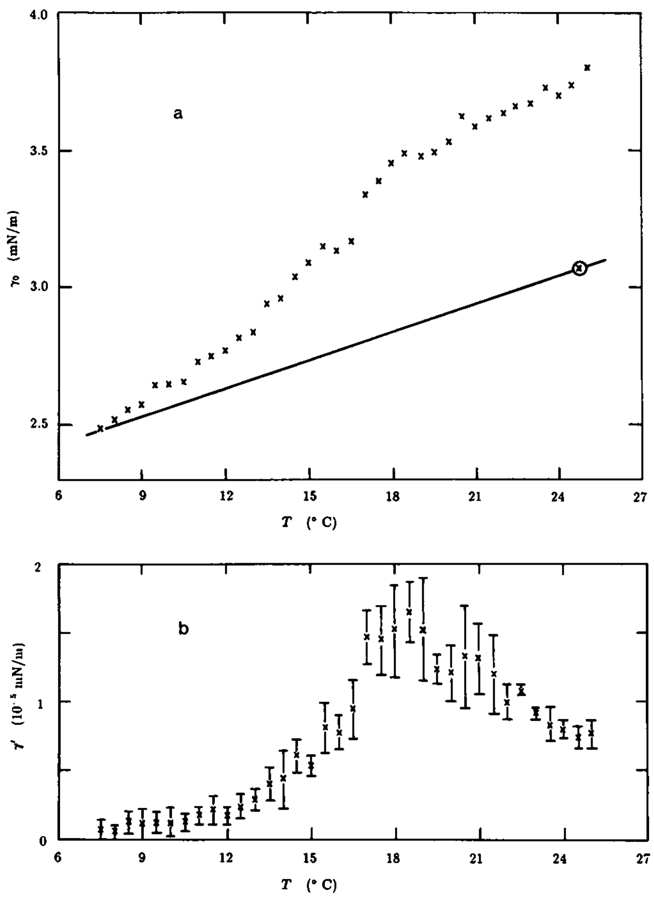

FIGURE 1 (*a*) Average values (x) of the tension of a solvent-free GMO bilayer membrane measured by light scattering from capillary waves of $q = 1{,}275\ \mathrm{cm}^{-1}$. The value of the equilibrium bilayer tension inferred for another, similar, BLM at 24.7°C is shown (⊗), together with the hypothesised temperature variation of the equilibrium tension (———). (*b*) The membrane transverse shear viscosity for the same membrane, averaged over 0.5°C intervals.

small decrease in the gradient of the temperature dependence below about T_t. Such changes in slope would tend to imply equilibrium tensions above the high frequency data at low T: this would, of course, be contrary to accepted viscoelastic ideas (4). The physical plausibility of the presently hypothesised behavior of the equilibrium tension is clear, as it nowhere exceeds the high frequency, light scattering values. This variation for $\gamma_0(\omega \rightarrow 0)$ permits $\Delta\gamma$ to be calculated, enabling τ to be evaluated.

Values of τ found from the data of Figs. 1 and 2 are plotted in Fig. 3 as a function of T. For completeness, the variation of G found from either Eqs. 3 or 4 with these τ data (and the experimental values of $\Delta\gamma$ or γ' as appropriate) are plotted in Fig. 4. Fig. 3 shows that for this BLM, $\tau \sim 37\ \mu s$ at $T = 24.5°C$. The agreement of this τ (found from data for a single wavenumber) with that deduced from the frequency variation of γ for a different solvent-free BLM at this T (2) indicates both the validity of the present analysis, and the reproducibility of these BLM. The form of the variation of τ with T is very striking, despite the fluctuations evident at the lower temperatures. These arise from the small magnitudes, and thus the large fractional errors, of both $\Delta\gamma$ and γ' in that region, making their ratio unreliable (at higher T the accuracy of τ is dominated by the errors upon γ'). In particular, the three small values of $\Delta\gamma$ at the lowest T are

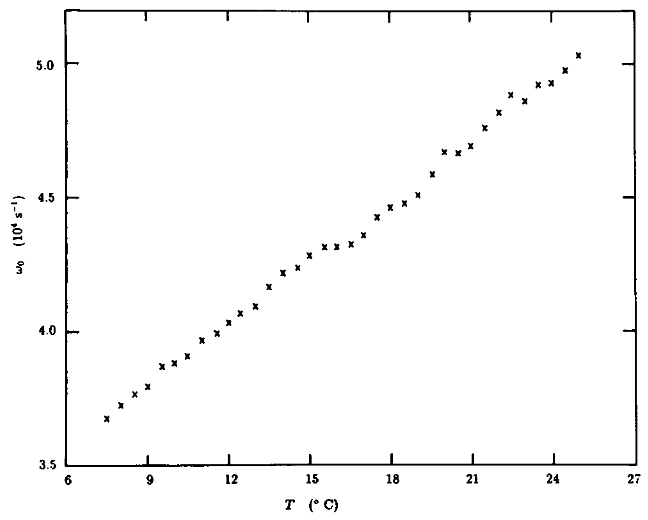

FIGURE 2 The propagation frequencies of capillary waves of $q = 1{,}275\ \mathrm{cm}^{-1}$ upon the GMO bilayer of Fig. 1, averaged over 0.5°C intervals.

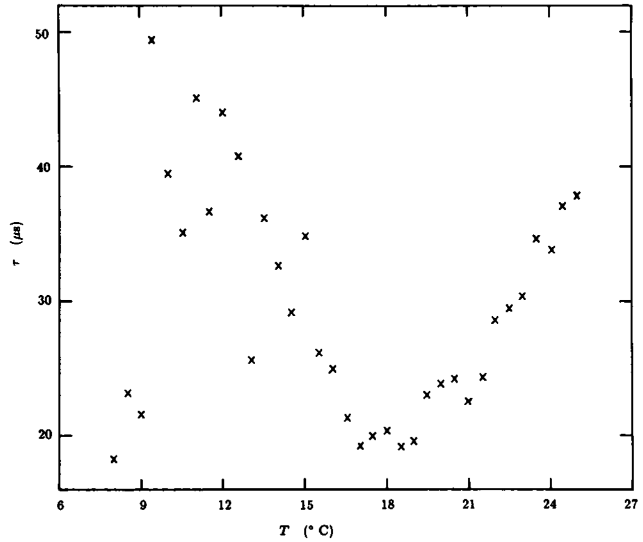

FIGURE 3 The variation of τ inferred from the data of Figs. 1 and 2. See text for discussion.

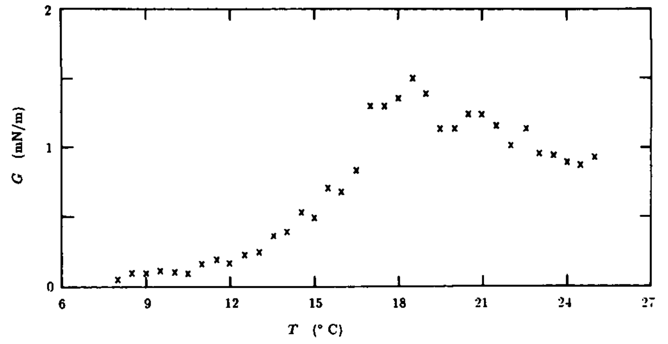

FIGURE 4 G found by substituting the τ data of Fig. 3 into Eqs. 3 or 4 with the observed $\Delta\gamma$ or γ' values.

probably unreliable, as they derive from the greatest extrapolation of the equilibrium tension.

DISCUSSION

No comparable observations of the temperature variation of the relaxation of a membrane viscoelastic modulus appear to exist. However, the present results initially appear to be in marked contrast with certain other data. Rather than cataloging the various studies of the relaxation of different model membrane systems, we concentrate upon one very comprehensive investigation (9), which verified and greatly extended earlier work. In a series of temperature jump experiments Holzwarth et al have shown that suspensions of phospholipid vesicles display five separate relaxation times, ranging from nanoseconds to milliseconds, as observed in the turbidity (9) or fluorescence (10) response. The faster relaxation processes were not cooperative, whereas those between 5 μs and 5 ms increased markedly at the lipid transition temperature, showing considerable cooperativity.

The contrast between these established results, showing a transitional slowing down of the relaxation of various processes, and the present data, showing a transition-associated decrease in relaxation time, demands a critical appraisal of the present data. Several assumptions are involved, the validity of which must be considered: (*a*) Only one exponential relaxation process is involved at all T. Above T_t this has been explicitly demonstrated, both for BLM (at 24.7°C [2]) and for monolayers (at 20°C [3]). It has similarly been verified below T_t (13°C) for the monolayer case (3). Thus the assumption is well founded except perhaps in the immediate neighborhood of T_t. As the variation of τ of Fig. 3 is not confined to that neighborhood, it cannot be ascribed to breakdown of this assumption. (*b*) The variation of the equilibrium bilayer tension resembles that found for the interface of a bulk solution of GMO on *n*-decane and 0.1 M NaCl. BLM formed from solutions of GMO in *n*-decane show no apparent viscoelastic relaxation (2) so that light scattering BLM tensions should equal the equilibrium values. The high frequency tensions of such BLM do indeed vary with T in a manner very similar to the equilibrium tensions of monolayers at oil-water interfaces (1). The use of monolayer results to suggest the temperature variations of the classical tensions thus seems reasonable. The extrapolation below 15°C cannot cause the minimum of τ, which occurs above this temperature. The use of data for GMO/*n*-decane for solvent-free BLM may be questioned, but in practice the variation found for τ is implicit in the behavior of $\omega_0^2\gamma'$. To remove the transitional minimum of τ would require $\gamma_0(\omega \rightarrow 0)$ to behave most peculiarly. For τ to be constant, the equilibrium bilayer tension would have to decrease rather fast ($d\gamma_0/dT \sim 0.126$ mN/m/°C) until 18°C, increase (~ -0.23 mN/m/°C) until 14°C and then decrease again more slowly ($\sim$0.041 mN/m/°C). Such a variation would contradict all the available data for temperature variation of interfacial tensions (S. H. White, private communication, also [8]).

In the absence of detailed studies of the frequency dependence of γ at a series of different temperatures we thus accept the reality of the variation of τ of Fig. 3.

There is no clear understanding of the differences in the behavior of the present relaxation and those in earlier studies (9, 10). This is the first investigation of these phenomena, and comparisons with studies involving the relaxation of different stresses in different membrane systems (vesicles versus BLM) of different lipids (lecithins versus GMO) may not be valid. Indeed, there may be no real conflict between the results.

The relaxation of transverse stress in a bilayer, like the passive ionic permeability (11), may be easiest at boundaries between gel and fluid domains. It has recently been suggested (11) that the transitional maximum in ionic permeability of phospholipid vesicles (12) arises from the changing population of such boundary lipid molecules, which may find it easier than the molecules in pure phase regions to undergo the transverse motions, associated with permeation (13, 14), involved in capillary waves on membranes. The variation of τ of Fig. 3 would thus be related to the population of boundary lipid, which is apparently peaked at T_t (11).

The significance of the variation of G is less clear: strictly, G is simply a quantity, having the dimensions of an interfacial elastic modulus, which combines with τ to yield the (maximum) low frequency value of the membrane viscosity γ' (6). It may be that the variation of Fig. 4 reflects the fractional population of interfacial lipid. The relatively weak variation of G may reflect some indirectness in this association or it may reflect the rather uncooperative nature of the transition in GMO bilayers:

fluid domains seem to persist well below T_t (1). More work is required to substantiate these points.

In conclusion, the temperature variation of a BLM viscoelastic relaxation process has been measured for the first time. The time scale for this relaxation appears to reach a minimum close to the membrane transition. It has been suggested here that the reduction in relaxation time at T_t may be connected with changing populations of lipid molecules at boundaries between gel and fluid domains. There is a need for computer modeling of the dynamics of such processes. Further experiments to substantiate the present results by explicit observation of the frequency dependence of γ at various temperatures are desirable.

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