VISCOELASTIC RELAXATION OF BILAYER LIPID MEMBRANES

Frequency-dependent Tension and Membrane Viscosity

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ABSTRACT Photon correlation spectroscopy has been used to study capillary waves on black lipid membranes of glycerol monooleate at temperatures above the lipid transition. For the first time the tension and viscosity of solvent-free bilayers have been observed to display a frequency dependence. The variations of both parameters can be accounted for by a Maxwell viscoelastic fluid model having a relaxation time of 37 μs. The equilibrium ($\omega = 0$) tension is compatible with literature values. The present results do not suffice to precisely define the specific molecular processes involved, but relaxation times similar to the present are associated with certain phenomena in phospholipid vesicles. Bilayers containing hydrocarbon solvent do not show such relaxation, presumably due to their weaker intermolecular interactions.

INTRODUCTION

Biological membranes are complex structures of lipid and other molecules. They are also very dynamic, the molecules being continually agitated by thermal excitation. Several recent computer simulations have shown that these molecular motions lead to roughening and flexing of model bilayer systems (Leermakers et al., 1983; Owenson and Pratt, 1984): the head groups of the molecules in the two constituent monolayers by no means lie in well-defined planar arrangements, but show considerable fluctuations about average or equilibrium planes. Many of the vital functions of biomembranes arise from or are modulated by such molecular fluctuations. While the time-scales of these motions are significant, the microscopic theories mentioned have not, to date, addressed the dynamics of the membrane fluctuations. This paper demonstrates that experimental observations of long-wavelength fluctuations in lipid bilayers yield information on the molecular time-scales involved. The systems studied (black lipid membranes) are in a state of tension, so that bending modes (cf. Engelhardt et al., 1985) are suppressed.

The dynamics of membrane fluctuations will be governed by molecular interactions, expressed as membrane elastic moduli and viscosities. A fluctuation from the equilibrium state will be followed by a relaxation towards that state: such relaxation processes, usually characterized by a relaxation time, might be expected to cause time (or frequency) dependent membrane viscoelastic behavior. For example, such variations are well established for systems such as polymer solutions (e.g., Ferry 1980), where different viscoelastic regimes have been associated with different types of molecular response. However, there do not seem to have been any observations of frequency-dependent viscoelasticity for insoluble molecular films (such as membranes).

The existence of relaxation processes within insoluble monolayers at air/water interfaces has been inferred. Observations of longitudinal waves (f = 200 Hz) in a cholesterol/lecithin monolayer (Lucassen, 1968) suggest a dilational molulus several times greater than that derived from the equilibrium π–A plot. Also, for the case of a polymer monolayer of low surface coverage, thermally excited capillary waves ($f \sim$ kHz) behaved as if the surface tension were greater than that measured classically (Kawaguchi et al., 1986). Both observations are explicable by relaxation processes on time scales short compared with the classical measurement but long compared with the period of the oscillatory motions. We are not aware, however, of any reports of an explicitly frequency-dependent viscoelasticity for insoluble molecular films. Monolayers of soluble surfactants do display such behavior (Van den Tempel and Lucassen-Reynders, 1983), but diffusional exchange between film and bulk solution can explain the observations: intramonolayer relaxation has not been invoked in that case.

This paper presents results of a light-scattering study of thermally excited membrane fluctuations (in the hydrody-

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namic limit). For certain membrane types frequency-dependent membrane viscoelasticity is observed, and the corresponding relaxation times can be estimated.

THEORETICAL BACKGROUND

The response of a membrane to perturbation cannot be described by a single interfacial elasticity or viscosity (Goodrich, 1962) and thus depends upon the motions probed experimentally. Various modes of fluctuation exist (and will be thermally excited), each corresponding to a different, independent viscoelastic modulus. The modes of a bilayer membrane somewhat resemble those observed for soap films (e.g., Young and Clark, 1981). However, for membranes the transverse compression modulus is so high that the "squeezing" modes observed for soap films are essentially unobservable, being restricted to wave-numbers far above the present experimentally accessible range (Hladky and Gruen, 1982). In the present experiments only the transverse (capillary) waves are observed.

The theoretical description of various types of interfacial fluctuation that can occur for a membrane is well established (e.g., Kramer, 1971). We briefly rehearse the relevant arguments before making connections with membrane relaxation processes. This approach is valid in the hydrodynamic limit, far removed from the molecular scale. However, viscoelastic relaxation must arise from molecular processes within the membrane, which manifest themselves as observable properties in the hydrodynamic limit.

For symmetric bilayers (those separating identical fluids), the transverse capillary waves are decoupled from other modes. The displacement of the membrane from its equilibrium plane can be written

$$\xi = \xi_0 e^{i(qx+\omega t)}, \tag{1}$$

where q, the real wave-number ($2\pi/\Lambda$) and ω, the complex frequency ($-\omega_0 + i\Gamma$), are related by the dispersion equation (Kramer, 1971):

$$i\omega - \gamma q^3(q - m)/2i\rho m\omega = 0. \tag{2}$$

Here γ is the membrane tension. The quantity m is defined by

$$m = (q^2 + i\,\omega\rho/\eta)^{1/2} \quad Re(m) > 0, \tag{3}$$

where η and ρ are the viscosity and density, respectively, of the aqueous medium bathing the membrane.

The frequencies found by solution of Eq. 2 are shown in Fig. 1. Approximate analytic solutions have been derived (Kramer, 1971; Crilly and Earnshaw, 1983), particularly for the case of negligible membrane viscosity where η provides the only dissipation. In the low-damping limit the capillary waves propagate:

$$\omega \simeq -(\gamma q^3/2\rho)^{1/2} + i\eta q^2/\rho. \tag{4}$$

For high damping two overdamped modes exist, ω being pure imaginary:

$$\Gamma_1 \simeq \gamma\, q/4\eta \quad \text{and} \quad \Gamma_2 \simeq 2\eta q^2/\rho. \tag{5}$$

The first mode (Γ_1) governs the slow recovery of the membrane towards the equilibrium plane at a rate governed by capillary forces and viscosity, whereas the second mode (Γ_2) is determined by the inertia of the system. To this approximation, the inertia arises from the ambient fluid (ρ).

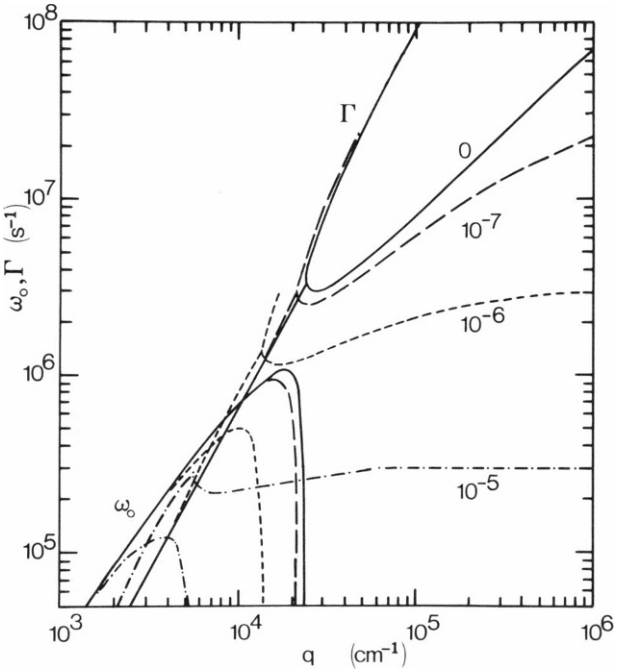

FIGURE 1 The dispersion behavior of transverse waves on a symmetric membrane of tension 3 mN/m immersed in water. Note the change-over from propagation to overdamping as q increases. The parameter of the curves is membrane viscosity, expressed in mN·s/m. The q value corresponding to overdamping decreases as γ' increases.

Membrane Viscoelasticity

Goodrich (1981) has shown from first principles that up to four separate interfacial viscosities may exist, each forming the dissipative portion of a viscoelastic modulus. The four moduli constitute two pairs, one acting in the interface and the other normal to it. Each pair comprises a dilational and a shear modulus. Capillary waves upon a membrane involve shearing motions transverse to the membrane plane, the appropriate modulus being

$$\gamma = \gamma_0 + i\omega\gamma', \tag{6}$$

where γ_0 is the membrane tension and γ' is the transverse shear viscosity of the membrane. This is not the conventional membrane viscosity, which involves shear in the membrane plane.

A usual rheological convention has been used in Eq. 6 to display γ as a complex dynamic modulus relating oscillatory stress ($T(t) = T^* e^{i\omega t}$) and strain ($u(t) = u^* e^{i\omega t}$). In

conventional notation (Ferry, 1980),

$$T^*/u^* = G^*(\omega), \tag{7}$$

where the dynamic modulus can be written

$$G^*(\omega) = G'(\omega) + iG''(\omega). \tag{8}$$

In the present instance we identify the storage modulus $G'(\omega) \equiv \gamma_0$ and the loss modulus $G''(\omega) \equiv \omega\gamma'$.

In previous consideration of membrane modes, the tension γ_0 and the viscosity γ' have been considered as single-valued membrane properties. This may be unnecessarily restrictive: the membrane viscoelasticity may depend upon the strain rate involved. In general $G'(\omega)$ and $G''(\omega)$ may be arbitrary functions of frequency, reflecting relaxation processes within the membrane. There is as yet no molecular theory of membrane viscoelasticity, such as exists for polymer solutions. We consider here only the simplest linear viscoelastic models, the Voigt or Kelvin solid having one retardation time and the Maxwell fluid with a single relaxation time. We emphasize that these models have no molecular basis, but represent the simplest possible combinations of discrete elastic and viscous elements. Such combinations (and elaborations thereon) display behavior that closely resembles that found for linear viscoelasticity.

A membrane comprising a Voigt solid would form a linear retarded elastic medium (having both γ_0 and γ' constant, i.e., $G'(\omega)$ constant and $G''(\omega)$ linearly increasing with ω). Such a system displays two characteristic times:

$$\tau_1 = \gamma'/\gamma_0 \tag{9}$$

$$\tau_2 = (\gamma' + 4\eta/q)/\gamma_0. \tag{10}$$

At high shear rates the system is dominated by the membrane retardation time τ_1.

For a Voigt model γ' modifies the propagation of the capillary waves as shown in Fig. 1. For low damping the modification is slight, whereas in the high-damping limit the effects are greater. The extra dissipation in the system shifts the critical damping to longer wavelength and slows down the slower, capillary mode (Γ_1). For high damping Γ_1 is dominated by γ', being asymptotic to $1/\tau_1$. The approach to this limit is slow: $1/\tau_1$ fails to satisfy the original dispersion equation (Eq. 2) even for extreme values of q. Γ_1 always satisfies Eq. 2: the membrane will always relax to equilibrium after a fluctuation (provided it is stable). The faster, inertial mode (Γ_2) only exists over a limited range of q (Fig. 1), disappearing when Γ_2 exceeds the inverse of the retardation time ($1/\tau_1$). The disappearance of the inertial mode is due to the rigidity of a Voigt body for large strain rates. Lacking analytic approximations for Γ_2 valid over wide ranges of q and γ', Kramer's (1971) estimate that cut-off occurs when

$$1/\tau_1 < \eta q^2/\rho$$

(correcting an obvious misprint) offers a general guide.

The Maxwell fluid membrane comprises a viscous fluid system having a linear elastic resistance to shear. The dynamic modulus is frequency dependent:

$$G'(\omega) = G_e + G\frac{\omega^2\tau^2}{1 + \omega^2\tau^2} \tag{11}$$

$$G''(\omega) = G\frac{\omega\tau}{1 + \omega^2\tau^2}, \tag{12}$$

where G_e is the equilibrium ($t = \infty$) storage modulus, τ is the relaxation time, and G the strength of the relaxation process. Both membrane tension and viscosity will be frequency dependent: γ_0 rising from G_e ($\omega = 0$) to $G_e + G$ ($\omega = \infty$), and γ' falling from $G\tau$ ($\omega = 0$) to 0 ($\omega = \infty$).

The propagation of capillary waves on a membrane constituting a Maxwell fluid system will differ from that shown in Fig. 1: for $\omega \ll 1/\tau$ it will resemble the case for constant γ_0 ($=G_e$) and γ' ($=G\tau$), whereas for $\omega \gg 1/\tau$ the behavior will be pure elastic ($\gamma' = 0$) with increased γ_0 ($=G_e + G$). If $1/\tau$ is less than the maximum value of ω_0 the critical damping will be moved to higher q, due to the increase in tension. These effects are illustrated in Fig. 2:

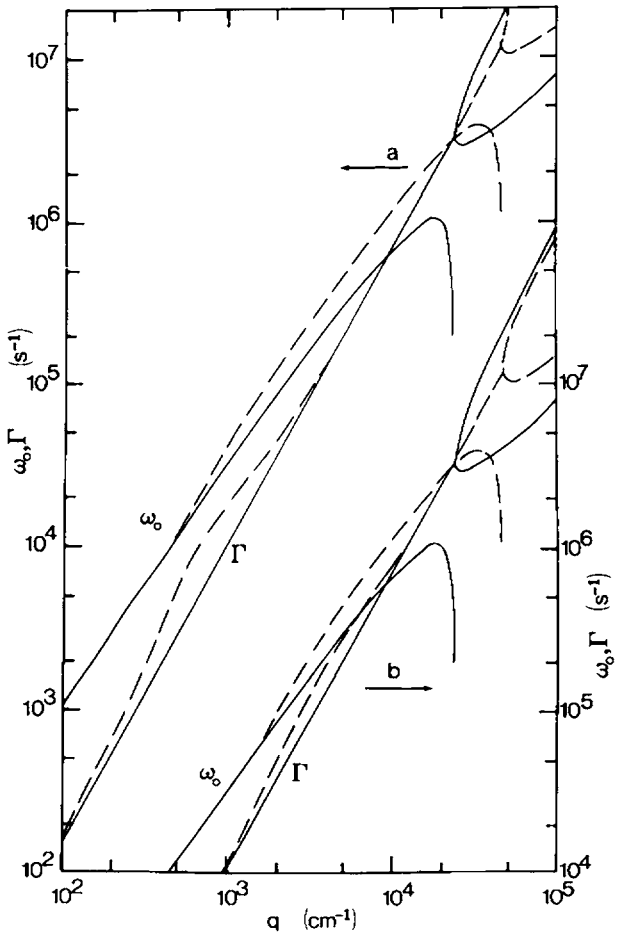

FIGURE 2 As Fig. 1, but for frequency-dependent membrane tension and viscosity (as Eqs. 11 and 12). Values used were $G_e = 3.0$ mN/m with $G = 0$ (——) or 2.7 mN/m (– – –). Relaxation times were 37 μs (*a*) and 3.7 μs (*b*); note the different ω scales.

ω_0 and Γ values were derived from the dispersion equation (Eq. 2) using γ_0 and γ' dependent upon frequency, as in Eqs. 11 and 12. The effects of different τ values are shown (for constants G_e and G). For direct comparison with Fig. 1, G_e was taken as 3.0 mN/m, while values of G and τ were chosen to give maximum γ' values of 10^{-5} and 10^{-4} mN·s/m. At low q, the steadily increasing effect upon Γ of constant γ' is evident, while at high q the two overdamped modes appropriate to $\gamma' = 0$ are seen. In between these limits a smooth transition occurs, as γ' decreases and γ_0 increases.

EXPERIMENTAL METHODS

The methods used in bilayer lipid membrane (BLM) formation and in light scattering have been fully described elsewhere (see Crawford and Earnshaw, 1986 and references therein).

Two types of glycerol monooleate (GMO) membranes were studied: BLM formed from lipid dissolved in *n*-decane (known to incorporate quantities of solvent in their structure) and those formed from lipid dispersed in squalane ($C_{30}H_{62}$, yielding essentially solvent-free bilayers). In all film-forming solutions the lipid concentration was 10 mg/ml. All experiments were carried out at room temperature, well above the lipid transition at T_t = 16.6°C (Crawford and Earnshaw, 1986).

A laser beam fell upon the BLM, light scattered at small angles from the specular reflection being selected. Stray flare light provided a heterodyne reference beam. The detector output was analyzed by photon correlation.

The wave-number q of the capillary waves observed is the projection of the usual scattering vector on the membrane plane. The scattering angle was determined from the separation of the detector pinhole from the specular reflection (error in $q = \pm 30$ cm^{-1}). Scanning the pinhole position permitted membrane properties to be observed over a limited range of q (~700–1,800 cm^{-1}). The upper limit was set by the q^{-2} decrease in scattered intensity, while the lower limit arose from the diffuse nature of the specular reflection from the BLM. The latter was particularly apparent for the GMO/decane case, where results (particularly Γ values) became somewhat unreliable for $q \leq 1{,}000$ cm^{-1}.

ω_0 and Γ were estimated (with errors 1.7 and 3.7%, respectively) by fitting the measured correlation functions by the form

$$G(\tau) = B + A \cos(\omega_0 \tau + \phi)\, e^{-\Gamma\tau}\, e^{-\beta^2\tau^2}. \qquad (13)$$

The observed correlation functions correspond well to this form (Crawford and Earnshaw, 1986): no extraneous scattering processes contribute. The observed time dependence is thus unambiguously associated with the temporal evolution of the capillary waves upon the BLM.

The physical properties of the system were derived from the fitted values of ω_0 and Γ by substituting these values into Eq. 2 and solving for γ_0 and η in the first instance. If the viscosity η agreed with the accepted value for the fluid bathing the lipid bilayer, the membrane viscosity γ' was assumed to be negligible. If η exceeded its accepted value, Eq. 2 was re-solved for γ_0 and γ', with η held at this value.

RESULTS

Results are presented for one membrane of each type examined. Data from different membranes were not averaged. Qualitatively similar results were obtained from other membranes. Small quantitative differences arose from inevitable membrane variability. Two consecutive light-scattering observations were made at each q value for a given membrane, the ω_0 and Γ data being averaged. In all cases the two ω_0 and Γ values agreed within the errors quoted above.

We first discuss the results for GMO/*n*-decane bilayers (studied at 21.2°C). For $q < 1{,}050$ cm^{-1} the problems already discussed caused the ω_0 values to be somewhat scattered (Fig. 3) and the Γ values to be both scattered and rather increased. We thus concentrate upon $q > 1{,}050$ cm^{-1}. The γ_0 values found (Fig. 4 *a*) were consistent with a constant membrane tension, while the η values agreed with the accepted viscosity of the 0.1 M NaCl solution bathing the BLM.

Various statistical tests were used to investigate any q dependence of γ_0. (*a*) Polynomials of increasing degree in q were fitted to the data. No significant improvement of the fit was obtained by inclusion of other than a constant tension (using Fisher's F test). (*b*) γ_0 and η were averaged for $q > 1{,}050$ cm^{-1} (to avoid possible biases): $\bar{\gamma}_0 = 6.72 \pm 0.20$ mN/m and $\bar{\eta} = 1.011 \pm 0.005$ mPa·s. The average tension found for $q < 1{,}050$ cm^{-1} was 6.90 mN/m, emphasizing the lack of q dependence. $\bar{\eta}$ agrees well with the accepted value of 1.003 mPa·s. (*c*) Using $\bar{\gamma}_0$ and the accepted η, the q variations of ω_0 and Γ were found from the dispersion equation. The observed ω_0 data (Fig. 3) are compatible with the predicted variation (runs test at 5% significant level). The Γ data (for $q > 1{,}050$ cm^{-1}) gave a

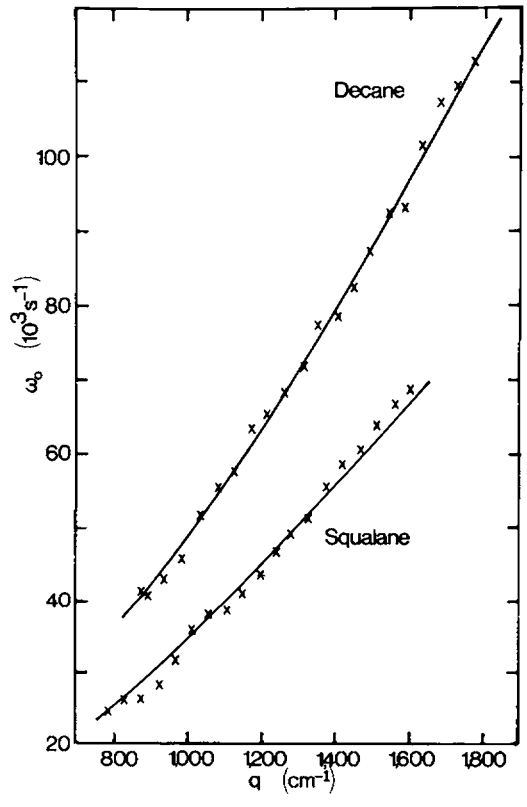

FIGURE 3 Measured values of ω_0 for GMO/*n*-decane and solvent-free bilayers. The lines show the expected behavior for constant viscoelastic properties, as discussed in the text.

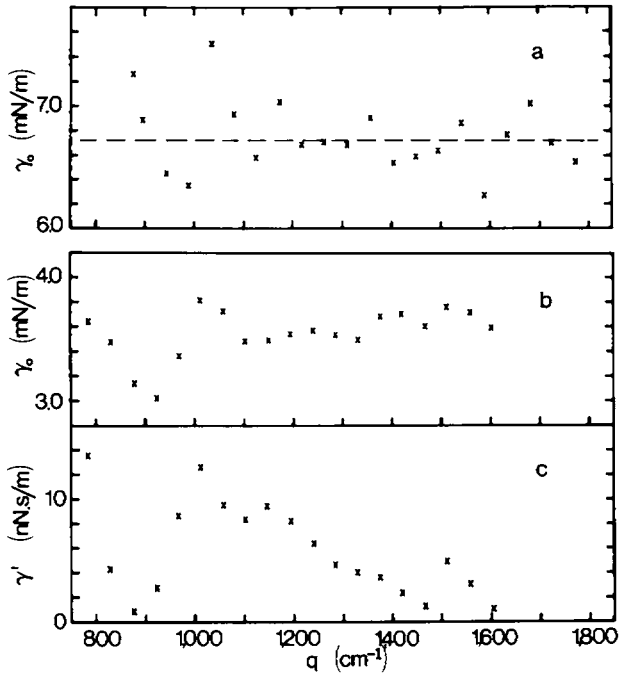

FIGURE 4 Membrane tension and viscosity values found for different membranes. (*a*) γ_0 for GMO/*n*-decane bilayer; the dashed line represents the average tension. (*b*) γ_0 and (*c*) γ' for solvent-free bilayer.

TABLE I
RESULTS FOR SOLVENT-FREE GMO MEMBRANES

q	ω_0	Γ	γ_0	γ'
cm^{-1}	*kHz*	*kHz*	*mN/m*	$\times$ *10 nN·s/m*
786.3	24.56	7.296	3.652	1.373
831.6	26.25	7.092	3.480	0.440
877.0	26.47	7.258	3.141	0.091
922.3	28.22	8.185	3.027	0.283
967.6	31.71	10.12	3.371	0.877
1,013	35.99	12.07	3.815	1.269
1,058	38.01	12.46	3.727	0.957
1,104	38.94	13.14	3.488	0.845
1,149	41.08	14.54	3.496	0.957
1,194	43.86	15.35	3.545	0.828
1,240	46.70	15.96	3.570	0.648
1,285	49.12	16.41	3.536	0.469
1,330	51.36	17.24	3.498	0.405
1,376	55.63	18.39	3.689	0.365
1,421	58.64	18.95	3.706	0.243
1,466	60.62	19.40	3.608	0.136
1,512	63.90	22.92	3.766	0.495
1,557	66.60	23.04	3.716	0.317
1,602	68.73	22.68	3.599	0.109

less clear-cut result, due to the lower precision of Γ, but were not inconsistent with expectation.

These various tests concur: the evolution of capillary waves upon GMO/*n*-decane BLM is entirely consistent with a constant membrane tension and negligible membrane viscosity. This conclusion is confirmed by independent data on similar bilayers (Crilly and Earnshaw, 1983). The average BLM tension in the present study is compatible with literature values (see Crawford and Earnshaw, 1986). The agreement of $\bar{\eta}$ with the accepted value shows that no systematic biases exist in our experiment or data analysis procedure. In particular the q values (central to data interpretation) cannot be significantly in error.

These GMO/*n*-decane results cannot be unambiguously interpreted in terms of the viscoelastic models quoted above. Either a Voigt solid with negligible γ' or a Maxwell fluid with small G or large τ could explain the data. Given the solvent content of the BLM and the fact that our experiments were carried out well above T_t, we favor the latter alternative. The included solvent tends to separate the apposing monolayers and reduce the lipid packing density within these monolayers (Lea, 1979), rather weakening the intermolecular interactions. The acyl chains would thus be free to move, and it seems likely that G would be small for such BLM.

Results for solvent-free BLM (studied at 24.7°C) were qualitatively different (Table I). The apparent viscosity η clearly exceeded the accepted value (0.924 mPa·s), indicating non-negligible γ'. Tension and membrane viscosity data derived from ω_0 and Γ are shown in Table I and Fig. 4, *b* and *c*. These data were analyzed in the same way as the GMO/*n*-decane results. (*a*) The tension increases with q, the preferred degree of polynomial approximation (Fisher's F test, 5% significance level) being $\gamma_0 = \alpha_0 + \alpha_1 q$. (*b*) The membrane viscosity is not constant: at $q > 1{,}000$ cm^{-1} the decrease of γ' with q is very evident (Fig. 4 *c*). We do not regard the fluctuations in γ' about $q = 1{,}500$ cm^{-1} as very significant. Crudely speaking γ' is found from the deviation of Γ from that value expected from the ambient viscosity η: small values of γ' are rather uncertain. (*c*) Using data for $q > 1{,}000$ cm^{-1}, γ_0 and γ' were averaged. The dispersion behavior of ω_0 (see Fig. 3) and Γ based on these average values were clearly incompatible with the observed variations (runs test at 5% level).

The behavior observed for solvent-free bilayers of GMO is thus not consistent with constant membrane tension or viscosity. The difference from the GMO/*n*-decane case does not arise from the methods used to evaluate γ_0 and γ': the observed frequencies ($\omega = -\omega_0 + i\Gamma$) do not vary with q as expected for constant tension and viscosity. Independent data for similar BLM (Crilly and Earnshaw, 1983) again confirm both this conclusion and the general trends of the present results.

The variations of γ_0 and γ' are inconsistent with a Voigt solid model, but are suggestive of a Maxwell fluid behavior. In examining the frequency dependences of γ_0 and γ' (Fig. 5), the measured capillary wave frequencies (ω_0) were used; frequencies computed for assumed variations of γ_0 and γ' would introduce bias.

The data of Fig. 5 were fitted to the variations of $G'(\omega)$ and $G''(\omega)/\omega$ for a Maxwell fluid (Eqs. 11 and 12) using a nonlinear least-squares procedure. The free parameters of

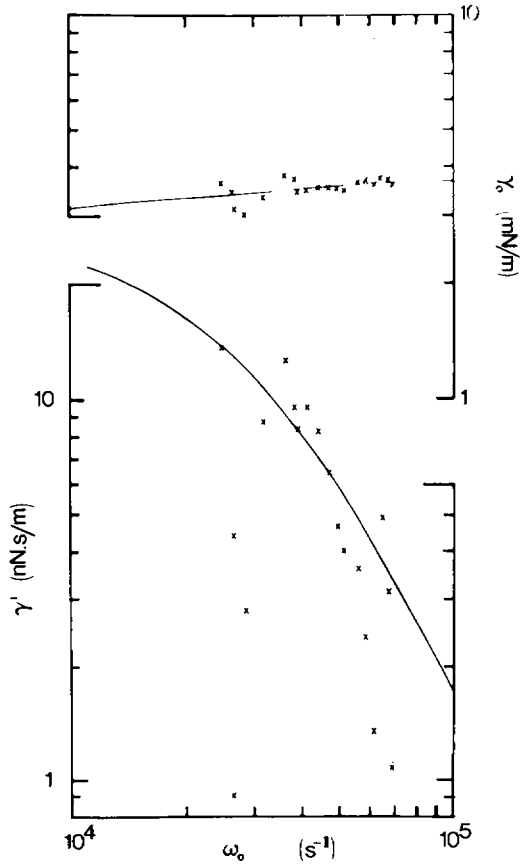

FIGURE 5 Membrane tension and viscosity (as Fig. 4, *b* and *c*) as functions of capillary wave frequency. The lines show the best-fit functions for the Maxwell model (fit C of Table II): $G'(\omega)$ and $G''(\omega)/\omega$, respectively.

the fit were G_e, G, and τ. To avoid bias all data were included in the fitting, but γ' data at $q < 1{,}000\ \text{cm}^{-1}$ were given smaller weights (arbitrarily $\times 0.2$) than the other γ' data. The γ_0 and γ' data gave very different contributions to S, the sum of squared deviations. Various weighting schemes were used (Table II): fit A was heavily biased towards γ_0, fit B towards γ', while in fit C γ_0 and γ' gave roughly equal contributions to S. It is noticeable that the parameters of the fit (G_e, G, and τ) do not vary greatly with the weighting scheme used. The agreement of fits based (essentially) on γ_0 and γ' alone increases our confidence in the appropriateness of the Maxwell fluid model. Small differences between the fits are unsurprising in view of the scatter of the data. Fit C is here taken as the best: the variations from fit A to B may indicate the range of the parameters.

TABLE II
ANALYSIS OF VISCOELASTICITY OF SOLVENT-FREE GMO BILAYERS

Fit	W_1*	W_2‡	G_e	G	τ
			mN/m	*mN/m*	*μs*
A	1	1	3.04	0.771	34.0
B	1	10^8	3.02	0.710	42.3
C	1	10^5	3.07	0.684	37.2

*W_1 is weight of γ_0 data in fit.
‡W_2 is weight of γ' data in fit.

Alternative explanations of the q-dependent properties of solvent-free BLM either fail to explain all of the data or involve physically impossible results: (*a*) Systematic errors in q could explain either the γ_0 or the γ' data. Together the two sets of data are incompatible with this hypothesis: γ_0 demands an error in q which increases with q, whereas γ' would require a decreasing error in q. (*b*) Capillary waves involve membrane bending, which increases the apparent tension as $\gamma_0 + Kq^2$ (Fan, 1973). The value of the bending modulus K ($\sim 10^{-13}$ J) inferred from the present tension data is about seven orders of magnitude greater than values reported for other lipid membranes (e.g., Engelhardt et al., 1985). This mechanism could not explain the γ' data. (*c*) It has recently been shown (for a rather different system; Lipowsky, 1985) that local inhomogeneities in a fluid interface can affect capillary waves as $\omega_0^2 \simeq \gamma_0 q(\xi^{-2} + q^2)/\rho$, where ξ is the lateral correlation length of the interface. No predictions exist for Γ. The present ω_0 values yield an unphysical negative ξ^2, with small errors. All of these alternative explanations are inadequate and are thus rejected.

We conclude that solvent-free bilayers of GMO respond to transverse shear with a dynamic viscoelastic modulus, which depends upon frequency in a manner that agrees (within the data scatter) with a particularly simple linear model, the Maxwell viscoelastic fluid. Our confidence in this conclusion is reinforced by the quite similar results obtained by fitting the γ_0 data and the γ' data separately and by the agreement with previous independent results.

DISCUSSION

The viscoelastic behavior of BLM containing quantities of *n*-decane and those that are essentially solvent-free are very different, specifically in the frequency dependence of the dynamic modulus γ for the solvent-free case. This discussion will largely concern these latter data.

There are few data comparable with our results (Table II) except classical observations of the equilibrium ($\omega = 0$) tension, G_e, of Eq. 11. However, no data are available for the GMO/squalane case. Hladky and Gruen (1982) suggest a range from twice the surface tension (i.e., 3.0 mN/m) to twice the tension of a squalene–water interface supporting a GMO monolayer (i.e., 4.0 mN/m, using γ_{interf} from Elliott et al., 1983). The present best fit value of G_e = 3.07 mN/m is compatible with the lower limit of this range.

The limited experimental frequency range makes it difficult to exclude more complicated viscoelastic models. However, if the present data were fitted by a more general model (including several parallel Maxwell elements), the fitted G_e would necessarily be reduced. Any large decrease

in G_e would lead to conflict with the lower limit quoted above.

Other experimental approaches to BLM viscoelasticity involve considerably longer time-scales than the present, making positive identification of the molecular processes involved in the observed relaxation difficult: either the membrane or structured vicinal water layers could contribute. However, a relaxation time of 37 μs seems a priori much too long for any process in water (cf. the observed dielectric relaxation time of 1.11×10^{-10} s for water in the presence of a monolayer of oleyl alcohol [Hühnerfuss and Alpers, 1983]). We thus associate the present relaxation with the lipid membrane.

Other evidence yields some insight into the processes involved. Thermal relaxation of lipid vesicles has been studied (Holtzwarth et al., 1984); for dipalmitoylphosphatidylcholine (DPPC) a series of five time-scales (τ_1–τ_5) ranging from 10^{-9} s to 1 s were found. The temperature dependence of the amplitudes and time-scales of the various processes showed varying degrees of cooperativity at the lipid transition. The present data were taken well above T_t for GMO and should thus be compared with thermal relaxation above the DPPC transition. A relaxation time of 37 μs falls between τ_3 (~5 μs), associated with the formation of *gauche* conformations in the lipid acyl chains, and τ_4 (~100 μs), apparently arising from the formation of ordered clusters within the bilayers. A time scale ~μs for the formation of *gauche* conformations is supported by recent computer simulations (Lookman et al., 1982). The GMO and DPPC molecules are somewhat different, as are vesicles and BLM. Closer identification of the present relaxation with specific intra- or intermolecular processes is thus somewhat difficult as yet but it seems clear that intramembrane processes are involved.

Identification might be aided by experimental refinements. A more precise determination of q would improve the accuracy of γ. This, together with an extension of the q range to $q < 700$ cm^{-1} would enable the viscoelastic behavior to be established with much greater assurance.

The present experiments were conducted well above T_t for GMO, where fluid behavior is not unexpected. At or below T_t changes in the viscoelastic behavior would be expected. Observation of such thermotropic changes would extend the experimental basis for molecular understanding of the viscoelastic relaxation and further elucidate the lipid transitions.

The present demonstration of viscoelastic fluid behavior (above T_t) vitiates the extraction of a characteristic time from the γ_0 and γ' values observed in a recent light-scattering study for the transitions of GMO bilayers (Crawford and Earnshaw, 1986). For a Voigt solid, this time ($=\gamma'/\gamma_0$) could be identified as the retardation time. Unfortunately in that study capillary waves were observed at a single q so the parameters of a Maxwell model cannot be fully specified. Thus the observed temperature variations of γ_0 and γ' cannot be simply interpreted in terms of variations of G_e, G, and τ. The most notable feature was a peak in γ' close to T_t. This probably arises from simultaneous variations of G and τ. If the relaxation time τ has a maximum at T_t, like Holtzwarth et al.'s (1984) τ_3 and τ_4, a concomitant and large maximum in G would be required to reproduce the observed peak in γ'. The amplitude of the signals observed by Holtzwarth et al. showed relatively large maxima: if the amplitudes can be associated with the (relative) strengths of the corresponding relaxation processes (G), the observed temperature variation of γ' could be qualitatively explained. This interpretation is conjectural and studies of the q dependences of γ_0 and γ' at various temperatures are required.

Other approaches to membrane viscoelasticity have been reported, the viscosity usually being investigated via the time-dependence of membrane deformation or relaxation. Lipid vesicles have been found (e.g., Waugh, 1982) to display membrane viscosities that do not vary with time and are rather greater in magnitude than the maximum ($\omega = 0$) value of γ' given above. The time-scales involved in these experiments are quite long ($\gtrsim$ ms), and fast relaxation processes would not have been discernable. To our knowledge, the only case in which viscoelastic fluid behavior has been found is (Chien et al., 1978) the membranes of red blood cells ($\tau \sim$ 20–200 ms). These are very complex systems with interactions both within the membrane and with the underlying cytoskeleton. It is not surprising that such systems exhibit relaxation time-scales very different from that of a pure lipid bilayer (cf. Engelhardt et al., 1984). Furthermore, the perturbations observed for these cells are governed by bending elasticity, so that the effective tension (Kq^2) is low. The fluctuations will characteristically be slow and unaffected by fast molecular relaxation processes (cf. Fig. 2, where no effects are seen for $\omega_0 \ll \tau^{-1}$).

As already noted, a system such as a lipid bilayer possesses, in principle, various separate viscoelastic moduli. It is unlikely that these would display the same characteristic relaxation time. An arbitrary membrane process might well be influenced by several or all of these moduli to some extent. The consequent frequency dependence of the membrane response could be quite complex. However, depending upon the relaxation times involved, it is conceivable that at the time-scales characteristic of the process only one viscosity could be effective.

CONCLUSIONS

Quasi-elastic light scattering from capillary waves on bilayer lipid membranes of GMO has been shown to yield viscoelastic data from which the rate-limiting relaxation times can be estimated. The data suggest that the dynamic molecular behavior underlying the relaxation of the capillary modes can be probed by observations in the hydrodynamic limit. This is apparently the first demonstration of viscoelastic relaxation for insoluble molecular films. The

observed data are quite well accounted for by a Maxwell fluid model; other models would require arbitrarily frequency dependent tension and viscosity. As already noted, more precise determination of both γ_0 and γ' over a wider frequency range than the present results would permit a more rigorous validation of the appropriate viscoelastic model. The equilibrium membrane tension deduced from the Maxwell model is compatible with literature values. The maximum possible membrane viscosity is probably too low to have discernible effects in other experiments. The relaxation time found, 37 μs, is clearly consistent with a membrane phenomenon but association with any specific molecular process is not, at present, entirely secure. As yet no microscopic models exist for viscoelastic relaxation in lipid bilayers.

Light scattering has various advantages: it covers a rather different time-scale from other methods, it is essentially nonperturbative, and membrane properties probed are averaged over areas (illuminated by the laser beam) that are much greater than the molecular scale but much smaller than the entire membrane. In all these respects light scattering contrasts with other techniques, involving either slow macroscopic perturbations of the membrane or more rapid motion of molecular probes, influenced by the immediate molecular environment.

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