Eur Biophys J (1984) 11:25–33

European Biophysics Journal

Photon correlation spectroscopy as a probe of planar lipid bilayer phase transitions

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Received February 7, 1984/Accepted April 9, 1984

Abstract. Photon correlation spectroscopy has been applied to study phase transitions of planar bilayer membranes. The membrane tension and one specific membrane viscosity are probed. Difficulties arising in the measurement of the temperature dependence of these properties are discussed and a servo-control system to overcome them is described. Typical data are presented for monoglyceride bilayers. Membranes incorporating cholesterol display effects below the lipid transition temperature which are interpreted in terms of separation within the membrane into cholesterol-rich fluid regions and regions of lipid in the gel phase. Some of the cholesterol-rich regions are apparently of macroscopic extent.

Key words: Photon correlation spectroscopy, Planar bilayer membranes, Phase transitions, Monoglyceride bilayers, Cholesterol

Introduction

Amongst the most interesting and significant properties of biomembranes are their thermotropic phase transitions. These transitions have been extensively studied in a variety of model membrane systems. The techniques which have been applied range from the thermodynamic (DSC, DTA) to the spectroscopic (ESR, NMR, Raman). Most of these methods are suitable only for application to multilamellar phases, since they rely upon multiple membranes to provide sufficient signal.

These studies have provided a considerable amount of information concerning the transitions of membranes formed from a variety of lipids. A general picture has emerged linking the transitions with the co-operative trans-isomerization of the hydrocarbon chains of the lipid molecules. At temperatures above the phase transition the molecules, which may possess several gauche configurations per chain, pack together to form a relatively disordered 'liquid-crystalline' state. As the temperature decreases the chains assume an all-trans configuration, leading to a more ordered 'gel' phase. However, there exists no unified theoretical model of such a transition capable of predicting the behaviour of all the membrane properties. Recent reviews provide a summary of both the experimental and theoretical work on membrane transitions (e.g., Baret 1981; Chapman 1975).

There is a place, then, for an experimental technique capable of providing new information about membrane transitions. This paper describes a novel spectroscopic technique for the investigation of the phase transitions of a single bimolecular lipid membrane. The potential of the method is illustrated by the results of a preliminary series of experiments on glycerol monooleate bilayers, both with and without cholesterol.

Photon correlation spectroscopy is an accepted biophysical technique for the study of molecular behaviour (Bloomfield 1981). It has recently been used to study the viscoelasticity of molecular monolayers (e.g., Langevin 1981; Crilly and Earnshaw 1982) and more recently bimolecular lipid membranes (Crilly and Earnshaw 1983a, b). It probes fluctuations (capillary waves) of the membrane which are excited by thermal agitation of the molecules of the membrane or the adjoining fluid. The technique is thus essentially non-perturbative. The membrane properties which govern the capillary wave propagation – the membrane tension and one specific membrane viscosity – can be deduced from the PCS[1] experiment.

1 Abbreviations used: BLM: bimolecular lipid membrane, GMO: glycerol monooleate, PCS: photon correlation spectroscopy

Theoretical background

The theory of the scattering of light by thermally excited capillary waves upon membranes has been discussed previously (Kramer 1971; see also Crilly and Earnshaw 1983a). Here only a few useful facts and relationships will be summarized.

The spectrum of the scattered light may be described approximately as Lorentzian, having a peak frequency ω_0 and a line width Γ. These parameters form the real and imaginary parts of a complex frequency $\omega\ (= \omega_0 + i\Gamma)$. In the present work the spectral information has been measured using photon correlation (Cummins and Pike 1974). The observed correlation function is the Fourier transform of the optical spectrum of the scattered light; it can be described by the form (Byrne and Earnshaw 1977)

$$G(t) = A + B \cos(\omega_0 t + \phi)\, e^{-\Gamma t}\, e^{-\beta^2 t^2} . \qquad (1)$$

The phase term ϕ is included to allow for the deviation of the exact theoretical spectrum from a Lorentzian form. Various experimental effects contribute additional broadening to the measured spectrum compared to that expected (Byrne and Earnshaw 1977). These effects are largely accounted for by a Gaussian instrumental function, the second exponential term in Eq. (1). The amplitude B reflects the intensity of light scattered by thermally excited capillary waves of wave number q (Bouchiat and Langevin 1978):

$$I_s \propto \frac{kT}{\gamma_0 q^2} , \qquad (2)$$

where γ_0 is the membrane tension.

The parameters ω_0 and Γ depend upon the physical properties of the interface and the adjoining fluid. In the general case (Earnshaw 1983) up to four interfacial properties may effect ω_0 and Γ; a single measurement of these two parameters does not suffice to determine all the properties. However, for symmetric BLM (those separating identical fluids) only two interfacial properties can affect the capillary waves observed (Crilly and Earnshaw 1983a). These are the membrane tension and an associated membrane viscosity, γ' (relating to shear stress normal to the membrane plane), which form a response function.

$$\gamma = \gamma_0 - i\omega\gamma' . \qquad (3)$$

In the remainder of this paper, reference to 'membrane viscosity' will refer specifically to the quantity γ'. For capillary waves of given q the frequency ω is related to γ and to the properties of the adjoining medium (dynamic viscosity η and density ϱ) via the dispersion equation (Kramer 1971)

$$D(\omega) = i\omega - (\gamma/2\varrho)(q^3/m)(q - m)/i\omega = 0 . \qquad (4)$$

Here m is the positive root $\sqrt[+]{q^2 - (i\omega\varrho/\eta)}$.

Thus, for symmetric BLM, numerical solution of the dispersion equation (by substitution of the measured values of ω_0 and Γ and demanding that the real and imaginary parts of Eq. (4) simultaneously equal zero) yields values of γ_0 and η. Initially γ' is taken to be zero. In some cases the apparent viscosity η inferred in this way systematically exceeds the accepted value. This may be due to non-negligible membrane viscosity γ' (Crilly and Earnshaw 1983a). If the viscosity η is assumed to have its accepted value, solution of the dispersion equation gives γ_0 and γ'. It is useful to note the existence of approximate solutions of Eq. (4); to first order (Crilly and Earnshaw 1983a)

$$\omega_0 = \sqrt{\gamma_0 q^3/2.3\varrho} , \qquad \Gamma = \eta q^2/\varrho . \qquad (5)$$

Materials and methods

The experimental methods are refinements of techniques already published (Crilly and Earnshaw 1983a). The present description will thus be limited to the modifications relevant for phase transition studies.

a. Temperature-controlled membrane cell

Membranes were formed by the Van den Berg method (Van den Berg 1965), using a PTFE septum with a 4-mm hole. The film-forming solution comprised 10 mg/ml glycerol monooleate (GMO, Sigma, ~ 99% purity) in n-decane (Koch-Light, ≥ 99% purity). In the preliminary experiments described here the aqueous medium surrounding the BLM was ultra-pure water (Millipore Milli-Q). In this environment membranes appeared to be more robust than in NaCl solution.

The cell used in BLM formation was a standard 1-cm^2 cross-section spectrophotometer cuvette (Fig. 1). This was held in position by a brass heat-sink (55 cm^3), which acted as a large thermal reservoir, avoiding temperature fluctuations. The heat-sink was

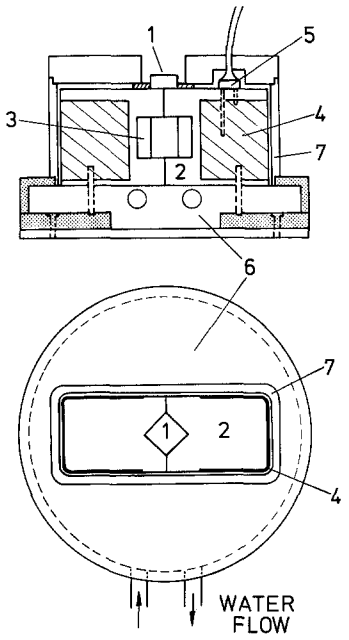

Fig. 1. The thermostatted membrane cell (see text). Key: *1* – cuvette, *2* – heat-sink, *3* – aperture for laser beams, *4* – thermofoil heaters, *5* – temperature sensor i.c., *6* – base for thermostatted water circulation, *7* – PTFE insulation

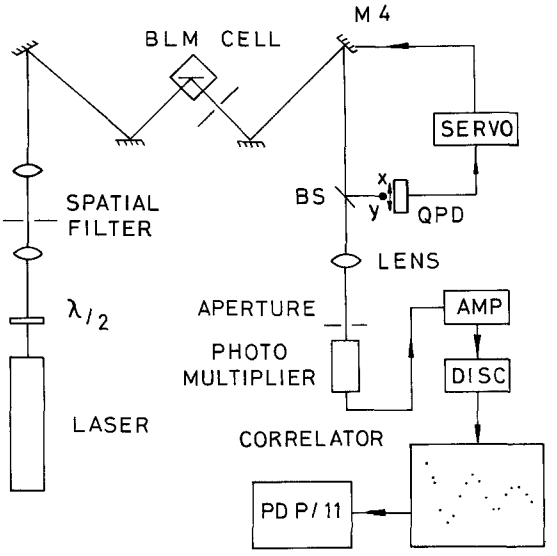

Fig. 2. A sketch of the optical arrangement (not to scale). The quadrant photodetector is marked as QPD and the x and y directions of its sensitivity are indicated

provided with apertures to permit visual inspection of the thinning film and for passage of the incident and scattered laser beams. Thermostatted water from a temperature-controlled bath (Grant SB 35 Q) circulating through a brass base provided coarse heat control. Fine control was governed by a temperature sensor integrated circuit (LM 3911N) mounted on the top of the heat-sink. The output of this i.c. was used to regulate the current flow to two thermofoil heaters (Minco Products Inc.) bonded to the sides of the heat-sink. The balance of heating and cooling was adjustable to give any required rate of temperature change. The thermal capacity of the system ensured minimal local variation of temperature within the cuvette. The temperature of the BLM was measured to better than 0.1° C using a digital thermometer (Comark 5215) and a pre-calibrated Ni-Cr/Ni-Al thermocouple placed in the water, close to the BLM. The thermostatted system was surrounded by PTFE insulation ranging from 6 to 15 mm in thickness. During light scattering measurements the only gap in this insulation was that for the laser beams, that for visual observations being blocked by a PTFE plug. This insulation had the added advantage of improving the acoustic isolation of the BLM cell. The temperature-controlled cell was mounted on a prism table with additional translational x-y-z degrees of freedom to permit precise alignment of the membrane in the optical system.

b. The light scattering spectrometer

Our heterodyne apparatus is sketched in Fig. 2. Briefly, a TEM_{00} laser beam of Gaussian profile from an argon ion laser ($\lambda = 488$ nm) was incident upon the membrane at an angle (θ) which was set between 20° and 30° to the normal. Light was scattered by the capillary waves in directions close to the specularly reflected beam from the BLM. A detector placed far (~ 1 m) from the BLM was illuminated by light scattered at a particular angle ($\delta\theta$) and by stray flare light due to scattering from inhomogeneities in the glass cell or optical components. This flare light provided a reference beam for heterodyne detection of the scattered light. The amplifier and discriminator provided standardized pulses for subsequent post-detection spectral analysis. The entire optical system was mounted on a massive vibration-isolated table.

The wave number q of the capillary waves observed is given by the projection onto the plane of the membrane of the scattering vector k of the scattered light. Using the well-known formula for k,

$$q = \frac{4\pi}{\lambda} \sin(\delta\Theta/2) \cos\Theta \,. \tag{6}$$

The scattering angle $\delta\theta$ was determined from careful measurement of the distance of the detector pinhole

from the point of maximum intensity of the specularly reflected beam. The angle of incidence θ was measured using an angular scale on the brass base upon which the cuvette was mounted. Previous versions of this spectrometer (Byrne and Earnshaw 1977; Crilly and Earnshaw 1983a) used a diffraction grating placed close to the fluid interface (or BLM) to generate a heterodyne reference beam. This had an additional advantage of precisely defining q. Unfortunately the bulky thermostating arrangements in the present apparatus prevent a grating being placed sufficiently close to the BLM for statisfactory operation. Correct determination of the q value appropriate to a particular experiment can be checked by comparison, where possible, of measured physical properties with accepted values (see below).

Various modifications have been made to the light scattering system, as previously described (Crilly and Earnshaw 1983a). Several of these have improved the signal-to-noise ratio, permitting statistically significant correlation functions to be acquired in briefer experiments. Consequently data can be taken more frequently, yielding more detailed information on dynamic variations. The changes include the following:

(*1*) Increased laser power (to ~ 200 mW) has been achieved by removal of the intra-cavity etalon of our Lexel model 85 laser, with no observable degradation in the quality of the correlation functions. This fortuitous observation has subsequently been substantiated independently (Pusey et al. 1983). Our laser output beam was restricted to the TEM_{00} mode, avoiding inter-mode beating. The coherence length of the beam without the etalon (~ 5 cm) exceeds any optical path differences in the apparatus, so no loss of coherence between reference and scattered beams occurs.

(*2*) A photon-counting photomultiplier (EMI 9863KB), specially selected for low spurious correlation, was used. The dark count was more than two orders of magnitude better than before.

(*3*) A 128 channel multi-bit correlator (Malvern Instruments K7025) was used for signal analysis. Multi-bit correlation offers significant statistical advantages in heterodyne experiments (Lehmann 1981).

Correlation functions were recorded (and subsequently analysed) on-line with a minicomputer. Several hundred correlation functions can be recorded in a single experimental session.

c. Servo system

A typical observed correlation function is shown in Fig. 3. A non-linear least squares routine was used to fit a functional form as Eq. (1) to the data, yielding estimates of the frequency (ω_0) and the temporal damping constant (Γ) of the capillary wave of selected q value. The indication of the correct functioning of the light-scattering system is the variation of these parameters with q in accord with the theoretical dispersion behaviour (Eq. 4). Spontaneous bulk movements of the membrane will somewhat obscure this dispersion behaviour (Crilly and Earnshaw 1983a): if the plane of fixation of the membrane

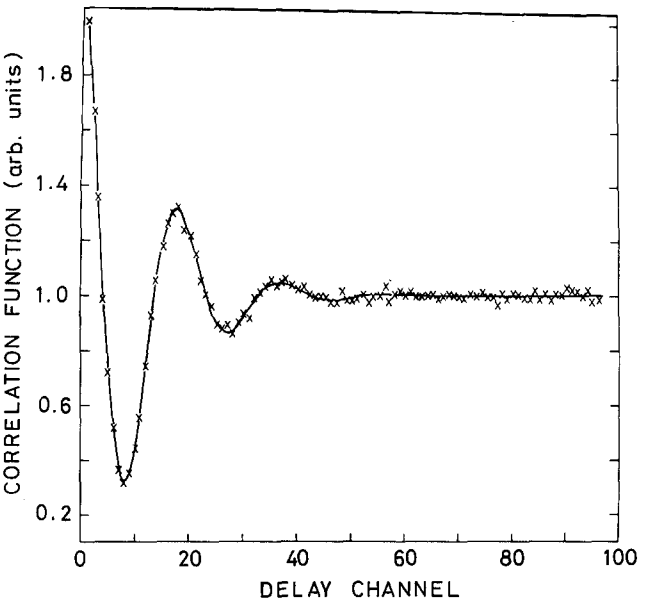

Fig. 3. A typical correlation function measured for a GMO/decane membrane. The sample time per channel was 4.0 μs. The solid line indicates the best fit function of the form of Eq. (1)

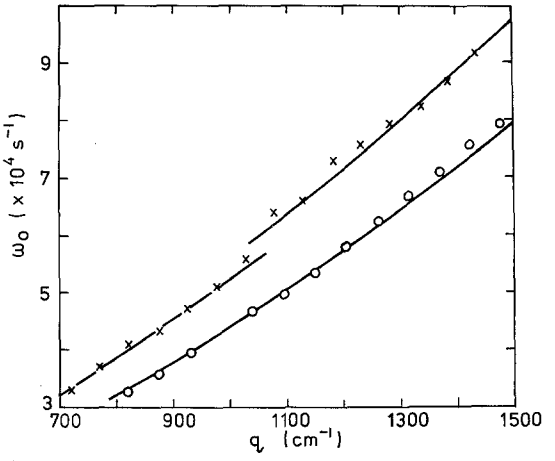

Fig. 4. The variation of ω_0 with wave number q for two membranes with (o) and without (x) servo system. The lines represent the average theoretical dispersion behaviour (Eq. 4) for the membranes. These are based on averages of the values of γ_0 and η estimated for each observation as described in the text

changes slightly, both the angle of incidence of the laser beam and the angle of scattering will be affected, changing the q value corresponding to a set detector position (Eq. 6). Figure 4 illustrates this effect quite clearly for ω_0. A similar shift was perceptible in the corresponding Γ values.

Unfortunately, it appears that such changes in the plane of fixation tend to be promoted by variations in temperature. The shifts observed for the specular reflection tended to be relatively gradual changes in the one direction (Crawford and Earnshaw 1983). Large effects seem to occur at or near the lipid transition temperature. Early results (Crawford and Earnshaw 1983) are thus suspect.

To offset these changes in the plane of fixation a servo-control system has been devised. Initially it appears possible to control the membrane orientation relative to the incident laser beam directly, using the prism table mounting. There are two reasons for not doing this. Firstly, any motion of the BLM cell might transmit undesirable vibrations to the membrane. Secondly, rapid or excessive tilting of the cell could cause bulging of the BLM, leading to non-planarity, changed lipid packing densities etc. The servo system as implemented therefore involves control of the mirror M4 (Fig. 2).

A pellicle beam splitter (Ealing) diverted ~ 25% of the light incident upon the photomultiplier through an angle of 90° to a quadrant photodetector, placed at the same distance from the BLM as the photomultiplier. The quadrant detector (Silicon Detector Corp.) has four active elements, each occupying one quadrant of a circle. It was oriented so that the two pairs of opposite quadrants are sensitive to vertical (y) and horizontal (x) motions of the incident light beam. The outputs from each pair of quadrants were fed to two identical difference amplifiers. Initially the system was aligned so that the specular reflection from the BLM gave balanced outputs from each member of a pair of quadrants. Any movement of the reflected beam caused out-of-balance signals from the difference amplifiers which drove DC motors on the micrometer controls of the mirror mount M4 to restore balanced outputs from the quadrant detector.

In use this system afforded a marked improvement. Figure 4 includes a typical dispersion plot obtained using the servo system. The data accord well with the expected dispersion behaviour, lacking any evidence for abrupt shifts of q value. However, large deviations of the membrane orientation may not be perfectly compensated, as changes in the total intensity of light at the quadrant detector (due to variations with angle of the reflectivity of the beam splitter) upset its operation. In practice these reservations seemed not to be significant.

Results and discussion

Membranes were formed at ambient temperature, well above the lipid phase transitions of GMO (White 1975; Pagano et al.1973), and allowed to thin to the bimolecular state over ~ 3 h. Observations were then taken as the membrane cooled slowly ($\lesssim 0.1°$ C/min) to temperatures well below the transitions. The slow cooling ensured that the temperature did not change significantly during acquisition of each correlation function. The temperature of the system was noted as each light scattering observation was started. Typically each correlation function was acquired over 30 s, some 200–300 functions being recorded in a cooling run. The experiment lasted for several hours. Many membranes did not survive so long. If they did survive, the process was continued in a heating run.

The correlation functions (Fig. 3) were of higher quality than those of previous PCS studies of membranes (Crilly and Earnshaw 1983a, b). The rms noise on the correlation functions, estimated from the final flat portion, was typically $< 1.5\%$. This good quality translates into high precision in the fitted variables ω_0 and Γ. Several correlation functions were observed for a BLM at constant temperature; the ω_0 and Γ values are plotted in Fig. 5. The standard deviations of the parameters for this data were 1.7% for ω_0 and 3.7% for Γ, considerably better than in previous work. These figures are typical of other scatter plots we have taken. The improvements are sufficient to permit observations of the subtle changes in membrane properties during thermotropic phase transitions.

Figures 6 and 7 show typical data from a series of experiments on GMO membranes. Some 240 data

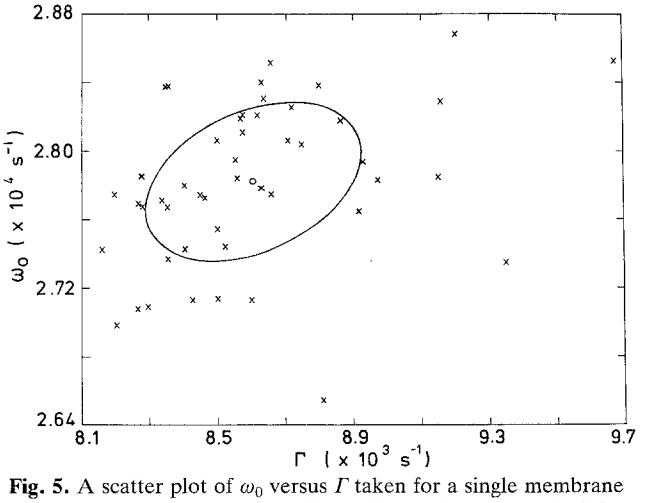

Fig. 5. A scatter plot of ω_0 versus Γ taken for a single membrane under constant conditions. The line indicates the one standard-deviation likelihood contour

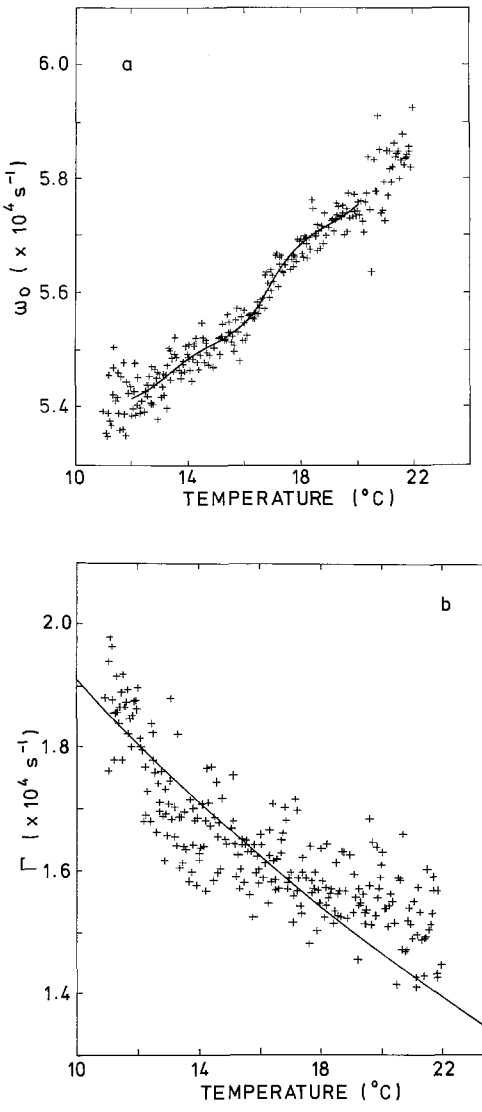

Fig. 6a and b. Variation with temperature of frequency (**a**) and damping constant (**b**) for a GMO/decane membrane (q = 1,209 cm^{-1}). The line in (**a**) is a cubic spline approximation to the data. The maximum gradient occurs at T = 16.8° C. That in (**b**) is the behaviour expected (Eq. 5) for damping due to the viscosity of the aqueous medium alone

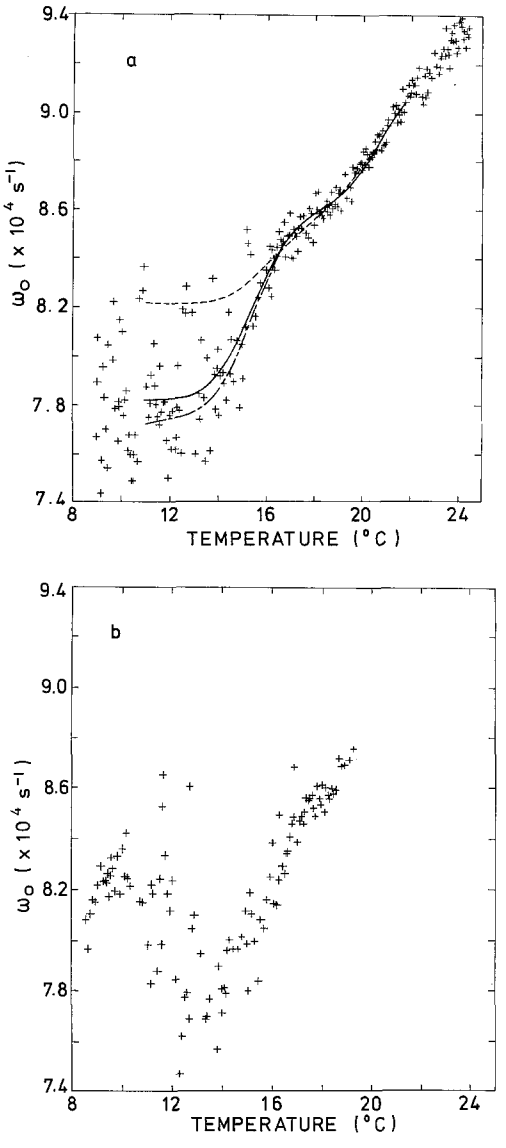

Fig. 7a and b. Variation with temperature of the frequency observed for a GMO/cholesterol/decane membrane (q = 1,400 cm^{-1}). Data for both cooling (**a**) and heating (**b**) are shown for comparison. The curves on (a) are discussed in the text: the maximum gradient of the solid line is at T = 15.5° C

points from a cooling run on a GMO/decane membrane are shown in Fig. 6; this membrane ruptured before a heating run could be undertaken. The smooth curve in Fig. 6a is a cubic B-spline approximation to the frequency data, derived with five knots at optimised positions (de Boor 1978). The curve through the damping data (Fig. 6b) represents the approximate theoretical behaviour of Γ (using the accepted variation of η for water with temperature).

From such data as that of Fig. 6a it is conceivable that the variation of ω_0 with temperature might not derive from a variation in membrane tension. A shift of membrane orientation, only partially compensated by the servo system, could cause such changes. We do not believe this to be the case. The damping data affords some reassurance, following as it does the accepted variation of η. From 22° C to 11° C ω_0 decreased by 8.2%. If this decrease were caused by membrane movements, the necessary change in q would be 5.5%. This would cause a corresponding 11% reduction in Γ, whereas the change seen is a 29% increase. Further support for the association of the changes in ω_0 with the membrane transition is afforded by other membranes for which a full cooling/heating cycle has been examined. In these

cases the data at high temperatures on the heating run coincide with those taken several hours previously at the start of the cooling run. Careful inspection of Fig. 7a and b illustrates this point. Such agreement would be most unlikely after a series of random changes in q value.

Some slight deviations between the cooling and the heating data might be expected due to (a) the ageing of the membrane over the several hours of an experimental run and (b) heating effects of a fairly powerful laser beam. The general accord between the heating and cooling data discussed above suggests that such effects are not significant, at least within our experimental precision. The absence of any systematic hysteresis effects, unlike preliminary reports (Crawford and Earnshaw 1983), is a powerful argument for the satisfactory functioning of our servo-control system.

Returning to the data of Fig. 6, the plot of ω_0 shows a clear change of slope in the region of the lipid phase transition, whereas that for Γ is apparently a smooth variation in general accord with the dependence of the kinematic viscosity of water on temperature. The detailed analysis of ω_0 and Γ in terms of membrane properties and the subsequent considerations of molecular mechanisms will not be presented now. Here we just note that the variations in gradient in the ω_0 versus temperature plots are common features of all our experimental data.

As noted above [Eq. (5)], the frequency ω_0 relates to the membrane tension, whereas the damping constant Γ relates to the viscosity of the ambient fluid (in the absence of significant membrane viscosity). The agreement of the Γ data of Fig. 6 with values expected for damping dominated by the ambient medium suggests that the GMO/decane membranes display no measurable membrane viscosity. This agrees with previous results (Crilly and Earnshaw 1983a). The membrane tension values derived from ω_0 vary from 5.5 mN/m at 20° C to 5.15 mN/m at 15° C. An increase in the interfacial concentration of GMO molecules as the BLM changes from the liquid-crystalline phase to the gel phase would give rise to the observed decrease in tension. Away from the transition region the membrane tension varies at about 0.04 (mN/m)/° C, both above and below the transition. This is in good agreement with a figure of 0.034 (mN/m)/° C from measurements of the interfacial tension of a solution of GMO in n-decane against 0.1 M NaCl (S.H. White, personal communication).

The tension values quoted exceed those found for similar GMO/decane membranes by Crilly and Earnshaw (1983a). In that work values close to the interfacial tensions of solutions of GMO in decane against water were found. The present tensions are roughly twice as large. We have no clear understanding of this discrepancy: the measured frequencies *are* systematically different. It appears that the present experiment is measuring membrane tension (film tension $\gamma_f \simeq 2\gamma_i$ the interfacial tension). It may be that if the two interfaces of the BLM were sufficiently separated they could respond to thermal disturbance as two separate though coupled interfaces. In this case (Crilly 1981) light scattering would probe interfacial tension. The conclusion that membrane tension is probed is supported by the only independent light scattering study of bilayer fluctuations (Grabowski and Cowen 1977), in which tensions in fair accord with literature values for *bifacial* tension (Tien 1974) were found. Reinterpretation of Grabowski and Cowen's data markedly improved the agreement (Crilly and Earnshaw 1983a).

As part of the investigation of the utility of PCS as a probe of membrane transitions, membranes of GMO incorporating various proportions of cholesterol have been studied. The data of Figs. 7 and 8 derive from a BLM formed from a solution of 10 mg/ml GMO, to which had been added 8.82 mg/ml cholesterol. At high temperature the measured ω_0 and Γ data display spreads comparable to those of Fig. 6. Below about 15° C the fluctuations in both become much greater. The temperature at which the behaviour changes is essentially the same for both cooling and heating runs. Other membranes incorporating substantial amounts of cholesterol showed similar variability at low temperature and also, on heating, reverted to the same high temperature values of ω_0 and Γ as initially found. This change in fluctuations is not noticeable for BLM containing low levels of cholesterol. Unfortunately our present data are not sufficiently comprehensive for us to estimate a threshold for this effect.

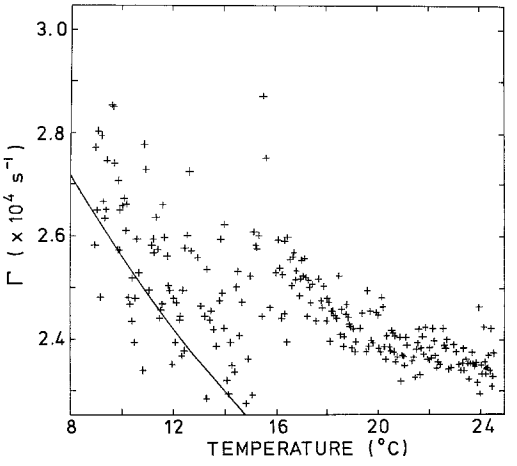

Fig. 8. The damping constants for the cooling run on the same membrane as in Fig. 7. The line is the behaviour expected based on the viscosity of the aqueous medium

We do not believe that these effects are instrumental in origin. While further work is required to substantiate the details, a tentative explanation is already at hand. These two points are considered in turn.

To cause fluctuations in ω_0 such as those shown, several large random changes in BLM orientation would be needed. The arguments already presented about the significance of the agreement of cooling and heating data at high temperature would be even stronger in this case. As already noted, the shifts evident without the servo system were gradual, in the one sense, rather than the random changes required to explain these data. Again, such variations have only been seen in BLM incorporating cholesterol, which is hardly likely for an experimental artefact. As discussed below, the Γ values at low temperature (Fig. 8) tend to agree with expectation [Eq. (5)]; this is most improbable for any explanation involving membrane orientation. We thus accept that the variations are rooted in some real variability, at low temperatures, of the properties of membranes incorporating substantial amounts of cholesterol.

We believe that the data of Figs. 7 and 8 may reflect a lateral phase separation within these BLM. Various aspects of the data support such an interpretation.

Careful inspection of Figs. 7 and 8 indicate that some few adjacent points tend to lie together above the general trend. This grouping of the data is particularly apparent for the more precise ω_0 data (Fig. 7a). At temperatures below 16° C ω_0 and Γ are somewhat correlated (correlation coefficient 0.29, significant at the 0.5% level). Data points which have high ω_0 values tend to have high Γ values.

The solid curve in Fig. 7a is a spline fit to all the data. The broken curves are spline fits derived by omitting the groups of points lying conspicuously above the general trend (chain curve) and by omitting all data below 16° C *except* these high points (dashed curve). The form of the chain curve suggests a phase transition similar to the GMO/decane membrane of Fig. 6. The dashed curve suggests the association of the high groups of points with the high temperature behaviour of ω_0.

The most obvious feature of Fig. 8 is a sharp decrease in Γ at about 15° C. Above this temperature, whilst the spread in Γ is comparatively low, the actual values markedly exceed the prediction of Eq. (5) for damping due to the aqueous medium alone. This is expected, as previous PCS studies (Crilly and Earnshaw 1983b) have shown that cholesterol-containing GMO membranes display a significant membrane viscosity (γ'). However, below 15° C, where the fluctuations in Γ are much increased, the lower range of Γ observed is more nearly compatible with the behaviour of Eq. (5), implying the absence of significant membrane viscosity. Again, the data corresponding to the groups of high points of Fig. 7a lie on a reasonable extrapolation of the high temperature data.

The local inhibition of the transition from the liquid-crystalline phase incorporating gauche configurations to the all-trans gel phase by cholesterol (e.g., Baret 1981) affords a natural explanation of these features of our data. Regions of the BLM may arise comprising a cholesterol-rich fluid phase while other areas, poor in cholesterol, transform to pure GMO gel phase. Light scattering probes an area of the membrane defined by the laser beam, averaging the local BLM properties over this area. Averaging over several small regions of separated phases would lead to values of γ_0 and γ' intermediate between those appropriate to the GMO gel phase and the cholesterol-rich fluid regions, as observed.

Two points are noteworthy. The present results suggest that the fluid behaviour of the cholesterol-rich regions persists to temperatures well below the phase transition. Secondly, it is apparent that at least some of these regions must be of macroscopic extent. The laser beam at the membrane had a diameter ($1/e^2$ point of intensity profile) of 0.3 mm. To perceive fluctuations in the spectrum of the scattered light such as those evident in Figs. 7 and 8 the laser beam must illuminate an area largely comprising one phase at any one time. The existence within membranes of separated phase regions of macroscopic extent has been suggested before (Pagano et al. 1973).

Returning to the phase transitions as revealed in this work, the points of maximum slope of the spline approximations to the ω_0 – T plots occur at temperatures close to the accepted transition temperature of GMO (White 1975; Pagano et al. 1973). The temperature of maximum gradient appears to decrease somewhat with increasing cholesterol content of the film-forming solution. This is in general agreement with published observations (e.g., Ladbrooke et al. 1968). These temperatures are not dependent upon the spline approximation used; they are essentially independent of the number of knots used and of whether or not their positions are optimised.

Summary

Techniques of laser light scattering, recently shown to be a useful probe of the viscoelasticity of planar lipid membranes, have been refined to permit subtle changes in these properties at the lipid phase

transition to be followed for the first time. Servo-controlled optics form an essential part of the refined system. Light scattering yields *local* average values for the membrane properties (averaged over the extent of the laser beam) rather than the bulk averages determined by thermodynamic techniques (e.g., DSC). This local averaging contrasts with information derived using molecular probes, which refer to the microenvironment surrounding the probe. Indeed the environment sensed may be created by the probe itself (Azzi 1975). The light scattering approach requires neither probe nor any gross perturbation of the membrane to observe viscoelastic properties (cf. Tien 1974).

The viability of photon correlation spectroscopy for phase transition studies has been demonstrated in an exploratory series of experiments on GMO bilayers formed in water. Below the lipid phase transition, membranes incorporating substantial amounts of cholesterol behaved very differently from those lacking cholesterol. While much work is needed to substantiate the picture, the effects receive a coherent explanation in terms of a cholesterol-induced lateral phase separation within the membrane. As a probe of local average membrane properties, light scattering has provided indications of the size and mobility of the fluid regions incorporating cholesterol within the gel phase of the membrane.

Acknowledgements. This work has been supported by grants from the Science and Engineering Research Council. One of us (GEC) is grateful for financial support by the Department of Education for Northern Ireland.

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