THERMAL EXCITATIONS OF A BILIPID MEMBRANE

E. F. GRABOWSKI *and* J. A. COWEN, *Department of Physics, Michigan State University, East Lansing, Michigan 48824*

ABSTRACT Propagating modes of vibration of a bilipid membrane have been detected with light beating spectroscopy. The dependence of ω on q is consistent with a model of a fluid film of surface tension $\sigma = 2.5 \pm 0.5$ dyn cm^{-1} surrounded by a medium with $\rho = 1$ g cm^{-3} and $\eta = 1.01 \times 10^{-2}$ P.

In this note we report the first measurement of the dynamic properties of a bilipid membrane (BLM) using light beating spectroscopy and discuss in some detail the limitations of the technique. Among the various possible models (1–3), our rather crude results seem best fit by the very simple model of an interface with surface tension σ, immersed in a medium of density ρ and viscosity η.

Thermally excited fluctuations of the membrane surface may be Fourier analyzed into a set of traveling waves—ripplons—of wave vector $q = 2\pi/\lambda$ (λ is the wave length of the mode). A collimated, monochromatic light beam which illuminates the membrane will be specularly reflected by the $q = 0$ excitations and will be diffracted and frequency shifted by the $q \neq 0$ excitations. A graph of the frequency shift, ω, vs. q gives the dispersion law, which can be related to the restoring forces in the membrane. In the absence of instrumental or configurational factors, the line width of the signal depends on the decay time of the excitations and thus on the viscosity of the medium. In principle, then, we can obtain information about the elastic forces or surface tension and the viscosity of the membrane. The BLM is, of course, surrounded by an aqueous medium in which the interfacial excitation extends as an evanescent wave to a depth of approximately λ. We observe signals for 500 cm$^{-1} < q < 1{,}500$ cm^{-1}, corresponding to 40 μm $< \lambda \leq 125$ μm. Since the BLM is at most 10^{-2}-μm thick, clearly the principle contribution to viscous losses is from the surrounding medium.

We have attempted to produce membranes of egg lecithin, glycerol monooleate, and oxidized cholesterol but find that only the cholesterol will form the large, stable membranes necessary for these measurements. The most serious limitations on the configuration are: (*a*) The diameter of the membrane should be larger than the diameter of the beam, which should, in turn, be larger than the wavelength of the excitation. (*b*) The macroscopic surface should be flat over the total illuminated area so that the reflected light does not diverge due to deviations in the surface normal. (*c*) The illuminated spot should be sufficiently large so that diffraction does not seriously effect the observed line width.

The membrane is formed in the following fashion: A few drops of oxidized choles-

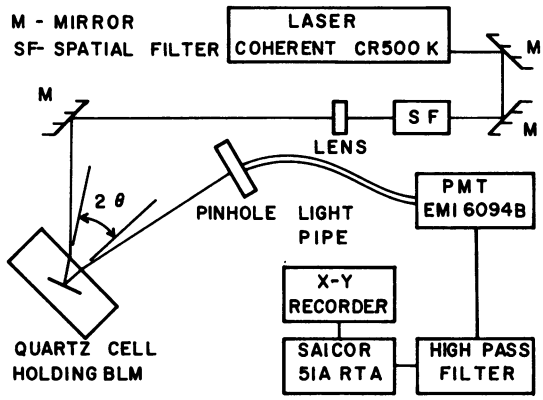

FIGURE 1 Block diagram of the experimental system.

terol[1] dissolved in normal octane are deposited on the surface of a 0.1 N solution of KCl in water. A thin Teflon form that has a polished 0.6-cm-diameter hole is carefully lowered through the surface. A V-shaped groove is cut in the periphery of the hole to orient and stabilize the membrane. A film forms in the hole which thins like a soap film until it becomes black and stable for up to 48 hs. At this point it is presumed to be essentially bimolecular. The statics of the formation of the vertical film are not well understood. It is readily inspected with a low-power eyepiece under illumination with a bright white light source or an expanded laser beam. Inspection demonstrates that it is never flat. The surface is puckered and convoluted on a macroscopic scale. However, the membrane diameter is much larger than the ripplon wavelengths of interest and we can always find a spot on the membrane that looks locally flat for laser beam diameters of 0.02 cm or less.

The membrane also undergoes low-frequency vibrations due to acoustic and mechanical excitations in the room. We mount our apparatus on a pneumatically supported 2,000-kg granite table for isolation from building vibrations.

The membrane is illuminated with the spatially filtered beam from a Coherent Radiation CR500K krypton ion laser (Coherent Radiation, Palo Alto, Calif.), focussed to a 0.02-cm-diameter spot on the membrane with a convergence of approximately 5 mrad (Fig. 1).

The scattered light passes through a movable pinhole onto a fiber optic light pipe, which guides it to the active surface of the photomultiplier. The audio frequencies that result from the mixing of the scattered light with the "local oscillator" light elastically scattered from the window of the cell pass through a high-pass filter and a preamplifier and are analyzed by a SAICOR 51A real time analyzer (SAICOR, Honeywell, Hauppauge, N.Y.). The circles in Fig. 2 are a "typical" signal taken at an angle of incidence of 6° with light of wavelength 476.2 nm. We have observed signals

[1]The samples were kindly provided by Professor H. T. Tien of the Michigan State University Biophysics Department, East Lansing, Mich.

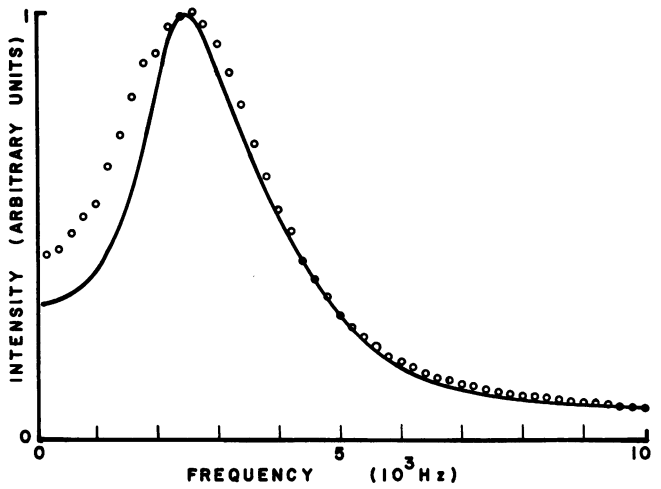

FIGURE 2 "Typical" data taken at $\lambda = 476.2$ nm. The vertical scale is arbitrary and the horizontal scale is 0–10 kHz. $q = 813$ cm^{-1}. The solid line is the convolution of the theory with a rectangular instrument function of width 500 cm^{-1}.

with light of wavelength 568.2, 520.8, 482.5, and 476.2 nm at several angles of incidence between 6 and 25°. The signal-to-noise ratio improves with decreasing wavelength.

Two sources of noise with amplitude much larger than the signals of interest complicate our measurements. The water jacket of our laser tube introduces noise at frequencies below 100 Hz. There is also a Rayleigh line from light scattered by particles suspended in the liquid (4). We pass all our solutions through a 0.5-μm filter to minimize this effect. There are also several mechanical resonances of the lipid system driven by acoustic excitations. A high-pass filter with an adjustable corner frequency (100–1,000 Hz) eliminates these signals before we integrate. Their presence, however, prevents examination of low-frequency signals.

Data acquisition is complicated by periodic shifting of the direction of the surface normal to the lipid. The V groove successfully "pins" the edges of the BLM but the convolutions of the surface change with time. Large shifts (5°–10°) of the average direction occur on a time scale of 15 min–2 h. The surface normal also exhibits small-amplitude ($\sim$2 mrad) oscillations about a given average direction with periods of 1–5 min. This motion contributes considerably to the scatter in our data points, as we measured the position of the pinhole relative to the position of the specular reflected beam that corresponds to $q = 0$. The amplitude of these oscillations decreases as the membrane grows older. The reflected $q = 0$ beam can be as large as 0.2 cm at the pinhole, making the accurate location of $q = 0$ difficult. The large shifts can be eliminated by reducing the membrane diameter to 0.1 cm but the surface is still curved and it is difficult to find flat regions on these small membranes.

To minimize the error due to this uncertainty in position and shifting of the surface normal, we took data on both sides of $q = 0$ in the plane of incidence. A typical run of 25 points took 1 h. We used data symmetric about a point consistent with the mea-

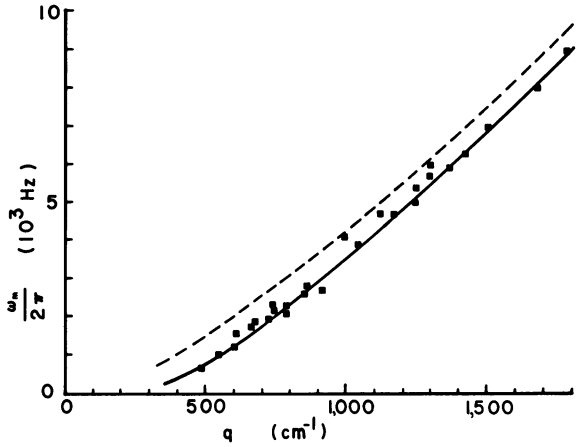

FIGURE 3 $\omega_{max}/2\pi$ vs. q taken at $\lambda = 476.2$ nm, $\theta_{incidence} = 6°$, and $T = 18°C$ with a solid angle of collection of 5×10^{-6} steradians. The dashed line represents the theory for $\sigma = 2.5$ dyn cm^{-1}, $\eta = 1.01 \times 10^{-2}$ P and $\rho = 1$ g cm^{-3}. The solid line is the convolution of this theory with a rectangular instrument function of width 500 cm^{-1}.

sured $q = 0$. At the end of a run we retook several points near the start to make sure the membrane had not shifted. The data in Fig. 3 were taken from a membrane about 14 h old.

Among the possible theoretical models we have found that our results are best fit by expressions given by Herpin and Meunier (3) for the power spectral density of the light scattered by thermal fluctuations of the interface between two liquids of densities ρ and ρ' and viscosities η and η' with interfacial tension σ. This theory has been successfully applied to the free surface of water (5) and to the interface between a liquid and its vapor near a critical point (6).

Under our assumption, the power spectral density $P(q, \omega)$ reduces to:

$$P(q,\omega) = (Y\kappa T/\sigma\pi\omega q^2)\, Im[1/D(i\omega\tau_0)],$$

$$Y = (\sigma\rho/8\eta^2 q), \quad \tau_0 = (\rho/2\eta q^2),$$

and

$$D(S) = Y + (1/2)[S(1 + 2S)^{1/2}][1 + (1 + 2S)^{1/2}].$$

κ is Boltzman's constant, T the temperature, and Im signifies the imaginary part. The dashed line in Fig. 3 represents numerical solutions for the frequency corresponding to the peak in $P(q, \omega)$ as a function of q with $\rho = 1$ g cm^{-3}, $\eta = 1.01 \times 10^{-2}$ P, and $\sigma = 2.5$ dyn cm^{-1}. The accepted value for the surface tension of oxidized cholesterol is 1.9 ± 0.5 dyn cm^{-1} (7).

The theoretical dependence of ω_{max} on q was obtained by assuming that the density and viscosity of the system were essentially that of water and then varying the surface tension (and thus the curvature and slope) until the dashed curve in Fig. 3 was ob-

tained. At a particular q the width of $P(\omega)$ is approximately 50% of the observed signal width. The remainder of the width must be due to instrumental factors that arise from pinhole size, beam divergence, local curvature in the membrane surface, and the fact that we illuminated between 2 and 10 "waves" to produce the signal. Some of these factors, such as beam divergence, are constant and calculable, others, such as local curvature, vary and are not calculable. In the absence of a better approach, we assumed a rectangular instrument function whose width was a free parameter and convoluted it with the theory to produce a "theoretical" curve, with which we compared our data. The best fit for all data was obtained with a width of 500 cm^{-1}. Since the theoretical intensity varies approximately as q^2, the effect of the instrument function was to shift the peak to lower frequencies as well as to broaden the signal. The solid line in Fig. 3 is the resultant dispersion law while that in Fig. 2 is the numerical fit to a particular piece of data. Note that the effect of the instrument function on the dispersion law was to shift the curve parallel to itself while the effect of the surface tension is to change the slope and curvature. It is therefore relatively easy to vary these two parameters independently and in so doing detect a change in σ of less than ± 0.5 dyn cm^{-1}, so that our experimental value of σ is 2.5 ± 0.5 dyn cm^{-1}. One further check of the instrument function was made with a year-old sample of cholesterol whose state of oxidation had very likely changed. The results gave a best fit with $\sigma = 1.9$ dyn cm^{-1} but with the same instrument function width.

To show that our signals were characteristic of the interface and not Doppler shifts from convection currents due to local heating, we varied the incident laser power by a factor of five, holding q constant. No shifts in frequency were observed. When we replaced the membrane with a plastic microscope cover slip no signals were observed. In addition, the symmetry of the observed signals on both sides of the specularly reflected beam ($q = 0$) and the identical results for angles of incidence between 6 and 25° place considerable constraints on possible convection currents. We also made membranes in solutions of 0.1 N KCl and glycerine. We observe no signals displaced from $\omega = 0$ with a 28% glycerine solution having $\eta = 2.75 \times 10^{-2}$ P and $\rho = 1.07$ g cm^{-3}. We would not expect this total damping of the signals if we were observing Doppler shifts from convection currents of thermally driven cholesterol molecules moving along the membrane surface.

If the only effect of the glycerine were to change the viscosity of the liquid, the theory predicts propagating modes only for q less than 1,400 cm^{-1} with no peak frequencies above 2,000 Hz. The intensity of these signals would be near our detection threshold. Any simultaneous lowering of the surface tension would push the signals into the noise.

Our original motivation for performing the experiments was to study the effect of lipid phase transitions on the surface tension and especially on the viscosity. As is clear from our discussion, we are unable to obtain information on the viscosity of the membrane since the principal contribution to the viscosity comes from the evanescent wave in the aqueous solution. The accuracy of our results is limited by our inability to produce the large ($d > 0.2$ cm), flat membranes with good borders with any of the

lipid materials that we used. Nonetheless we have demonstrated that the technique is useful for detecting ripplons on a BLM, and we feel that with somewhat better techniques for producing large BLM's it could be used to monitor changes in surface tension arising from the incorporation of proteins or other molecules into the lipid or even the effect of phase transitions.

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