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THE EFFECT OF TEMPERATURE ON CAPACITANCE CHANGES IN AN OSCILLATING MODEL MEMBRANE

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Summary

The electrical properties of model membranes are altered during stretching or pressure pulses. We have used a mechanico-electric transduction model to interpret the temperature dependence of capacitance changes produced in oxidized cholesterol membranes during mechanical oscillation. The relative contribution of the torus and bilayer portions of the membrane to the capacitance change is identified. The difference in elasticity between the bilayer and torus decreases rapidly with decreasing temperature and ultimately the torus becomes as solid as the bilayer portion of the model membrane.

Introduction

Many cells produce electrical changes in response to mechanical stimuli. In mechanico-receptors, such as stretch receptors in muscle, this property is highly developed and these electrical changes can give rise to nerve impulses [1]. The mechanism by which mechanico-electric transduction takes place is not well understood; however, transduction can take place in simple model membranes without specific proteins present [2—6]. Several authors have described those changes in electrical properties of model membranes during mechanical stretching or pressure pulses. They have described their observations as pressure-dependent conductance changes [3,4], permeability changes due to membrane stretching [5] or capacitance changes [2,6]. One of the most informative studies of mechanico-electric transduction is that of Wobschall [2] who measured capacitance changes in an oscillating cholesterol-hexadecyltrimethylammonium chloride membrane and described the capaci-

tance changes in terms of a linear model of membrane and border elasticity. A mechanically induced electrical oscillation is interpreted as a capacitance change caused by alterations in the relative proportion of the bilayer and torus and their thickness.

We have found this model appropriate for other membrane systems, and have used it here to describe the effect of temperature on the elastic properties of oxidized cholesterol membranes. These changes with temperature are of interest in understanding the factors that control membrane fluidity since permeability properties, activity of certain membrane-bound enzymes, and transport systems, all may depend upon the fluidity and physical state of the membrane lipids [7].

Methods

Model membrane. The membrane-forming solution was made by oxidizing a 4% solution of cholesterol (Primary standard grade, Eastman) in octane according to the method of Tien et al [8]. The solution was divided into 1 ml samples, dried under vacuum, sealed, and stored under liquid nitrogen until used. The working solution was reconstituted by adding 1 ml of decane to the dried material.

The bathing medium in the cell was 0.1 M KCl plus 10 mM Tris buffer (pH 7.4) in distilled water.

Experimental cell and electronics. The experimental cell, which was machined from a 7.56 cm diameter plexiglass rod, is shown in Fig. 1 along with a schematic diagram of the electronics. Membranes were formed by the brush method across the horizontal septum (2 mm diameter) milled into a piece of Rulon® (Johnston Industrial Plastics) rod. The membrane thus separates the upper chamber, which is open to the atmosphere, from the lower, which is sealed by O-rings to a stainless steel diaphragm. The diaphragm is coated with Silastic® (Dow Chemical Co.) on its inner surface and is oscillated by a rod attached to a 2.5 inch (nominal) speaker. Shape and amplitude of the oscillations are monitored by a Hewlett Packard 24DCDT-100 displacement transducer, whose output signal is measured with a voltmeter or oscilloscope. The speaker is driven by an EXACT Electronics model 250 function generator in the frequency range 0.001—10 Hz. The membrane oscillations can be made symmetric about the flat or any bulged membrane position. Membranes are bulged a fixed amount by applying an appropriate DC voltage to the speaker in addition to the oscillating voltage.

The basic capacitance of the resting membrane and the capacitance change during oscillating are measured with an AC bridge circuit in which the membrane appears as one arm opposite a parallel-connected resistance and capacitance box. Electrical connection between the membrane and the bridge is maintained by platinum-platinum black electrodes. The driver voltage applied across the bridge was the reference signal from a P.A.R. HR-8 lock-in-amplifier. The driver frequency is 10 kHz and the signal applied to the bridge is 60 mV. The output signal of the bridge is fed into the lock-in-amplifier and is nulled with the capacitance and resistance boxes. Once the bridge is balanced and the phase adjusted on the lock-in-amplifier, oscillations in capacitance appear as

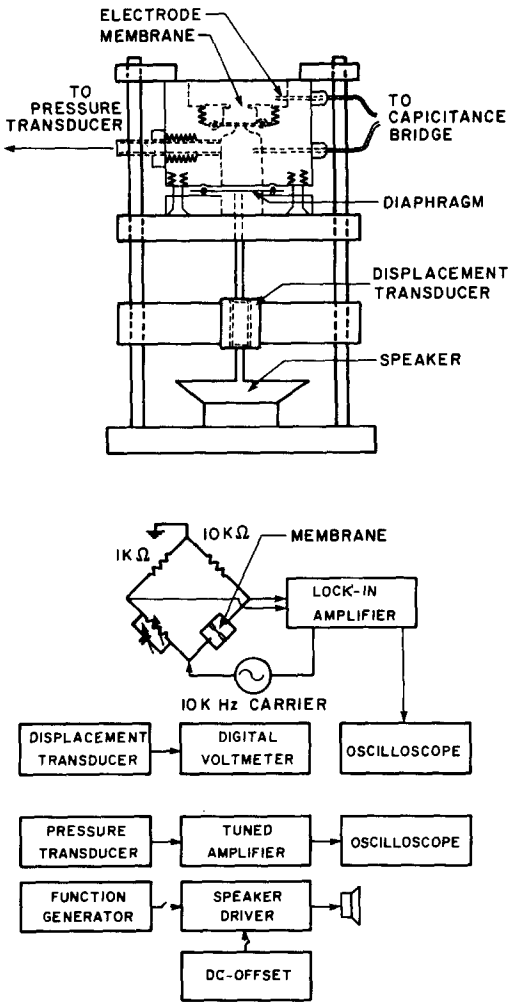

Fig. 1. The cell clamped firmly into place on the metal and plexiglass stand. Pressure and displacement transducers were attached during alignment to monitor the mechanical oscillations applied to the membrane.

voltage oscillations, and can be measured on an oscilloscope or an AC voltmeter. This small relative capacitance signal, which appears upon mechanical oscillation, will not be affected by frequency dispersion caused by stray capacitance within the cell.

Pressure oscillations in the cell can also be measured by attaching a Pace P90D pressure transducer across the upper and lower chamber of the cell.

Differential scanning calorimetry. Thermal analyses of the membrane-forming solution were performed on a Perkin-Elmer DCS-1B differential scanning calorimeter equipped with a cooling attachment for subambient operation. Samples were sealed in aluminium pans and heated at 5 K/min.

Results

Typical oscilloscope traces of the pressure, displacement and capacitance signals are shown in Fig. 2. In the measurements reported here, the membranes were bulged a fixed amount at all frequencies, so the capacitance signal appears as in Fig. 2d.

The analysis of capacitance amplitude in membranes undergoing sinusoidal oscillation has been made by Wobschall [2]. His expression for the relative capacitance change $\Delta C/C$ in a bulged membrane (Fig. 2d) is:

$$\frac{\Delta C}{C} = 2X_0\left[\frac{(Q_1/2-\omega^2)^2+Q_3^2\omega^2}{(Q_1-\omega^2)^2+Q_2^2\omega^2}\right]\sin(\omega t+\phi) \tag{1}$$

Bilayer stretching is represented by a spring of value g_1, thinning by a dashpot of value h_1. The torus is represented by a spring g_2, which describes the torus' tendency to return to equilibrium, and a viscosity element, dashpot h_2. The equivalent mechanical circuit of the model membranes is a parallel spring g_2 and a dashpot h_2 in series with a spring g_1 and dashpot h_1. In terms of these elements $Q_1 = g_1g_2/h_1h_2$, $Q_2 = g_2/h_2 + g_1/h_2 + g_1/h_1$, $Q_3 = g_1/2\,h_1 + g_2/h_1$, while X_0 is the relative area change $\Delta A/A$ as the frequency approaches zero, ω is

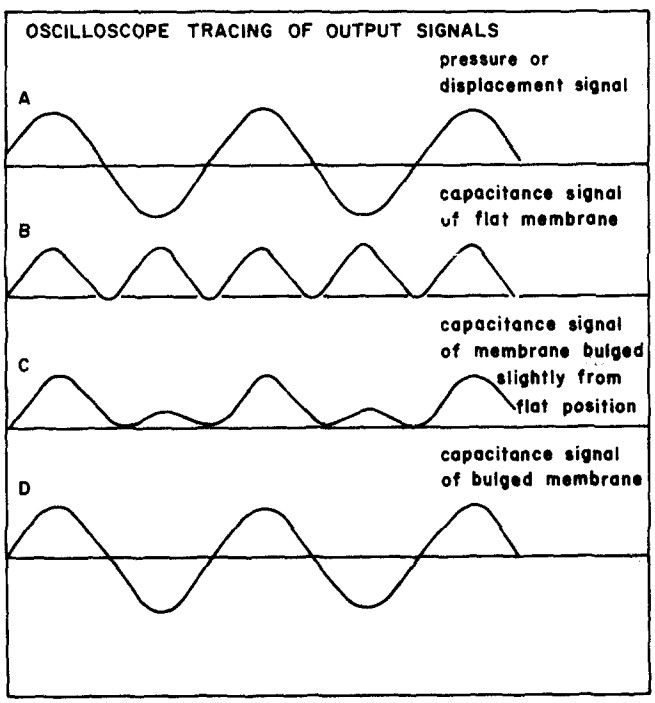

Fig. 2. Typical oscilloscope traces. (A) Signal applied to speaker by function generator. This also is the output signal of the pressure and displacement transducers. Signals at output of lock-in-amplifier: (B) The membrane is flat before oscillation begins. In this case the capacitance increases regardless of whether the membrane is stretched upward or downward. (C) Signal from a membrane which is slightly bulged. (D) Signal from a membrane bulged further than the oscillation amplitude. The membrane does not pass through the flat position.

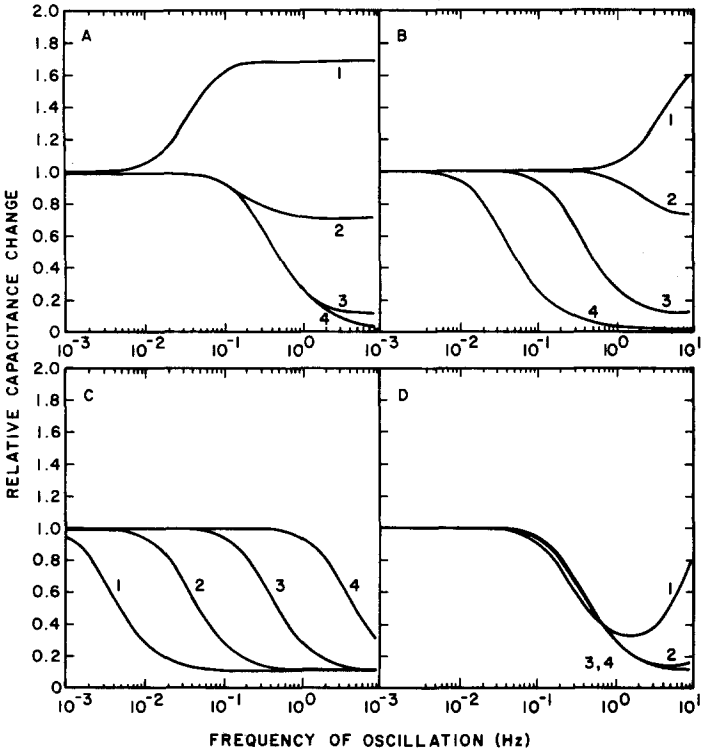

Fig. 3. The effects on membrane capacitance of varying the parameters in Eqn. 1. (A) $g_2 = 11.0$, $h_1 = 6.0$, $h_2 = 0.02$; curve 1, $g_1 = 2.0$; curve 2, $g_1 = 20.0$; curve 3, $g_1 = 200.0$; curve 4, $g_1 = 2000$. (B) $g_1 = 200.0$, $h_1 = 6.0$, $h_2 = 0.02$; curve 1, $g_2 = 1100.0$; curve 2, $g_2 = 110.0$; curve 3, $g_2 = 11.0$; curve 4, $g_2 = 1.1$. (C) $g_1 = 200.0$, $g_2 = 11.0$, $h_2 = 0.02$; curve 1, $h_1 = 600.0$; curve 2, $h_1 = 60.0$. curve 3, $h_1 = 6.0$; curve 4, $h_1 = 0.6$; (D) $g_1 = 200.0$, $g_2 = 20.0$, $h_1 = 6.0$; curve 1, $h_2 = 2.0$, curve 2, $h_2 = 0.2$; curve 3, $h_2 = 0.02$; curve 4, $h_2 = 0.002$.

the angular frequency, t is time and ϕ is the phase angle.

The effects of varying each parameter independently are shown in Fig. 3. Measurements were made in the frequency range 0.01—10 Hz. A comparison of the curves in Fig. 3 with the experimental curves (Figs. 4 and 5) shows that the relative size of g_1 and g_2 are the most important parameters in this frequency range. The value of h_2 has little effect in the frequency range studied. For small values of h_2, Eqn. 1 can be simplified. Defining the ratios $X = g_1/g_2$ and $Y = h_1/g_1$, we then have:

$$\frac{\Delta C}{C} = 2X_0\left[\frac{\frac{1}{4} + (Y\omega)^2}{1 + \left(\frac{Y\omega}{\alpha}\right)^2}\right]^{1/2} \sin(\omega t + \phi) \tag{2}$$

where $\alpha = 1/(1 + X)$.

Fig. 4 shows a plot of $\Delta C/C$ for membranes made from a cholesterol solution which was oxidized for 10 h. The low frequency-specific capacitance of membranes used in these experiments were in the range 0.52—0.41 $\mu F/cm^2$, when calculated as capacitance/septum area, and averaged 0.47 $\mu F/cm^2$. The curves

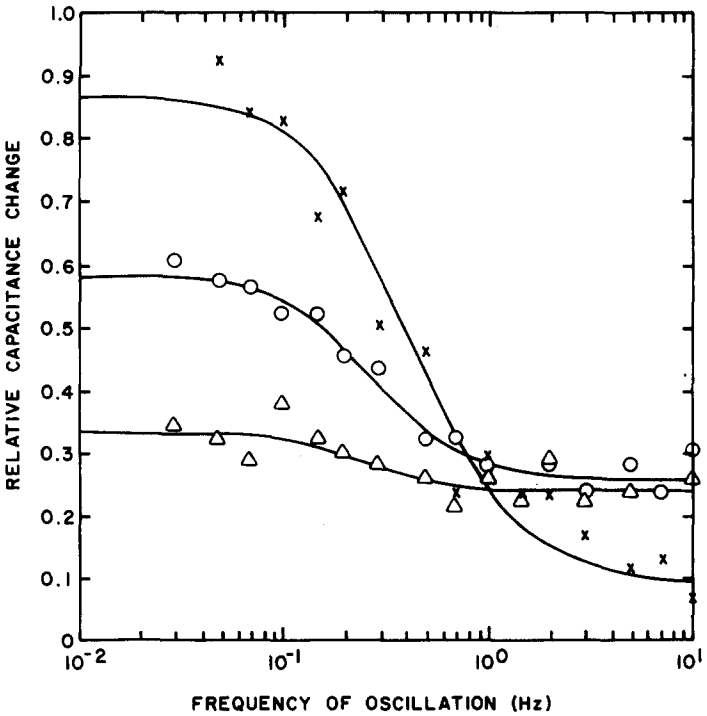

Fig. 4. The relative capacitance change for oxidized cholesterol membranes from the 10 h sample are shown as a function of frequency of oscillation. X, $T = 22°C$. The value of $g_1/g_2 = 17.9$ and $h_1/g_1 = 0.0308$. ○, $T = 13°C$, $g_1/g_2 = 3.58$ and $h_1/g_1 = 0.152$. △, $T = 8°C$. $g_1/g_2 = 1.82$ and $h_1/g_1 = 0.242$. The values of g_1/g_2 and h_1/g_1 are found by fitting Eqn. 2 to the experimental points, using a combination of the gradient and Newton-Raphson procedures [13].

are from measurements at different temperatures along with the least-squares fit of Eqn. 2 to the data points. It is clear that the elastic behaviour of the membrane is very temperature dependent. The ratio g_1/g_2 (the spring constant of the bilayer to that of the torus) decreases rapidly with temperature. Thus, g_2 which represents the tendency of the torus to return to its equilibrium position, becomes larger than g_1, the resistance to bilayer stretching. As expected, the ratio h_1/g_1 generally increases, implying a decreased rate of thinning with decreasing temperature. At low frequency a very small amount of thinning takes place. However, at higher frequencies the oscillations are too rapid for thinning to take place, only the elastic stretch of the bilayer contributes to the relative capacitance change $\Delta C/C$.

As is usual in work with model membranes made from oxidized cholesterol, there was a variation in the values obtained between membranes. For example, four different membranes made from the solution shown in Fig. 5 gave values of g_1/g_2 and h_1/g_1, at 23°C, which averaged 2.99 and 0.184, with S.D. of 1.33 and 0.049, respectively. However, when measurements were repeated on one membrane at various temperatures, the results were always qualitatively the same, a decrease in g_1/g_2 and an increase in h_1/g_1 with decreasing temperature.

Fig. 5 shows the behaviour of membranes made from a second membrane-forming solution which was oxidized for 8 h. A typical membrane made from

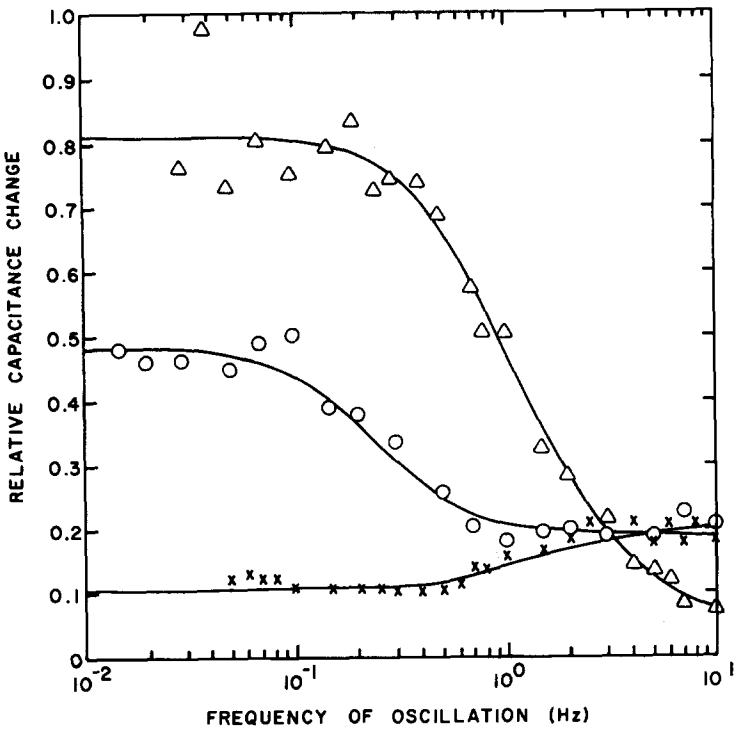

Fig. 5. The relative capacitance change for oxidized cholesterol membranes from the 8-h sample are shown as a function of frequency of oscillation. △, $T = 35°C$, $g_1/g_2 = 39.4$, $h_1/g_1 = 0.00545$ ○, $T = 23°C$, $g_1/g_2 = 4.12$, $h_1/g_1 = 0.156$; X, $T = 20°C$, $g_1/g_2 = 0.0765$, $h_1/g_1 = 0.995$.

this solution gave a change in shape of the capacitance vs. frequency curve as the temperature was lowered to 20°C. The temperature at which the curve changes shape varied from membrane to membrane, although the transition always occurred in the range 16—20°C.

We originally considered that a phase transition might be taking place in these membranes at approx. 20°C. Transitions have been noted in glycerol monoleate and glycerol monstearate planar model membranes [9] as well as in many other model and biological membrane systems [10].

A phase transition has been observed in pure cholesterol [11,12]. It occurs at approx. 40°C, although the cooling curve occasionally shows a peak extending near 20°C. Differential scanning calorimetry measurements of this 8-h-oxidized cholesterol membrane-forming solution, when dried or in decane, also show the transition near 40°C, probably due to the large proportion of unoxidized cholesterol always present in the membrane-forming solutions. No additional phase transitions were seen at or near 20°C. Thus, the change in shape of the capacitance amplitude curve seen in Fig. 5 near 20°C is not caused by a phase transition, but can be explained by a continuous variation in the values of g_1/g_2 and h_1/g_1. The 20°C curve in Fig. 5 can be described by Eqn. 2 with the proper choice of parameters, $g_1/g_2 < 1$. This implies that the torus appears less elastic than the bilayer at lower temperatures.

Discussion

We have shown that the frequency vs. induced capacitance change has a strong temperature dependence consistent with changes in mechanical properties of model membranes. When a sinusoidal volume change is applied to the closed side of the experimental cell to oscillate the membrane about its resting position at low oscillation frequencies, the bilayer increases its area at the expense of the torus and hence the capacitance, which is proportional to the area of bilayer, also increases. As the frequency increases, however, the membrane begins to oscillate too quickly for thinning to occur. The capacitance increase is then due to the elastic stretching of the bilayer only. Thus, the mechanical properties of the membrane are defined by the curve of capacitance amplitude vs. frequency. We have thus interpreted changes in the capacitance-frequency curve with temperature as a variation in the mechanical parameters of the bilayer and torus.

The curves of the relative capacitance change during oscillation, $\Delta C/C$, obtained at all temperatures, are adequately described by Wobschall's model, with suitable choice of parameters. In addition, this approach can be useful in studying the effect of other physical or chemical agents, such as X-rays or antibiotics, on the mechanical properties of bilayers, since the relative influence of bilayer and torus can now be identified.

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