PRELIMINARY NOTE

Thickness changes in lipid bilayer membranes

The application of an electric field to a lipid bilayer membrane can cause an increase in the total electrical capacitance of the membrane[1-3]. The fractional increase has been shown by several authors[1,3] to be proportional to the square of the applied potential. LÄUGER et al.[2] attribute the increase to a thickness change while BABAKOV et al.[1] attribute it to an area change. A decrease in thickness due to the compressive effect of the electric field was predicted by HAYDON AND OVERBEEK[13]. REDWOOD AND HAYDON[4] have observed that the specific capacitance of lecithin–cholesterol–hydrocarbon membranes is inversely related to temperature. This temperature effect was interpreted on the basis of previous work[5] as resulting from changes in the amount of cholesterol present in the bilayer which in turn leads to thickness and dielectric coefficient changes.

A technique for the accurate determination of specific capacitance has been developed in this laboratory and used to determine the effects of temperature and voltage on the specific capacitance (C_m) of bilayer membranes formed from n-decane saturated with oxidized cholesterol[6]. The data show variations in C_m which are best explained by changes in thickness.

The lipid solution was spread with a fine brush across a 2-mm diameter aperture in a Teflon partition which separated a plexiglass chamber into two compartments. The compartments were filled with 0.01 M KCl buffered to pH 7.1 with 0.2 mM phosphate. Any temperature between 15 and 40° could be maintained to within ± 0.05° by water circulating through a jacket surrounding the chamber. An a.c. wheatstone bridge designed by the author was used to determine the total capacitance from measurements made at low frequencies[7]. Flat platinized Ag–AgCl electrodes developed from the method of COLE AND KISHIMOTO[8] connected the electrolyte–membrane system to the bridge. These electrodes have the advantage of low a.c. impedance and negligible polarization (≤ 0.5 mV). d.c. potentials of up to 200 mV could be applied through the unknown arm of the bridge without affecting bridge balance. The area of black membrane was determined from photographs of membranes made with transmitted light. The calculated specific capacitance has an estimated error of only 1 %.

The specific capacitance of freshly thinned membranes increases exponentially in time to values 10–15 % higher than the initial one. The time constant is 15 min. The mean initial value is 0.432 ± 0.021 (S.D.) $\mu F/cm^2$ (15 membranes). The standard deviation of 5 % is larger than the 1 % experimental error and is attributed to inherent variations in the membranes. Although the reason for the increase is not understood, it cannot be due to a loss of decane from the membrane to the electrolyte since saturation of the electrolyte with decane had no effect on the time constant.

Final state membranes were used for the measurements of the effects of temperature and voltage on C_m. A final state membrane is produced when a 100-mV d.c. potential is placed across a freshly thinned membrane for 10–20 min. The procedure eliminates time variations in C_m since C_m (measured with a 7-mV root mean square a.c. voltage) remained constant for more than 45 min after the potential was removed.

Fig. 1 is a plot of C_m *versus* the square of the applied voltage for one membrane. The mean values fall close to a straight line so that C_m is given by

$$C_m = C_o + \beta V^2{}_a. \tag{1}$$

The applied potential usually consisted of a d.c. "bias" potential and a.c. signal for the bridge measurements. Experimentally, the total applied potential (V_a) was obtained from $V_a = V_{d.c.} + \sqrt{\overline{V^2}_{a.c.}}$.
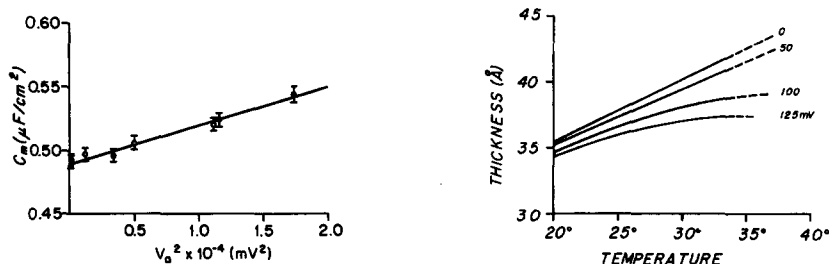

Fig. 1. The voltage dependence of C_m for a final state membrane. C_m is plotted against $V^2{}_a$. The data fall near a straight line. O, a.c. voltages; ●, d.c. voltages. An a.c. signal (7 mV, root mean square) is always present for determining capacitance. V_a is defined as $V_a = V_{d.c.} + \sqrt{\overline{V^2}_{a.c.}}$. Temp., 27.5°.

Fig. 2. Membrane thickness as a function of temperature. These curves were derived by assuming that all variations in C_m result from changes in δ. ε_m was taken as 2.24. The thermal expansion effect becomes smaller as the voltage across the membrane increases. The curvature in the 100-mV and 125-mV curves may or may not be significant.

Table I shows the effects of temperature changes on C_o and β. These results were obtained by fitting data gathered at three different temperatures to Eqn. 1 by the method of least squares. Note that C_o decreases and β increases with increasing temperature. Plots of C_o and β against temperature give a straight line within experimental error.

TABLE I

LEAST SQUARES FIT OF DATA TO $C_m = C_o + \beta V^2{}_a$

C_o and β depend on the temperature. The standard deviation in C_o is approx. 2 % while the deviation in β is approx. 15 %. When the temperature is raised from 20 to 34°, C_o decreases by approx. 15 % while β increases by 200 %.

Temp.	C_o ($\mu F/cm^2$)	$\beta \times 10^4$ ($\mu F/cm^2$ per mV^2)	Number of points	Number of membranes
20.0°	0.559 ± 0.01	0.0123 ± 0.0036	20	5
27.5°	0.511 ± 0.01	0.0203 ± 0.0034	21	5
34.0°	0.472 ± 0.01	0.0382 ± 0.0039	20	5

The measured capacitance of a lipid bilayer membrane represents the geometric capacitance $(C_g/A = \varepsilon_0\varepsilon_m/\delta)$ of the bilayer in series with the capacitance of the electrical diffuse layers at the membrane's surfaces[2,9]. Thus, field and temperature dependent capacitance effects might arise from membrane thickness (δ) and dielectric coefficient (ε_m) changes or from changes in the composition and geometry of the diffuse layers. The possibilities that the variations of C_m with temperature and voltage arise primarily from changes in the diffuse layers or membrane dielectric coefficient were considered and were discarded as quantitatively untenable. It is concluded that the primary variable is δ although the possibility of secondary effects on ε_m due to volume and composition changes cannot be completely excluded. This conclusion is supported by the square dependence of C_m on V_a since an electric field exerts a compressive force on a parallel plate capacitor which is proportional to the square of the applied potential[10].

Assuming that δ is the primary variable and equals 40 Å at 30° (ref. 6), membrane thickness as a function of temperature with V_a as a parameter can be calculated. The result of this calculation is shown in Fig. 2. The value of ε_m from these calculations is 2.24. This is lower than the value of 2.4–2.8 calculated for cholesterol–lecithin membranes by HANAI et al.[5].

The mechanism by which temperature and electric fields cause δ to change is not entirely clear at this time. However, some progress has been made by constructing, from the data, potential energy (U) versus δ curves based on the concepts of membrane stability advanced by HAYDON AND TAYLOR[11,12]. In the absence of an electric field, an attractive potential energy (U_L) due to London–Van der Waals forces between the separated aqueous phases proportional to $(1/\delta)^2$ is balanced by a repulsive potential energy (U_s) arising from steric interference between surfactant molecules. U_s is not significant until the surfactant molecules begin to overlap and then it rises very steeply with further decreases in thickness. The application of an electric field introduces an additional attractive potential energy term (U_V) given by $U_V = -(\varepsilon_m\varepsilon_0/2\delta)\cdot V_a^2$ (U_V is the electrical potential energy of the membrane capacitor[10]). The electric field modifies the shape of the asymmetric U–δ curve and therefore the stability conditions for the membrane. Since thickness increases with increasing temperature, simple thermal expansion may suffice to explain the temperature effects observed. This is a particularly attractive hypothesis since in general thermal expansion is the result of an asymmetric U–δ function.

Department of Physiology and Biophysics,   STEPHEN H. WHITE*
University of Washington,
Seattle, Wash. 98105 (U.S.A.)

* Present address: Physical Science Division, Fort Detrick, Frederick, Md. 21701 (U.S.A.).

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