ELECTRICAL CHARACTERISTICS OF SPHINGOMYELIN BILAYER MEMBRANES

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ABSTRACT Current-voltage characteristics and the conductivity temperature dependence of sphingomyelin bilayer membranes have been determined. The resistances were of the order of 10^8 Ω-cm² and exhibited ohmic behavior up to approximately 25 mv followed by increasing conductivity with applied voltage. The current is found to be proportional to a hyperbolic sine function of the voltage. The temperature dependence indicates a thermally activated conduction mechanism. The observed behavior closely follows a kinetic model involving a barrier modified by the applied electric field, the rate-limiting process being the surmounting of the barrier by the impinging ions. The model allows predictions to be made over a wide range of conditions.

INTRODUCTION

Although a great deal of attention has been directed to model membrane systems, little work has centered on the nature of the conduction mechanism of the bilayer system without additives. Fundamental questions such as the relative importance of barrier limitation vs. bulk limitation of current remain obscure. We report some experiments on the electrical conduction in high resistance membranes and a barrier model which gives calculated behavior very close to that observed. The model allows predictions to be made over a wide set of conditions. The nature of the barriers is reflected in the parameter, α, which enters into the model as described below.

EXPERIMENTAL PROCEDURE AND RESULTS

The system chosen was a 4% solution of sphingomyelin in α-tocopherol, chloroform, methanol (3:2:1). The solution on both sides of the membrane was 0.1 M NaCl in doubly distilled water. At this salt concentration the ion injection effect is not significant (Walz, Bamberg, and Laüger, 1969). The membranes were formed in a teflon orifice by standard methods (Mueller and Rudin, 1969). Resistances of these membranes were generally of the order of 10^8 Ω-cm². Care was taken to avoid contaminations which would lower membrane resistance. All measurements were DC using constant current in the range 10^{-7}–10^{-10} amp/cm² supplied by a modified Keithley 610C electrometer (Keithley Instruments, Inc., Cleveland, Ohio) with corresponding voltage between 1 and 180 mv. Greatest accuracy was obtained for voltages above 10 mv. Temperature was monitored to ±0.1°C. In all current-voltage measurements each point was

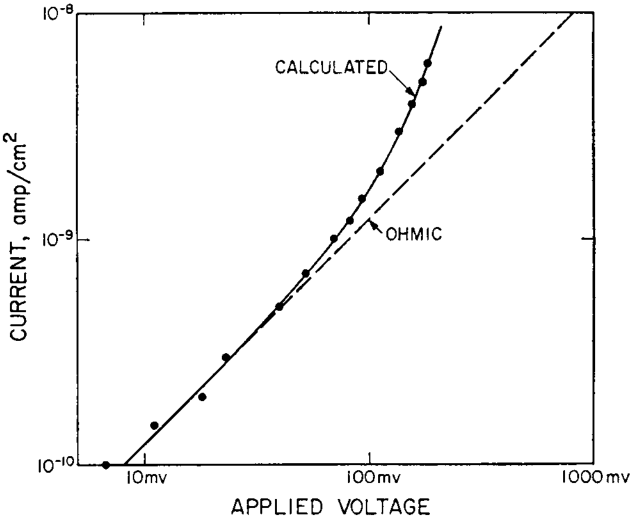

FIGURE 1 Typical current-voltage curve, 0.1 M NaCl, pH = 7.0. Solid curve is calculated using $\alpha = 0.38$ and $I = 8.52(10)^{-10} \sinh (\alpha V)/(kT)$.

checked in regard to drift, requiring membrane life to be several hours. For some experiments 0.005 M histidine was used to maintain a pH level of 7. All runs were made within the pH range 6–7, and no significant variations for different membranes were found within this range. These membranes had a small torus and no change in membrane area could be measured throughout the course of the experiments.

A representative current-voltage curve, one of many obtained, is shown in Fig. 1. Typical ohmic behavior is seen up to about 25 mv as reported by others (Hanai, Haydon, and Taylor, 1965; Laüger, Lesslauer, Marti, and J. Richter, 1967). Above 25 mv we found a nonlinear behavior which approaches an exponential in the higher voltage range. The complete curves can be accurately fitted with a hyperbolic sine function.

The conductivity dependence on temperature over the 30–65°C range (Fig. 2) is seen to consist of two branches, each showing exponential behavior. The exponential temperature dependence suggests a thermally activated conduction process. The average (between heating and cooling) activation energies are 1.09 ev below about 48°C and 0.74 ev above. Considerable additional data are required in order to evaluate the significance of the numerical values obtained for the activation energies. In both heating and cooling phases, a transition is evident near 48°C and appears to extend over only a few degrees of temperature.

The temperature behavior of the narrow thermal transition is not surprising in view of results of differential thermal analysis reported for a number of lipids which show transformations extending over such narrow ranges. These have been interpreted as an onset of increased motion of the hydrocarbon chains. We are not aware of any such results for the case of sphingomyelin. However, X-ray diffraction results (Finean, 1953) show an onset of contraction of the long spacing of sphingomyelin that occurs between 40°C and 50°C. This contraction may be due to increased disorder of the hydrocarbon chains. The cause of the observed hysteresis is not known. Recently Bean and Chan (1969) reported similar behavior for a 6% sphingomyelin and 40% tocopherol membrane; a transition was seen slightly above 40°C with hysteresis mainly above the transition. In addition a small lateral shift was observed at the transition.

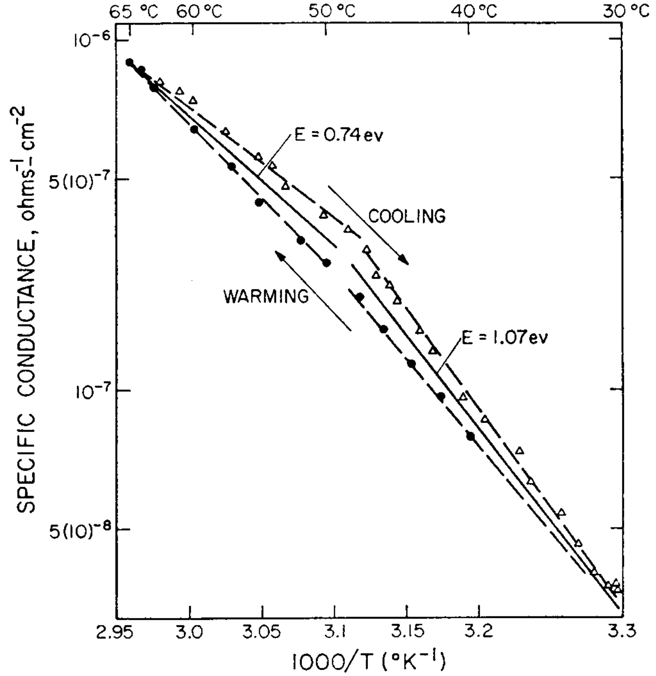

FIGURE 2 Temperature variation of conductance, log specific conductance vs. $1/T$. Membrane formed at 40°C. The conductance determined at 45 mv.

DISCUSSION

Various barrier models can account for the above general functional relations. Two of these are shown in Fig. 3. For the same ionic concentrations on each side of the membrane, the Boltzmann factors for surmounting the barrier give for the current density, I, for both Figs. 3 a and b,

$$I = 2e_0Be^{-E/kT} \sinh\left(\frac{\alpha e_0 V}{kT}\right), \tag{1}$$

where E is the barrier height, e_0 the charge, V the applied voltage, B the effective ion bombardment rate, and α is in general proportional to the fraction by which the applied voltage is effective in altering the significant barrier. For the case in Fig. 3 a, if the voltage drop in the electrodes and bath can be neglected up to the membrane, then $\alpha = 1/2$ and for the case in Fig. 3 b, $\alpha = 1/4$. In Fig. 1, $\alpha = 0.38$.

The above hyperbolic sine relation is not uncommon and was perhaps first obtained (see for a general discussion, Mott and Gurney, 1940) by considering currents resulting from the drift due to an applied electric field of ions over barriers within ionic crystals, the applied field modifying the periodic lattice potential and giving rise to an ionic drift velocity analogous to the above case. Ciani (1965) has

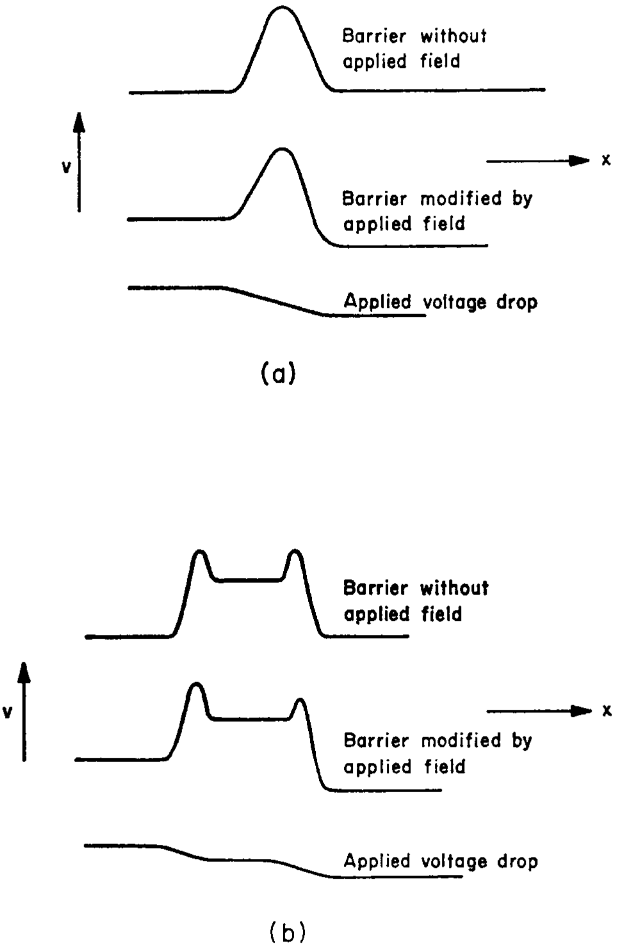

FIGURE 3 Two examples of barriers giving rise to the current-voltage dependence of equation 1. $\alpha = \frac{1}{2}$ for case 3 *a*. For 3 *b* if the interior region of the membrane has a much higher conductivity than the two outer peaks, then the applied potential drop occurs mainly within these peaks and $\alpha \simeq \frac{1}{4}$.

obtained an identical expression in considering the rate theory approach compared to the Nernst-Plank equation for describing ionic flow across membranes.

A rough estimate of B can be obtained from elementary kinetic theory where $B = 3.8(10)^{25}(\gamma/M)$ ions cm^{-2} sec^{-1} for γ moles/liter and molecular weight M. It is perhaps most significant to consider the calculated value of B in relation to the barrier height E. For sodium a value of $E = 0.9$ ev would give currents as observed. This value is consistent with the range observed (Fig. 2).

Unfortunately due to lack of published data of the type here presented, it is not possible to discuss thoroughly the generality of the observed characteristics. However, current-voltage data (Laüger et al., 1967) for a dioleyl phosphatidyl choline membrane in 10^{-2} M KI having a specific resistance of the order of 10^4 Ω-cm^2 was satisfactorily fitted by $I = 8(10)^{-6} \sinh (e_L V)/(2kT)$. In unpublished work (Gabor Szabo, 1969) current-voltage relations at room temperature for purified soy bean lecithin in *n*-decane were found to accurately follow the empirical formula $I = A \sinh mv$, $(m = 17)$. Excellent agreement is reported for all points using Ca^+, Rb^+, and K^+ salts and 10^{-3} M dicyclohexyl-18-crown-6 solutions for $m = 17$, all curves differing only in the value of A. In terms of the present model the above results correspond to $\alpha = 0.43$, which considering the differences in membrane and aqueous solutions, is in very good agreement with the present results. This agreement over such a wide set of conditions would further indicate that spurious effects such as variation of membrane area or thickness with voltage should not be significant.

The physical origin of the barrier stems primarily from the electrostatic field difference of a charge in an aqueous solution of dielectric constant, $K \simeq 80$ and within the membrane $K \simeq 2$, giving an energy difference of $(e_0^2/2r)(1/2 - 1/80) \simeq e_0^2/4r$. A monovalent charge with effective radius, for example, of 3 A results in a barrier of 1.17 ev. This value is lowered due to the multiple image effect by only a few per cent. The system is viewed as a continuum for these effects. The microscopic structure is expected to be important but is difficult to take into account. Since the charge carrier is unknown, it is not possible at this time to attempt a meaningful calculation of the barrier height. An additional contribution to the barrier comes from the dipolar ion head groups of the phospholipid and is expected to vary with pH.

The problem of current flow across barriers has been extensively studied in regard to electronic flow through semiconductor contacts where the above barrier model has been used. An alternative formulation involving diffusion and drift of carriers is often used, which makes use of the diffusion equation and the Einstein relation which together give rise to the Nernst-Planck equation:

$$I = \frac{-ne_0^2 D}{kT}\frac{dV_a}{dx} + eD\frac{dn}{dx}, \tag{2}$$

where D is the diffusion coefficient, n the carrier concentration, and V_a the sum of the applied potential and barrier potential. As pointed out by Ciani (1965), the Nernst-Planck equation results from a linearization of a more rigorously derived result using rate theory. This linearization is of the drift term and results from using the Einstein relation where the drift velocity is assumed to increase linearly with applied field. For a general discussion in relation to semiconductor contacts see Henisch (1957). In any case, the present results indicate that the exponential dependence due to barrier modification by the applied voltage overwhelms any nonlinear preexponential terms. Under these conditions the experimental value of α is a direct measure of the fractional change in barrier height per unit applied voltage.

CONCLUSIONS

(*a*) The measured current accurately follows a hyperbolic sine function of applied voltage giving ohmic behavior up to about 25 mv and increasing exponentially with voltage in the higher range.

(*b*) The conductivity increases exponentially with temperature implying a thermally activated process for the conduction mechanism.

(*c*) A transition is observed near 48°C. Its nature is yet to be determined. It is possibly associated with the onset of increased motion in the hydrocarbon chains.

(*d*) A field-modified barrier conduction mechanism gives calculated results in close agreement with measured values. The values obtained for the constants B, E, and α are reasonable. However, additional measurements are needed to determine the general validity of the model and to further evaluate and interpret the constants B, E, and α. Work directed toward further elucidation of these parameters is now in progress.

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