THICKNESS DEPENDENCE OF MONOGLYCERIDE BILAYER MEMBRANE CONDUCTANCE

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ABSTRACT We have studied the conductance properties of unmodified monoglyceride membranes as a function of monoglyceride chain length. As membrane thickness decreases from 31 to 20 nm, the steepness of the current-voltage (I-V) curve increases from 80 mV per *e*-fold current increase to 52 mV per *e*-fold current increase. The zero-voltage conductance increases more than 1,000-fold and the apparent activation energy of conductance decreases from 18.4 to 14.2 kcal/mol. We have analyzed our results using both the Nernst-Planck equation and absolute rate theory. Both approaches are consistent with our results and give consistent values for the parameters describing the I-V curves. We conclude that both the surface ion concentration and the distance from the surface of the membrane at which the energy of an ion rises appreciably above its value in solution (position of the barrier) are invariant with thickness.

INTRODUCTION

Theories of passive ion transport across a bilayer lipid membrane are very well developed (for a review see Schwartz, 1971; Markin and Chizmadjev, 1974). Ion transport is usually described by the generalized Nernst-Planck equation or the Eyring rate model with a series of barriers. An ion's energy depends on its position in the membrane. This dependence can be estimated using image forces as described by Neumcke and Läuger (1969). The Nernst-Planck equation with the image force energy profile predicts the nonlinear current-voltage curve commonly observed in passive ion transport. Absolute rate theory is usually used to describe kinetics of a carrier transport or transport of ions through ionic channels. Both approaches have special advantages, and comparisons between them can provide useful information which neither alone could give.

Dependence on membrane thickness has been invoked to explain the changes in the time constant of the gramicidin-induced membrane conductance relaxations (Hladky and Haydon, 1972) and to explain the effects of some general anesthetics (Haydon et al., 1977), but the effect of membrane thickness on passive ion transport has, rather surprisingly, not been systematically investigated.

In this experimental work, we varied membrane thickness systematically using monoglycerides with different chain lengths. The solvent content (squalene), specific capacitance, and thickness of these membranes have been well characterized by Waldbillig and Szabo (1979). Varying the membrane thickness causes a systematic variation in the parameters of the barrier.

We found that the shape of the current-voltage curve can be described by the Nernst-Planck equation with a simple rectangular potential energy barrier. Absolute rate theory is also applicable to our results and describes the current-voltage curves over a wide range of voltages. A comparison between the Nernst-Planck formulation and absolute rate theory establishes the constancy of surface ion concentration as thickness is varied. The parameters of our simplified barrier can be calculated from the image force barrier form for different thickness membranes, but quantitative agreement with the experimental values is poor. The nature of the disagreement suggests that there is a surface layer of the membrane that is invariant with thickness.

MATERIALS AND METHODS

The monoglycerides, monopalmitolein, monoolein, monoeicosaeinoin, and monoerucin, were purchased from Sigma Chemical Co. (St. Louis, MO). Corresponding catalogue numbers are M 4630, M 1378, M 6130, and M 5880. All of them were 99% purity, grade 1. Monomyristolein was obtained from Nu Chek Prep. Inc. (Elysian, MN), code M 209, purity 99%.

All monoglycerides were shipped on dry ice and stored on receipt at −10°C. Monoglyceride chloroform stock solutions (20 mg/ml) were prepared and stored under argon at −10°C prior to use. Membrane-forming solutions (10 mg/ml in *n*-pentane) were prepared by evaporation of chloroform under an argon stream and resuspension in *n*-pentane. These membrane-forming solutions were used immediately after preparation. Chloroform stock solutions were stored for no more than 36 h before use.

Squalene was purchased from Atomergic Chemetals Corp. (Plainview, NY), catalogue number 8197-00-JT. We used *n*-pentane and salts from Mallinckrodt Inc. (St. Louis, MO). Initially, pentane was passed through an alumina column to remove surface active impurites, but this precaution was found unnecessary and was not used in later experiments.

The membrane formation method used for monoglycerides with fewer than 18 carbons in the chain was similar to that described by Montal and Mueller (1972) and slightly modified by Vodyanoy et al. (1983). A small drop of squalene was placed in the hole on which the membrane was to be formed. With the water level below the hole, monolayers of the monoglyceride were spread from the 10-mg/ml pentane solution. The membrane was formed by raising each monolayer above the hole by injection of water into each chamber. The hole diameter punched in the thin Teflon partition was 0.3 mm, corresponding to an area of 7.07×10^{-4} cm^2. Membrane formation was monitored by change in capacitance, and membranes with a noisy or unusually high conductance were rejected.

For membranes formed from monoglycerides with 18, 20, and 22 carbons in the chain, we used a modified Montal-Mueller method using a transfer of two monolayers onto an aperture in a closed chamber (Vodyanoy and Murphy, 1982). This method allowed us to increase membrane area to ~1 mm^2, a necessary expedient for membranes having very low specific conductance. Temperature was controlled by a specially designed bridge circuit using Peltier thermoelectric elements (Cambion, Cambridge, MA) and measured by a Yellow Springs thermistor (45387; Yellow Springs Instrument Co., Yellow Springs, OH) in a 3-mm glass tube immersed in the aqueous solution.

Voltage for current-voltage curve measurements was generated by a computer-controlled 12-bit digital-to-analog converter (AD5782, Analog Devices, Norwood, MA). Current was measured by an operational amplifier (AD42k, Analog Devices) used as a current to voltage converter. We used an X-Y recorder (HP7037A, Hewlett-Packard Co., Palo Alto, CA) to record current-voltage curves.

RESULTS

Membrane thickness was determined by capacitance measurements at fixed frequency (Vodyanoy and Hall, 1982) and was in good agreement with the data of Waldbillig and Szabo (1979), Benz et al. (1975), and White (1978). The total membrane capacitance was divided by the membrane area to evaluate the specific geometric capacitance of the membrane. (For our membranes, the membrane area was taken to be the geometric area of the hole.) We used the monoglyceride dielectric constants estimated by Requena and Haydon (1975) to calculate the membrane "dielectric thickness" (as a thickness of a parallel plate capacitor). Capacitance measurements for all monoglycerides were carried out at room temperature, 23°C. Results are shown in Table I. Membrane "dielectric thickness" is proportional to the number of carbons in the fatty acid chain.

Membrane current-voltage curves were measured for all monoglycerides in unbuffered 1 M KCl (pH 5.4–5.6) at temperatures ranging from 15 to 35°C. Our results indicate that current-voltage (I-V) curve steepness increases from an *e*-fold change in current for 80 mV potential change in the thickest membrane to an *e*-fold change for 52 mV in the thinnest. Fig. 1 shows typical I-V curves for monoerucin (top curve) and for monomyristolein (lower curve) at room temperature (23°C). Both I-V curves are nonlinear, and the monomyristolein (14 carbons in the chain) membrane I-V curve is a much steeper function of voltage than the I-V curve of monoerucin (22 carbons in the chain).

About 10–20 I-V curves were taken for each monoglyceride to evaluate the averaged zero-voltage conductance, G_0. G_0 was taken as the slope of the current-voltage curve at zero volts. Fig. 2 shows the thickness dependence of G_0 at 23°C. As dielectric thickness changes from 2 (14 carbons) to 3.1 nm (22 carbons), G_0, changes from 3.1×10^{-5} to 2.2×10^{-8} S/cm^2.

The apparent activation energy of conductance also decreases from 18.4 kcal/mol in the thickest membrane to 14.2 kcal/mol in the thinnest. Zero-voltage conductance, G_0, as a function of temperature is shown in Fig. 3 for all monoglycerides studied. Apparent activation energies of conductance derived from the data of Fig. 3 are compiled in Table I. The curves in Fig. 3 were obtained by least-squares fitting. The points are measured G_0 values from I-V curves taken at fixed temperatures. Monoolein (18 carbons) and monoeicosaeinoin (20 carbons) membrane conductances are average values from several membranes. The points shown for the other thicknesses are from one membrane for each thickness. However, at least two membranes of each thickness were measured over the entire temperature range, and these curves had slopes nearly the same (within 10%) as those of the curves shown for the same thickness, although the conductance points were taken at different temperatures from those of the curves shown. The activation energies shown in Table I include the values from all the curves, both those shown and those not shown in Fig. 3. Uncertainty in the slopes for 18 and 20 carbon thicknesses is $< \pm 0.6$ kcal/mol. For the others, the standard errors are <10% (see Table I for individual standard errors.) The temperature dependence of G_0 can be described as

$$G_0 = (7.7 \pm 2) \times 10^5 \exp\left(-\Delta E_i/kT\right)$$

TABLE I
PROPERTIES AND THEORY-DEPENDENT PARAMETERS OF MONOGLYCERIDE BILAYERS

	Monomyristolein	Monopalmitolein	Monoolein	Monoeicosaeinoin	Monoerucin
Number of carbons in the chain	14	16	18	20	22
Double bond position	9-*cis*	9-*cis*	9-*cis*	11-*cis*	13-*cis*
Capacitance (nF/cm^2)	1,150 ± 60	985 ± 45	852 ± 43	788 ± 40	700 ± 48
Dielectric thickness (nm)	2.00 ± 0.15	2.23 ± 0.11	2.58 ± 0.13	2.79 ± 0.19	3.13 ± 0.24
G_0 ($\times 10^7$ S/cm^2)	310 ± 62	19 ± 3.8	5.4 ± 0.08	0.93 ± 0.014	0.22 ± 0.044
i_0 (nA/cm^2)	890 ± 20	31 ± 4.7	9.2 ± 1.3	3.2 ± 0.35	0.75 ± 0.11
α'	0.481	0.409	0.344	0.321	0.305
E (kcal/mol)	14.21 ± 0.8	14.60 ± 0.4	16.40 ± 0.5	17.72 ± 0.4	18.39 ± 0.9

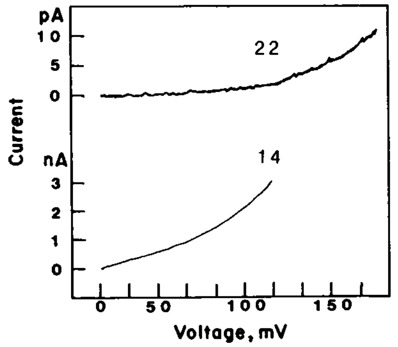

FIGURE 1 Typical monoglyceride membrane current-voltage curves. Taken at 23°C, in 1 M KCl (pH ~5.5), voltage sweep was 5 mV/s. *Upper* curve: monoerucin membrane (22 carbons in the chain). *Lower* curve: monomyristolein membrane (14 carbons in the chain).

(regression coefficient $r^2 = 0.969$), where ΔE_i is the activation energy in a particular thickness membrane, G_0 is measured in siemens per square centimeter, and kT has its usual meaning. This type of temperature dependence where conductance vs. temperature curves intersect at the same temperature has been reported for a variety of organic semiconductors and polymers (Boguslavskii and Vannikov, 1970). The conductance of "thick phospholipid films" (mixture of organic solvent and lipid) modified by various impurities also shows this type of dependence. The temperature at which the curves intersect is characteristic of the film material and is not altered by small amounts of impurity (Rosenberg and Bhowmik, 1969).

The large change in conductance with thickness and the change in I-V curve steepness cannot be explained by an increase in electric field in the thinner membranes. However, if surface reactions are unaffected by thickness, a reasonable explanation of our observation is a change in potential energy profile with thickness. The thinner the membrane, the narrower and lower the barrier (Neumcke and Läuger, 1969). The change in the barrier profile can also explain the decrease in apparent activation energy of conductance from 18.4 kcal/mol in the thickest membrane to 14.2 kcal/mol in the thinnest. Our results thus demonstrate that decreasing the thickness increases conductance, increases the steepness of the current-voltage curve, and decreases the apparent activation energy of ion transport.

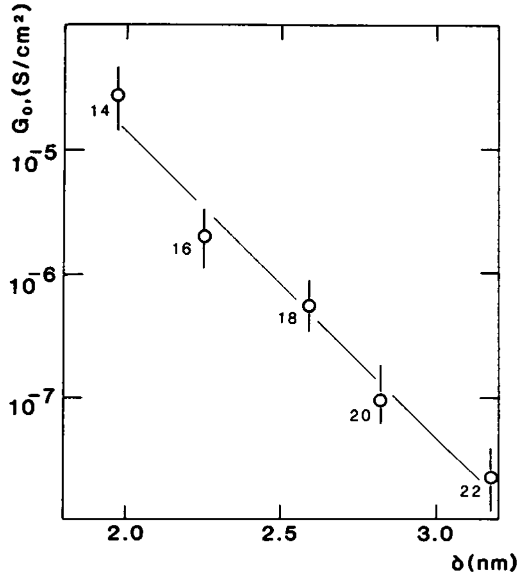

FIGURE 2 Dependence of G_0 on membrane thickness. Numbers near every point show the number of carbons in the monoglyceride chain.

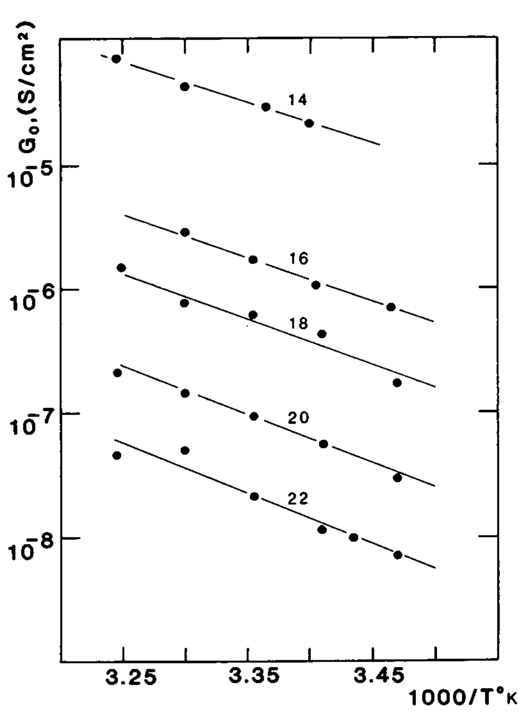

FIGURE 3 Dependence of G_0 on temperature. Numbers above each curve correspond to a number of carbons in the monoglyceride. Regression coefficients r^2: C14:1–0.999; C16:1–0.964; C18:1–0.987; C20:1–0.999; C22:1–0.975.

ANALYSIS AND DISCUSSION

In this analysis, we first apply the Nernst-Planck formalism with rectangular symmetrical barrier (first section) and then apply absolute rate theory (second section). Finally, in the third section, we derive the parameters of the rectangular barrier using both approaches.

Nernst-Planck Equation

If the membrane is a homogeneous medium and ion transport in it is due to diffusion and drift, then the electrical current due to ions of type k is

$$i_k = z_k u_k RT\left(\frac{F}{RT} z_k c_k E - \frac{\partial c_k}{\partial x}\right), \qquad (1)$$

where z_k is the ionic charge, u_k is the mobility (related to the diffusion coefficient by $D_k = u_k RT/F$), E is the electric field, and c_k is the concentration of the diffusing ions. The total current, i, then is

$$i = \sum_{k-1}^{n} i_k.$$

For i_k constant in time, Eq. 1 is a first-order nonlinear differential equation with unknown functions of time c_k and E. Following Neumcke and Läuger (1969), we assume that the chemical potential of the ion depends on its position inside the membrane, and is of the form

$$\mu_k^0 = W_k(x) + c,$$

where $W_k(x)$ is zero at both membrane surfaces $[W_k(0) = 0,\ W_k(\delta) = 0]$, δ is the membrane thickness, and c is a constant proportional to the standard chemical potential.

If two ions differ appreciably in radius, as K^+ and Cl^- do, the membrane will present a considerably higher barrier to the smaller ion than to the larger. Thus, the observed current will be almost entirely due to the larger ion. Because the chloride ion is considerably smaller than the potassium ion, the current in our experiments is probably carried principally by potassium. This view is supported by the observation that bare bilayers are cation selective in sodium and potassium chloride solutions (Lev et al., 1966). We will accordingly treat the case for only a single ionic species.

We also assume that the ion concentration in the membrane is small, the applied potential is constant and that the ion concentration a long way from the membrane is the same on both sides and constant. Then for a single ion species, membrane current is

$$i = zFD\gamma c(e^{z\phi} - 1) \Big/ \int_0^{\delta} \exp\left[z\phi\left(\frac{\delta - x}{\delta}\right) + w(x)\right] dx \quad (2)$$

where γ is the partition coefficient, and $\phi = FV/RT$ (V is the applied potential). The integral here is an analogue of membrane resistance and is determined by the potential energy barrier profile $w(x)$.

The principal contribution to the integral in Eq. 2 comes from the region where the potential energy is large, i.e., the top of the barrier. We will thus approximate the exact barrier by a symmetrical rectangular barrier (see Fig. 4) (Markin and Chizmadjev, 1974). The barrier's coordinates are ξ and $\delta - \xi$. The height of a barrier is fixed and equal to w_0. If we put this barrier in the integral in Eq. 2, the expression for the current-voltage curve becomes

$$i = \frac{z^2FD\gamma c}{\delta e^{w_0}} \phi \frac{e^{z\phi} - 1}{e^{z\phi(1-\alpha)} - e^{z\phi\alpha}}; \quad (3)$$

where $\alpha = \xi/\delta$.

For a small voltage ($\phi << 1$),

$$i = \frac{z^2FD\gamma c}{\delta e^{w_0}} \phi \frac{1}{1 - 2\alpha}. \quad (4)$$

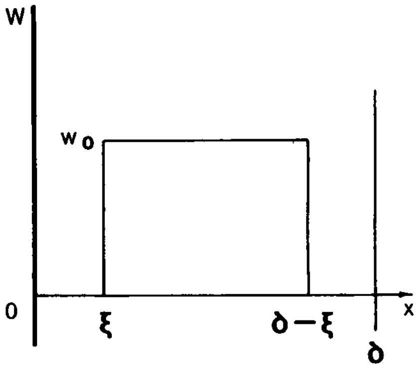

FIGURE 4 Membrane potential energy profile. The origin is at the left membrane surface. δ is a membrane thickness. ξ is the distance between the barrier and membrane surface. w_0 is the energy at the top of the barrier.

The conductance at low voltage is then

$$G_0 = \frac{z^2FD\gamma c}{\delta e^{w_0}} \frac{F}{RT} \frac{1}{1 - 2\alpha}. \quad (5)$$

For a voltage $\phi >> 1$,

$$i = \frac{z^2FD\gamma c}{e^{w_0}} \phi \frac{1}{\delta} e^{z\phi\alpha}. \quad (6)$$

Then

$$G/G_0 = (1 - 2\alpha) \exp(z\phi\alpha). \quad (7)$$

Absolute Rate Theory

The current-voltage curve can be also described by absolute rate theory (Eyring et al., 1939). Following Delahey (1965), i, the total membrane current, can be written in the form

$$i = i_0 (e^{-z\phi\alpha'} - e^{z(1-\alpha')\phi}), \quad (8)$$

where i_0 is the exchange current, α' is the transfer coefficient, and $\phi = FV/RT$. The net current is the difference between the currents in the forward and backward directions. The exchange current is the unidirectional current through the membrane with zero applied potential. It is thus a measure of how fast ions jump across the barrier.

For analysis of data, Eq. 8 can be recast in the form (Allen and Hickling, 1957):

$$\frac{i}{1 - e^{-z\phi}} = i_0 e^{z\phi\alpha'}. \quad (9)$$

Hence

$$\ln \frac{i}{1 - e^{-z\phi}} = \ln i_0 + z\phi\alpha', \quad (10)$$

where i is taken with its sign. The slope of Eq. 10 yields α' and the intercept at $\phi = 0$ gives the exchange current, i_0.

Fig. 5 shows the current-voltage curves for all monoglycerides linearized according to the above Eq. 10. The fits are very good over a wide range of voltages. Corresponding parameters, i_0, and transfer coefficients, αs, are shown in Table I.

DISCUSSION

Absolute rate theory treatment (Eq. 10) allowed us to fit I-V curves for all the membrane thicknesses and derive the corresponding transfer coefficients, α' (see Table I). In the Nernst-Planck formalism, a simple rectangular barrier treatment determines the I-V curve slope as $\alpha = \xi/\delta$ (where ξ and δ are the barrier parameters). Thus, ξ can be calculated from the membrane thickness and the I-V curve slope, assuming $\alpha' = \alpha$.

Number of carbons:	14	16	18	20	22
$\xi = \delta\alpha'$	0.96	0.91	0.88	0.89	0.95

$$\xi = (0.92 \pm 0.03)\ \text{nm}.$$

Thus, using α' derived from Eq. 10 of absolute rate theory we have obtained the parameter ξ, the distance between the barrier and the membrane surface.

Eq. 5 can be rewritten as

$$G_0 = B\frac{1}{\delta - 2\xi},\ \text{where}\ B \equiv \frac{z^2FD\gamma c}{e^{w_0}}. \tag{11}$$

Thus, in the rectangular barrier model, membrane conductance is inversely proportional to the barrier width $\delta - 2\xi$ (see Fig. 4). If we plot log G_0 vs. log $(\delta - 2\xi)$, the slope should be equal to -1, given B, and thus the diffusion coefficient, barrier height, and partition coefficient remain constant with changing membrane thickness.

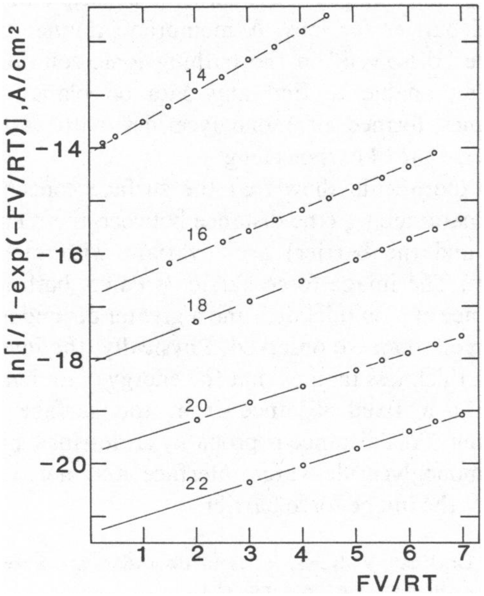

FIGURE 5 Linearized current-voltage curves. I-V curves were taken at 23°C in 1 M KCl, and then linearized according to Eq. 10; numbers above each of the curves correspond to the number of carbons in the monoglyceride chain.

Fig. 6 shows a plot of log G_0 vs. log$(\delta - 2\xi)$ for $\xi = 0.9$ nm. Its slope is not equal -1 and therefore Eq. 11 does not properly describe the experimental data with constant B. The failure of Eq. 11 to describe the data indicates that B changes with thickness. Thus, the diffusion constant, barrier height, and partition coefficient all may depend on thickness, but the most likely of these to depend on thickness is barrier height. Dependence of barrier height on thickness is also suggested by the experimental dependence of activation energy on membrane thickness.

If activation energy is determined by the barrier height and the barrier height is the only component of B that is thickness dependent, we can find B for each membrane thickness from

$$B_i = G_{i0}\,(\delta_i - 2\xi),$$

where the subscripts refer to different thicknesses. Then, because $B_i \sim 1/e^{w_i}$,

$$\ln\left(\frac{B_i}{B_j}\right) = w_i - w_j,$$

where i is subscript for one fixed thickness, j ranges through all thicknesses, and w_i is given in kT units of energy. Taking ξ as 0.9 nm, the height of the barrier calculated in this way changes ~5.3 ± 0.8 kT from the thinnest to the thickest membrane. This value is not far from the 6.1 ± 0.9 kT observed difference in apparent activation energies.

We chose the monoglycerides for this study because we wanted to vary only thickness and not surface properties. But even though the head group is the same for all the lipids we used, we have no a priori assurance that the surface properties of the monoglycerides might not change with thickness. We can, however, test whether

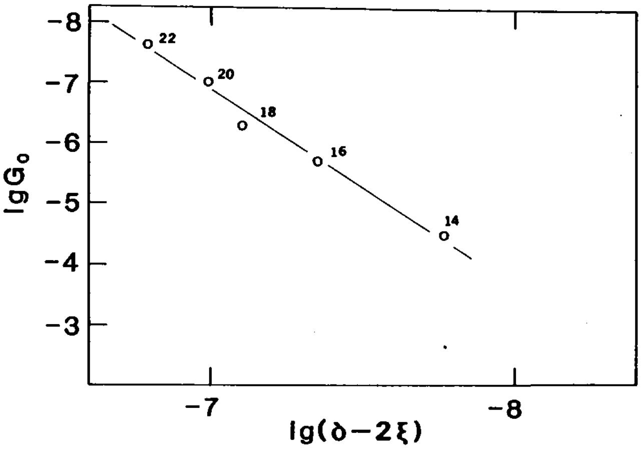

FIGURE 6 Log-log plot of membrane conductance against barrier width. Numbers correspond to the number of carbons in the monoglyceride chain.

the surface properties change with thickness in such a way that the surface concentration of potassium is affected. To do this, we use the magnitude of the exchange current as a function of thickness to estimate the surface concentration for each membrane thickness.

The exchange current is given by (Johnson et al., 1954)

$$i_0 = q\frac{kT}{h}\exp\left(\Delta G^{\neq}/RT\right)\lambda c_1 \exp\left(-\alpha'\phi_0 F/RT\right), \qquad (12)$$

where k and h are the Boltzmann and Planck constants, respectively; $\Delta G^{\neq}$ is the free energy of the transition of the ions from the membrane into the solution; $\Delta G^{\neq} = \Delta H^{\neq} - T\Delta S^{\neq}$, where $\Delta H^{\neq}$ and $\Delta S^{\neq}$ are activation enthalpy and entropy, respectively; q is the electronic charge; c_1 is the ion concentration in the surface layer; λ is the thickness of the surface layer of ions that can jump across the barrier; and ϕ_0 is the potential at which no net current flows. The product λc_1 is thus the surface concentration of permeant ions which we would like to estimate.

To estimate λc_1 from the measured values of i_0, we need an estimate of the value of $\Delta G^{\neq}$ and therefore of values for both the entropy and enthalpy of activation. We have been unable to find a value for the entropy of activation for the potassium ion at the membrane electrolyte boundary. However, a reasonable assumption is that the entropy of activation is very close to the dehydration entropy of potassium ions. This is ~8 cal/degree mol (Robinson and Stokes, 1970).

The apparent activation energy, ΔE, is determined by differentiation of Eq. 12 with respect to $1/T$ at constant voltage:

$$\Delta E \equiv -R\left[\frac{\partial \ln i_0}{\partial\left(\frac{1}{T}\right)}\right]_V . \qquad (13)$$

Then from Eqs. 12 and 13 we have

$$G_0 = \frac{F}{RT}q\frac{kT}{h}\lambda c_1\left(e^{\Delta S/R-\Delta E/RT}\right). \qquad (14)$$

Using the data of Table I for activation enthalpy and taking S as 8 cal/degree mol, we can calculate the potassium ion surface concentration for each membrane thickness. It is independent of thickness and has a value of $(3.5 \pm 0.6) \times 10^8$ ions/cm^2. This is much less than the concentration of the lipid at the membrane surface (~10^{14} molecules/cm^2). Thus, our earlier assumption of low permeant ion concentration is justified. The constancy of surface concentration with thickness is important because it demonstrates that we are dealing with an effect of thickness that takes place inside the membrane and not at the surface.

TABLE II

PARAMETERS FOR SIMPLE RECTANGULAR BARRIER, ξ, α, AND w_0 DERIVED FROM THE IMAGE FORCE BARRIER*

Membrane thickness	ξ	α	w_0
nm	*nm*		*kT*
34	11.4	0.33	39.8
30	10.3	0.34	39.1
26	9.2	0.35	38.2
22	8.0	0.36	36.9

*With ϵ for the water equal to 78.5 and for the membrane equal to 2 with an ionic radius of 3 Å.

Finally, we consider the physical reasons behind the change in barrier height and shape with thickness. We tested the ability of the image force barrier to predict the changes in shape and activation energy by using it to calculate a potential barrier for each membrane thickness. Then we approximated this barrier using a rectangular barrier that just fit inside of the image force barrier with its top 1 kT below the top of the image force barrier. The results of these calculations, assuming a dielectric constant of 78.5 for water and 2 for the membrane and using an ionic radius of 3 Å, are shown in Table II. They show that the image force alone is inadequate to explain the observed changes in barrier parameters with thickness, although the general trends and magnitudes are properly predicted. Further, the increase in α with decreasing thickness to nearly 0.5 implies that the core of the membrane is the location of most of the energy change. The core becomes very thin compared with the surface layer in thin membranes. In fact, the constancy of the parameter ξ implies that a membrane thinner than 2ξ would be unstable because of the collapse of the core and consequently of the potential barrier for ions. A membrane thinner than 2ξ would be "dissolved" in the bathing ionic solutions. We have been unable to find any data on planar bilayer membranes formed of monoglycerides with fatty acid chains less than14 carbons long.

Our experiments show that the surface concentration and the parameter ξ (the distance between the membrane surface and the barrier) are invariant with membrane thickness. The image force barrier predicts both a lesser dependence of α on thickness and a greater dependence of ξ on thickness than we observed. Physically, the invariance of ξ with thickness implies that the energy of an ion begins to rise at a fixed distance from the surface of the membrane. This distance is probably an intrinsic property of the monoglyceride-water interface and not a consequence of the image force barrier.

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