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Displacement Currents in Lipid Bilayer Membranes

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ABSTRACT

The processes of charge redistribution in phospholipid bilayer membranes have been examined by voltage clamping. Different types of phospholipids dissolved in n-decane were used as membranes and the membranes were made conducting by adding ionophores. The relaxation processes were found to be composed of at least two exponential decays of which the fast process can be explained in terms of a charge redistribution between the surface layers and the interior of the membrane, following the step in potential. The effect on this of varying the lipid composition, the external solution conditions and the applied potential are described in terms of changes in the capacitance C_P and the resistance R_P of the surface layers. A model in which a structural rearrangement of the lipid polar groups is responsible for the observed changes in C_P and R_P is also discussed.

INTRODUCTION

The structure and orientation of the lipid polar groups is not only essential to the formation and stability of lipid bilayer membranes but also an important factor determining the electrical and permeability properties of the membranes. It is well established, theoretically as well as experimentally, that the surface charge and polarizability of the polar groups depend on several external factors such as ionic environment, pH and temperature, all of which are known to influence the membrane conductance and rate constants determining the transfer of ions across the membrane (for review see ref. 6). It has therefore been possible, through the use of ionophores, to correlate the over all electrical properties of lipid bilayer membranes to the sign and magnitude of the corresponding surface charge (10).

Coster and Smith (4) have measured the charge rearrangement in the membrane using impedance methods and interpreted these results in terms of a three

capacitor model (see Fig. 1) where the outer capacitors correspond to the polar groups and where the inner capacitor represents the hydrocarbon region. Their model was later extended to include an intermediate region between the hydrocarbon and polar parts of the membrane (1).

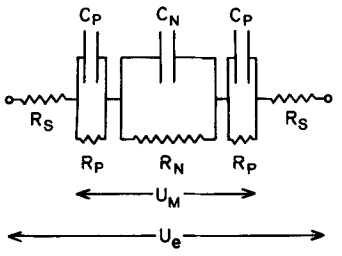

Fig. 1. Equivalent circuit of a membrane showing the external solution resistances (R_S), the electrical parameters of the surfaces (R_P, C_P) and of the nonpolar region (R_N, C_N). U_M is the potential difference across the membrane and U_e is the total potential difference between the electrodes.

The three capacitor model was also used by Sandblom et al. (11) in order to explain the fast relaxation process seen in voltage clamp experiments with brain lipid extracts in the presence of ionophores (nonactin). The voltage clamp method can therefore be used to characterize the lipid polar region in different lipids and the results of such studies will be reported here.

If a feed back circuit is used to clamp the voltage U_M, the initial charging process will supply the capacitors C_P and C_N with equal amounts of charge. Following this short period the resulting charge on the capacitors will redistribute to conform with the new steady state potential profile which in turn will depend on the values of R_N and R_P. This gives rise to a displacement current with a time constant τ and initial G_0 [▫ I (t ▫ 0)/U_M] given by:

$$\tau = \left(C_N + \frac{C_P}{2}\right) \cdot \frac{R_N \cdot 2R_P}{R_N + 2R_P} \qquad (1)$$

$$G_0 = \left[\frac{C_N \cdot \frac{C_P}{2}}{C_N + \frac{C_P}{2}}\right] \cdot \left[\frac{2}{R_P C_P^2} + \frac{1}{R_N C_N^2}\right] \qquad (2)$$

If the following conditions are satisfied:

$$R_P \ll R_N \qquad (3)$$

$$C_N \ll C_P \qquad (4)$$

Eqs. (1) and (2) reduce to:

$$\tau_f \approx R_P C_P \tag{5}$$

$$G_{0f} \approx \frac{2C_N^2}{R_P C_P^2} \tag{6}$$

The index f has been introduced to indicate that the process is fast compared to the interior charging time ($\tau = R_N C_N$).

If, instead, the circuit of Fig. 1 is subjected to a current step (current clamp) the potential will approach its steady state value with a time constant τ and final conductance G_∞ given by:

$$\tau \approx R_N C_N \tag{7}$$

$$G_\infty \approx \frac{1}{R_N} \tag{8}$$

where the inequalities, Eqs. (3) and (4) have been assumed to apply.

A comparison between Eqs. (5) and (7) shows that the circuit parameters can be conveniently evaluated by a combination of voltage and current clamp experiments. We will use this method to compare the properties of the circuit parameters of Fig. 1 in different lipids.

The presence of carrier molecules used to increase the conductances in the circuit of Fig. 1 will also introduce additional time constants associated with the kinetics of ion binding to the carrier (13). As will be shown, however, the conductance of the polar region is much higher than the corresponding apparent conductance of the carrier kinetics which makes it possible to study the two phenomena separately.

MATERIAL AND METHODS

Lipids

The phospholipids used were phosphatidyl ethanolamine (PE), phosphatidyl choline (PC), phosphatidyl serine (PS) and phosphatidyl inositol (PI) with fatty acid chains consisting of oleic acid in all cases. The phosphatidyl ethanolamine was obtained from bovine brain extract (Sigma). All other lipids were purchased from Supelco Inc.. The membranes were formed from 2% (w/w) solution of lipid in n-decane and applied to a hole (2 mm in diameter) in a Teflon diaphragm. In some cases the phospholipids were combined with choles-

terol (C) to give lipid solutions of 1% (w/w) with respect to cholesterol and 2% (w/w) with respect to phospholipids.

Aqueous solutions

The membrane was surrounded by solutions having the same concentration on the two sides. Members of the macrotetrolides (actins) were used as ionophores. These are macrocyclic polypeptides exhibiting a high degree of alkali cation specificity and where the individual members differ from one another by the substitution of the backbone with methyl and ethyl groups. Nonactin or a mixture of monoactin and dinactin in proportions of 70% and 30% respectively (kindly provided by Dr. Barbara Stearns of Squibb) were added in various concentrations to the aqueous solutions. The chamber and aqueous solutions were maintained at a temperature of 30°C by means of a Peltier cell on which the chamber was mounted (11).

Electrical measurements

The circuit used for clamping either the potenital or the current has been described by Eriksson et al. (5), and its performance has been tested on dummy circuits of the kind shown in Fig. 1. The method was found to give values, accurate to within 30%, for the electrical parameters of relaxation processes with time constants down to about 1 μs.

In order to determine the inital change in the conductance following an applied potential step across the membrane, it is necessary to estimate the time it takes for the membrane to be effectively clamped. A rapid charging of the membrane capacitance has been achieved by the use of a positive current feed back which compensates the series resistance R_S. Figs. 2a and 2b show the recordings from an actual experiment using the voltage clamp circuit, and

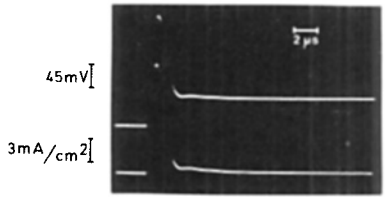

Fig. 2a. Potential (upper) and current (lower) records from a voltage clamp experiment with 0.1 M KCl, 10^{-6} M monactin-dinactin mixture and 2% PE.

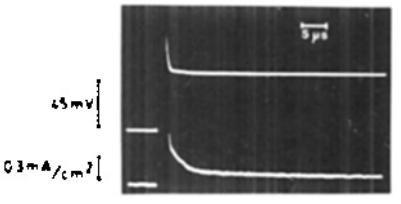

Fig. 2b. Same records as in Fig. 2a, but with different amplitude and time scales.

the large initial transients seen in the voltage records are due to potential drops across the solution series resistances R_S (see Fig. 1) caused by the charging current.

The intial conductance is evaluated by extrapolating the conductance values, measured subsequent to the charging period, back to 1 μs (5, 11). This procedure is illustrated in Figs. 2b and 3. The potential and current trans-

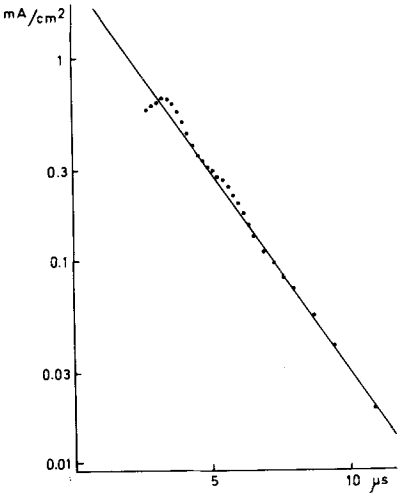

Fig. 3. Logarithmic plot of the current record in Fig. 2b., which includes only the early part of the record with the fast process.

ients seen in Fig. 2b are the same as those in Fig. 2a although recorded with different amplitude and time scales. Fig. 3 shows record plotted in a logarithmic diagram from which the values of τ_f for the process is calculated from the slope of this line and the initial conductance G_{0f} is obtained by extrapolating the line back to 1 μs.

In order to obtain a complete set of electrical parameters characterizing the membrane it is necessary to carry out a current clamp experiment under the same conditions as the voltage clamp experiment. This is done with the same electric equipment simply by eliminating the voltage feed back in the circuit. Fig. 4 shows the potential and current records from such an experiment and a logarithmic plot of U_M (Fig. 5) gives the capacitance C_N and the resistance R_N according to Eqs. (7) and (8).

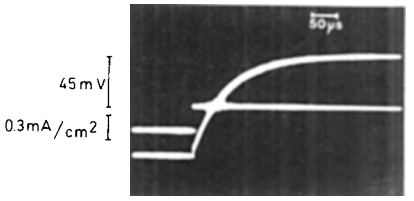

Fig. 4. Time course of electrode potential U_e obtained from a current clamp experiment with 0.1 M KCl, 10^{-6} M Nonactin and 2% PE.

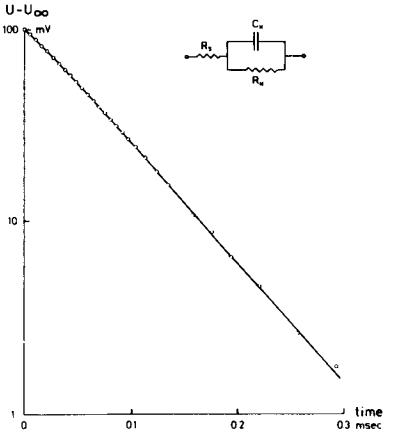

Fig. 5. Logarithmic plot of the potential record in Fig. 4. The contribution from R_p and C_p (Fig. 1) can be neglected in a current clamp experiment.

The values of C_N for PC measured by this method are equal to 0.45 $\mu F/cm^2$. The corresponding values for PS and PE were found to be 0.32 $\mu F/cm^2$ and 0,25 $\mu F/cm^2$ respectively. For PI the value was 0.42 $\mu F/cm^2$ calculated from the data reported by Stark et al. (12). These values have been used in the calculations of C_p and R_p according to Eqs. (5) and (6).

The results of the voltage clamp experiments will be presented in a series of figures and tables. The figures are taken from typical experiments whereas the results presented in the tables are average values calculated from 1-3 experiments. Unless otherwise stated, the magnitude of the applied potential step has been kept between 60-70 mV.

RESULTS

In response to an applied voltage step the current relaxes towards a new steady state with a time course which is composed of at least two different exponential decays. This is seen from Fig. 6, which shows the logarithmic plots of such records for the four phospholipids used. The two processes exhibit quite different patterns of behaviour and Fig. 7 showing the potential dependence of the two processes, gives an example of this. The fast process is seen to be independent of the applied potential suggesting that it is independent of the kinetics of carrier transport and associated with the dielectric properties of the boundary layers of the lipid membrane. The second process has a time constant which decreases with increasing applied potential in agreement with the observed behavior of the kinetics of carrier transport (7, 8, 12).

Table 1 summarizes the measured time constants τ_f, τ_s and the initial conductances G_{0f} and G_{0s} for the fast and slow processes respectively at different solution conditions with respect to concentration and ionic strength. It is seen that C_p changes more with the ionic strength than with the concentration

Table 1. Ionic strength dependence. Membranes were made of 2% PS and 1% C in the presence of 10^{-6} M Nonactin.

Solutions	0.1 M KCl		0.1 M KCl+ 1.0 M LiCl		1.0 M KCl*	
τ_f	3.1	μs	2.4	μs	1.9	μs
τ_f	10	μs	13	μs	8.3	μs
G_{0f}	11	mS/cm^2	30	mS/cm^2	58	mS/cm^2
G_{0s}	1.4	mS/cm^2	1.4	mS/cm^2	4.5	mS/cm^2
G_∞	0.19	mS/cm^2	0.83	mS/cm^2	2.9	mS/cm^2
C_p	6.0	μF/cm^2	2.8	μF/cm^2	1.9	μF/cm^2
R_p	0.52	Ω cm^2	0.86	Ω cm^2	1.0	Ω cm^2

*U_M = 110 mV

of the solution. G_p on the other hand remains more constant than C_p consistent with the observation made by Coster and Smith (4) that G_p shows a weak dependence on the concentration of the electrolyte.

The effect of varying the nonactin concentration is summarized in Table 2 for two different lipids. The fast time constant is relatively independent of the nonactin concentration whereas the slow time constant increases at the higher concentration with PE, consistent with carrier kinetics (10).

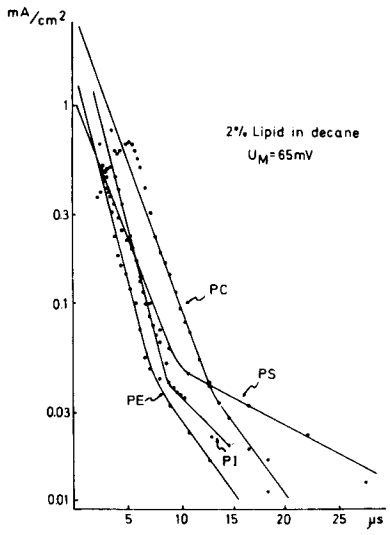

Fig. 6. Logarithmic plot of the current for a 2% PS membrane together with the corresponding curves for 2% PE, 2% PI and 2% PC obtained under similar experimental conditions.

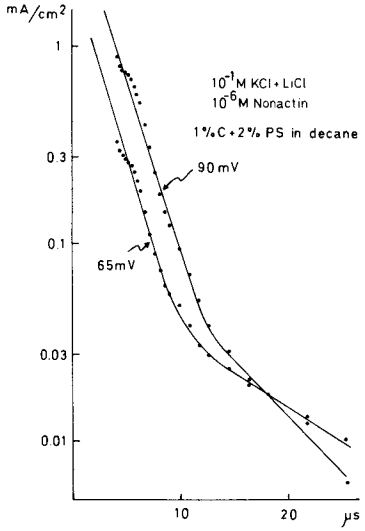

Fig. 7. Logarithmic plots of current vs time at two different applied potentials with 0.1 M KCl + 1.0 M LiCl, 10^{-6} M Nonactin and 2% PS + 1% C.

Table 2. Ionophoric dependence. Aqueous solution: 0.1 M KCl.

Lipids	2% PS				2% PE			
Ionophore	10^{-7} M Nonactin		10^{-6} M Nonactin		$0.5 \cdot 10^{-7}$ M Nonactin		10^{-6} M Nonactin	
τ_f	2.9	μs	2.8	μs	2.0	μs	2.0	μs
τ_s	16	μs	15	μs	3.2	μs	5.7	μs
G_{0f}	13	mS/cm^2	22	mS/cm^2	13	mS/cm^2	18	mS/cm^2
G_{0s}	1.3	mS/cm^2	4.3	mS/cm^2	2,9	mS/cm^2	5.1	mS/cm^2
G_∞	0.45	mS/cm^2	2.9	mS/cm^2	1.9	mS/cm^2	3.2	mS/cm^2
C_P	5.4	μF/cm^2	3.3	μF/cm^2	5.6	μF/cm^2	4.1	μF/cm^2
R_P	0.54	Ω cm^2	0.85	Ω cm^2	0.36	Ω cm^2	0.49	Ω cm^2

Comparing Tables 1 and 2 shows that an increase in the ionic strength has the same effect on C_P and R_P as an increase in the nonactin concentration namely a decrease in the capicitance and an increase in the resistance. This suggests an increase in the thickness of the surface layer as the charge density increases.

Table 3. Lipidic dependence. Aqueous solution: 0.1 M KCl and 10^{-6} M Nonactin.

Lipids	2% PE		2% PC		2% PS		2% PI	
τ_f	2.0	μs	2.7	μs	2.8	μs	2.1	μs
τ_s	5.7	μs	5.8	μs	15	μs	8.0	μs
G_{0f}	18	mS/cm^2	33	mS/cm^2	22	mS/cm^2	28	mS/cm^2
G_{0s}	5.1	mS/cm^2	4.5	mS/cm^2	4.3	mS/cm^2	1.6	mS/cm^2
G_∞	3.2	mS/cm^2	1.5	mS/cm^2	2.9	mS/cm^2	0.5	mS/cm^2
C_P	4.1	μF/cm^2	4.5	μF/cm^2	3.3	μF/cm^2	6.0	μF/cm^2
R_P	0.49	Ω cm^2	0.60	Ω cm^2	0.85	Ω cm^2	0.35	Ω cm^2

Table 3 is based on experiments with identical external solution conditions to allow a comparison between the four different phospholipids used. The time constants for the fast process show a smaller dispersion than in the case of the slow process. This could imply, in all of the cases, that the fast process takes place either between the aqueous solution and the surface layers of the membrane or in the polar groups of the lipids or between the surface layers and the interior of the membrane.

DISCUSSION

Despite the uncertainty involved in estimating the electrical parameters C_P and R_P the results permit some general conclusions to be drawn. Firstly, it is established, from the general dependence of relaxation processes on the ionic strength, nonactin concentration and the applied potential, that the two processes behave differently. The fast process might be due to a charge redistribution between the surface layers and the interior of the membrane. The most obvious reason for interpreting the rapid, process in terms of a charge redistribution process is the value of the initial conductance which seems far too large to be explained by a net transfer of charge across the membrane (2, 4). In addition the values obtained for C_P and R_P seem to be reasonable figures for the polar layers of the membrane. Assuming that the polar layers accupy about 1/5 of the total membrane thickness, the dielectric constant of the polar layer must be about twice that of the nonpolar region in order to satisfy the experimental values of C_P. The values of C_P in the experiments reported by Sandblom et al. (11), and by Coster and Smith (4) are about 3 to 6 times higher than those given here. Since phospholipids have been used in all cases, the difference must be due to the additives [α-tocopherol (11), tetradecane (4) and decane respectively].

The values of R_P obtained in the present experiments are similar to those found previously with nonactin (11) and are about 3 orders of magnitude lower than the values obtained with the bare lipid bilayer (4). The voltage clamp technique does not allow an accurate determination of displacement current at the low conductance level of the bare lipid membrane and is advantageous for resolving fast processes with higher conductances.

The possibility that the measured parameters could be those of the electric double layers adjacent to the membrane water interfaces has been considered (3, 11) although calculations show that either G_{0f} or τ_f are much too small to give any measurable effects in these experiments. Another possibility would be the presence of aqueous surface layers with specialized structures, a model suggested by Coster and Simons (3) to account for the high frequency dispersion regions appearing in the impedance curves. The same mechanism may be responsible for both the high frequency dispersion and the rapid step response of the conductance although the explanation offered here seems more adequate in accounting for the effects of lipid composition and concentration of ionophore.

A second conclusion which can be drawn from the results is that the capacitance C_P decreases with increasing ionic strength, whether this occurs on the aqueous side or on the hydrocarbon side of the polar groups. This is

also accompanied by a slight increase in the resistance R_p. These findings suggest a variation in the thickness of the polar layer, possibly by screening the charge on the phosphate or the amino groups. Adding salt would then cause the two charged sites to separate producing the observed changes in the electrical parameters of the polar region. An independent evidence for this structural change is obtained from NMR studies on lamellar mesophases in the presence of various salt concentrations (9).

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