The Effect of Valinomycin on the
Electrical Properties of Solutions
of Red Cell Lipids in $n$-Decane

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ABSTRACT This paper reports the electrical properties of thick lipid membranes
in the absence and presence of valinomycin. The thick lipid membranes were
formed by placing a solution of sheep red cell lipids in decane between two
cellophane partitions which formed the interfaces between the membrane and
the two aqueous bathing solutions. The DC electrical resistance of these structures
was found to be directly proportional to the reciprocal of the concentration of
lipids in the decane ($C_L$). The limiting resistance, as ($C_L^{-1}$) approached zero,
was $3 \times 10^8$ ohm-cm$^2$. Resistance was also found to be linearly related to mem-
brane thickness. The limiting resistance at zero thickness was again $1-3 \times 10^8$
ohm-cm$^2$. These data are interpreted to indicate that the DC resistance of thick
lipid membranes comprises two surface resistances ($R_s$) at each interface with
the aqueous bathing solutions, and a bulk resistance ($R_B$) of the lipid-decane
solution, arranged in series. Measurements of the effect of variations of area on
resistance were consistent with this interpretation. Valinomycin reduced
$R_s$ but had no effect on $R_B$. Under certain conditions, thick lipid membranes containing
valinomycin behaved like highly selective $K^+$ electrodes.

INTRODUCTION

Previous reports from this laboratory (1) described the electrical properties
of optically black, thin lipid membranes, formed from sheep red blood cell
lipids dissolved in decane, which separated two aqueous phases. Our ob-
servations, in agreement with the reports of others (2), indicated that such
membranes had an extremely high DC resistance (approximately $10^8$ ohm-cm$^2$)
approaching that of an equivalent layer of pure hydrocarbon.

However, the specific resistivity of bulk solutions of sheep red cell lipids in
decane, identical to those used to form membranes, was approximately
$2 \times 10^4$ ohm-cm, as estimated by an AC method (3). For a layer of about the
same thickness as a thin lipid membrane (circa 100 Å), this value predicted a DC membrane resistance considerably lower ($\sim 10^2$ ohm-cm$^2$) than that observed experimentally ($\sim 10^6$ ohm-cm$^2$). This discrepancy was rationalized by assuming that phospholipids organized in lamellae, as they are in the surface monolayers of the bilayer membranes, have a relatively high resistance (2, 3). In order to evaluate this hypothesis, we constructed a system which permitted an estimation of the electrical properties of the surface layers bounding lipid phases at their interfaces with aqueous solutions.

The macrocyclic depsipeptide antibiotic, valinomycin, has been shown to enhance strikingly the $K^+$, but not the $Na^+$, permeability of thin lipid membranes (3–5). However, these observations did not rigorously exclude the possibility that the $K^+$-dependent reduction in DC membrane resistance was referable to an increase in potassium conductance through the bulk lipid torus (6). In addition, with respect to the mechanisms by which valinomycin increases the $K^+$ permeability of thin lipid membranes, it seemed pertinent to inquire whether the effect of valinomycin was restricted to optically black lipid membranes (5), or whether the antibiotic could increase the $K^+$ permeability of bulk lipid phases as well.

This paper describes the formation and electrical properties of red cell lipid-decane solutions separating two aqueous phases (referred to below as thick lipid membranes), and the effect of valinomycin on these electrical properties. In agreement with previous hypotheses (2, 3), the results indicate that the high electrical resistances of thin lipid membranes are referable primarily to the properties of their surface monolayers, and that valinomycin can reduce the electrical resistance and concomitantly increase the potassium permeability of such monolayers. In addition, the data suggest that the electrical properties of these surface monolayers are largely independent of the thickness of the lipid medium which separates them. A summary of some of the studies described in this paper has been presented elsewhere (7).

**METHODS**

Lipids were extracted from high potassium (HK) sheep red blood cells according to a method described previously (1), and were dissolved in decane at the various concentrations indicated in the text. Hence, the lipid solutions used to form thick lipid membranes were at least qualitatively, and frequently quantitatively, identical to the solutions used to form thin lipid membranes (3).

The apparatus used for the formation of thick membranes, illustrated schematically in Fig. 1, is similar in general design to the setup used in thin lipid membrane studies (3). Lucite cells of varying thickness (1–9 mm), having a central aperture, 9.6 mm in diameter (unless otherwise specified), were covered on each facing side with a sheet of cellophane dialysis tubing and clamped between two chambers (front and rear), each having an approximate volume of 1.0 ml. The lipid solutions indicated in the text
were introduced into the Lucite cell through a narrow input channel (0.5 mm diameter), and air bubbles were flushed out through an output channel (0.5 mm diameter). When the cell had been completely filled with a lipid solution, both the input and output channels were sealed, and the aqueous solutions indicated in the text were introduced into the front and rear chambers. The solutions in these chambers were changed during the experiments by perfusion through intake and output channels (not shown in Fig. 1). As in prior studies (1, 3), the aqueous solutions were unbuffered (pH $\geq 5.8$), and the experiments were carried out at room temperature (22°–24°C).

The electrical circuit, utilized in the measurement of the $\text{DC}$ resistances and potentials in the aqueous phases bathing the thick lipid membranes, was identical to one previously described (1) except for the following modifications. The input resistors ($R_i$) varied from $10^7$ to $10^{14}$ ohms (Hi-Meg; Victoreen Instrument Co., Cleveland, Ohio); their tolerance was $\pm 2\%$ ($\leq 10^9$ ohms) or $\pm 10\%$ ($\geq 10^{11}$ ohms). Whenever possible, the value of the input resistor was approximately the same as the resistance of the thick lipid membrane. A vibrating reed electrometer (Cary Model 31; Applied Physics Corp., Monrovia, Calif.), having an input impedance of $\geq 10^{16}$ ohm, was used to measure the electrical potential difference across the thick lipid membranes.

The validity of the electrical measurements depended primarily on the absence of electrical "leakage" pathways in the apparatus. The results of an experiment designed to evaluate this possibility are illustrated in Fig. 2. When the lipid cell contained decane, the $\text{DC}$ membrane resistance was $\geq 10^{15}$ ohms, and was stable for at least 90 min (it should be noted that $10^{15}$ ohms is a minimum estimate, and represents the maximum value which could be assigned under the present experimental conditions). In all experiments, the cells were first filled with decane and checked for stable, high $\text{DC}$ resistances ($\geq 10^{15}$ ohms for at least 15 min) prior to introducing decane solutions containing sheep red cell lipids. Fig. 2 also provides a measure of the reproducibility of the resistance values. A sample containing 25 mg/ml HK sheep red cell lipids per ml decane was injected into the cell and the resistance values were recorded (Fig. 2, open squares). Subsequently, the sample was removed, the lipid cell was flushed with approximately 10 ml decane, and the same sample of sheep red cell lipids in decane was reintroduced into the cell and the $\text{DC}$ resistances were measured (Fig. 2, open
triangles). As shown in Fig. 2, the results were independent of time (for a particular lipid sample) and reproducible within an approximate range of \( \pm 20\% \).

Naturally occurring valinomycin (mol wt = 1111) was kindly furnished by Dr. J. C. McDonald, Prairie Regional Laboratory, Saskatoon, Saskatchewan, Canada. Stock solutions of the antibiotic in 95% ethanol (1-2 mg/ml) or redistilled chloroform (approximately 20 mg/ml) were prepared and stored at 4°C. In the experiments, aliquots of the stock ethanolic or chloroform solutions were added to aqueous or lipid solutions, respectively. Appropriate control solutions, at the same or greater concentrations of ethanol (aqueous concentration \(<0.1\%\)) or of chloroform (lipid concentration \(\leq 5\%\)) did not affect the DC resistance or ionic permeability properties of the thick lipid membranes. The reagents and analytical determinations were the same as those used in previous studies (1, 3).

RESULTS

The Effect of Lipid Concentration on DC Resistance

When the lipid cell contained only decane, the DC membrane resistance was \( \geq 10^{15} \) ohms (Fig. 2). Fig. 3 illustrates the effects of varying the concentration of sheep red cell lipids in the decane solution on the DC resistance of

**FIGURE 2**

The time course of the DC resistance of thick lipid membranes under different experimental conditions. The aqueous phase in each chamber was 0.1 M KCl. The lipid cell contained decane (open circles) or 25 mg sheep red cell lipids per ml decane (open squares, open triangles). Experimental details are in Methods.

**FIGURE 3**

The effect of lipid concentration (abscissa, mg sheep red cell lipids per ml decane) in the bulk lipid solution on the DC resistance \( (R_{cm}) \) of thick lipid membranes. The cell thickness (path length) and the composition of the aqueous solution are indicated in the figure. Experimental details are given in Methods.
thick lipid membranes. As shown in the figure, relatively low lipid concentrations (approximately 0.5 mg/ml) in the bulk lipid phases reduced the DC resistance of the thick lipid membranes to less than $10^{10}$ ohms. When the lipid concentration of the solutions was increased to 25 mg/ml, the DC membrane resistance approached $10^8$ ohms as a limiting asymptote. This phenomenon is illustrated more clearly in Fig. 4, where the DC resistance of the thick lipid membranes is plotted as a function of the reciprocal of the red cell lipid concentration in the decane solutions. An approximately linear relationship was obtained in the concentration range from 0.5 to 25 mg sheep red cell lipids/ml decane. When extrapolated to infinitely high lipid concentration (i.e., the zero intercept on the ordinate), the curve in Fig. 4 reached a limiting value of approximately $3 \times 10^8$ ohms. Since the area of the thick lipid membranes was 0.725 cm$^2$, the limiting resistance of these membranes, normalized for area but not thickness, was approximately $2.2 \times 10^8$ ohm-cm$^2$, i.e., similar to the DC resistance values (from 1 to $3 \times 10^8$ ohms-cm$^2$) obtained on thin lipid membranes formed from identical lipids dissolved in decane at a concentration of approximately 30 mg per ml (1, 3).

As shown in Fig. 5, the DC resistance of the thick lipid membranes was constant over a minimum range of $\pm 150$ mv, and was independent of the cation ($\text{Na}^+$ or $\text{K}^+$) in the aqueous phase.
Effect of Cell Geometry and Valinomycin on DC Resistance

It seems reasonable to attribute the limiting resistance of thick lipid membranes at infinite lipid concentration to the properties of the surface monolayers at the interfaces between the lipid and aqueous phases. According to this view, the electrical resistance of these membranes (when the concentration of sheep red cell lipids in the decane solutions was greater than 0.5 mg/ml) might be described by an equation having the form:

$$ R_T = \frac{\rho_B l_B}{A} + 2R_s $$  \hspace{1cm} (1) 

where $R_T$, the DC resistance of the membrane (ohms), is the sum of $R_B$, the bulk resistance ($R_B = \frac{\rho_B l_B}{A}$ where $\rho_B$ = specific resistivity of the bulk lipid phase, $A$ = area, and $l_B$ = thickness of the bulk lipid phase), and $2R_s$, the sum of the surface resistances of the monolayers bounding the membrane at its two aqueous interfaces. The results of a set of experiments designed to evaluate this possibility are illustrated in Fig. 6. As the lipid concentration in the bulk lipid phase was increased from 2.6 to 25 mg/ml, the slope of the relation between the DC resistance of the thick lipid membranes (Fig. 6, ordinate) and the thickness of the lipid cell (Fig. 6, abscissa) became progres-
sively smaller while the zero intercept (i.e., $2R_s$ in equation 1) did not change by more than a factor of 2. In agreement with Fig. 5 and the results with thin lipid membranes (1, 2), the zero intercept for each of the lines in Fig. 6 was in the range from 2 to $4 \times 10^8$ ohms, or approximately $1.5-3.0 \times 10^8$ ohm-cm$^2$.

Furthermore, as shown in Table I, the DC resistance of these thick lipid membranes was proportional to the area, rather than to the circumference of the cell, over at least a threefold difference in area. Consequently, the possibility of shunt conductances (6) along the edges of the lipid cells rather than through the phases seems unlikely.

The macrocyclic antibiotic, valinomycin, reduces considerably the DC resistance of thin lipid membranes when the aqueous phases bathing the membranes contain 0.1 M KCl but not when they contain 0.1 M NaCl (3-5). As indicated in Fig. 7, the addition of valinomycin ($3 \times 10^{-4}$ M) to the bulk lipid phases produced no change in the slope but at least a 10-fold reduction in the zero thickness intercept of a plot of $R_s$ vs. $L_s$. Interpreted according to equation 1, these results indicate that valinomycin reduces $R_s$, the surface resistivity but not $\rho_s$, the specific resistivity of the bulk lipid phase. It should be noted that the zero intercept in Fig. 7, when valinomycin ($3 \times 10^{-4}$ M) was in the bulk lipid phase, could have any value between zero and $10^7$ ohms.
since this range of values could not be distinguished with the present experimental setup. Hence, although these experiments indicate clearly that valinomycin (lipid phase) reduces the surface resistance of these thick lipid membranes, they do not provide an accurate estimate of the magnitude of the reduction.

**TABLE I**

**THE EFFECT OF CELL AREA ON THE DC RESISTANCE OF THICK LIPID MEMBRANES**

Thick lipid membranes were formed in Lucite cells having the indicated areas and thickness from solutions containing 25 mg sheep red cell lipids per ml decane. The aqueous phase contained 0.1 M KCl. The dc total membrane resistance of the thick lipid membrane ($R_T$) was measured as indicated in Methods. $R_m$ is the resistance normalized for area. The results are the mean values observed and the numbers in parentheses refer to the number of membranes on which the observations were carried out.

| Cell area (cm$^2$) | Cell thickness (cm) | $R_T$ (ohms) | $R_m$ (ohm-cm$^2$) |
|---------------------|---------------------|--------------|-------------------|
| 0.72                | 0.32                | 4.6 x 10$^8$ (6) | 3.3 x 10$^8$ (3) |
| 2.54                | 0.32                | 1.7 x 10$^8$ (6) | 4.3 x 10$^8$ (3) |

**Ionic Selectivity Properties**

When the solutions in the two aqueous phases bathing the thick lipid membranes contained unequal concentrations of salt, the membrane potentials ($V_m$) observed under these conditions presumably reflected the sum of the potential at each interface between lipid and aqueous phases, and a diffusion potential through the lipid phases (8, 9). In the measurements of zero current potential reported in Fig. 8, the composition of one aqueous phase (rear chamber) was kept constant, while the aqueous solutions in the front chamber were changed. Thus, it seems likely, in view of the thickness of the lipid phases, that the membrane potential under these circumstances was determined largely by shifts in the distribution of ions at the interface between the lipid membranes and the aqueous solutions in the front chamber. When the solution in the front chamber was changed, the potential difference developed by the cell changed to a new steady value during the next 1–2 min. Although no extensive study was made of the time course of the potential difference after the initial transient was completed, it remained constant (±1 mv) for at least 15 min.

As shown in Fig. 8, a linear relation was observed between the electrical potential difference in the two aqueous phases bathing the membrane and the log of the activity of K$_2$SO$_4$ in the front chamber (aqueous phase) over approximately a 400-fold range of salt concentration. Furthermore, the sign and
magnitude of the potentials indicate that the surface layers at the interfaces between the thick lipid membranes and the aqueous solutions in the front chamber were more permeable to K\(^+\) than to \(\text{SO}_4^{2-}\), and that the K\(^+\), with respect to \(\text{SO}_4^{2-}\) permeability, was consistently enhanced by the addition of valinomycin \(3 \times 10^{-4} \text{ M}\) to the lipid solutions. A similar series of experiments in which Cl\(^-\) salts were present in the aqueous phases is illustrated in Table II. When the lipid phases did not contain valinomycin, the thick lipid mem-

![Figure 8](image)

**Figure 8.** The relationship of membrane potential \((V_m)\) to negative logarithm of the activity of K\(_2\)SO\(_4\) \((a_{K_2\text{SO}_4})\) in the aqueous phase (front chamber). The rear chamber contained 0.2 M K\(_2\)SO\(_4\). The lipid cell was 3.18 mm thick. The lipid solution contained 25 mg/ml sheep red cell lipids (open squares), or 25 mg/ml sheep red cell lipids and \(3 \times 10^{-4} \text{ M}\) valinomycin (open circles). The lipid solvent was 95% decane-5% chloroform (v/v) in all cases. Experimental details are given in Methods.

branes were cation (K\(^+\) or Na\(^+\)) selective, in agreement with the observations on thin lipid membranes formed from identical lipids (1, 2). However, in contrast to the observations with thin lipid membranes, the bulk phases exhibited an appreciable degree of K\(^+\), with respect to Na\(^+\), selectivity, even in the absence of valinomycin (Table II). When valinomycin was present in the lipid phases, the increase in \(V_m\) (when the aqueous phases contained a single salt) was considerably greater when K\(^+\) rather than Na\(^+\) was the cation in the aqueous phase. Furthermore, when the aqueous phases contained both K\(^+\) and Na\(^+\), the membrane potential was nearly the same as when K\(^+\) alone was present (Table II). Thus, the addition of valinomycin to the lipid solutions produced a striking increase in the K\(^+\), with respect to Na\(^+\), selectivity.
of the thick lipid membranes. However, it should be noted that the value of $V_m$ (Fig. 8, Table II), when the lipid phases contained valinomycin and the aqueous phases contained K+, was invariably less than the value that would be predicted (approximately 55 mv) if coion exclusion (i.e., Cl$^-$ or SO$_4^{2-}$) were complete.

### TABLE II

**THE EFFECT OF VALINOMYCIN ON THE IONIC SELECTIVITY OF THICK LIPID MEMBRANES**

The aqueous phases (front and rear chambers) contained the indicated concentrations of salt. The lipid cell was 3.2 mm thick. The lipid solutions contained 25 mg/ml sheep red cell lipids in 95% decane-5% chloroform (v/v) and the indicated concentrations of valinomycin. The membrane potential ($V_m$) was measured as described in Methods.

| Experiment | Front chamber | Rear chamber | Valinomycin | $V_m$ |
|------------|---------------|--------------|-------------|-------|
| X-16-2     | 0.1 KCl       | 0.01 KCl     | 0           | -26   |
| X-16-8     | 0.1 KCl       | 0.01 KCl     | $3 \times 10^{-4}$ | -43   |
| X-16-3     | 0.1 NaCl      | 0.01 NaCl    | 0           | -12   |
| X-16-7     | 0.1 NaCl      | 0.01 NaCl    | $3 \times 10^{-4}$ | -16   |
| X-16-4     | 0.1 KCl       | 0.01 KCl     | 0           | -13   |
| X-16-6     | 0.01 NaCl     | 0.1 NaCl     | $3 \times 10^{-4}$ | -40   |

**DISCUSSION**

The experiments described in this paper provide additional support for the hypothesis (2, 3) that the high $\text{dc}$ resistance of thin lipid membranes is determined primarily by the properties of the monolayers at the two interfaces between aqueous and lipid phases. Thus, the resistances of the surfaces of the thick lipid membranes, estimated from extrapolation either to infinitely high bulk lipid concentrations (Figs. 3 and 4) or to infinitely short path length (Fig. 5) were in the range from 1 to $3 \times 10^8$ ohm-cm$^2$, in remarkably good agreement with the observed $\text{dc}$ resistances of thin lipid membranes formed from identical lipids dissolved in decane (1, 2, 4).

The origin of this high $\text{dc}$ electrical resistance across the interfaces between aqueous solutions and the interior of thin or thick lipid membranes is not clear. Two general classes of explanation may be entertained. In order to define these classes, it is useful to consider the interface as consisting of two regions: (a) polar region containing water and ions from the aqueous phase, and phosphoryl, amino, quaternary, ammonium, hydroxyl, and other groups on phospholipid and other membrane molecules, and (b) a nonpolar region of low dielectric constant containing only the aliphatic hydrocarbon chains of membrane components.
For class I, it is assumed that equilibrium exists at all times between the regions and, therefore, between the bulk aqueous phase and the nonpolar region of the membrane. In this case, the resistance of the membrane depends primarily on the concentrations and mobilities of all charge-carrying components in the nonpolar region. An example of a model of this class is the treatment developed by Ciani et al. (14).

For class II, it is assumed that equilibrium does not exist between the regions when electrical current is passing through the system. In this case, the resistance of the membrane is determined by the conductance of both the polar and nonpolar regions of the interface. The conductance across the polar region is a complex process which involves several factors (13, 15). First, the water in the immediate vicinity of the membrane surface may have different kinetic properties from bulk water (16). Second, if the penetrating ions must be dehydrated before entering the membrane, the rate of the substitution reaction of membrane ligands for water molecules in the hydration shell of the ions may be of kinetic significance. Third, the entry of ions into the interior of the membrane may depend in part on the rotational mobility of membrane counterions, e.g. phospholipids. Rotation of these molecules may be restricted when they are arranged as a lamella at the surface of the membrane. Finally, the dielectric constant in the nonpolar region of the interface is probably between 2 (3, 11) and 4.5 (12) so that many of the ions which enter may do so as undissociated ion pairs incapable of carrying charge. The data presented in this and other relevant papers (2, 11) do not permit a clear decision between these two classes of explanation.

Under the conditions of the experiments described in this paper, valinomycin reduced the surface resistance but did not influence the bulk resistance of thick lipid membranes. This result does not rule out the possibility that valinomycin could increase the conductance of bulk lipid phases under other conditions. In the experiments described above, the lipid-decane solutions were not equilibrated with solid KCl or aqueous solutions containing KCl in the absence and presence of the antibiotic. Exposure of the lipid-decane phase to aqueous solutions occurred only during the time of the measurements of electrical properties of the thick lipid membranes. When such an equilibration is carried out, it is possible for the antibiotic to increase substantially the K\(^+\) content of the nonaqueous phase and thus, presumably, its conductance (17). If the total amount of K\(^+\) in a nonaqueous phase is kept constant, the conductance can actually be reduced by addition of valinomycin (19). This effect is due to the fact that the mobility of the K\(^+\)-valinomycin complex in ethanol is less than the mobility of K\(^+\) alone. Indeed, this fact has been used in an attempt to measure the association constant of K\(^+\) with valinomycin (19).

The mechanism by which valinomycin increases selectively the conductance and alters the zero current potentials at the surface of thick lipid membranes
(and thin lipid membranes) is probably related to its capacity to form complexes with cations (13, 18, 19). The formation of such complexes could increase the conductance of lipid membranes by either of the classes of mechanism described above. If class I obtains, the presence of cation-valinomycin complexes at equilibrium in the nonpolar region of the interface could increase the concentration of charge-carrying elements and thus the conductance. If class II holds, the antibiotic could also exert its effect by altering one or more of the kinetic steps involved in the transition of ions from polar to nonpolar region. With the actin series of macrocyclic antibiotic compounds, the relationships between membrane resistance and salt concentration and direct measurements of K+ fluxes are difficult to rationalize with explanations of class I (20-22).

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