NONLINEAR ELECTRICAL EFFECTS IN LIPID BILAYER MEMBRANES

I. ION INJECTION

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ABSTRACT In this and the following papers a theoretical analysis of the current-voltage characteristic of lipid bilayer membranes is presented. In order to explain the observed nonlinearity, three different mechanisms have to be considered: the injection of ions into the membrane, the distortion of the potential energy profile, and the dissociation field (or Wien) effect. In this first part of the paper, the ion injection effect is analyzed. As both the ion concentration and the thickness of the membrane are extremely small, the constant field approximation can be introduced into the Nernst-Planck equations. In this way the general treatment of Bruner can be greatly simplified, so that the solution is obtained in closed form. The result shows that the ion injection effect gives a contribution to the current-voltage characteristic only at low ionic strength ($< 10^{-3}$ M) of the aqueous solution.

INTRODUCTION

Artificial bimolecular lipid films are now widely studied as models for biological membranes (for a review see Tien and Diana, 1968). One of the most easily accessible properties is the electrical conductivity of the membrane in aqueous electrolyte solutions. In most cases the membrane conductivity λ_0 is very small; under carefully controlled conditions values of λ_0 as low as $0.5 \cdot 10^{-9}$ mho/cm^2 may be observed with a phosphatidyl choline membrane in 10^{-2} M NaCl (Hanai et al., 1965). As the thickness of these membranes is about 70 A, this corresponds to a bulk conductivity in the order of 10^{-16} mho/cm. The low value of λ_0 is consistent with the concept that the interior of the membrane is formed by the apolar side chains of the lipid molecules and has essentialy the properties of a thin hydrocarbon film. The membrane conductivity is increased, in some cases by many orders of magnitude, when certain lipid soluble acids like 2,4-dinitrophenol or picric acid are present in the aqueous phase (Bielawski et al., 1966; Hopfer et al., 1968; Liberman and Topaly, 1968).

There is much evidence that in these cases the charge transport in a lipid membrane is ionic. However, the mechanism by which ions pass across a lipid film about 100 A thick is as yet not well understood. Experimentally it is found that the current-voltage characteristic of lipid bilayer membranes is strongly nonlinear (Läuger et al., 1967; Miyamoto and Thompson, 1967; Liberman et al., 1968). Ohmic behavior is observed only at voltages below about 30 mv; above 30 mv the current increases much more rapidly than the voltage.

The aim of this and the following papers is to develop a theoretical description of the ion transport mechanism in thin lipid membranes and to give an explanation of their nonlinear current-voltage characteristic. We restrict ourself to the transport of simple ions across the unmodified membrane, i.e. we do not take into consideration cases in which ions are transported by lipid-soluble carriers like certain macrocyclic antibiotics (see, for reference, Finkelstein and Cass, 1968). Even in this case the situation is rather complicated. There exist at least three different mechanisms each of which may lead to a nonlinear electrical behavior of the membrane:

Ion Injection. As long as the system is in equilibrium (i.e. both external solutions are at the same electrical potential) ions are distributed between the membrane phase and the outer aqueous phases according to their partition coefficients. In most cases the equilibrium ion concentrations within the membrane are extremely small, owing to the low dielectric constant of the membrane phase. When a voltage is applied across the membrane, part of the potential drop lies in the aqueous phases just outside the membrane. As a consequence, the concentration of positive ions on one interface, and of negative ions on the other are raised by a Boltzmann factor. This leads to an injection of ions into the membrane and to an increase in the membrane conductivity.

Distortion of the Potential Energy Profile by the Electric Field. Because the dielectric constant of the membrane is much lower than that of the surrounding aqueous medium, an ion located in the membrane is in general subjected to strong image forces. The potential energy w of the ion in the membrane is therefore dependent on the position x; in the absence of an external electric field, $w(x)$ has the shape of a symmetrical barrier with a peak in the middle of the membrane. When an external voltage is applied across the membrane, the potential barrier is changed by an additive term originating from the coulombic energy of the ion. This effect may be described by a generalized form of the Nernst-Planck equations It also leads to a nonlinear current-voltage characteristic of the membrane.

Field Dissociation (*Wien Effect*). As a consequence of the low dielectric constant, the attractive force between oppositely charged ions in the membrane is many times greater than in an aqueous solution. An appreciable fraction of the ions is therefore present in the form of neutral ion pairs which cannot contribute to the electric current. However, in a 70 A thick membrane the electric field strength becomes very high even at moderate voltages (about 10^5 V/cm at a voltage of 100 mv). In the presence of such high fields the number of free ions in the membrane is markedly increased by field dissociation of ion pairs. Accordingly, the current becomes a nonlinear function of the voltage. This effect has been frequently studied with macroscopic phases and has already been mentioned as a possible source for the nonohmic behaviour of thin lipid membranes (Miyamoto and Thompson, 1967; Bass and Moore, 1968).

In general, these three effects occur simultaneously when a voltage is applied across a thin lipid membrane. However, their relative contributions to the current-voltage curve of the membrane are difficult to estimate a priori. The three mechanisms are therefore considered separately. This will be possible by a suitable choice of the model.

In this paper we treat the injection of ions into the membrane. We show that this effect is significant only if the ionic strength of the aqueous phase is low. This offers the possibility to separate experimentally the ion injection mechanism from the two other effects.

Mathematical Treatment of the Model

As a model for the lipid membrane we consider a homogenous film of thickness d and dielectric constant ϵ_m interposed between two aqueous electrolyte solutions of identical composition (see Fig. 1). The solutions contain a completely dissociated electrolyte A^+B^- of concentration c, the ions of which have a finite, but very low solubility in the membrane phase. In addition, a second electrolyte X^+Y^- of concentration c^* which is completely insoluble in the membrane is assumed to be present. Both concentrations are held constant by continuous renewal of the solutions.

Recently, in an excellent series of papers, Bruner (1965, 1967) has treated the steady-state ion transport in a similar three phase system. The result of Bruner's analysis is a set of nonlinear differential equations which have to be evaluated numerically. In the present treatment, which is of a less general character, certain properties of the lipid film, in particular its low solubility for ions, are explicitly introduced into the theory. In this way the formal analysis is greatly simplified, and the result can be obtained in closed form.

The model is further defined by the following assumptions:

I. The electrolyte A^+B^- is completely dissociated in the membrane. Accordingly, the field dissociation effect can be neglected.

II. The passage of the permeable ion species A^+ and B^- across the membrane/solution interface is completely unrestrained.

III. The ionic concentrations are discontinuous at the membrane/solution interfaces and can be characterised by partition coefficients γ_+ and γ_- :

$$\gamma_+ \equiv \frac{c_+^m(d/2)}{c_+(d/2)} = \frac{c_+^m(-d/2)}{c_+(-d/2)} \qquad (1)$$

$$\gamma_- \equiv \frac{c_-^m(d/2)}{c_-(d/2)} = \frac{c_-^m(-d/2)}{c_-(-d/2)} \qquad (2)$$

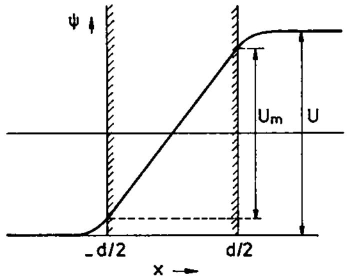

FIGURE 1 Homogeneous membrane of dielectric constant ϵ_m which is in contact with aqueous electrolyte solutions at $x = -d/2$ and $x = d/2$. The profile of the electrical potential ψ in the presence of an external voltage U is drawn schematically.

(c_+, c_- = concentrations of A^+ and B^-, respectively, in the aqueous phases; c_+^m and c_-^m are the corresponding concentrations in the membrane). It follows from assumption II that equations 1 and 2 are valid not only in the equilibrium, but also in the case of nonvanishing current.

Assumption III is a rather severe simplification. In a molecular scale, the ionic concentrations are continuous functions of position even at the interface between two different media. This is a consequence of the image forces which, in the aqueous medium, tend to repell the ions from the interface, but attract them toward the interphase in the hydrocarbon medium. This problem will be analyzed in more detail in the subsequent paper.

The first step in the calculation of the membrane conductance is the evaluation of the electric potential $\psi(x)$ in the presence of an external voltage U (see Fig. 1). We begin with the limiting case that the membrane is an ideal dielectric with zero conductivity (γ_+, $\gamma_- = 0$). Then even at $U \neq 0$ the system remains in an equilibrium state, and the ionic concentrations in the aqueous phase at $x \geqq d/2$ are given by the Boltzmann law

$$c_+ + c_+^* = c_0 \cdot e^{-\varphi(x)} \qquad (x \geqq d/2) \tag{3}$$

$$c_- + c_-^* = c_0 \cdot e^{\varphi(x)} \tag{4}$$

with

$$\varphi(x) \equiv [\psi(x) - U/2]F/RT \tag{5}$$

(F = Faraday constant, R = gas constant, T = absolute temperature, c_+, c_+^*, c_-, c_-^* = concentrations of A^+, X^+, B^-, Y^-, respectively, $c_0 \equiv c + c^*$). Inserting equations 3 and 4 into the Poisson equation

$$\frac{d^2\psi}{dx^2} = -\frac{4\pi F}{\epsilon}(c_+ + c_+^* - c_- - c_-^*) \tag{6}$$

(ϵ = dielectric constant of water), one obtains

$$\frac{d^2\varphi}{dx^2} = \kappa^2 \sinh \varphi, \quad \kappa^2 \equiv \frac{8\pi}{\epsilon}\frac{c_0 F^2}{RT} \tag{7}$$

where $1/\kappa$ is the Debye-Hückel length. The solution satisfying the boundary conditions

$$\varphi(\infty) = 0 \tag{8}$$

and

$$\left(\frac{d\varphi}{dx}\right)_{x=\infty} = 0 \tag{9}$$

is given by

$$\varphi(x) = 2 \ln \frac{1 + \tanh(\varphi_i/4)\cdot e^{-\kappa(x-d/2)}}{1 - \tanh(\varphi_i/4)/e^{-\kappa(x-d/2)}}$$

$$(x \geqq d/2) \tag{10}$$

where $\varphi_i \equiv \varphi(d/2)$ is the reduced potential (equation 5) at the interface.

If the membrane has a finite conductivity, equation 10 is no longer valid. In practice, however, the observed membrane conductivities are very small. A typical value for artificial bilayer membranes is $\lambda_0 \simeq 10^{-6}$ mhos/cm². This corresponds (with a membrane thickness of 10^{-6} cm) to a bulk conductivity of 10^{-12} mhos/cm, a value which is many orders of magnitude lower than the conductivity of the aqueous phase. As for all practical values of the voltage U the current density is extremely small, the potential (and concentration) profile in the aqueous phase is almost unchanged by the current flow. We may therefore assume that even at nonzero current the state of the aqueous phase remains practically near equilibrium and is approximately described by equation 10.[1]

The fluxes Φ_+ and Φ_- of the permeable ion species A^+ and B^- across the membrane in the steady state are given by the Nernst-Planck equations:

$$\Phi_+ = -D_+^m\left(\frac{dc_+^m}{dx} + c_+^m \frac{F}{RT}\frac{d\psi_m}{dx}\right) \tag{11}$$

$$\Phi_- = -D_-^m\left(\frac{dc_-^m}{dx} - c_-^m \frac{F}{RT}\frac{d\psi_m}{dx}\right) \tag{12}$$

where D_+^m and D_-^m are the diffusion coefficients of A^+ and B^-, respectively, in the

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[1] A closer examination shows that assumption IV is a very good approximation in all cases in which the membrane conductance λ_0 can be determined by direct current methods. If the membrane conductance is high and the concentration c of the permeable ion species very low, the DC method is no longer applicable (Läuger et al., 1967). This is a consequence of the concentration polarization caused by insufficient stirring of the solutions. The polarization effect is observed in the conductance range $(\lambda_0)_{\text{mhos/cm}^2} \geqq 2\cdot(c)_{\text{moles/liter}}$.

membrane. The electric potential ψ_m in the membrane is connected with the ion concentrations by the Poisson equation

$$\frac{d^2\psi_m}{dx^2} = -\frac{4\pi}{\epsilon_m}\rho = -\frac{4\pi}{\epsilon_m}F(c_+^m - c_-^m) \tag{13}$$

(ρ = charge density). By equations 11, 12 and 13 and the appropriate boundary conditions, the functions $c_+^m(x)$, $c_-^m(x)$, and $\psi_m(x)$ are completely determined. In general, c_+^m, c_-^m, and ψ_m cannot be obtained in closed form. However, the solution is greatly simplified by consideration of the fact that the ionic concentrations and hence the charge density $\rho = F(c_+^m - c_-^m)$ in the membrane are extremely small. If the membrane conductance, λ_0, is 10^{-6} mhos/cm², the concentration of permeable ions must be in the order of 10^{-13} moles/cm³, if the ion mobility in the membrane is taken to be about ten times lower than in the aqueous solution. In the limiting case that all permeable ions are of the same sign ($\gamma_+ \gg \gamma_-$ or $\gamma_+ \ll \gamma_-$), the charge density ρ would then be 10^{-8} coul/cm³. At this low value of ρ the curvature of the potential profile according to the Poisson equation is quite negligible. This is illustrated by the following consideration. If in a film of thickness d = 100 A and dielectric constant $\epsilon_m = 2$ charge is homogeneously distributed with the density $\rho = 10^{-8}$ coul/cm³, the potential difference $\Delta\psi$ between the center and the surface of the film, as obtained by integration of equation 13, is given by $\Delta\psi = \pi\rho d^2/2\epsilon_m \simeq 7 \cdot 10^{-9} V$. We may therefore replace equation 13 by the approximation

$$\frac{d^2\psi_m}{dx^2} \approx 0 \tag{13 a}$$

This is equivalent to the postulate (Goldman, 1943; Schlögl, 1966) that the electric field in the membrane is constant:

$$\frac{d\psi_m}{dx} = \text{constant} = \frac{U_m}{d} \tag{14}$$

The voltage drop U_m in the membrane (fig. 1) is related to φ_i by eqn. 5:

$$U_m = U + 2(RT/F)\varphi_i \tag{15}$$

After introduction of relation 14, the equations 11 and 12 can be easily integrated. The solution satisfying the boundary conditions

$$c_+^m(d/2) = \gamma_+ c_+(d/2) = \gamma_+ c \cdot e^{-\varphi_i} \tag{16}$$

$$c_-^m(d/2) = \gamma_- c_-(d/2) = \gamma_- c \cdot e^{\varphi_i} \tag{17}$$

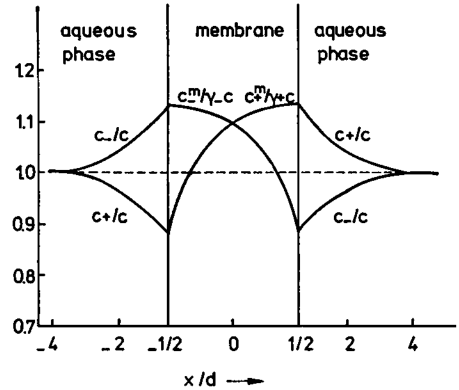

FIGURE 2 Concentration profiles at a voltage $U = 100$ mv. $\epsilon_m = 2$, $\epsilon = 78.5$, $d = 70$ A, $1/\kappa = 96$ A ($c_0 = 10^{-3}$ M). Dashed line: concentrations at equilibrium ($U = 0$). The length scale is dilated between $x = -d/2$ and $x = d/2$.

is given by

$$\frac{c^m_+(x)}{\gamma_+ c} = p - q \cdot e^{-u_m x/d} \tag{18}$$

$$\frac{c^m_-(x)}{\gamma_- c} = p - q \cdot e^{u_m x/d} \tag{19}$$

$$\Phi_+ = -\gamma_+ c D^m_+ p u_m/d \tag{20}$$

$$\Phi_- = \gamma_- c D^m_- p u_m/d \tag{21}$$

with

$$p \equiv \frac{\sinh(u/2)}{\sinh(u_m/2)}; \qquad q \equiv \frac{\sinh\left(\dfrac{u - u_m}{2}\right)}{\sinh(u_m/2)}$$

$$u \equiv \frac{U}{RT/F} \quad ; \quad u_m \equiv \frac{U_m}{RT/F}$$

From the boundary condition for the electric field:

$$\epsilon \left(\frac{d\psi}{dx}\right)_{x=d/2} = \epsilon_m \left(\frac{d\psi_m}{dx}\right)_{x=d/2} \tag{22}$$

we obtain the following relation between u and u_m :

$$\frac{\epsilon_m}{2\epsilon\kappa d} u_m = \sinh \frac{u - u_m}{4} \tag{23}$$

The concentration profiles of the permeable ions A^+ and B^- are drawn in Fig. 2

according to equations 18, 19, and 23 (the concentrations $c_+ = c \cdot e^{-\varphi(x)}$ and $c_- = c \cdot e^{-\varphi(x)}$ in the aqueous phases have been calculated using equation 10). By this representation the ion injection phenomenon is clearly shown. Under the influence of the external voltage U the mean ion concentration in the membrane is substantially increased compared with the equilibrium level.

The current density

$$J = -\lambda U = F(\Phi_+ - \Phi_-)$$

is given by the following expression:

$$J = -\frac{cF}{d}(\gamma_+ D_+^m + \gamma_- D_-^m) u_m \frac{\sinh(u/2)}{\sinh(u_m/2)} \tag{24}$$

If the voltage u is small ($|u| \ll 1$), equation 24 reduces to

$$(J)_{U \to 0} = -\lambda_0 U = \frac{cF^2}{dRT}(\gamma_+ D_+^m + \gamma_- D_-^m) U \tag{24 a}$$

Thus, for small values of U, the current-voltage curve of the membrane becomes linear and may be characterised by an ohmic conductance λ_0. The conductance function, $\lambda(u)/\lambda_0$, of the membrane is then obtained in the form

$$\frac{\lambda(u)}{\lambda_0} = \frac{u_m}{u} \frac{\sinh(u/2)}{\sinh(u_m/2)} \tag{25}$$

with

$$\lambda_0 = \frac{cF^2}{dRT}(\gamma_+ D_+^m + \gamma_- D_-^m) \tag{26}$$

DISCUSSION

The final result is contained in the equations 23, 25, and 26 and may be interpreted as follows: The ratio λ/λ_0 which describes the degree of nonlinearity is determined by the two dielectric constants ϵ and ϵ_m, the membrane thickness d, and the Debye-Hückel length $1/\kappa$, but is independent of the ionic mobilities and the partition coefficients. The fact that λ/λ_0 depends on the Debye-Hückel length leads to an interesting conclusion. As κ is a function of the total ion concentration c_0, the shape of the conductance function λ/λ_0 should depend on the concentration of the impermeable electrolyte if the concentration of the permeable ions is held constant. This is illustrated by Fig. 3 in which $\lambda(u)/\lambda_0$ is drawn for a fixed concentration, c, of permeable ions, but different values of the total concentration c_0. At high ionic strength the membrane conductance is almost independent of the voltage U, but increases markedly with U at low electrolyte concentrations.

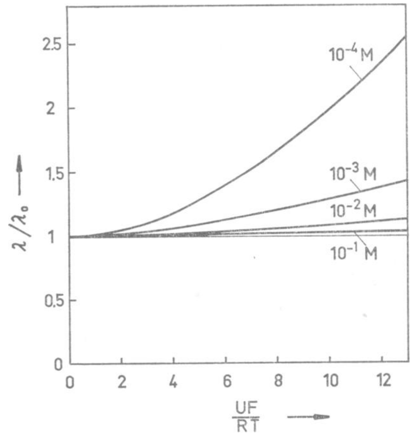

FIGURE 3 Membrane conductivity λ as a function of the voltage U, calculated from equation 25 with $\epsilon_m = 2$, $\epsilon = 78.5$, $d = 70$ A. ($RT/F = 25.6$ mv at 25° C) λ_0 = conductivity in the limit $U \approx 0$.

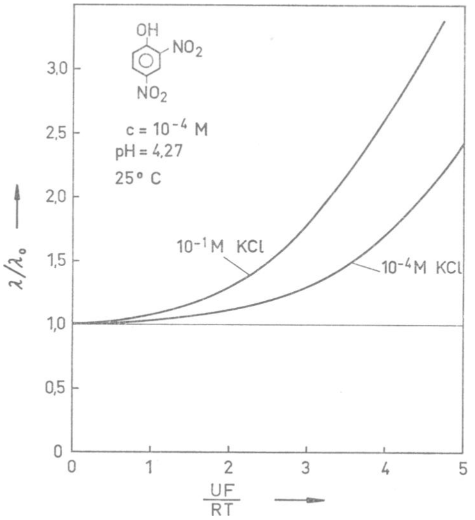

FIGURE 4 Measured conductance function of a dioleoyl lecithin membrane in aqueous solutions of 2,4-dinitrophenol + KCl (25° C). At pH = 4.27 about 60% of the 2,4-dinitrophenol is dissociated.

It is seen from Fig. 3 that the ion injection effect should be observable only at ionic strengths below about 10^{-3} M. In experiments with lipid bilayer membranes, however, a strongly nonlinear current-voltage characteristic was found at much higher electrolyte concentrations. An example is given in Fig. 4 in which the measured conductance function $\lambda(u)/\lambda_0$ of a dioleoyl lecithin membrane in aqueous solutions of KCl + 2,4-dinitrophenol is plotted (the experimental procedure was similar to that described by Läuger et al., 1967 *b*; experimental details will be published elsewhere). The permeable ions here are H^+ and the dinitrophenolate ion (Hopfer et al., 1968; Liberman et al., 1968), whereas the contribution of K^+ and Cl^- to the conductivity is relatively small. A comparison between Figs. 3 and 4 shows that the rise of the experimental λ/λ_0-curve at low ionic strength with increasing voltage U is steeper than predicted by the theory. Moreover, the nonlinearity does not vanish at high ionic strength, but becomes even more pronounced. We therefore conclude that the contribution of the ion injection to the current-voltage characteristic of the membrane is small compared with the two other mechanisms, the distortion of the potential energy barrier, and the Wien effect. These effects will be treated in the two subsequent papers.

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