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THE RATE CONSTANTS OF VALINOMYCIN-MEDIATED ION TRANSPORT THROUGH THIN LIPID MEMBRANES

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ABSTRACT Electrical relaxation experiments have been performed with phosphatidylinositol bilayer membranes in the presence of the ion carrier valinomycin. After a sudden change of the voltage a relaxation of the membrane current with a time constant of about 20 μsec is observed. Together with previous stationary conductance data, the relaxation amplitude and the relaxation time are used to evaluate the rate constants of valinomycin-mediated potassium transport across the lipid membrane. It is found that the rate constants of translocation of the free carrier S and the carrier-ion complex MS^+ are nearly equal ($2 \cdot 10^4$ sec^{-1}) and are of the same order as the dissociation rate constant of MS^+ in the membrane-solution interface ($5 \cdot 10^4$ sec^{-1}). The equilibrium constant of the heterogeneous association reaction M^+ (solution) + S (membrane) $\rightarrow$ MS^+ (membrane) is found to be $\sim$ 1 M^{-1}, about 10^6 times smaller than the association constant in ethanolic solution.

INTRODUCTION

The classical concept of carrier-mediated ion transport is based on the idea that a membrane-bound carrier molecule combines with an ion at one membrane surface, then moves to the other surface and releases the ion into the aqueous solution. Electrochemical experiments with lipid bilayer membranes in the presence of monactin or valinomycin carried out in different laboratories have led to the conclusion that these molecules behave like carriers in the above sense (Lev and Buzhinsky, 1967; Mueller and Rudin, 1967; Andreoli et al., 1967; Liberman and Topaly, 1968; Szabo et al., 1969; Shemyakin et al., 1969; Stark and Benz, 1971). A formal kinetic analysis of the mechanism of carrier-mediated ion transport has been worked out (Markin et al., 1969; Läuger and Stark, 1970); this mechanism is depicted in Fig. 1. It has been shown previously (Stark and Benz, 1971) that the proposed theory adequately describes the steady-state conductance behavior of lipid membranes in the presence of monactin or valinomycin.

Important questions related to the carrier transport of ions still need to be

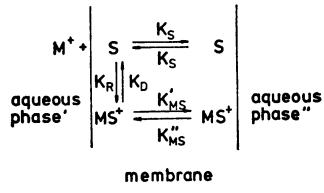

FIGURE 1 Mechanism of carrier-mediated ion transport through the bilayer membrane. The transport takes place in three steps: (*a*) recombination of ion M^+ and neutral carrier S at the left-hand interface ('), (*b*) translocation of the complex to interface ("), and (*c*) dissociation of the complex and release of the ion into the solution.

answered, however. Do the single transport reactions (complex formation, translocation, dissociation) take place at comparable rates or is there a rate-limiting step? Is the ion specificity of the carrier determined by equilibrium properties (stability constant) of the complex alone or also by kinetic parameters (rate constants)? For a detailed understanding of these questions the single parameters of the model have to be determined experimentally. At first sight, this seems to be a rather difficult task, because not only do the four rate constants k_R, k_D, k_S, and k_{MS} (Fig. 1) have to be evaluated, but also the concentrations of free carrier and complex in the bilayer membrane. From steady-state conductance measurements only certain combinations of the constants can be obtained; however, as we will show in this paper, the full set of parameters can be determined if the electrical relaxation of the membrane current after a voltage jump is measured in addition to the stationary conductance. Because of experimental limitations, such an analysis could be carried out until now only in the case of a phosphatidylinositol membrane in the presence of valinomycin and K^+. With further experimental refinements, however, we hope to perform similar measurements also with other systems.

THEORY

Steady-State Membrane Conductance

In this section we summarize some theoretical results which will be used later for the evaluation of experimental data; for details the reader should refer to the earlier papers (Läuger and Stark, 1970; Stark and Benz, 1971). We describe the heterogeneous reaction taking place in the membrane-solution interface between an ion M^+ from the aqueous phase (*a*) and a carrier molecule from the membrane (*m*) by rate constants k_R and k_D:

$$M^+(a) + S(m) \underset{k_D}{\overset{k_R}{\rightleftarrows}} MS^+(m). \qquad (1)$$

For zero current this reaction is in equilibrium:

$$K_h = \frac{k_E}{k_D} = \frac{N_{MS}}{c_M N_S}. \tag{2}$$

K_h is the heterogeneous equilibrium constant, c_M the concentration of M^+ in the aqueous solution, and N_S and N_{MS} are the interfacial concentrations (moles per square centimeter) of S and MS^+ at equilibrium. If the concentrations of S and MS^+ in the aqueous phase are denoted by c_S and c_{MS}, the equilibrium constant in the solution is given by

$$K = \frac{c_{MS}}{c_M c_S}. \tag{3}$$

We define dimensionless partition coefficients γ_S and γ_{MS} by

$$\gamma_S = \frac{2}{d}\frac{N_S}{c_S}, \tag{4}$$

$$\gamma_{MS} = \frac{2}{d}\frac{N_{MS}}{c_{MS}}, \tag{5}$$

(d = membrane thickness) so that N_S may be expressed by

$$N_S = \frac{d}{2}\frac{c_0\gamma_S}{1 + c_M K}, \tag{6}$$

$$c_0 = c_S + c_{MS}. \tag{7}$$

c_0 is the total concentration of carrier in the aqueous phase. It has been shown (Stark and Benz, 1971) that $K \ll 1\ M^{-1}$ for valinomycin-K^+; this means that N_S is independent of ion concentration c_M up to the highest experimental values of c_M. The net fluxes Φ_S and Φ_{MS} (moles per square centimeter $\times$ seconds), of S and MS^+ through the membrane are equal to

$$\Phi_S = k_S(N_S' - N_S''), \tag{8}$$

$$\Phi_{MS} = k_{MS}'N_{MS}' - k_{MS}''N_{MS}'', \tag{9}$$

where N_S', N_S'', N_{MS}', and N_{MS}'' are the concentrations of S and MS^+ at the left-hand and right-hand interfaces, respectively (Fig. 1). The electrical current density I is then simply given by (F = Faraday constant):

$$I = F\Phi_{MS}. \tag{10}$$

The rate constant k_S for the translocation of the neutral carrier S is the same in both directions. In the case of the charged complex MS^+, however, the rate con-

stants for jumps from left to right (k'_{MS}) and from right to left (k''_{MS}) are different in the presence of an external voltage U:

$$k'_{MS} = k_{MS}e^{u/2}, \tag{11}$$

$$k''_{MS} = k_{MS}e^{-u/2}, \tag{12}$$

$$u \equiv \frac{U}{RT/F} = \frac{\psi' - \psi''}{RT/F}. \tag{13}$$

(k_{MS} = rate constant for zero voltage, R = gas constant, T = absolute temperature, ψ', ψ'' = electrical potential in the left-hand and right-hand aqueous phases, respectively.) For the representation of steady-state conductance measurements we introduce the membrane conductivity

$$\lambda \equiv \frac{I}{U}, \tag{14}$$

as well as the ohmic limit of the membrane conductivity

$$\lambda_o \equiv \left(\frac{I}{U}\right)_{U\approx 0}, \tag{15}$$

which is reached for $| U | \ll RT/F \simeq 26$ mv. For negligible exchange of S and MS^+ across the interface, the following relations hold (Stark and Benz, 1971):

$$\frac{\lambda}{\lambda_o} = \frac{2}{u}(1 + A)\frac{\sinh(u/2)}{1 + A\cosh(u/2)}, \tag{16}$$

$$A \equiv \frac{2k_{MS}}{k_D} + \frac{c_M k_R}{k_D}\frac{k_{MS}}{k_S}, \tag{17}$$

$$\lambda_o = \frac{F^2}{RT} N_S k_{MS} \frac{c_M k_R/k_D}{1 + A}, \tag{18}$$

$$\approx \frac{F^2 d}{2RT} c_0 \gamma_S k_{MS} \frac{c_M k_R/k_D}{1 + A}, \qquad (c_M K \ll 1). \tag{19}$$

The last two relations are obtained from equations 7 and 14 of the paper of Stark and Benz combined with equation 6 from this paper. According to equation 16, the quantity A may be directly obtained from the current-voltage characteristic of the membrane. A plotted as a function of c_M then gives $2k_{MS}/k_D$ and $k_R k_{MS}/k_D k_S$. Furthermore, the quantity $\gamma_S k_{MS} k_R/k_D$ may be calculated from the experimental value of λ_o (equation 19).

Thus, only three independent combinations of the five constants γ_S, k_S, k_{MS}, k_R, and k_D may be obtained from the steady-state conductance. A complete analysis, however, is possible if the electrical relaxation of the membrane is measured in addition.

Relaxation of the Membrane Current

We assume that at times $t < 0$ the voltage across the membrane is zero so that the membrane is in equilibrium with the aqueous solutions. The interfacial concentrations of S and MS^+ are then given by $N'_S = N''_S = N_S$, $N'_{MS} = N''_{MS} = N_{MS}$. At time $t = 0$ a constant voltage U is applied across the membrane; as a consequence, the interfacial concentrations shift to new stationary values $\bar{N}'_S$, $\bar{N}''_S$, $\bar{N}'_{MS}$, and $\bar{N}''_{MS}$. The rate of change is given by the sum of the chemical production (equation 1) and the fluxes across the membrane (equations 8 and 9):

$$\frac{dN'_S}{dt} = -k_R c_M N'_S + k_D N'_{MS} - k_S N'_S + k_S N''_S , \tag{20}$$

$$\frac{dN''_S}{dt} = -k_R c_M N''_S + k_D N''_{MS} - k_S N''_S + k_S N'_S , \tag{21}$$

$$\frac{dN'_{MS}}{dt} = k_R c_M N'_S - k_D N'_{MS} - k'_{MS} N'_{MS} + k''_{MS} N''_{MS} , \tag{22}$$

$$\frac{dN''_{MS}}{dt} = k_R c_M N''_S - k_D N''_{MS} - k''_{MS} N''_{MS} + k'_{MS} N'_{MS} \tag{23}$$

Implicit in these equations is the assumption that the exchange of S and MS^+ across the membrane-solution interface is slow compared with the other transport processes. As has been shown previously (Stark and Benz, 1971), this assumption is supported by the results of stationary conductance measurements in the presence of valinomycin and K^+. It is seen from equations 20–23 that

$$\frac{dN'_S}{dt} + \frac{dN''_S}{dt} + \frac{dN'_{MS}}{dt} + \frac{dN''_{MS}}{dt} = 0 . \tag{24}$$

This means that the total number of carrier molecules per unit area of the membrane,

$$N_0 = N'_S + N''_S + N'_{MS} + N''_{MS} , \tag{25}$$

is independent of time. (It can be shown that this is still true in the more general case where the exchange of S and MS^+ across the interface can no longer be neglected.) If equation 25 is introduced into equations 20–23, a set of three inhomogeneous linear differential equations for N'_S, N''_S, N'_{MS} is obtained (N''_{MS} is determined by equation 25):

$$\frac{dN'_S}{dt} = -(k_R c_M + k_S) N'_S + k_S N''_S + k_D N'_{MS} , \tag{26}$$

$$\frac{dN''_S}{dt} = (k_S - k_D) N'_S - (k_R c_M + k_D + k_S) N''_S - k_D N'_{MS} + k_D N_0 , \tag{27}$$

$$\frac{dN'_{MS}}{dt} = (k_R c_M - k''_{MS})N'_S - k''_{MS}N''_S - (k_D + k'_{MS} + k''_{MS})N'_{MS} + k''_{MS}N_0 , \quad (28)$$

with the condition that for $t = 0$:

$$N'_S = N''_S = N_S = \frac{N_0}{2}\frac{k_D}{k_R c_M + k_D}, \quad (29)$$

$$N'_{MS} = N''_{MS} = N_{MS} = \frac{N_0}{2}\frac{k_R c_M}{k_R c_M + k_D}. \quad (30)$$

(compare equations 2 and 25). The solution of equations 25–28 may be represented by the solution of the corresponding homogeneous system (equations 26–28 without the constant terms $k_D N_0$ and $k''_{MS}N_0$) plus a particular solution of the inhomogeneous system. The solution of the homogeneous systems reads

$$N_i = \sum_{k=1}^{3} A_i^k e^{-t/\tau_k}, \qquad i = S', S'', MS', MS''. \quad (31)$$

The constants $-1/\tau_k$ are the roots of the characteristic polynomial of the homogeneous system. A particular solution of the inhomogeneous system is given by the steady-state concentrations $\bar{N}_i$ which are obtained by inserting $dN_i/dt = 0$ $(t \to \infty)$ into equations 26–28. The complete solution therefore reads

$$N_i = \sum_{k=1}^{3} A_i^k e^{-t/\tau_k} + \bar{N}_i . \quad (32)$$

The twelve coefficients A_i^k are evaluated in the following way. First, the solutions N_i (equation 31) are introduced into the homogeneous system. This gives the A_i^k apart from factors h_k. The constants h_k are then obtained from equation 32 for $t = 0$ using the boundary conditions 29 and 30.

As MS^+ is practically the only charge carrier, the electrical current density is given by

$$I = F(k'_{MS} N'_{MS} - k''_{MS} N''_{MS}). \quad (33)$$

From equation 32 we see that $I(t)$ has the form

$$I = I_\infty(1 + \sum_{k=1}^{3} \alpha_k e^{-t/\tau_k}), \quad (34)$$

where $I_\infty = F(k'_{MS} \bar{N}'_{MS} - k''_{MS} \bar{N}''_{MS})$ is the stationary current in the limit $t \to \infty$. After a lengthy calculation the following expressions for τ_k, α_k, and I_∞ are obtained:

$$\tau_1 = (a - b)^{-1}, \quad (35)$$

$$\tau_2 = (a + b)^{-1}, \tag{36}$$

$$\tau_3 = (c_M k_R + k_D)^{-1}, \tag{37}$$

$$a \equiv \frac{1}{2}\left(c_M k_R + k_D + 2k_S + 2k_{MS}\cosh\frac{u}{2}\right), \tag{38}$$

$$b \equiv \frac{1}{2}\left[\left(c_M k_R - k_D + 2k_S - 2k_{MS}\cosh\frac{u}{2}\right)^2 + 4c_M k_R k_D\right]^{1/2}, \tag{39}$$

$$\alpha_1 = \frac{A}{2}\cosh\frac{u}{2} + B, \tag{40}$$

$$\alpha_2 = \frac{A}{2}\cosh\frac{u}{2} - B, \tag{41}$$

$$\alpha_3 = 0, \tag{42}$$

$$B \equiv \frac{\cosh(u/2)}{4b}\left[A\left(c_M k_R + k_D + 2k_S - 2k_{MS}\cosh\frac{u}{2}\right) - 4k_{MS}\right], \tag{43}$$

$$I_\infty = 2FN_S\frac{c_M k_R}{k_D}k_{MS}\frac{\sinh(u/2)}{1 + A\cosh(u/2)}.$$

The quantity A is given by equation 17. As the third relaxation amplitude α_3 vanishes, the current $I(t)$ is determined by the time constants τ_1 and τ_2 alone:

$$I(t) = I_\infty(1 + \alpha_1 e^{-t/\tau_1} + \alpha_2 e^{-t/\tau_2}). \tag{44}$$

Because of limitations in the time resolution, in many cases only the larger relaxation time τ_1 can be detected experimentally. If, however, relaxation process 2 can be resolved, too, a simple procedure may be used for the evaluation of the rate constant k_{MS}. For this purpose the tangent to the experimental $I(t)$ curve is drawn in point $t = 0$. The intercept t^* of the tangent with t-axis is then given by the relation

$$-\left(\frac{dI}{dt}\right)_{t=0} = \frac{I(0)}{t^*}. \tag{45}$$

$I(0)$ and $(dI/dt)_{t=0}$ are obtained from equations 22, 23, 29, 30, and 33. The result is

$$t^* = \frac{1}{2k_{MS}\cosh(u/2)}. \tag{46}$$

Thus, k_{MS} may be directly obtained from the intercept t^*.

EXPERIMENTAL METHODS

Phosphatidylinositol was purchased from Supelco Inc., Bellafonte, Pa. Dioleoyllecithin was synthesized according to the method of Robles and van den Berg (1969). The purity of both lipids was checked by thin-layer chromatography. Valinomycin was obtained from Calbiochem (Los Angeles, Calif.).

Bilayer membranes were formed from a 0.5% (w/v) lipid solution in *n*-decane on a Teflon diaphragm with a circular hole of 1.2 mm diameter (Läuger et al., 1967). The measuring cell was thermostated at 25°C. In order to maintain a constant surface potential of the membrane, the ionic strength of the aqueous solution was held constant at 1 M in all experiments. This was done by addition of LiCl which has a negligible influence on the membrane conductivity in the presence of valinomycin (McLaughlin et al., 1970).

The experimental arrangement for the electrical relaxation experiments was the same as described previously (Ketterer et al., 1971); however, in order to increase the time resolution of the system, the membrane area was reduced to 1.2 mm^2 so that the membrane capacitance was $C_m \simeq 5$ nF. With a cell resistance (electrodes plus solutions) of $R_S \simeq 100\,\Omega$ and an external measuring resistance $R_e = 100\,\Omega$, the charging time of the circuit was $\tau_c \approx C_m(R_e + R_S) \simeq 1$ μsec.

RESULTS

With a phosphatidylinositol membrane in the presence of 1 M K^+ and 10^{-7} M valinomycin in the external phases, a relaxation of the membrane current I with a time constant of 23 μsec is observed (Fig. 2); after the exponential decay the current remains constant up to at least 10 sec. No such relaxation is found if the membrane is made from the neutral lipid dioleoyllecithin; i.e., in this case only the capacitative current decaying with $\tau_c \simeq 1$ μsec is observed after the voltage jump. If log $(I - I_\infty)$ is plotted as a function of time t (I_∞ is the stationary current in the limit $t \to \infty$) a straight line is obtained for $t > \tau_c$ within the limits of error, indicating that the relaxation process is governed by a single time constant τ in the time range $t > 5$ μsec. (It will be shown below that the other relaxation process has a smaller time constant and also a smaller amplitude and is therefore not resolved.)

As predicted by equations 38 and 39, the relaxation time τ depends on both the ion concentration c_M and the voltage u. Experimental values of $\tau(c_M)$ and $\tau(u)$ are shown in Figs. 3 and 4.

The experimental relaxation amplitude α is defined as the ratio

$$\alpha \equiv \frac{I_0 - I_\infty}{I_\infty}, \qquad (47)$$

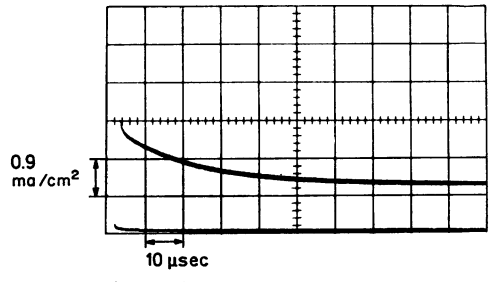

FIGURE 2 Relaxation of the electrical current for a phosphatidylinositol membrane in the presence of 10^{-7} M valinomycin and 1 M KCl. At $t = 0$ a voltage jump of $U = 38$ mv is applied to the membrane. After the initial charging of the membrane capacitance, the current decays with a time constant of about 20 μsec toward a stationary value $I_\infty \simeq 1.2$ ma/cm². I_∞ remains constant up to at least $t = 10$ sec.

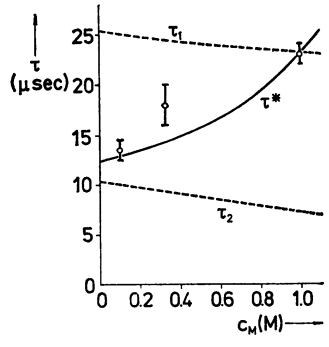

FIGURE 3

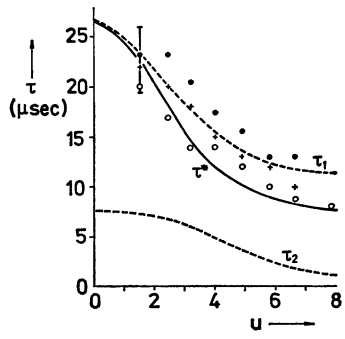

FIGURE 4

FIGURE 3 Relaxation time τ as a function of potassium concentration c_M (10^{-7} M valinomycin, U = 38 mv). The ionic strength has been held constant at 1 M by addition of appropriate amounts of LiCl. The experimental points are the mean of 3–12 membranes and are given together with the standard error. For the meaning of τ_1, τ_2, and τ^*, see text.

FIGURE 4 Relaxation time τ as a function of reduced voltage $u \equiv UF/RT = U/25.6$ mv (10^{-7} M valinomycin, 1 M KCl). ● ○ +, experimental values from three different membranes. A larger number (12) of measurements have been made at u = 1.5; the range of the observed values is indicated by ├──┤.

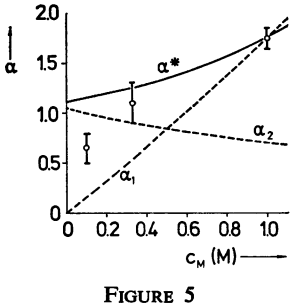

FIGURE 5

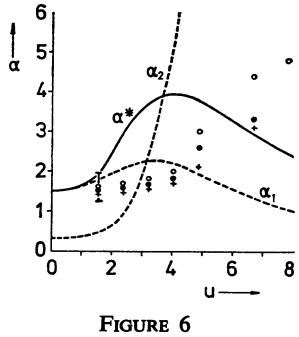

FIGURE 6

FIGURE 5 Experimental relaxation amplitude $\alpha \equiv (I_o - I_\infty)/I_\infty$ as a function of potassium concentration c_M (10^{-7} M valinomycin, U = 38 mv). The ionic strength has been held constant at 1 M by addition of LiCl. The experimental points represent mean values from 4 to 12 membranes. The meaning of α_1, α_2, and α^* is explained in the text.

FIGURE 6 Experimental relaxation amplitude $\alpha \equiv (I_o - I_\infty)/I_\infty$ as a function of reduced voltage u (10^{-7} M valinomycin, 1 M KCl). ● ○ +, experimental values from three different membranes. A larger number (12) of measurements have been made at u = 1.5; the range of the observed values is indicated by ├──┤. The evalvation of the rate constants was performed using $\alpha = \alpha_1 = 1.7$ at u = 1.5.

where $I_0 - I_\infty$ is obtained by extrapolation of the linear part of log $(I - I_\infty)$ to $t = 0$. α is plotted in Figs. 5 and 6 as a function of potassium concentration and of voltage. As may be seen from equations 35–41, the relaxation time τ as well as the amplitude α should be independent of the carrier concentration c_0 in the aqueous phase. Therefore, a few experiments have been carried out with $c_0 = 10^{-8}$ M (in-

stead of 10^{-7} M). Indeed it has been found that both τ and α were insensitive to a change in c_0.

EVALUATION OF THE RATE CONSTANTS

As described previously (Stark and Benz, 1971) the following combinations of rate constants can be obtained from steady-state conductance measurements with a phosphatidylinositol membrane in the presence of valinomycin and K^+:

$$\gamma_{MS} k_{MS} K = \gamma_S k_{MS} k_R / k_D \simeq 1.2 \cdot 10^9 \text{ M}^{-1} \text{ sec}^{-1}, \quad (48)$$

$$\frac{k_{MS} k_R}{k_S k_D} \simeq 1.0 \text{ M}^{-1}, \quad (49)$$

$$\frac{k_{MS}}{k_D} \simeq 0.4. \quad (50)$$

k_{MS}/k_D has been determined from the voltage dependence of the membrane conductance (λ/λ_o, equation 16) at $c_M \leq 10^{-2}$ M; at these concentrations the term $c_M k_R k_{MS}/k_D k_S$ in A is negligibly small, so that $A \approx 2\, k_{MS}/k_D$. On the other hand, from a measurement of λ/λ_o at $c_M = 1$ M, $k_R k_{MS}/k_D k_S \simeq 1.0$ M^{-1} is obtained.[1] The quantity $\gamma_S k_{MS} k_R/k_D$ has been calculated from the linear part of $\lambda_o(c_M)$ using equation 19.

As described above, the observed time course of the current after the voltage jump may be described by a single time constant τ. For the interpretation of this result there exist three different possibilities:

(*a*) τ is equal to the longer relaxation time $\tau_1 = 1/(a - b)$; correspondingly, the measured amplitude α has to be identified with α_1. This implies that τ_2 or α_2 (or both) are so small that the second relaxation process cannot be detected.

(*b*) τ is equal to the shorter relaxation time $\tau_2 = 1/(a + b)$; relaxation process 1 is not resolved because $|\alpha_1| \ll \alpha_2 = \alpha$.

(*c*) $\tau_1 \approx \tau_2$, so that $\alpha \approx \alpha_1 + \alpha_2$.

With the experimental values of τ and α at $c_M = 1$ M all three cases have been analyzed separately. It is found that only case *a* leads to results consistent with the experiments. (In case *b* negative values of the rate constants are obtained, whereas case *c* would lead to the wrong sign of the concentration dependence of τ.) Thus,

$$\tau_1 = 23 \cdot 10^{-6} \text{ sec}, \quad (51)$$

[1] A higher value ($k_R k_{MS}/k_D k_S \simeq 7$ M^{-1}) has been calculated from the nonlinearity of $\lambda_o(c_M)$ at large c_M (Stark and Benz, 1971). This value, however, is not consistent with the results of the relaxation experiments and is not used here. The origin of the discrepancy is not clear; possibly it arises from a change in the surface charge of the membrane at high c_M due to the formation of MS^+ in the interface.

$$\alpha_1 = 1.7, \tag{52}$$

(values at $c_M = 1$ M and $U = 38$ mv). Together with equations 48–50, relations 51 and 52 are sufficient to evaluate the five constants k_R, k_D, k_{MS}, k_S, and γ_S. The results are

$$k_R = 5 \cdot 10^4 \text{ M}^{-1} \text{ sec}^{-1},$$

$$k_D = 5 \cdot 10^4 \text{ sec}^{-1},$$

$$k_S = 2 \cdot 10^4 \text{ sec}^{-1},$$

$$k_{MS} = 2 \cdot 10^4 \text{ sec}^{-1},$$

$$\gamma_S = 6 \cdot 10^4.$$

Furthermore, with $\gamma_{MS} k_{MS} K \simeq 1.2 \cdot 10^9$ M^{-1} sec^{-1} (equation 48) and $K < 0.1$ M^{-1} (Stark and Benz, 1971) the inequality

$$\gamma_{MS} > 6 \cdot 10^5$$

is obtained.

The partition coefficient γ_S of valinomycin between the black film and the aqueous phase may be compared with the corresponding macroscopic partition coefficient Γ_S. Γ_S is defined as the equilibrium concentration ratio of valinomycin in the bulk lipid solution and water. With the same method as described previously (Stark and Benz, 1971), $\Gamma_S \simeq 1.5 \cdot 10^4$ is found for phosphatidylinositol in *n*-decane.

For a check of the consistency of the model the above values of the rate constants have been used to calculate τ and α at various concentrations and voltages. For this purpose first the separate values of τ_1, τ_2, α_1, and α_2 have been evaluated according to equations 35–41.[2] The results are shown in Figs. 3 and 5. It is seen that at $c_M = 1$ M relaxation process 2 has a shorter time constant and also a smaller amplitude than process 1, so that the time course of the current is determined only by process 1 within the experimental accuracy; however, this is no longer true at lower concentrations c_M where the amplitudes and time constants are such that $I(t)$ becomes a mixture of both relaxation processes. As a consequence, mean amplitudes and mean time constants are observed in the experiment. Therefore, the exact shape of $I(t)$ has been calculated according to equation 44 using τ_1, τ_2, α_1, and α_2 from Figs. 3 and 5. If $\log [I(t) - I_\infty]$ is plotted as a function of t, the resulting curve is nearly a straight line for $t > 5$ μsec (the experimental limit of resolution). From the intercept of this straight line with the ordinate, the mean relaxation amplitude α^* is

[2] For the calculation of τ_1, τ_2, α_1, and α_2, the exact numerical values of the rate constants ($k_R = 4.88 \cdot 10^4$ M^{-1} sec^{-1}, $k_D = 4.55 \cdot 10^4$ sec^{-1}, $k_{MS} = 1.82 \cdot 10^4$ sec^{-1}, $k_S = 1.95 \cdot 10^4$ sec^{-1}) as obtained from the measured relaxation time and amplitude at $c_M = 1$ M have been used instead of the rounded values given in the text.

obtained; likewise the slope of the line gives the mean relaxation time τ^*. These theoretical values of τ^* and α^* are also given in Figs. 3 and 5. It is seen that there is at least qualitative agreement with the measured values. By the same procedure values of τ_1, τ_2, τ^*, α_1, α_2, and α^* have been calculated as functions of voltage u at a fixed ion concentration $c_M = 1$ M (Figs. 4 and 6).

Fig. 4 shows that the calculated relaxation time τ^* approximately describes the experimental results. In the case of the amplitude α the theoretical curve (α^*) deviates from the measured values at larger voltages. The reason for this discrepancy is not known; however, there is evidence also from the current-voltage characteristic of the membrane (Stark and Benz, 1971) that additional effects occur at large voltages which are not included in the model.

DISCUSSION

From the numerical values of the rate constants a number of interesting conclusions may be drawn. First, it is seen that k_D, k_S, and k_{MS} are of comparable magnitude. Especially at a potassium ion concentration in the vicinity of 1 M ($c_M k_R = 5 \cdot 10^4$ sec^{-1}) all transport steps (association and dissociation of MS^+ in the interface, translocation of S and MS^+ across the membrane) take place at approximately the same rate ($\sim 10^4$ sec^{-1}). At lower c_M the association step is rate limiting.

The finding that k_S and k_{MS} are nearly equal seems at first surprising in view of the fact that the charged complex MS^+ has to surmount a high dielectric activation energy barrier in the center of the membrane (Neumcke and Läuger, 1970); however, the result $k_S \simeq k_{MS}$ may be understood on the basis of the mechanism proposed by Shemyakin et al. (1969), who assume that the highly surface-active valinomycin molecule is adsorbed to the membrane-solution interface with the polar carbonyl groups in contact with the aqueous solution and the apolar parts of the molecule pointing toward the membrane interior. A jump of the uncomplexed carrier S across the membrane therefore involves the desorption from the interface and requires a relatively high activation energy. On the other hand, if S forms a complex with M^+, the surface activity of the molecule is lost because of a conformational change which turns the carbonyl groups to the interior of the complex; this means that MS^+ is readily desorbed from the interface. Thus, from $k_S \simeq k_{MS}$ we may conclude that the activation energy for the desorption of S and for the jump of MS^+ over the dielectric barrier are of similar magnitude.

The rate of formation of MS^+ in the interface is described by the rate constant k_R. It is important to note that k_R depends on the surface potential ψ of the membrane, which in turn is a function of the surface charge and the ionic strength of the aqueous solution. This is a consequence of the definition of k_R. The number of complexes which are formed per square centimeter and second is given by $\nu_R = k_R c_M N_S$. c_M is the alkali ion concentration in the bulk aqueous phase and is related to the ion concentration $\tilde{c}_M$ at the membrane surface by

$$\tilde{c}_M = c_M e^{-\psi F/RT}. \tag{53}$$

For the negatively charged phosphatidylinositol membrane ($\psi < 0$) $\tilde{c}_M$ is larger than c_M. The formation rate of MS^+ may then be described by a "true" association rate constant k_R^0 which is, to a first approximation, independent of voltage:

$$\nu_R = k_R^0 \tilde{c}_M N_S , \tag{54}$$

$$k_R = k_R^0 e^{-\psi F/RT}. \tag{55}$$

It has been found previously (Stark and Benz, 1971) that the factor $\exp(-\psi F/RT)$ is about 20 for a phosphatidylinositol membrane at ionic strength 1 M, corresponding to 1 elementary charge per 60 A^2. From this we estimate a value of $3 \cdot 10^3$ M^{-1} sec^{-1} for k_R^0. (In view of the drastic simplifications introduced in the calculation of ψ, this value of k_R^0 should be considered as a rough approximation.) With the macrocyclic ion carrier monactin a much higher recombination rate constant ($k_R \simeq 3 \cdot 10^8$ M^{-1} sec^{-1}) has been found in methanol (Diebler et al., 1969). As kinetic data on the valinomycin-K^+ system in methanol have not yet been published, it is not clear whether the drastic difference between the k_R values merely reflects the difference in the reaction rates of monactin and valinomycin, or whether the complexation rate of the carrier molecule bound to the membrane-solution interface is so much lower than in methanol.[3]

As the dissociation rate constant k_D may be considered as independent of ψ to a first approximation, the "true" equilibrium constant of the valinomycin-K^+ complex in the interface is given by $K_h^0 = N_{MS}/N_S c_M = k_R^0/k_D \simeq 0.1$ M^{-1}. This value is much lower than the equilibrium constant for valinomycin-K^+ in ethanol ($K \simeq 2 \cdot 10^6$ M^{-1}) reported by Shemyakin et al. (1969). From measurements with ethanol-water mixtures, Shemyakin et al. have shown that K strongly decreases with increasing polarity of the solvent (the value extrapolated to pure water would be of the order of 1 M^{-1}). Therefore it is possible that the low value of K_h^0 comes from two effects: the polar environment of the carrier molecule in the interface, and the fact that the adsorbed carrier is forced into a conformation which is less favorable for complexation than the conformation in ethanolic solution.

As mentioned above, no relaxation of the current could be detected with a phosphatidylcholine membrane in the presence of valinomycin and K^+. Probably the relaxation amplitude is too small in this case. From equations 40 and 41 one obtains for the sum of both relaxation amplitudes $\alpha_1 + \alpha_2 = A \cosh(u/2)$. Equations 17 and 55 (see also Stark and Benz, 1971) show that A depends on the surface charge of the membrane and is smaller for a neutral compared with a negatively charged membrane.

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[3] An association rate constant $k_R = 5 \cdot 10^7$ M^{-1} sec^{-1} for the system valinomycin-K^+ in methanol has been reported recently by Funck et at. (1971). (Note added in proof).

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