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KINETICS OF CARRIER-MEDIATED ION TRANSPORT ACROSS LIPID BILAYER MEMBRANES

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SUMMARY

A theoretical treatment of the electrical conductance and tracer permeability of lipid bilayer membranes in the presence of macrocyclic ion carriers is given. The analysis is based on the assumption that the complex between the ion and the carrier is formed in the membrane–solution interface. The translocation of the complex across the membrane is described as a transport over an activation energy barrier. It is shown that some information on the rate constants may be obtained from the existing conductance measurements. For instance, the current–voltage characteristic of lecithin membranes in the presence of monactin indicates that the rate-determining step in the ion transport is the translocation of the complex across the interior of the membrane.

INTRODUCTION

A number of macrocyclic antibiotics, such as valinomycin, enniatin B and monactin, are able to increase the cation permeability of artificial lipid membranes by many orders of magnitude[1–6]. Common to these macrocyclic compounds is the structural peculiarity that the interior of the ring is hydrophilic whereas the exterior is hydrophobic due to the presence of apolar side chains. Within a lipid membrane such a molecule offers to an ion a polar environment similar to the normal environment of the ion in aqueous solution. An alkali ion which is extremely insoluble in the unmodified membrane may cross the membrane in the form of a complex with the lipid-soluble macrocyclic compound. Complex formation presumably occurs at the membrane–solution interphase where the inner hydration shell of the ion is replaced by the carbonyl oxygens of the ring. This description of carrier-mediated ion transport is supported by recent X-ray analyses of the alkali ion complexes of nonactin[7], enniatin B[8] and valinomycin[9]. With other macrocyclic compounds like nystatin[10] or alamethicin[11] more than one molecule seems to be involved in the ion transport. These cases in which pore-like structures are possibly formed are excluded in the following treatment.

However, even in the simple case of the classical carrier mechanism several questions remain unanswered. The most important problem is the nature of the rate-determining step. The passage of the ion across the membrane involves three distinct steps: (i) formation of the ion–carrier complex in the membrane surface,

(ii) translocation of the complex to the opposite surface and (iii) release of the ion into the solution. Depending on the given system, the overall rate of ion transport may be determined either by the rate of complex formation (and dissociation) or by the rate of translocation across the membrane. DIEBLER *et al.*[12] studied the kinetics of the reaction between Na^+ and monactin in methanol with relaxation methods and found that the rate of formation of the complex is extremely fast ($k_R \simeq 3\ 10^8$ $M^{-1} \cdot sec^{-1}$). It is not known, however, whether such a high reaction rate is also possible in the membrane–solution interface where the carrier molecule may be constrained to a conformation unfavorable for complex formation.

In most studies of carrier-mediated ion transport across lipid membranes the electrical conductivity of the membrane has been measured. From these experiments some information can be obtained on the rate constants of the single transport steps. This will be shown in the present paper which gives a theoretical treatment of the membrane conductivity in the presence of ion carriers. In addition, the tracer permeability coefficient is calculated. The treatment is based on the mathematical analysis of a model in which the translocation of the ion–carrier complex across the membrane is described as a migration over an activation energy barrier.

An analysis of the electrical properties of bilayer membranes containing ion carriers has also been given by CIANI *et al.*[13], but with the assumption that the equilibrium between free carrier and complex in the interface is not disturbed by the electric current. The influence of a finite association–dissociation rate on the carrier-mediated transport of nonelectrolytes has been analyzed by BLUMENTHAL AND KATCHALSKY[14]. Recently, MARKIN and co-workers [15–17] examined the carrier transport of ions on the basis of an electrodiffusion model, which is an alternative to our model but leads to similar results.

GENERAL DESCRIPTION OF THE SYSTEM

We consider a bilayer membrane which is in contact on both sides with aqueous solutions of an univalent cation M^+ of concentration c_M (see Fig. 1). In the aqueous phase the neutral carrier S and the ion M^+ may form a complex MS^+ with an association constant

$$K = \frac{c_{MS}}{c_M c_S} \tag{1}$$

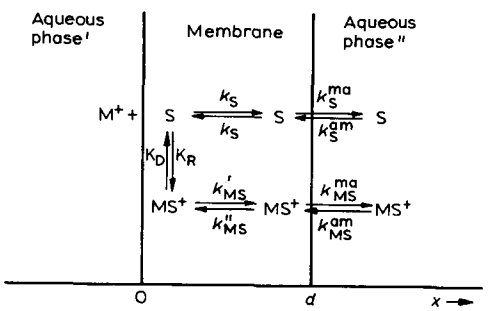

Fig. 1. Transport of the cation M^+ mediated by a neutral carrier.

(c_S, c_{MS} = concentrations of S and MS^+ in the aqueous phase). As the electrical conductivity of lipid bilayer membranes is extremely low in the absence of carriers, we may assume that the uncomplexed ion M^+ is practically excluded from the membrane. However, both S and MS^+ may be exchanged between aqueous phase (a) and membrane (m) according to

$$S\,(a) \underset{k_S^{ma}}{\overset{k_S^{am}}{\rightleftharpoons}} S\,(m) \tag{2}$$

$$MS^+\,(a) \underset{k_{MS}^{ma}}{\overset{k_{MS}^{am}}{\rightleftharpoons}} MS^+\,(m) \tag{3}$$

Besides this, a chemical reaction may take place at the interface between an ion M^+ from the solution and a carrier S in the membrane. This heterogeneous recombination–dissociation reaction is described by rate constants k_R and k_D:

$$M^+\,(a) + S\,(m) \underset{k_D}{\overset{k_R}{\rightleftarrows}} MS^+\,(m) \tag{4}$$

When a voltage is applied across the membrane, the charge transport through the interface may proceed in principle both by reaction (3) and by reaction (4). There are some hints, however, that of these two parallel ways only reaction (4) is important. First, the association constant, K, is very small, at least in the case of valinomycin[18]. This means that the concentration of MS^+ in the aqueous solution is extremely low. Second, the concentration of the carrier S is much higher in the membrane than in the water phase; for instance, the distribution coefficient of monactin between lipid and water was determined by SZABO *et al.*[6] to be about $5 \cdot 10^3$. We therefore assume that the rate of the chemical reaction (4) is high compared with the rate of the exchange reactions (2) and (3). For greater generality, however, we will first derive the general relations including reactions (2) and (3) and will later specialize the result for the case of vanishing exchange.

FORMAL ANALYSIS OF THE MODEL

Specific for the model is the assumption that the potential energy of MS^+ has a minimum inside the membrane near the interface and that MS^+ may jump in a single step from one interphase to the other over a symmetrical energy barrier. The same assumption is made for S. If we denote the interfacial concentrations of S and MS^+ at the left-hand and right-hand interface by N_S', N_S'', N_{MS}', N_{MS}'', respectively, then the fluxes of S and MS^+ across the membrane are given by

$$\Phi_S = k_S(N_S' - N_S'') \tag{5}$$

$$\Phi_{MS} = k_{MS}' N_{MS}' - k_{MS}'' N_{MS}'' \tag{6}$$

As the only charge carrier in the membrane is the complex, the current density J is directly related to Φ_{MS}:

$$J = F\Phi_{MS} \tag{7}$$

(F = Faraday constant). For the neutral carrier S the rate constants for a jump from left to right and from right to left are the same. For the charged carrier, however, the rate constants k_{MS}' and k_{MS}'' depend on the external voltage U. For $U = 0$:

$$k_{MS}' = k_{MS}'' = Ae^{-E/RT} \equiv k_{MS} \tag{8}$$

E is the energy (per mole) for zero voltage at the top of the barrier (R = gas constant, T = absolute temperature). For $U \neq 0$ the barrier height is changed by the electrostatic energy of MS^+. If we assume that the electric potential $\psi(x)$ in the center of the membrane ($x = d/2$) is equal to $U/2$, the barrier height becomes $E + FU/2$ for the transport from left to right and $E - FU/2$ for the transport from right to left. Therefore

$$k_{MS}' = k_{MS}e^{-u/2} \quad \left(u = \frac{U}{RT/F}\right) \tag{9}$$

$$k_{MS}'' = k_{MS}e^{u/2} \tag{10}$$

$\psi(d/2) = U/2$ is valid under the assumption of a constant field strength in the membrane. It has previously been shown[19] that this assumption which neglects space charge effects is a good approximation for a lipid bilayer membrane under most experimental conditions.

For each particle at each interface the sum of the net chemical production and of the fluxes toward the interface must vanish in the stationary state:

$$\frac{dN_S'}{dt} = -k_R c_M N_S' + k_D N_{MS}' - \Phi_S + k_S^{am} c_S - k_S^{ma} N_S' = 0 \tag{11}$$

$$\frac{dN_S''}{dt} = -k_R c_M N_S'' + k_D N_{MS}'' + \Phi_S + k_S^{am} c_S - k_S^{ma} N_S'' = 0 \tag{12}$$

$$\frac{dN_{MS}'}{dt} = k_R c_M N_S' - k_D N_{MS}' - \Phi_{MS} + k_{MS}^{am} c_{MS} - k_{MS}^{ma} N_{MS}' = 0 \tag{13}$$

$$\frac{dN_{MS}''}{dt} = k_R c_M N_S'' - k_D N_{MS}'' + \Phi_{MS} + k_{MS}^{ma} c_{MS} - k_{MS}^{am} N_{MS}'' = 0 \tag{14}$$

For $U = 0$ the chemical reaction and both exchange reactions are at equilibrium separately. In this case the relations $N_S' = N_S'' = N_S$ and $N_{MS}' = N_{MS}'' = N_{MS}$ hold. We may characterize the equilibrium state of the system by the partition coefficients γ_S and γ_{MS} of the free carrier and the complex, respectively:

$$\gamma_S \equiv \frac{2}{d}\frac{k_S^{am}}{k_S^{ma}} = \frac{2N_S/d}{c_S} \tag{15}$$

$$\gamma_{MS} \equiv \frac{2}{d}\frac{k_{MS}^{am}}{k_{MS}^{ma}} = \frac{2N_{MS}/d}{c_{MS}} \tag{16}$$

If we further denote the equilibrium constant of the heterogeneous reaction (4) by K_h, we obtain

$$K_h = \frac{k_R}{k_D} = \frac{N_{MS}}{c_M N_S} = \frac{\gamma_{MS}}{\gamma_S} K \tag{17}$$

The six unknown quantities Φ_S, Φ_{MS}, N_S', N_S'', N_{MS}', N_{MS}'' are determined by the six equations 5, 6, 11–14. Solution of this system and introduction of Φ_{SM} into Eqn. 7 gives the result

$$J = -Fc_0\gamma_{MS}k_{MS}d\frac{c_MK}{c_MK+1}\cdot\frac{[s(c_M+q)-c_M]\sinh(u/2)}{(c_M+q)\left[s+\frac{2k_{MS}}{k_D}\cosh(u/2)\right]-c_M} \tag{18}$$

$$q = \frac{2k_S+k_S^{ma}}{k_R}$$

$$s = 1+\frac{k_{MS}^{ma}}{k_D}$$

$c_0 = c_S + c_{MS}$ is the total concentration of the carrier in the aqueous solution. If the exchange of S and MS^+ across the interface is slow ($k_S^{ma} \approx k_{MS}^{ma} = 0$) we get

$$J = -Fc_0\gamma_{MS}d\frac{c_MK}{c_MK+1}\cdot\frac{\sinh(u/2)}{\frac{1}{k_{MS}}+\left(\frac{c_MK_h}{k_S}+\frac{2}{k_D}\right)\cosh(u/2)} \tag{19}$$

DISCUSSION OF THE CURRENT–VOLTAGE CHARACTERISTIC

(a) *Ohmic conductance*

The membrane conductance in the limit of small voltages is defined by

$$\lambda_0 = -\left(\frac{J}{U}\right)_{U\approx 0} \tag{20}$$

Introducing the approximations $\sinh(u/2) \approx u/2$, $\cosh(u/2) \approx 1$, $\coth(u/2) \cong 2/u$ which are valid for $|u| \ll 1$ into Eqn. (19) we obtain

$$\lambda_0 = a\frac{c_MK}{(c_MK+1)(c_MK+b)} \tag{21}$$

$$a = \frac{F^2c_0\gamma_Sk_Sd}{2RT}$$

$$b = \frac{\gamma_Sk_S}{\gamma_{MS}k_{MS}}\left(1+\frac{2k_{MS}}{k_D}\right)$$

It is interesting to note that Eqn. 21 has the same general form as the conductance equation derived by MARKIN *et al.*[15] on the basis of the electrodiffusion model. The only difference between the two equations consists in the meaning of the concentration independent parameters a and b. It is easily seen from Eqn. 21 that the conductivity becomes zero in the limit of low and high ion concentration c_M. The maximum of λ_0 is reached at

$$c_{M,max} = \sqrt{b}/K \tag{22}$$

and is equal to

$$\lambda_{0,max} = \frac{a}{(1+\sqrt{b})^2} \tag{23}$$

The conductivity maximum occurs because at high c_M the number of free carrier molecules in the membrane is low, so that the back transport of free carrier is blocked. It may be expected, however, that in many cases $c_{M,max}$ is outside the experimentally accessible concentration range.

(b) Limiting current

If the voltage becomes large compared with $RT/F \simeq 25$ mV ($u \gg 1$), the approximations $\sinh(u/2) \gg 1$, $\cosh(u/2) \gg 1$ may be introduced into Eqn. 19. In this case the current becomes independent of voltage. This limiting current is given by

$$J^* = -Fc_0\gamma_S k_S d \frac{c_M K}{(c_M K + 1)(c_M K + 2\gamma_S k_S/\gamma_{MS} k_D)} \tag{24}$$

so that Eqn. (19) may be written as

$$J = J^* \frac{(c_M K + 2\gamma_S k_S/\gamma_{MS} k_D) \sinh(u/2)}{(\gamma_S k_S/\gamma_{MS} k_{MS}) + (c_M K + 2\gamma_S k_S/\gamma_{MS} k_D) \cosh(u/2)} \tag{25}$$

Whether a limiting current occurs in the experimentally accessible range of u depends on the relative magnitude of the two terms in the denominator of Eqn. 25. When no limiting current is observed for, *e.g.* $u \leqslant 10$ ($U \leqslant 250$ mV), we may infer that

$$\gamma_S k_S/\gamma_{MS} k_{MS} \gg c_M K + 2\gamma_S k_S/\gamma_{MS} k_D \tag{26}$$

and, *a forteriori*,

$$\gamma_S k_S/\gamma_{MS} k_{MS} \gg 2\gamma_S k_S/\gamma_{MS} k_D \text{ or } k_D \gg k_{MS} \tag{27}$$

From the absence of a limiting current we may therefore conclude that the slowest step in the ion transport is the migration of the complex across the interior of the membrane.

In the same way, we may deduce from Eqn. 26 the inequality

$$\gamma_S k_S/\gamma_{MS} k_{MS} \gg c_M K$$

which is equivalent to

$$c_M K_h = \frac{N_{MS}}{N_S} \ll \frac{k_S}{k_{MS}} \tag{28}$$

As k_S/k_{MS} is of the order of unity in most cases, this inequality signifies that the carrier is far from saturation.

TRACER PERMEABILITY COEFFICIENT

To calculate the tracer permeability coefficient, we assume that part of the cations M^+ in the left-hand solution are replaced by tracer ions $\overset{*}{M}{}^+$. Φ_{MS} is then the total flux of the complex (MS^+ *plus* $\overset{*}{M}S^+$), and N_{MS}', N_{MS}'', c_M the corresponding total concentrations; the quantities Φ_{MS}^*, $N_{MS}^{*\prime}$, $N_{MS}^{*\prime\prime}$, c_M^*, c_{MS}^* refer to the labeled components. We further assume that the rate constants of labeled and unlabeled components are equal. Thus, for zero voltage, the following relations hold:

$$k_{MS}' = k_{MS}'' = k_{MS}$$
$$\Phi_S = \Phi_{MS} = 0$$
$$N_S' = N_S'' = N_S$$
$$N_{MS}' = N_{MS}'' = N_{MS}$$

In the stationary state, the surface concentrations of MS^+ are constant. Therefore,

$$\frac{dN_{MS}^{*\prime}}{dt} = k_R c_M^* N_S - k_D N_{MS}^{*\prime} - \Phi_{MS}^* + k_{MS}^{ma}\left(\frac{d}{2}\gamma_{MS} c_{MS}^* - N_{MS}^{*\prime}\right) = 0 \tag{29}$$

$$\frac{dN_{MS}^{*\prime\prime}}{dt} = -k_D N_{MS}^{*\prime\prime} + \Phi_{MS}^* - k_{MS}^{ma} N_{MS}^{*\prime\prime} = 0 \tag{30}$$

$$\Phi_{MS}^* = k_{MS}(N_{MS}^{*\prime} - N_{MS}^{*\prime\prime}) \tag{31}$$

(compare Eqns. 6, 13 en 14). The tracer permeability coefficient P is defined by

$$\Phi_{MS}^* = P c_M^* \tag{32}$$

Elimination of Φ_{MS}^*, $N_{MS}^{*\prime}$, $N_{MS}^{*\prime\prime}$ from Eqns. 29–31 and introduction of Φ_{MS}^* into Eqn. 32 gives the following relation for the tracer permeability coefficient:

$$P = \frac{d}{2}\frac{c_0 K}{1 + c_M K}\gamma_{MS} k_{MS}\frac{k_D + k_{MS}^{ma}}{k_D + 2k_{MS} + k_{MS}^{ma}} \tag{33}$$

For negligible exchange of MS^+ across the interface ($k_{MS}^{ma} \approx 0$), this equation becomes

$$P = \frac{d}{2}\frac{c_0 K}{1 + c_M K}\left(\frac{\gamma_{MS} k_{MS}}{1 + 2k_{MS}/k_D}\right) \tag{34}$$

By comparison with Eqn. 21 we may derive the following relation between the tracer permeability coefficient P and the ohmic conductivity λ_0:

$$P = \frac{RT}{F^2}\frac{\lambda_0}{c_M}\left(1 - \frac{k_{MS}}{k_S}\frac{c_M k_R}{k_D + 2k_{MS}}\right) \tag{35}$$

Thus, a measurement of P in addition to λ_0 gives further information on the rate constants of the system.

ESTIMATION OF NUMERICAL VALUES

The diffusion time of a molecule with diffusion coefficient D across the membrane is equal to $d^2/2D$. Thus, an upper limit for the rate constants $k_S \simeq 2\,D_S/d^2$ and $k_{MS} \simeq 2\,D_{MS}/d^2$ may be estimated if the membrane is considered as a fluid phase of viscosity η in which the migration of S and MS^+ may be described by diffusion coefficients D_S and D_{MS} respectively. Then D_{MS} may be calculated from the Einstein–Stokes relation

$$D_{MS} \simeq \frac{kT}{6\pi\eta r_{MS}} \tag{36}$$

($\mathbf{k}$ = Boltzmann constant, r_{MS} = hydrodynamic radius of the complex). If we regard the viscosity of triolein at 20° as representative for the membrane ($\eta \simeq 1.2$ erg·cm^{-3}·sec) and use $r_{MS} = 7$ Å, we obtain $D_{MS} \simeq 3 \cdot 10^{-8}$ cm^2·sec^{-1}, or (with $d = 70$ Å) $k_{MS} \simeq 1 \cdot 10^5$ sec^{-1}. As the hydrodynamic radii of the complex and of the free carrier should not be very different, we may regard

$$k_S \simeq k_{MS} \simeq 1 \cdot 10^5 \text{ sec}^{-1}$$

as an upper limit also for the free carrier. The real values of k_S and k_{MS} may be much lower due to the liquid crystalline nature of the membrane.

COMPARISON WITH EXPERIMENTS

SZABO *et al.*[6] studied the electrochemical properties of lipid bilayer membranes in the presence of monactin. The current–voltage characteristic of the membrane in a solution of $2.2 \cdot 10^{-7}$ M monactin and $1 \cdot 10^{-2}$ M CsCl shows no limiting current up to at least 200 mV. The same is true for solutions of KCl, LiCl, NaCl and RbCl instead of CsCl (R. BENZ AND G. STARK, unpublished). From the foregoing, we may therefore conclude for the system monactin–M^+: (a) that the rate-limiting step is the migration of the complex across the membrane ($k_D \gg k_{MS}$, Eqn. 27) and (b) that the complex formation in the membrane is small up to at least $c_M = 1 \cdot 10^{-2}$ M ($c_M K_h \ll 1$, Eqn. 28).

In addition, SZABO *et al.*[6] found that the ohmic conductivity is a linear function of the ion concentration in the range $1 \cdot 10^{-4}$ M $\leqslant c_M \leqslant 1 \cdot 10^{-1}$ M, if they kept the total ionic strength constant. With this experimental finding it follows from Eqn. 21 that

(i) $c_M K \ll 1$

(ii) $c_M K \ll b = \dfrac{\gamma_S k_S}{\gamma_{MS} k_{MS}} \left(1 + \dfrac{2k_{MS}}{k_D}\right)$

(i) means that complex formation in the aqueous phase is negligible up to $c_M = 1 \cdot 10^{-1}$ M. With $k_S \simeq k_{MS}$ and $k_D \gg k_{MS}$ (see above), (ii) reduces to $c_M K_h \ll 1$, a result which has already been deduced from the current–voltage characteristic. In the range $1 \cdot 10^{-4}$ M $\leqslant c_M \leqslant 1 \cdot 10^{-1}$ M the ohmic conductivity is then given by

$$\lambda_0 \approx \frac{a}{b} c_M K \approx \frac{F^2 c_0 c_M k_S d}{2RT} \gamma_{MS} K \tag{37}$$

This result is in agreement with the observation by SZABO *et al.*[6] and EISENMAN *et al.*[20] that for two different alkali ions i and j the ratio of the ohmic conductivities is approximately equal to the ratio of the "bulk extraction constants" (for fixed c_0 and c_M), *i.e.* that

$$\frac{\lambda_0^i}{\lambda_0^j} \approx \frac{\gamma_{MS}^i K_i}{\gamma_{MS}^j K_j}$$

The approximation (37) corresponds to the relation for the membrane conductance used by CIANI *et al.* (Eqn. 59 of ref. 13), if the mobility u^* of ref. 13 is replaced by $k_S d^2/2RT \simeq D_S/RT$.

CONCLUSION

The preceding analysis shows that some information on the kinetics of carrier-mediated ion transport may be obtained from stationary conductance experiments. For instance, the nature of the rate-determining step may be elucidated from the shape of the current–voltage characteristic under favorable conditions. However, measurements of the membrane conductance and of the tracer permeability are not sufficient to determine the values of the individual rate constants k_R, k_D, k_{MS}, k_S. For a more detailed insight into the kinetics of carrier transport relaxation methods are necessary.

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