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A THEORY FOR THE EFFECTS OF DIFFUSION-REACTION COUPLING ON THE CARRIER-MEDIATED IONIC PERMEABILITY IN LIPID BILAYERS

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The zero-current membrane potential and the current-voltage relations are discussed theoretically for the case in which ionic transport is mediated by carriers that form complexes with ions in the aqueous phase ('solution complexation' mechanism). Interest for this topic originated partly from the finding that gradients of the neutral cyclic peptide PV, cyclo (DVal-LPro-LVal-DPro)$_3$, commonly thought to act as a carrier via 'solution complexation', generate Nernstian potentials across lipid bilayers separating solutions of identical ion composition. It is shown that the general expression for the potential in a gradient of carriers reduces to the Nernst equation under any of the following conditions: slow aqueous reaction; impermeability of the membrane to the neutral carriers; high concentration of the complexing ions in solution; finite permeability of the membrane to the neutral carrier, but faster rate of movement from the membrane surface into the torus than across the middle or out of the membrane. In symmetrical solutions, the conductance is most typically characterized by a quantity that we designate by $\delta^\star$, which has the dimensions of a length and is generally a complex function of ion activity. Comparing the thory with previous data on dioleoylphosphatidylcholine membranes in the presence of PV and K$^+$, the order of magnitude of the rates of the aqueous reaction and of the membrane permeability to the neutral carriers is tentatively estimated.

Introduction

The mechanism of carrier-mediated transport of ions across lipid bilayers requires that the carriers associate with ions to form membrane-soluble complexes. For the simple case of one-to-one complexation, it is generally postulated that the reaction occurs either at the interfaces (between ions in the aqueous phases and carrier and complexes in the membrane), or in the aqueous solution, with subsequent transfer of both carriers and complexes into the membrane. Using a nomenclature introduced by Benz et al. [1], the two cases will be referred to as 'interfacial complexation' and 'solution complexation', respectively. The former has been discussed extensively in the literature [2–5], since there is evidence that it applies to valinomycin and to the macrotetrolide actins, at least for certain membrane lipids and for the most effectively complexed cations [6,7]. By contrast, 'solution complexation', which is probably applicable to a different class of carriers, notably the cyclic peptice PV, cyclo(DVal-LPro-LVal-DPro)$_3$ [1,8] and some negatively charged uncouplers [9–11], has been analyzed less thoroughly. Of the existing theories, some neglect the effects of the unstirred layers altogether [3,5], while others, either assume equilibrium of the aqueous reactions at the outset [4], or do allow for the kinetics of such reactions, but restrict the analysis to the ohmic portion of the conductance near zero current [7,10,11]. This paper extends those theories to derive explicit expressions for the zero-current membrane potential in gradients of carriers and for the current-voltage relations in symmetrical solutions. In addition, by comparing the results of the theory with the existing data for PV and K$^+$ [1], and assuming that 'solution complexation' is indeed applicable to this system, an attempt is made to estimate the order of magnitude of important parameters, such as the rate constants of the aqueous reaction and the overall permeability of the membrane to the neutral carriers.

Description of the model and assumptions

All the kinetic steps are illustrated in Fig. 1, the main point being that formation and dissociation of the complexes occur in the aqueous phases via the homogeneous reaction

$$i + S \underset{k_d^a}{\overset{k_r^a}{\rightleftharpoons}} iS \; ; \qquad \frac{k_r^a}{k_d^a} = K = \frac{C_{is}}{C_s a_i} \tag{1}$$

where C_{is} and C_s are the concentrations of the complexes and of the carriers at equilibrium in the bulk solutions and a_i is the activity of the complexing ion. Partitioning of the carriers and of the complexes across the interfaces is described by

$$S^a \underset{k_s^{ma}}{\overset{k_s^{am}}{\rightleftharpoons}} S^m \; ; \qquad \frac{k_s^{am}}{k_s^{ma}} = \gamma_s \frac{d}{2} = \frac{N_s}{C_s} \tag{2}$$

and

$$iS^a \underset{k_{is}^{ma}}{\overset{k_{is}^{am}}{\rightleftharpoons}} iS^m \; ; \qquad \frac{k_{is}^{am}}{k_{is}^{ma}} = \gamma_{is} \frac{d}{2} = \frac{N_{is}}{C_{is}} \tag{3}$$

where γ_s and γ_{is} are dimensionless partition coefficients, d is the membrane thickness, and N_s and N_{is} are the interfacial densities of carriers and complexes at equilibrium. Since an exchange of neutral carriers between the membrane and the surrounding torus is also allowed for, we shall consider the additional process,

$$S^m \underset{k_s^{tm}}{\overset{k_s^{mt}}{\rightleftharpoons}} S^t \tag{4}$$

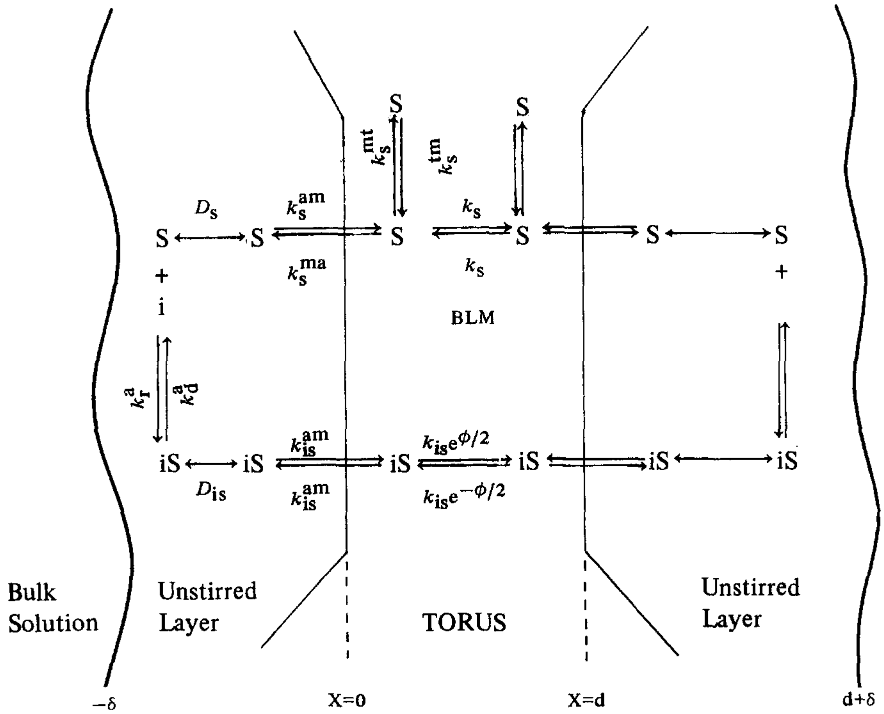

Fig. 1. Kinetic diagram for the mechanism of 'solution complexation'. The essential feature, which distinguishes it from the alternative mechanism of 'interfacial complexation', is that the current across the interfaces is carried by the complexes formed in solution.

The main assumptions and some of their consequences on the description of the overall transport across the membrane can be summarized as follows:

(1) Similarly to the early model by Läuger et al. [3], the membrane is assumed symmetrical to its center and is represented schematically by three Eyring barriers and two lateral wells.

(2) The two wells, though located within the membranes, are assumed to be sufficiently close to the boundaries that the potential drop across the lateral barriers is negligible compared to that across the central one *. Consequently, translocation of the complexes across the interfacial barriers will be characterized by the voltage-independent rate constants defined in Eqn. (3), k_{is}^{am} and k_{is}^{ma}, while translocation across the center will be described by

$$iS_1^m \underset{k_{is}e^{-z_{is}\phi/2}}{\overset{k_{is}e^{z_{is}\phi/2}}{\rightleftharpoons}} iS_2^m \tag{5}$$

The subscripts 1 and 2 denote the position of the two lateral wells and ϕ is the potential difference between them in units of RT/F ($\phi \equiv (V_1 - V_2)F/RT$). The corresponding transmembrane movement of the neutral carriers is described by

$$S_1 \underset{k_s}{\overset{k_s}{\rightleftharpoons}} S_2 \tag{6}$$

(3) Following standard conventions, the unstirred layers are represented as aqueous regions of thickness δ, at the outer boundary of which the concentrations of all species have their equilibrium values, as in the bulk solutions.

(4) The potential drop in the unstirred layers is assumed negligible, so that ϕ, in Eqn. 5, can be identified with the total potential difference between the two solutions.

As a consequence of assumption (4), all the fluxes in the unstirred layers will be described by diffusion equations with no electrical terms. Thus,

$$J_s = -D_s \frac{dC_s}{dx}; \qquad J_{is} = -D_{is} \frac{dC_{is}}{dx} \tag{7}$$

Due to Reaction 1, the stready-state fluxes (Eqn. 7) vary in the direction perpendicular to the membrane surface according to the equations

$$-\frac{dJ_s}{dx} = \frac{dJ_{is}}{dx} = k_r^a a_i C_s - k_d^a C_{is} \tag{8}$$

(5) The aqueous activity of the free ions, which in most practical cases is much higher than the concentrations of both carriers and complexes, will be assumed constant throughout the unstirred layers.

Theoretical results

For simplicity, the results presented in this section are restricted to the case in which the exchange with the torus is negligible ($k_s^{mt} = k_s^{tm} = 0$). Their extension to the case in which this limitation is relaxed is however easily accomplished, and some of the generalized equations that are useful for the analysis of the data are given in the Appendix.

A. Zero-current potential in a gradient of carriers

Let the membrane separate solutions with identical ion compositions, but different concentrations of carriers *. To shorten the expressions for the results, it is expedient to introduce the following auxiliary parameters:

$$\frac{1}{L} = \left[\frac{k_d^a}{D_{is}}\left(1 + \frac{D_{is}}{D_s} K a_i\right)\right]^{1/2} \tag{9}$$

$$P_s = k_s^{am} k_s / (k_s^{ma} + 2k_s) \tag{10}$$

$$N = \frac{(\delta/L)\coth(\delta/L) - 1}{1 + \dfrac{D_{is}}{D_s} K a_i} \tag{11}$$

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* Assumptions (1) and (2) are unnecessary from a mathematical standpoint, and are made here mainly to simplify some equations. However, a more realistic description of the transmembrane fluxes, such as that used by Hladky [12] or Ciani [13], could be easily incorporated in the present treatment, and would somehow affect the voltage-dependence of the current-voltage relationship, but would leave the results for the zero-current potential unchanged.

* The more complicated expressions for the case in which the concentrations of ions, or those of both carriers and ions, are different in the two solutions, are not given here, but could be derived easily using the procedure outlined in the Appendix.

and

$$e^{\phi_0^M} = 1 + \frac{1}{N}\left(2 + \frac{D_s}{LP_s}\coth(\delta/L)\right) \tag{12}$$

where δ in Eqns. 11 and 12 is the thickness of the unstirred layers. Following the procedure outlined in the Appendix, and recalling that $z_{is} = 1$, the zero-current potential in units of RT/F is given by

$$\phi_0 = \phi_0' - \phi_0'' = \ln \frac{e^{\phi_0^M} C_s^{T''} + C_s^{T'}}{e^{\phi_0^M} C_s^{T'} + C_s^{T''}} \tag{13}$$

where $C_s^{T'}$ and $C_s^{T''}$ denote the total concentrations of carriers on each side ($C_s^T = C_s + C_{is}$). ϕ_0^M, defined in Eqn. 12, represents the maximum absolute value of the potential obtainable with a gradient of carriers for a given ion activity. For example, Eqn. 13 shows that $|\phi_0|$ coincides with ϕ_0^M when the concentration of carriers is zero in one of the two bulk solutions (either $C_s^{T'} = 0$, or $C_s^{T''} = 0$). It is also clear that, the greater $\exp(\phi_0^M)$, the better Eqn. 13 is approximated by the Nernst equation

$$\phi_0 = \ln(C_s^{T''}/C_s^{T'}) \tag{14}$$

It is easy to identify at least three independent conditions that would give large values for $\exp(\phi_0^M)$ and would thus extend the range of the gradient of carriers for which Eqn. 13 can be approximated by Eqn. 14.

(a) Low values of the rate constant k_d^a, such that the condition, $\delta < L$, is satisfied in the whole experimental range of ion activities. In this case, $\coth[\delta/L] \simeq L/\delta$, $(\delta/L)\coth(\delta/L) - 1 \simeq \delta^2/3L^2$, and Eqn. 12 can be approximated by

$$e^{\phi_0^M} \simeq 1 + \frac{3D_{is}}{k_d^a\delta^2}\left[2 + \frac{D_s}{P_s\delta}\right] \tag{15}$$

Eqn. 15 shows clearly that, the smaller the rate constant k_d^a, the larger $\exp(\phi_0^M)$. Note from Eqn. 9 that the condition for slow aqueous reaction, $\delta < L$, requires that $k_d^a < D_{is}/\delta^2$,. Choosing reasonable values for D_{is} and δ, such as $D_{is} \simeq 2 \cdot 10^{-6}$ cm²/s and $\delta \simeq 0.02$ cm, the condition above gives, $k_d^a < 5 \cdot 10^{-3}\ \text{s}^{-1}$, which implies lifetimes for the complexes longer than 200 s.

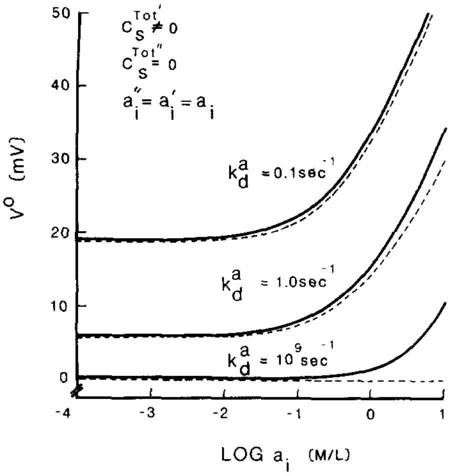

Fig. 2. Theoretical dependence on ion activity of the membrane potential in the case in which the neutral carrier is present in one bulk solution only, and the ion activities are identical on both sides. All the curves have been calculated for $K = 10\ \text{M}^{-1}$, $D_s = D_{is} = 5 \cdot 10^{-6}$ cm²/s and $\delta = 0.02$ cm. In each pair of curves, corresponding to the indicated value of k_d^a, the solid line refers to $P_s = 0.05$ cm/s, the dashed one to $P_s = 10^2$ cm/s.

(b) Negligible permeability (defined Eqn. 10) of the membrane to the neutral carriers. Eqn. 12 shows clearly that the smaller P_s, the larger $\exp(\phi_0^M)$.

(c) High activity of the complexing cations. It is easy to realize from Eqns. 9, 11 and 12 that $\exp(\phi_0^M)$ increases unlimitedly with a_i. This behavior is also illustrated in Fig, 2.

It will be shown later that an exchange of neutral carriers between the membrane and the torus may also give rise to high values for $\exp(\phi_0^M)$. This point is of some importance, since it bears on the conclusions that one can draw from a comparison of the theory with the data.

B. *Current-voltage in symmetrical solutions*

The current-voltage relation, derived according to the methods outlined in the Appendix, is given by

$$I = z_{is}Fk_{is}N_{is}^0 \frac{2\sinh(z_{is}\phi/2)}{1 + 2A\cosh(z_{is}\phi/2)} \tag{16}$$

where I is the current per unit area of membrane, F the Faraday constant, k_{is} the rate constant defined in

Eqn. 5, and N_{is}^0 the interfacial density of complexes at zero current, namely

$$N_{is}^0 = \frac{\gamma_{is} d}{2} \cdot \frac{K a_i C_s^T}{1 + K a_i} \tag{17}$$

The quantity, A, in the denominator of Eqn. 16 is equal to

$$A = \frac{k_{is}}{k_{is}^{ma}} + \frac{k_{is} \gamma_{is} d}{2} \cdot \frac{\delta^*}{D_{is}} \tag{18}$$

where

$$\delta^* = \delta \cdot \frac{1 + \dfrac{2P_s \delta}{D_s} + N \dfrac{D_{is}}{D_s} K a_i}{(\delta/L) \coth(\delta/L) + \dfrac{2P_s \delta}{D_s}(1 + N)}, \tag{19}$$

the parameters L, P_s and N being defined in Eqns. 9, 10 and 11, respectively. δ^* has the dimension of a length, is independent of the concentration of carriers, but depends on the ion activity and on several rate and equilibrium constants. Since δ^* is in general a complicated function of ion activity, we shall consider some of its properties in the two limits of 'slow' and 'fast' reactions.

Slow chemical reactions. As was already mentioned in discussing the potential, the aqueous reaction is considered slow when the condition $\delta < L$, is satisfied. In this case, $\delta/L \coth(\delta/L) \simeq 1$ and $N \simeq 0$, so that Eqn. 19 reduces to

$$\delta^* \simeq \delta \tag{20}$$

δ^* is thus a constant, equal to the actual thickness of the unstirred layers.

Fast chemical reactions. When the reaction is sufficiently fast that the opposite condition, $\delta > L$, is satisfied in the whole range of the ion activity, it can be shown from Eqn. 19 that δ^* is an increasing function of a_i, and varies between the two limits,

$$\lim_{a_i \to 0} \delta^* = [D_{is}/k_d^a]^{1/2} \qquad \text{and} \qquad \lim_{a_i \to \infty} \delta^* = \delta \tag{21}$$

It is interesting, and not immediately intuitive, that in the limit of high ion activity the value of δ^* becomes identical to that corresponding to slow reactions, namely to the actual thickness of the aqueous layer. Note also that, although the overall behavior of δ^* depends on the permeability, P_s, neither limit in Eqn. 21 does so.

The limiting case of 'equilibrium' of the aqueous reactions (e.g., see Ref. 4) corresponds to the assumption that the reaction rates are so large that not only the inequality, $\delta \gg L$, but also the following, $N K a_i D_{is}/D_s \gg 1 + 2P_s\delta/D_s$, is statisfied in Eqn. 19, even for the lowest experimental values of a_i. With the approximations implied by these two conditions, Eqn. 19 becomes

$$\delta^* = \delta \, \frac{D_{is} K a_i}{D_{is} K a_i + D_s + 2P_s \delta} \tag{22}$$

Substituting Eqn. 22 in Eqns. 18 and 16 yields an equation for the current-voltage relationship which is identical to that obtained when equilibrium of the aqueous reaction is assumed at the outset.

Maximum conductance near zero voltage. A clear illustration of the effects on the electrical properties of the processes in the aqueous layers is provided by the 'maximum' conductance, defined as the conductance in the case in which the membrane is assumed to be 'unlimitedly' permeable to both complexes and neutral carriers, namely: $k_{is} d \simeq k_{is}^{ma} d \simeq k_{is}^{am} \simeq P_s \to \infty$. If k_{is}^{ma}d and k_{is}^{am} are infinities of the same order, Eqns. 16, 17 and 18 give, at small voltages,

$$\lambda(0) = \lim_{V \to 0} \frac{I}{V} = \frac{z_{is}^2 F^2}{2RT} \cdot \frac{D_{is}}{\delta^*} \cdot \frac{K a_i C_s^T}{1 + K a_i} \tag{23}$$

where δ^*, recalling Eqn. 19 and the assumption, $P_s \to \infty$, simplifies into

$$\delta^* \simeq \delta/(1 + N) \tag{24}$$

For slow reactions ($\delta < L$, $N \simeq 0$, $\delta^* \simeq \delta$), Eqns. 23 and 24 give a simple result, which indicates that the whole resistance is due to the diffusional barrier provided by the aqueous layers, and that the conductance saturates when all the carriers in solution are complexed ($K a_i > 1$). However, if the reaction is fast ($\delta > L$), and if the reasonable assumption is made that $D_{is} \simeq D_s \equiv D$, Eqns. 9,11,24 and 23 yield the interesting expression

$$\lambda(0) = \frac{z_{is}^2 F^2}{2RT} [K k_r^a D]^{1/2} \frac{a_i C_s^T}{(1 + K a_i)^{3/2}} \times \left\{ 1 + \left(\frac{D}{k_d^a \delta^2} \right)^{1/2} \frac{K a_i}{(1 + K a_i)^{1/2}} \right\} \tag{25}$$

Note in Eqn. 25 that the last term within parentheses becomes significant (and $\lambda(0)$ saturates) only when $Ka_i > k_d^a \delta^2/D$. Since the assumed condition of fast reaction, $\delta > L$, implies only that $k_d^a \delta^2/D > 1$, it is possible that Ka_i, while capable of becoming greater than one, will nevertheless remain smaller than $k_d^a \delta^2/D$ in the whole experimental range of ion activities. If so, Eqn. 25 simplifies into the expression

$$\lambda(0) = \frac{z_{is}^2 F^2}{2RT} (Kk_r^a D)^{1/2} \frac{a_i C_s^T}{(1 + Ka_i)^{3/2}} \tag{26}$$

which is independent of the unstirred layer thickness and shows a maximum for $a_i = 2/K$. The physical reasons for this maximum are not immediately intuitive and are, at any rate, considerably different from those pertaining to the alternative mechanism of 'interfacial complexation'. For example, in the latter mechanism the occurrence of a maximum requires a finite permeability to the neutral carriers, while Eqns. 25 and 26 have a maximum also for infinitely high permeability ($P_s \to \infty$ is in fact one of the conditions under which Eqns. 25 and 26 were derived).

To illustrate the behavior of the conductance in the general case in which all the reaction rates and P_s have finite values, numerical calculations have been carried out, and few theoretical curves are plotted in Fig. 3.

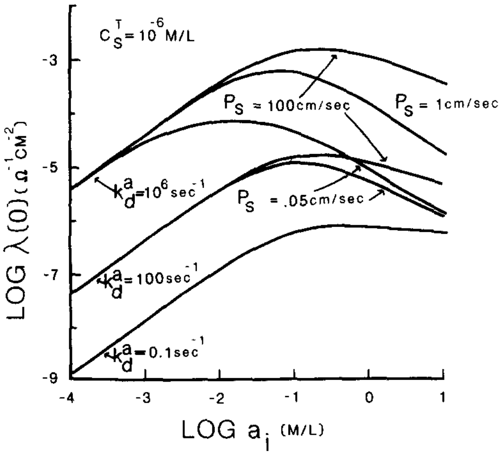

Fig. 3. Theoretical dependence on ion activity of the zero-voltage membrane conductance for different values of the parameters, k_d^a and P_s. All the curves were calculated for $K = 10$ M^{-1}, $D_s = D_{is} = 5 \cdot 10^{-6}$ cm^2/s and $\delta = 0.02$ cm.

C. Numerical estimates from a comparison of the present theory with previously obtained data on PV, K^+ and dioleoylphosphatidylcholine membranes

In accordance with the three-barrier-model for the membrane used in the present treatment, as well as with the assumption that the two lateral wells are located immediately close to the interfaces, the simplest interpretation of the initial current following a step of voltage is that it is due to transfer of the complexes across the central energy barrier [14]. Using Eyring's formalism to describe the flux of complexes across such barrier, the instantaneous conductance, after a voltage jump from 0 to ϕ_1, is given by

$$\lambda^0(\phi_1) = \frac{F^2}{RT} k_{is} N_{is}^0 \frac{\sinh(\phi_1/2)}{\phi_1/2} \tag{27}$$

where z_{is} has been placed equal to 1, and the superscript (0) is used to denote that λ^0 is the conductance measured at the time $t = 0$. Combining Eqn. 27 with the steady-state conductance, $\lambda(\phi_2)$, at the normalized potential, ϕ_2 (deducible from Eqn. 16 by simply dividing it by $V_2 = RT\phi_2/F$), one finds, after simple rearrangements,

$$A = \left\{ \frac{\phi_1 \lambda^0(\phi_1)}{\phi_2 \lambda(\phi_2)} \cdot \frac{\sinh(\phi_2/2)}{\sinh(\phi_1/2)} - 1 \right\} \Big/ 2\cosh(\phi_2/2) \tag{28}$$

This relation shows that A, defined in Eqn. 18, can be deduced directly from instantaneous and steady-state current-voltage data. Using the data by Benz et al. [1] for 10^{-6} M PV and five KCl concentrations (0.01, 0.03, 0.1, 0.3 and 1 M), and taking into account the fact that the two conductances, λ^0 and λ, were measured at different voltages, the values for A were calculated from Eqn. 28, and are listed in Table I. The fact that A increases with the KCl concentration suggests that the aqueous reaction cannot be slow. In fact, Eqns. 18 and 20 show unambiguously that in the domain of slow reactions A should be a constant, independent of the ion activity. Therefore, (although aware of the paucity of the steady-state data, but nevertheless considering that PV samples are presently unavailable for additional studies), we have tried to fit the existing data with the theory to estimate the rate constant, k_d^a, and the permability, P_s. Substituting the values of A in Eqn. 18, δ^*/D_{is} was calculated for each of the five KCl concentrations, and subsequently fitted with Eqn. 19. Note that, in order to calculated δ^*/D_{is} from A using Eqn. 18, the

TABLE I

Values of the parameters A and δ^*/D, deduced from Eqns. 28 and 18 using the data of Benz et al. [1] for dioleoylphosphatidylcholine membranes, PV and K^+. See section C of the Results for details. The concentrations are assumed equal to the activities. It is also assumed that $D_{is} = D_s \equiv D$.

a_i (M)	A (dimensionless)	$\frac{\delta^*}{D}$ (s/cm) × 10^{-2}	
		k_{is}^{ma} 7.5 s^{-1}	k_{is}^{ma} 75 s^{-1}
0.01	239	0.92	3.09
0.03	289	0.93	3.48
0.1	595	5.49	7.80
0.3	880	8.83	10.7
1	2042	19.8	21.8

TABLE II

Values of the constants, k_d^a, P_s and D, estimated by fitting Eqn. 30 to the data of Table I and postulating the reasonable value of 0.02 cm for the thickness of unstirred layers, δ. The numbers in the two rows correspond to two values for k_{is}^{ma}. The first, 7.5 s^{-1}, is the value determined from the experiments [1], although is referred to as tentative; the second has been chosen for reasons explained in the text (see section C of the Results).

	k_d^a (s^{-1})	P_s (cm/s) × 10^4	D (cm^2/s) × 10^6
$k_{is}^{ma} = 7.5\ s^{-1}$	2980	8.1	6.6
$k_{is}^{ma} = 75\ s^{-1}$	2.7	12.2	5

knowledge of the three parameters, k_{is}, $\gamma_{is}d/2$ and k_{is}^{ma}, is required. The numbers used for k_{is} are the mean values given in Table I of Ref. 1. $\gamma_{is}d/2$ was calculated from Eqn. 17, using the mean values for N_{is}^0 found in the same Table I of Ref. 1, $C_s^T = 10^{-6}$ M and $K \simeq 10\ M^{-1}$ (see Fig. 10 of Ref. 1). The ionic activities, a_i, were assumed identical to the concentrations. According to the estimate of Benz et al. [1], k_{is}^{ma} was taken equal to 7.5 s^{-1}. However, since the latter number is referred to as tentative, and should probably be regarded as a lower limit, we have also repeated the whole fitting procedure using a tenfold higher value for k_{is}^{ma}, the main purpose being to assess the sensitivity of the fitted parameters to variations of this poorly known constant. The two sets of values for δ^*/D_{is}, corresponding to these two choices for k_{is}^{ma}, are listed in Table II. From inspection of Eqn. 19, one can realize that, if the equilibrium constant of the aqueous reaction, K, and the ionic activities, a_i, are known, and if the reasonable assumption is made that $D_{is} = D_s \equiv D$, the quantity δ^*/D can be expressed as a function of the four independent quantities:

$$\Omega \equiv (1 + Ka_i)^{1/2}\ ; \qquad \delta/D\ ; \qquad \rho_1 \equiv \left(\frac{k_d^a\,\delta^2}{D}\right)^{1/2}\ ;$$

$$\rho_2 \equiv 2(P_s\,\delta/D) \tag{29}$$

Ω is known, while the remaining three must be determined. Eqn. 19 can thus be rewritten in the form,

$$\frac{\delta^*}{D} = \frac{\delta}{D} \cdot \frac{\{\rho_1\Omega \coth[\rho_1\Omega]\}\,(\Omega^2 - 1) + 1 + \rho_2\Omega^2}{\{\rho_1\Omega \coth[\rho_1\Omega]\}\,(\Omega^2 + \rho_2) + \rho_2(\Omega^2 - 1)} \tag{30}$$

Using the values of δ^*/D listed in Table I, the parameters were calculated using a computerized algorithm for fitting non-linear equations [15] *. The values in Table II correspond to $\delta = 0.02$ cm, a reasonable guess for well stirred solutions. The numbers in the first row of Table II correspond to $k_{is}^{ma} = 7.5\ s^{-1}$ (Mean value from the data found in Ref. 1), those in the second to a 10-fold larger value, chosen for reasons discussed previously. The quality of the fit is illustrated in Fig. 4. As shown in Table II, the fitting value for the rate constant, k_d^a, is extremely sensitive to k_{is}^{ma}. A 10-fold change of k_{is}^{ma} causes k_d^a to vary by three orders of magnitude, while the other two constants, P_s and D, are affected much less. The large discrepancy in the two fitting values of k_d^a in Table II is due mainly to the following reasons: k_d^a is proportional to the square of ρ_1, so that a 10-fold change of ρ_1 implies a 100-fold change of k_d^a. More-

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* The computer fitting program was actually set to calculate ρ_1 and ρ_2 for assigned values of δ/D. δ was fixed at 0.02 cm, a reasonable guess for well stirred solutions, and D was given initially the value of $2 \cdot 10^{-6}\ cm^2/s$, deducible theoretically from Stokes law and the known radius of PV [1]. D was then varied around such number to search for the value that optimized the fit by yielding the smallest sum of the squared errors.

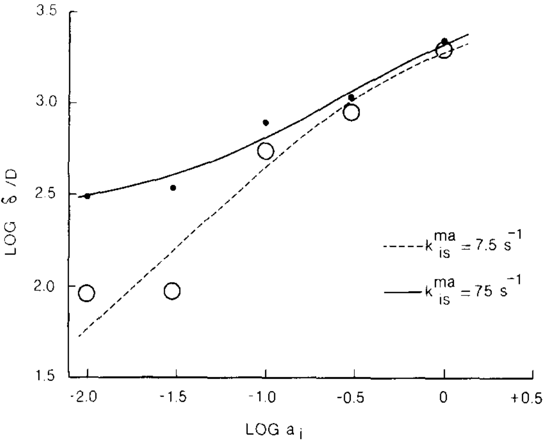

Fig. 4. Flits of Eqn. 30 (which is merely a convenient rearrangement of Eqn. 19) to the data for δ^*/D shown in Table I. The open circles refer to the values in the third column, the black dots to those in the fourth. The logatirhms are base 10 in both abscissa and ordinate. The activities are expressed in mol/l, δ^*/D in s/cm. The best fitting values of the parameters are shown in Table II for δ = 0.02 cm.

over, the data for δ^*/D in the two columns of Table I, while close at high ion activities, are quite different at the lower ones, implying a change in shape of the 'δ^*/D versus a_i' curves, as well as a change of the limit of δ^*/D for $a_i \to 0$ (see Fig. 4). Since this limit is shown by Eqn. 30 to be equal to $\delta/\rho_1 D$, it is not surprising that the values of ρ_1 that fit the two sets of data are substantially different.

Discussion

Are the parameters deduced from the conductance consistent with the zero-current potential data?

Zero-current potential experiments are presented in [1], where the concentrations of several cations, including K^+, were equal to 1 M in both solutions, while those of the carrier PV were kept constant in one solution and varied in the other. According to Eqn. 13, the value of the potential can never become greater than $|V_0^M| = RT\phi_0^M/F$, where ϕ_0^M is defined in Eqn. 12. If we now substitute the values of Table II in Eqn. 12, recalling that for K^+ the aqueous complexation constant is $K \simeq 10\ M^{-1}$, and using a_i = 1 M, we find, for T = 300 K,

$$|V_0^M| \simeq 44\ \text{mV} \quad (k_{is}^{ma} = 7.5\ s^{-1})\ ;$$

$$|V_0^M| \simeq 33\ \text{mV} \quad (k_{is}^{ma} = 75\ s^{-1})$$

Both these numbers, which represent the maximum value of the potential expected for an infinite gradient of carriers, are inconsistent with the data of Ref. 1, since they are less then half of the highest experimental values (see Fig. 11 of Ref. 1). Smaller values for k_d^a and P_s than those of Table II would clearly give larger values of V_0^M, and would surely help to resolve this inconsistency. However, it was found that lowering them enough to obtain theoretical potentials as high as the experimental ones would make the fitting of the conductance data in Fig. 4 impossible. One reasonable explanation fot his discrepancy might be that, in the calculations and in the equations reported in the text, we have neglected to consider an exchange of neutral carriers between the membrane and the torus, such as, for example, that found in previous work with valinomycin [16]. Some relevant consequences of allowing for this exchange are given quantitatively in Eqns. 6A and 7A of the Appendix, and can be summarized verbally as follows: If the rate constant, k_s^{mt}, describing the movement of carriers from the membrane into the torus, is greater than either k_s^{ma}, or $2k_s$, or both, the maximum potential, ϕ_0^M can become very large, while P_s remains always smaller than $k_s^{am}/2$ (see Eqns. 6A and 7A). It is then clear that, by choosing an appropriately high value for k_s^{mt}, the zero-current potential data could be fitted with values of P_s and k_d^a as high as those reported in Table II, and the discrepancy would thus be resolved. Given these considerations based on the theory, it would be clearly important to establish experimentally whether any exchange between the membrane and the torus occurs indeed in the case of PV. One pertinent experiment might be to compare the zero-current potentials between the case in which the membrane is pre-loaded with carriers (e.g. by dissolving them in the membrane-forming solution) and the case in which it is not. It seems plausible to argue that, if there is an efficient exchange with the torus, the flux of carriers from the torus into the membrane will tend to reduce the trans-membrane gradient of carriers induced by the different carrier concentrations of the solutions, and will, consequently, reduce also the value of the potential with respect to that observed when the membrane is not pre-loaded with carriers. Another experiment bearing on the same problem might be the measurement of the specific conductance (conductance per unit area) for membranes with different areas. In the case of valinomycin, it was found that the specific conductance decreased with decreasing membrane area [16], the explanation being that a depletion of carriers from the membrane via absorption by the torus, with a consequent decrease of the specific conductance, was likely to be more pronounced in membranes with smaller areas. From a formal viewpoint, one could say that the rate constant, k_s^{mt}, is actually a function of the membrane area and, more specifically, increases with decreasing membrane area. Even though valinomycin acts by 'interfacial complexation', in which case the relationship between the density of carriers on the membrane and the membrane conductance is more intuitive, it can be shown that similar effects of varying k_s^{mt} are expected also for 'solution complexation'.

A different experiment that (although unrelated to the quenstion of exchange of carriers with the torus) might be useful to test the applicability of the present theory to PV, would be that of measuring ϕ_0^M (or V_0^M) as a function of the ion activity, a_i. From Eqns. 12 and 15 it is easy to deduce that ϕ_0^M generally increases with a_i, and that the only condition under which it is expected to be independent of a_i is when the aqueous reaction is so slow that the condition, $\delta < L$, is satisfied. However, on the basis of the behavior of the conductance, as well on the basis of the values in Table II, we have already argued against the applicability of the condition, and we would therefore expect ϕ_0^M to be a varying function of the ion activity.

Appendix

Outline of the derivation of the equation for the zero-current potential and the current-voltage relation in the case of 'solution complexation'

Combining Eqns. 7 and 8 yields two differential equations which can be integrated by standard methods to give explicit profiles of the concentrations $C_{is}(x)$ and $C_s(x)$ in the unstirred layers. The fluxes of such two species at any point in the layers can then be calculated by substituting the equations for $C_{is}(x)$ and $C_s(x)$ in Eqns. 7. Thus, in the immediate vicinity of the interfaces ($x = 0$ and $x = d$), the four fluxes, $J_{is}(0)$, $J_{is}(d)$, $J_s(0)$ and $J_s(d)$, can be expressed as functions of the known concentrations of ions and carriers in the bulk solutions, as well as of the unknown aqueous concentrations near the boundaries, $C_{is}(0)$, $C_s(0)$, $C_{is}(d)$ and $C_s(d)$. For example, $J_{is}(0)$ is given by

$$J_{is}(0) = \frac{D_{is}}{\delta}\,[K a_i' N' C_s(0) - (1 + N')\,C_{is}(0) + C_{is}'] \tag{1A}$$

where N is defined in Eqn. 11 and the superscript (′) is used to denote quantities referring to the left bulk solution. Similar expressions are found for the other three fluxes, $J_{is}(d)$, $J_s(0)$ and $J_s(d)$. Peculiar to the mechanism of 'solution complexation' at steady state is that the two fluxes of the complexes, $J_{is}(0)$ and $J_{is}(d)$, must be equal to the two corresponding fluxes across the interfaces, as well as to the flux of complexes across the interior. Recalling the kinetic processes (3) and (5), we can thus write

$$\begin{aligned} J_{is}(0) &= k_{is}^{am} C_{is}(0) - k_s^{ma} N_{is}(0) \\ &= k_{is}\,[e^{\phi/2} N_{is}(1) - e^{-\phi/2} N_{is}(2)] \\ &= k_{is}^{ma} N_{is}(2) - k_{is}^{am} C_{is}(d) = J_{is}(d) \end{aligned} \tag{2A}$$

As to the neutral carriers, the conservation equations are slightly more complicated, due to the presence of the torus. Recalling the kinetic steps described by Eqns. 2, 4 and 6, as well as the fact that the torus is schematized as a sink for neutral carriers, one can easily realize that the following relationships apply:

$$\begin{aligned} J_s(0) &= k_s^{am} C_s(0) - k_s^{ma} N_s(1) \\ &= k_s\,[N_s(1) - N_s(2)] + k_s^{mt} N_s(1) - k_s^{tm} C_s^t \end{aligned} \tag{3A}$$

and

$$\begin{aligned} &k_s\,[N_s(1) - N_s(2)] + k_s^{tm} C_s^t - k_s^{mt} N_s(2) \\ &= k_s^{ma} N_s(2) - k_s^{am} C_s(0) = J_s(d) \end{aligned} \tag{4A}$$

Since $J_{is}(0)$, $J_{is}(d)$, $J_s(0)$ and $J_s(d)$ are all linear functions of $C_{is}(0)$, $C_{is}(d)$, $C_s(0)$ and $C_s(d)$, it is clear that Eqns. 2A, 3A and 4A represent a system of eight linear equations in the eight unknowns: $C_s(0)$, $N_s(1)$, $N_s(2)$, $C_s(d)$, $C_{is}(0)$, $N_{is}(1)$, $N_{is}(2)$ and $C_{is}(d)$. One way of getting a general expression for the current-voltage relations would then be to solve such a system for $N_{is}(1)$ and $N_{is}(2)$ and substitute them in the equation

$$J_{is}(0) = \frac{I}{z_{is}F} = k_{is}\left[e^{\phi/2}N_{is}(1) - e^{-\phi/2}N_{is}(2)\right] \qquad (5A)$$

The results for the zero-current potential, corresponding to the case in which the torus can be viewed as a reservoir with negligible concentration of carriers for the time of the experiment, are obtained from Eqn. 5A (in which $N_{is}(1)$ and $N_{is}(2)$ are substituted by their explicit expression), placing $I = 0$, $C_s^t = 0$ and $a_i' = a_i'' = a_i$. By so doing, one finds an expression identical to Eqn. 13, where

$$e^{\phi_0^M} = 1 + \frac{1}{N\sigma_1\sigma_2}\left\{2 + \frac{D_s}{LP_s}\coth\left(\frac{\delta}{L}\right)\right\} \qquad (6A)$$

with

$$\sigma_1 = \frac{k_s^{ma}}{k_s^{ma} + k_s^{mt}}, \qquad \sigma_2 = \frac{2k_s}{2k_s + k_s^{mt}},$$

$$P_s = \frac{k_s^{am}}{2} \cdot \frac{k_s^{mt} + 2k_s}{k_s^{ma} + k_s^{mt} + 2k_s} \qquad (7A)$$

As was already stated in the discussion, large values of k_s^{mt}, or, equivalently, low values of σ_1 and σ_2, raise the value of ϕ_0^M, while P_s remains always smaller than $k_s^{am}/2$. Eqns. 10 and 12 of the text are clearly obtained from Eqns. 6A and 7A when $k_s^{mt} = 0$.

The expression for the steady-state current-voltage relation in symmetrical solutions is also obtained by substituting the explicit expressions of $N_{is}(1)$ and $N_{is}(2)$ in Eqn. 5A, imposing $a_i' = a_i'' \equiv a_i$ and $C_s^{T'} = C_s^{T''} \equiv C_s^T$. If the exchange of carriers with the torus is allowed for, and if the carrier concentration in the torus is assumed negligible ($C_s^t = 0$ in Eqns. 3A and 4A), one still finds an expression formally similar to Eqn. 16. However, N_{is}^0 is now given by

$$N_{is}^0 = \frac{\gamma_{is}d}{2} \cdot \frac{Ka_iC_s^T}{1 + Ka_i} \times \left\{1 - k_s^{mt}\frac{k_s^{am}}{k_s^{ma}} \cdot \frac{N\sigma_1}{(D_s/L)\coth(\delta/L) + k_s^{am}(1 - \sigma_1)(1 + N)}\right\} \qquad (8A)$$

where σ_1 is defined in Eqn. 7A. Eqns. 18 and 19 of the text remain identical, except for the fact that P_s must now be redefined as in Eqn. 7A.

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