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STEADY-STATE ION TRANSPORT BY NONACTIN AND TRINACTIN

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SUMMARY

The steady-state fluxes of Na^+, K^+, and NH_4^+ carried by nonactin and trinactin across thin lipid membranes have been measured as functions of ion activity, carrier concentration, and the applied potential. In agreement with earlier studies the conductance, $G(O)$, is found to be proportional to the carrier concentration and, for low activities, to the ion activity. The determination of the dependence of $G(O)$ on activity at high activities is, however, apparently obscured by changes in the concentration of carrier in the membrane. Using the values for the rate constants at zero potential which were determined in the preceding paper, it is possible to adjust the potential dependence of the constants so as to achieve a reasonable fit to the current–voltage relations. The data presented provide further evidence that a single molecule of nonactin or trinactin acts cyclicly as a carrier of univalent cations.

INTRODUCTION

It is generally accepted that nonactin and trinactin increase the fluxes of ions across thin lipid membranes by forming one carrier–one ion complexes which are "soluble" in the hydrophobic portion of the membrane. In the preceding paper it was shown that a simple version of the carrier model correctly predicts the relationship between the initial and steady-state currents after the abrupt application of a small potential. From that and other data, it was possible to estimate the values for the various rate constants. In this paper the shape of the steady-state current–voltage relation and the dependence of the conductance on the concentrations of carrier and electrolyte will be compared with the predictions of the same model. These results and some from the preceding paper have been discussed in a less formal manner in a recent review [1].

THEORY

The simple version of the carrier model has been described in detail on many occasions and references were given in earlier papers of this series [2–5]. In one con-

* Most of the work reported in this paper was carried out while the author was a Beit Memorial Research Fellow.

ducting cycle an ion on the left at activity a'_i combines with a carrier on the same side of the membrane at surface concentration N'_s to form a complex at surface concentration N'_{is}. The forward reaction proceeds at a rate $k'_{Ri}a'_iN'_s$ while the reverse rate is $k'_{Di}N'_{is}$. The complex may then diffuse or be driven across the membrane*, $k'_{is}N'_{is}$, where it may dissociate, $k''_{Di}N''_{is}$ releasing the ion into the right aqueous phase. The carrier is then free to recombine, $k''_{Ri}a''_iN''_s$ or to diffuse back across the membrane, $k''_sN''_s$. In the steady-state, after the carriers and complexes have been redistributed across the membrane but before the total concentration of carrier in the membrane N_T has changed, the current is given by [2, 7],

$$I = z_{is}FN_T(K'_ik'_{is}-K''_ik''_{is})/\{(2+K'_i+K''_i)+(k'_{is}/k'_{Di})(2+K'_ik'_{Di}/k''_{Di}+K''_i)+$$
$$(k''_{is}/k''_{Di})(2+K'_i+K''_ik'_{Di}/k'_{Di})+(k'_{is}K'_i/k_s)(1+K''_i)+(k''_{is}K''_i/k_s)(1+K'_i)\} \quad (1)$$

where it has been assumed that there is only one permeable species of cation and where $K'_i = k'_{Ri}a'_i/k'_{Di}$ is the equilibrium constant for ion carrier association on the left at a particular applied potential and K''_i is the equivalent on the right. The rate constants for the transfer of free carrier are assumed to be the same in each direction but not, necessarily, constant. The total concentration of carrier in the membrane, N_T, may depend on a'_i and a''_i. In the limit of zero applied potential and with $a'_i = a''_i$ Eqn 1 reduces to

$$G(0) = \lim_{\Delta V\to 0} I/\Delta V = \frac{z_{is}F^2}{RT}\,\frac{N_T}{(1+k_{Ri}a_i/k_{Di})}\,\frac{k_{Ri}a_ik_{is}/(k_{Di}+2k_{is})}{1+k_{Ri}a_ik_{is}/k_s(k_{Di}+2k_{is})} \quad (2)$$

The concentration of carrier in the membrane is determined by the concentration in the aqueous phase, by the concentration in the lipid phase contained in the Plateau border, or by some compromise depending on the relative rates at which the carriers can be exchanged between the membrane and the two phases [2, 3, 8, 9]. Small membrane areas, high aqueous phase to lipid phase partition coefficients for the carrier ($\Gamma = c_s^l/c_s^{aq}$ at equilibrium), low aqueous phase concentration of complexes weak stirring of the aqueous phases, and low longitudinal membrane viscosity favour equilibrium of the carrier between the membrane and the Plateau border while the opposite conditions favour equilibrium between the membrane and the aqueous phase.

When the carrier in the membrane is in equilibrium with that in the lipid phase, then for $a'_i = a''_i$ and $\Delta V = 0$,

$$N'_s = N''_s = \xi_s c_s \quad (3)$$

where ξ_s is the lipid phase to membrane adsorption constant. The total concentration of carrier, free and complexed, in the membrane is then

$$N_T = 2(1+k_{Ri}a_i/k_{Di})\xi_s c_s \quad (4)$$

and the expression for the conductance (2) becomes

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* The flux data provide little information about the nature of the process which allows a charged complex to cross the membrane except to show that the associated energy barrier is high [4, 6]. In particular the empirical relations used here assume neither the presence nor the absence of gross distortion of the membrane.

$$G = \frac{z_{is}F^2}{RT}\,\xi_s c_s^l\,\frac{k_{Ri}\,a_i\,k_{is}/(k_{Di}+2k_{is})}{1+k_{Ri}\,a_i k_{is}/k_s(k_{Di}+2k_{is})} \tag{5}$$

Thus, under this assumption, as the electrolyte activity is increased, the conductance should first increase linearly then approach a plateau.

When the carrier in the membrane is in equilibrium with the aqueous phases, the same on both sides, then for $\Delta V = 0$,

$$N_s' = N_s'' = \beta_s c_s^{aq} \tag{6}$$

and

$$N_T = 2\beta_s(1+k_{Ri}\,a_i/k_{Di})c_s^T/(1+K_i^{aq}a_i) \tag{7}$$

where β_s is the aqueous phase to membrane adsorption constant, K_i^{aq} is the aqueous association constant for the ion and carrier, and c_s^T is the total concentration of carrier in the aqueous phase. Under these circumstances the conductance becomes

$$G = \frac{z_{is}F^2}{RT}\,\frac{\beta_s c_s^T}{(1+K_i^{aq}a_i)}\,\frac{k_{Ri}\,a_i\,k_{is}/(k_{Di}+2k_{is})}{1+k_{Ri}\,a_i\,k_{is}/k_s(k_{Di}+2k_{is})} \tag{8}$$

Thus the conductance now goes through a maximum and eventually declines as the electrolyte activity is increased.

METHODS

Membranes were formed from glyceryl monooleate (Sigma)+*n*-hexadecane or from bacterial phosphatidylethanolamine (Supelco)+*n*-decane as described previously [4, 5, 10]. Current–voltage relations have been measured both by imposing successive stationary potentials (as described in [4]) and by superimposing steps of approx. 10 ms duration onto a steady potential of 100 mV. The latter procedure allows the use of higher potentials without breaking the membrane and avoids complications due to variations in membrane area and the amount of carrier in unit area of the membrane since these both change slowly.

The nonactin (gift of Dr B. Stearns, Squibb) and trinactin (gift of Dr H. Bickel, Ciba-Geigy) were normally dissolved in ethanol at concentrations between 10^{-5} and 10^{-3} M. Using an appropriate concentration either 2 μl was added to the lipid phase or an appropriate volume less than 100 μl was added to the aqueous phase.

Pre-equilibrations of trinactin between NH_4Cl solutions and hexadecane were attempted by adding 50–100 ml of an NH_4Cl solution (2 or 6 molal), 10–20 ml of hexadecane, and trinactin (10^{-3} M in ethanol) to a stoppered separating funnel. The mixture was shaken by hand and allowed to stand for several hours before use.

RESULTS

In agreement with earlier results [8, 11, 12] the conductances reported in Figs 1–4 for glyceryl monooleate+*n*-hexadecane membranes were proportional to the carrier concentration in the lipid phase except, as mentioned in the previous paper, for simultaneously high carrier and permeant electrolyte concentrations. Most curves have

been repeated at 10^{-5} M and at more than one other concentration and no discrepancies have been observed. Two experiments were tried in which trinactin was preloaded into the aqueous phase by equilibration with lipid phase solvent already containing the trinactin. These results are indicated in Fig. 4. A few experiments have been carried out with trinactin added to bacterial phosphatidylethanolamine+*n*-decane and with trinactin added solely to the aqueous phases for both types of membrane and these results are listed in Table II.

The current–voltage relations are given in Figs 5–8. The shape of these curves is independent of carrier concentration up to at least 10^{-5} M.

DISCUSSION

Zero-potential conductances

As summarized for nonactin in an earlier paper [3], it is reasonable to assume for membrane areas approx. $5 \cdot 10^{-3}$ cm^2, carrier added to the lipid phase, and low electrolyte activities, that the concentration of carrier in the membrane is nearly at equilibrium with the carrier concentration in the lipid and hence is given by $2N_s = 2\xi_s c_s^l$. Thus from the position of the linear portions of the conductance–activity curves it is possible to determine $\xi_s k_{Ri} k_{is}/(k_{Di}+2k_{is})$. The solid curves in Figs 1–4 have been drawn according to Eqn 5 using these values and those of $k_{Ri}k_{is}/k_s(k_{Di}+2k_{is})$ based on the relaxation experiments in the preceding paper (see Table I).

At high electrolyte activities there is no available evidence for equilibrium of the carrier in the membrane with either the aqueous or lipid phases. If significant complexing occurs between carriers and ions in the aqueous phase, then, as the electro-

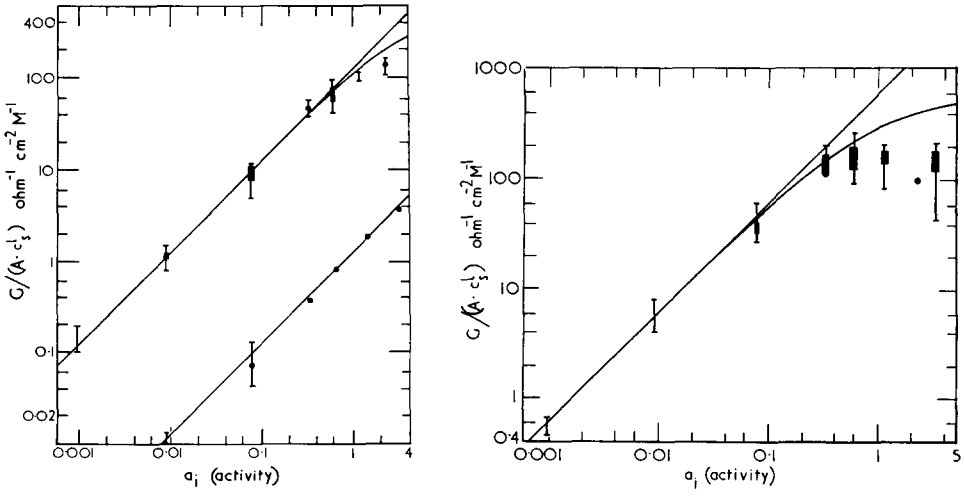

Fig. 1. The specific conductance (G/A) of membranes made from glyceryl monooleate+*n*-hexadecane containing nonactin at concentration c_s^l (M). Error bars designate the complete spread of the experimental observations. Solid bars indicate the extent of a cluster of values. The upper curve is for KCl, the lower for NaCl and both are drawn according to (Eqn 5) using the constants listed in Table I.

Fig. 2. Specific conductances for nonactin with NH_4Cl. See Fig. 1 for further details.

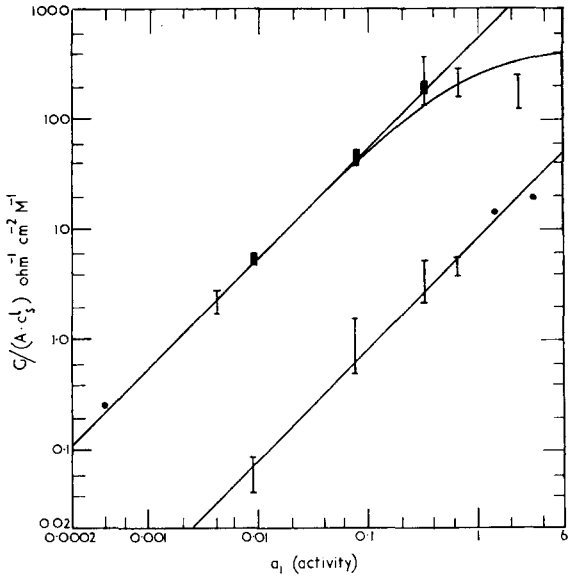

Fig. 3. Specific conductances for trinactin with NaCl (lower) and KCl (upper). See Fig. 1 for more details.

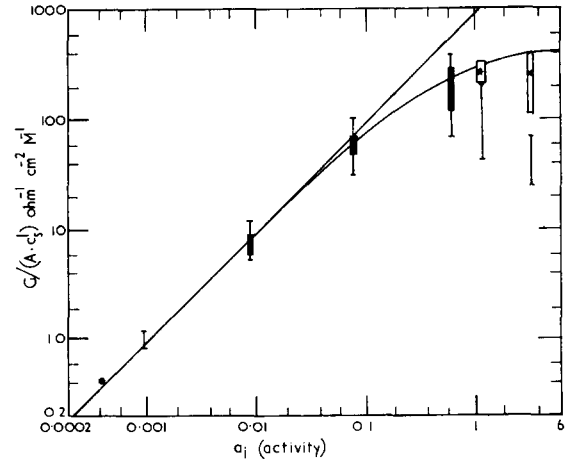

Fig. 4. Specific conductances for trinactin with NH_4Cl. See Fig. 1 for further details. The hollow bars indicate the range of conductances observed with trinactin preloaded into the aqueous phase as described in the text.

lyte activity is increased, a given lipid concentration of carrier will be at equilibrium with an increasing total concentration of carrier plus complexes in the aqueous phase. As a result, if carrier is added only to the lipid phase, the rate at which it is lost across the unstirred layers into the aqueous phase will increase. Thus the carrier concentration in a small membrane will become less than the equilibrium value to an extent which increases with electrolyte activity. Such an effect probably explains why the

TABLE I

THE COMBINATIONS OF RATE CONSTANTS NECESSARY TO PREDICT THE CONDUCTANCE–ACTIVITY RELATION

The observed conductance per unit area at low activities (Figs 1–4) divided by the concentration of carrier (M) and activity of the electrolyte (molar activity units) are listed in the first column while the associated rate constants (Eqn 5) obtained by fitting to the data are in the second column. The combination in the third column which also occurs in Eqn 5 has been taken from Table V of the preceding paper [5].

	$(G/A \cdot c_s^t)/a_i$ $(\text{ohm}^{-1} \cdot \text{cm}^{-2} \cdot \text{M}^{-1} \cdot [\text{act}]^{-1})$	$\xi_s k_{RI} k_{is}/(k_{DI}+2k_{is})$ $([\text{act}]^{-1} \cdot \text{cm} \cdot \text{s}^{-1})$	$k_{RI} k_{is}/k_s(k_{DI}+2k_{is})$ $([\text{act}]^{-1})$
Nonactin			
NaCl	1.25	$3.2 \cdot 10^{-4}$	Approx. 0.01
KCl	125	$3.2 \cdot 10^{-2}$	0.21
NH_4Cl	600	$1.6 \cdot 10^{-1}$	1.1
Trinactin			
NaCl	8.3	$2.1 \cdot 10^{-3}$	0.02
KCl	540	$1.4 \cdot 10^{-1}$	1.25
NH_4Cl	900	$2.3 \cdot 10^{-1}$	2.0

conductances in the present experiments are less than predicted using Eqn 5.

The difficulty in determining the concentration of carrier in the membrane is even more acute when the carrier is added only to the aqueous phase [3, 8, 9]. For nonactin which partitions comparatively weakly into the lipid (Γ is approx. 5000, [11] and unpublished results), it is possible to achieve "equilibrium" conductances using small membranes. However for valinomycin (Γ is approx. 25 000, [8]) and trinactin (Γ is approx. 20 000, see below) which partition more strongly into the lipid, larger membranes are required [9]. Since the membranes used to obtain the data in section b of Table II were $1.8 \cdot 10^{-2}$–$2 \cdot 10^{-2}$ cm^2 in area, which may not be sufficiently large, the observed conductances are indicated as lower limits. The apparent value of the partition coefficient at each electrolyte activity, $\Gamma(a)$, may be estimated from the data in Table II as the ratio of the conductances for the same concentration of carrier added to the aqueous and lipid phases. Thus the partition coefficient for trinactin at low electrolyte activity is 20 000 or larger as compared to 5000 for nonactin. This is hardly surprising since trinactin differs from nonactin by the addition of three hydrophobic $-CH_2-$ groups. The data in Table II also indicate that some interaction occurs between NH_4Cl and the carrier in the aqueous phase. If, in spite of the anomalous behaviour (see second footnote of Table II), the decline in the apparent value of $\Gamma(a)$ at high activities is taken to be entirely due to formation of 1–1 complexes in the aqueous phase, then the association constant, K_i^{aq}, is of the order of $(20\,000/500-1)/3.23 = 12\ [\text{act}]^{-1}$*. (Compare the discussion in [9].) There is, however, no evidence available concerning the stoichiometry, structure, or solubility of the aqueous complexes.

Less ambiguous data than that in Table II could be obtained in principle by pre-equilibrating the carrier between the aqueous phase and an excess of the lipid

* The symbol [act] is used to denote the units of molal activity.

TABLE II

VARIOUS SPECIFIC MEMBRANE CONDUCTANCES (G/A) DIVIDED BY THE CONCENTRATION OF TRINACTIN

Salt concentration (M)	Types of lipid and cation			
	PE-C_{10}, NH_4^+	PE-C_{10}, Na^+	gmo-C_{16}, Na^+	gmo-C_{16}, NH_4^+
A. Trinactin added to the lipid phase ($G/(A \cdot c_s^l)$ ($\Omega^{-1} \cdot cm^{-2} \cdot M^{-1}$)				
0.001				1.0
0.01	0.02–0.035★		0.07	8
0.1	0.14–0.15★		0.8	60
1	1–1.7	0.007–0.017	5	200
6 (molal)	2.5–10		20	45
B. Trinactin added to the aqueous phase ($G/(A \cdot c_s^{aq})$($\Omega^{-1} \cdot cm^{-2} \cdot M^{-1}$)				
0.001		40★,★★★		≧ 10 000
0.01	≧ 640★	300★,★★★		≧100 000
0.1	≧3500★	1000★,★★★		≧250 000
1	≧6300★★	3000★★★	≧110 000	≧200 000★★
6 (molal)	≧4500★★	4000★★★		≧17 000★★
C. The apparent value of the aqueous phase to lipid phase partition coefficient, $\Gamma = G/(A \cdot c_s^{aq})/G/(A \cdot c_s^l))$				
0.001				≧10 000
0.01	≧21 000			≧12 000
0.1	≧24 200			≧ 4 200
1	≧ 3 700		≧22 000	≧ 1 000
6 (molal)	≧ 450			≧ 380

Abbreviations: PE, bacterial phosphatidylethanolamine; gmo, glyceryl monooleate; C_{10}, *n*-decane; C_{16}, *n*-hexadecane.

★ Ionic strength raised to 1 M using LiCl.

★★ These are not stable, reproducible conductances. At low electrolyte activities successive membranes formed from lipid which remains in the hole reach the same steady-state conductance. At high activities the conductance of the second membrane can be as little as one-tenth that of the first.

★★★ Data taken from Fig. 6 of [13]. These conductances, if correct, would suggest a smaller apparent value of K_i^{aq} (see [1]).

phase thus allowing the membrane to be in equilibrium with both phases simultaneously. However, this procedure assumes that the activity of the carrier in the lipid can be kept constant which in turn assumes that the formation of oil in water and water in oil dispersions (with high surface areas) can be avoided. In the presence of surface active lipids this condition is unlikely to be fulfilled. Experiments have, however, been tried in which hexadecane containing 10^{-5} M trinactin was equilibrated against 10 times the volume of either 2 M NH_4Cl or 2.5 times the volume of 6 M NH_4Cl. These aqueous phases were then used together with glyceryl monooleate+*n*-hexadecane+10^{-5} M trinactin. The conductances (Fig. 4) were indeed higher than obtained by adding carrier to the lipid alone. As the aqueous properties of the carrier are peripheral to the present study and it is only required to find $\xi_s k_{Ri} k_{is}/(k_{Di}+2k_{is})$ from the conductance measurements at low electrolyte activities, the matter has not been pursued further. It would clearly be of value to have an independent means of measuring the concentrations of free and complexed carrier in the membrane.

Current-voltage relations

If the potential dependence of the various steps in the transport process were known, it would be possible to determine k_{is}/k_{Di} by fitting Eqn 1 to the shape of the current–voltage relation at low electrolyte activity [4] and $k_{is}k_{Ri}/k_{Di}k_s$ by fitting to the shape at high electrolyte activity. However, the values can vary considerably from one set of assumptions for the potential dependences to another. As a first approximation, it would be reasonable to use the simplest assumptions [8]:

k_{Ri}, k_{Di}, and k_s, independent of potential

$$k'_{is} = k_{is} \exp(-0.5\, F\Delta V/RT)$$

$$k''_{is} = k_{is} \exp(0.5\, F\Delta V/RT). \qquad (9)$$

The values of k_{is}/k_{Di} and $k_{Ri}k_{is}/k_{Di}k_s$ based on fitting Eqn 1 to the data using these assumptions (the dashed curves in Figs 5–8) are listed in Table III. The values of $k_{is}/$

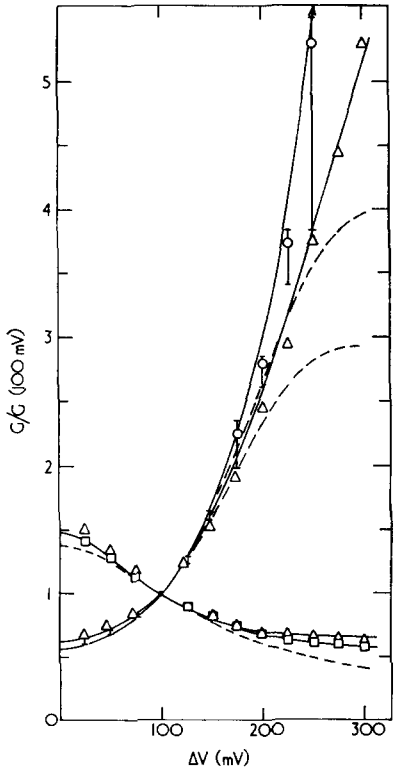

Fig. 5. Current–voltage relations for nonactin with NaCl at 0.1 M (○) and 2 M (△) and for trinactin with NH_4Cl at 0.001 M (□) and 6 molal (△). The data are reported as $G(\Delta V)/G(100) = [I(\Delta V)/\Delta V]/[I(100)/100]$, i.e. as the conductance at the potential ΔV relative to the value at 100 mV. The data for nonactin and 0.1 M NaCl are identical to those reported earlier [4] and have been used with Eqn 1 to determine the potential dependence of $k'_{Ri}k'_{is}/k'_{Di}$. Similarly the fit to the data for trinactin and 6 molal NH_4Cl has been used to determine the potential dependence of k'_{is} while that for trinactin and 0.001 M NH_4^+ is used for k'_{Ri}. The dashed curves are based on a priori assumptions (Eqn 9) for the potential dependence of the rate constants as described in the text.

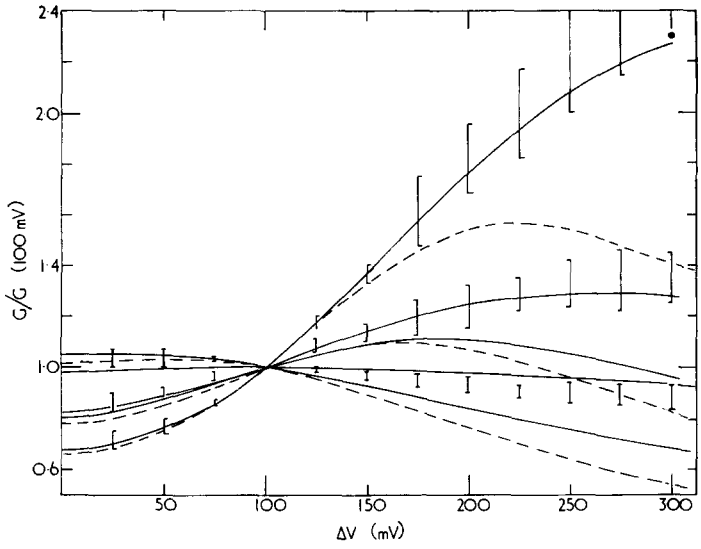

Fig. 6. Current–voltage relations for nonactin and KCl at 0.01 M ([), 1 M (]), and 4 molal (I). The error bars indicate the complete range of the experimental observations. The theoretical curves drawn according to Eqn 1 using the constants in Table III are described in the text. In descending order at 200 mV the solid curves correspond to: 1, $k_{Ri}/k_s \ll 1$; 2, $k_{Ri}k_{is}a_i/k_{Di}k_s = 0.1$, $k_s/k_{is} = 20$; 3, $k_{Ri}k_{is}a_i/k_{Di}k_s = 0.1$, $k_s/k_{is} = 2$; 4, $k_{Ri}k_{is}a_i/k_{Di}k_s = 0.37$, $k_s/k_{is} = 20$; 5, $k_{Ri}k_{is}a_i/k_{Di}k_s = 0.37$, $k_s/k_{is} = 2$

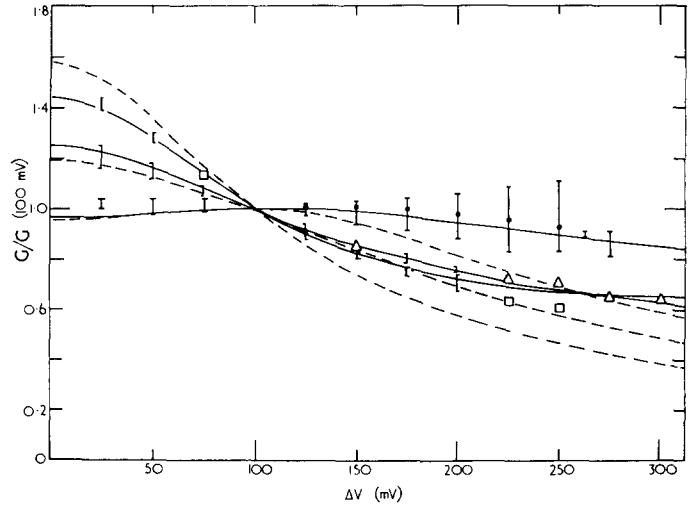

Fig. 7. Current–voltage relations for nonactin and NH_4Cl at 0.01 M (I); 1 M (] and △); and 6 molal ([and □). The discrepancy at low potentials between the dashed curve and the experimental points for 6 M electrolyte is evidence, that the assumptions in Eqn 9 are inadequate.

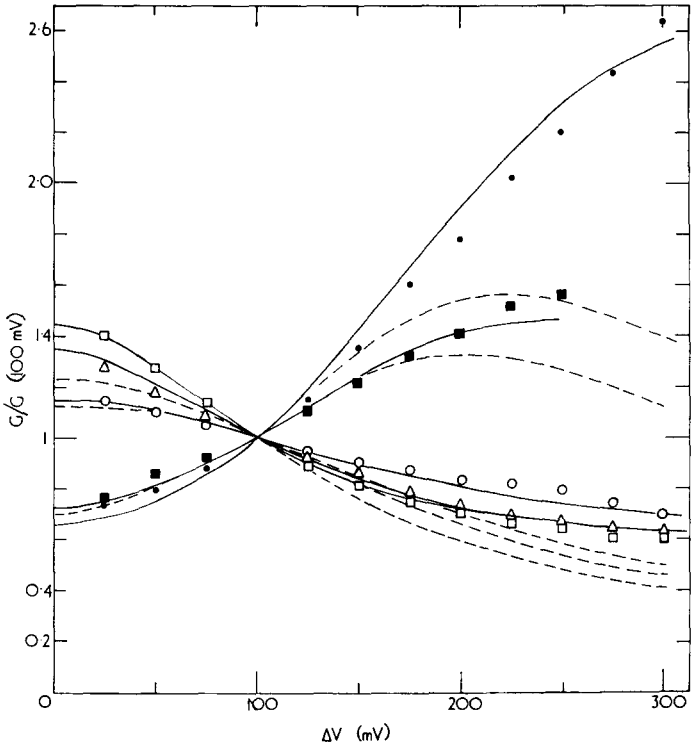

Fig. 8. Current–voltage relations for trinactin with NaCl at 1 M (●) and 4 molal (■) and with KCl at 0.001 M (○), 1 M (△), and 4 molal (□). The theoretical curves, drawn according to Eqn 1 using the constants in Table III, are described in the text. The solid and dashed curves coincide at low potentials for 1 M NaCl and 4 M KCl.

TABLE III

RATIOS OF RATE CONSTANTS

	Based on the simplest a priori assumptions (see text)		Based on empirical potential dependences and current relaxation measurements (see preceding paper)	
	$\frac{k_{is}}{k_{Di}}$	$\frac{k_{Ri}k_{is}}{k_{Di}k_s}$	$\frac{k_{is}}{k_{Di}}$	$\frac{k_{Ri}k_{is}}{k_{Di}k_s}$
Nonactin				
NaCl	0.01	Approx. 0.003	$\leq$0.01 (set equal to 0)	0.01
KCl	0.04	0.16	0.04	0.23
NH_4Cl	0.18	0.5	0.25	1.6
Trinactin				
NaCl	0.037	0.012	0.02	0.02
KCl	0.3	0.37	0.5	2.5
NH_4Cl	0.6	$<$0.24	2	10

k_{Di} for trinactin are in approximate agreement with those for glyceryl oleate+*n*-decane membranes reported by Ciani et al. [13] based on the same assumptions [4] but a different experimental procedure.

Unfortunately there are at least three aspects of the data which cannot be fitted using these simple assumptions. Firstly the shapes of the current–voltage relations at low electrolyte activities require that k'_{Ri} and k''_{Ri} depend on potential since the current continues to increase with potential even when limited by the rate of association [4]. Secondly at high electrolyte activities, the current should reach a limiting value for potentials as low as 100–150 mV (specifically $I \propto I_0 \tanh(\Delta V/50\text{ mV})$, while the observed current continues to increase at all potentials. Thirdly the values of k_{is}/k_{Di} and $k_{is}k_{Ri}/k_{Di}k_s$ calculated on the basis of the a priori assumptions (Table III) predict transient currents after the abrupt application of an applied which are much smaller than those observed. Thus the simplest assumptions (Eqn 9) do not allow the current–voltage relations to be fitted and result in apparent values for the rate constants which are inconsistent with the relaxation data.

In order to derive quantitative information from the current–voltage relations, it is necessary to devise some means for determining the potential dependence of the various rate constants. In an earlier paper of this series [4] it was shown that the current voltage relations for nonactin at low electrolyte activities could be fitted if

$$\frac{k'_{is}k'_{Ri}}{k'_{Di}} = \frac{k_{is}k_{Ri}}{k_{Di}} \exp\{+0.5F\Delta V/RT - b(F\Delta V/RT)^2\}, \quad b \cong 0.005 \tag{10}$$

and

$$k'_{Ri} = k_{Ri} \exp\{-\eta F\Delta V/RT\}, \quad 0.05 \leqq \eta \leqslant 0.1 \tag{11}$$

for large negative applied potentials. The same analysis applied to the data in Figs 5 and 8 for trinactin requires $0.03 \leqq \eta \leqq 0.08$. It is assumed here that these expressions apply for all potentials, and that the doubly primed expressions are obtained by changing the sign of the applied potential. One possible interpretation of these potential dependences was offered in the earlier paper, but here they are regarded merely as empirical relations which must be determined or assumed before other portions of the data can be fitted and before values can be assigned to the various constants★.

Using these functions, Eqns 10 and 11, values for k_{is}/k_{Di} may be specified for each value of η. The overlap between these and the values determined directly from the relaxation amplitudes at low electrolyte activities [5] provides support for the model. Furthermore the comparison allows more precise assignments of k_{is}/k_{Di} (Table III) and of η ($\cong 0.05$).

In the preceding paper [5] it was shown by measuring the initial current-voltage relation that if

$$k'_{is} = k_{is} \exp\{-(0.5-\xi)F\Delta V/RT - b(F\Delta V/RT)^2\} \tag{12}$$

with $b = 0.005$ then the value of ξ★★ should be approx. 0.1. The data in this paper for

★ The assumption that k'_{Ri} and k''_{Ri} are related by changing the sign of the potential is arbitrary. If the potential dependence were indirect and such that $k'_{Ri} = k''_{Ri}$, then it would be necessary for k_s to increase with potential.

★★ The symbols ξ and ξ_s should be distinguished.

trinactin with K^+ or NH_4^+ at high activity may be fitted using any value of $\xi < 0.08$ by adjusting the potential dependence of k_s. The predicted curves at high activity depend on ξ since

$$K_i' = k_{Ri}'/k_{Di}' = k_{Ri}/k_{Di} \exp(-\xi F \Delta V/RT).$$

If k_s is a constant, $\xi \cong 0.08$ and thus this value has been used.

Values of $k_{Ri}k_{is}/k_{Di}k_s$ were obtained from the relaxation data at high electrolyte activities reported in the preceding paper. Unfortunately in order to determine $k_{Ri}k_{is}/k_{Di}k_s$ from the current–voltage relations it is also necessary to determine an approximate value for k_{Ri}/k_{Di} unless this latter ratio is independent of potential. The data available are not adequate to make the determinations convincing. It is, however, possible to show approximate agreement between the measured current-voltage relations and curves predicted using values of $k_{Ri}k_{is}/k_{Di}k_s$ (Table III) and k_s/k_{is} (or k_{Ri}/k_{Di}) consistent with those already derived [5]. The values of k_s/k_{is} are not known accurately. For simplicity 2 has been assumed and with this value the fit is satisfactory for all ion–carrier combinations except nonactin–K^+ (see Figs 5–8). A better fit to those data (Fig. 6) is possible if either $k_s/k_{is} = 10$–20 (in which case $k_{Ri}k_{is}/k_{Di}k_s$ should be somewhat larger for nonactin–NH_4^+ and trinactin–K^+) or $k_{Ri}k_{is}/k_{Di}k_s$ for nonactin-K^+ is approx. 30 % smaller.

The principle success of the treatment of the steady-state data given in this paper is that the dependence on ion activity of the shape of the current–voltage relations at low potentials can be predicted correctly using values for the rate constants determined in independent experiments and potential functions obtained by curve fitting to the extreme cases (Fig. 5).

CONCLUSION

The evidence set out in this paper and the one preceding it provides additional support for the contention that a single molecule of nonactin or trinactin acts cyclicly as a carrier of univalent cations in the most pictorial sense of the term (see Fig. 1 of the preceding paper). Thus the rate constant for the transfer of complexes across the membrane has been shown to be independent of the concentrations of the ions and the carrier while the association reaction between ions and carriers has been shown to be first order in the concentrations of each. However, the measurements of the fluxes cannot provide a complete description of the mechanism. For instance there is nothing in these data which requires k_{Ri} to be the rate constant for formation of a complex with the same structure as that which crosses the membrane. Thus if N_{is} represents the concentration of some form of pre-crossing complex, then the value of k_{is} would reflect a rearrangement of the complex as well as movement from one side of the membrane to the other. Similarly there is nothing in the data which establishes the nature of the process for moving a complex except in that it has been shown to be strongly dependent on the applied potential. In summary the flux data now establish the magnitude and concentration dependence of the rates, but not their physical explanations.

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