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Turnover Numbers for Ionophore-Catalyzed Cation Transport across the Mitochondrial Membrane

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Summary. The turnover numbers of the ionophores valinomycin, the macrolide actins, enniatin B and dicyclohexyl 18-crown-6 for translocation of cations through the mitochondrial membrane have been compared quantitatively. The rank order of decreasing maximum turnover number for K^+ transport calculated on the basis of the ionophore concentration within the membrane is trinactin > dinactin ~ monactin ~ valinomycin > nonactin > 18-crown-6 > enniatin B. The strength of binding of the ionophores to the mitochondria has the following rank order: valinomycin > macrolide actins > enniatin B > 18-crown-6.

A rough proportionality was observed between the transport rate of K^+, Rb^+, Cs^+ or Na^+ with a given ionophore and the heterogeneous complexation constant of the corresponding ionophore-cation pair in two-phase extraction experiments. However, the proportionality constants between transport and the heterogeneous complexation constant differ between the ionophores. These comparisons indicate that valinomycin and enniatin B transport cations about 10 times slower than would be expected from their two-phase complexation behavior, using the complexation and transport reactions of the macrolide actins as a basis for comparison. Transport with 18-crown-6 was about 1,000 times slower than predicted. These observations are discussed in terms of partitioning of the ionophores between various regions of the mitochondrial membrane.

The data are discussed in terms of a carrier model involving hydrophilic complexes on the membrane surface in addition to hydrophobic complexes which cross the membrane.

Since our initial report that valinomycin- and nigericin-type antibiotics function as mobile carriers in biological membranes [21], we have continued with attempts to elucidate the mechanism action of these two large classes of carriers. The major conclusions of these studies were that (a) the ionophores can extract cations from an aqueous phase into a nonpolar organic

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phase [20–23]; (b) the cation specificity for the extraction reaction is generally the same as that of the transport reaction [20, 22], indicating that the rate of transport is proportional to the ability of the ionophore to make a lipophilic complex; (c) the kinetics of the complexation and decomplexation reactions of the ionophores in organic solvents are fast [11, 13, 15, 22], consistent with the mobile carrier hypothesis.

The mobile carrier has been endorsed as applicable to lipid bilayer membranes by the Eisenman group [2] in studies which confirmed observations (a) and (b) for the macrolide actins [4, 26]. The present communication extends the previous studies by comparing the ion transport activity of valinomycin, the macrolide actins, enniatin B, and dicyclohexyl 18-crown-6 for four monovalent cations on the basis of their turnover numbers in rat liver mitochondria. We have recently made this type of comparison for several representatives of the negatively charged ionophores [23].

Materials and Methods

The ionophores and reagents used in this study are the same as in the companion paper [14]. Erythrocytes obtained from outdated human blood were washed at least four times in buffered sucrose or NaCl solutions in which the experiments were carried out. In the red cell experiments, ion movements were monitored by use of a Beckman 49047 cation-sensitive glass electrode [19] and by atomic absorption spectrometry of the centrifically cleared reaction medium. The cells could be spun down quantitatively in 15 sec in a Coleman microcentrifuge, giving a time resolution of about 2.0 sec for sampling. Thick suspensions of cells were used, with a packed (Hematocrit) volume constituting 15 to 30% of the volume of the system. Concentrations of K^+ in the pellet were determined on extracts obtained by resuspending and hemolyzing the cells in distilled water, followed by deproteinizing with trichloroacetic acid. The intracellular K^+ concentration was calculated, correcting for the volume fraction of the pellet occupied by interstitial water by means of ^{14}C dextran.

Rat liver mitochondria were isolated essentially as described by Schneider [25] with sucrose containing 200 μM EDTA, pH 7.4. Respiratory assays of the ionophores were made in a standard medium of 125 mM sucrose, 10 mM glutamate, 10 mM malate and 1.0 mM phosphate, all tris salts adjusted to pH 7.4, 0.5 mM $MgCl_2$ and 50 mM alkali cation chloride at mitochondrial Biuret protein concentrations of 1 mg/ml as bovine serum albumin. The rate of respiration was measured by use of a rotating oxygen electrode [5].

Results

The stoichiometry between mitochondrial respiration and ionophore-mediated K^+ uptake below saturating levels of ionophore has been reported to be fixed under given conditions of pH, substrate and permeant anion concentration [3, 8]. Based on these data we have assumed that a fixed stoichiometry held for the four cations and the four groups of ionophores we have studied. The relative rates of cation transport were calculated from

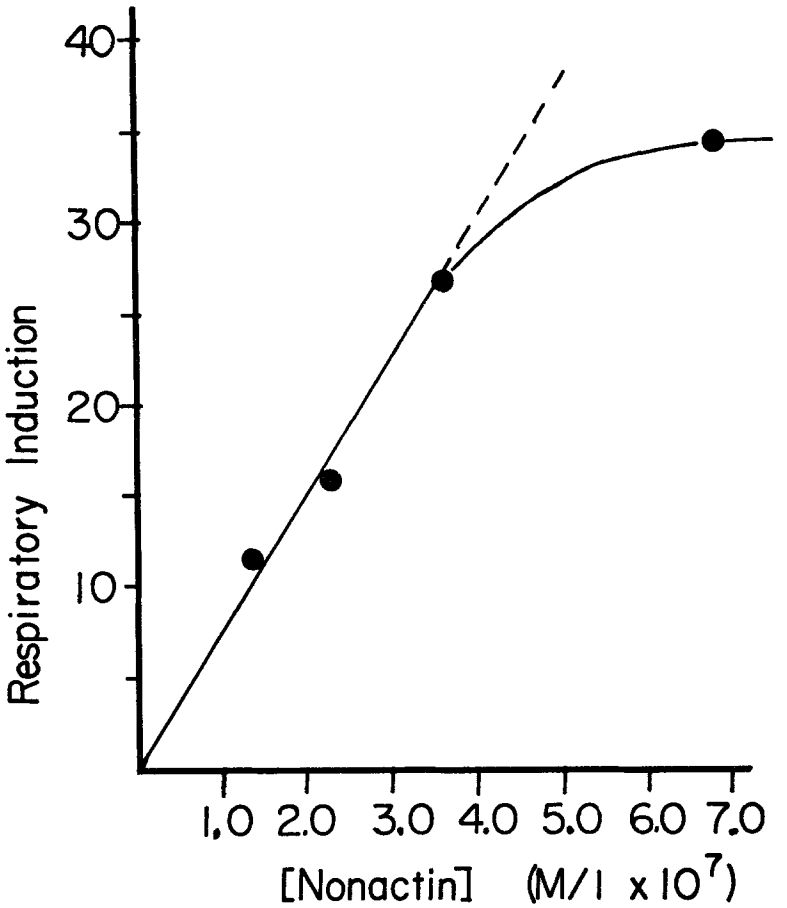

Fig. 1. Relationship between the increase in respiration and the monactin concentration. $\Delta(d[O_2]/dt)$ is plotted against the concentration of nonactin in the medium. Reaction conditions were identical to those in the text, with the mitochondrial protein concentration held constant below the saturating level and KCl concentration held constant at 50 mM

respiratory rates measured using the oxygen electrode. Since no effects of ionophore addition on respiration were found at the ionophore concentrations used in this study in the absence of cations, it seems likely that uncoupling due to an effect of "surface activity" [24] of the ionophores did not contribute to the observed increases in respiration.

The increment $\Delta(d[O_2]/dt)$ to the State IV respiration rate upon addition of the ionophore was determined for the standard test medium. For fixed concentrations of cations, permeant anions and mitochondria, a linear relationship was found between the respiratory response and ionophore concentration, as shown in Fig. 1. In the linear range of ionophore con-

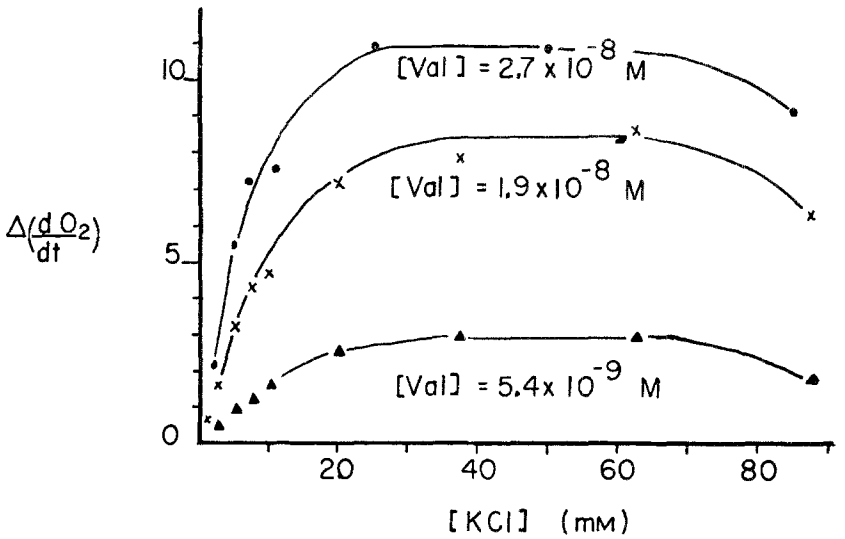

Fig. 2. Saturation of the valinomycin-induced mitochondrial respiratory response with respect to K^+. Reaction conditions were identical to those of Fig. 1 except that KCl was varied. The osmolarity of the medium was kept constant according to: 2(KCl) + (sucrose) = 250 mM

centration, the rate of transport is considered to be ionophore-limited, while at higher concentrations, the mitochondrial energy supply or the ability of the intrinsic carriers to transport the phosphate anion was probably limiting. All of the results reported in the present communication were obtained under the former condition, in order to ensure that the behavior reported was intrinsic to the ionophore. For low ionophore concentrations, a first power dependence of $\Delta(d[O_2]/dt)$ on ionophore concentration was observed for all the ionophores studied.

As had been demonstrated previously, the respiratory response was found to be saturable in terms of both the cation, anion and ionophore concentration [3]. In Fig. 2 ($d[O_2]/dt$) has been plotted against [KCl] in the external medium for three valinomycin concentrations at a constant mitochondrial protein concentration. The respiratory response shows saturation with increasing concentrations of K^+. Full saturation was observed for all cations and ionophores studied at cation concentrations of 50 mM, and the cation concentration giving half-maximal respiratory response (K_m) ranged from 8 to 12 mM. The large ion specificity of the ionophores is thus not reflected in the K_m values. That the saturation effect was not the result of an ionic strength effect on the mitochondrial energetics or the membrane surface was demonstrated in experiments which showed that variations of

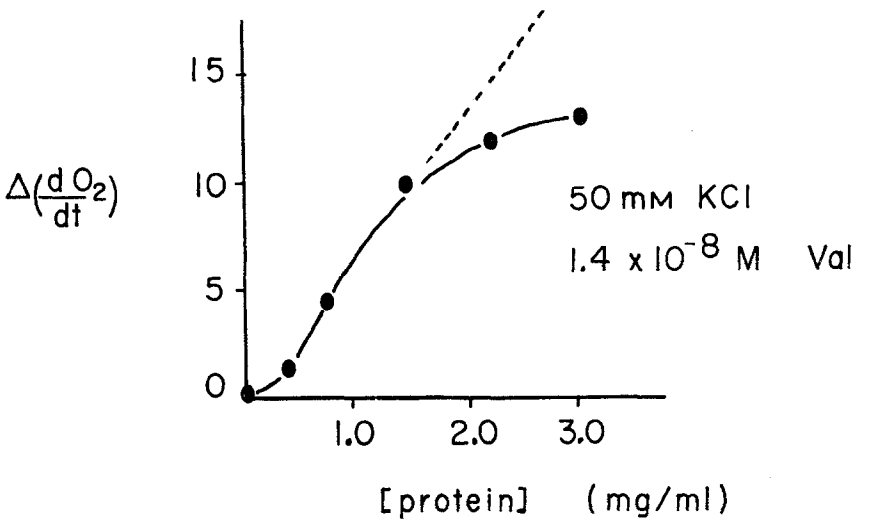

Fig. 3. Saturation of the respiratory response by the mitochondrial protein concentration. Reaction conditions were identical to those of Fig. 1 except that the protein concentration was varied

concentrations of Li^+ and tris had little effect on the transport rates of the other cations. On the other hand, Na^+ addition decreased $\Delta(d[O_2]/dt)$ and increased the K_m values for the ionophores with K^+, Rb^+ and Cs^+, suggesting that Na^+ can compete with these cations under conditions where it is itself not transported.

Fig. 3 shows the effect of the concentration of mitochondria on the respiratory response for a fixed and saturating KCl concentration and a fixed but not saturating valinomycin concentration. The experiments reported below support the interpretation that this saturation phenomenon represents the absorption of all of the ionophore into the membrane according to the process:

$$I_{aq} + \text{Membrane} \overset{P}{\leftrightarrow} \text{I-Membrane} \quad (1)$$

where I represents the ionophore and P is the equilibrium constant for this process, expressed here in units of reciprocal mitochondrial protein concentration. To test this interpretation, direct binding studies were carried out.

Aliquots of ionophore were mixed with 0.5 ml of the mitochondrial suspension and were spun down in a microcentrifuge. The ionophore concentration in the supernate was then determined by the respiratory assay. Table 1 gives a comparison of the partition ratios $(P)^{-1}$, determined by this method and those determined from experiments such as in Fig. 3.

Table 1. Partition ratios of ionophores between mitochondria and solution

Ionophore	$(P_1)^{-1}$ [a]	$(P_2)^{-1}$ [b]
Macrolide actins	3 ± 1 mg/ml (1×10^{-7} M) [c]	—
18-crown-6	>4 mg/ml (1×10^{-4} M)	100 ± 50 mg/ml (1×10^{-4} M)
Enniatin B	>4 mg/ml (6×10^{-6} M)	12 ± 2 mg/ml (6×10^{-6} M)
Valinomycin	1.4 ± 0.3 mg/ml (1.2×10^{-8} M)	—

[a] Determined as the concentration of mitochondrial protein giving half-maximal respiratory response from plots similar to Fig. 3.

[b] Calculated as $\frac{[I]_{supernate}}{[I]_{total} - [I]_{supernate}} \times$ mitochondrial protein from centrifugation experiment.

[c] Concentration of ionophore in test system.

Table 2. Specific respiratory induction [a] as a function of ionophore and cation

Ionophore	Cs^+	Rb^+	K^+	Na^+	Li^+	No additions
Valinomycin	2300	4000	4500	1.1	0.4	0.4
Nonactin	170	860	930	20	0.5	0.7
Monactin	310	1600	2800	110	1.7	1.6
Dinactin	480	2300	2800	200	2.4	2.1
Trinactin	690	3000	3100	400	6.0	2.5
Enniatin B	2.1	6.0	10	0.9	0.2	0.08
Dicyclohexyl ether	0.06	0.09	0.2	$\leqq 0.02$	0.0	0.0

[a] Specific Respiratory Induction $= \frac{\mu M\ O_2}{(mg/ml)\ (min)\ (\mu M\ ionophore)}$.

A series of experiments was performed to compare the transport-inducing ability of all of the cation-ionophore combinations. The values of the "specific respiratory induction" (SRI) defined as the slope of the type of plot shown in Fig. 1 were determined for all cations and ionophores at mitochondrial protein concentrations below the saturating level (e.g. $\leqq$ 1 mg/ml). The SRI can be considered as a measure of the efficiency of the ionophore for partitioning into the membrane and to mediation of cation translocation at saturating cation concentrations. Table 2 shows that the order of cation selectivity based on SRI is $K^+ \geqq Rb > Cs^+ > Na^+$ for all the ionophores tested and that the order of decreasing activity for K^+ was

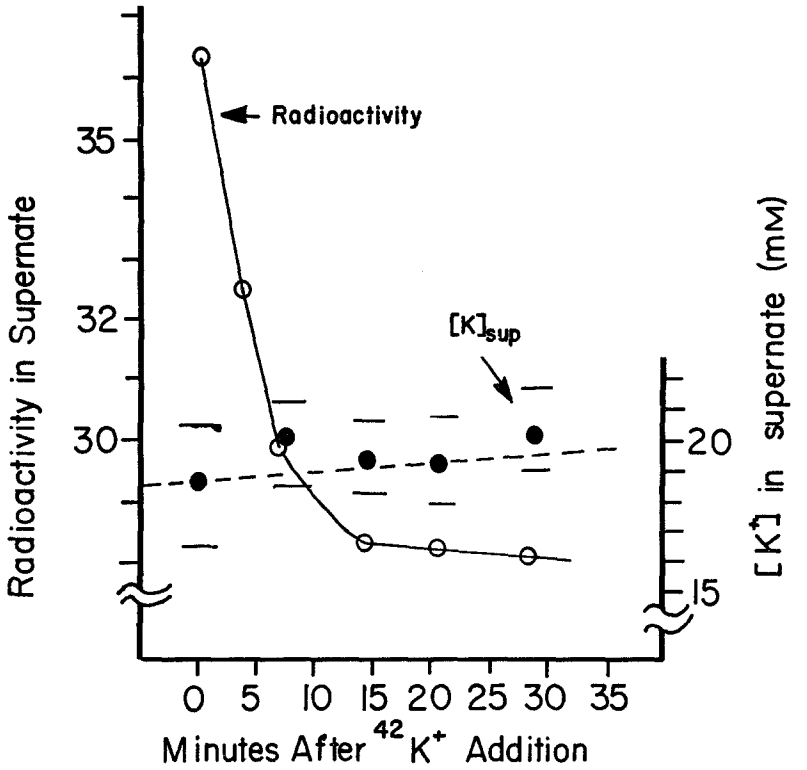

Fig. 4. Valinomycin-induced K^+ flux in human red cells. The experiment was carried out under the condition of no net flux, as described in the text. The bottom trace represents the constant reading from a K^+ electrode calibrated by atomic absorption spectrophotometric assays of the external K^+ (uncertainty indicated by error bars). The upper portion of the figure shows the disappearance of $^{42}K^+$ from the external medium as the result of the exchange reaction

valinomycin > trinactin ≧ dinactin > monactin > nonactin ≫ enniatin B ≫ 18-crown-6.

Tracer flux experiments were performed with human erythrocytes in the presence and absence of net efflux. The former condition was studied by adding $^{42}K^+$ to a suspension of cells, then adding 10 to 60 μliters of 2×10^{-4} M valinomycin in methanol to 12 ml of cell suspension, and determining the change in the flux rates. For experiments performed in the absence of net flux, valinomycin was added and then $^{42}K^+$ was added after the net flux had ended. An experiment of the second type is shown in Fig. 4. Table 3 shows the flux rates determined for two preparations of human red cells in the presence and absence of external Na^+. The turnover numbers for efflux range from 2 to 40 sec^{-1}, with an average value of about 14 sec^{-1}.

Table 3. Valinomycin-induced flux rates in human red cells

Exp. no.	$[K^+]_{ext}$ (mM)	$[K^+]_{int}$ (mM)	$[Na^+]_{ext}$ (mM)	Turnover number [a] for net efflux (sec^{-1})	Turnover number [a] for net influx (sec^{-1})	Turnover number for efflux (sec^{-1})
37A	5.1	78	150	1.7	2.2	3.9
37B	9.6	29	150	0 [b]	1.7	1.7
37C	5.0	78	150	5.6	9.6	14
54–1	15	80	0	31	9.6	41
54–2	20	67	0	15	5.3	20
54–3	6.0	72	0	7.3	0.8	8.1
54–4	11	68	0	16.3	1.3	18
54–5	20	40	0	0 [b]	8.3	8.3

[a] Turnover number = Cations translocated per valinomycin per second.
[b] Experiment performed in absence of net flux.

Discussion

Mitochondrial Cation Transport

The results of the present study, taken together with the results of Cockrell *et al.* [3] and Harris *et al.* [8] indicate that the rate of ionophore-induced cation transport in rat liver mitochondria is dependent upon both the concentration of K^+ in the external medium and also on the concentration and type of permeant anion which accompanies the accumulated K^+. In the present study, the permeant phosphate anion [8] was used at an optimal nonlimiting concentration, the ionophores were used in limiting concentrations, and the concentration of the transported cation was varied. It would thus be expected that the ionophore-mediated cation transport would represent the rate-limiting step in the transport reaction sequence and that such processes as anion transport and energy production were not limiting. The SRI is thus a measure for the maximum turnover number of cation transport by the ionophore in the mitochondrial system, acting as monomeric carriers.

Evidence has been given for (enniatin $B)_2 - M^+$ [9, 16] and (18-crown-$6)_2 - M^+$ [6] complexes at high ionophore concentrations in organic solvents. Furthermore, nuclear magnetic resonance (NMR) experiments [9] with 23 mM enniatin B showed fast exchange between the ionophore in the free form and in the 2:1 and 1:1 forms. These fast exchange reactions occur even in the nonpolar solvent $CHCl_3$ [9] in which the exchange between the complexed and uncomplexed forms of valinomycin is immeasurably slow [13]. Recently, Ivanov *et al.* [16] have combined NMR evidence for enniatin B complexes in organic solvents with evidence for a second power concentra-

Table 4. Calculation of the maximal turnover numbers for the ionophores with K^+

Ionophore	SRI[a]	$(P)^{-1}$[b]	SRI/P[c]	Turnover number (sec^{-1})
Valinomycin	4500	1.4±0.03	6300±1350	2020±430
Nonactin	930	3±1	2790±930	890±300
Monactin	2800	3±1	8400±2800	2690±900
Dinactin	2800	3±1	8400±2800	2690±900
Trinactin	3100	3±1	9300±3100	2980±990
Enniatin B	10	12±2	120±20	38±6
Dicyclohexyl 18-crown-6	6.2	100±25	620±155	200±50

[a] M (O_2)/((mg/ml) × min × M (ionophore)).
[b] (mg/ml).
[c] M (O_2)/(min M (ionophore)).

tion dependence of bilayer conductivity induced by enniatin B to show that 2:1 complexes can play a role in transport with this ionophore. However, in the present experiments conducted with low ionophore concentrations within the membrane (10^{-3} moles ionophore per liter membrane volume) we could find no evidence for such complexes. Since the membrane concentrations of ionophore are much lower than the dissociation constant of (enniatin B)$_2$ – M^+ in ethanol (ca. 10^{-2} M; [16]) these complexes would also not be expected.

Turnover Numbers of Ionophores in the Membrane

In Table 4, the SRI data of Table 2 is recalculated using the ionophore partition data in Table 1, to give SRI values specific for the ionophore in the membrane. Using this corrected data together with the M^+/P value of 3.2 and a P/O value of 3.0 ([1], *cf.* [3]) the turnover numbers based on net flux of K^+ through the membrane are calculated, as shown in Table 4. The value calculated for valinomycin is larger than the lower limit previously reported by us [21] due to the correction for the partition behavior of the ionophore.

Comparison of Mitochondrial Transport and Complexation Properties

If the ionophores act as monomeric carriers in biological membranes, their mediation of transport activity might be, to a first approximation, proportional to their complexing ability. Fig. 5 is a plot of log(SRI/P) for mitochondria against log(K_3), the heterogeneous complexation constants measured in the toluene-butanol/water system [14]. The plot includes the

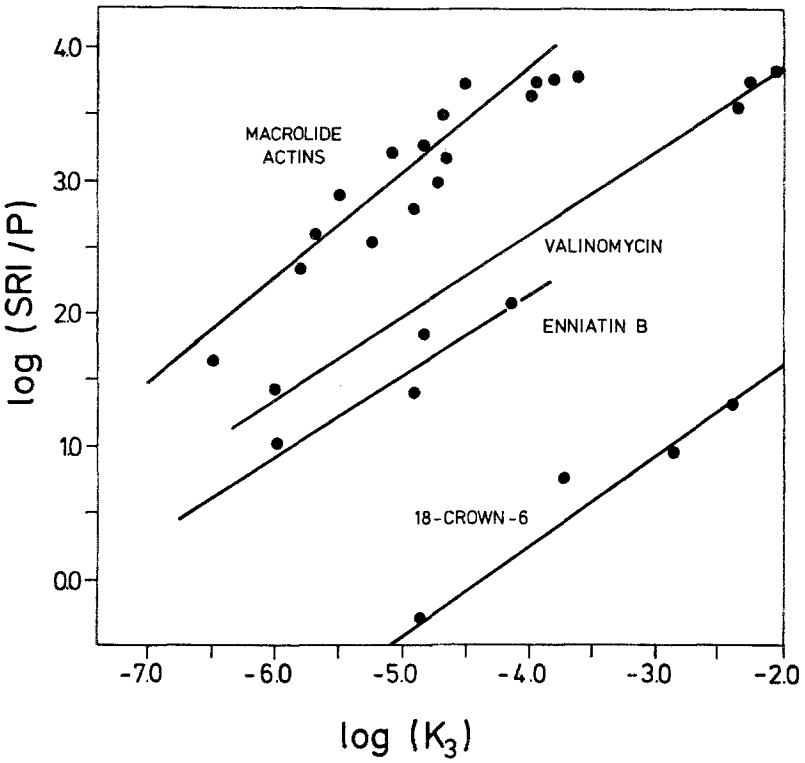

Fig. 5. Correlation between SRI/P and K_3 for 70% toluene-30% n-butanol

data for Na^+, K^+, Rb^+ and Cs^+ complexation of all of the ionophores studied. If the transport activity were proportional to the ability to complex ions in organic phases, then the data should obey the relationship:

$$\log(\mathrm{SRI}/P) = m \log(K_3) + C \tag{2}$$

where m is the slope of the line on the log-log plot (equal to 1.0 for strict proportionality) and where C can be considered as the logarithm of the proportionality constant between the two experimental parameters. For the ionophores studied here, the value of m was between 0.7 and 0.9, indicating that strict proportionality between SRI/P and K_3 does not obtain, and that the weakly complexed cations are transported more rapidly than would be expected on the basis of their two-phase complexation behavior. This discrepancy is not diminished when the present SRI/P data is plotted against the K_3 values obtained in CH_2Cl_2 [4].

The term C in Eq. (2) is the logarithm of the proportionality constant between the transport rate for an ionophore-cation combination and its two-phase complexation constant, under conditions where the total ionophore is in the membrane or oil phase, respectively. The value of this para-

meter shows the ionophore specificity macrolide actins > valinomycin ≈ enniatin B > 18-crown-6. The variation of C shown in Fig. 5 indicates that in the membrane valinomycin and enniatin B transport cations about 10 times slower, and 18-crown-6 transports cations about 1,000 times slower than would be expected from their two-phase complexation behavior, using the complexation and transport reactions of macrolide actins as a basis for comparison. These differences between the ionophores could arise from differences in the partitioning of the ionophores or their complexes between the membrane's interface and the hydrophobic center of the membrane.

Carrier Model

The model for the neutral ionophore-mediated transport reaction must explain the following observations: (a) The rate of the net transport reaction obeys the Michaelas-Menton equation, saturating with respect to the cation concentration in the medium. A similar observation has been made for valinomycin in thylakoid membranes [17]. (b) The K_m value for this saturation process is essentially independent of the cation and ionophore used, although the maximum turnover number at saturation varies over orders of magnitude with the choice of cation and ionophore. (c) Na^+ can inhibit K^+ transport with the ionophores under conditions where Na^+ itself is not transported. (d) The maximum turnover number of an ionophore with a particular cation in the mitochondrial system is nevertheless proportional to the heterogeneous complexation in the oil/water system. We have attempted to fit these observations to a carrier model under the assumption that observation (d) indicates that the two-phase association constants give a true measure of the propensity for the formation of hydrophobic complexes capable of transversing the membrane.

Attempts to fit the data with carrier models involving only complexation at the membrane surface, permeation of the complex, decomplexation at the membrane surface, and permeation of the free ionophore were unsuccessful in explaining observation (b). A second step in the complexation reaction is necessary to explain the data. All three observations can be explained if it is assumed that the ionophore first reacts to form a "hydrophilic" complex on the membrane surface, that this complex can transform to a "hydrophobic" complex, and that only the "hydrophobic" complex can cross the membrane. It is assumed the complexation, decomplexation and transformation reactions are faster than the permeation reactions (*cf.* [12]), and that the formation of the "hydrophilic" complex corresponds to the process giving rise to saturation behavior with a negligible ion or ionophore specificity. The process of transformation of the "hydrophilic"

complex to the "hydrophobic" complex would determine the maximal turnover number at cation saturation, and would thus be proportional to the heterogeneous complexation constant in the two-phase system. The formal properties of this model have been discussed previously [12]. We emphasize that this analysis depends on our conclusion that the behavior observed in the mitochondrial system reflects the intrinsic properties of the ionophores themselves, and the properties of the mitochondrial membrane and active transport system are not influenced by the ionophore. The reasoning for this conclusion was given in a previous section.

Additional evidence for hydrophilic complexes is found in studies of ionophores in model systems. Studies of the complexation kinetics of valinomycin in methanol [7] have indicated the formation of loose complexes with low K^+/Na^+ specificity and with diffusion-controlled rates of formation. The formation of a loose K^+ complex, with $K_s = 4\ M^{-1}$ (E. Grell, *unpublished observation*), or a loose Na^+ complex with $K_s = 3.5\ M^{-1}$ [7] is the first step in the formation of the tighter complexes, with $K_s = 3 \times 10^4\ M^{-1}$ and $4.7\ M^{-1}$, respectively. The ion specificity of valinomycin thus arises from the difference in energy for transforming these loose complexes into tight complexes.

The existence of loose "hydrophilic" complexes is also indicated from studies in phospholipid membranes. Complexes of valinomycin, the macrolide actins, enniatin B and 18-crown-6 were detected on the surface of dimyristoyl and dipalmitoyl lecithin [10, 12] by use of their electrostatic influence on theb inding constant of the anionic fluorescent probe 1-anilino-8-naphthalenesulfonate. The complexation reaction on the surface had the rank order of ion specificity $Rb^+ \approx K^+ > Cs^+ > Na^+$, as in the case of the biological reaction, but the K^+/Na^+ specificity ratios were low (<10). This is similar to the behavior of the K_m values in the present study which also have low ion specificity, indicating that the surface complexes in the two types of membrane may be similar. The experiments with the phospholipid vesicles were performed below the crystalline-liquid crystalline phase transition temperature of the membrane, a condition under which the ionophore could not penetrate the membrane [10, 12], and the formation of the surface complex was favored.

Turnover Number in Erythrocyte

Although the ionophore-mediated transport in red cells has not been studied as extensively as in the mitochondrial system, it is possible to compare the behavior of valinomycin in the two systems. The turnover numbers for valinomycin based on efflux of K^+ from cells ranged between 1 and

40 sec^{-1} with an average value of about 14 sec^{-1}. This value is two orders of magnitude larger than values we have calculated from published data [27]. This difference arises from the differences in red cell concentrations in the two experiments. The previous data were obtained at high dilution where probably only a fraction of the added valinomycin was in the membrane. The present data were obtained for a cell volume to system volume of about 0.2 where 100% of the added valinomycin was bound, whereas the previous data [27] was for much lower ratios, where this condition did not obtain.

The turnover numbers for valinomycin and K^+ measured in the present study are still two orders of magnitude lower than the maximal value obtained with this combination in rat liver mitochondria. This difference might be explained as the result of the high cholesterol content of the erythrocyte membrane being able to decrease the partitioning of the ionophore from the membrane surface to the hydrocarbon chain region of the membrane, or to decrease the mobility of the ionophore in the membrane interior. Absorption of the ionophore to the hemoglobin or to membrane proteins may account for part of this difference.

Ion Specificity

From the discussion of the present and the companion paper on ion extraction it is clear that the ion specificity of the complexation and of the transport reactions of the ionophores represents more than the intrinsic properties of the ionophores themselves. The quantitative ion specificity will vary somewhat with the environment in which these processes occur, and with the type of complex studied. Table 5 summarizes all of the ion specificity data of the present study and that reported for two-phase extraction and cation transport through membranes for the ionophores of the present study. It can be seen that although all studies are in good qualitative agreement, that differences in ion specificity ratio of as much as a factor 10 can result from comparison of the behavior of the ionophore in different systems. The excellent agreement reported between lipid bilayer conductivity [26] induced by the macrolide actins and their equilibrium constants for heterogeneous complexation in one solvent system [4] should not be expected when ionophore behavior in diverse membrane and solvent systems is compared. As was shown in the previous communication [14], the ion specificity observed in the two-phase extraction system varies somewhat with the choice of solvent, and the comparisons in Table 5 show that the ion specificity ratios observed in membranes depend somewhat upon the choice of membrane.

Table 5. Ion specificity ratios for ionophores determined in several membrane systems

Membrane	Ref.	Cs^+	Rb^+	K^+	Na^+
A. Valinomycin					
Mitochondria	present	0.51	0.89	1.0	0.00024
70% toluene-30% *n*-butanol	[14]	0.48	0.60	1.0	0.00011
Lipid bilayer, (*P*)	[18]	0.53	2.3	1.0	0.0035
Lipid bilayer, (*G*)	[18]	<0.25	—	1.0	$\leqq 0.012$
B. Nonactin					
Mitochondria	present	0.18	0.92	1.0	0.022
70% toluene-30% *n*-butanol	[14]	0.43	0.54	1.0	0.021
Lipid bilayer, (*P*)	[26]	0.037	0.48	1.0	0.0071
Lipid bilayer, (*G*)	[26]	0.039	0.48	1.0	0.0067
C. Monactin					
Mitochondria	present	0.11	0.57	1.0	0.039
70% toluene-30% *n*-butanol	[14]	0.40	0.67	1.0	0.050
Lipid bilayer, (*P*)	[26]	0.023	0.50	1.0	0.0075
Lipid bilayer, (*G*)	[26]	0.015	0.34	1.0	0.0048
D. Dinactin					
Mitochondria	present	0.17	0.82	1.0	0.071
70% toluene-30% *n*-butanol	[14]	0.21	1.0	1.0	0.021
Lipid bilayer, (*P*)	[18]	0.14	2.7	1.0	0.04
Lipid bilayer, (*P*)	[26]	0.014	0.42	1.0	0.0067
Lipid bilayer, (*G*)	[26]	0.013	0.48	1.0	0.0081
E. Trinactin					
Mitochondria	present	0.22	0.97	1.0	0.13
70% toluene-30% *n*-butanol	[14]	0.088	0.64	1.0	0.0120
Lipid bilayer, (*P*)	[26]	0.015	0.32	1.0	0.0099
Lipid bilayer, (*G*)	[26]	0.013	0.38	1.0	0.0042
F. Enniatin B					
Mitochondria	present	0.21	0.60	1.0	0.09
70% toluene-30% *n*-butanol	[14]	0.13	0.15	1.0	0.011
Lipid bilayer, (*P*)	[18]	0.062	—	1.0	0.027
G. 18-crown-6					
Mitochondria	present	0.30	0.45	1.0	$\leqq 0.1$
70% toluene-30% *n*-butanol	[14]	0.047	0.38	1.0	0.0035

(*P*) refers to values based on membrane potential measurements; (*G*) refers to membrane conductance measurements.

Conclusions

The maximal turnover numbers of the ionophores valinomycin, the macrolide actins, enniatin B and dicyclohexyl 18-crown-6 for the transport of the cations K^+, Rb^+, Cs^+ and Na^+ through the mitochondrial membrane have been compared with the equilibrium constants for the corresponding ionophore-cation combinations in the two-phase system. For a given ionophore, the turnover numbers and two-phase complexation constants are roughly proportional. However, the proportionality constants differ over about three orders of magnitude, showing the ionophore specificity macrolide actins > valinomycin ~ enniatin B > dicyclohexyl 18-crown-6. These observations may be explained on the basis of partitioning of the uncomplexed and complexed forms of the ionophore between various regions of the membrane.

Considerations of the cation concentration dependence of the transport phenomenon and the approximate proportionality mentioned above indicate that the net transport reactions of the ionophores may involve hydrophilic complexes as well as hydrophobic complexes capable of crossing the membrane.

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