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Two-Phase Partition Studies of Alkali Cation Complexation by Ionophores

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Summary. The partition of alkali cations and anions between an aqueous and an immiscible organic phase has been studied in the absence and presence of neutral and carboxylic ionophores of the valinomycin and nigericin types, respectively. Cation extraction into the organic phase was augmented considerably by the ionophores, and a cation specificity of $K^+ \geqq Rb^+ > Cs^+ \gg Na^+$ was found for all the neutral ionophores tested. Evidence is given that the actual values of ion specificity are a function of the solvent polarity, especially for valinomycin where an inversion of the K^+/Rb^+ specificity was observed. The ionophores examined have the following rank order of effectiveness for K^+ extraction into a standard organic phase consisting of 70% toluene-30% *n*-butanol: valinomycin > 18-crown-6 ≫ trinactin > enniatin B ≈ dinactin > monactin > nonactin. The ion affinity and selectivity data thus obtained have been compared with data previously reported.

In a toluene-butanol solvent, extraction of cations in the absence of ionophores occurs as ion pairs. On the other hand, the neutral ionophores extract the cations by the mechanism of complexation, with the lipophilic anions coextracted as free gegenionic species at lower ionophore complex concentrations. When the concentration of extracted cations exceeds 1×10^{-4} M, ion pairing between the ionophore complex and the anion occurs, and this tendency increases with increasing concentration and decreasing polarity of the organic phase. Anion pairing with the complexed cations is much less than for the free cations and this effect appears to be due to the larger distance of closest approach of the anion for the complexed cation.

The mobile carrier concept of ionophore-mediated cation transport [2, 17, 18] implies that the efficiency of the cation-transport reaction will depend strongly upon the equilibrium constant of the complexation reaction within the membrane and the kinetics of the complexation [3, 7, 9, 10, 19] and transport reactions. In order to facilitate predictions about complexation affinities in biological membranes, the ability of various ionophores

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to extract cations from an aqueous phase into an organic phase has been measured [4, 6, 17, 19].

It was shown that the negatively-charged, carboxylic ionophores, e.g. nigericin, could extract ions directly into the organic phase [17]. The neutral, valinomycin-type ionophores extract alkali cations from water most effectively when lipophilic anions such as CNS^- are present and thus able to accompany the ionophore-cation complex as a gegenanion [17, 19]. The $Na^+/K^+/Rb^+/Cs^+$ ion specificity ratios of several ionophores were compared with the ion specificity ratios for ionophore-facilitated transport through the mitochondrial membrane and good qualitative agreement was found [19]. This was taken as evidence that the efficiency of a given ionophore to act as a carrier was determined primarily by the stability constant of the complex in the hydrophobic interior of the membrane [19], and that the interfacial complexation and dissociation processes are generally too fast to constitute the rate-limiting steps of the transport reaction (*cf.* [9]).

Further support for the above interpretation has been provided by the studies of Eisenman and co-workers [2, 4, 21] in which proportionality was found between the equilibrium constants for the heterogeneous extraction process for the macrolide actin ionophores and their transport reactions as measured by lipid bilayer conductance values extrapolated to zero current.

The present communication extends the previous studies of ionophore-mediated cation extraction [4, 6, 17, 19]. We have now provided a comprehensive quantitative comparison of the Na^+, K^+, Rb^+ and Cs^+ extraction reactions of the ionophores valinomycin, the macrolide actin series, enniatin B, and 18-crown-6 in one solvent system. These data have been compared to the transport efficiencies of these ionophores for cations reported in the companion paper [11]. The effect of solvent on the complexation reactions and on the ion specificity of these reactions is discussed in terms of the interrelation of the cation complexation constant and changes in ionophore conformation.

Materials and Methods

Cation extraction was measured by shaking 1.0 ml of the organic phase with 0.5 ml of an aqueous phase, centrifuging, and sampling the organic phase. The aqueous phase consisted of 20 mM glycine and tricine, pH 7.4, 50 mM $Mg(CNS)_2$, a variable concentration of alkali cation, added as the sulfate salt, and a constant amount of carrier-free radioisotope of the test cations $^{22}Na^+$, $^{42}K^+$, $^{86}Rb^+$, $^{137}Cs^+$. The concentrations of cations in the organic phase were determined by liquid scintillation counting.

When the organic phase tested was denser than water (CCl_4 and CH_2Cl_2), the volumes of the aqueous and organic phases were each 0.5 ml, and the centrifugations were performed with a Coleman microcentrifuge with polyethylene tubes. The organic

phase was isolated, free of contamination from the aqueous phase, by puncturing the bottom of the tube.

The purity of the radioisotopes was established by three criteria: (1) The amount of extracted radioactivity decreased appropriately with increasing carrier concentration. (2) The isotope distribution coefficients for isotope extraction in a given system were constant with successive extractions with a given type of nonpolar phase. (3) In the case of $^{42}K^+$, decay curves gave the theoretical half-life of about 12.5 hr.

The amount of cation extraction attributable to the ionophore was determined as the total quantity extracted in presence of the ionophore minus the total extraction in absence of the ionophore. The ionophore concentration was usually 1.0×10^{-4} M in the organic phase, but sometimes as high as 5.0×10^{-4} M for ionophore-cation combinations with poorer affinities. All experiments were carried out at 21 °C unless otherwise specified. The sources and purity of the ionophores have been described in a previous communication [9]. Magnesium butyrate, valerate and heptanoate were formed by adding a stoichiometric amount of fatty acid to a suspension of MgO in water. The products were recrystallized twice at pH 7.0. Ammonium 1-anilino-8-naphthalenesulfonate (ANS^-) and bromothymol blue (BTB^-) were obtained from Pierce Chemical Co. Their extraction was measured by their fluorescent and absorption signals, respectively.

Results

Ion Extraction

The process of salt extraction into an organic phase will now be considered and compared with the process of ion extraction by ionophores in the next section. When an aqueous salt solution is shaken with an organic phase, a small fraction of the salt is extracted into the latter. The degree of extraction depends upon the nature of the anionic and cationic species, their concentrations in the aqueous phase, and the composition of the organic phase. It has been found here that ions with low valency and low hydration energy at high aqueous concentrations are extracted well. For example, when alkali cations (M^+) are supplied to the aqueous phase as SO_4^{2-} salts and when the lipid-soluble anion CNS^- is added as the Mg^{2+} salt, stoichiometric quantities of M^+ and CNS^- can be extracted into an organic phase consisting of 70% toluene, 30% *n*-butanol. The doubly charged spectator ions Mg^{2+} and SO_4^{2-} are not extracted appreciably and variations in their concentrations or in the concentrations of less lipophilic anions such as Cl^- do not appreciably affect the results. The degree of extraction increases with increasing aqueous concentration of the extracted ions and increases with decreasing polarity and increasing water content of the organic phase.

Extraction can be represented as the sum of two processes. The cation and anion can be extracted as free species:

$$M^+_{aq} + A^-_{aq} \overset{K_1}{\leftrightarrow} M^+_{org} + A^-_{org} \qquad (1)$$

where aq and org denote the aqueous and organic phases, respectively. The equilibrium constant for this process, K_1, is given by:

$$K_1 = \frac{[M^+]_{org}[A^-]_{org}}{[M^+]_{aq}[A^-]_{aq}}. \tag{2}$$

Within the organic phase it is possible for the two species to ion pair [6]:

$$M^+_{org} + A^-_{org} \overset{K_2}{\leftrightarrow} M^+A^-_{org} \tag{3}$$

with an equilibrium constant K_2 given by:

$$K_2 = \frac{[M^+A^-]_{org}}{[M^+]_{org}[A^-]_{org}}. \tag{4}$$

Under the experimentally verified conditions that $[M^+]_{org} = [A^-]_{org}$ (electrical neutrality) and that only the anion under consideration accompanies the extracted cation, the total concentration of extracted cation $[M^+]_{org(t)}$ is given by [13]:

$$\log[M^+]_{org(t)} = \log([A^-]_{aq}K_1) + \log\left(K_2[M^+]_{aq} + \frac{[M^+]_{aq}^{\frac{1}{2}}}{(K_1[A^-]_{aq}^{\frac{1}{2}})}\right). \tag{5}$$

Eq. (5) predicts that a plot of $\log[M^+]_{org(t)}$ against $\log[M^+]_{aq}$ for the condition $[A^-]_{aq} = \text{constant}$, will have a slope approaching 0.5 for the case $[M^+]_{aq} < (K_2^2 K_1 [A^-]_{aq})^{-1}$ (Condition I), where only the mechanism of Eq. (1) contributes to the extraction. At higher concentrations, $[M^+]_{aq} > (K_2^2 K_1 [A^-]_{aq})^{-1}$ (Condition II), when the contribution of Eq. (3) becomes significant, the slope of the log-log plot will increase, approaching a value of 1.0.

In the extraction systems reported in this study, Condition II proved applicable and the anions and cations were thus extracted predominantly as ion pairs. The results of a typical experiment are shown in Fig. 1, for the case of Na^+ extraction into 56% toluene – 44% *n*-butanol in the presence of 0.100 M CNS^- in the aqueous phase. A linear relationship is observed on the log-log plot with a slope of 0.93, indicating that the term in Eq. (5) involving the cation and anion concentrations to the one-half power made only a negligible contribution to $[M^+]_{org(t)}$. The value of the product $K_1 K_2$ was thus calculated as $[M^+]_{org(t)}/([A^-]_{aq}[M^+]_{aq})$. For the case where intermediate values of slope in the log-log plots were observed, the values of K_1 and K_2 were evaluated by curve fitting according to Eq. (5). Table 1 gives

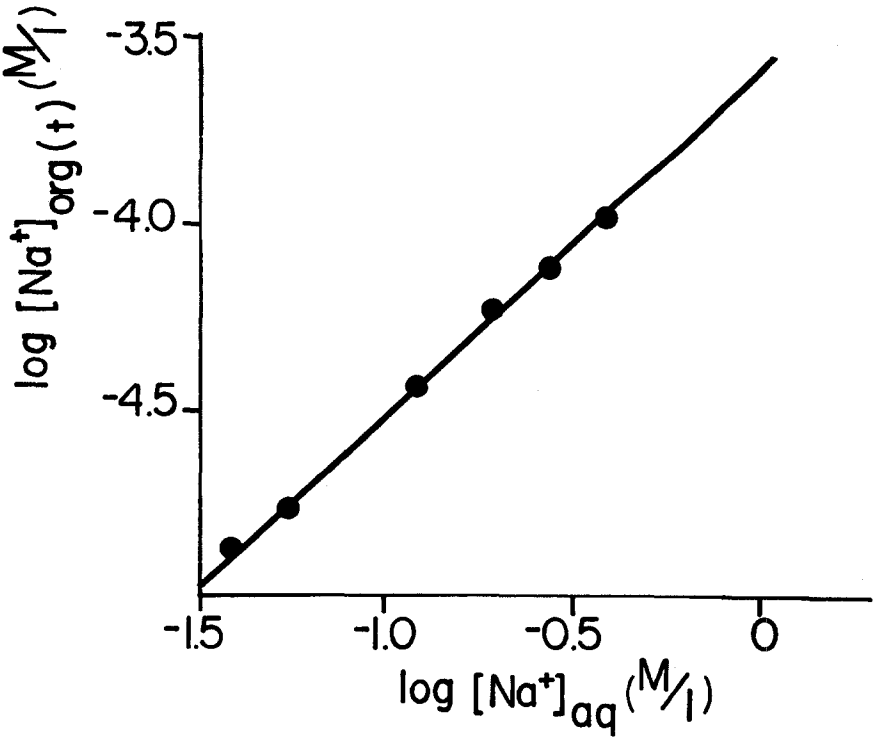

Fig. 1. The dependence of $[Na^+]_{org(t)}$ on $[Na^+]_{aq}$ for extraction into 56% toluene-44% *n*-butanol. The experiment was carried out as described in the text with $[CNS^-]_{aq} = 0.100$ M

the approximate values of K_1 and K_2 and their more accurately measured product, $K_1 K_2$, for the alkali cations with several different anions for a variety of solvent systems. Corrections for the variation of the activity coefficients of the ions in both the organic and aqueous phases as a function of the ionic strengths in these phases were made (*cf.* [8]) using the Debye-Hückel theory.

As has been pointed out in a previous communication the efficiency of a solvent for ion extraction is paralleled by its efficiency for extracting water [19]. Table 1 shows that the water concentrations in the organic phases at equilibrium with unit activity water vary from 1.1 M in 56% toluene – 44% *n*-butanol to 0.0024 M in hexane. Control experiments in which the water content and 90° light scattering of the organic phase were measured before and after extraction both in the absence and in the presence of ionophores indicate that the extraction involves only ionic and molecular species, and that no aqueous micelles were formed in the organic phase. This was not the case when extraction studies were attempted with the toluene-*n*-butanol

Table 1. Effect of cation and solvent on the distribution behavior in the two-phase system

Ions	Solvent [a]	K_1K_2 [b]	Slope [c]	K_1 [d]	K_2 [d]	ε		[H_2O] (moles/liter)
RbCNS	Cyclohexane	2.9×10^{-7}	1.0	$<10^{-16}$	$>10^{9}$	2.052		0.0024 [f]
RbCNS	CCl_4	6.8×10^{-6}	0.98	$<10^{-16}$	$>6\times10^{8}$	2.238		0.0087 [f]
K-ANS	Toluene	2×10^{-6}	—	—	—	2.438		0.011
K-BTB	Toluene	3×10^{-6}	—	—	—	2.438		—
K-ANS	Toluene/$CHCl_3$	3×10^{-7}	—	—	—			—
K-BTB	Toluene/$CHCl_3$	8×10^{-7}	—	—	—			—
RbCNS	CH_2Cl_2	1.3×10^{-5}	0.75	$6\times10^{-11\pm1}$	$2\times10^{5\pm1}$	9.08		
NaCNS	70:30	5.6×10^{-4}	0.87	$10^{-9\pm1}$	$10^{6\pm1}$	3.57	(3.08) [e]	0.54
KCNS	70:30	7.5×10^{-4}	0.86	$10^{-9\pm1}$	$10^{6\pm1}$			0.54
RbCNS	70:30	1.1×10^{-3}	0.82	$10^{-9\pm1}$	$10^{6\pm1}$			0.54
CsCNS	70:30	1.1×10^{-3}	0.79	$10^{-9\pm1}$	$10^{6\pm1}$			0.54
NaCNS	56:44	2.1×10^{-3}	0.91	$10^{-7\pm1}$	$10^{4\pm1}$	7.59	(6.87) [e]	1.1
KCNS	56:44	4.2×10^{-3}	0.82	$10^{-7\pm1}$	$10^{4\pm1}$			1.1
RbCNS	56:44	6.6×10^{-3}	0.89	$10^{-7\pm1}$	$10^{4\pm1}$			1.1
CsCNS	56:44	1.1×10^{-2}	0.88	$10^{-7\pm1}$	$10^{4\pm1}$			1.1

[a] Toluene/$CHCl_3$ is 1:1 (v/v); 56:44 and 70:30 refer to volume fractions of toluene and *n*-butanol.

[b] Evaluated for the condition $[M^+]_{aq}=[CNS^-]_{aq}=0.100$ M. In the [K-ANS] experiments $[ANS]_{aq}=1\times10^{-2}$ M and $[K^+]_{aq}$ varied between 0.01 and 0.1 M. In the BTB experiments $[BTB]_{aq}=7\times10^{-3}$ M and $[K^+]_{aq}$ varied between 0.01 and 0.1 M. These anions could not be used in the toluene-*n*-butanol systems since they caused micellarization.

[c] Slope of log-log plot as in Fig. 1, evaluated for $[M^+]_{aq}=0.10$ M.

[d] Calculated by fitting data as in Fig. 1 to Eq. (5). The order of magnitude uncertainty in the exact value of K_1 and K_2 results from the small variation in the slope over the range of concentrations in the experiment and from the rather large corrections for activity coefficient of the ions in both phases.

[e] Values given for solvent saturated with water.

[f] Ref. [13].

mixtures in the presence of about 5×10^{-3} M ANS^- or BTB^-. Micellarization and flocculation of material at the interface made this system unusable.

Table 1 shows that the value of K_1 increases with increasing dielectric constant of the solvent whereas the value of K_2 decreases. The product $K_1 K_2$ also increases with increasing solvent polarity, although neither the value of K_1, K_2, nor $K_1 K_2$ shows a great cation specificity. These effects will be discussed in terms of chemical and electrostatic interaction below.

Extraction with Ionophores

The more efficient process of extraction of alkali cations by neutral (I) and carboxylic (I^-) ionophores has been treated in a manner analogous to the above. The mechanism of extraction depends upon the charge of the ionophore, the polarity of the solvent and the total ionophore concentration.

Extraction with I^- ionophores, which mediate a M^+ for H^+ exchange [17], occurs by the following mechanism:

$$I^- - H^+_{org} + M^+_{aq} \overset{K_n}{\leftrightarrow} I^- - M^+_{org} + H^+_{aq} \tag{6}$$

with

$$K_n = \frac{[I^- - M^+]_{org}[H^+]_{aq}}{[I^- - H^+]_{org}[M^+]_{aq}}. \tag{7}$$

An apparent association constant $K_{n\,app}$ (equal to 10^{-7} K_n) can be defined as the reciprocal cation concentration required to produce half-maximal saturation with the cation at pH 7.0. Extraction experiments in which the ionophores nigericin and monensin were titrated with M^+ for differing ratios of the organic and aqueous phases showed that the extraction proceeded according to Eq. (6) and that only insignificant amounts of the ionophore were distributed into the aqueous phase. The ion specificity of nigericin and monensin has been reported previously [19]. The solvent dependence of the ion specificity is not very large. Furthermore, large changes in solvent polarity which have a large influence on the values of K_1, K_2 and $K_1 K_2$ (*cf.* Table 1) leave the extraction constants K_n for nigericin and monensin relatively unchanged. This is shown in Table 2.

The extraction reaction with the neutral I-type ionophores differs from that of the I^- ionophores in both mechanism and solvent dependence. The extraction mechanism can be considered as the sum of two separate reactions [4, 6]: (a) The cation and anion can be extracted as separate species by the mechanism:

$$M^+_{aq} + A^-_{aq} + I_{org} \overset{K_3}{\leftrightarrow} M - I^+_{org} + A^-_{org} \tag{8}$$

Table 2. Effect of solvent on the complexation constants of nigericin and monensin for Rb^+

Solvent	$K_{n(app)}$, Nigericin	$K_{n(app)}$, Monensin
Cyclohexane	2.0×10^2	1.5×10^3
Toluene	3.0×10^2	2.2×10^3
90% Toluene-10% *n*-Butanol	1.6×10^3	1.2×10^4
70% Toluene-30% *n*-Butanol	1.8×10^3	1.6×10^4
50% Toluene-50% *n*-Butanol	1.8×10^3	8.7×10^3
n-Butanol	2.4×10^3	5.5×10^3

with the equilibrium constant K_3 given by:

$$K_3 = \frac{[M-I^+]_{org}[A^-]_{org}}{[M^+]_{aq}[A^-]_{aq}[I]_{org}}; \tag{9}$$

(b) The ionophore-cation complex and the anion can form an ion-pair according to:

$$M-I^+_{org} + A^-_{org} \overset{K_4}{\leftrightarrow} M^+ - I - A^-_{org} \tag{10}$$

in a manner directly analogous to the reaction in Eq. (3). The equilibrium constant for this reaction is given as:

$$K_4 = \frac{[M^+ - I - A^-]_{org}}{[M-I^+]_{org}[A^-]_{org}}. \tag{11}$$

As has been shown previously [4, 6], the contributions of reactions (8) and (10) can be distinguished by their influence on the concentration dependence of the net extraction process. Under conditions in which the amount of free anion extracted in the absence of the ionophore is negligible compared with the amount of anion extracted in the presence of the ionophore, $[A^-]_{org}$ can be equated with $[M-I^+]_{org}$. This was the case in the present study. Eq. (9) can thus be rewritten as:

$$K_3 = \frac{[M-I^+]^2_{org}}{[M^+]_{aq}[A^-]_{aq}[I]_{org}}. \tag{12}$$

Evaluation of the ion extraction data according to Eq. (12) indicates that the mechanism of Eq. (8) is predominant for low levels of extraction, as

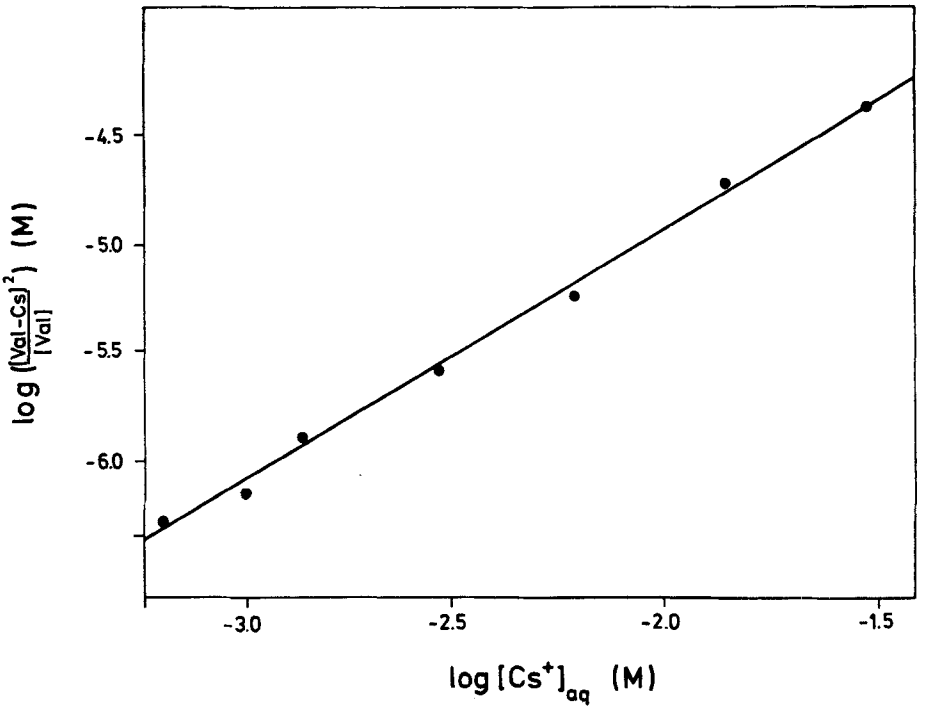

Fig. 2. Extraction of Cs^+ into 56% toluene-44% *n*-butanol by valinomycin. The experiment was carried out as described in the text with $[CNS^-]_{aq} = 0.066$ M and $[\text{valinomycin}]_{org} = 1.0 \times 10^{-4}$ M. $[I - M^+]$ was taken as the difference in the total cation concentration in the presence and in the absence of the ionophore

shown for the extraction of Cs^+ by valinomycin into 56% toluene – 44% *n*-butanol in Fig. 2. A linear relationship with a slope of 1.1 was observed between $\log([M - I^+]^2_{org})$ and $\log([M^+]_{aq})$. The upward deviation in the region of higher extraction is due to the contribution of ion pairing. Further discussion of this phenomenon will be given in a following section.

Experiments were performed to determine the values of K_3 for the neutral ionophores with the alkali cations Na^+, K^+, Rb^+ and Cs^+. Table 2 gives K_3 values for all the ionophores and cations studied for the solvent 70% toluene – 30% butanol. The K_3 values for K^+ give the rank order valinomycin > 18-crown-6 $\gg$ trinactin > enniatin B $\approx$ dinactin > monactin > nonactin for efficacy of the complexation reaction. The values K_3 were all independent of the total ionophore concentration, indicating that there was no tendency for formation of complexes with stoichiometry other than 1:1.

The data of Table 3 also give the ion specificity of the ionophores of this study for the nonpolar solvent 70% toluene- 30% *n*-butanol. The rank order $K^+ \geqq Rb^+ > Cs^+ \gg Na^+$ was found for the neutral ionophores.

Table 3. The complexing constant K_3 as a function of cation and ionophore

Ionophore	K_3 value ($\times 10^4$ M^{-1})			
	Cs^+	Rb^+	K^+	Na^+
Valinomycin	43.5	54.5	91.0	10^{-2}
	200[a]	1100[a]	400[a]	1.0[a]
Nonactin	0.06	0.075	0.14	0.003
Monactin	0.12	0.20	0.30	~0.015
Dinactin	0.19	0.91	0.91	~0.020
Trinactin	0.22	1.6	2.5	~0.030
Enniatin B	0.125	0.140	0.95	0.01
Dicyclohexyl 18-crown-6[b]	1.87	15.2	40.0	0.14

Values of K_3(M^{-1}) were determined for 70% toluene-30% *n*-butanol with CNS^- serving as the lipophilic anion. The values are from at least 3 separate experiments each involving at least 7 different cation concentrations. The total ionophore concentrations were chosen between 1×10^{-4} M and 5×10^{-4} M. The standard deviation of the mean of the K_3 values was ca. 25%.

[a] Obtained for 56% toluene-44% *n*-butanol.

[b] A mixture of the A and B isomers (*cf.* [6]).

Table 4. Influence of solvent on K_3 for valinomycin with ANS^-

Solvent	K_3 values (M^{-1})			
	Cs^+	Rb^+	K^+	Na^+
Toluene	3×10^{-4}	2×10^{-2}	2×10^{-2}	2×10^{-5}
50% Toluene-50% $CHCl_3$ (v/v)	—	3×10^{-2}	3×10^{-2}	4.9×10^{-4}

The degree of extraction was measured by isolating an aliquot of the organic phase, evaporating, redissolving in MeOH and measuring the ANS fluorescence signal. When the extraction was made with the absorption indicator BTB^-, the constants were 4 to 6 times larger. The concentration of valinomycin was varied between 1×10^{-5} and 3×10^{-5} M.

However, in 56% toluene-44% *n*-butanol the K^+/Rb^+ specificity of valinomycin is reversed, indicating that the ion specificity of this ionophore is definitely a function of solvent. Quantitative differences in the valinomycin ion specificity are also found in Table 4, which presents data for cation extraction into the less polar solvents toluene and 50% toluene $CHCl_3$ with the absorption and fluorescent probes BTB^- and ANS^- serving as gegenanions. These comparisons indicate that the ion specificity is not completely intrinsic to the ionophore, but can be influenced by the solvation of the ionophore complex in the organic phase.

Table 5 Effect of the fatty acid anion on the cation extraction process

Anion	K_1K_2(M^{-1})	K_3(M^{-1})	$\frac{K_3}{K_1K_2}$
Butyrate	1.5×10^{-3}	5.9×10^{-3}	4.0
Valerate	4.6×10^{-3}	2.5×10^{-2}	5.55
Heptanoate	7.6×10^{-2}	2.86×10^{-1}	3.85

The experiments were performed in 56% toluene-44% *n*-butanol with 1.0×10^{-4} M valinomycin and 0.100 M fatty acid anion, added to the aqueous phase as the Mg^{2+} salt.

The absolute value of K_3 depends upon the hydrophobicity of the accompanying anion, consistent with Eq. (8). Thus anions, or solvents, which give higher values of K_1 or K_1K_2 will also give higher values for the heterogeneous complexation constant for I [19]. Table 5 gives the effect of chain-length of fatty acid anions on their ability to act as gegenanions for K^+ and the valinomycin-K^+ complex. Both K_3 and K_1K_2 increase with increasing chain-length, but their ratio is constant, indicating that there is no direct interaction between the anion and the ionophore complex. A plot of $\log(K_1K_2)$ or $\log(K_3)$ against the number of CH_2 units in the fatty acid gave a value of -0.76 Kcal per CH_2 group for the free energy of transfer of the anion from an aqueous to a hydrophobic environment, in agreement with Traub's rule [22].

A significant degree of ion pairing between $I-M^+_{org}$ and A^-_{org} is observed when the concentration of the charged species in the organic phase is large, or when the organic phase is less polar. When ion pairing occurs, the calculated values of K_3 ($K_{3\,(app)}$) will actually be given as:

$$K_{3(app)}=\frac{([M-I^+]_{org}+K_4[M-I^+]^2)^2}{[I]_{org}[M^+]_{aq}[A^-]_{org}}. \tag{13}$$

Ion pairing serves to increase $K_{3\,(app)}$ above the value of K_3, determined for low degrees of extraction, according to the following relationship:

$$\frac{K_{3(app)}}{K_3}=(1+K_4[M-I^+])^2. \tag{14}$$

The value of K_4 for the ionophore was evaluated for low degrees of ion pairing by use of Eq. (14). Table 6 gives a comparison of the K_4 values obtained by this method with the ionophores for several combinations of the alkali cations, anions and organic solvents. It is noteworthy that the

Table 6. Ion pairing constants for the ionophore-cation complexes as a function of anion and solvent

Ionophore	Solvent	Anion	$K_4(M^{-1})$
Valinomycin	cyclohexane	CNS^-	$>1.4\times10^6$
Valinomycin	CH_2Cl_2	CNS^-	$\sim1\times10^4$
Valinomycin	56% toluene-44% *n*-butanol	CNS^-	1.7×10^4
Valinomycin	toluene	ANS^-	$\sim6\times10^4$
Valinomycin	toluene	BTB^-	$\sim6\times10^4$
Valinomycin	50% toluene-50% $CHCl_3$	ANS^- BTB^-	$<1\times10^4$ $<1\times10^4$
Valinomycin	70% toluene-30% *n*-butanol	CNS^-	$<3\times10^4$
Macrolide actins	70% toluene-30% *n*-butanol	CNS^-	1.5×10^4
Enniatin B	70% toluene-30% *n*-butanol	CNS^-	$\sim2\times10^4$
18-crown-6	70% toluene-30% *n*-butanol	CNS^-	$\sim1.5\times10^4$

value of K_4 is essentially independent of the ionophore and cation, but is quite sensitive to the composition of the organic solvent. The K_4 values are also much lower than the corresponding K_2 values.

At much higher ionophore concentrations, ion pair formation dominates, and the extraction reaction is described by:

$$K_3K_4=\frac{[M^+-I-A^-]_{org}}{[M^+]_{aq}[A^-]_{aq}[I]_{org}}. \tag{15}$$

Eq. (15) predicts a linear relationship between $[M^+-I-A^-]_{org}^{-1}$ and $[M^+]_{aq}$, as shown for the case of extraction of Rb^+ into CH_2Cl_2 with valinomycin shown in Fig. 3. A value of $5.5\times10^3\ M^{-2}$ was obtained in this experiment for K_3K_4. A similar result was obtained for the solvent CCl_4 with $K_3K_4=0.25\ M^{-2}$ for RbCNS extraction with 4×10^{-4} M valinomycin. The corresponding constant for RbCNS extraction into cyclohexane with 3×10^{-4} M valinomycin was $0.75\ M^{-2}$. The ratio of ion specificity for ionophore extraction of cations into 70% toluene-30% *n*-butanol based on K_3K_4 values, evaluated at high degrees of saturation have been given in previous communications [17, 19]. The specificity values based on K_3K_4 parallel those based on K_3 as would be expected from the above observation of the constancy of K_4. Thus, the tendency of $M-I_{org}^+$ and A_{org}^- to ion pair does not depend greatly upon whether M is Na^+, K^+, Rb^+ or Cs^+.

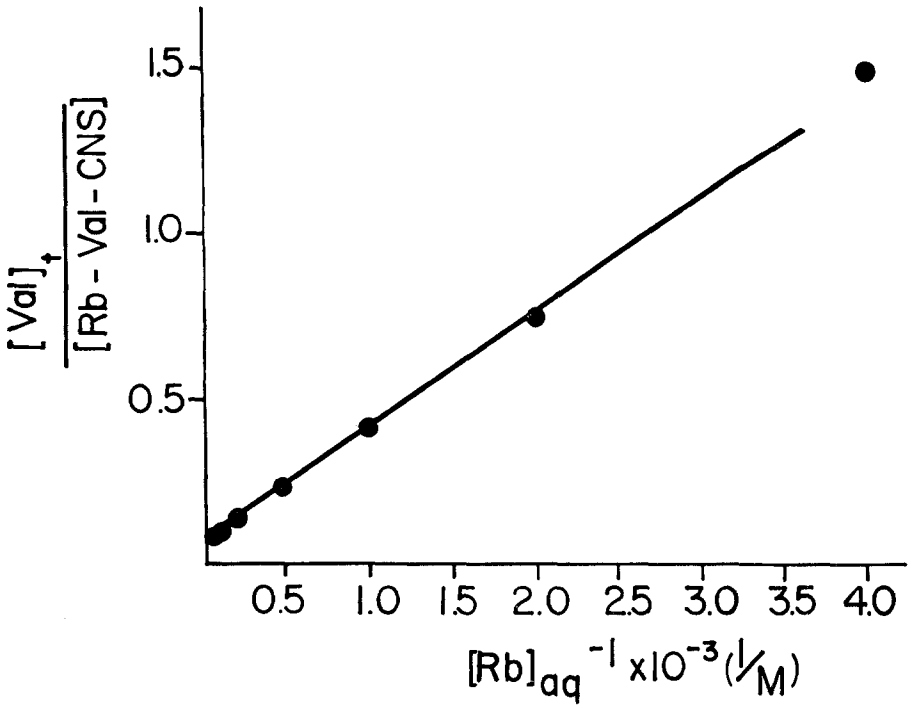

Fig. 3. Saturation behavior of valinomycin with RbCNS in CH_2Cl_2. The experiment was similar to that of Fig. 2 except that the organic phase was less polar and $[\text{valinomycin}]_{org} = 1.0 \times 10^{-3}$ M and $[CNS^-]_{aq} = 0.062$ M. A value of 5.5×10^{-3} M^{-2} was obtained for K_3K_4

Discussion

This study has catalogued the heterogeneous complexation constants of valinomycin, the macrolide actins, enniatin B, and 18-crown-6 under a common set of conditions. For the neutral ionophores, the rank order of cation specificity remains $K^+ \geqq Rb^+ > Cs^+ \gg Na^+$. These values are compared with literature values in Table 7. The rank order of ionophore specificity for the K^+ complexation reaction is valinomycin $\approx$ 18-crown-6 $\gg$ trinactin > enniatin B $\approx$ dinactin > monactin > nonactin. It is shown in the following paper [11] that these observations of the present study are in good agreement with the rank orders of cation and ionophore specificity of these ionophores operating as mobile carriers in a biological membrane, i.e. rat liver mitochondria, and that semi-quantitative agreement is obtained between the heterogeneous complexation constants and the actual rates for the ionophores and cations in this study.

The data of Table 7 show that the specificity of the ionophores can be influenced by the solvent composition and polarity of the organic phase.

Table 7. Ion specificity ratios for ionophores determined in several solvents

Solvent	Ref.	Cs^+	Rb^+	K^+	Na^+
A. Valinomycin					
70% toluene-30% *n*-butanol	(present, [8])	0.48	0.60	1.0	0.00011
56% toluene-44% *n*-butanol	(present, [8])	0.50	2.75	1.0	0.0025
toluene	(present)	0.015	1.0	1.0	0.001
50% toluene-50% $CHCl_3$	(present)	—	1.0	1.0	0.024
CH_2Cl_2	[5]	0.62	1.95	1.0	0.000017
B. Nonactin					
70% toluene-30% *n*-butanol	(present, [8])	0.43	0.54	1.0	~0.021
CH_2Cl_2	[4]	0.052[a]	0.43[a]	1.0	0.015[a]
C. Monactin					
70% toluene-30% *n*-butanol	(present, [8])	0.40	0.67	1.0	~0.050
hexane	[4]	0.19[b]	0.53[b]	1.0	0.0035[b]
64% hexane-36% CH_2Cl_2	[4]	0.011	0.22	1.0	0.0046
CH_2Cl_2	[4]	0.029	0.34	1.0	0.0094
D. Dinactin					
70% toluene-30% *n*-butanol	(present, [8])	0.21	1.0	1.0	~0.021
CH_2Cl_2	[4]	0.023	0.40	1.0	0.013
E. Trinactin					
70% toluene-30% *n*-butanol	(present, [8])	0.088	0.64	1.0	~0.0120
CH_2Cl_2	[4]	0.019	0.29	1.0	0.011
F. Enniatin B					
70% toluene-30% *n*-butanol	(present, [8])	0.13	0.15	1.0	0.011
G. Dicyclohexyl 18-crown-6[c]					
70% toluene-30% *n*-butanol	(present, [8])	0.047	0.38	1.0	0.0035
CH_2Cl_2	[6]	—	—	1.0	0.019[d]

[a] Average of values given in Table (15) of Ref. [4].
[b] Based on reported K_3 values in which the authors [4] indicated that the results may have been influenced by ion pairing in the organic phase.
[c] Mixture of the A and B isomers (*cf.* Ref. [6]).
[d] K_3 was calculated as $K_e \cdot K_d$ from Table 2 of Ref. [6].

As will be discussed below, this implies that either the complex conformation or the solvation energy of the complex is influenced by solvent composition, and that this in turn affects the binding constant for the cation. This reasoning is discussed at greater length below, following a consideration of the effect of dielectric constant on the extraction process.

Influence of Dielectric Constant on the Extraction Process

The electrostatic energy of partition of ions and ionophore complexes into membranes or media of low dielectric constant has been evaluated according to the Born equation [16]. The ionophore-cation extraction reactions have also recently been discussed in the context of the Born equation [5], but the applicability of the Born equation to these systems was not dealt with numerically. It was thus of interest to compare the present extraction data with the predictions of this model.

The electrostatic energy for introducing a charge into a homogeneous medium is given by:

$$E = e^2/2\varepsilon r \tag{16}$$

where e is the electronic charge, ε is the dielectric constant of the medium and r is the ionic radius. The electrostatic energy for the process corresponding to Eq. (1) would be given as:

$$E = (e^2/2r_1)(1/\varepsilon_2 - 1/\varepsilon_1) + (e^2/2r_2)(1/\varepsilon_2 - 1/\varepsilon_1) \tag{17}$$

where r_1 and r_2 refer to the cation and anion radii, respectively, and where ε_1 and ε_2 refer to the dielectric constants of the aqueous and organic phases, respectively. Eq. (17) was tested with the experimental data on KCNS extraction into 70% toluene and 30% *n*-butanol. Using a value of 1.33 Å for the radii of K^+ and CNS^- and a value of 3.57 for the dielectric constant of the organic phase saturated with water, an electrostatic energy of +57 kcal is calculated for the extraction process. This corresponds to a K_1 value of about 3×10^{-43}, which is about 34 orders of magnitude smaller than the measured value! A further difficulty with the application of the Born equation is that it does not give the proper ion specificity of the cation extraction reaction. Using crystallographic radii and data for this same solvent system, Cs^+/Na^+ specificity values of the order of 10^{12} are calculated whereas the experimentally measured upper limit for this selectivity is 10^1. This gross disagreement between the experimental data and the Born

model indicates that the latter does not have a great deal of predictive value in these solvent systems, and that specific solvation effects must be operating in the two-phase extraction system.

Flexibility of Ionophore Complexes

From the forgoing discussion, it is clear that the process of ion extraction is not determined by electrostatic energy alone, and that ion hydration makes a large energetic contribution. This is especially true for the case of extraction with the ionophores, in which the interaction of the cation and the complexing groups of the ionophore can result in a large ion specificity of complexation. The conformation of the complexed ionophore can either be thought of as a rigid charge into which the cation can be placed, or as a flexible ligand capable of adapting its cavity size and conformation to fit the complexed cation. These two models are similar to the "lock and key" and "induced fit" models for enzyme substrate interaction. The first model would produce a complex whose properties such as conformation and binding relative to that for other cations are essentially independent of solvent. The second model, which allows for conformational change of the complex under the influence of the solvent, temperature and other parameters would predict that the relationship of binding constants for the different cations could vary with these external parameters.

Evidence for or against these two models can be obtained either from studies of the complex conformation as a function of cation, solvent and temperature, or can be deduced indirectly from the behavior of the ion or ionophore specificity with solvent composition.

Experiments of the first type have been carried out for nonactin, valinomycin and enniatin B. Prestegard and Chan [20] presented evidence based on 200 MHz nuclear magnetic resonance (NMR) data that the Na^+, K^+ and Cs^+ complexes of nonactin in acetone-d_6 are slightly different in structure. NMR experiments [8, 10] and circular dichroism (CD) experiments [7] give evidence for subtle differences between the K^+, Rb^+ and Cs^+ complexes of valinomycin, and indicate a larger difference with the Na^+ complex. Ovchinnikov *et al.* [15] have shown that the Li^+, Na^+, K^+ and Cs^+ complexes of enniatin B differ greatly with respect to the orientation of the complexing carbonyl groups about the complexed cation. These differences in complex conformation could easily express themselves in different solvent dependencies of the cation binding constants, and thus a solvent-dependent ion specificity.

A second argument for conformational flexibility of the complexes comes from consideration of the solvent dependence of the cation specificity and the ionophore specificity. The free energy of the complexation reaction can be artificially divided into electrostatic contributions (ΔG_{el}) and chemical contributions such that:

$$-RT\ln K_3 = \Delta G_{el} + \Delta G_{solv,A^-} + \Delta G_{solv,M^+} + \Delta G_{solv,I} + \Delta G_{conf,I} \tag{18}$$

where the last four ΔG terms represent the free energies of chemical interactions. $\Delta G_{solv,A^-}$ is the change in solvation energy of A^- upon transfer from the aqueous phase to the organic phase. $\Delta G_{solv,M^+}$ is the change in solvation energy of M^+ upon transfer from the aqueous phase into the cavity of the ionophore. $\Delta G_{solv,I}$ is the change of solvation energy of the ionophore upon complexation of the cation, and $\Delta G_{conf,I}$ is the change in energy of the ionophore associated with a change in its conformation upon complexation of the cation. The ratio of ion specificity for cation M_1^+ and M_2^+ can be developed from Eq. (18) from the difference in free energies according to:

$$-RT\ln(K_{3,M_1^+}/K_{3,M_2^+}) = \Delta G_{solv,M_1^+} - \Delta G_{solv,M_2^+} + \Delta G_{solv,I(M_1^+)} - \Delta G_{solv,I(M_2^+)} + \Delta G_{conf,I(M_1^+)} - \Delta G_{conf,I(M_2^+)}. \tag{19}$$

A similar formulation has recently been given by Eisenman *et al.* [5]. The rigid cage model would assume that all of the terms on the right in Eq. (19) are independent of the choice of nonpolar solvent. This assumption, termed the "isosteric postulate" by Eisenman *et al.* [4], predicts that the ion specificity will be independent of solvent. The data of Table 7 show that for valinomycin and monactin this is clearly not the case.[1] The flexible ligand model would allow any of the terms on the right side of Eq. (19) to be a function of the solvent.

The solvent dependence of the ion specificity of valinomycin and monactin resulting from conformational flexibility can be considered in terms of two limiting cases: (a) The conformation of any given ionophore-cation complex may be completely dependent upon the cation and completely independent of the solvent. In this case, the solvent dependence of the ion

1 Actually, evidence against the "isosteric principle" can be found in the paper in which this principle was elaborated (*cf.* Table 5, ref. [5]).

specificity would be due to differences in the solvent dependencies of $\Delta G_{\text{solv, I}(M_1^+)}$ and $\Delta G_{\text{solv, I}(M_2^+)}$. (b) In the second limiting case it is assumed that the conformation of a given ionophore-cation complex may vary with solvent composition. In this case, the changes in ion specificity would be accounted for by differences in the solvent dependencies of $\Delta G_{\text{solv, }M_1^+}$ and $\Delta G_{\text{solv, }M_2^+}$. Either case indicates that there will be small differences in the conformation of the complexed form dependent on the size of the complexed cation, a conclusion supported by the NMR and CD studies cited above.

It is shown by comparison of Tables 1 and 5 that the ionophore cation complexes have less of a tendency to form ion pairs with available gegenanions than does the free cation. This is consistent with the larger distance of closest approach of the anion for the cation when the latter is bound by the ionophore molecule. Boone and Kowalsky [1] have given evidence for extensive ion pairing of valinomycin-M^+ and (18-crown-6)-M^+ complexes with $CoBr_4^{2-}$ and $Co(SCN)_4^{2-}$ in organic solvents using an NMR technique In the present study, the ion pairing constants do not differ greatly for the different ionophores, indicating that the distances of closest approach are large and essentially independent of the complexed cation. The subtle conformation differences in the ionophore-cation complexes do not alter the accessibility of the anion to the bound cation.

Ionophore Structure Alterations

The effects of solvation and conformational variability of the ionophore complexes can also be considered from the effect of subtle structural alterations of the ionophore on its complexation constant for a given ion. The homologs of the macrolide actin series differ only in variations of substitution of CH_3 for H at sites five bonds removed from the liganding oxygen atoms. The ratio of the heterogeneous complexation constants (enhancement ratio) for two derivatives of a given ionophore with a given cation, anion and solvent can be expressed in a formulation such as in Eq. (19) in which the subscripts I and M are exchanged. Exactly the same arguments apply regarding ionophore conformation and solvation as in the considerations of ion specificity discussed above. As observed in previous studies [4, 8, 23], the complexation constants for the macrolide actins increase with increasing methyl substitution for the ionophore (nonactin to trinactin). The values of the trinactin/nonactin enhancement ratios calculated for Na^+, K^+, Rb^+ and Cs^+ are 10, 18, 21 and 37, respectively, in the present study. Eisenman *et al.* [4] reported corresponding ratios of 14, 16, 11 and 6.4. Both sets of

data show that increasing methyl substitution serves to increase the complexing ability of the ionophore much more for K^+ and Rb^+ than for Cs^+ and Na^+. The monactin/nonactin enhancement ratio also varies with the organic solvent. For Na^+ complexation it is 8.8 in methanol [23], 2.6 in CH_2Cl_2 [4], and about 5 in 70% toluene-30% *n*-butanol. The corresponding values for K^+ complexation are 51 to 82 in methanol [23], 3.8 in CH_2Cl_2 [4], and 2.1 in 70% toluene-30% *n*-butanol. This obvious effect of solvent on the enhancement ratio is further evidence that changes in solvent result in changes in the conformational or solvation energy of the ionophore upon achievement of a form fit between the cation and the molecule.

The enhancement effect of methyl substitution was considered by Eisenman *et al.* [4] to be the result of "electron repelling action of the methyl group increasing the dipole moments of the ligand oxygens." It is difficult to imagine how this effect which must be conducted through five chemical bonds could result in a 50-fold change in the complexation constant. It is also unclear why such an inductive effect should show an ion specificity.

In view of the solvent dependencies of the complexation constants and ion specificities of the ionophores, it is not surprising that the ratios of the complexation constants of a single cation for two different ionophores can also vary with solvent composition. This can be shown by comparison of the K^+ binding constants for valinomycin and nonactin in methanol (3×10^4 for valinomycin [7], and 3.9 to 6.3×10^3 for nonactin [23]), with the corresponding K_3 values of the present study. In methanol the ratio of the valinomycin to nonactin binding constant is 4.8 to 7.6 while in 70% toluene-30% *n*-butanol (*cf.* Table 3) this ratio is increased to 303. The increase in the valinomycin complexation constant compared with the nonactin constant as the solvent polarity is lowered can be explained on the basis of stabilization of the complexed conformation in the lower polarity solvent by the formation of intramolecular hydrogen bonds [12, 14].

Conclusions

The extraction of ions from an aqueous phase into an organic phase both in the presence and in the absence of neutral ionophores is strongly dependent upon the polarity or dielectric constant of the organic phase, while extraction by carbonylic ionophores which form neutral complexes is less so. The ion specificity of complexation in this study was in qualitative agreement with the ion specificities determined in other studies. However, quantitative differences in the ion specificity and the relative strength of

complexation of different ionophores have been noted as a function of solvent polarity. These differences have been attributed to differences in the solvation energies of, and to differences in the conformation of, the complexed form. These differences appear to be more pronounced in the case of valinomycin.

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