Sodium Ion–Proton Exchange Reactions of the Carboxylic Acid Ionophore Monensin

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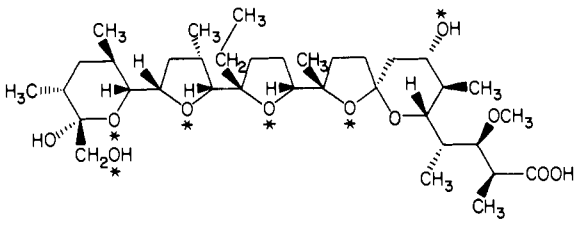
Abstract: The reaction between sodium monensin (NaMon) and the acid–base indicator 2,4-dinitrophenol has been investigated in ethanol at various temperatures between −15 and 25 °C. The overall reaction, viz., the replacement of Na^+ by H^+ on monensin to give monensic acid (MonH), occurs by two mechanisms: (a) a rate-determining dissociation of Na^+ from NaMon, followed by rapid protonation of Mon^-, or (b) a direct exchange involving pre-equilibrium protonation of NaMon to give $NaMonH^+$, followed by dissociation of Na^+ from $NaMonH^+$. Under limiting high acid conditions, direct exchange of H^+ for Na^+ via $NaMonH^+$ occurs about 400 times more rapidly than that via the monensin anion, Mon^-. The pK_a of $NaMonH^+$ and the kinetics, equilibria, and activation parameters of $NaMonH^+$ with respect to dissociation of Na^+ have been measured. The stability constant of the Na^+ complex of MonH, $NaMonH^+$, is almost 10^5 times lower than that of Mon^-. This results from a decrease in formation rate constant (200-fold) and an increase in dissociation rate constant (400-fold) relative to NaMon. Large negative entropies of activation are observed for both formation and dissociation reactions of $NaMonH^+$, suggesting that Na^+ is strongly solvated in the transition state.

Monesin (MonH, I) is a member of the nigericin group of carboxylic acid ionophores or polyether antibiotics, which are characterized by their ability to form electrically neutral 1:1 or 2:1 ligand–cation complexes.[2,3] These ionophores possess multiple ether linkages, usually in the form of tetrahydrofuran or tetrahydropyran rings, and a carboxylic acid group which is partially dissociated at physiological pH values. The transport properties of the ionophores are known to involve exchange of M^+ for H^+, or cation for cation, via the electrically neutral complexed or protonated species.[3,4] However, evidence for the importance of charged, dimeric species of the form $(HL_2)^-$, $(CaHL_2)^+$, where L^- represents the antibiotic anion, has also been presented.[5]



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Stability constants of alkali metal and alkaline earth metal complexes of monensin have been investigated in several solvents[6-11] and have shown that in addition to the normal acid–base dissociation (eq 1) and complexation reactions (eq 2), complexes

$$\mathrm{MonH} \overset{K_a(\mathrm{MonH})}{\rightleftharpoons} \mathrm{Mon^-} + \mathrm{H^+} \qquad (1)$$

$$\mathrm{M^+} + \mathrm{Mon^-} \overset{K_s(\mathrm{M{\cdot}Mon})}{\rightleftharpoons} \mathrm{M{\cdot}Mon} \qquad (2)$$

involving the neutral monensin molecule are also formed in acid solution (eq 3).[6,12] Similar behavior has been reported for the structurally related ionophores grisorixin[13] and nigericin.[14]

$$\mathrm{M^+} + \mathrm{MonH} \overset{K_s(\mathrm{M{\cdot}MonH^+})}{\rightleftharpoons} \mathrm{M{\cdot}Mon\ H^+} \qquad (3)$$

It is highly likely that complexes of the form shown in eq 3 will be involved in H^+/M^+ exchange reactions of monensin, as the protonated complexes ($M{\cdot}MonH^+$) should be more labile than the thermodynamically more stable neutral complexes, M·Mon. It is known, for example, that dissociation of alkali metal and alkaline earth metal ions from synthetic macrocyclic cryptand and diazapolyether ligands, which contain basic nitrogen donor atoms, is often strongly catalyzed by acids.[15-18] Recently Krause et al.[18] have also shown that the dissociation of calcium and magnesium from the antibiotic A 23187 (calcimycin) is very sensitive to pH. At low pH values complex dissociation preferentially occurs via the protonated AHM^{2+} complex, whereas in the neutral or alkaline pH range direct dissociation from AM^+ is observed (A^- = calcimycin anion).

In the present paper we report a kinetic and thermodynamic study of the proton/Na^+ exchange reaction between sodium monensin and the acid–base indicator 2,4-dinitrophenol (2,4-DNPH,HA) in ethanol (eq 4). 2,4-Dinitrophenol was chosen because it is a sufficiently strong acid to complete effectively with

$$\mathrm{NaMon} + \mathrm{HA} \overset{K_e}{\rightleftharpoons} \mathrm{MonH} + \mathrm{Na^+} + \mathrm{A^-} \qquad (4)$$

Na^+ for the monensin anion, and the strong absorption of the 2,4-dinitrophenoxide ion in the UV/visible region provides a convenient means of monitoring the reaction. The stability constant and rates of formation and dissociation of NaMon (the most stable of the alkali metal monensin complexes) in ethanol are available from an earlier study.[10]

Experimental and Results

Sodium monensin (Sigma or Calbiochem Corp.) was purified by recrystallization from methanol/water as previously described[10] or used as

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obtained. No difference in kinetic behavior of the different samples was observed. MonH was perpared from NaMon by stirring in a two-phase aqueous $HClO_4$/chloroform system as described previously.[10] Ethanol (BDH "AristaR" or May and Baker, <0.1% H_2O) was used without further purification.

2,4-Dinitrophenol solutions were prepared from high-purity commercial samples (BDH, Merck) and standardized by titration against standard alkali or by spectrophotometric measurements at λ = 390 nm. This was necessary because of variable amounts of water added to stabilize solid 2,4-dinitrophenol. At the concentrations used in kinetic and thermodynamic measurements ($\leq 5 \times 10^{-3}$ M), the amount of water introduced to the ethanol was negligible.

Tetrabutylammonium ethoxide solutions were prepared by dilution of a methanolic solution of tetrabutylammonium methoxide (Aldrich, 1 M). Final solutions used contained ≤0.1% v/v MeOH. Sodium ethoxide solutions were prepared by dissolving either metallic sodium or solid NaOH in ethanol. Again the quantity of H_2O introduced into ethanol was less than that contained in the pure solvent used (i.e., ≤0.1% v/v).

pK_a Determinations. The acid-dissociation constants of monensin and 2,4-dinitrophenol were determined at 25 °C with standard techniques of pH measurements with glass electrodes in ethanol[19] as in cell A. The

glass electrode	HA $\rightleftharpoons$ $H^+ + A^-$ in EtOH	KCl sat. aqueous solution	calomel

A

salt bridge connecting the two half-cells contained 0.1 M Bu_4NBr in ethanol. A Radiometer PM 82 pH meter was used for all measurements. The true pH is related to the observed pH, $(pH)_{obsd}$ (based on calibration of the glass electrode–calomel system in aqueous buffers), by eq 5, in which δ is a correction factor for the change in hydrogen ion activity and

$$pH = (pH)_{obsd} + \delta \quad (5)$$

liquid junction potential on transfer from H_2O to EtOH. A value of δ = 2.28 (±0.03) was obtained from measurements in dilute $HClO_4$ solutions (8×10^{-5} to 1×10^{-3} M). pH values were determined for a number of HA/A^- ratios obtained by titrating Bu_4NOEt–EtOH into HA solutions in ethanol and used in conjunction with eq 6 to determine pK_a(HA). The activity coefficient of HA was assumed to be unity, and $\gamma_\pm$ values

$$pK_a(HA) = pH + \log \frac{[HA]\gamma_{HA}}{[A^-]\gamma_\pm} \quad (6)$$

$$\log \gamma_\pm = -\frac{Z^2 A I^{1/2}}{1 + I^{1/2}} + \frac{Z^2 A I}{3} \quad (7)$$

were calculated from the Davies eq 7,[20] where A = 2.916 for ethanol at 25 °C and I = ionic strength. At the low ionic strengths used ($\leq 8 \times 10^{-4}$ M) activity coefficient corrections were small. The results obtained were as follows:

$$pK_a(MonH) = 11.45\ (\pm 0.07)$$

$$pK_a(2,4\text{-dinitrophenol}) = 8.32\ (\pm 0.15)$$

The stability constant, K_e, in eq 4 is related to the stability constant of NaMon and the above pK_a values by eq 8. Using the present results together with the earlier reported log K_s(NaMon) = 8.80 (±0.1)[10] gives log K_e = −5.70 (±0.30).

$$\log K_e = pK_a(MonH) - pK_a(2,4\text{-dinitrophenol}) - \log K_s(NaMon) \quad (8)$$

Calorimetric Measurements. The enthalpy change for reaction 4 was measured at 25 °C with a Tronac 457 titration calorimeter. To ensure complete reaction, the reaction was carried out in the reverse direction to that shown in eq 4, by titrating MonH into excess sodium 2,4-dinitrophenoxide (Na-2,4-DNP)/2,4-dinitrophenol (total concentration 10^{-2} M). The result obtained was

$$\Delta H(\text{eq } 4) = 28.5\ (\pm 0.3)\ \text{kJ mol}^{-1}$$

This value, which refers to an ionic strength of 10^{-2} M, was independent of the concentration of MonH used in the titration (5×10^{-4} to 5×10^{-3} M) or the relative concentrations of Na-2,4-DNP and 2,4-DNPH. The presence of excess Na-2,4-DNP prevented reaction of 2,4-DNPH with NaMon to generate $NaMonH^+ + 2,4\text{-}DNP^-$ under the conditions used in the calorimetric titrations (see below).

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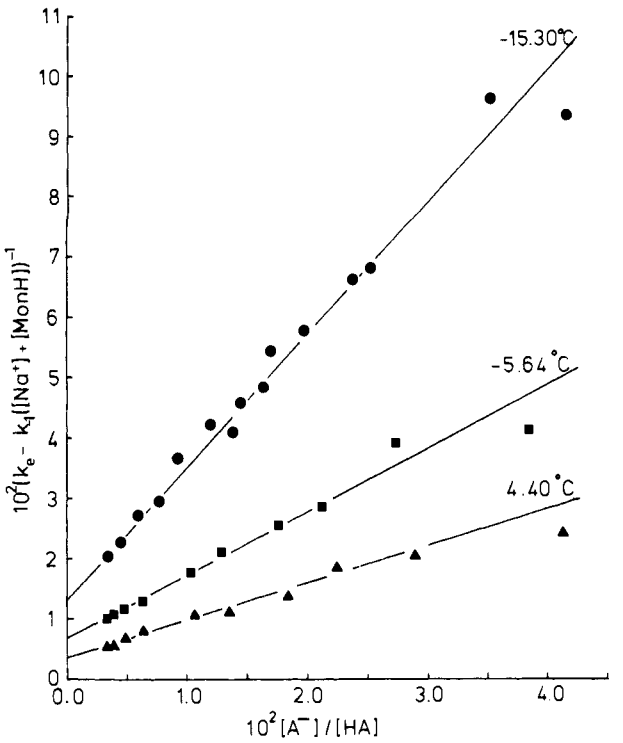

Figure 1. Rate constants for the reaction of sodium monensin with 2,4-dinitrophenol in ethanol plotted according to eq 12.

Kinetic Measurements. Kinetic measurements were carried out at seven different temperatures ranging from −15.3 to 25 °C by monitoring the appearance of the 2,4-dinitrophenoxide anion, A^-, at λ = 405 nm, using a home-built, all-glass, stopped-flow apparatus with optical detection. Reactions were normally initiated by mixing NaMon (1×10^{-5} M before mixing) with an excess of HA (1.5×10^{-4} to 3.2×10^{-3} M before mixing), but in some cases known amounts of $NaClO_4$ or Me_4NOH (to generate $Me_4N^+A^-$) were also added. Qualitatively, in all cases, an immediate increase in absorbance occurred during the mixing time of the apparatus (ca. 2 ms) and this was followed by a first-order approach to equilibrium. The observed first-order rate constant, k_e, (i) increased with [HA], showing saturation at higher concentrations, (ii) increased with added $[Na^+]$, (iii) decreased with added $[A^-]$, and (iv) increased slightly with increasing [NaMon] (depending upon [HA]).

This behavior is consistent with a kinetic scheme as shown in eq 9 and 10, in which the proton-transfer reaction 9 is rapid on the stopped-flow time scale. It may be shown that a simple first-order approach to

$$NaMon + HA \overset{K}{\rightleftharpoons} NaMonH^+ + A^- \quad (9)$$

$$NaMonH^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Na^+ + MonH \quad (10)$$

equilibrium for eq 4 will be observed under two limiting conditions: (a) when HA is in sufficiently large excess or is sufficiently strong that the reverse reaction is negligible, and (b) when the reaction is observed close to the final equilibrium position (typical relaxation conditions).[21] Under the latter conditions, if the final equilibrium concentration of the intermediate complex $NaMonH^+$ is negligible (see below), the observed first-order rate constant is given by eq 11, where the concentrations refer to the equilibrium concentrations obtained after mixing.[22] The results

$$k_e = \frac{k_1 K[HA]}{[A^-] + K[HA]} + k_{-1}([Na^+] + [MonH]) \quad (11)$$

were found to be quantitatively consistent with eq 11, using the fitting procedure as follows. Starting from the measured value of K_e at 25 °C extrapolated to −15.3 °C (where the most comprehensive data set was obtained) with the measured ΔH value, concentrations of $[Na^+]$,

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(22) The full expression including $[NaMonH^+]$ is given by:

$$k_e = \frac{k_1(K[HA] + [NaMonH^+])}{[A^-] + [NaMonH^+] + K[HA]} + k_{-1}([Na^+] + [MonH])$$

Table I. Rates and Equilibria of Reaction between 2,4-Dinitrophenol and Sodium Monensin in Ethanol at 25 °C

T, °C	k_1,[a] s^{-1}	k_{-1},[b] $M^{-1}\,s^{-1}$	log K_s(NaMonH$^+$)[c]	log K[d]	log K_e[e]
−15.30	75.2	1.7×10^6	4.36	−2.26	−6.61
−10.55	97.1	2.0×10^6	4.31	−2.23	−6.52
−5.64	145	2.1×10^6	4.16	−2.24	−6.40
−0.72	199	2.5×10^6	4.11	−2.22	−6.31
4.40	274	2.8×10^6	4.01	−2.20	−6.22
10.40	371	3.0×10^6	3.91	−2.19	−6.10
25.00	810	4.8×10^6	3.77	−2.10	−5.84

[a] Rate constant for dissociation of Na$^+$ from NaMonH$^+$, ±10%. [b] Rate constant for formation of NaMonH$^+$, ±20%, see text. [c] $K_s = k_{-1}/k_1$, ±0.10. [d] ±/0.10, eq 9. [e] ±0.20, eq 4.

[HMon], and [A$^-$] (all equal in the absence of added Na$^+$ or A$^-$) were calculated. Then by using eq 12, obtained rearranging eq 11, plots of $\{k_e - k_{-1}([Na^+] + [MonH])\}^{-1}$ vs. [A$^-$]/[HA] were made for various k_{-1}

$$\{k_e - k_{-1}([Na^+] + [MonH])\}^{-1} = \frac{1}{k_1} + \frac{1}{k_1 K} \cdot \frac{[A^-]}{[HA]} \qquad (12)$$

values giving slope $(k_1K)^{-1}$ and intercept $(k_1)^{-1}$. The resulting parameters, k_1, k_{-1}, and K, were then compared with the original value of K_e ($=K_1K$ where $K_1 = k_1/k_{-1}$). The value of K_e was then varied until the best overall fit was obtained. This was repeated at the various temperatures studied, taking into consideration also the measured enthalpy of reaction. A summary of the results obtained is given in Table I, and examples of plots according to equation 12 are given in Figure 1. A full set of data together with k_e values calculated from the parameters given in Table I is included in the Supplementary Material. Several comments may be made on the results and the fitting procedure as follows:

(i) The k_1 values, i.e., the rate constant for dissociation of Na$^+$ from the protonated complex, NaMonH$^+$, are very insensitive to variations in K_e, e.g., even a twofold variation in K_e from those listed in Table I leads to only a 10% variation in k_1. This is because at high [HA], K[HA] ≫ [A$^-$] and k_{-1} ([Na$^+$] + [HMon]) approaches a constant, $2k_{-1}$[NaMon]$_T$, where [NaMon]$_T$ is the stoichiometric concentration of NaMon added.

(ii) The K_e value at 25 °C, which corresponds to log K_e = −5.84, agrees within the experimental uncertainty with the value measured potentiometrically (log K_e = −5.70 ± 0.30). However, it is difficult to fix K_e precisely from the kinetic data as it is possible by using appropriate combinations of k_{-1} and K to obtain reasonable fits to the data for a range of K_e values. For this reason k_{-1} and K values could not be established to better than ±20%.

(iii) Excellent agreement (normally to ±5%) between measured rate constants and those calculated from eq 11 and the parameters in Table I was obtained for all [HA] ≥ 1×10^{-4} M. At concentrations below ca. 5×10^{-5} M, the equilibrium concentration of NaMonH$^+$ (see below) was not negligible with respect to K[HA] and the full kinetic equation[22] is required in treating such results. Data obtained at these very low concentrations of HA were not used in the fitting procedure but are included in the supplementary data.

The enthalpies and entropies of reaction and activation derived from the results in Table I are listed in Table II. It should be noted that the stability constant for formation of NaMonH$^+$ (eq 3) is given by K_s(NaMonH$^+$) = k_{-1}/k_1, and this value and associated enthalpies and entropies of complexation are also reported in Tables I. and II.

In terms of the kinetic treatment it may readily be shown from the equilibrium constants in Table II that at the concentrations of NaMon used (normally 5×10^{-6} M) the equilibrium concentration of NaMonH$^+$ will be ≤5% of the concentrations of Na$^+$, MonH, and A$^-$, even at the highest concentrations of 2,4-dinitrophenol used. However, they are not negligible with respect to K[HA] values[22] at very low concentrations of HA (≤5×10^{-5} M).

Discussion

The rates and equilibria of complexation of Na$^+$ with Mon$^-$ and MonH, together with the pK's of MonH and NaMonH$^+$, are summarized in Table III. It is clear that the intermediate complex NaMonH$^+$ formed in the direct exchange of Na$^+$ for H$^+$ on Mon$^-$ has quite a high stability, viewed either as a complex between Na$^+$ and MonH (log K_s = 3.77) or as an acid (pK_a = 6.40) which may dissociate to give H$^+$ and NaMon. However, Mon$^-$ has a considerably higher affinity (10^5-fold) than NaMon or MonH respectively for H$^+$ and Na$^+$. This must be attributable at least partially to simple electrostatic factors favoring interactions of Mon$^-$ with H$^+$ or Na$^+$. Another important factor may be structural rearrangements required when, for example, Na$^+$ is added to MonH. Crystallographic studies of NaMon,[23] MonH,[24] and NaMonH$^+$ Br$^-$[12] reveal that although (in the solid state) all three involve similar cyclic structures in which the ends of the monensin chain are linked by head-to-tail hydrogen bonds, there are important differences in the hydrogen-bonding pattern of the carboxylic acid or carboxylate groups with the OH groups. In addition there are significant conformational changes, the most important of which occur between the free acid form on the one hand and the Na$^+$ complex of either Mon$^-$ or MonH on the other.[23] A possible complexation sequence for the addition of Na$^+$ to MonH has been suggested on the basis of the changes observed in the crystal structures,[23] but it is difficult to know how the structural measurements will translate to solution, especially in hydrogen-bonding media such as ethanol. It is possible, for example, that the free acid in ethanol has an open-chain form stabilized by intermolecular hydrogen bonds with the solvent, as has been suggested for nigericin in methanol.[14]

Stability constants for NaMon and NaMonH$^+$ in methanol have been reported by Hoogerheide and Popov,[6,7] who used simultaneous potentiometric measurements with glass and cationic selective electrodes. The values obtained (log K_s(NaMon) = 6.4; log K_s(NaMonH$^+$) = 2.5; pK_a(MonH) = 10.30) are lower than those found in ethanol, but they show the same general pattern. This is as expected, at least in qualitative terms, from the known differences in solvation energies of electrolytes in methanol[25] and ethanol.[26,28] Thus, for example, the free energies of NaCl and HCl increase by 10.5 and 6.9 kJ mol^{-1}, respectively, on transfer from methanol to ethanol, which correspond to changes of 1.8 and 1.2 log units in solubility product and acidity constant (pK_a), respectively.

Compared with NaMon, the reduction in stability of NaMonH$^+$ results almost equally form a 400-fold increase in the rate constant for dissociation of Na$^+$ and a 200-fold decrease in the formation rate constant. The increase in the dissociation rate constant is very similar to those obtained for the dissociation of Ca^{2+} (300-fold) and Mg^{2+} (314-fold) from the anionic and protonated forms of calcimycin.[36] The large decrease in k_f on protonation of Mon$^-$ is perhaps unexpected as changes in the nature of ligands are normally reflected more strongly in the dissociation rate constants for macrocyclic complexes.[2,10,29,30] However, if as suggested by the crystallographic studies changes in ligand structure, especially in the hydrogen-bonding pattern, accompany addition of Na$^+$ to MonH, a relatively low formation rate constant is not unreasonable. The neutral macrocyclic antibiotics valinomycin and antamanide, where independent evidence from ultrasonic absorption studies of conformational changes is available, are characterized by low formation rate constants for their complexes (1×10^7 and 1×10^5 M^{-1} s^{-1} for Na$^+$ complexes respectively in methanol).[31,32]

The activation parameters for formation and dissociation of NaMonH$^+$ (Table II) are of some interest. Although the overall entropy change is close to zero, the activation entropies for both the formation and dissociation reactions have very large negative values. This can most simply be rationalized if it is assumed that in the transition state Na$^+$, although associated with MonH, still retains most of its solvation. Thus in the formation reaction the negative activation entropy will result largely from a decrease in translational entropy on association of the reactants. In the reverse

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Table II. Enthalpies and Entropies of Reaction and Activation[a] for Reaction between 2,4-Dinitrophenol and Sodium Monensin in Ethanol

ΔH_e, kJ mol^{-1}	ΔS_e, kJ mol^{-1}	ΔH_1,[b] kJ mol^{-1}	ΔS_1,[b] J K^{-1} mol^{-1}	ΔH_{-1},[c] kJ mol^{-1}	ΔS_{-1},[c] J K^{-1} mol^{-1}	ΔH(NaMonH$^+$),[d] kJ mol^{-1}	ΔS(NaMonH$^+$),[d] kJ mol^{-1}
28.8	−15.1	35.2	−70.8	13.8	−71.0	−21.4	−0.2

[a] Derived from data in Table i. [b] Dissociation of Na^+ from $NaMonH^+$. [c] Formation of $NaMonH^+$. [d] Enthalpy and entropy of reaction $Na^+ + MonH \rightleftharpoons NaMonH^+$.

Table III. Complexation of Na^+ with Mon^- and MonH in Ethanol at 25 °C[a]

1. $Na^+ + Mon^- \underset{2.2\ s^{-1}}{\overset{1.1 \times 10^9\ M^{-1}\ s^{-1}}{\rightleftharpoons}}$ NaMon; log K_s = 8.8
2. $Na^+ + MonH \underset{810\ s^{-1}}{\overset{4.8 \times 10^6\ M^{-1}\ s^{-1}}{\rightleftharpoons}}$ $NaMonH^+$; log K_s = 3.77
3. $MonH \rightleftharpoons Mon^- + H^+$; pK_a = 11.45
4. $NaMonH^+ \rightleftharpoons NaMon + H^+$; pK_a = 6.40[b]

[a] Results for NaMon from ref 10. [b] $pK_a(NaMonH^+) = pK_a(MonH) + \log K_s(NaMonH^+) - \log K_s(NaMon)$.

direction the resolvation of Na^+ as it emerges from the ligand cavity will result in a loss of entropy (and a relatively low activation energy); this will subsequently be compensated for by an increase in translational entropy as Na^+ and MonH separate completely.

Comparable data for other naturally occurring ionophores are scarce, but the available evidence from other macrocyclic ligand systems suggests that negative activation entropies for formation and dissociation may be fairly general, especially for complexation of highly solvated cations. Wilkins and co-workers[33] found large negative activation entropies for dissociation of alkaline earth metal ion complexes of macrobicyclic cryptand[30] ligands (cryptates) in water and similar results have also been reported for alkaline earth complexes of diaza-crown ethers in methanol.[34] Sodium ion complexes of cryptands in methanol show small entropies of reaction but large negative activation entropies of formation and dissociation ($\Delta S_f^{\ddagger}$ and $\Delta S_d^{\ddagger}$ values ca. −35 to −70 J K^{-1} mol^{-1}).[35,36] Within the alkali metal ion series Li^+–Rb^+, $\Delta S_f^{\ddagger}$ values cryptate complexes show no definite trends, all of them being large and negative, but $\Delta S_d^{\ddagger}$ values become increasingly less negative with increasing cation size and in some cases (benzo-substituted ligands) become positive.[35,36] All of these results may be interpreted in a manner similar to that given above for $NaMonH^+$. Detailed studies of the solvent effects on cryptate formation and dissociation rates[29] also suggest that in the transition state the cation retains most of its solvation.

Finally, the implications of the results for Na^+/H^+ exchange reactions of monensin (eq 13) may be considered. The reaction may proceed via the monensin anion followed by rapid reaction with H^+ or Na^+, or via $NaMonH^+$ followed by dissociation of

$$H^+(HA) + NaMon \rightleftharpoons HMon + Na^+ (+A^-) \qquad (13)$$

either Na^+ or H^+, depending upon the direction. In the latter case, the dissociation of NaMon (or HMon) is avoided and is replaced by the rather more rapid dissociation of $NaMonH^+$; a rate increase of up to 400-fold may be achieved for dissociation of Na^+ in ethanol as solvent. This may be a considerable advantage as dissociation rate constants of monensin complexes are generally much lower than those of other antibiotic ionophores studied under comparable conditions.[2] The rate constant for dissociation of MonH is unknown, but for the known pK_a values in methanol and ethanol, even allowing for a diffusion-controlled formation rate, it is clear that it must be lower than that for NaMon. However, the relatively low stability of $NaMonH^+$ means that significant concentrations of the incoming cation (H^+ or Na^+) will be required to achieve the maximum rate enhancement available for reaction by way of $NaMonH^+$.

With regard to ion transport in membrane systems, a crucial difference is that Na^+ and H^+ will be confined essentially to the aqueous phase whereas NaMon and HMon, which are very poorly soluble in water, will partition preferentially into the lipid phase. This might be expected to enhance the trends observed here, i.e., the dissociation rate constants of $NaMonH^+$ compared to those of NaMon or MonH should be higher, but the higher solvation of Na^+ and H^+, and hence the lower stability of $NaMonH^+$, means that for a given [Na^+] (or [H^+]) a lower fraction of monensin will be in the more reactive $NaMonH^+$ form. It is difficult to predict that the net effect on the relative importance of the two exchange pathways would be.

Registry No. NaMon, 22373-78-0; 2,4-DNHp, 51-28-5.

Supplementary Material Available: Tables of k_e data at several temperatures (4 pages). Ordering information is given on any current masthead page.

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