Thermodynamics of Complexation of Monensin A and Monensin B in Methanol by Titration Calorimetry

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In the first reported study of the complexation behaviour of monensin B, careful titration calorimetry experiments show the following: (i) monesin B tetrabutylammonium salt ($MonB^-Bu_4N^+$) is a weaker binder than monensin A anion ($MonA^-Bu_4N^+$) of both Na^+ and K^+ in methanol; (ii) the Na^+/K^+ selectivity exhibited by $MonB^-$ is diminished relative to $MonA^-$; and (iii) the observed differences are chiefly due to differences in entropic contributions to the binding free energy.

Monensin A (MonA: **1**, R=Et) is one of the most extensively studied members of the large and growing class of naturally occurring monocarboxylic acid ionophores.[1] MonA is particularly interesting, showing selectivity for Na^+ over K^+ in both complexation and transport. This selectivity pattern is quite rare, indeed unique to MonA among all the monocarboxylic acid ionophores.

One explanation for the selectivity exhibited by MonA involves a 'hole size' argument, stating in essence that the conformational energy of the ligand in the K^+ complex is raised relative to that in the Na^+ complex. This argument seems consistent with the observed enthalpic contributions to the free energy of binding of MonA to Na^+ and K^+ (slight preference for Na^+, see below), since in competition with methanol solvent, K^+ should be favoured over Na^+ in complexation with a 'non-selecting' host binding site.

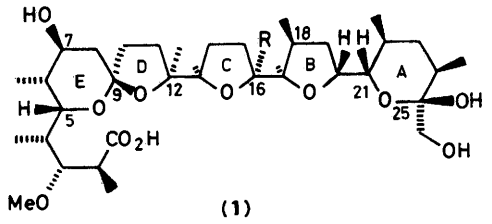
Synthetic technology is now close to the point where analogues of MonA may be prepared efficiently. It is attractive to consider the possibility that, by relatively minor changes in the monensin structure, the conformational energy surface could be tuned to increase Na^+ selectivity. Analysis of a considerable body of *X*-ray data led Duax *et al.*[2] to postulate that MonA accommodates larger cations by a mechanism in which the C-ring acts as a hinge. They also suggested that changes in alkylation pattern about the C-ring could affect complexation properties.[2]

In fact, monensin B (MonB; **1**, R=Me) is a readily available analogue of MonA differing only with respect to the alkyl

Table 1. Thermodynamic data for the binding of Na^+ and K^+ to MonA and MonB.[a]

Cation	Ionophore	ΔH	$-T\Delta S$	ΔG
Na^+	$MonA^-$	-3.62 ± 0.10	-5.06 ± 0.23	-8.68 ± 0.20
K^+	$MonA^-$	-3.38 ± 0.10	-3.89 ± 0.13	-7.27 ± 0.08
Na^+	$MonB^-$	-3.92 ± 0.05	-4.06 ± 0.17	-7.98 ± 0.16
K^+	$MonB^-$	-3.38 ± 0.05	-3.61 ± 0.09	-6.99 ± 0.07

[a] All values are kcal/mol (1 cal = 4.184 J). All runs were made in triplicate. Error limits represent a 95% confidence interval and are the larger of the errors determined from propagation of errors or statistical scatter. Error limits given in text for differences between these quantities are the square root of the sum of the squares of the two errors given in the table.



(1)

MonA: R = Et
MonB: R = Me

substituent at C-16.[3] In light of the Duax proposal, we were especially intrigued by the dramatically different behaviour of MonA and MonB sodium salts on both silica gel and C-18 reversed phase chromatography.† Since to our knowledge no data concerning the complexation or transport properties of MonB have been reported, we felt it would be interesting to determine whether such a small change in the structure of MonA could indeed change binding and selectivity patterns.

MonA and MonB tetrabutylammonium salts were prepared by titration of the free acids with tetrabutylammonium hydroxide. The enthalpy, entropy, and free energy of complexation of both MonA$^-$Bu$_4$N$^+$ and MonB$^-$Bu$_4$N$^+$ with Na$^+$ and K$^+$ trifluoromethanesulphonates (triflates) were measured in methanol at 25 °C by careful titration calorimetry[4] using a Tronac isoperibol titration calorimeter controlled by an HP 85 microcomputer. The thermodynamic parameters were extracted from the titration curves by a non-linear least-squares fitting technique assuming 1 : 1 complexation in each case. The values obtained for MonA differ slightly from those reported by Simon for the MonA tributylammonium salt in methanol–tributylamine solvent.[5] We have obtained evidence that use of tributylamine as cosolvent and base leads to systematically lowered free energies of complexation owing to incomplete deprotonation of the MonA free acid. This is consistent with the observed differences between the values reported herein and the values of Simon.[5]

As shown in Table 1, MonA$^-$Bu$_4$N$^+$ and MonB$^-$Bu$_4$N$^+$ do indeed differ in both binding and selectivity pattern for Na$^+$ and K$^+$. MonA$^-$ is selective for Na$^+$ over K$^+$ by $\Delta\Delta G$ (Na$^+$–K$^+$) = −1.41 ± 0.22 kcal/mol, with a free energy of complexation for Na$^+$ of ΔG (Na$^+$) = −8.68 kcal/mol. Changing the ethyl grouping at C-16 to methyl affects both absolute binding strength and selectivity. MonB$^-$ is selective for Na$^+$ over K$^+$ by $\Delta\Delta G$ (Na$^+$–K$^+$) = −0.99 ± 0.17 kcal/mol, with a free energy of complexation for Na$^+$ of ΔG (Na$^+$) = −7.98 kcal/mol. MonB$^-$ thus has diminished affinity for both K$^+$ and Na$^+$ relative to MonA$^-$, and binding free energy for Na$^+$ is lowered more than binding free energy for K$^+$. The *difference in selectivity* for the two ionophores [$\Delta\Delta G$ MonA (Na$^+$–K$^+$)−$\Delta\Delta G$ MonB (Na$^+$–K$^+$)] is −0.42 ± 0.28 kcal/mol. Put another way, the selectivity of MonA$^-$ is increased relative to MonB$^-$ by about 40%. While not a large effect, it is clear that small structural changes in the monensin framework can have a measurable effect on binding and selectivity.

It is interesting to consider the origins of the observed differences between MonA$^-$ and MonB$^-$. Note that enthalpically, MonB$^-$ is actually slightly more selective than MonA$^-$ for Na$^+$. The enthalpic driving force for complexation of Na$^+$ by MonB$^-$ is increased by 0.30 ± 0.11 kcal/mol relative to MonA$^-$, while the two ionophores have equal heats of binding for K$^+$. The diminished selectivity and binding observed for MonB$^-$ is a reflection of rather dramatic differences in the entropic contribution to the free energy of complexation. Thus, the entropic driving force for binding of MonB$^-$ with Na$^+$ ($-T\Delta S$) is diminished by 1.00 ± 0.29 kcal/mol, or 20%, relative to MonA$^-$, while the entropic driving force for binding of K$^+$ is only lowered by 0.28 ± 0.16 kcal/mol (7%) for MonB$^-$ relative to MonA$^-$.

A useful interpretation of these results is not possible at this time. The described study does, however, serve to define an absolute lower limit on the possible changes in binding and selectivity that may be achieved by simple manipulation of alkylation pattern about the monensin backbone.

Acknowledgment is made to the Donors of The Petroleum Research Fund, administered by the American Chemical Society, for partial support of this research. We also thank the Colorado Heart Assocation and Research Corporation for financial assistance, and the Eli Lilly Company for a generous gift of the monensin complex. D. M. W. is a Fellow of the A.P. Sloan Foundation (1982—84) and a Dreyfus Teacher–Scholar (1983—85).

Received, 6th November 1984; Com. 1576

† Separation of MonA and MonB on silica gel is described in reference 3. In our hands, utilizing Merk glass-backed silica gel 60 F254 t.l.c. plates (0.25 mm layer thickness), MonA$^-$Na$^+$ has an R_f of 0.31 and MonB$^-$Na$^+$ has an R_f of 0.24 with hexane–EtOAc–MeOH (78 : 20 : 2) as eluting solvent. Preparative separations of MonA and MonB sodium salts are easily achieved utilizing flash chromatography. Interestingly, if MonA free acid is passed through a flash silica gel column, the sodium salt is isolated after the chromatography. Analytical and preparative separations of MonA and MonB sodium salts by C-18 reversed phase chromatography are reported by M. Beran, J. Tax, V. Schon, Z. Vanek, and M. Podojil, *J. Chromatogr.*, 1983, **268**, 315. On reversed phase chromatography, MonB is the faster moving component utilizing methanol–water as the mobile phase.

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