Biophysical Chemistry 6 (1977) 345–349

KINETICS OF MONENSIN COMPLEXATION WITH SODIUM IONS BY ^{23}Na NMR SPECTROSCOPY

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Received 26 November 1976

The kinetics of the sodium binding to the ionophore monensin (Mon) in methanol has been studied by ^{23}Na NMR spectroscopy. Fast quadrupole relaxation of the bound sodium affected the relaxation rate of the free sodium through an exchange process between these two species. The exchange was found to be dominated by the reaction: $Na^+ + Mon^- \rightleftharpoons MonNa$. The dissociation rate constant at 25°C is 63 s^{-1}, with an activation enthalpy of 10.3 kcal/mol and activation entropy of −15.8 cal/mol deg. These results indicate that the specificity of the binding of sodium ions to monensin is reflected in the relatively slow dissociation process. The entropy changes indicate that the activated monensin–sodium complex undergoes a conformational change, but the existence of a conformational change in monensin anion prior to complexation is excluded.

1. Introduction

The carboxylic ionophore monensin [1] (Mon) has been shown selectively to bind and transport sodium ions in biological systems [2,3], and artificial membrane systems [3]. The actual mechanism of ion transport by carrier ionophores involves the association and dissociation of the ion–carrier complex. Characterization of the thermodynamics and kinetics of the complexation process in homogeneous solutions should provide a deeper understanding of the mechanism of the overall transport process.

The equilibrium solution chemistry of monensin and its sodium complex (MonNa) in methanol has been studied previously [4,5]. This includes the acidity constant of monensic acid [4], the equilibrium constant and free enthalpy and entropy of the sodium complex formation [5], and the identification of two MonNa complexes whose existence depends on the pH of the methanolic solution [4].

The attempt of Haynes et al. [6] to study the kinetics of monensin and sodium interactions by ^{23}Na NMR shift measurements failed due to the small resonance frequency difference between the solvated and complexed sodium ion. Very few kinetic studies of the complexation in solution of metal ions with other ionophores have been determined. Those studied include valinomycin–alkali metal ion interactions [7,8], nonactin–sodium complexation [9] and X-537A interactions with barium [10] and transition metal ions [11].

Here the kinetics of the monensin–sodium complexation process in methanol is reported. ^{23}Na NMR spectroscopy was found to be the most appropriate technique for this study. This method has been successfully applied to study the kinetics of the interactions of sodium with the crown ethers [12].

The method is based on the fact that ^{23}Na complexed with monensin exhibits a fast quadrupole relaxation and slow rotational motion, thus causing the ^{23}Na relaxation rate to be much faster than that of free sodium. The kinetic parameters can therefore be determined by studying the effect of the exchange between free and complexed sodium upon the nuclear relaxation of the sodium.

2. Experimental

2.1. Materials

The monensin sodium salt was obtained from Eli Lilly and Co. The salt was purified by successive re-

crystallizations from methanol until clean, clear crystals were obtained. The purified salt was dried in a vacuum oven at 60°C for 24 hours.

Sodium bromide (analytical grade) was dried in a vacuum oven at 100°C for 48 hours. Spectroscopic grade methanol was dried over molecular sieves.

The methanolic solutions containing MonNa and NaBr were prepared prior to each experiment. They were found to be stable for at least 2 months. The addition of the pure sodium salt of monensin ensured the existence in solution of only the basic species of the complex [4].

2.2. Methods

The ^{23}Na cw experiments were performed on a Varian DP-60 NMR spectrometer with a V-4210A variable rf unit operating at 15.9 MHz. The transverse relaxation time (T_2) was calculated from the measured width of the derivative of the absorption mode, taking into account the correction due to modulation broadening [13].

Longitudinal relaxation measurements of ^{23}Na were made by the 180°–τ–90° pulse sequence, using a Bruker 322S pulse spectrometer operating at 15.8 MHz or 16.8 MHz, with dead time less than 30 μs. The signal to noise ratio of the free induction decay (FID) was enhanced by a HP 5480A signal analyser. The longitudinal relaxation times (T_1) were calculated from a semilog plot of the intensity of the FID immediately after the 90° pulse as a function of τ.

There were cases when two decaying components were observed, a fast one of the complexed species and a slow one of the uncomplexed ^{23}Na. The FID of the slow component immediately after the 90° pulse was extrapolated from that part of the curve showing total decay of the fast component. The longitudinal relaxation rates are accurate within 10%.

3. Results and discussion

3.1. ^{23}Na NMR studies

The dominant mechanism of the sodium relaxation in diamagnetic solutions, in the absence of the exchange, is the modulation of the quadrupole interaction by molecular tumbling. The nuclear relaxation

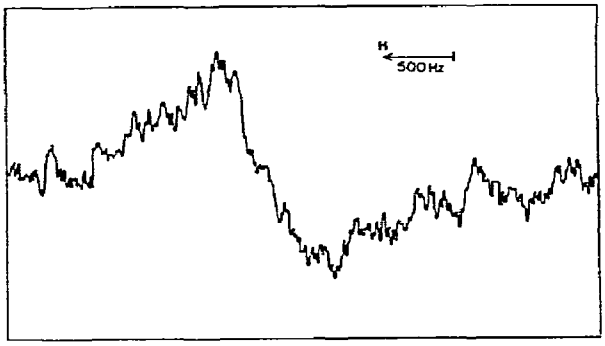

Fig. 1. ^{23}Na magnetic resonance spectrum (the derivative of the absorption mode) of 0.58 M MonNa in methanol at 25°C. RF frequency 15.95 MHz, modulation frequency 130 Hz and modulation amplitude 0.272 G.

rates (at the extreme narrowing range) is then given by [14]

$$1/T_2 = 1/T_1 = \tfrac{1}{10}(e^2qQ/\hbar)^2\tau_r, \qquad (1)$$

where $eqQ/\hbar$ is the quadrupole interaction and τ_r is the correlation time for the rotation of the complex.

For a molecule with a MW and shape as MonNa τ_r is adequately given by the Debye equation (MW = 692)

$$\tau_r = (4\pi r^3/3k)\eta/T, \qquad (2)$$

where r is the mean radius of the complex and η is the viscosity.

In fig. 1 a ^{23}Na spectrum of the MonNa complex (0.58 M in methanol) is recorded. The transverse relaxation rate calculated from the observed line width was found to be (2400 ± 200) s^{-1} at 25°C. This value (within experimental error) was determined for the longitudinal relaxation rate under the same conditions, as expected from eq. (1).

The longitudinal relaxation rates of the complexed sodium exhibited a concentration dependence which could be due to changes in the viscosity of the solution. A similar concentration effect has been observed for the transferse relaxation rate of dibenzo-18-crown-6–Na^+ in DMF [12]. It should be noted that since no concentration specifications have been given for previous line width measurements of the MonNa complex, it is not possible to compare these results with the previously published reports [4,6].

The temperature dependence of the relaxation rates

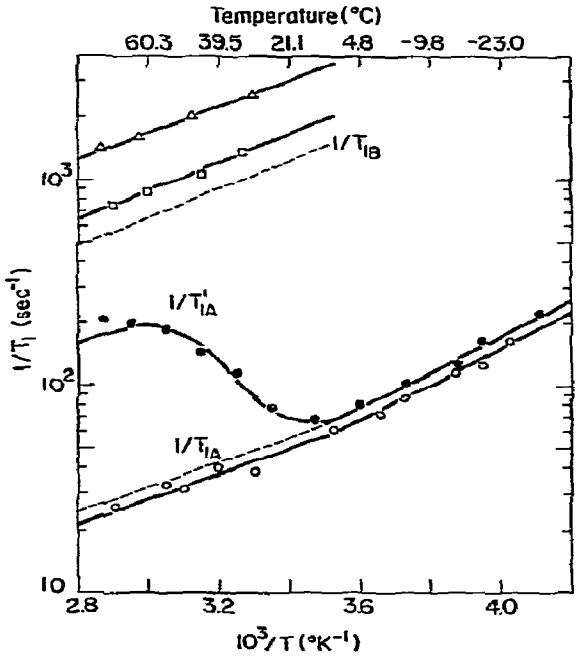

Fig. 2. Semilog plot of the longitudinal relaxation rate for ^{23}Na in methanol versus reciprocal temperature. Solutions composition: ○ 0.5 M NaBr, ● 0.15 M MonNa and 0.35 M Na NaBr, □ 0.3 M MonNa, △ 0.65 M MonNa. $1/T_{1B}$(– – –) 0.15M MonNa extrapolated from higher concentrations. $1/T_{1A}$(– · –) 0.5 M NaBr corrected for the change in viscosity as explained in the text. $1/T'_{1A}$(——) calculated from eq. (5a).

of MonNa and the solvated sodium correlates with changes in viscosity with respect to temperature (fig. 2).

3.2. Kinetic studies

The kinetic parameters for the exchange of sodium ions between monensin (state B) and methanol (state A) were derived from the effect of the exchange upon the longitudinal relaxation rates of the solvated species ($1/T'_{1A}$ in fig. 2). A theoretical description of the relaxation of a nucleus which is transfered between two states, A and B, having different relaxation times and resonance frequencies has been previously derived [15]. The equations which are essential for the analysis of these results written in Wassner's notation [15] are as follows:

The solution of the Bloch–McConnell equations for the magnetization in the z direction (M_z) describes the time dependence of this longitudinal magnetization. For a nucleus transfered between two different magnetic environments, A and B, in the absence of an rf field, the M_z decay is given by [15]:

$$F(\tau) = P'_A \exp(-\tau/T'_{1A}) + P'_B \exp(-\tau/T'_{1B}). \tag{3}$$

The apparent population fractions P'_A and P'_B and the apparent relaxation times T'_{1A} and T'_{1B} are given in the following equations in terms of: (1) P_A and P_B – the fractions of nuclei in states A and B, respectively, (2) T_{1A} and T_{1B} – the longitudinal relaxation times, without exchange, of the nuclei at A and B, respectively, (3) τ_A and τ_B – the life times of the nuclei at state A and B, respectively.

$$P'_A = \tfrac{1}{2} + \frac{\tfrac{1}{2}[(P_A - P_B)(1/T_{1B} - 1/T_{1A}) + 1/\tau_B + 1/\tau_A]}{[(1/T_{1B} - 1/T_{1A} + 1/\tau_B - 1/\tau_A)^2 + 4/\tau_A\tau_B]^{1/2}} \tag{4a}$$

and

$$P'_B = 1 - P'_A; \tag{4b}$$

$$\frac{1}{T'_{1A}} = \frac{1}{2}\left\{\left(\frac{1}{T_{1B}} + \frac{1}{T_{1A}} + \frac{1}{\tau_B} + \frac{1}{\tau_A}\right) - \left[\left(\frac{1}{T_{1B}} - \frac{1}{T_{1A}} + \frac{1}{\tau_B} - \frac{1}{\tau_A}\right)^2 + \frac{4}{\tau_A\tau_B}\right]^{1/2}\right\} \tag{5a}$$

and

$$\frac{1}{T'_{1B}} = \frac{1}{2}\left\{\left(\frac{1}{T_{1B}} + \frac{1}{T_{1A}} + \frac{1}{\tau_B} + \frac{1}{\tau_A}\right) + \left[\left(\frac{1}{T_{1B}} - \frac{1}{T_{1A}} + \frac{1}{\tau_B} - \frac{1}{\tau_A}\right)^2 + \frac{4}{\tau_A\tau_B}\right]^{1/2}\right\}. \tag{5b}$$

Rearrangement of eq. (5a) based on the equilibrium conditions $P_A/\tau_A = P_B/\tau_B$ yields an expression for the exchange rate [12]

$$\frac{1}{\tau_A} = \frac{(1/T_{1B} - 1/T'_{1A})(1/T'_{1A} - 1/T_{1A})P_B}{P_A/T_{1A} + P_B/T_{1B} - 1/T'_{1A}}. \tag{6}$$

By analysing the temperature dependence of the longitudinal relaxation rate $(T'_{1A})^{-1}$ of the solvated sodium in exchanging solution it can be seen that

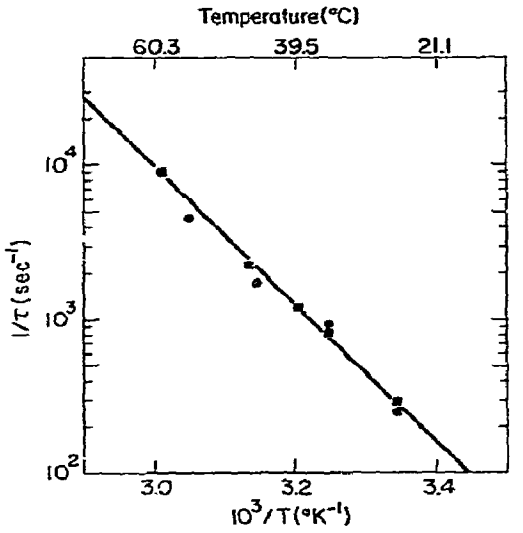

Fig. 3. Arrhenius plot of $1/\tau_A$. ▪ 0.15 M MonNa and 0.35 M NaBr, ▪ 0.3 M MonNa and 0.7 M NaBr.

below 10°C the exchange rates $1/\tau_A$ and $1/\tau_B$ are very slow, relative to the nuclear relaxation rates $1/T_{1A}$ and $1/T_{1B}$; hence, the effect of the exchange is practically unobservable. At this slow exchange region the relaxation of $^{23}Na^+$ bound to monensin was too fast to be detected, and only the decay of the solvated species was observed. This decay is to some extent faster than the decay of a monensin free solution in the same temperature range ($1/T_{1A}$) probably because of the change in viscosity caused by the addition of monensin [12].

In measurements taken from 20°C to 60°C two decaying components were observed in the FID; (1) an underpopulated fast apparent relaxation of the complexed sodium ions (P'_B changes from 0.3 to 0.0) and (2) a highly populated slow apparent relaxation of the solvated sodium ions. The slow relaxation component dominated the observed FID except for immediately after the first 200 μs where a small contribution of the fast component distorted the slow exponential decay. The exchange rate $1/\tau_A$ was calculated from the measured parameters: T'_{1A}, T'_{1B} and T_{1A} at this range according to eq. (6).

The temperature dependence to this exchange rate has the usual Arrhenius form with an appreciable activation energy (fig. 3).

In general the exchange of monensin with sodium ions can proceed according to the following possible reactions:

$$Na^+ + Mon^- \underset{k_d}{\overset{k_a}{\rightleftharpoons}} MonNa, \tag{7}$$

$$^*Na^+ + MonNa \overset{k_1}{\rightleftharpoons} Mon^*Na + Na^+. \tag{8}$$

Hence, the exchange rate in terms of the kinetic constants is given by

$$1/\tau_A = k_1[MonNa] + k_d[MonNa]/[Na^+]. \tag{9}$$

In order to determine the contribution of each reaction to the observed exchange, the exchange rates were measured at 35°C for different MonNa concentrations (0.125 M to 0.3 M) and with a constant concentration ratio ([MonNa]/[Na^+] = 3/7). These exchange rates were found to be the same within experimental error ($1/\tau_A = 80 \pm 10\ s^{-1}$). This indicates according to eq. (9) that the dominant exchange route is via a first order dissociative mechanism. This mechanism was shown also to be the dominant one for other ionophores [7,8,11] as well as for some crown ethers [12,16].

The resulting kinetic coefficients for reaction (7) are given in table 1.

3.3. Comparison with the kinetics of complexation of other ionophores

As indicated in the introduction there is a lack of kinetic data for ionophore–ion interactions. This discussion of monensin–sodium interactions is therefore limited to a comparison with the kinetics of sodium complexation with valinomycin (Val) and Dicyclo-18-crown-6 (DCC) in methanol. The data are summarized in table 1.

It can be seen that the association rate constants for all three complexes differ by about one order of magnitude while the dissociation rate constants differ by several orders of magnitude going from MonNa to ValNa$^+$. The specificity of monensin to sodium is thus mainly reflected in the slow dissociation rate of this complex.

In observing the changes in the entropies of activations it can be seen that a very small decrease in

Table 1
Formation and dissociation rate data for the complexation with sodium ions in methanol at 25°C

Compound	$k_a/10^7$ (s^{-1})	k_d (s^{-1})	$\Delta H_d^{\ddagger}$ (kcal/mol)	$\Delta S_d^{\ddagger}$ (cal/mol deg)	$\Delta H_a^{\ddagger}$ (kcal/mol)	$\Delta S_a^{\ddagger}$ (cal/mol deg)
monensin	6.3	63	10.3	−15.8	−0.8	−3.9
valinomycin [a]	1.4	2×10^6	9.5	−7.3		
DCC [b]	26	5.2×10^4	8.3	−21	2.7	−23

a) The rate constants have been taken from ref. [8]. $\Delta H_d^{\ddagger}$ and $\Delta S_d^{\ddagger}$ have been calculated from ref. [7]. The activation enthalpy and entropy for the association of $ValNa^+$ complex have not been calculated due to the lack of the thermodynamic equilibrium parameters for this process in methanol.
b) The parameters have been calculated from ref. [16];

entropy occurs when the activated sodium–monensin complex is formed from the free species. In contrast, the formation of the DCC–Na^+ complex is accompanied by an appreciable negative activation entropy. This indicates that the crown ether conformation is most likely altered before association with sodium occurs, as has been previously observed for dicyclo-30-crown-10 [17]. Therefore the difference between the association activation entropies of the two complexes shows that the monensin anion does not undergo a conformational change prior to interaction with the sodium. The increase in entropy which occurs while going from the activated state to the complex is similar for both complexes and may indicate that a conformational change occurs after the sodium has interacted with the ionophore as has been shown previously to occur in the Val–sodium complexation process [8].

This research is being extended to other ionophores and other media, as well as membrane model systems and biological systems.

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