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ION BINDING BY X-537A. EQUILIBRIUM AND RATE OF COMPLEXATION OF Co^{2+} IN METHANOL ★

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The reaction of X-537A (XH) with Co^{2+} in methanol to form the complex CoX^+ has been studied fluorometrically to determine the equilibrium constant as a function of temperature. The effect of complexation on the proton NMR spectrum of the X-537A was studied to determine the kinetics of complex formation. Comparing the data for the reaction $XM^+ \to X^- + M^{2+}$ in methanol at 25° for several M^{2+} we find that the equilibrium constants increase in the order $CoX^+ < MnX^+ < NiX^+$ and span only a factor of 5 while the rate constants increase in the order $NiX^+ < CoX^+ < MnX^+$ and span a factor of more than 100.

1. Introduction

After the completion of a study of the stoichiometric, spectral, and equilibrium characteristics [1] and kinetics [2] of complexes of certain cations with the ionophorous antibiotic X-537A (Lasolacic acid), it became clear the method for studying the kinetics would probably give definitive results for Co^{2+} as it had for Ni^{2+} in the original study. In the other case for which the rates were studied, namely the complex of Mn^{2+}, the data we were able to obtain were sufficient to determine the rate of complexation only within an order of magnitude. Because of the possible applicability of results of this kind to the study of X-537A-mediated transport of ions through membranes [2] the original studies have been extended to include the determination of the equilibrium and rate of the reaction (X-537A = XH)

$$MX^+ \to M^{2+} + X^- \qquad (1)$$

in methanol for the case $M^{2+} = Co^{2+}$.

2. Experimental section

The materials and the experimental procedures were the same as in the work reported earlier [1,2] except as noted here. Anhydrous cobaltous chloride was obtained from Alfa Inorganics. The NMR measurements were made with a JEOLCO MH-100 instrument. The ratio of stoichiometric concentrations $[Co^{2+}]/[XH]$ was varied from 0.01 to 0.05 in the rate measurements. In this range there is no detectible variation of the rate constant calculated for eq. (1); other possibilities for the complexation kinetics were described by Degani and Friedman [2].

3. Fluorometric and circular dichroism studies of solutions in methanol

The concentration dependence of the fluorescence of solutions of Co^{2+} and XH showed that CoX^+ is the dominant complex in solutions in which the free X^- concentration ranges up to 200 μM and the ratio of stoichiometric concentrations $[XH]/[Co^{2+}]$ is as small as 0.01. The fluorescence characteristics of CoX^+ are very similar to those reported by Degani and Friedman [2] for NiX^+ and MnX^+. For example the relative fluorescence of the complex is 0.05 ± 0.02. The equi-

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Table 1
Temperature dependence of the dissociation constant of CoX^+ in methanol

Temperature (°C):	1.5	26	41
pK [a]	4.52	4.63	4.89

[a] For hyp. 1M standard states.

Table 2
Data for $MX^+ \rightarrow M^{2+} + X^-$ in methanol at 25°

M	$\Delta G°$ (kcal/mol)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/mol deg)	k_d $(10^3\ s^{-1})$	$\Delta H^{\ddagger}$ (kcal/mol)	$\Delta S^{\ddagger}$ (cal/mol deg)	$k_f = k_d/K$ $(10^8\ s^{-1}\ M^{-1})$
Mn [a]	6.02			14 to 1000 [b]			3.5 to 2500 [b]
Co	6.28 ±0.04	−2.0 ±0.3	−28 ± 1	12 ± 2	1.9 ±0.5	−33 ± 4	4.0
Ni [a]	5.40 ±0.02	−2.3 ±0.1	−26 ± 1	0.24 ± 0.04	4.5 ±1.5	−32 ± 6	0.022

[a] Data from refs. [1,2].
[b] Most probable: near 100.

librium constant, defined as $1/K$, for reaction (1) was deduced from the data with the results given in tables 1 and 2. The thermodynamic data for reaction (1), which are needed for the interpretation of the rate experiments described below, are rather similar to those for NiX^+ and MnX^+.

The CD spectrum of CoX^+ is similar to the spectra of MgX^+ and NiX^+ reported by Degani and Friedman [2]. For CoX^+ we find $\Delta\epsilon = -2.1 \pm 0.3$ at 295 nm and -1.0 ± 0.15 at 245 nm.

Taken together with the other results [1] these thermodynamic and spectral results imply that the conformation of the X-537A anion in CoX^+ is similar to its conformation in MgX^+, NiX^+, MnX^+, and CaX^+.

Table 3
Data for H_m in CoX^+ in MeOH [a]

Temperature	k_2 (ms^{-1})		*s* (rad/ms)	
	expt.	calc.	expt.	calc.
−66	1.4	1.6	0.79	0.74
−50	2.2	1.9	1.4	1.4
−30	2.2	1.8	2.4	2.3
−6	1.1	1.3	2.8	2.8
10	1.0	0.97	2.9	2.9
30	0.77	0.67	2.8	2.8

[a] Composition of solution: 4×10^{-2} M X-537A, 2×10^{-3} M $CoCl_2$, pH adjusted with LiOH to 7.3.

4. Magnetic resonance studies and rates

Degani and Friedman [2] designate the para, meta, and methyl-group protons of the salicylate moiety of the X-537A as H_p, H_m, and H_{mm}, respectively. These are the protons which are most readily identified and studied in the NMR spectra of the solutions. Shifts and broadenings of these proton lines at 220 MHz and 100 MHz were found when Ni^{2+} or Mn^{2+} was added to solutions of X-537A, the effects being largest for H_m. For CoX^+ neither shifts nor broadenings were observed for H_p and H_{mm} at 100 MHz. Therefore the rate studies are based upon the H_m line alone. The data are given in table 3 in the notation of ref. [2].

The molar broadening is

$$k_2 \equiv (1/T_2 - 1/T_{20})\,[XH]/(MX^+) \qquad (3)$$

and the molar shift is

$$s = (\omega - \omega_0)\,[XH]/(MX^+)\,. \qquad (4)$$

These coefficients are defined in such a way that if reaction (1) were very fast then they would be, respectively, the $1/T_2$ rate and the shift of the proton in the complex MX^+. The actual shift of the proton in the complex, relative to the free, uncomplexed species ($X^- + HX$) is called $\Delta\omega_{\mathrm{m}}$. When reaction (1) is not too fast and when the data are obtained for an adequate range of the experimental variables then the theory of Swift and Connick [3] may be applied to determine the rate constant k_{d} of reaction (1).

The following analysis leads to the conclusion that the data in table 3 are in the range in which the following simplified form of the Swift–Connick equation is applicable without sifnificant error

$$k_2/s = \Delta w_{\mathrm{m}}/k_{\mathrm{d}} \,. \tag{5}$$

Reference should be made to Degani and Friedman [2] for definitions of all of the notation used in the rest of this paragraph as well as for the full Swift–Connick equations and the Solomon–Bloembergen equations which govern the proton relaxation within the complex. For $Co(CH_3OH)_6^{2+}$ in methanol $T_{1\mathrm{e}}$ is about 0.5 ps (Luz and Meiboom [4]) and it is expected to be no longer in CoX^+. We assume $T_{2\mathrm{e}} = T_{1\mathrm{e}}$; if $T_{2\mathrm{e}} < T_{1\mathrm{e}}$ the conclusion of this paragraph is not changed but the discussion is more complicated. In this case the Solomon–Bloembergen equations reduce to

$$k_{1\mathrm{m}} = k_{2\mathrm{m}} = (2/3)S(S+1)(2A_{\mathrm{a}}^2 + A_{\mathrm{i}}^2)T_{1\mathrm{e}} \,. \tag{6}$$

The data in table 3 in the range where s is independent of temperature are presumed to be in the range in which k_{d} is large enough so that $s = \Delta\omega_{\mathrm{m}}$. From this value of $\Delta\omega_{\mathrm{m}}$ we estimate (Bloembergen [5]) for the scalar interaction coefficient $A_{\mathrm{i}} \approx 7 \times 10^4$ rad/s. We assume that in CoX^+ the metal is coordinated to the carboxylate group in the salicylate moiety [1] in which case the metal, H_{m} distance is in the range from 0.5 to 0.7 nm and the dipolar coefficient $A_{\mathrm{a}} = \hbar\gamma_S\gamma_I/r^3$ is in the range from 4×10^6 to 1×10^6 rad/s. Now we find from eq. (6) that we have

$$k_{1\mathrm{m}} = k_{2\mathrm{m}} = 8 \times 10^{13}\, T_{1\mathrm{e}}/s^2 \leqslant 40/\mathrm{sec} \text{ at } 25^\circ \,. \tag{7}$$

Thus at 25° we have $k_{2\mathrm{m}} \ll \Delta\omega_{\mathrm{m}}$. The temperature dependence of $T_{1\mathrm{e}}$, and hence of $k_{2\mathrm{m}}$ is very likely small enough so that $k_{2\mathrm{m}} \ll \Delta\omega_{\mathrm{m}}$ over the whole temperature range of table 3. Then over this range the Swift–Connick equations reduce to eq. (5).

We assume the usual forms for the temperature dependences:

$$\Delta\omega_{\mathrm{m}} = c/T \,, \tag{8}$$

$$k_{\mathrm{d}} = c' T^{-1} \exp(-\Delta H^{\ddagger}/RT) \,, \tag{9}$$

and then determine c/c' and $\Delta H^{\ddagger}$ by a least-squares fit of eq. (5) to the experimental data for k_2/s. When eq. (5) is valid we also have [2]

$$s = k_{\mathrm{d}}^2 \Delta\omega_{\mathrm{m}}/(k_{\mathrm{d}}^2 + \Delta\omega_{\mathrm{m}}^2) \,. \tag{10}$$

We determine c by fitting eq. (10), using eqs. (8) and (9), to the datum for s at 30°. Now c, c', and $\Delta H^{\ddagger}$ all are fixed, and k_2 and s can be calculated from eqs. (5), (8), (9) and (10). The calculated values given in table 3 are in satisfactory agreement with the experimental ones.

The equation for k_{d} can be written in the standard form

$$k_{\mathrm{d}} = (k_{\mathrm{B}}T/h)\exp(\Delta S^{\ddagger} + \Delta H^{\ddagger}/RT) \,, \tag{11}$$

where k_{B} and h are Boltzmann's and Planck's constants and where the parameters determined above give $\Delta S^{\ddagger}$, $\Delta H^{\ddagger}$, and k_{d} at 25° as given in table 2 for comparison with earlier results.

5. Discussion

The interpretation of the rate data in table 2 is most conveniently made in terms of the formation rate constants k_{f} which also are given there. We need add only briefly to the discussion already given for the Mn^{2+} and Ni^{2+} data [2].

Rather often it is found that the formation of complexes of metal ions proceeds by the so-called I_{d} mechanism of Eigen and Tamm [6]. In this case we have

$$k_{\mathrm{f}} = K_{\mathrm{ss}}/\tau_{\mathrm{m}} \,, \tag{12}$$

where K_{ss} is the formation constant for the solvent-separated ion pair (in our case $M(MeOH)_6^{2+}X^-$) and τ_{m} is the lifetime of a solvent molecule in the coordination shell of the metal ion (Basolo and Pearson [7], Frey and Stuehr [8]). For the systems in table 2 it is very likely that K_{ss} is the same for each. If the value of τ_{m} in the outer-sphere complex is the same as the measured rate for $M(MeOH)_6^{2+}$ in bulk methanol, then we can calculate any two of the k_f values in table 2 from the third. The τ_m values in bulk methanol at 25° are in μs, 1000 for Ni^{2+}, 55 for Co^{2+} (Luz and Meiboom [4]), and 1.05 for Mn^{2+} (Levanon and Luz [9]). Then if the I_d mechanism applies we may calculate the k_f values for Co^{2+} and Mn^{2+} from the one for Ni^{2+} with the results: $k_f = 0.4$ for Co^{2+} and 21 for Mn^{2+}, both in the units in table 2, i.e., $10^8\ s^{-1}\ M^{-1}$. The calculation for Co^{2+} thus fails by an order of magnitude, suggesting that the Eigen–Tamm mechanism is at best a qualitative guide to the actual complexation process in these systems.

The large rate differences of the complexes in table 2, together with the relatively small differences in stabilities, should make them useful in the study of mechanisms of transport of ions through phospholipid membranes by X-537A. Of course more needs to be done in terms of varying the solvent and studying the kinetics of MX_2 complexes, but the regularities mentioned above make it seem likely that the kinetic differences and thermodynamic similarities of the complexes of Mn^{2+}, Co^{2+}, and Ni^{2+} will also be characteristic of the actual membrane processes.

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