Complexes of the Nickel(II) Ion with Purine Bases: Relaxation Spectra with Theophylline

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Temperature-jump experiments with theophylline (HL) and Ni^{2+} yield spectra characterized by a single relaxation process. Similar experiments with caffeine and Ni^{2+} in the concentration range 10^{-3}—10^{-2}M, pH 3·85—6·10, do not yield detectable effects. Potentiometric determination of the number and stability of the theophylline complexes formed at 25 ± 0·1 °C and variable ionic strength in the range 0·010—0·018M gave $K_1 = [NiL^+]/[Ni^{2+}][L^-] = (1{\cdot}8 \pm 0{\cdot}3) \times 10^3$ l mol^{-1} and $K_2 = [NiL_2]/[NiL^+][L^-] = (1{\cdot}0 \pm 0{\cdot}4) \times 10^3$ l mol^{-1}. Evaluation of the relaxation data, based on the formation of two theophylline complexes, results in the values $k_1 \approx 1 \times 10^2$ l mol^{-1} s^{-1} and $k_{-1} \approx 10^7$ l mol^{-1} s^{-1} for the reaction $Ni^{2+} + HL \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} NiL^+ + H^+$, and $k_2 \approx 1 \times 10^2$ l mol^{-1} s^{-1} and $k_{-2} \approx 2 \times 10^7$ l mol^{-1} s^{-1} for the reaction $NiL^+ + HL \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} NiL_2 + H^+$. It is concluded that the formation rate constant is smaller than that which would be expected for a neutral ligand. A possible explanation of this retardation may be hindrance to the loss of the dissociable proton, due to internal H-bond formation in theophylline.

IN the course of investigating the kinetic behaviour of purines as metal-binding ligands,[1,2] it became obvious that the numerous possible binding sites on these molecules make a straightforward interpretation of chemical relaxation data very difficult. Substitution of, for example, methyl groups at one or more of the possible binding sites serves to inhibit complexation, often resulting in simpler kinetic behaviour.[2] A number of naturally occurring purines can also serve this purpose. However, experiments with hypoxanthine and caffeine yielded neither interpretable relaxation spectra,[2] nor any observable effect, respectively. In this paper we report the results of a temperature-jump study on the reaction of the nickel(II)

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[1] R. L. Karpel, K. Kustin, and M. A. Wolff, *Israel J. Chem.*, submitted for publication.

[2] R. L. Karpel, K. Kustin, and M. A. Wolff, *J. Phys. Chem.*, 1971, **75**, 799.

ion with theophylline (for structure see below), a purine base which yields a detectable and interpretable relaxation spectrum.

EXPERIMENTAL

Theophylline (Mann Research Laboratories) was used without further purification, as were all other chemicals. $Ni(NO_3)_2,6H_2O$ (Fisher Scientific) and KNO_3 (Baker) were both of reagent grade purity. Indicators used were Chlorophenol Red (Fisher) and Bromochlorophenol Blue (Matheson, Coleman, and Bell). For the pH titration fresh NaOH (Fisher certified, 1N) solution was used.

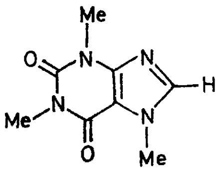
Solutions were prepared by dissolving weighed amounts of solid nickel(II) nitrate and/or theophylline in distilled water. Indicator concentration and ionic strength were adjusted by the addition of appropriate amounts of stock solutions of indicator and KNO_3, respectively. The pH was adjusted by dropwise addition of dilute NaOH and/or HNO_3.

A Sargent–Welch model NX pH-meter was used to measure the pH values to ±0·01 pH units. The measured hydrogen-ion activity for the temperature-jump experiments was divided by $\gamma_{\pm} = 0·79$ in order to obtain the hydrogen-ion concentration. Ionic strength in these experiments was 0·1M except where noted. The pH titrations were carried out under a nitrogen atmosphere in a cell (100 ml) thermostatted to 25 ± 0·1 °C and equipped with a magnetic, Teflon-coated stirring bar. Changes in pH were read after each incremental addition of titrant (NaOH) until the solution became turbid or a fall in pH was registered indicating the start of precipitation, presumably of the bis complex.

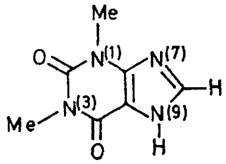
The temperature-jump apparatus has been described elsewhere.[3] The magnitude of the temperature perturbation was 10 °C. The temperature in all the experiments was 25 ± 1 °C. All reported data fulfilled the requirement that blank experiments with ligand and indicator did not show any relaxation effects.

RESULTS

Caffeine.—In caffeine all but one of the nitrogen atoms are blocked by a methyl group. Experiments were carried out to see if this nitrogen atom would enable caffeine to serve as a ligand for the nickel(II) ion. The nickel(II) and caffeine concentrations were varied in the range 1×10^{-3}—1×10^{-2}M, the pH from 3·85 to 6·1, and temperature-jump experiments were performed on these solutions. No observable relaxation effects could be detected; we therefore conclude that complex formation between caffeine and Ni^{2+} is non-existent or very slight. This result is in agreement with previous findings that, although mercury and copper form complexes with other purine bases, they do not do so with caffeine.[4,5]



Caffeine



Theophylline

Theophylline.—Another naturally occurring purine base of similar structure is theophylline. It differs from caffeine only by having a dissociable proton on the imidazole ring at N(9) instead of a methyl group. The acid dissociation constants of theophylline are pK_{a1} 1 and pK_{a2} 8·6.[6] Temperature-jump experiments with this ligand yielded relaxation effects. Results of 15 experiments with the nickel(II) ion and theophylline over a range of concentration and pH are summarized in the Table.

Relaxation spectra of nickel(II)–theophylline complexes

Expt.	$10^3[Ni]_0$ [a] M	10^3[Theophylline]$_0$ [a] M	pH	$\tau_{obs.}$ ms	$\tau_{+calc.}$ ms	$\tau_{-calc.}$ ms
(1)	10	10	3·87 [b]	No detectable effect		
(2)	10	10	5·97	3·7	2·2	17·9
(3)	10	10	5·47	5·1	1·1	5·8
(4)	10	10	6·34	5·0	4·5	40·2
(5)	10	5	6·20	5·3	3·7	30·6
(6)	10	2·5	6·20	5·0	4·3	31·1
(7)	5	2·5	6·22	7·0	8·2	32·5
(8)	2·5	2·5	6·20	13·1	14·0	31·1
(9)	2·5	5	6·18	12·5	11·9	29·3
(10)	33	5	6·20	2·9	1·2	30·7
(11)	66·7	5	5·24	$\geqslant$3·5 [c]	0·1	3·3
(12)	33	2·5	6·22	3·6	1·4	32
(13)	2·5	10	6·20	8·4	11·4	29·6
(14)	5	20	5·68	4·9	2·2	9·2
(15)	5	20	6·20	7·5	6·0	28·0

[a] Total stoicheiometric concentration. [b] Indicator: Bromochlorophenol Blue, total concentration $1·0 \times 10^{-5}$M, pK_a 4·00. In all the other experiments the indicator was Chlorophenol Red, total concentration $1·2 \times 10^{-5}$M, pK_a 6·00. (Indicator pK values from I. M. Kolthoff, *J. Phys. Chem.*, 1930, **34**, 1466.) [c] Ionic strength 0·2M.

MECHANISM AND TREATMENT OF DATA

Mechanism.—Single-exponential relaxation effects were recorded in all experiments, wherein the theophylline ligand is present in its neutral form. The absence of complex formation with caffeine, and previous experiments with mercury[4] and copper,[7] indicate that the theophylline ligand binds to the metal ion in the anionic form. (There is one report in which a different conclusion is reached.[5]) The simplest possible mechanism would therefore be a

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[3] P. Hurwitz and K. Kustin, *Inorg. Chem.*, 1964, **3**, 823.

[4] I. Bayer, E. Pasgay, and P. Majlat, *Pharm. Zentralhalle*, 1962, **101**, 476.

[5] A. T. Tu and C. G. Friedrick, *Biochemistry*, 1968, **7**, 4367.

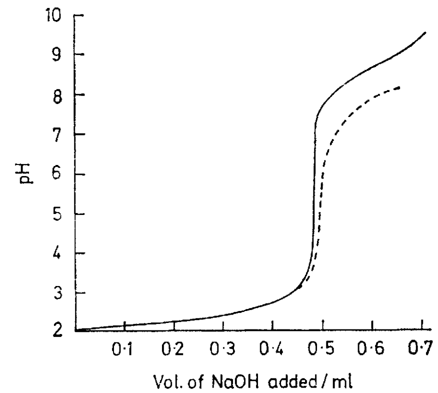
[6] D. D. Perrin, 'Dissociation Constants of Organic Bases in Aqueous Solutions,' Butterworth and Co., Ltd., London, 1965.

[7] R. Weiss, *Z. physiol. Chem.*, 1965, **340**, 138.

one-step reaction, *e.g.* that in equation (1), where HL is

$$Ni^{2+} + HL \rightleftharpoons NiL^{+} + H^{+} \qquad (1)$$

the neutral form of the ligand. The equilibrium constant for this reaction has not been reported in the literature, so that both the forward and the reverse rate constants had to be found independently. This process, carried out by computer fit of the rate constants, was only partially successful. The largest deviation between the experimentally measured relaxation times, τ_{obs}, and the calculated τ values obtained from the fitted rate constants were at low metal-to-ligand ratios. These deviations are consistent with the presence of a higher order complex.



Plot of pH against added 1N-NaOH (ml) for the potentiometric titration of theophylline: (——), titration of 50 ml solution 0·005M in theophylline and 0·01M in HCl; (— — —), as above with 0·0025M-$Ni(NO_3)_2$. The presence of chloride ions is not important as the degree of nickel(II) chloride complex formation is insignificant under these conditions (L. G. Sillén and A. E. Martell, 'Stability Constants of Metal-ion Complexes,' Chem. Soc. Special Publ. No. 17, London, 1964, p. 282)

The existence of such a complex implies a two-step mechanism which usually involves a second relaxation time. In none of the experiments was a second relaxation time detected, but this lack of an observed second effect does not necessarily preclude a two-step mechanism, as the second relaxation time might be out of the instrument's range or its amplitude might be very small.[8]

To find the two forward and two reverse rate constants of a two-step mechanism [equations (2) and (3)] by an

$$Ni^{2+} + HL \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} NiL^{+} + H^{+} \qquad (2)$$

$$NiL^{+} + HL \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} NiL_2 + H^{+} \qquad (3)$$

iterative method to any degree of accuracy is not possible because of the limited range and accuracy of the experimental data. Consequently, an independent determination of the two stability constants was attempted by potentiometric titration.[9] The useful range of the titration curve lies between pH 3·5 and 8·0. There is no detectable complexation below the lower limit and precipitation of the bis complex occurs above the upper limit. Since precipitation occurs at lower pH values as the ionic strength increases, the titration was not performed under controlled and constant ionic strength. Instead, the ionic strength varied from an initial value of $1{\cdot}00 \times 10^{-2}$M to *ca.* $1{\cdot}75 \times 10^{-2}$M. The use of stability constants determined at one ionic strength had no appreciable effect on the calculation of rate constants at a different ionic strength; a two-fold variation in stability constant showed a negligibly small change in the rate constants. As it seemed more important to demonstrate evidence for the existence of the bis complex by allowing the fullest range for collection of the potentiometric data, than to control the ionic strength, the latter was allowed to vary. The data are shown graphically in the Figure; the values obtained are $K_1 = [NiL^+]/[Ni^{2+}][L^-] = (1{\cdot}8 \pm 0{\cdot}3) \times 10^3$ l mol^{-1} and $K_2 = [NiL_2]/[NiL^+][L^-] = (1{\cdot}0 \pm 0{\cdot}4) \times 10^3$ l mol^{-1}.

Treatment of Relaxation Data.—The exact treatment of the relaxation spectrum for a coupled two-step process has been reported previously.[8,10] Evaluation of the secular determinant applicable to these spectra yields the reciprocal of τ, the relaxation time [equation (4)]. The

$$\left(\frac{1}{\tau}\right)^2 - (a_{11} + a_{22})\left(\frac{1}{\tau}\right) + (a_{11}a_{22} - a_{12}a_{21}) = 0 \qquad (4)$$

a_{ij} terms in equation (4) are known functions of the rate constants and equilibrium concentrations, the exact forms of which depend upon the mechanism. Such terms for the two-step mechanism were inserted into a trial and error computer program for finding rate constants that give the best fit between calculated and experimentally measured relaxation times.[1] In this method of calculation we obtain two different relaxation times for every experiment (conforming to the two solutions of the quadratic equation). We would therefore expect only one set of solutions, the τ_+ or the τ_- terms, to fit the experimental values. The results of such a fit are given in the last two columns of the Table. Values used for the rate constants are: $k_1 = 1 \times 10^2$; $k_{-1} = 1 \times 10^7$; $k_2 = 1 \times 10^2$; and $k_{-2} = 2 \times 10^7$ l mol^{-1} s^{-1}.

Comparison of the experimental with the calculated τ_+ values shows that most of the individual τ_+ values correlate quite closely with the experimental values. Relative to each other, the τ_+ values display the proper correspondence with concentration variation. This agreement is as good as can be expected, considering that rate and equilibrium constants are correct only to a factor of *ca.* 1·5. Notable exceptions are experiments (3), (11), and (14), a common factor of which is that all were conducted at lower pH. For experiments (3) and (11), the τ_- values match the experimental τ values within experimental error. In experiment (14) neither τ_+ nor τ_- fits the experimental τ value, but the latter is rather close to the average of τ_+ and τ_-. The pH in this experiment is also midway between the pH in experiments (3) and (11) and that in the rest of the experiments. An explanation of this behaviour is that, in the pH range 5·2—6·2, we are witnessing a magnitude inversion of the two relaxation times. Such an exchange in magnitudes has been observed previously.[11]

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[8] G. G. Hammes and J. I. Steinfeld, *J. Amer. Chem. Soc.*, 1962, **84**, 4639.

[9] A. Albert and E. P. Serjeant, 'Ionization Constants of Acids and Bases,' John Wiley and Sons, Inc., New York, 1962, p. 154.

[10] M. Eigen, *Angew. Chem. Internat. Ed.*, 1964, **3**, 1.

[11] R. L. Karpel, K. Kustin, A. Kowalak, and R. F. Pasternack, *J. Amer. Chem. Soc.*, 1971, **93**, 1085.

DISCUSSION

The dissociative mechanism has been well documented for substitution reactions of the nickel(II) ion.[12] The essence of this mechanism is that the rate-determining step for penetration of an incoming ligand into the metal ion's inner co-ordination shell is the rate of release of a co-ordinated solvent molecule. For most neutral ligands, the bimolecular nickel(II) ion substitution rate constant is 1—5 × 10^3 l mol^{-1} s^{-1}. Rate constants lower than those predicted by this mechanism have not been observed as frequently for nickel(II) as for other divalent metal ions. One effect which lowers the observed rate constant below the 'normal' value may be associated with certain multidentate ligands. In the latter case, the rate-determining step is chelate-ring closure, which must be slower than the loss of the first, bound water molecule in order to be noticeable.[12] The effect is most pronounced for ions of inherently greater lability than nickel, *e.g.* manganese [13] or copper.[14,15]

The chemical evidence previously cited indicates that the most probable binding site on the theophylline ligand is N(9). Two possible explanations for the retardation therefore suggest themselves: a chelate is formed between N(9) and the nearest carbonyl oxygen atom, formation of which is relatively slow, or proton loss is rate determining. It is, however, unlikely that a chelate is formed. Moreover, the ring geometry in such a chelate would not be expected to show strain and normal substitution would be expected. Let us, therefore, consider the mechanism of proton loss. Formally, this mechanism is identical with that of water loss [equation (5)]. The observed bimolecular

$$Ni^{2+} + HL \overset{K}{\rightleftharpoons} Ni,HL^{2+} \xrightarrow{k} NiL^{+} + H^{+} \quad (5)$$

rate constant k_{obs}, is given by $k_{obs} = Kk$. For this mechanism, k is not identified with the characteristic nickel(II) ion water-dissociation rate constant, but would instead be assigned to a different rate-limiting step. (As long as $d[Ni,HL^{2+}]/dt \cong 0$ holds, only one relaxation process will be observed.) Rate-limiting proton loss has been previously observed in other systems. For example, this effect has been discussed in connection with the formation of vanadium(IV)–tartrate complexes.[16] In the present case, it is possible that hydrogen bonding between the carbonyl oxygen atom and the N(9) hydrogen atom hinders expulsion of the proton upon metal binding at N(9). The effect should also be present during formation of the bis complex.

The results in this study indicate that $k_2 \approx k_1$. If theophylline binds as a negative ion, it would be expected that $k_2 > k_1$. That is, since the charge product of the partners is the same for formation of both mono- and bis-complexes, only the water-loss rate constant should control the rate of reaction. It has, however, been shown that co-ordination of a negatively charged species in a metal ion's inner hydration sheath enhances the rate of water loss.[8,12] That this effect is not observed here, is also suggestive of a departure from the normal substitution mechanism for theophylline.

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[2/2010 *Received, 24th August,* 1972]

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[12] K. Kustin and J. Swinehart, *Progr. Inorg. Chem.*, vol. 13 'Mechanisms of Inorganic Reactions,' John Wiley and Sons, Inc., New York, 1970, p. 107.

[13] K. Kustin, R. F. Pasternack, and E. W. Weinstock, *J. Amer. Chem. Soc.*, 1966, **88**, 4610.

[14] R. F. Pasternack and K. Kustin, *J. Amer. Chem. Soc.*, 1968, **90**, 2295.

[15] R. G. Pearson and O. P. Anderson, *Inorg. Chem.*, 1970, **9**, 39.

[16] K. Kustin and R. Pizer, *Inorg. Chem.*, 1970, **9**, 1536.