# Purines. Part I. Kinetics of Interaction of Nickel(II) with some Purine Bases and Nucleosides

By **Anne Casper** and **G. Victor Fazakerley,*** Department of Inorganic Chemistry, University of Cape Town, Rondebosch 7700, S. Africa

The interaction of $Ni^{II}$ with adenine, 4-aminopyrazolo[3,4-*d*]pyrimidine (app), adenosine, hypoxanthine, and inosine have been studied by stopped-flow techniques. Adenine and app show a complex reaction pattern with both the neutral (HL) and protonated ($H_2L^+$) species attacking the nickel ion with subsequent deprotonation, the forward rate constants for adenine being 336 and $1.44 \times 10^3$ l $mol^{-1}$ $s^{-1}$ respectively. The preference for the protonated ligand may be explained in terms of the neutral ligand hydrogen bonding with a water molecule in the metal-ion co-ordination sphere, poorly aligning the ligand for attack on $Ni^{II}$. Hypoxanthine and inosine show no pH dependence in the region pH 2—6 and give rates within the range of ' normal ' substitution.

RELATIVELY few kinetic studies have been reported on the interaction of purines, nucleosides, and nucleotides [1-8] with first-row transition-metal ions despite the importance of such studies in understanding the role of these ions in biological systems. The nickel(II)–adenine system was studied by Boivin and Zador [1] who observed a pH-independent reaction and by Kustin [4] who postulated a pH-dependent reaction involving only the neutral ligand which deprotonates after attack. It has been suggested that linkage isomerism occurs, giving rise to observed non-exponential traces because this behaviour was absent with theophylline (1,3-dihydro-2,6-dioxopurine). Our own results suggest that a bad fit of the data is due to the presence of a more complex reaction scheme than previously considered.

## EXPERIMENTAL

Adenine, 4-aminopyrazolo[3,4-*d*]pyrimidine (app), hypoxanthine, and inosine were obtained from Aldrich Chemical Co. Nickel(II) perchlorate was prepared from AnalaR $Ni[SO_4]$ and $Na[HCO_3]$ and treating the $Ni[HCO_3]_2$ produced with AnalaR $HClO_4$. Excess of $Ni[HCO_3]_2$ was filtered off and $Ni[ClO_4]_2$ crystallized. Solutions of $Ni[ClO_4]_2$ were standardized by adding a known concentration of ethylenediaminetetra-acetate (edta) and back titrating with standard $Mg[SO_4]$ using Eriochrome Black T as indicator.

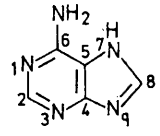
Dissociation constants were determined by potentiometric titration of acidified ligand solutions, using standardized carbonate-free NaOH (*ca* 0.1M) as titrant.† The solutions were 0.4M in $Na[ClO_4]$. Measurements were made at 283 K on an Orion 801 digital pH meter. A Beckman 39000 glass electrode was used with a Wilhelm-type bridge reference electrode. Hydrogen-ion activities were divided by 0.683 [9] to obtain $[H^+]$. To calculate $OH^-$ concentrations it was necessary to evaluate $K_w$ under the conditions of the experiments. This was achieved by titrating NaOH against $HClO_4$ at $I = 0.4$M ($Na[ClO_4]$) and taking points in the region pH 10—11.5; $K_w$ was found to be $1.22 \times 10^{-14}$ $mol^2$ $l^{-2}$.

Reactions were followed at 280 nm for adenine and inosine, 290nm for app, 270nm for hypoxanthine, and 310nm for adenosine on a Durrum D-110 stopped-flow spectrophotometer at 283 ± 0.5 K. An acetate buffer was used ($5 \times 10^{-2}$M) and solutions were made up to a constant ionic strength of 0.4M with $Na[ClO_4]$. Ligand concentrations were $5 \times 10^{-4}$M in the reaction mixture. Although acetate ions act as ligands towards $Ni^{II}$,[10] the reaction is too fast to be observed by stopped-flow techniques and the formation constant is relatively small. Test runs in the presence and absence of acetate established that the experimental rate constants were not affected by its presence. Reactions were run under pseudo-first-order conditions with $Ni^{II}$ concentrations in 10—100-fold excess to ensure that only the 1 : 1 species was formed. Oscilloscope traces yielded excellent first-order rate constants, linear for at least 90% completion of reaction. Values of $k_{obs.}$ used in evaluating further parameters were an average of 3—5 individual determinations.

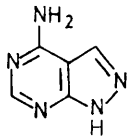
† 1M = 1 mol $dm^{-3}$.

## RESULTS

*Protolytic Dissociation Constants.*—Both $pK_{a1}$ and $pK_{a2}$ of adenine and app were determined. Values of $pK_{a1}$ and $pK_{a3}$ of hypoxanthine and inosine were too low and too high, respectively, to warrant consideration, but the $pK_{a2}$ values were determined. The results are listed in Table 1 with available literature values.



Adenine



app

The reactions were first order with respect to metal-ion concentration, linear plots being obtained of $k_{obs.}$ against $[Ni^{2+}]$. These plots did not pass through the origin, showing that the kinetics of complex formation obey the reversible mixed second- and first-order rate equation (1) where

$$-\mathrm{d}\,[\mathrm{L}]_\mathrm{T}/\mathrm{d}t = k_\mathrm{f}\,[\mathrm{Ni}^{2+}]\,[\mathrm{L}]_\mathrm{T} - k_\mathrm{d}\,[\mathrm{Product}] \qquad (1)$$

$k_f$ and $k_d$ are the observed formation and dissociation rate constants, respectively.

Adenine and app behaved unusually towards $Ni^{II}$ in that, when the $Ni^{2+}$ concentration was held constant and the pH

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varied, $k_{obs.}$ increased with increasing hydrogen-ion concentration. The pH effect indicates that hydrogen ions are released on complex formation and/or that more than one ligand species is reactive towards the metal, the fastest

TABLE 1

Protolytic dissociation constants

| Ligand | $pK_{a1}$ | $pK_{a2}$ | Conditions ($\theta_c$/°C, $I$/M) | Ref. |
|---|---|---|---|---|
| Adenine | 4.26 ± 0.03 | 9.90 ± 0.02 | 10, 0.4 | *a* |
| | 4.22 | 9.80 | 25, 0.05 | 11 |
| | 4.25 | 9.83 | 20 | *b* |
| | 4.18 | 9.70 | 25, 0.05 | 12 |
| app | 4.57 ± 0.03 | 11.0 ± 0.1 | 10, 0.4 | *a* |
| Hypoxanthine | | 8.76 ± 0.04 | 10, 0.4 | *a* |
| | | 8.83 | 25, 0.05 | 11 |
| | 1.98 | 8.94 | 20 | *c* |
| Inosine | | 8.68 ± 0.04 | 10, 0.4 | *a* |
| | *ca.* 1.5 | 8.82 | 20 | *c* |

[a] This work. [b] A. Albert and E. P. Serjeant, *Biochem. J.*, 1960, **76**, 621. [c] A. Albert, *Biochem. J.*, 1953, **54**, 646.

reacting species being that which increases in concentration as the pH is lowered. In the pH range over which this study was conducted (6.5—4.2 for adenine and 6.2—4.6 for app) this species is the protonated form of the ligand, $H_2L^+$. The coulombic repulsion produced by two positively charged species interacting causes the extent of reaction to decrease considerably, decreasing the total optical absorbance change, and resulting in unfavourable signal to noise ratios; hence any measurements made at pH values below the $pK_{a1}$ values of the ligands have a high uncertainty. Nevertheless, although it was not possible to study the reactions in a region where $H_2L^+$ is the dominant species, a clear pattern emerged making the following mechanism, in which all three ligand species are postulated as reacting to give identical products, the most likely.

$$H_2L^+ + Ni^{2+} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [NiL]^+ + 2H^+ \quad (2)$$

$$HL + Ni^{2+} \underset{k_4}{\overset{k_3}{\rightleftharpoons}} [NiL]^+ + H^+ \quad (3)$$

$$L^- + Ni^{2+} \underset{k_6}{\overset{k_5}{\rightleftharpoons}} [NiL]^+ \quad (4)$$

In the pH range studied the concentration of $L^-$ is negligible; hence the $k_5$ path need not be considered. The rate equation resulting from this mechanism is (5). Since

$$k_{obs.} = \frac{[Ni^{2+}]}{K_{a1} + [H^+]}(k_1[H^+] + k_3K_{a1}) + k_2[H^+]^2 + k_4[H^+] + k_6 \quad (5)$$

it is likely that $k_2[H^+]^2 \ll k_4[H^+]$ or $k_6$, this equation reduces to (6). Plots of $k_{obs.}$ against $[Ni^{2+}]/(K_{a1} + [H^+])$

$$k_{obs.} = \frac{[Ni^{2+}]}{K_{a1} + [H^+]}(k_1[H^+] + k_3K_{a1}) + k_4[H^+] + k_6 \quad (6)$$

should be linear, with gradient $(k_1[H^+] + k_3K_{a1})$ and intercept $(k_4[H^+] + k_6)$. These constant-pH studies were conducted for both ligands and families of straight lines were obtained, each family consisting of four lines corresponding to four pH values. Values of the gradients and intercepts (Table 2) were then plotted against $[H^+]$ to yield $k_1$ and $k_3$ and $k_4$ and $k_6$ respectively (Table 3).

TABLE 2

Gradients and intercepts obtained by plotting $k_{obs.}$ against $[Ni^{2+}]/(K_{a1} + [H^+])$

| Ligand | $10^5[H^+]$/M | Gradient/$s^{-1}$ | Intercept/$s^{-1}$ |
|---|---|---|---|
| Adenine | 0.583 | 0.0268 ± 0.002 | 26.8 ± 0.3 |
| | 1.46 | 0.0390 ± 0.002 | 27.4 ± 0.4 |
| | 2.93 | 0.0608 ± 0.003 | 28.4 ± 0.6 |
| | 4.63 | 0.0842 ± 0.006 | 29.5 ± 1.1 |
| app | 0.158 | 0.0147 ± 0.005 | 79.5 ± 1.5 |
| | 0.583 | 0.0480 ± 0.006 | 82.3 ± 1.8 |
| | 1.46 | 0.1132 ± 0.007 | 87.0 ± 1.1 |
| | 2.32 | 0.1838 ± 0.014 | 92.8 ± 2.8 |

The good fit of the data to the rate equation adds confidence to the proposed mechanism. A further test was applied, more rigorous in that it encompassed a wider pH range. This consisted of rearranging equation(6) to give a function of $k_{obs.}$ linear in $[H^+]$ when the $Ni^{2+}$ concentration is held constant [equation (7)]. The value of $k_4$ obtained

$$k_{obs.}([H^+] + K_{a1}) - k_4[H^+]^2 = [H^+]([Ni^{2+}]k_1 + k_4K_{a1} + k_6) + [Ni^{2+}]k_3K_{a1} + k_6K_{a1} \quad (7)$$

from the $[Ni^{2+}]$-dependence studies was used to calculate the left-hand side of equation (7) corresponding to each $[H^+]$ value. This was then plotted against $[H^+]$. As seen in Figures 1 and 2 the points fall on a straight line, as anticipated. The gradient of the line is $([Ni^{2+}]k_1 + k_4K_{a1} + k_6)$ and the intercept $([Ni^{2+}]k_3K_{a1} + k_6K_{a1})$. Table 4 shows the experimental gradients and intercepts together with those derived from the data in Table 3. The agreement, both for the adenine and app systems, is very satisfactory.

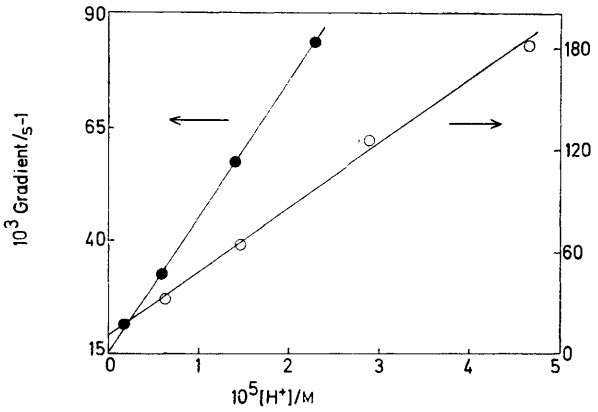


FIGURE 1 Determination of $k_1$ and $k_3$ for adenine (●) and app (○)

*Hypoxanthine and Inosine.*—Hypoxanthine and inosine were also found to obey the reversible mixed second- and first-order rate equation in their reactions with $Ni^{II}$. However, unlike adenine and app, no change in $k_{obs.}$ was discerned on varying $[H^+]$ over three pH units. This indicates that only one ligand species is reactive towards the metal; this is assumed to be the neutral species ($H_2L$), which is predominant in the range studied.

The mechanism proposed is as in (8), for which the rate

$$H_2L + Ni^{2+} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [Ni(H_2L)]^{2+} \quad (8)$$

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TABLE 3

Summary of results obtained on $Ni^{II}$–adenine and –app studies

| Equilibrium | Conditions | Rate constants | Ref. |
|---|---|---|---|
| (*a*) Adenine | | | |
| $Ni^{2+} + HL \underset{k_d}{\overset{k_f}{\rightleftharpoons}} [Ni(HL)]^{2+}$ | pH 4.2, $I$ = 1.2M ($Na[ClO_4]$)<br>(*i*) 8 °C<br>(*ii*) 31 °C | <br>$k_f$ = 33 l mol$^{-1}$ s$^{-1}$, $k_d$ = 18 s$^{-1}$<br>$k_f$ = 500 l mol$^{-1}$ s$^{-1}$, $k_d$ = 130 s$^{-1}$ | 1 |
| $Ni^{2+} + HL \underset{k_d}{\overset{k_f}{\rightleftharpoons}} [NiL]^{+} + H^{+}$ | pH 4.9—6, $I$ = 0.1M ($K[NO_3]$), 25 °C | $k_f$ = 300 l mol$^{-1}$ s$^{-1}$, $k_d = 10^7$ l mol$^{-1}$ s$^{-1}$ | 4 |
| $Ni^{2+} + H_2L^{+} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [NiL]^{+} + 2H^{+}$ | pH 4.2—6.5, $I$ = 0.4M ($Na[ClO_4]$), 10 °C | $k_1 = (1.44 \pm 0.08) \times 10^3$ l mol$^{-1}$ s$^{-1}$ | This work |
| $Ni^{2+} + HL \underset{k_4}{\overset{k_3}{\rightleftharpoons}} [NiL]^{+} + H^{+}$ | | $k_3$ = 336 ± 30 l mol$^{-1}$ s$^{-1}$, $k_4$ = (6.64 ± 1.5) × 10$^4$ l mol$^{-1}$ s$^{-1}$ | |
| $Ni^{2+} + L^{-} \underset{k_6}{\overset{k_5}{\rightleftharpoons}} [NiL]^{+}$ | | $k_6$ = 26.44 ± 0.2 s$^{-1}$ | |
| (*b*) App | | | |
| $Ni^{2+} + H_2L^{+} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [NiL]^{+} + 2H^{+}$ | pH 4.6—6.2, $I$ = 0.4M ($Na[ClO_4]$), 10 °C | $k_1 = (7.67 \pm 0.4) \times 10^3$ l mol$^{-1}$ s$^{-1}$ | This work |
| $Ni^{2+} + HL \underset{k_4}{\overset{k_3}{\rightleftharpoons}} [NiL]^{+} + H^{+}$ | | $k_3$ = 96.6 ± 50 l mol$^{-1}$ s$^{-1}$, $k_4$ = (6.09 ± 0.6) × 10$^5$ l mol$^{-1}$ s$^{-1}$ | |
| $Ni^{2} + L^{-} \underset{k_6}{\overset{k_5}{\rightleftharpoons}} [NiL]^{+}$ | | $k_6$ = 78.5 ± 1.3 s$^{-1}$ | |

TABLE 4

Comparison of observed gradient and intercept obtained on plotting $\{k_{obs.}([H^+] + K_{a1}) - k_4[H^+]^2\}$ against $[H^+]$ and those predicted using equation 7 and Figures 1 and 2

| | Gradient/s$^{-1}$ | | 10$^3$ Intercept/l mol$^{-1}$ s$^{-1}$ | |
|---|---|---|---|---|
| Ligand | obs. | calc.[a] | obs. | calc.[b] |
| Adenine | 52.0 ± 2.0 | 47.8 ± 2.0 | 1.7 ± 0.1 | 1.68 ± 0.04 |
| app | (2.23 ± 0.1) × 10$^2$ | (2.05 ± 0.12) × 10$^2$ | 2.3 ± 0.15 | 2.15 ± 0.05 |

[a] Gradient = $[Ni^{2+}]k_1 + k_4K_{a1} + k_6$. [b] Intercept = $[Ni^{2+}]k_3K_{a1} + k_6K_{a1}$.

equation (9) applies. In order to determine $k_1$ and $k_2$, $k_{obs.}$

$$k_{obs.} = k_1[Ni^{2+}] + k_2 \quad (9)$$

was measured as a function of metal-ion concentration. Linear plots were obtained and from the gradients and

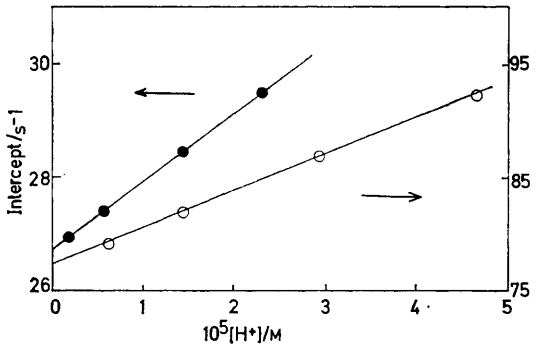


FIGURE 2 Determination of $k_4$ and $k_6$ for adenine (●) and app (○)

intercepts, respectively, the calculated rate constants are: hypoxanthine, $k_1 = (1.90 \pm 0.1) \times 10^3$ l mol$^{-1}$ s$^{-1}$, $k_2$ = 32.1 ± 2.0 s$^{-1}$; inosine, $k_1 = (1.12 \pm 0.10) \times 10^3$ l mol$^{-1}$ s$^{-1}$ $k_2$ = 59.5 ± 1.5 s$^{-1}$.

## DISCUSSION

*Adenine and app.*—The mechanism postulated here differs from those proposed previously [1,4] where only the neutral ligand, HL, was the reactive species. The results are summarized in Table 3. Although the experimental conditions vary widely, the previous results can be questioned. Boivin and Zador found no evidence for reaction at pH values greater than 5 where the neutral species predominates, and yet propose this as the reacting species.[1] Kustin[4] quotes a rather high experimental deviation of ±25% on relaxation data because of ligand instability. However, under the conditions of our reactions the ligand spectrum remains unchanged over several days. The poor fit arises because the protonated ligand species also reacts with $Ni^{II}$, followed by deprotonation of the intermediate species. It is interesting to note the preference of $Ni^{II}$ for $H_2L^+$ rather than HL. The reaction with HL is unusually slow, being about an order of magnitude less than that for 'normal' substitution. This is not a temperature effect, as Kustin [4] found anomalously slow behaviour at room temperature.

We suggest that the neutral ligand initially forms a hydrogen bond from N(1) to a water molecule in the metal's inner co-ordination sphere [N(1) is involved in hydrogen bonding between adenine and thymine in the Watson–Crick model of base pairing]. Metal binding at N(1) seems unlikely in adenine; none of the studies in solution or in the solid state have indicated an N(1)–metal co-ordinate bond.[11-19] This misorientation caused

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[19] L. Srinivasan and M. R. Taylor, *Chem. Comm.*, 1970, 1668.

by hydrogen bonding slows the reaction. If N(1) is protonated[20] in $H_2L^+$ hydrogen bonding cannot occur and 'normal' substitution is observed. The app–$Ni^{II}$ system is rationalized in the same way. The differences in $k_3$ and $k_1$ for the two ligands results from N(1) in app being more basic resulting in a stronger hydrogen-bonding effect.

The binding site on adenine and app is proposed to be N(9). This may explain why the $Ni^{II}$–adenosine reaction was too fast to be measured on the stopped-flow apparatus as N(9) is blocked and another mechanism must be operative. The formation rate constant for this reaction is reported [1] to be about five times greater than for adenine. Most crystal-structure studies show N(9) and N(3) as binding sites, but this may not be so in solution. It seems unlikely that a chelate structure involving N(7) and the amino-group occurs because of the large value of $k_1$. This should be much smaller if an SCS (sterically controlled substitution) mechanism were operative. The steric strain involved in distorting the orbitals would almost certainly slow down the reaction.

*Hypoxanthine and Inosine.*—Both ligands gave formation rate constants ($k_1$) with $Ni^{II}$ within the range of 'normal' substitution with a neutral ligand. Kustin [4] attempted to study the hypoxanthine–$Ni^{II}$ system but observed non-exponential relaxation traces and suggested that this might be due to linkage isomerism. The excellent exponential traces observed in this study indicate that if linkage isomers do coexist the various binding sites on the ligand must have either near-identical affinities for the metal ion or their affinities are so different only one effect can be observed.

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