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Kinetics and mechanism of complexation of *trans*-$[PtCl(NH_3)_2(H_2O)]^+$ with inosine and 1-methylinosine in aqueous solution at different pH values

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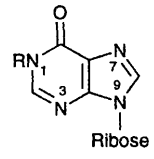
The kinetics of complexation of *trans*-$[PtCl(NH_3)_2(H_2O)]^+$ with inosine and its 1-methyl derivative has been studied at 298.2 K in aqueous solution (pH 2.8–8.4) by employing HPLC as an analytical tool. Under these conditions *trans*-$[PtCl(NH_3)_2(H_2O)]^+$ behaves like a monofunctional platinum(II) species. Throughout the pH range studied the complex formation can be explained by substitution of the aqua ligand by the incoming nucleoside, the OH group appearing to be inert toward substitution. 1-Methylinosine forms only a N^7-bound 1:1 complex. This binding mode is favoured also with inosine when pH < 6, whereas above this pH N^1 becomes an additional binding site which facilitates the formation of a N^1,N^7-bonded diplatinum species, too. Rate parameters obtained for the formation of the inosine 1:1 complexes show that the N^7 site is preferred over the N^1 site, whereas in the binding of the second platinum(II) unit to the different 1:1 complexes the N^1 site is slightly more favourable than the N^7 site. Co-ordination of *trans*-$[PtCl(NH_3)_2(H_2O)]^+$ to the inosine N^7 site lowers the basicity of N^1H site by about 1.3 log units.

In aqueous solution hydrolysis of the first chloride ligand is usually the rate-determining step in the complexation of isomeric $[PtCl_2(NH_3)_2]$ with biologically relevant compounds, *e.g.* nucleic acid constituents, unless their concentration is very high.[1] Under acidic conditions hydrolysis is, however, strongly reversible and chloride anation of the first hydrolysis products efficiently blocks the complexation.[2,3] In basic solution, instead, complete hydrolysis occurs which gives substitution-inert dihydroxo species.[3–5] Quantitative studies on various binding modes of the monochloromonoaqua species thus become difficult if the dichloro compounds are used as starting materials. Owing to the biological activity of *cis*-$[PtCl_2(NH_3)_2]$ much attention has been paid to the isolation and properties of *cis*-$[PtCl(NH_3)_2(H_2O)]^+$,[2,5,6] whereas studies dealing with the corresponding *trans* derivative are rather limited.[3,7,8]

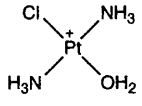
We have recently reported the preparation and structural characterization of *trans*-$[PtCl(OH)(NH_3)_2]\cdot H_2O$.[9] In acidic aqueous solution it provides the *in situ* generation of *trans*-$[PtCl(NH_3)_2(H_2O)]^+$ facilitating detailed investigations of the reactions of this ion without the strong intervening influence of Cl^- ion. As an extension to our previous studies,[3] we now report on the kinetics of complexation of *trans*-$[PtCl(NH_3)_2(H_2O)]^+$ with the 6-oxopurine derivatives inosine and its 1-methyl derivative in aqueous solution in the range pH 2.7–8.4 at 298.2 K.

Experimental

The complex *trans*-$[PtCl(OH)(NH_3)_2]\cdot H_2O$ was prepared as previously reported.[9] For the preparation of *trans*-$[PtCl(NH_3)_2(Hino\text{-}N^7)]^+$ **1**, inosine (40 mg, 0.15 mmol) dissolved in 0.5 mol dm^{-3} HNO_3 (0.32 cm^3) was added to a solution of *trans*-$[PtCl(OH)(NH_3)_2]\cdot H_2O$ (45 mg, 0.15 mmol) in water (0.5 cm^3). Any insoluble material was immediately removed by centrifugation, and water (1 cm^3) was added to the supernatant. The white voluminous solid *trans*-$[PtCl(NH_3)_2(Hino\text{-}N^7)]NO_3$, which appeared quite soon was filtered off after cooling the mixture on ice. It was washed with a small amount of cold water, then with cold ethanol and finally air-dried. Yield: 60 mg (65%) (Found: C, 19.4; H, 3.00; N, 15.9. $C_{10}H_{18}ClN_7O_8Pt$ requires C, 20.2; H, 3.05; N, 16.5%). Other materials and the general procedure for kinetic measurements were as described earlier.[10]



Inosine, (Hino, R = H)
1-Methylinosine (mino, R = CH_3)



trans-$[PtCl(NH_3)_2(H_2O)]^+$ ion

Kinetic runs were started by adding the desired amount of a fresh solution of Pt^{II} to a pre-thermostatted reaction mixture ($[Pt]_T > 20[L]_T$). Samples withdrawn from the reaction mixture at suitable time intervals were immediately analysed by HPLC using an RP-18 column (Merck LiChrospher 100 endcapped, 5 μm) and aqueous 0.05 mol dm^{-3} $NaClO_4$–methanol mixtures (pH 3) as eluents. Peak areas at 260 nm were used as the measure of the concentration by employing 1,3-dimethyluracil as an internal standard.

Pseudo-first-order rate constants, k_i', for the disappearance of the free nucleoside or the N^7-bound 1:1 complex were calculated by use of equation (1), where $[X]_0$ denotes the initial

$$\ln[X]_t = -k_i' t + \ln[X]_0 \qquad (1)$$

concentration of the nucleoside or the complex and $[X]_t$ is the concentration at time t. The reactions were followed at least for two half-lives, which gave strictly linear plots of $\ln[L]_t$ *vs.* t in each case. Above pH 6.3 the time-dependent concentration of the N^1- and N^7-bound 1:1 inosine complexes ($[ML]_t$) gave the rate constants k_d' for the formation of the 2:1 complex from these species in the presence of excess of Pt by use of equation (2).

$$[ML]_t = [L]_T \frac{k_f'}{k_d' - k_1'} (e^{-k_1' t} - e^{k_d' t}) \qquad (2)$$

Here k_1' refers to the rate constant of the formation of all 1:1 complexes obtained by equation (1) from the disappearance of the free nucleoside, and k_f' denotes the individual rate constant for the formation of the N^1- or N^7-bound species. The latter were found from the ratio of the areas of the 1:1 complexes determined chromatographically at the initial stage of the reaction. The apparent ratio was corrected by transforming the

peak area of **1** into the concentration from the consumption of the free nucleoside by employing an internal standard, and by taking into account that **1** was the only detectable species formed below pH 5.8.

The protonation constant of *trans*-$[PtCl(OH)(NH_3)_2]$ was determined potentiometrically at 298.2 K in 0.1 mol dm^{-3} $NaClO_4$ under nitrogen by titrating with a standard 0.1 mol dm^{-3} $HClO_4$ solution using a Metrohm combined glass electrode. The pH meter was calibrated with Metrohm standard buffer solutions (pH 4.00 and 7.00).

The NMR measurements were carried out in D_2O on a JEOL JNM-A500 spectrometer; ^1H spectra were recorded at 500 MHz by using Bu^{t}OH as internal standard (δ_H 1.24 from sodium 4,4-dimethyl-4-silapentane-1-sulfonate), ^{195}Pt spectra at 107.11 MHz ($[PtCl_4]^{2-}$ as external reference, δ_{Pt} −1625 from $[PtCl_6]^{2-}$).

Results and Discussion

1-Methylinosine

Complexation of *trans*-$[PtCl(NH_3)_2(H_2O)]^+$ with 1-methylinosine appeared to follow a similar pattern to that found earlier for monofunctional $[Pt(dien)(H_2O)]^{2+}$ (dien = diethylenetriamine) with this nucleoside,[10] *i.e.* chromatographic analysis throughout the pH range studied revealed the formation of a single reaction product which most probably refers to the N^7 bound 1:1 complex. As seen in Table 1, the observed second-order rate constant for the formation of this species decreases with increasing pH. Since 1-methylinosine is neutral in the pH range studied (pK_a 1.39),[11] the decrease in $k_{1,obs}$ must be attributed to deprotonation of the aqua ligand in *trans*-$[PtCl(NH_3)_2(H_2O)]^+$ ion which gives the substitution-inert OH group.[10,12] Accordingly, the proposed reaction mechanism may be depicted by Scheme 1 and the pH-dependent rate constant, $k_{1,obs}$, by equation (3). Here k_1

$$k_{1,obs} = k_1[H^+]/([H^+] + K_1) \qquad (3)$$

represents the second-order rate constant for the formation of *trans*-$[PtCl(NH_3)_2(mino\text{-}N^7)]^+$ ion and K_1 is the acidity constant of *trans*-$[PtCl(NH_3)_2(H_2O)]^+$ ion. A least-squares fit to the rate data gave $k_1 = 0.352 \pm 0.007$ dm^3 mol^{-1} s^{-1} and $K_1 = (1.28 \pm 0.04) \times 10^{-6}$ mol dm^{-3}.* The latter is in good agreement with the pK_1 value of 5.94 ± 0.02 found potentiometrically. In the literature a pK_a of 5.63 has been reported for this species at 298 K from NMR measurements.[7] It is known that concentrated acidic aqueous solutions of *trans*-$[PtCl(NH_3)_2(H_2O)]^+$ are not infinitely stable, but slowly disproportionate into dichloro and diaqua species.[3,7] At pH 4.5–6 dinuclear species may also be formed.[7] To prevent these side reactions only fresh solutions of *trans*-$[PtCl(NH_3)_2(H_2O)]^+$ were employed, and the total concentration of Pt^{II} in kinetic runs was kept as low as possible (< 0.02 mol dm^{-3}) by taking into account that the aquation rate of *trans*-$[PtCl(NH_3)_2(H_2O)]^+$ increases upon deprotonation of the aqua ligand.[3] The linearity of plots of $\ln[L]_t$ *vs.* *t* (correlation coefficients > 0.998) showed that no significant changes in $[Pt]_T$ had occurred during the complex formation. However, near pH 6 rather low platinum(II) concentrations (≈0.001 mol dm^{-3}) were required to give satisfactory kinetics. At higher $[Pt]_T$ values plots of $\ln[L]$ *vs.* *t* showed upfield curvature, which suggests a decrease in the effective platinum(II) concentration during the complexation probably due to the formation of OH-bridged species.

* The data quoted were obtained using a logarithmic scale for $k_{1,obs}$ and equation (3) in logarithmic form to avoid domination by the larger $k_{1,obs}$ values. However, the values obtained on the normal scale were practically identical, *viz.* $k_1 = 0.350 \pm 0.004$ dm^3 mol^{-1} s^{-1} and $K_a = (1.22 \pm 0.07) \times 10^{-6}$ mol dm^{-3}.

Table 1 Observed second-order rate constants for the complexation of *trans*-$[PtCl(NH_3)_2(H_2O)]^+$ with 1-methylinosine in aqueous solution of different pH at 298.2 K[a]

pH	$10^3 k_{obs}$/dm^3 mol^{-1} s^{-1}
2.70	355
3.80	349
4.25	344
5.05	280
5.35	272
5.75	217
6.25	114
6.40	80
7.45	8.9
7.65	6.3
7.80	4.3[b]

[a] I = 0.1 mol dm^{-3}. [b] Refers to the initial value.

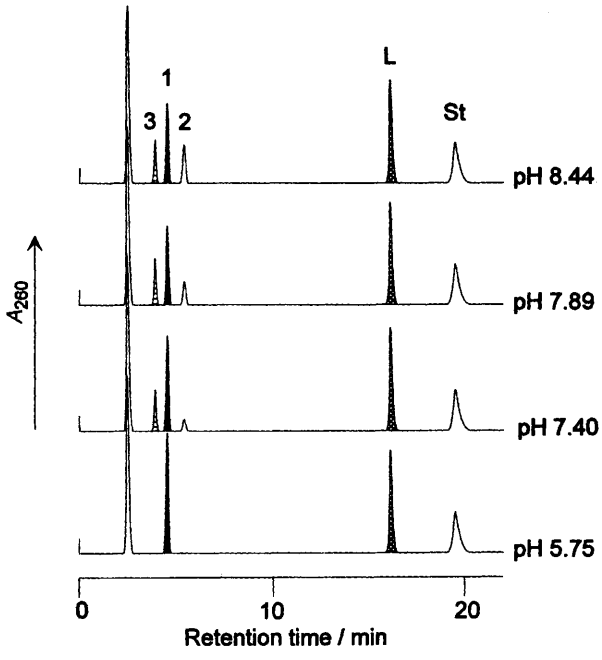


Fig. 1 Smoothed HPLC traces of the system *trans*-$[PtCl(NH_3)_2(H_2O)]^+$–inosine (L) after one half-life at different pH values using aqueous 0.05 mol dm^{-3} $NaClO_4$–methanol (pH 3) as eluent (linear gradient from 100:0 to 91:9 in 6 min, 3 min initial delay, flow rate 1.0 cm^3 min^{-1}. Notation: **1**, N^7-bound 1:1 complex; **2**, N^1-bound 1:1 complex; **3**, N^1,N^7-bound diplatinum complex; St = 1,3-dimethyluracil. The A_{260} scale is arbitrary

$$[PtCl(NH_3)_2(H_2O)]^+ \underset{+L}{\overset{k_1}{\longrightarrow}} [PtCl(NH_3)_2(L)]^+$$
$$+H^+ \uparrow\downarrow -H^+; K_1$$
$$[PtCl(OH)(NH_3)_2]$$

Scheme 1

Inosine

Fig. 1 depicts typical chromatograms of the system *trans*-$[PtCl(NH_3)_2(H_2O)]^+$–Hino recorded after one half-life at different pH values. Below pH 5.8 only one product is detected which may be assigned to the N^7-bound 1:1 complex **1**, analogously to that found earlier with aquated Pt^{II}(dien).[10] The NMR spectroscopic data for **1** are consistent with N^7-co-ordination [δ 8.97 (H^8) and 8.33 (H^2) indicate N^7-bound platinum species;[10] δ_{Pt} −2303 is typical for a PtN_3Cl

chromophore[13]]. Above this pH two additional products appear, and their relative amount increases with increasing pH. On the basis of their time-dependent formation these are assigned to a N^1,N^7-bound diplatinum complex **3** and an N^1-bound 1:1 complex **2** according to their order of elution. Evidently the appearance of these signals results from the deprotonation of inosine N^1H, which is an additional binding site for Pt^{II} and facilitates the formation of the 2:1 complex, too. Consequently, the complexation pathway of inosine with excess of Pt^{II} may be depicted as in Scheme 2. After longer reaction times additional products begin to appear, which have shorter retention times than that of **1** (not shown in Fig. 1). It is assumed that these refer to the aqua derivatives of species **1–3**, which are expected to elute earlier than the corresponding chloro complexes.[3] Since the areas of these peaks were less than 15% of the area of the minor species **2** at its maximum, these possible side-reactions were not taken into account in the calculations, except when pH > 8. In these cases the contribution of the aquation reaction to the disappearance of **1** and **2** was approximated using the value of 1.0×10^{-5} s^{-1} found for the aquation of **1** at 298.2 K (pH 10). Other side-reactions, *e.g.* migration of Pt^{II} between the N^1 and N^7 sites,[14] were not detected.

Rate constants, k_1', for the formation of complexes **1** and **2** were calculated by equation (1) from the disappearance of the free nucleoside. Below pH 7 the data obtained directly give the observed rate constant for the formation of **1**, when divided by $[Pt]_T$ (Table 2). By contrast, above this pH a similar treatment yields the sum of the rate constants for **1** and **2**, which were resolved by employing the product distribution obtained chromatographically. According to Scheme 2 the observed rate constant, $k_{(1+2),obs}$, for the formation of **1** may be expressed by equation (4). Here K_1 and K_L ($= 1.58 \times 10^{-9}$ mol dm^{-3})[15] are

$$k_{(1+2),obs} = \frac{(k_1[H^+] + k_2K_L)[H^+]}{(K_1 + [H^+])(K_L + [H^+])} \quad (4)$$

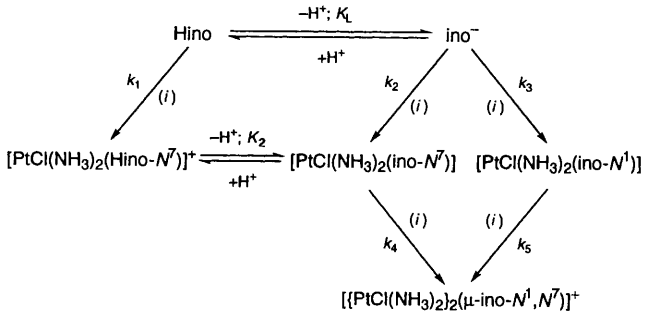
the known acidity constants of *trans*-$[PtCl(NH_3)_2(H_2O)]^+$ and inosine, respectively. Least-squares fitting to the data using logarithmic values for $k_{(1+2),obs}$ and equation (4) in logarithmic form gave $k_1 = 0.302 \pm 0.009$ dm^3 mol^{-1} s^{-1} and $k_2 = 2.2 \pm 0.2$ dm^3 mol^{-1} s^{-1} (normal-scale fitting gave $k_1 = 0.307 \pm 0.001$ and $k_2 = 2.2 \pm 1.0$ dm^3 mol^{-1} s^{-1}). Thus, *trans*-$[PtCl(NH_3)_2(H_2O)]^+$ seems to have an almost equal affinity for the N^7 sites of neutral inosine and its 1-methyl derivative, analogously to what was found earlier with $[Pt(dien)(H_2O)]^{2+}$.[10] The employment of normal-scale fitting to the data obtained for **2** gave $k_3 = 1.6 \pm 0.1$ dm^3 mol^{-1} s^{-1} by equation (4), when k_2 is replaced by k_3 and $k_1 = 0$. The $N^1:N^7$ binding ratio of about 0.7:1 is in agreement with earlier findings[10] for $[Pt(dien)(H_2O)]^{2+}$ and shows that deprotonation of inosine N^1H increases the affinity of N^7 for Pt^{II} also in this case.

As seen in Table 2, the rate data obtained for the formation of complex **3** from **1** using either *trans*-$[PtCl(NH_3)_2(H_2O)]^+$ or isolated **1** as starting materials are compatible, which supports the validity of the data. According to Scheme 2 the observed rate constant $k_{4,obs}$ may be expressed by equation (5), which

$$k_{4,obs} = \frac{k_4K_2[H^+]}{(K_2 + [H^+])(K_1 + [H^+])} \quad (5)$$

gives $k_4 = 0.86 \pm 0.04$ dm^3 mol^{-1} s^{-1} and $K_2 = (3.0 \pm 0.2) \times 10^{-8}$ mol dm^{-3} by least-squares fit. The latter indicates that the binding of *trans*-$[PtCl(NH_3)_2(H_2O)]^+$ to the N^7 site of inosine lowers the basicity of the N^1 site about 1.3 log units. This is about 0.3 log units less than that induced by $[Pt(dien)(H_2O)]^{2+}$,[10] which is in line with charge effects of these platinum(II) species. For comparison, a difference of 0.67 log units has recently been reported for the acidity constants of the N^1H sites of the two 9-ethylguanines (egua) in *cis*-$[Pt(NH_3)_2(egua)_2]^{2+}$.[16] Treatment of the data found for step **2** ⟶ **3** gave $k_5 = 0.66 \pm 0.01$ dm^3 mol^{-1} s^{-1} by equation (5), in which k_5 stands for k_4 and the term $K_2/(K_2 + [H^+]) \approx 1$ (the protonation constant of **2** is expected to be near 5×10^{-3} mol dm^{-3}).[10] These rate parameters show that the N^1 site is slightly preferred over the N^7 site in the second complexation step.

In summary, the complexation pattern of *trans*-$[PtCl(NH_3)_2(H_2O)]^+$ with the studied nucleosides appears to be very similar to that found earlier for $[Pt(dien)(H_2O)]^{2+}$, *i.e.* *trans*.-$[PtCl(NH_3)_2(H_2O)]^+$ behaves under these conditions like a monofunctional platinum(II) species, in agreement with the *trans* effect $Cl^- > H_2O$.[3,17] Moreover, the magnitudes of the corresponding rate parameters of these platinum(II) compounds roughly match each other suggesting that the *trans* effect of Cl^- is similar to that of the NH group of the terdentate dien ligand. Throughout the pH range studied the complexation of *trans*-$[PtCl(NH_3)_2(H_2O)]^+$ can be fully explained by substitution of the aqua ligand with the incoming nucleoside, which indicates that the OH group bound to Pt^{II} is inert toward substitution relative to the co-ordinated water molecule. Rate parameters obtained for the formation of inosine 1:1 complexes show that the N^7 site is preferred over the N^1 site, whereas in the binding of the second platinum(II) unit to the different 1:1 complexes the N^1 site is slightly more favourable than the N^7 site. Co-ordination of *trans*-$[PtCl(NH_3)_2(H_2O)]^+$ to the inosine N^7 site lowers the basicity of the N^1H site by about 1.3 log units.



Scheme 2 (*i*) *trans*-$[PtCl(NH_3)_2(H_2O)]^+$

Table 2 Observed second-order rate constants, 10^3 $k_{i,obs}$/dm^3 mol^{-1} s^{-1}, for the complexation of *trans*-$[PtCl(NH_3)_2(H_2O)]^+$ with inosine in aqueous solution of different pH at 298.2 K[a]

pH	$k_{(1+2),obs}$	$k_{3,obs}$	$k_{4,obs}$	$k_{5,obs}$
2.80	304			
3.25	301			
4.35	300			
5.10	270			
5.35	250			
5.75	190			
5.81			9.6[b]	
6.14			13.0[b]	
6.30	95.0		14.0	
6.60			16.4[b]	
7.03	25.0		16.0	
7.25			13.6[b]	
7.42	10.9	1.9 (2.0)[c]	12.0	22
7.89	4.65	1.8 (1.9)	6.8	7.7
8.24	3.80	1.8 (1.7)	3.6	3.5
8.44	3.02	1.6 (1.5)	2.4	2.1

[a] $I = 0.1$ mol dm^{-3}. Rate constants obtained by equations (1) and (2). [b] Obtained by equation (1) using complex **1** as a starting material. [c] The data in parentheses refer to values calculated by equation (5), see text.

Acknowledgements

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