Kinetics and Mechanism of the Complexation of *cis*-Diamminedichloroplatinum(II) with the Purine Nucleoside Inosine in Aqueous Solution

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Kinetics of the complexation of *cis*-$Pt(NH_3)_2Cl_2$ (**1**), and its hydrolysis products *cis*-$[Pt(NH_3)_2Cl(H_2O)]^+$ (**2**) and *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ (**3**), with the purine nucleoside inosine (L) has been studied by HPLC in aqueous solution at 318.2 K (pH = 3.5–4.0, I = 0.1 M). The relative ability of cisplatin and its hydrolysis products to bind inosine is about 1:70:350, as given by the ratio of the second-order rate constants $k_3 = (1.9 \pm 0.4) \times 10^{-3}$ M^{-1} s^{-1}, $k_4 = 0.14 \pm 0.01$ M^{-1} s^{-1}, and $k_5 = 0.67 \pm 0.03$ M^{-1} s^{-1} for compounds **1**–**3**, respectively. Excess of ligand gives stepwise formation of the 1:2 complex. When [L] ranges from 0.002 to 0.01 M, hydrolysis of the first chloro ligand is the rate-limiting step in the binding of inosine to cisplatin (**1**) ($k_1 = (1.9 \pm 0.2) \times 10^{-4}$ s^{-1}) as well as to the 1:1 complex (**4**) ($k_6 = (2.1 \pm 0.1) \times 10^{-4}$ s^{-1}), in which the fourth ligand is Cl^-. Direct substitution of the chloro ligand becomes significant in higher ligand concentration ([L] > 0.01 M). By contrast, when [L] < 0.002 M second hydrolysis of **1** ($k_2 = (2.3 \pm 0.3) \times 10^{-4}$ s^{-1}) competes with the formation of **4**, thus giving rise to the formation of the 1:1 complex (**5**), which bears coordinated water molecule as the fourth ligand. The second-order rate constant for the chloride anation of **5** is $k_{-6} = 0.75 \pm 0.07$ M^{-1} s^{-1}, which gives the value $K_6 = (2.8 \pm 0.4) \times 10^{-4}$ M for the equilibrium constant of the reaction between **4** and **5**. Competition of inosine and Cl^- for **2** and **3** were used to study the rate of chloride anation of aquated Pt(II) species. The second-order rate constants are $k_{-1} = 0.060 \pm 0.015$ M^{-1} s^{-1} for **2** and $k_{-2} = 0.98 \pm 0.14$ M^{-1} s^{-1} for **3**. Thus, the equilibrium constants for the stepwise hydrolysis of **1** are $K_1 = (3.2 \pm 1.5) \times 10^{-3}$ M and $K_2 = (2.3 \pm 0.9) \times 10^{-4}$ M. The kinetic data presented in this study are in line with the proposal that the first hydrolysis product is the active intermediate in the action of cisplatin, whereas the direct substitution of the chloro ligand as well as the role of the second hydrolysis product seem to be relative unimportant.

Introduction

Coordination properties of the anticancer drug cisplatin, *cis*-$Pt(NH_3)_2Cl_2$ (**1**), and related compounds are currently of considerable interest. Much attention has been paid especially to the interactions of platinum with DNA and its constituents, the importance of which in the chemotherapeutic activity of these compounds is generally accepted.[1] Binding studies with mono- and oligonucleotides, nucleosides, and model compounds by X-ray crystallography and NMR spectroscopy have given valuable information about the available coordination sites.[1a] Besides the available binding sites, knowledge also about the kinetics of the different binding modes is important in explaining the working mechanism of the drug. In fact, it has been suggested that only the kinetically preferred binding modes are important in biological systems.[2,3] It is well documented that in aqueous solution the labile chloro ligands in **1** are easily replaced by water molecules to give *cis*-$[Pt(NH_3)_2Cl(H_2O)]^+$ (**2**) and *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ (**3**), both of which exist in aqua/hydroxo species depending on the pH.[2,4] Under acidic conditions hydrolysis is reversible, yielding an equilibrium system,[4] whereas in strongly basic solution complete hydrolysis occurs with the formation of dihydroxo derivative.[5] In neutral solution the hydrolysis products of **1** may form dimers and higher aggregates through OH bridges.[6] The existence of such a variety of different Pt species makes the complex formation exceedingly complicated, particularly, because the leaving properties of the non-amine ligands largely determine the reaction rate in these cases.

It is known that the coordinated water molecule is a better leaving group than the chloro ligand in square planar Pt(II) compounds.[7] In addition, it has been shown that the hydroxo group is inert toward substitution relative to the aqua ligand in aquated $Pt^{II}(NH_3)_2$,[8] as well as in monofunctionally binding Pt^{II}(dien).[9] In these respects, the coordination properties of **2** with nucleobases are particularly interesting, because the pK_a of the aqua ligand is 6.41.[10] However, most of the kinetic studies deal with the complexation behavior of **3**,[11] whereas relative little is known about the coordination abilities of **1** and **2**. In addition, the available kinetic data for these species is somewhat controversial. Treatment of DNA with hydrolyzed and unhydrolyzed cisplatin has led to the suggestion that the hydrolysis products are the active intermediates in Pt–DNA interactions.[12] Recent study has shown that the rate of the initial binding of **1** to DNA is the same as the rate of hydrolysis of the first chloro ligand.[3] On the other hand, in the presence of KCl the complexation of **1** with mononucleotides has been explained to proceed significantly

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(2) Reedijk, J. *Pure Appl. Chem.* **1987**, *59*, 181–192.
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(4) Miller, S. E.; House, D. A. *Inorg. Chim. Acta* **1989**, *161*, 131–137 and references therein.
(5) Miller, S. E.; House, D. A. *Inorg. Chim. Acta* **1989**, *166*, 189–197 and references therein.
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(11) For a recent review, see: Green, M.; Garner, M.; Orton, D. M. *Transition Met. Chem. (Weinheim, Ger.)* **1992**, *17*, 164–176.
(12) Johnson, N. P.; Hoeschele, J. D.; Rahn, R. O. *Chem.-Biol. Interact.* **1980**, *30*, 151–169.

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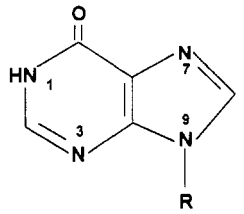
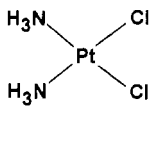
Chart I



Inosine (L)



Cisplatin (1)

via direct substitution of the chloro ligand.[13] Complex formation of guanosine 5'-monophosphate with **1** or its ethylenediamine analogue has been interpreted to occur via the dichloro compound and the corresponding monoaquated species at pH 6.5, but not via the diaqua species.[14] In the case of adenosine, instead, **2** has been reported to react 15 times faster than **1** at pH 3.[15]

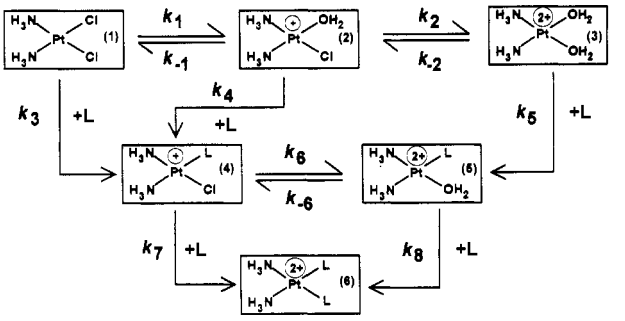
In order to extend our knowledge of the Pt–nucleobase interactions, we have undertaken an HPLC study of the kinetics and mechanism of the complex formation of **1** and its hydrolysis products with inosine in slightly acidic aqueous solution at 318.2 K. Inosine (Chart I) was chosen for the model nucleobase because in acidic medium the predominant binding site is the ring nitrogen N7,[8,9] analogous to that of the guanine residue, which is the main target of platinum in DNA.[1] In addition, when compared with guanosine, inosine is far more soluble in aqueous solution. The main purposes of the work were to study quantitatively the leaving properties of the aqua and/or chloro ligands in compounds **1–3**. Special emphasis was given to the complexation ability of **2** as well as to the equilibrium between 1:1 Pt–inosine complexes, in which the fourth ligand is either Cl^- or a water molecule. In addition, competition of inosine and Cl^- for **2** and **3** was used to estimate the anation rate of these species.

Experimental Section

Materials and Solutions. Inosine and 1-methylthymine were purchased from Sigma, and they were used as received.[16] DMF (E. Merck AG) was dried over 5 Å molecular sieves. *cis*-$Pt(NH_3)_2Cl_2$ was prepared and its purity checked as reported earlier.[18] This was converted to the corresponding diaqua species by $AgNO_3$ treatment.[19] A stock solution containing a 1:1 mixture of the hydrolysis products of cisplatin was prepared by the following procedure. 25 μmol of aquated $Pt^{II}(NH_3)_2$ and 12.5 μmol of NaCl were mixed, the whole was brought to 1 mL, and the equilibrium was allowed to settle at 298.2 K for 4 days.[20] Stock solutions of the 1:1 complexes, *cis*-$[Pt(NH_3)_2(Ino\text{-}N7)(H_2O)]^{2+}$ and *cis*-$[Pt(NH_3)_2(Ino\text{-}N7)Cl]^+$, were obtained by LC-fractionation of the mixture of aquated $Pt^{II}(NH_3)_2$ (0.1 mmol) and inosine (0.06 mmol) at about pH 4.[19] In the case of the chloro complex, 1 mmol of NaCl was added to the Pt–inosine mixture, after which the mixture was fractionated by LC.

Kinetic Measurements. Kinetics of the complexation of inosine with *cis*-$Pt(NH_3)_2Cl_2$ and its hydrolysis products in unbuffered aqueous solution (pH 3.5–4.0) at 318.2 K was studied by HPLC as previously described.[9] The pH of the reaction mixtures remained practically constant (within 0.1 unit) in each measurement. In all cases signal height was used as the measure of the concentration. The reactions were started by adding a known amount of the desired platinum compound to a prethermostated reaction mixture.[22] In the case of *cis*-$Pt(NH_3)_2Cl_2$, about 10 mg of the compound was rapidly dissolved in water by sonication at about 310 K to give a 5 mM solution. The reaction was started immediately after the dissolution, which took less than 1 min. For control purposes, **1** was dissolved in dry DMF to give a 0.02 M solution and the desired amount (<50 μL) of fresh solution was added to the reaction mixture. Samples withdrawn from the reaction mixture at suitable time intervals were made alkaline (pH > 12) in order to convert reactive aqua ligands into inert hydroxo groups. To prevent aquation of the chloro derivatives, samples were stored in an ice bath (generally less than 1 h) before injection into the chromatograph. Peak heights of the 1:1 complexes were transformed into the concentration by employing 1-methylthymine as an internal standard both in the reaction mixtures and in the calibration samples. In the case of **5** the calibration sample was prepared from a known amount of inosine in excess of **3**. Addition of a large excess of Cl^- to this mixture converted **5** into **4**. The employment of known amounts of **3** or isolated **4** as a starting material in excess of inosine gave calibration samples for **6**.

Scheme I



Results and Discussion

Scheme I depicts the assumed reaction pathway for the complexation of inosine (L) with cisplatin and its hydrolysis products in slightly acidic aqueous solution. Under these conditions Pt(II) binds inosine through the N7 position in all cases,[8] whereas coordination to 1-methylthymine employed as an internal standard is unlikely. In the latter compound the single available coordination site (N3) is protonated at pH 4, which efficiently prevents platination at this site.[23] According to chromatographic analysis the concentration of 1-methylthymine remained constant in each kinetic run. At pH 4 deprotonation of the aqua ligands in **2**, **3**, and **5** can be neglected (reported pK_a values: 6.41,[10] 5.64,[8] and 5.78,[8] respectively). In general, the chloride anation reactions of the aquated platinum species were prevented by choosing the experimental conditions properly; i.e. $[L]_T \gg [Pt]_T$. Only the interconversion of the 1:1 complexes **4** and **5** was studied under reversible conditions. The low molar absorptivity of compounds **1–3** prevents the accurate determination of these species by UV detector in these cases.[5] Hence, the measurement of the individual rate constants was based on the time-dependent concentration of **4** and **5** as well as **6**. Due to the complexity of the reaction pattern, the different steps were studied independently.

Formation of 1:1 Complexes. The employment of an excess of an equimolar mixture of **2** and **3** provided pseudo-first-order conditions for the formation of both 1:1 complexes ($[Pt]_T$:$[L]_T \geq 30$:1).[24] The observed rate constant, k'_{obs}, for the disappearance of a known amount inosine was calculated by the first-order rate equation (eq 1, see Appendix). It is assumed that the ratio of

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(15) Murakami, S.; Saito, K.; Muromatsu, A.; Moriyasu, M.; Kato, A.; Hashimoto, Y. *Inorg. Chim. Acta* **1988**, *152*, 91–99.
(16) Inosine was found to be free from contaminants of crystallization liquids.[17]
(17) Arpalahti, J.; Lippert, B. *Inorg. Chem.* **1990**, *29*, 104–110.
(18) Arpalahti, J.; Lippert, B. *Inorg. Chim. Acta* **1987**, *138*, 171–173.
(19) Arpalahti, J.; Lehikoinen, P. *Inorg. Chim. Acta* **1989**, *159*, 115–120.
(20) The actual ratio of **3**:**2** is about 1.05 by using the equilibrium constants $K_1 = 3.3 \times 10^{-3}$ and $K_2 = 4 \times 10^{-5}$ reported at 298.2 K for the first and second hydrolysis reactions of **1**, respectively.[21]
(21) Reishus, J. W.; Martin, D. S. *J. Am. Chem. Soc.* **1961**, *83*, 2457–2462.
(22) The ionic strength was adjusted to 0.1 M with $NaClO_4$.
(23) pK_a = 9.6 for thymidine: Martin, R. B. *Acc. Chem. Res.* **1985**, *18*, 32–38.
(24) Chromatographically isolable *cis*-$[Pt(NH_3)_2Cl(H_2O)]^+$ (reported purity >90%[4]) may contain small amounts of the diaqua derivative, which can seriously affect the kinetics by fulfilling the pseudo-first-order requirements unsatisfactorily.

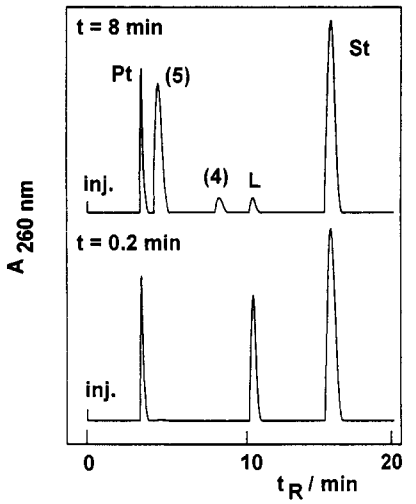

Figure 1. Chromatographic analysis of the formation of 1:1 complexes **4** and **5** from a known amount of inosine ($[L]_T = 1 \times 10^{-4}$ M) in excess of a mixture of **2** and **3** ($[Pt]_T = 5 \times 10^{-3}$ M) at selected time intervals using 0.05 M $NaClO_4$ and 0.001 M HNO_3 in a water–methanol mixture (96/4) as an eluent (flow rate 0.8 mL/min). St denotes 1-methylthymine (2.5×10^{-4} M).

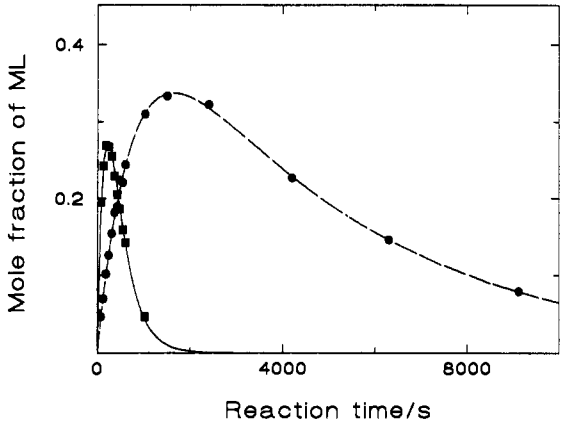

Figure 2. Time-dependent mole fraction (from the total amount of Pt, 4×10^{-4} M) of the 1:1 complexes **4** (●) and **5** (■) in excess of inosine (0.01 M). Both the solid line[26] and the dashed line[28] represent computer simulations using eq 2.

2 and **3** does not markedly change during the complexation. As seen in Figure 1, the half-life for the disappearance of the free ligand is about 2 min, during which less than 3% from the compound **2** has been hydrolyzed (vide infra). Under these conditions $k'_{obs} = k'_4 + k'_5$, where k'_4 and k'_5 represent pseudo-first-order rate constants. Chromatographic analysis showed that **4** and **5** are formed in a constant ratio of 0.22 ($=k'_4:k'_5$) over 2 half-lives, which gave the values 0.14 ± 0.01 M^{-1} s^{-1} and 0.63 ± 0.04 M^{-1} s^{-1} for the corresponding second-order constants k_4 and k_5, respectively.[25] The latter agrees well with that found for the formation of **5** in excess of unmixed **3**; viz., $k_5 = 0.67$ M^{-1} s^{-1}. Rate constant of 0.019 M^{-1} s^{-1} (308.2 K, pH 3, *I* not specified) has been reported for the complexation of **2** with adenosine.[15] This is in reasonable agreement with our k_4 value, because inosine is known to react with Pt(II) about 8 times faster than adenosine.[17]

The formation of 1:1 complexes was studied also in ligand excess by using a known amount of the mixture of **2** and **3** as a starting material. The time-dependent concentration of the 1:1 complexes is shown in Figure 2. Both the formation and disappearance of the aqua complex **5** are much faster than those of the chloro complex **4**. At maximum, the former reaches the mole fraction 0.28 from the total amount of platinum in about 10 min, whereas the maximum value 0.35 of the latter appears

(25) Mean values of several measurements.

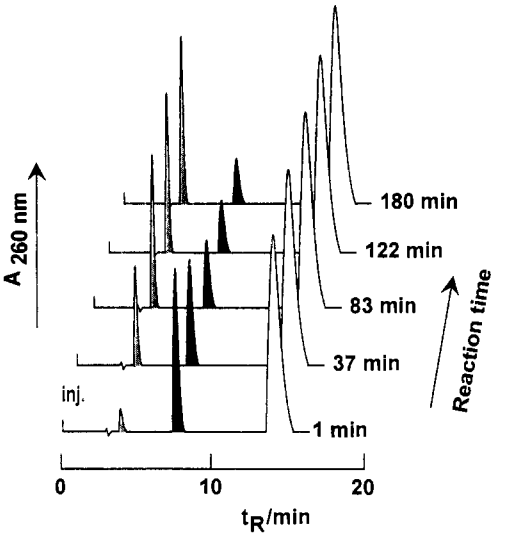

Figure 3. LC elution profiles of an aqueous solution of **4** (1×10^{-4} M, dark signal) at selected time intervals using 0.05 M $NaClO_4$ and 0.001 M HNO_3 in a water–methanol mixture (96/4) as an eluent (flow rate 0.8 mL/min). A hatched signal denotes compound **5**, and an open signal, 1-methylthymine (2×10^{-4} M) employed as an internal standard.

at 40 min. In the case of **5**, least-squares fit to eq 2 (see Appendix) gave the values $k_5 = 0.61 \pm 0.05$ M^{-1} s^{-1} and $k_8 = 0.34 \pm 0.02$ M^{-1} s^{-1} for the second-order rate constants, which are in good agreement with those obtained by employing only **3** as a starting material; viz., $k_5 = 0.70$ M^{-1} s^{-1} and $k_8 = 0.31$ M^{-1} s^{-1}.[26] In addition, this data agrees well with the values reported earlier for the complexation of *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ with inosine at 298.2 K.[27] A similar treatment of the data for **4** gave the values 0.13 M^{-1} s^{-1} for k_4 and (2.1–2.2) × 10^{-4} s^{-1} for the sum constant $k_6 + k_7[L]_T$.[28] Accordingly, an excess of ligand or Pt(II) gives practically the same values for the rate constant k_4 and the same holds true also for k_5, which lends support to the validity of the data and the assumptions made above. The reliability of the obtained rate constants is further supported by the nice correlation between the fitted curve and experimental data (Figure 2). The results show that the aqua ligand is displaced about 5 times faster in **3** than in **2**. Deprotonation of one aqua ligand in **3** has been previously noted to affect the leaving properties of the remaining one in the same manner.[8]

Interconversion of 1:1 Inosine Complexes. As seen in Figure 3, HPLC offers a convenient method for the simultaneous analysis of the concentration of both 1:1 complexes **4** and **5**. In aqueous solution hydrolysis or aquation of the compound **4** obeys a first-order rate law, while the reverse reaction is of second order; i.e., anation of the compound **5** depends also on the concentration of Cl^- ions. By employment of isolated **4** as a starting material, the rate law for the formation of **5** may be expressed by eq 3, which yields eq 4 upon integration (see Appendix).[29] The employment of least-squares fit to eq 4 gave $k_6 = (2.1 \pm 0.1) \times 10^{-4}$ s^{-1} and $k_{-6} = 0.78 \pm 0.02$ M^{-1} s^{-1}.[25] On the other hand, also the concentration of **4** can be used to calculate both rate constants by taking into account that $[MLCl]_t = [MLCl]_T - [ML(H_2O)]_t$, which gave the values (2.1 ± 0.1) × 10^{-4} s^{-1} and 0.80 ± 0.02 M^{-1} s^{-1} for k_6 and k_{-6}, respectively.[25]

The large value obtained for k_{-6} indicates that the anation reaction is very sensitive to the concentration of free Cl^- ions. The

(26) In eq 2 k'_5 stands for both k'_7 and k'_M, k'_8 for k'_d and $[M(H_2O)_2]_T$ for $[M]_T$.

(27) $k_5 = 0.135$ M^{-1} s^{-1} and $k_8 = 0.074$ M^{-1} s^{-1}, which should be multiplied by a factor of 4.7 in order to correct the temperature effect; see ref 17.

(28) $[L]_T = 0.004$–0.01 M. In this case k'_4 stands for both k'_7 and k'_M, $k_6 + k_7[L]$ for k'_8 and $[MCl(H_2O)]_T$ for $[M]_T$ in eq 2.

(29) Initial hydrolysis of **4** was taken into account by extrapolating the total concentration of **4** to represent the "real" value, viz. $[MLCl]_T = [MLCl]_t + [ML(H_2O)]_t$.

Table I. Observed Rate Constant, k_{obs}, for the Formation of the 1:2 Pt–Inosine Complex **6** at 318.2 K[a]

$[L]_T$/M	k_{obs}[b]/10^{-4} s^{-1}	$[L]_T$/M	k_{obs}[b]/10^{-4} s^{-1}
0.013	2.22 (2.08)	0.100	3.06 (2.79)
0.026	2.33 (2.46)	0.112	3.07 (2.95)
0.051	2.61 (2.54)		

[a] I = 0.1 M. [b] Calculated by eq 1 from the disappearance of **4**. The values in parentheses are obtained from the formation of **6**.

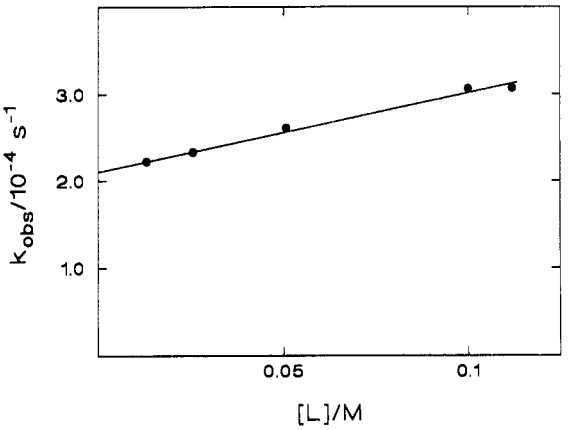

Figure 4. Observed rate constant, k_{obs}, for the formation of the 1:2 complex **6** as a function of the ligand concentration.

semipreparative HPLC method employed for the isolation of **4** is assumed to give a compound essentially free from unbound Cl^-. However, the measurement of free Cl^- ions is difficult because these are slowly released from **4** even when stored in an ice bath. Therefore the equilibrium between **4** and **5** was studied also in high Cl^- excess by using isolated **5** as a starting material. Under these conditions the time-dependent concentration of **5** may be expressed by eq **5** (Appendix). Least-squares fit to the kinetic data gave $k_6 = (2.5 \pm 0.2) \times 10^{-4}$ s^{-1} and $k_{-6} = 0.72 \pm 0.04$ M^{-1} s^{-1},[25] which are in reasonable agreement with the values reported above.

Formation of 1:2 Complex. The formation of **6** was studied by using isolated **4** as a starting material and a high ligand excess, which prevents the chloride anation reaction of **5** provided that $k_8[ML(H_2O)][L]_T \gg k_{-6}[ML(H_2O)][Cl]$.[30] In addition, it is assumed that the conversion of **5** into **6** is much faster than the hydrolysis of **4**. Hence, aquation of **4** becomes the rate-limiting step in the reaction sequence **4** → **5** → **6**. In each case the values obtained for k_{obs} from the disappearance of **4** and from the formation of **6** by first-order rate equation were compatible, as can be seen in Table I. Inspection of Scheme I reveals that the observed rate constant obeys the biphasic rate equation $k_{obs} = k_6 + k_7[L]_T$. Thus, a linear correlation is expected between k_{obs} and [L] (Figure 4). The slope of the plot k_{obs} vs [L] gives $k_7 = (8 \pm 1) \times 10^{-4}$ M^{-1} s^{-1}, while the intercept is $k_6 = (2.1 \pm 0.1) \times 10^{-4}$ s^{-1}. The latter is in excellent agreement with the value obtained above for k_6.

Complexation and Hydrolysis of Cisplatin. The time-dependent concentration of **4** plays a key role in determining the rate constants for *cis*-$Pt(NH_3)_2Cl_2$. According to Scheme I, the importance of steps **1** → **4**, **2** → **4**, and **4** → **6** depend on the concentration of the ligand. When the ligand concentration is small ($[L]_T = (1-2) \times 10^{-3}$ M; $[L]_T$: $[Pt]_T = 30$–80) aquation of **1** and **4** is assumed to predominante over steps **1** → **4** and **4** → **6**, respectively. The rate law for the formation and disappearance of **4** may then be expressed by eq 6, and the concentration of **4** by eq 7 (Appendix). The employment of the values reported above for k_4 and k_6 leaves two unknown parameters in eq 7, which can be obtained by least-squares fit; viz., $k_1 = (1.9 \pm 0.2) \times 10^{-4}$ s^{-1} and $k_2 = (2.3 \pm 0.3)$

(30) This is a reasonable assumption, since $[L]_T > 150$ $[MLCl]_T$ and $k_8 = 0.31$ M^{-1} s^{-1}.

Table II. Observed Rate Constants, $k_l/10^{-4}$ s^{-1}, for the Hydrolysis of **1** and **2** in the Presence of Inosine (L) at 318.2 K[a]

$[L]_T/10^{-3}$ M	$[Pt]_T/10^{-3}$ M	k_1[b]	$k_1 + k_3[L]$[c]	k_2[b]
0.98	0.025	1.74		1.97
1.13	0.031	1.73		2.46
1.98	0.025	2.06		2.25
2.00	0.050	1.89		2.30
2.52	0.050[d]	1.67		1.92
76.6	0.200		3.48	
90.5	0.200		3.55	
105	0.200		3.75	

[a] I = 0.1 M. [b] Calculated by eq 7. [c] Obtained by eq 2. [d] Dissolved in dry DMF.

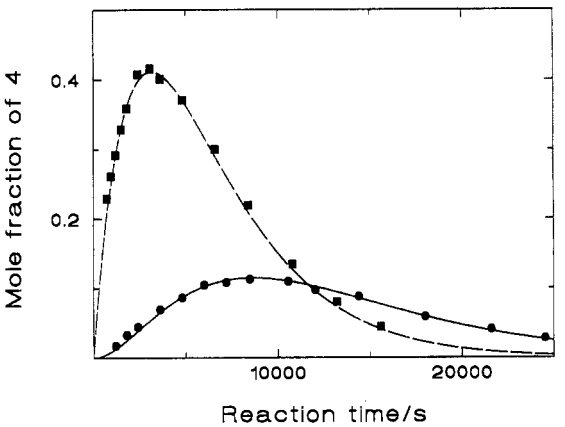

Figure 5. Time-dependent mole fraction of the 1:1 complex **4** (from the total amount of Pt) formed in the reaction of **1** with excess of inosine; (●) [L] = 9.8×10^{-4}, (■) [L] = 9.05×10^{-2} M. Both lines represent computer simulations: solid line obtained by eq 7; dashed line obtained by eq 2.

$\times 10^{-4}$ s^{-1}.[25] Accordingly, hydrolysis of Cl^- occurs at the same rate in both cases within the limits of experimental accuracy, including also the complex **4**. The value obtained for k_1 is, however, sensibly different from those given in literature at 318.2 K; viz., 5.21×10^{-4} s^{-1} (0.1 M $HClO_4$)[4], 3.3×10^{-4} (0.01 M HNO_3).[31] It should be noted, however, that the k_1 values reported in these two papers at various temperatures are considerably greater than those in other published data.[4] By contrast, $k_1 = 1.6 \times 10^{-4}$ s^{-1} ($T = 308.2$ K, pH = 7.0, $I = 0.5$ M),[32] as well as the rate data given by Reishus and Martin,[33] are quite close to our value. In the case of k_2, direct comparison with the literature data is difficult, because k_2 is usually obtained indirectly from the equilibrium constant K_2 and the anation rate constant k_{-2}. Instead, measurements in basic solution have given $k_2 = 1.13 \times 10^{-4}$ ($T = 318.2$ K; $I = 0.1$ M),[5] which correlates rather poorly with our value. The discrepancy between the data presented in this study and those reported in literature is difficult to explain. Control experiments employing freshly dissolved **1** in DMF gave compatible values for k_1 and k_2 under identical conditions, which rules out the possible interference of solvolysis of **1** (Table II).[34] In addition, the reliability of the k_1 and k_2 values is further supported by the nice correlation between the observed and calculated values for $[MLCl]_t$, as seen in Figure 5.

In a very high ligand concentration ($[L]_T > 0.07$ M; $[L]_T$: $[Pt]_T > 350$), in contrast, step **2** → **4** predominates over step **2** → **3**. In other words, practically all of **2** formed converts directly to **4**. Moreover, the rate data obtained reveals that $k_4[L]_T \gg k_1$. Hence, aquation of **1** becomes the rate-limiting step in the sequence

(31) Panasyuk, V. D.; Malashok, N. F. *Russ. J. Inorg. Chem.* (*Engl. Transl.*) **1968**, *13*, 1405–1408.
(32) Bose, R. N.; Cornelius, R. D.; Viola, R. E. *J. Am. Chem. Soc.* **1986**, *108*, 4403–4408.
(33) $k_1 = 2.5 \times 10^{-5}$ s^{-1} at 298.2 K; 7.6×10^{-5} s^{-1} at 308.2 K (I = 0.318); see ref 21.
(34) Prolonged standing of **1** in DMF (>48 h at 25 °C) reduced the reactivity of **1** and introduced additional products detectable by HPLC.

1 → **2** → **4**. Under these conditions the concentration of **4** may be given by eq 2, where $k_1 + k_3[L]_T$ stands for both k'_f and k'_M, $k_6 + k_7[L]$ for k'_d, and $[MCl_2]_T$ for $[M]_T$. Known values for the rate constants k_6 and k_7 were employed to compute $k_1 + k_3[L]_T$ by least-squares fit (Table II), which yielded $k_3 = (1.9 \pm 0.4) \times 10^{-3}$ M^{-1} s^{-1} by using the k_1 value given above. A very similar value has been reported for the complexation of **1** with adenosine, viz., 1.6×10^{-3} M^{-1} s^{-1} (313.2 K, I not specified),[15] which contradicts the behavior of **2** and the preference of Pt(II) for 6-oxopurine derivatives generally observed.[17] For comparison, reported rate constants for the direct reaction between **1** and adenine nucleotides range from 6.25×10^{-3} M^{-1} s^{-1} to 9.33×10^{-3} M^{-1} s^{-1},[32] whereas that between **1** and 5′-GMP is 0.14 M^{-1} s^{-1}.[14]

Anation of Hydrolysis Products of Cisplatin. Competition of inosine and Cl^- for **2** and **3** were used to study the rate of chloride anation of hydrolyzed Pt(II) species. In order to efficiently prevent the aquation of **4**, the concentration of Cl^- was adjusted so that $k_{-6}[Cl^-] > 30k_6$. When $[Cl^-]$ was 2–3 times larger than [L] the chromatographic analysis revealed the formation of both **4** and **5** by using **3** as a starting material. The formation and disappearance of **5** obeys the rate equation for consecutive first-order reactions (eq 2).[35] Introduction of known values for k_5, k_{-6}, and k_8 in eq 2 gave $k_{-2} = 0.98 \pm 0.14$ M^{-1} s^{-1} by least-squares fit.[25] Although the individual measurements deviated less than 5% from each other by using the mean values for k_5, k_{-6}, and k_8, introduction of the limiting values for these constants increased the deviation of k_{-2}. The k_{-2} value computed is compatible with the k_{-6} value given above for the anation of **5** as well as with the rate constant for the anation of $Pt(en)(H_2O)_2$,[36] but agrees rather poorly with that given by Miller and House.[37] The rate constant k_{-1} for the chloride anation of **2** was estimated from the conversion of known amount of **3** into **4**. Under these conditions the formation of **4** obeys the rate equation of two parallel consecutive reactions (eq 8 in Appendix), as evident from Scheme I. This appeared to be the case even at high $[Cl^-]$ to [L] ratio, though the formation of **5** was negligible according to chromatographic analysis. The employment of known values for k_{-2}, k_4, k_5, k_{-6}, and k_8 leaves k_{-1} as the single unknown parameter in eq 8, which can be obtained numerically; viz., $k_{-1} = 0.060 \pm 0.015$ M^{-1} s^{-1}.[25] Also in this case the individual measurements gave k_{-1} values within 10% by using the mean values for k_{-2}, k_4, k_5, k_{-6}, and k_8. Instead, the employment of the limiting values for the rate constants k_{-2} and k_4, in particular, increased the deviation of k_{-1}. The value found for k_{-1} agrees very well with that reported recently by Miller et al.;[38] viz., $k_{-1} = 6.47 \times 10^{-2}$ M^{-1} s^{-1} (T = 319 K, I = 0.1 M).

The knowledge of the rate constants for the aquation and anation reactions gives the equilibrium constants for both hydrolysis steps of **1** as the ratio of k_i/k_{-i}; viz., $K_1 = (3.2 \pm 0.15) \times 10^{-3}$ M and $K_2 = (2.3 \pm 0.9) \times 10^{-4}$ M. Both of these values agree fairly well with the literature data despite some inconsistency with the individual rate constants. The former is slightly smaller than most of the reported values,[4] although these refer to lower temperatures. Changes in temperature do not, however, markedly affect the magnitude of the equilibrium constant.[4,21] In the case of K_2, the agreement is better.[5] Interestingly, the equilibrium constant $K_6 = (2.8 \pm 0.4) \times 10^{-4}$ M for the aquation of **4** is practically the same as K_2. Thus, substitution of the aqua ligand with a nucleobase affects only slightly the affinity of Pt(II) toward Cl^- in the case of the *cis* configuration.

Conclusions. The rate constants obtained are summarized in Table III. The relative ability of cisplatin and its hydrolysis products to bind inosine is 1:70:350, as given by the ratio of k_3:k_4:k_5. Expectedly, coordinated water molecule is far more better leaving group than the chloro ligand. Excess of ligand gives stepwise formation of 1:2 complex. When [L] ranges from 0.002 to 0.07 M, hydrolysis of the chloro ligand is the rate-limiting step in the binding of inosine to cisplatin (**1**) as well as to the 1:1 complex (**4**), in which the fourth ligand is Cl^-. Direct substitution of the chloro ligand becomes significant in higher ligand concentration ([L] > 0.07 M). By contrast, when [L] < 0.002 M second hydrolysis of **1** competes with the formation of **4** giving thus rise to the formation of the 1:1 complex (**5**), which bears coordinated water molecule as the fourth ligand. Recently, similar results have been observed for the binding of **1** to DNA.[39] With increasing pH the significance of the second hydrolysis increases, as the aqua ligand in **2** is deprotonated ($pK_a = 6.41$). For example, at cellular pH = 7.4 the complexing ability of **2** is reduced by a factor of 10, because the OH group is inert toward substitution relative to the H_2O group.[8,9] On the other hand, the high affinity of the diaqua derivative (**3**) for Cl^- reduces its ability to bind other ligands in the cell, where $[Cl^-]$ is about 4 mM. To sum up, the kinetic data presented in this study are in line with the proposal that the first hydrolysis product is the active intermediate in the action of cisplatin, whereas the direct substitution of the chloro ligand as well as the role of the second hydrolysis product seem to be relative unimportant.

Table III. Summary of the Rate Constants for the Stepwise Complexation of *cis*-$Pt(NH_3)_2Cl_2$ with Inosine at 318.2 K[a]

reaction step[b]	$k_i/10^{-4}$ s^{-1}	$k_j/10^{-3}$ M^{-1} s^{-1}
1 → **2**	1.9 ± 0.2 (5.21),[c] (3.3)[d]	
1 → **4**		1.9 ± 0.4
2 → **1**		60 ± 15 (64.8)[e]
2 → **3**	2.3 ± 0.3	
2 → **4**		140 ± 10
3 → **2**		980 ± 140 (580)[f]
3 → **5**		670 ± 30[g]
4 → **5**	2.1 ± 0.1[h]	
4 → **6**		0.8 ± 0.1
5 → **4**		750 ± 70
5 → **6**		31 ± 3[g]

[a] In 0.1 M $NaClO_4$. [b] See Scheme I for details. [c] In 0.1 M $HClO_4$; see ref 4. [d] In 0.01 M HNO_3; see ref 31. [e] In 1.0 M $HClO_4$; see ref 37. [f] T = 319 K; I = 0.1 M; see ref 38. [g] Obtained by using **3** as a starting material. [h] The k_6 value obtained from the chloride anation of **5** is excluded.

Appendix

Rate laws and integrated rate equations (charges are omitted for clarity) are given, when M denotes the $Pt(NH_3)_2$ entity, L denotes coordinated inosine, and k'_i refers to the pseudo-first-order rate constant. $[i]_T$ refers to the total concentration of the species i and $[i]_t$ to the concentration at the time t. In eq 2 $[M]_T$ is the total concentration of the relevant Pt(II) compound and $[ML]_t$ is the concentration of the proper 1:1 complex at the time t. The terms k'_f and k'_d denote the rate constants for the formation and disappearance of the relevant 1:1 complex, respectively, whereas the term k'_M includes the first generation rate constants of the parent Pt(II) compound.

$$\ln [L]_t = -k'_{obs}t + \ln [L]_0 \qquad (1)$$

$$[ML]_t = \frac{k'_f [M]_T}{k'_d - k'_M} (e^{-k'_M t} - e^{-k'_d t}) \qquad (2)$$

(35) In eq 2 $k_5[L]$ stands for k'_f, $k_{-2}[Cl]_T + k_5[L]_T$ for k'_M, $k_{-6}[Cl]_T + k_8[L]_T$ for k'_d, and $[M(H_2O)_2]_T$ for $[M]_T$.
(36) k_{-2} = 0.31 at 298.2 K; 0.52 at 308.2 K (I = 0.318), see: Coley, R. F.; Martin, D. S. *Inorg. Chim. Acta* **1973**, *7*, 573–577.
(37) k_{-2} = 0.58 M^{-1} s^{-1} (318.2 K) by using the temperature dependence of k_{-2}; see ref 5.
(38) Miller, S. E.; Gerard, K. J.; House, D. A. *Inorg. Chim. Acta* **1991**, *190*, 135–144.
(39) Bernges, F.; Dörner, G.; Holler, E. *Eur. J. Biochem.* **1990**, *191*, 743–753.

$$\frac{d[ML(H_2O)]_t}{dt} = k_6[MLCl]_t - k_{-6}[ML(H_2O)]_t^2 \quad (3)$$

$$[ML(H_2O)]_t = [MLCl]_T \frac{2k_6(1 - e^{ts})}{k_6(1 - e^{ts}) - s(1 + e^{ts})} \quad (4)$$

where

$$s = \sqrt{k_6^2 + 4k_6k_{-6}[MLCl]_T}$$

$$[ML(H_2O)]_t = [ML(H_2O)]_T \frac{k_6 + k_{-6}[Cl]_T e^{-(k_6 + k_{-6}[Cl]_T)t}}{k_6 + k_{-6}[Cl]_T} \quad (5)$$

$$\frac{d[MLCl]_t}{dt} = [MCl_2]_t \frac{k_1k'_4}{k_2 + k'_4 - k_1}(e^{-k_1t} - e^{-(k_2 + k'_4)t}) - k_6[MLCl]_t \quad (6)$$

$$[MLCl]_t = \frac{k_1k'_4[MCl_2]_T}{k_2 + k'_4 - k_1} \times \left\{\frac{e^{-k_1t} - e^{-k_6t}}{k_6 - k_1} - \frac{e^{-(k_2 + k'_4)t} - e^{-k_6t}}{k_6 - k_2 - k'_4}\right\} \quad (7)$$

$$[MLCl]_t = \frac{(A_1 + A_2)(1 - e^{-(k'_{-2} + k'_5)t})}{k'_{-2} + k'_5} - \frac{A_1(1 - e^{-(k'_{-6} + k'_8)t})}{k'_{-6} + k'_8} - \frac{A_2(1 - e^{-(k'_{-1} + k'_4)t})}{k'_{-1} + k'_4} \quad (8)$$

where

$$A_1 = \frac{k'_5k'_{-6}[M(H_2O)_2]_T}{k'_{-6} + k'_8 - k'_{-2} - k'_5}$$

$$A_2 = \frac{k'_{-2}k'_{-4}[M(H_2O)_2]_T}{k'_{-1} + k'_4 - k'_{-2} - k'_5}$$