Studies on the Reactions of PtCl2en, *cis*-Pt(NH3)2Cl2 and their Aqua Species with Adenosine, Deoxyadenosine and Adenine using Ion-pair HPLC

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Abstract

The reactions of PtCl$_2$en or *cis*-Pt(NH$_3$)$_2$Cl$_2$ and their aqua species with adenine and adenosine were studied by means of ion-pair HPLC. From the chromatograms, it was found that the first binding site of Pt(II) was the N(7) site of adenine under both acidic and neutral conditions. The rates of Pt(II) binding at the N(7) site of adenosine and deoxyadenosine were measured. The rate constants, k_1, were obtained for the reactions of PtCl$_2$en or *cis*-Pt(NH$_3$)$_2$Cl$_2$ with adenosine and deoxyadenosine at pH 3 and 7 over the temperature range 9–25 °C. The k_1 values were 6.8–7.7 $\times 10^{-4}$ dm^3 mol^{-1} s^{-1} at 25 °C. For the aqua species, the rate of [*cis*-Pt(NH$_3$)$_2$-ClH$_2$O]$^+$ with adenosine N(7) was measured. The rate constants, k_2, which were found to be smaller than those of hydrolysis, k_h, were calculated at pH 3 over the temperature range 25–40 °C. The k_2 value obtained at 25 °C was 1.1 $\times 10^{-2}$ dm^3 mol^{-1} s^{-1}, 15 times larger than k_1. The activation parameters were also calculated.

Introduction

It is well known that the main target of cisplatin (*cis*-diamminedichloroplatinum(II), hereafter abbreviated as *cis*-Pt(NH$_3$)$_2$Cl$_2$), is the N(7) site of guanine in DNA. Scovell and O'Conner investigated the interaction of aquated *cis*-Pt(NH$_3$)$_2$Cl$_2$ with ribonucleosides, and determined the apparent formation constants (log K) for the 1:1 complexes utilizing ultraviolet difference spectroscopy [1]. The log K values obtained for guanosine, adenosine and cytidine were 3.7, 3.6 and 3.5, respectively at 25 °C and pH 8. Since these values are very close to each other, it seems that the reaction of *cis*-Pt(NH$_3$)$_2$Cl$_2$ with nucleobases in DNA is kinetically selective rather than thermodynamically selective.

Many studies on the reactions of platinum complexes with nucleobases in DNA have been reported, but only a few kinetic investigations. In many cases, it is difficult to determine the rate constant because various reaction products coexist in solution. Some of the major kinetic investigations for these reactions so far reported are summarized below.

In 1980, Johnson *et al.* determined the rate constants for the reactions of *cis*-Pt(NH$_3$)$_2$Cl$_2$, *trans*-Pt(NH$_3$)$_2$Cl$_2$ and their various aqua species with DNA *in vitro* by means of a radioactive ^{195m}Pt-labeled compound [2]. In 1982 Clore and Gronenborn undertook a ^{1}H NMR study of the kinetics of the reactions of 5'-adenosine monophosphate and 5'-guanosine monophosphate with excess *cis*-Pt(NH$_3$)$_2$Cl$_2$ in the presence of KCl at 80 °C and pH 6.5 [3].

Van Hemelryck *et al.* investigated kinetic studies based on UV and HPLC monitoring of the reactions of *cis*-Pt(NH$_3$)$_2$Cl$_2$ and its diaqua derivatives with adenylyl(3'-5')guanosine and guanylyl(3'-5')adenosine [4].

Since the primary action of *cis*-Pt(NH$_3$)$_2$Cl$_2$ is preferentially guanine in DNA, the main focus is on the reaction of *cis*-Pt(NH$_3$)$_2$Cl$_2$ with guanine. Therefore, the amount of data for the reaction of *cis*-Pt(NH$_3$)$_2$Cl$_2$ with adenine is smaller than for *cis*-Pt(NH$_3$)$_2$Cl$_2$ with guanine. But the determination of the rate constants for the reaction of *cis*-Pt(NH$_3$)$_2$Cl$_2$ with adenine is important not only as fundamental data but also to elucidate why guanine is the main target of cisplatin.

In this paper, as a result of monitoring the reaction of Pt(II) with adenine by using ion-pair HPLC, it was found that the Pt(II) interactions with adenine are at the N(1) and N(7) sites of adenine, as reported generally. Furthermore, it was found that the N(7) site is the first binding site under both acidic and neutral conditions and that the N(7) complex is the primary reaction product. Therefore, the rates of binding of platinum to the N(7) site in adenine were measured. The activation parameters were also determined.

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Experimental

Adenosine (Ado), deoxyadenosine (d-Ado), adenine (Ade), tubercidin and *cis*-Pt(NH$_3$)$_2$Cl$_2$ were purchased from Sigma Chemical Co. Other reagents were analytical grade. PtCl$_2$en was prepared according to ref. 5.

PtCl$_2$en and *cis*-Pt(NH$_3$)$_2$Cl$_2$ solutions were prepared by dissolving 0.1 g of the dichloro complex in 100 cm^3 of 0.2 M NaCl solution overnight and then filtering. The monoaqua species [PtClH$_2$Oen]$^+$ and the [*cis*-Pt(NH$_3$)$_2$ClH$_2$O]$^+$ solution were prepared by dissolving 0.1 g of the dichloro complex in 100 cm^3 of solutions containing various amounts of NaCl and incubating the mixture overnight. These solutions contained 8–60% of the monoaqua species. The equilibrated solution was then filtered. The diaqua species [Pt(H$_2$O)$_2$en]$^{2+}$ and the [*cis*-Pt(NH$_3$)$_2$(H$_2$O)$_2$]$^{2+}$ solution were prepared by suspending the dichloro complex in 0.01 M HClO$_4$ solution of a stoichiometric amount of AgClO$_4$, incubating the suspended solution overnight and filtering AgCl through a 0.47 μm millipore filter. All solutions were stored and reacted with nucleobases in the dark. Pt(II) concentration in the stock solution was determined by measuring the absorbance at 405 nm by the addition of SnCl$_2$ solution to the sample [6]. In all measurements, the ionic strength was kept at 0.2 by using NaCl or NaClO$_4$. The pH of the solutions was adjusted with either HClO$_4$ or NaOH.

The qualitative measurements of the reactions of the dichloro complexes or their aqua species with Ado and Ade were carried out under the following conditions: Pt/nucleobases = 1 (4 × 10^{-4} M), pH 2, 3 and 7, at 25 ± 0.1 °C. The reactions were traced for about 1800 h after the start of the reaction. For the kinetic studies of the dichloro complexes with Ado and/or d-Ado, the conditions were Pt/nucleobases = 1 (2 × 10^{-4} M), pH 2 and 7, at 9–35 ± 0.1 °C. For the kinetic studies of [*cis*-Pt(NH$_3$)$_2$ClH$_2$O]$^+$ with Ado, the conditions were Pt/Ado = 10 (Ado = 2 × 10^{-4} M), pH 3, at 25–40 ± 0.1 °C. The concentration of NaCl was 0.2 M for the dichloro complexes. In the case of the aqua complexes, the concentration of NaCl was 0–0.04 M.

HPLC analyses were performed on a Kyowa Seimitsu Co. KHW-20 and a Kyowa Seimitsu Co. KLC 200A spectromonitor. The optimum HPLC conditions were as follows: column, Nucleosil 5C$_{18}$ (M. Nagel, 4.6 mm diameter, 15 cm length); eluent, acetonitrile:0.1 M tartaric acid:sodium dodecyl sulfate = 55 cm^3:45 cm^3:0.5 g; detection, UV 260 nm.

Since the peak heights of the reactants and products often differed markedly, the sensitivity of the UV detector was changed during the chromatographic run from 0.64 a.f.s ($R = 64$) to 0.04 a.f.s ($R = 4$).

The thermostated samples were mixed and as the time elapsed, an aliquot of the sample solution was supplied for the chromatograph. Since the molar extinction coefficient of Pt(II) was small and difficult to detect, the change of the peak height of Ado was followed. The calibration curve was made for the estimation of the Ado concentration at each measurement.

Results and Discussion

The Reactions of PtCl$_2$en, [PtClH$_2$Oen]$^+$ and [Pt(H$_2$O)$_2$en]$^{2+}$ with Ado

Preceding the kinetic run, the reaction of Pt(II) with Ado was qualitatively investigated and also the reaction of Pt(II) with Ade. The results are described below.

Several peaks corresponding to the reaction products grew and fell with the lapse of time, which suggests that the reactions proceed progressively. A few samples of chromatograms near the equilibrium state are shown in Fig. 1. The first peak is due to unreacted Ado. In the case of PtCl$_2$en at pH 2 and [PtClH$_2$Oen]$^+$ at pH 2 and 7, there are four peaks in which peak (1) is the main and the others are minor.
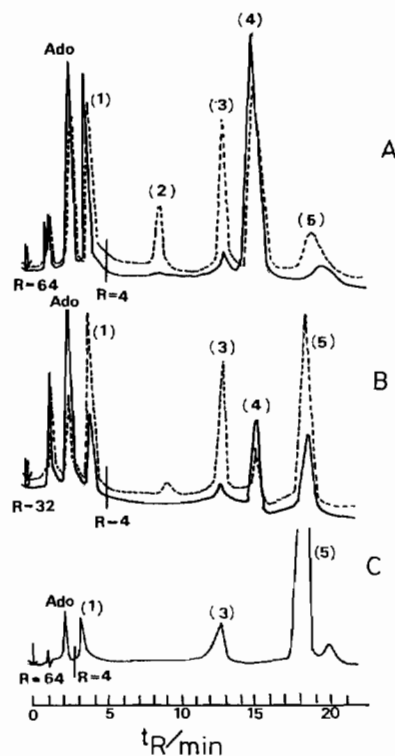

Fig. 1. Chromatograms of the reactions of PtCl$_2$en, [PtClH$_2$Oen]$^+$ and [Pt(H$_2$O)$_2$en]$^{2+}$ with Ado. Initial concentration: Pt(II) 4 × 10^{-4} M; Ado 4 × 10^{-4} M. (——) pH 2; (- - - -) pH 7. Temperature = 25 °C. A: PtCl$_2$en; B: [PtClH$_2$Oen]$^+$; C: [Pt(H$_2$O)$_2$en]$^{2+}$.
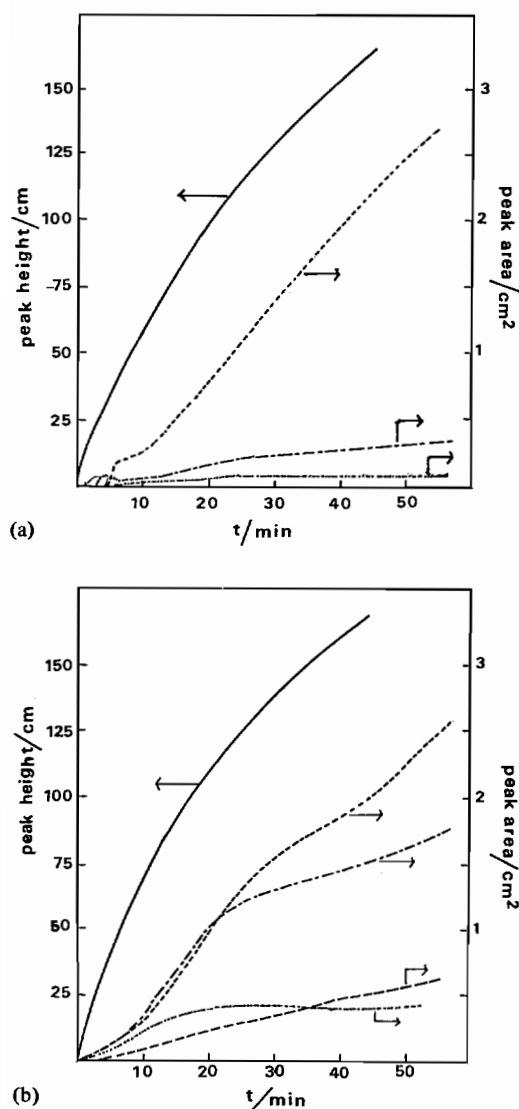
Fig. 2. (a) Change of peaks with time in chromatogram for PtCl$_2$en at pH 3. Initial concentration: Pt(II) 4 × 10^{-4} M; Ado 4 × 10^{-4} M. Temperature = 25 °C. (——) peak (1); (- - -) peak (3); (- - - -) peak (4); (·······) peak (5). (b) Change of peaks with time in chromatogram for PtCl$_2$en at pH 7. Initial concentration: Pt(II) 4 × 10^{-4} M; Ado 4 × 10^{-4} M. Temperature = 25 °C. (——) peak (1); (– – –) peak (2); (- - -) peak (3); (- - - -) peak (4); (·······) peak (5).

TABLE I. Time Required for Each Peak to Emerge in the Reactions of PtCl$_2$en, [PtClH$_2$Oen]$^+$ and [Pt(H$_2$O)$_2$en]$^{2+}$ with Ado at 25 °C. Initial concentration: Pt(II) 4 × 10^{-4} M, Ado 4 × 10^{-4} M

Species	pH	Time elapsed	Peak No.				
			(1)	(2)	(3)	(4)	(5)
PtCl$_2$en	2	d	0		2	4	4
	7	d	0	4.5	2.6	2.6	2.6
PtClH$_2$Oen	2	h	0		2	10	0.5
	7	h	0		0	7.5	0.5
Pt(H$_2$O)$_2$en	2	min			2.5		0

Five peaks appear for PtCl$_2$en at pH 7 in which peak (1) is the main, too. In the case of [Pt(H$_2$O)$_2$en]$^{2+}$ at pH 2, peak (5) predominates and peak (1) is only minor. These are different from the case of the chloro complex. The changes with time in each peak of the chromatogram for PtCl$_2$en are shown in Fig. 2(a) and (b). The times required for each peak to emerge are listed in Table I.

Each peak for PtCl$_2$en emerges in the order (1) → (3) → (4), (5) at pH 2 and (1) → (3), (4), (5) → (2) at pH 7, respectively. For peak (1), there is no differ-ence between pH 2 and pH 7 in the time required for the peak to emerge. For peak (3), the time required is almost the same at pH 2 and 7, but once it ap-peared, peak (3) grew more rapidly at pH 7 than at pH 2. Peak (4) emerges 2 days later at pH 2 than at pH 7, but the final amount is about the same after a lapse of 50 days. Peak (2) is small at pH 7 and is missing at pH 2. Peaks (4) and (5) emerge together at both pH 2 and 7, but the quantity of the reaction products of the former is larger than the latter at both pH values.

In the case of [PtClH$_2$Oen]$^+$, the peaks emerge in the order (1) → (5) → (3) → (4) at pH 2 and (1), (3) → (5) → (4) at pH 7, respectively. In this case, peak (1) also emerges first. Peak (3) emerges together with peak (1) at pH 7 which differs at pH 2. Peak (4) emerges a little later than peak (5) at both pH values.

As shown in Table I, the rates of the reactions increase markedly with an increase in the correspond-ing molar concentration of water in the complexes: PtCl$_2$en ≪ PtClH$_2$Oen ≪ Pt(H$_2$O)$_2$en.

The assignment of peaks (1), (3), (4) and (5) are described below. The basicity of nitrogen in Ado is in the order of N(1)(pK = 3.6) > N(7) ≫ N(3). The platination at the N(1) site is pH-dependent. Since the pK for the N(7) site is slightly lower than that for the N(1) site and it is almost zero, the complexa-tion at this site is pH-independent. Platination at the N(3) site occurs only slightly. When the N(1) site is occupied by H$^+$, the N(7) site is the most favored. In Table I, peak (1) emerges first of all. Thus peak (1) may be due to N(7)-platinated Ado. In order to con-firm this assumption, the reaction of [PtClH$_2$Oen]$^+$ with tubercidin (which does not have nitrogen at the 7 site) was examined. No peak emerged at pH 2 for 24 h after the reaction was initiated. On the other hand, as shown in Fig. 3, when the reaction was carried out at pH 6.6, a peak emerged (t_R = 12 min) and grew but the peak corresponding to peak (1) was not found. From these results, it is considered that peak (1) corresponds to N(7)-platinated Ado. From Fig. 2 and Table I, peaks (3) and (5) are pH-dependent. Accordingly, both peaks seem to corre-
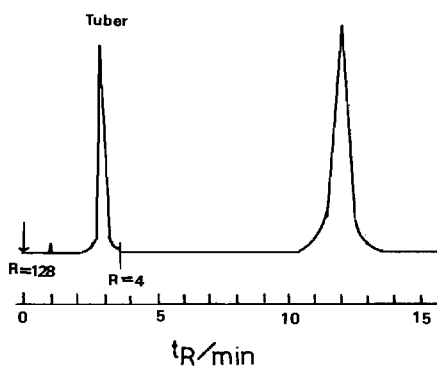
Fig. 3. Chromatogram for the reaction of [PtClH₂Oen]⁺ with tubercidine at pH 6.6. Initial concentration: Pt(II) 4×10^{-4} M; tubercidine 4×10^{-4} M. Temperature = 25 °C.
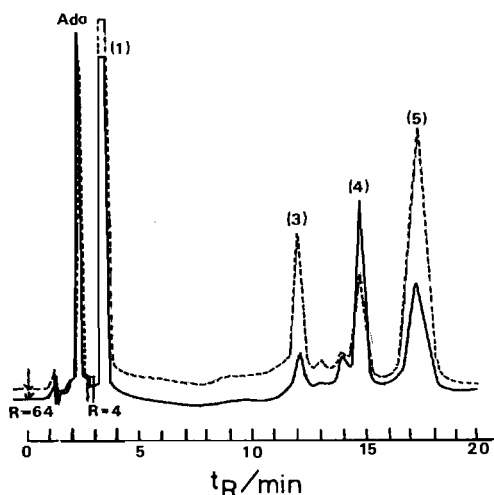
Fig. 4. Chromatograms for the reactions of [PtClH₂Oen]⁺ with Ado under the conditions of Pt/Ado = 0.5 and 1. (——) Pt/Ado = 1; (- - - -) Pt/Ado = 0.5.

Fig. 5. Chromatograms for the reactions of PtClH₂Oen with Ado under the conditions of Pt/Ado = 2 and 1. (——) Pt/Ado = 1; (- - - -) Pt/Ado = 2.

troscopy, according to the method reported in ref. 7. The results support the conclusion that peak (4) corresponds to the 2:1 N(1),N(7)-platinated Ado complex.

The Reactions of cis Pt(NH₃)₂Cl₂, [cis-Pt(NH₃)₂ClH₂O]⁺ and [cis-Pt(NH₃)₂(H₂O)₂]²⁺ with Ado

The experiments for the reactions of *cis*-Pt(NH₃)₂Cl₂, [*cis*-Pt(NH₃)₂ClH₂O]⁺ and [*cis*-Pt(NH₃)₂(H₂O)₂]²⁺ with Ado were also carried out. The chromatograms obtained are shown in Fig. 6. Each peak in the chromatograms is somewhat broad compared to Fig. 1, but peak (1) is as sharp as in Fig. 1. The main peaks at about 5000 h after the initiation of the reaction are described below. Peak (1) is the main peak for *cis*-Pt(NH₃)₂Cl₂ at pH 7. Peaks (1) and (4) for [*cis*-Pt(NH₃)₂ClH₂O]⁺ at pH 2 are the main peaks and the (4)/(1) ratio (area) is 0.52. At pH 7 peaks (1), (3) and (6) are the main peaks and the (3)/(1) ratio is 0.56 and the (6)/(1) ratio is 1.3. Peak (6) is the main peak for [*cis*-Pt(NH₃)₂(H₂O)₂]²⁺. Peak (1) for the ammine complex emerges first (t_R = 3.5 min) as well as for the en complexes. Thus, it is certain that peak (1) corresponds to N(7)-platinated Ado. The largest difference from the en complex is the ratios of the reaction products. For the en complexes the main product is the N(7) complex and the others are negligible. In contrast, for [*cis*-Pt(NH₃)₂ClH₂O]⁺ the other products cannot be neglected, as mentioned above. Another difference is in the time required for the second peak to emerge, especially in the case of *cis*-Pt(NH₃)₂Cl₂. Namely, 2 weeks, 5 h or more than 4 h are required for *cis*-Pt(NH₃)₂Cl₂, *cis*-Pt(NH₃)₂-

spond to the N(1)-platinated complex. Moreover, as shown in Fig. 4, both peaks are affected by the presence of excess Ado; t_R values for peaks (3) and (5) are 13 min and 19 min, respectively. In tubercidin, the possible binding site is the N(1) site. Therefore, there is no peak at pH 2. The peak in t_R 12 min at pH 6.6 is attributed to N(1)-platinated tubercidin owing to deprotonation at the N(1) site. These facts may support the hypothesis that peak (3) corresponds to N(1)-platinated Ado and peak (5) to 1:2 N(1),N(1) or N(1),N(7)-platinated Ado, (Pt)(Ado)₂. The chromatogram obtained for the reaction of [PtClH₂Oen]⁺ under the conditions Pt/Ado = 0.5 is shown in Fig. 4 and that for Pt/Ado = 2 is shown in Fig. 5. Peak (4), shown by the dotted line in Fig. 5, is fairly large but it is small in Fig. 4. From these facts, it is considered that peak (4) corresponds to the 2:1 (Pt)₂(Ado) complex. Then the fraction corresponding to peak (4) was collected and characterized by UV and differential spec-

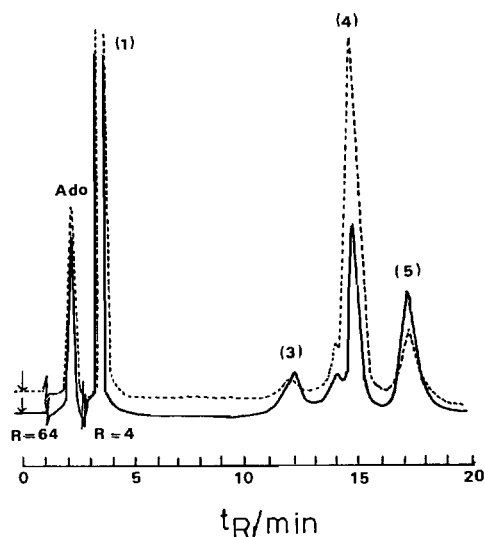Fig. 6. Chromatograms for the reactions of *cis*-Pt(NH₃)₂Cl₂, [*cis*-Pt(NH₃)₂ClH₂O]⁺ and [Pt(NH₃)₂(H₂O)₂]²⁺ with Ado. (A) *cis*-Pt(NH₃)₂Cl at pH 7; (B) [*cis*-Pt(NH₃)₂ClH₂O]⁺ at pH 2; (C) [*cis*-Pt(NH₃)₂ClH₂O]⁺ at pH 7; (D) [*cis*-Pt(NH₃)₂(H₂O)]²⁺ at pH 7. Initial concentration: Pt(II) 4×10^{-4} M; Ado 4×10^{-4} M. Temperature ≃ 25 °C.

Fig. 7. Chromatograms for the reactions of PtCl₂en, [PtClH₂-Oen]⁺ and [Pt(H₂O)₂en]²⁺ with Ade. (A) PtCl₂en at pH 2; (B) [PtClH₂Oen]⁺ (——) pH 2, (- - - -) pH 7; (C) [Pt(H₂O)₂en]²⁺ at pH 2. Initial concentration: Pt(II) 4×10^{-4} M; Ade 4×10^{-4} M. Temperature = 25 °C.

ClH₂O at pH 2 or *cis*-Pt(NH₃)₂ClH₂O at pH 7, respectively. The same behavior as for the en complex is found in the preference of binding site: *cis*-Pt(NH₃)₂-Cl₂ and [*cis*-Pt(NH₃)₂ClH₂O]⁺ Pt(II) bind through the N(7) site first, and [*cis*-Pt(NH₃)₂(H₂O)₂]²⁺ Pt(II) binds only slightly through the N(7) site.

The Reactions of PtCl₂en, [PtClH₂Oen]⁺ and [Pt(H₂O)₂en]²⁺ with Ade

These experiments were carried out at pH 2 for PtCl₂en, at pH 2 and 7 for PtClH₂Oen, and at pH 2 for [Pt(H₂O)₂en]²⁺, respectively. The chromatograms are shown in Fig. 7. The time required for each peak to emerge is listed in Table II. In the case of PtCl₂en,
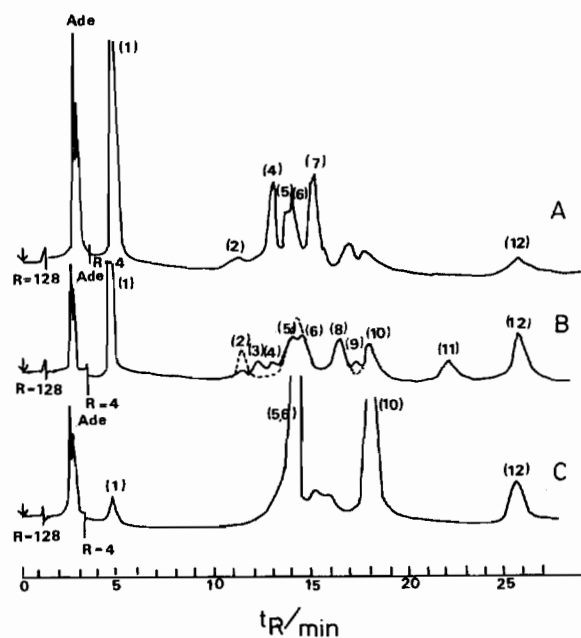
10 peaks appeared in which peak (1) was the main. In the case of PtClH₂Oen, 11 peaks appeared at pH 2, and 8 peaks at pH 7, in which peak (1) was the main. The chromatogram shows as a dotted line is that obtained at pH 7 and as a whole it is similar to that obtained at pH 2.

The number of peaks obtained for the reaction with Ade are larger than that with Ado. This may be attributed to the formation of polynuclear complexes, as shown below.

Since many peaks appeared, identification of each peak was difficult but some of them were estimated as follows. Peaks (1), (5) and (6) emerge in the first stage of the reaction, and are pH-dependent. Figure 8 shows the rate of growth of peaks (1), (5) and (6) at pH 2 and 7. Peak (1) predominates at pH 2 and peaks (5) and (6) predominate at pH 7. The pK_1 for Ade is equal to 4.2. Therefore, peak (1) is assigned as the

TABLE II. Time Required for Each Peak to Emerge in the Reactions of PtCl₂en, [PtClH₂Oen]⁺ and [Pt(H₂O)₂en]²⁺ with Ade at 25 °C. Initial Concentration: Pt(II) 4×10^{-4} M, Ade 4×10^{-4} M

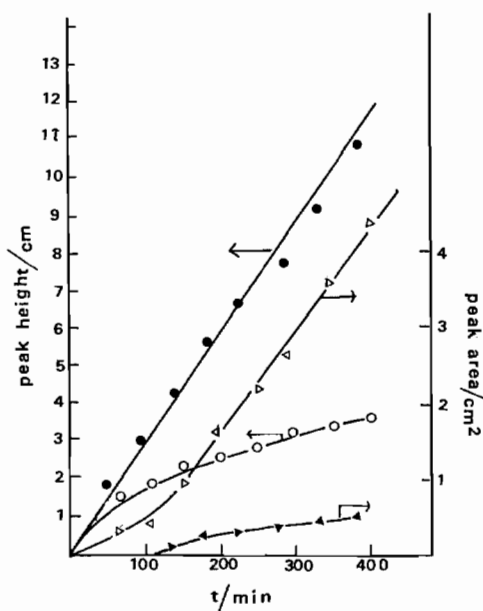| Species	pH	Time elapsed	Peak No.											
			(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
PtCl₂en	2	d	0	11	4		11	11	>30	65	19	19		11
PtClH₂Oen	2	h	0	23	8	8	1.5	1.5		10	23	1.5	1.5	4.5
	7	h	0	4.5			0	0		3		1.7	3	8
Pt(H₂O)₂en	2	min	40				30	30				0	30	50

Fig. 8. Plots of peak height *vs.* time *t* for peak (1) at pH 2 and 7, and plots of peak area for peaks (5) and (6) at pH 2 and 7. For peak (1): (●) pH 2. (○) pH 7; for peak (5)(6): (▲) pH 2, (△) pH 7.

N(7)-platinated complex. Peaks (5) or (6) are assigned to the N(1)-platinated complex. Peak (12) was assigned as follows. To 0.35 M [Pt(H₂O)₂en]²⁺ solution, Ade (0.7 M) was added and stirred for 4 days at 40 °C in the dark; the resulting solution contained the reaction product corresponding to peak (12) and a resinous product. The supernatant solution was used for the sample of ¹³C NMR and ESCA. The result obtained from ESCA shows that the ratio of Pt to N is 1:10, suggesting the formation of a 2:3 complex. The broad ¹³C NMR signal attributed to C(2) is obtained in the region of 151.6–155.2 ppm. It may be broadened by complexation of Pt(II) at the N(1) site. The peak attributed to C(8) is split into 144.8 and 146.4 ppm, the latter being twice as high as the former. This shows that the two C(8) atoms are equivalent and one carbon is not. These results show that two Pt(II) atoms bind Ado through N(7) and N(1) and another two Ado bind to each Pt(II) as N(1) to N(1) and N(7) to N(7).

From these results, it is clear that three Pt(II) species have respective selectivity for heterocyclic nitrogen. The dichloro complex prefers the N(7) site to the N(1) site and this fact agrees with the results reported by Inagaki *et al.* [7]. Diaqua species have a strong tendency to bind through the N(1) site, even when the N(1) site is occupied by H⁺ in acidic conditions. This is explained by the strong electrophilicity of the diaqua species. Chloroaqua species prefer both the N(1) and the N(7) site.

Rate of Reaction

The first stage of the reaction occurs at the N(7) site except for the case of the diaqua species. Thus, the reaction rate of Pt(II) with the N(7) site could be measured. As time proceeds the other reaction products are formed successively. Also, the dichloro complex as the starting material is hydrolysed in solution and many hydrolysed products, namely PtClH₂O, Pt(H₂O)₂, PtClOHH₂O and Pt(OH)₂ etc., are formed. To avoid complex factors and to simplify the reaction, the dichloro and chloroaqua species were chosen as the Pt(II) complex and the kinetics of their coordination reactions at the N(7) site of Ado and d-Ado were examined.

Rate Constants of PtCl₂en with Ado or d-Ado and cis-Pt(NH₃)₂Cl₂ with Ado or d-Ado

For the reaction of Ado with the dichloro complex, it takes two days for PtCl₂en and about two weeks for cis-Pt(NH₃)₂Cl₂ after the start of reaction until the second peak emerges at 25 °C. Thus the reaction rate at which Pt binds through the N(7) of Ado could be determined by measurement at the early stage of the reaction. The kinetic studies were performed with equimolar amounts of Pt(II) and Ado. The kinetic equation for this reaction is described as

$$\text{Pt complex} + \text{Ado} \xrightarrow{\ k_1\ } \text{Pt–AdoN(7)} \qquad (1)$$
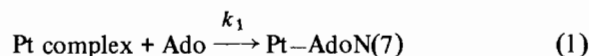
$$dx/dt = k_1(a_0 - x)^2 \qquad (2)$$

where a_0 denotes the initial concentration of Pt complex and Ado, x the concentration of reaction product, and k_1 the rate constant.

The peak height of Ado at time t corresponds to $(a_0 - x)$. Equation (2) was integrated in the usual way and $1/(a_0 - x)$ *versus* t were plotted. The plot gave a straight line and k_1 was calculated from the slope of this straight line. The k_1 values are listed in Table III.

The k_1 values for PtCl₂en with Ado at pH 6.7 and 3.0 are close to each other. For cis-Pt(NH₃)₂Cl₂, the k_1 values are nearly equal in the case of Ado and d-Ado. Moreover, all k_1 values in Table III at 25 °C are very close to each other.

Rate Constants of cis-Pt(NH₃)₂ClH₂O with Ado

The reaction of cis-Pt(NH₃)₂ClH₂O with Ado was carried out at pH 3 in solutions containing various concentrations of Cl⁻ and a 10-fold excess of Pt(II). The concentration of [cis-Pt(NH₃)₂ClH₂O]⁺ is dependent on [Cl⁻]. The reaction scheme can be described as:

cis-Pt(NH$_3$)$_2$Cl$_2$ + Ado

K_1 $\|$ k_h

k_1

Pt—AdoN(7)

k_2

[cis-Pt(NH$_3$)$_2$ClH$_2$O]$^+$ + Ado
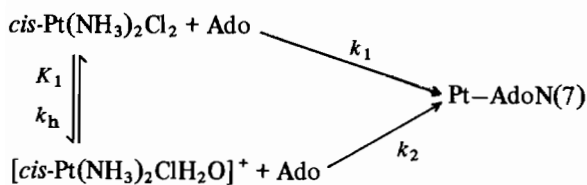
where K_1 is the equilibrium constant for the hydrolysis of cis-Pt(NH$_3$)$_2$Cl$_2$, k_h the rate constant for hydrolysis, k_1 the rate constant for the cis-Pt(NH$_3$)$_2$Cl$_2$ path and k_2 the rate constant for the [cis-Pt(NH$_3$)$_2$ClH$_2$O]$^+$ path. The kinetic equation for this reaction is described as:

$$Pt' + Ado \xrightarrow{k_{obs}} Pt-AdoN(7) \qquad (4)$$
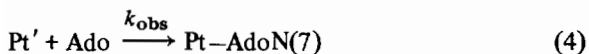
$$dx/dt = k_{obs}[Pt'][Ado] \qquad (5)$$

$$k_{obs}[Pt'][Ado] = k_{obs}[Ado]([cis\text{-}Pt(NH_3)_2Cl_2]$$
$$+ [cis\text{-}Pt(NH_3)_2ClH_2O]^+) \qquad (6)$$

where [Pt'] denotes the total concentration of Pt(II) not bound with Ado and k_{obs} is the conditional rate constant. Equation (5) was integrated in the usual way and $1/(a_0 - b_0) \ln b_0(a_0 - x)/a_0(b_0 - x)$ versus t

were plotted. Here a_0, b_0 and x denote the initial concentration of Pt, the initial concentration of Ado and the concentration of the reaction product, respectively. The plot shown in Fig. 9 gave a straight line. The reaction was run under very favorable conditions for the hydrolysis of cis-Pt(NH$_3$)$_2$Cl$_2$ because this was in excess with respect to Ado. In the case of $k_2 > k_h$, the reaction will be controlled by the slow hydrolysis step. Consequently, the reaction curve is not linear. Contrary to this, in the case of $k_2 < k_h$, the graph will be linear. The linearity shown in Fig. 9 confirms that the formation of Pt—AdoN(7) is slower than the hydrolysis reaction. From the straight line, k_{obs} values have been calculated and are shown in Table IV. If eqn. (6) is expressed by means of [Cl$^-$] and K_1, eqn. (6) results:

$$k_{obs}[Pt'][Ado]$$
$$= k_{obs}[Ado][cis\text{-}Pt(NH_3)_2Cl_2](1 + K_1/[Cl^-]) \quad (7)$$

$$k_{obs}[Pt'][Ado]$$
$$= [Ado](k_1[cis\text{-}Pt(NH_3)_2Cl_2]$$
$$+ k_2[cis\text{-}Pt(NH_3)_2ClH_2O]^+)$$
$$= [Ado][cis\text{-}Pt(NH_3)_2Cl_2](k_1 + k_2K_1/[Cl^-]) \quad (8)$$

TABLE III. Rate Constants (k_1) for the Reactions of PtCl$_2$en or cis-Pt(NH$_3$)$_2$Cl$_2$ with Ado and d-Ado

Reactants	pH	Temperature (°C)	$k_1 \times 10^4$ (dm^3 mol^{-1} s^{-1})	(S)[a]
Ado + PtCl$_2$en	3.0	9	1.4	(0.05)
		15	2.9	(0.11)
		20	4.7	(0.09)
		25	7.7	(0.21)
	6 7	11	2.7	(0.27)
		15	3.8	(0.26)
		20	5.6	(0.26)
		25	7.9	(0.23)
d-Ado + PtCl$_2$en	6.7	10	1.7	(0.09)
		15	2.5	(0.05)
		20	4.9	(0.05)
		25	7.7	(0.15)
Ado + cis-Pt(NH$_3$)$_2$Cl$_2$	3.0[b]	25	7.0	
		35	13	
		40	16	
	7.0	20	5.9	(0.27)
		25	7.8	(0.19)
		30	8.9	(1.2)
		35	13	(0.47)
d-Ado + cis-Pt(NH$_3$)$_2$Cl$_2$	7.0	20	4.7	(0.25)
		25	6.8	(0 11)
		30	10	(0.55)
		35	16	(0.02)

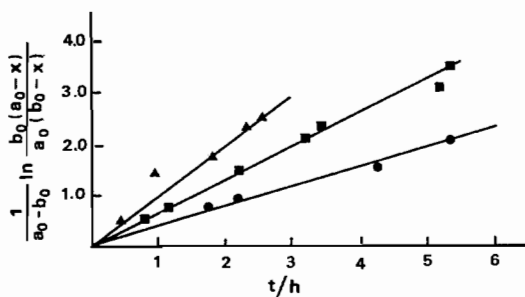[a](S) = standard deviation.   [b]Values were obtained from eqn. (8).

Fig. 9. Plots of $1/(a_0 - b_0) \ln b_0(a_0 - x)/a_0(b_0 - x)$ vs. time t at various temperatures: (●) 25 °C; (■) 35 °C; (▲) 40 °C.

TABLE IV. Conditional Rate Constants (k_{obs}) for the Reactions of cis-Pt(NH$_3$)$_2$Cl$_2$ and [cis-Pt(NH$_3$)ClH$_2$O]$^+$ with Ado in the Presence of Various Amounts of Cl$^-$ at pH 3

Temperature (°C)	$k_{obs} \times 10^3$ (dm^3 mol^{-1} s^{-1})(S)[a] at [Cl$^-$] (mol dm^{-3})			
	0.01	0.02	0.03	0.04
25	2.9 (0.09)	2.8 (0.38)	1.9 (0.04)	1.2 (0.02)
35	5.0 (0.31)	3.5 (0.21)	3.1 (0.11)	2.6 (0.05)
40	7.6 (0.28)	4.8 (0.14)	5.6 (0.44)	5.0 (0.13)

[a]S = standard deviation.
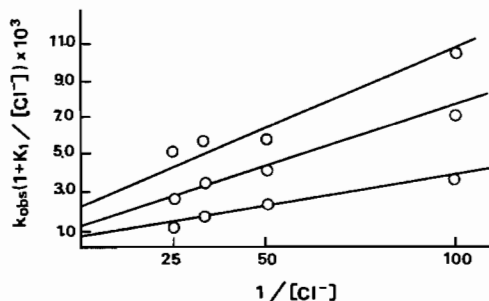

Fig. 10. Plots of $k_{obs}(1 + K_1/[Cl^-])$ vs. $1/[Cl^-]$ according to eqn. (8) at various temperatures.

Combining eqns. (7) and (8) and canceling [Ado] and cis-Pt(NH$_3$)$_2$Cl$_2$ gives

$$k_{obs}(1 + K_1/[Cl^-]) = k_1 + k_2 K_1/[Cl^-] \qquad (9)$$

The plots of $k_{obs}(1 + K_1/[Cl^-])$ *versus* $1/[Cl^-]$ are shown in Fig. 10. The rate constants (k_1) have been determined from the intercept of this straight line and are listed in Table IV. The k_2 values have been calculated from the slope of this straight line and are listed in Table V. It is clear that k_2 values are 12–15 times larger than k_1 values over the temperature range 25–40 °C. This indicates that cis-Pt(NH$_3$)$_2$ClH$_2$O reacts with Ado 12–15 times faster than cis-Pt(NH$_3$)$_2$Cl$_2$.

TABLE V. Rate Constants (k_2) for the Reaction of [cis-Pt(NH$_3$)$_2$ClH$_2$O]$^+$ with Ado

Reactants	pH	Temperature (°C)	$k_2 \times 10^2$ (dm^3 mol^{-1} s^{-1})
Ado + cis-Pt(NH$_3$)$_2$ClH$_2$O	3.0	25	1.1
		35	1.5
		40	1.9

TABLE VI. Activation Parameters for the Reactions of PtCl$_2$en, cis-Pt(NH$_3$)$_2$Cl$_2$ and [cis-Pt(NH$_3$)$_2$ClH$_2$O]$^+$ with Ado or d-Ado

Reactants	pH	E_a (kJ mol^{-1})	$\Delta H^{\neq}$ (kJ mol^{-1})	$\Delta S^{\neq}$ (J mol^{-1} K^{-1})
Ado + PtCl$_2$en	3.0	72	71	−67
	6.7	59	55	−118
d-Ado + PtCl$_2$en	6.7	74	72	−63
Ado + cis-Pt(NH$_3$)$_2$Cl$_2$	3.0	41	41	−169
	7.0	39	37	−184
d-Ado + cis-Pt(NH$_3$)$_2$Cl$_2$	7.0	63	54	−138
Ado + cis-Pt(NH$_3$)$_2$ClH$_2$O	3.0	31	28	−188

Activation Parameters

From the temperature dependence of the k_1 values, the activation parameters were calculated for the reaction of PtCl$_2$en or cis-Pt(NH$_3$)$_2$Cl$_2$ with Ado and d-Ado. From the temperature dependence of the k_2 values, the activation parameters for the reaction of [cis-Pt(NH$_3$)$_2$ClH$_2$O]$^+$ with Ado were also calculated. An Arrhenius plot was used for activation energy (E_a), and an Eyring plot for activation enthalpy ($\Delta H^{\neq}$) and for activation entropy ($\Delta S^{\neq}$), respectively. The values obtained are listed in Table VI.

In all cases $\Delta S^{\neq}$ has the characteristic large negative values as for the other cases of substitution reactions of Pt(II) [8].

Acknowledgements

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