Multinuclear NMR Studies and the Kinetics of Formation of Platinum(II)-Adenine Nucleotide Complexes

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Abstract: The reactions of adenosine 5'-monophosphate, adenosine 5'-diphosphate, and adenosine 5'-triphosphate with *cis*-diamminedichloroplatinum(II) exhibit biphasic kinetics at 40 °C and pH 7.0 in 0.5 M $NaClO_4$. The formation of the initial complexes follows a two-term rate law as a result of the direct reaction of the nucleotides with *cis*-diamminedichloroplatinum(II) and its corresponding aquated species. The reaction through the aquated pathway is limited by the rate of aquation; the value of the first-order rate constant is 1.60×10^{-4} s^{-1}. The values of the rate constants for the direct reaction between the nucleotides and *cis*-diamminedichloroplatinum(II) are 6.25×10^{-3} M^{-1} s^{-1} for ATP, 7.79×10^{-3} M^{-1} s^{-1} for ADP, and 9.33×10^{-3} M^{-1} s^{-1} for AMP. These initial complexes react further to yield final products through a first-order process apparently limited by the rate of aquation of these complexes. The rate constants for these aquation processes are evaluated to be 5.5×10^{-5} s^{-1} for the ATP complex, 1.12×10^{-4} s^{-1} for the ADP complex, and 1.04×10^{-4} s^{-1} for the AMP complex. The intermediates and final products of the reactions in solution were characterized by phosphorus-31, carbon-13, and proton NMR spectroscopy. The final products are proposed to be nucleotide complexes coordinated through both a phosphate group and N7 of the purine ring. The possible coordination sites for the nucleotides in the intermediate complexes are discussed.

Many nucleoside complexes of platinum(II) have been studied[1-3] in order to gain insight into the binding sites of *cis*-diamminedichloroplatinum(II) to DNA. X-ray crystallographic and NMR data have shown that when guanosine or adenosine reacts with *cis*-diamminedichloroplatinum(II), the binding site of the resulting complex is N7 of the purine ring.[2,3] However, under certain experimental conditions the binding site of adenosine complexes has been concluded on the basis of proton NMR[4] and other spectral data[1] to be N1. The X-ray crystal structure of a platinum(II) complex containing N1-bound adenine has also been reported.[5]

Nucleotide complexes of platinum(II) have also been investigated.[6-9] When these complexes are compared with those containing nucleosides, in most cases the presence of the phosphate group neither alters the binding site in the nucleoside nor creates a new site for coordination. For example, when inosine is the base, both the nucleoside and the monophosphate coordinate through N7.[6] The crystal structure of $[Pt(tn)(Me\text{-}5'\text{-}GMP)_2]$ (tn = triethylenediammine and Me-5'-GMP = guanosine 5'-monophosphate methyl ester) shows binding through the N7[6] as for guanosine. A recent study[8] dealing with the kinetics, proton NMR, and platinum-195 NMR of the reaction of adenosine 5'-monophosphate with *cis*-diamminedichloroplatinum(II) in 0.5 M NaCl has concluded that each nucleotide molecule binds two platinum ions through N7 and N1 of the adenine moiety. The kinetics of this reaction gave evidence for the presence of intermediates which were proposed to be N7-bound and N1-bound species.[8] In a few cases nucleotides were reported to exhibit phosphate coordination to platinum(II). For example, cytidine 5'-monophosphate reacts with *cis*-diaquadiammineplatinum(II) nitrate at pH 6.0 to form a bis(cytidine 5'-monophosphato)-bridged diplatinum(II,II) complex. In this complex, the phosphate group of cytidine 5'-monophosphate is coordinated to one platinum atom and N3 to the other platinum atom.[10]

Recently, we have demonstrated that the inorganic phosphate ligands ortho-, pyro-, and triphosphate are capable of reacting with *cis*-diamminedichloroplatinum(II) to form complexes that are stable at neutral pH for days.[11,12] Moreover, the rates of formation of these phosphato complexes are comparable to the rates observed for many nucleophiles containing nitrogen donor atoms. We have also observed that *cis*-diaquadiammineplatinum(II) in acidic aqueous solution promotes the hydrolysis of polyphosphate ligands.[12,13] The hydrolyzed products are blue in color[12,14] and have spectral and magnetic properties comparable to polyamide platinum blues.[15] From our results with the phosphato complexes of platinum(II), we concluded that phosphate coordination of nucleotides to platinum(II) may occur more frequently than has been reported. Here we present the results of an examination of the reactions of adenosine 5'-monophosphate, adenosine 5'-diphosphate, and adenosine 5'-triphosphate with *cis*-diamminedichloroplatinum(II).

Experimental Section

The nucleotides adenosine 5'-monophosphate (AMP), adenosine 5'-diphosphate (ADP), and adenosine 5'-triphosphate (ATP) were purchased from Sigma Chemical Co. (grade II). The deuteration of AMP

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at the H8 position[16] was accomplished by incubation at pD 6.5 in 100% D_2O for 72 h at 60 °C. The platinum substrate, *cis*-$PtCl_2(NH_3)_2$, was prepared as previously reported.[17] Aquated *cis*-$PtCl_2(NH_3)_2$ was prepared in situ by allowing the platinum substrate to stand for 15 h at pH 3.0 and 25 °C. The pH was adjusted with 0.5 M $HClO_4$. All other reagents were analytical reagent grade and were used without further purification.

The experimental details of the low-field NMR and other spectral measurements are the same as previously reported.[11] High-field proton NMR studies were conducted at 400 MHz on a Varian XL-400 NMR spectrophotometer. Data acquisition parameters were the following: 4000-Hz spectral width, 30 000 data points, 11-μs pulse width. Except for the high-field NMR studies, chemical shifts are reported with respect to external references of $(CH_3)_4Si$ for the proton and carbon-13 NMR spectra and 85% phosphoric acid for the phosphorus-31 NMR spectra. For the high-field NMR spectra the chemical shifts are referenced to DSS (sodium 2,2-dimethyl-2-silapentane-5-sulfonate) with tetramethylammonium chloride (3.18 ppm) used as the secondary standard.

Stoichiometries of the Reactions between the Nucleotides and *cis*-$PtCl_2(NH_3)_2$. The stoichiometries of the reactions between *cis*-$PtCl_2(NH_3)_2$ and the nucleotides were determined spectrophotometrically at pH 7.0 in 0.5 M $NaClO_4$. In a typical series of experiments, equal volumes of a solution containing a fixed concentration of platinum complex in the range of 0.5 to 2.5 mM were mixed with solutions having various concentrations of nucleotides such that the concentration ratio, [nucelotide]/[Pt], ranged from 0.4 to 15.0. These solutions were allowed to stand at 40 °C in a thermostated bath for 15 h. The absorbance of each of these mixtures was monitored at 370, 302, and 260 nm until no further change in absorbance was observed. The absorbance at both 260 and 302 nm for all three nucleotide reactions increased as the reactions progressed, while the absorbance at 330 nm decreased. The stoichiometries of the reactions were determined from plots of absorbance change vs. molar ratio.

Kinetics of Formation of the Nucleotide Complexes of Platinum(II). The kinetics of the reactions between *cis*-$PtCl_2(NH_3)_2$ and the nucleotides were followed at 302 and 370 nm. The ionic strength was maintained at 0.5 M with $NaClO_4$ and the pH was adjusted to 7.0 with 0.5 M NaOH. The concentrations of *cis*-$PtCl_2(NH_3)_2$ were in the range of 1.2–2.4 mM, and the concentrations of the nucleotides were at least tenfold excess over the platinum substrate. Plots of ln $(D_\infty - D)$ or ln $(D - D_\infty)$ deviated from linearity within the first half-life. The absorbance–time data can adequately be described by consecutive first-order reactions:

$$PtCl_2(NH_3)_2 + \text{nucleotide} \xrightarrow{k_0} (\text{intermediate}) \xrightarrow{k_1} \text{products} \quad (1)$$

The values of the rate constants k_0 and k_1 were evaluated with an iterative nonlinear least-squares fit of the integrated rate expression for consecutive first-order reactions[11] as given by eq 2. In this equation $[A]_0$

$$D = \epsilon_A[A]_0e^{-k_1t} + \frac{[A]_0\epsilon_1k_0}{k_1 - k_0}(e^{-k_0t} - e^{-k_1t}) + \frac{D_\infty}{k_1 - k_0}(k_0e^{-k_1t} - k_1e^{-k_0t}) + D_\infty \quad (2)$$

represents the initial concentration of the starting platinum complex, D and D_∞ are the values of the absorbance at time t and at infinite time, and ϵ_A and ϵ_I are the molar absorptivities of the starting platinum complex and the intermediate. In this fitting procedure, the known parameters, ϵ_A, $[A]_0$, and D_∞, were kept invariant and initial estimates were made for the two rate constants and ϵ_I. Usually 4–6 iterations were sufficient to simulate kinetic curves that are within the precision of the observed data. The reproducibility of the rate constants from replicate measurements was better than 7%.

Results

Kinetics. The spectral changes observed in the UV–vis spectra for solutions of *cis*-$PtCl_2(NH_3)_2$ in the presence of AMP, ADP, or ATP are markedly different from those for simple aquation of *cis*-$PtCl_2(NH_3)_2$ under identical experimental conditions. For example, in the presence of nucleotide the absorbance shoulder of *cis*-$PtCl_2(NH_3)_2$ at 370 nm completely disappears with a concomitant increase in absorbance at 302 nm. These changes in absorbance are due to the complexation of the nucleotides by *cis*-$PtCl_2(NH_3)_2$. At pH 7.0 and 40 °C in 0.5 M $NaClO_4$ no further changes in absorbance were observed after 20 h. Plots of the absorbance change vs. molar ratio, ([nucleotide]/[Pt]), at 370, 302, and 260 nm show break points at molar ratio values of 1.05 ± 0.05 for AMP, 1.1 ± 0.1 for ADP, and 0.95 ± 0.05 for ATP. A typical plot dealing with the change in absorbance vs. the concentration ratio of the two reactants is shown in Figure 1. These results indicate that the stoichiometries for the reactions of *cis*-$PtCl_2(NH_3)_2$ with the nucleotides are 1:1. The concentration ratios were selected to cover the conditions under which the kinetics were studied.

Under pseudo-first-order kinetic conditions, the first-order plots (ln$(D_\infty - D)$ vs. t) for all of the nucleotide reactions deviate from linearity within the first half-life of the reaction (Figure 2A). However, excellent agreement between the experimental and

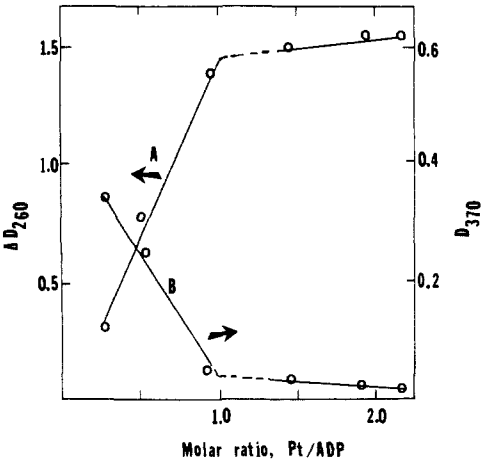

Figure 1. Plots of absorbance vs. molar ratio ([Pt]/[ADP]) for the reaction between ADP and *cis*-diamminedichloroplatinum(II) at 40 °C. For curve A the ordinate corresponds to the change in absorbance at 260 nm with use of a 0.1-cm cell and $[PtCl_2(NH_3)_2] = 5.0 \times 10^{-4}$ M. The ordinate for B represents the absolute absorbance at 370 nm with use of a 5-cm cell and $[PtCl_2(NH_3)_2] = 2.5 \times 10^{-3}$ M.

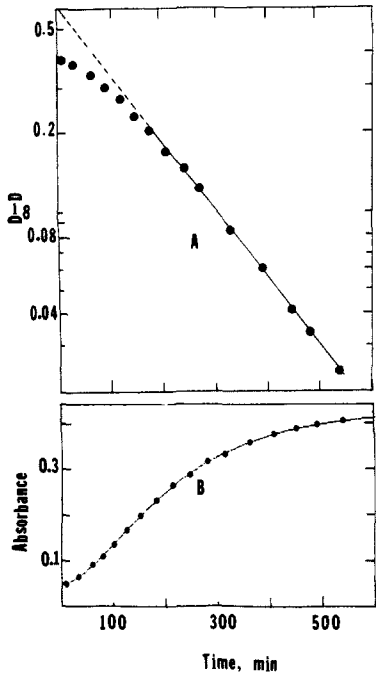

Figure 2. A typical absorbance–time profile and its first-order plot for the reaction of AMP (7.0 mM) and *cis*-diamminedichloroplatinum(II) (0.50 mM) at 40 °C, pH 6.8 in 0.5 M $NaClO_4$ at 302 nm. (A) Semilogarithmic plot of the absorbance change vs. time. (B) Observed and calculated kinetic profile; the closed circles represent the observed absorbance and the dotted line is the simulated profile using eq 1 with $k_0 = 2.05 \times 10^{-4}$ s^{-1}, $k_1 = 1.10 \times 10^{-4}$ s^{-1}, $\epsilon_A = 125$ M^{-1} cm^{-1}, $\epsilon_I = 138$ M^{-1} cm^{-1}, and $D_\infty = 0.43$.

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Table I. Rate Constants for the Reactions between Nucleotides and *cis*-Diamminedichloroplatinum(II) at 40 °C, pH 7.0, and I = 0.5 M ($NaClO_4$)

$[PtCl_2(NH_3)_2]$, mM	[nucleotide], mM	$k_0 \times 10^{-4}$, s^{-1}	$k_1 \times 10^{-4}$, s^{-1}
	AMP + *cis*-$PtCl_2(NH_3)_2$		
0.50	7.0	2.05	1.10
0.86	11.8	3.20	0.96
0.86	23.5	3.72	1.13
1.80	35.0	4.45	0.96
2.25	40.8	5.06	1.05
	ADP + *cis*-$PtCl_2(NH_3)_2$		
1.12	10.1	2.89	1.17
1.05	15.2	3.35	1.15
2.05	21.5	3.85	1.05
2.00	27.5	4.22	1.20
2.00	40.2	5.31	0.98
	ATP + *cis*-$PtCl_2(NH_3)_2$		
0.52	6.0	1.79	0.65
0.80	11.0	2.25	0.55
0.80	15.5	2.58	0.50
2.03	38.2	3.98	0.52

Table II. Values of k_s, k_y, and k_1 for the Reactions of Nucleotides with *cis*-$PtCl_2(NH_3)_2$ at 40 °C, pH 7.0, and I = 0.5 M ($NaClO_4$)

nucleotide	$k_s \times 10^{-4}$	$k_y \times 10^{-3}$	$k_1 \times 10^{-4}$
AMP	1.85	7.82	1.04
ADP	2.12	7.90	1.12
ATP	1.48	6.62	0.55

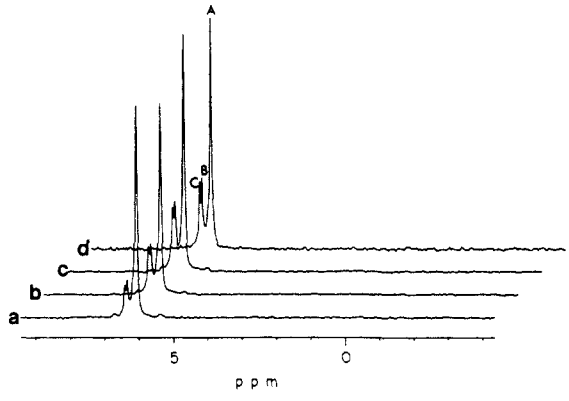


Figure 3. Ambient-temperature 36.3-MHz proton-decoupled phosphorus-31 NMR spectra of the reaction mixture of *cis*-diamminedichloroplatinum(II) (5 mM) and AMP (40 mM) at pH 6.8 at several time intervals: (a) 3 h; (b) 7 h; (c) 10.5 h; (d) 48 h. Peak A is due to free AMP, and peaks B and C are due to two products.

calculated absorbance values was observed when the kinetic profiles were treated as composites of two exponential curves. A typical computer fit of the data according to eq 1 is shown in Figure 2B. The two rate constants, k_0 and k_1, obtained from the fit are listed in Table I.

For each nucleotide the pseudo-first-order rate constant k_0 follows the rate law

$$k_0 = k_s + k_y[\text{nucleotide}] \quad (3)$$

The values of k_s and k_y were evaluated from a linear least-squares fit for eq 3 and are listed in Table II for the reactions of all three nucleotides. The values of the rate constant k_1 were independent of nucleotide concentration.

NMR Spectra. The phosphorus-31 NMR spectra of the reaction between AMP and *cis*-$PtCl_2(NH_3)_2$ under pseudo-first-order kinetic conditions were recorded at various time intervals as shown in Figure 3. The signal A at 6.09 ppm is due to free AMP while the two growing peaks, B and C, at 6.35 and 6.42 ppm, are due to product formation. The spectrum stopped changing within 24 h and no further changes were observed after 5 days. A plot of the intensities of the phosphorus-31 NMR peaks

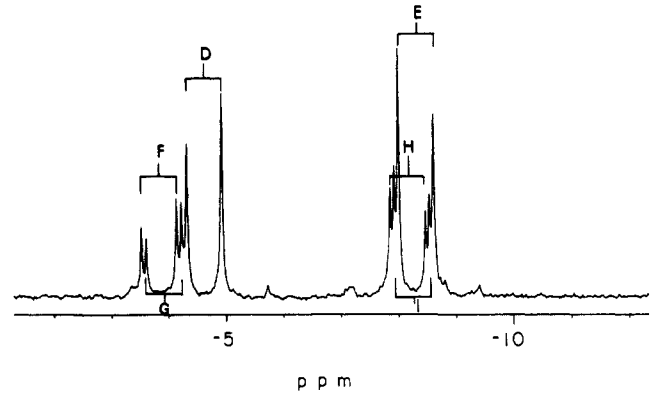


Figure 4. Ambient-temperature 36.3-MHz proton-decoupled phosphorus-31 NMR spectrum of the reaction mixture of *cis*-diamminedichloroplatinum(II) (5 mM) and ADP (40 mM) at pH 6.8 after 48 h. Doublets D and E are due to free ADP. The four sets of smaller doublets are due to two products.

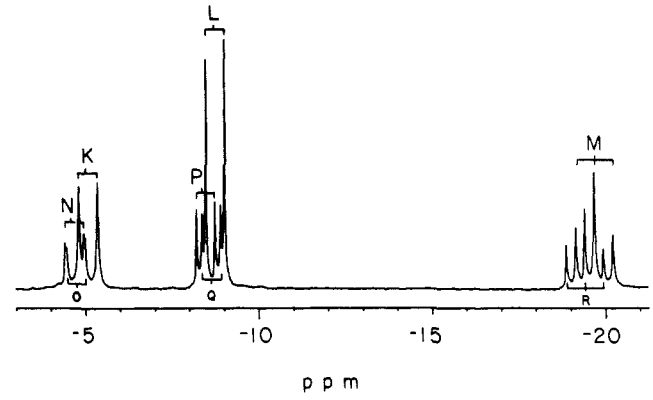


Figure 5. Ambient-temperature 36.3-MHz proton-decoupled phosphorus-31 NMR spectrum of the reaction mixture of *cis*-diamminedichloroplatinum(II) (5 mM) and ATP (40 mM) at pH 6.8 after 48 h. The doublets K and L and the apparent triplet M are due to free ATP. Two products each produce a pair of doublets and together produce the apparent triplet R.

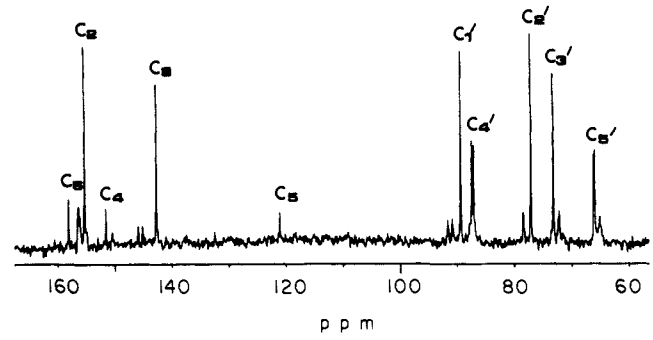


Figure 6. Ambient-temperature proton-decoupled carbon-13 NMR spectrum of the reaction mixture of *cis*-diamminedichloroplatinum(II) (5 mM) and AMP (40 mM) at pH 6.8 after 48 h. The taller peaks correspond to free AMP and the smaller peaks to two products.

of the products vs. time also exhibits the consecutive kinetic behavior that was observed when the kinetics were followed spectrophotometrically.

The progress of the reactions of ADP and ATP with *cis*-diamminedichloroplatinum(II) was also monitored by phosphorus-31 NMR spectroscopy at pH 7.0. Figure 4 presents the phosphorus-31 NMR spectrum at the end of the ADP reaction. Free ADP appears as the two doublets, D at −4.68 ppm (J = 22.2 Hz) and E at −8.31 ppm (J = 22.2 Hz). The new sets of doublets, F–I, are due to the formation of ADP complexes with the platinum substrate. The coupling constants and the positions of the various peaks are listed in Table III. Figure 5 exhibits the phosphorus-31 NMR spectrum at the end of the reaction between ATP and

Table III. Assignments of the Various Phosphorus-31 NMR Peaks of Platinum(II) Nucleotide Products

peak	position,[a] ppm	coupling constant, Hz	assignment	coord chemical shift, ppm
		AMP + *cis*-$PtCl_2(NH_3)_2$		
A	6.09 (s)		AMP	
B	6.35 (s)		Pt-AMP complex	+0.26
C	6.42 (s)		Pt-AMP complex	+0.33
		ADP + *cis*-$PtCl_2(NH_3)_2$		
D	−4.64 (d)	22.2	β-phosphate, ADP	
E	−8.31 (d)	22.2	α-phosphate, ADP	
F	−3.82 (d)	22.3	β-phosphate, Pt-ADP	+0.82
G	−3.92 (d)	22.2	β-phosphate, Pt-ADP	+0.72
H	−8.16 (d)	22.3	α-phosphate, Pt-ADP	+0.15
I	−8.23 (d)	22.2	α-phosphate, Pt-ADP	+0.09
		ATP + *cis*-$PtCl_2(NH_3)_2$		
K	−5.42 (d)	19.8	γ-phosphate, ATP	
L	−8.56 (d)	19.7	α-phosphate, ATP	
M	−19.69 (t)	19.7	β-phosphate, ATP	
N	−4.76 (d)	19.5	γ-phosphate, Pt-ATP	+0.66
O	−4.83 (d)	19.3	γ-phosphate, Pt-ATP	+0.59
P	−8.32 (d)	19.3	α-phosphate, Pt-ATP	+0.24
Q	−8.47 (d)	19.5	α-phosphate, Pt-ATP	+0.09
R	−19.36 (t)	19.4	β-phosphate, Pt-ATP	+0.25

[a] pH = 7.0; d, doublet; s, singlet, t, triplet.

cis-$PtCl_3(NH_3)_2$. The spectrum of free ATP consists of two doublets, K and L, at −5.42 ppm (J = 19.8 Hz) and −7.34 ppm (J = 19.7 Hz), and an apparent triplet, M, at −19.67 ppm (J = 19.7 Hz). Four other doublets, N–Q, and an apparent triplet, R, increased with time. After an initial 48-h period at room temperature no further changes were observed.

The carbon-13 and proton NMR spectrum of the products at the end of the reactions were also recorded. Figure 6 shows the carbon-13 NMR spectra of the AMP-containing products and the remaining unreacted nucleotide. The assignments of the various carbon atoms in the free nucleotide have been made previously.[18] The spectrum contains sets of additional signals near most of the peaks for the carbon atoms in the free nucleotide. Except for the peaks near the 3′- and 5′-carbon atoms all of these new signals appear downfield relative to the positions of the carbon atoms in the free nucleotide.

The proton NMR spectrum at the end of the ATP reaction was also recorded. Under our experimental conditions, the resonances of the H8 and H2 protons of the free adenine nucleotide appear at 8.40 and 8.11 ppm. In addition to these two resonances, four new signals are observed at 9.36, 9.26, 8.07, and 8.04 ppm. The signals at 9.36 and 9.26 ppm are 0.96 and 0.86 ppm downfield from that of the H8 proton. The other two peaks due to the formation of the products appear at 0.04 and 0.07 ppm upfield from the position of the H2 resonance. The proton NMR spectra of the products due to the reactions of AMP and ADP with *cis*-$PtCl_2(NH_3)_2$ are similar to those for the ATP reaction. The chemical shifts of the various products and free nucleotides are listed in Table IV.

Table IV. Chemical Shifts for the H2 and H8 Protons for the Complexed and Uncomplexed Adenine Nucleotides

compd	H8, ppm	coord chem shift, ppm	H_2, ppm	coord chem shift, ppm
AMP	8.42		8.14	
Pt-AMP	9.48	+1.06	8.10	−0.04
Pt-AMP	9.33	+0.91	8.08	−0.06
ADP	8.44		8.16	
Pt-ADP	9.42	+0.99	8.13	−0.03
Pt-ADP	9.32	+0.88	8.11	−0.06
ATP	8.40		8.11	
Pt-ATP	9.36	+0.96	8.07	−0.04
Pt-ATP	9.26	+0.86	8.04	−0.07

Table V. pH Dependence of the Proton Chemical Shifts Measured at High Field for Complexed and Uncomplexed Adenosine Diphosphate

	H8, ppm			H2, ppm		
pH	free	bound		free	bound	
0.48	8.56	9.46	9.39	8.39	8.48	8.46
0.96	8.60	9.51	9.43	8.41	8.48	8.46
1.48	8.62	9.51	9.42	8.42	8.44	8.42
2.08	8.62	9.47	9.37	8.42	8.36	8.31
2.27	8.63	9.46	9.36	8.41	8.33	8.28
2.66	8.62	9.43	9.32	8.41	8.28	8.23
3.33	8.60	9.42	9.29	8.38	8.23	8.19
3.42	8.60	9.42	9.29	8.37	8.22	8.18
4.29	8.53	9.42	9.28	8.29	8.20	8.17
5.12	8.49	9.42	9.29	8.23	8.20	8.17
6.03	8.49	9.46	9.34	8.22	8.19	8.16
7.04	8.50	9.51	9.41	8.22	8.19	8.15

To aid in the assignment of the new proton signals that are associated with platinum complexation, AMP was incubated in 100% D_2O under conditions that would promote exchange of the H8 proton with solvent.[16] After the proton NMR spectrum showed less than 4% residual proton, *cis*-$PtCl_2(NH_3)_2$ was added and the reaction was allowed to proceed as before. No new signals were observed in the region from 8.0 to 9.5 ppm where peaks from platinum complexation had previously been observed. When a partial deuterium exchange on H8 proton was carried out after the complexation reaction was completed, a significant decrease in the intensities was observed for the two furthest downfield signals and for the H8 signal of the free nucleotide.

The proton NMR spectra of the reaction mixtures were also recorded at high field in the pH range 1–8. Table V gives these chemical shift data for some of the resonances observed during the reaction between ADP and the platinum substrate. From the change in chemical shift with pH, pK values for the N1 site of free ADP are calculated to be 4.1 and 4.2 by using the H2 and H8 signals, respectively. From the chemical shift data for the two downfield signals of the ADP complexes, the pK values can be calculated as 2.2 and 2.3 by using the H8 signals and 2.1 and 2.2 by using the H2 signals.

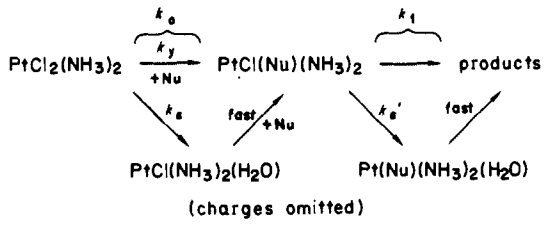
The final products of the reaction of ATP with aquated[18] *cis*-$PtCl_2(NH_3)_2$ exhibit the same phosphorus-31 NMR and proton NMR spectra as observed in the case of *cis*-$PtCl_2(NH_3)_2$ (without prior aquation). However, when the progress of the reaction with the aquated platinum substrate was monitored by phosphorus-31 NMR spectroscopy, two doublets appeared within 2 h of reaction as shoulders on the doublets (K and L) of the α- and γ-phosphate groups of the free ATP ion. These two shoulders disappeared after 7 h with the formation of the doublets N–Q. In the proton NMR spectra recorded at similar time intervals, the appearance of the

(18) Reishus, J. W.; Martin, D. S., Jr. *J. Am. Chem. Soc.* **1961**, *83*, 2453–2462. The preaquated *cis*-$Pt(NH_3)_2Cl_2$ contains 66% *cis*-$[Pt(NH_3)_2Cl(H_2O)]^+$, 7% *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$, and 27% *cis*-$Pt(NH_3)_2Cl_2$.

(19) Jones, A. J.; Grant, D. M.; Winkely, M. W.; Robins, R. K. *J. Am. Chem. Soc.* **1970**, *92*, 4079–4083.

(20) The absorbance vs. time profiles have been interpreted in terms of the formation and decay of an intermediate (eq 1). However, such an equation when rewritten in the form $D = ae^{-k_0t} + be^{-k_1t} + D_\infty$ could also represent a concurrent kinetic scheme where the preexponential factors, a and b, represent the concentrations of two slowly equilibrated reactive species. If it is assumed that the kinetic scheme is concurrent rather than consecutive, then *cis*-$PtCl_2(NH_3)_2$ and $PtCl(NH_3)_2(H_2O)^+$ could be visualized as the two reactive species. In such a situation one of the reactions should be a first-order rate process due to the aquation of the dichloro species and the other should be a second-order reaction between the nucleotides and the dichloro species itself. However, the values of k_0 and k_1 are not consistent with such a scheme, and therefore a concurrent kinetic scheme can be ruled out. We are grateful to Professor Edwin S. Gould for pointing out such a kinetic complication.

Scheme I



products peaks cannot be observed until 6 h after the start of the reaction.

Discussion

Kinetics. The kinetics of the reactions of nucleotides with *cis*-$PtCl_2(NH_3)_2$ suggest that an initial intermediate is formed and reacts further to form final products. The formation of the intermediate follows the two-term rate law invariably observed for the substitution kinetics of platinum(II) complexes.[21] The value found for the rate constant of the solvent pathway, k_s, is 1.8×10^{-4} s^{-1} and agrees well with the reported value[17] of 1.6×10^{-4} s^{-1}. The rate constants labeled K_y represent the direct reaction between *cis*-$PtCl_2(NH_3)_2$ and the nucleotides. The magnitude of the first-order rate constant for the decay of the intermediate for all three nucleotide reactions is within the range $(5-10) \times 10^{-5}$ s^{-1}. The value of the rate constant for the second aquation of *cis*-$PtCl_2(NH_3)_2$ at 35 °C was reported to be 10.4×10^{-5} s^{-1} by Reishus and Martin[18] on the basis of isotope exchange kinetics with use of chlorine-35. The rate constant for the aquation of monochloro(nucleotide)diammineplatinum(II) should not be much different from that of the second aquation of the starting dichloro–platinum complex. The first-order decay of the intermediate thus appears to correspond to the rate of aquation of a (nucleotide)platinum(II) intermediate. Therefore, one plausible reaction scheme is that an initial nucleotide complex is formed as an intermediate and is then converted to final products through a process limited by the rate of aquation. A mechanism[22] which is consistent with our kinetic data is shown in Scheme I. According to this scheme[20] the observed first-order rate constant, k_1, would be equal to k_s'.

Characterization of Products. The multinuclear NMR spectra[23] recorded during and after the reaction as presented in the Results section can be utilized to characterize the intermediate and final nucleotide products. The final products are discussed first and then the intermediates. The phosphorus-31 NMR spectra of the ATP reaction show downfield coordination chemical shifts of 0.2–0.7 ppm from free ATP for all the product peaks. This downfield shift for the product peaks indicates that the phosphate group is coordinated. Taking into consideration the coupling constants of the four doublets N–Q and the triplet R, it can be concluded that the doublets N and Q (J = 19.5 Hz) belong to one product while the doublets O and P (J = 19.3 Hz) are due to another product. The triplet R (J = 19.4 Hz) results from the concidence of triplets of the two products. Similarly, for the ADP reaction, the two doublets F and H (J = 22.2 Hz) belong to one product, and the remaining two doublets, G and I (J = 22.3 Hz), are due to the formation of a different product. The formation of two phosphate-bound products can be supported from the phosphorus-31 NMR spectra of the products of the AMP reaction. Since AMP contains only one phosphate group, the two singlets located 0.32 and 0.26 ppm downfield from the free AMP peak must originate from two different AMP molecules bound to the platinum substrate. Two molecules of AMP both bound to the two available sites of a single *cis*-$PtCl_2(NH_3)_2$ complex in a monodentate fashion would not result in two magnetically inequivalent AMP molecules. Moreover, such bis-complexation would be inconsistent with the observed 1:1 stoichiometry. The formation of phosphate-coordinated species is also supported from the carbon-13 NMR spectra. It has been reported that the carbon-13 NMR spectra of N7-bound adenosine complexes of platinum(II) exhibit downfield coordination chemical shift only for the adenine carbon atoms.[24] The chemical shifts of the ribose carbon atoms in such complexes were not changed from their positions for the free adenine nucleoside. In the present case, coordination chemical shifts are observed for carbon atoms in both the adenine and the ribose moieties. The coordination chemical shifts of the ribose carbon atoms are most likely due to the presence of phosphate coordination.

The proton NMR spectrum of the product for the AMP reaction shows four singlets in the adenine portion of the nucleotide. Two signals are 1.06 and 0.91 ppm downfield from H8 of the free nucleotide. The remaining two peaks appear 0.04 and 0.06 ppm upfield from the free H2. These results suggest that the nucleotide is coordinated not only through the phosphates but also through a nitrogen atom in adenine. The potential donor sites are N7 and N1, with N7 being the site found in many adenine and substituted adenine complexes of platinum(II).[4] For the N7 coordinated adenine complexes of platinum(II), a downfield coordination chemical shift of 0.8 to 1.0 ppm has been observed for H8.[4] The two singlets at 9.48 and 9.33 ppm exhibiting a downfield coordination chemical shift of 1.06 and 0.91 ppm would be consistent with two N7 bound species. The two signals upfield from the free H2 would then be due to the H2 of the two N7 coordinated species. The possibility that the signals at 9.48 and 9.33 ppm are due to formation of N1-coordinated species can be ruled out from our deuterium exchange experiments. When the partial deuteration was carried out at the H8 proton after the complexes were formed, a substantial decrease in the intensities of signals at 9.48 and 9.33 ppm and of the free H8 resonance was observed. The other two signals at 8.10 and 8.08 ppm did not show any detectable changes in intensity after partial deuteration. Moreover, complete specific deuteration of AMP at H8 is sufficient to eliminate any new signals in the proton NMR spectrum arising from platinum coordination. These results suggest that the product signals observed in the complexation of AMP to platinum(II) arise from the H8 proton, reflecting coordination at the adjacent nitrogen (N7).

The results of the pH dependent proton NMR spectra can also be used to support our conclusion that N7 (not N1) is the binding site.[25] The pK values of the Pt–ADP complexes are about 2.0 lower than the pK of the free ligand. These pK values correspond to the protonation at N1 of the complexed and free ADP molecules. These results are in accord with the observations by several workers[23b,26,27] that coordination to the N7 site lowers the pK for the N1 bound proton.

The phosphorus-31, carbon-13, and proton NMR spectra all show coordination chemical shifts for platinum bound species. The NMR and kinetic data are consistent with the existence of two products in solution.[28] Since both the phosphate group and N7 of the purine ring are coordinated to the platinum atoms, the structures of some of the likely complexes in solution that are

(21) For example: Wilkins, R. G. *The Study of Kinetics Mechanisms of Reactions of Transition Metal Complexes*; Allyn & Bacon, 1974; pp 223–229, and references therein.

(22) The extent of hydroxo-bridged dimer formation from the transient aquated intermediate can be considered very small in comparison with the nucleotide complexation to the same transient intermediate. See, for example, ref 11, footnote 27.

(23) (a) No coupling was observed between ^{31}P and either ^{1}H or ^{195}Pt. In similar instances other authors have explained the absence of coupling as due to the use of higher magnetic fields and a chemical anisotropic relaxation effect: Lallemard, J. Y.; Soulie, J.; Chottard, J. C. *J. Chem. Soc., Chem. Commun.* **1980**, 436–437. (b) Chottard, J. C.; Girault, J. P.; Chottard, G.; Lallemand, J. Y.; Mansuy, D. J. *J. Am. Chem. Soc.* **1980**, *102*, 5565–5572.

(24) Dehand, J.; Jordanov, J. *J. Chem. Soc., Dalton Trans.* **1977**, 1588–1593.

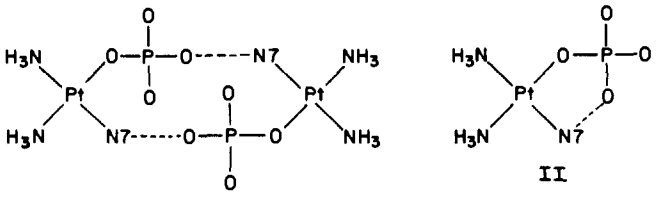
(25) We are grateful to one of the reviewers for suggesting the pH dependent chemical shift measurements.

(26) Dijt, F. J.; Canters, G. W.; Hartog, J. H. J. D.; Marcelis, A. T. M.; Reedijk, J. *J. Am. Chem. Soc.* **1984**, *106*, 3644–3647. Hartog, J. H. J. D.; Elst, H. V. D.; Reedijk, J. *J. Inorg. Biochem.* **1984**, *21*, 83–92.

(27) Martin, R. B. *Acc. Chem. Res.* **1985**, *18*, 32–38.

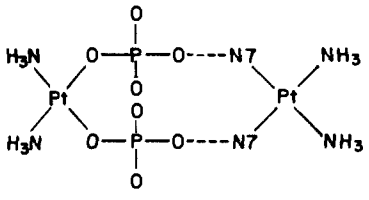
(28) From spectra on solutions at the end of the reaction between ATP and *cis*-$PtCl_2(NH_3)_2$, the integrated intensities of peaks for the two products were measured to be 1:0.86 for the α-phosphate and 1:0.77 for the γ-phosphate; the ratio of the concentrations of the two products can be written as 1:(0.80 ± 0.05).

consistent with the observed stoichiometry may be proposed as shown in I–III. In structure I, the two AMP ligands are bridged



I

II



III

between the two $Pt(NH_3)_2^{2+}$ units in a head-to-tail fashion such that each platinum(II) atom is coordinated to the N7 of one nucleotide and to the phosphate group of the other. The N7 sites for the two bridging nucleotides in structure III are bonded to one platinum while the two phosphate groups are coordinated to the other forming a head-to-head dimer. A chelate-type structure coordinating through the phosphate and N7 sites of the same nucleotide molecule is shown as structure II. The X-ray crystal structure of a dimer $[Pt(en)(5'\text{-CMP})]_2$ has been reported by Louie and Bau[10] in which N3 and phosphate oxygen are coordinated to platinum in a head-to-tail fashion. Strong hydrogen bonding between a phosphate oxygen and a hydrogen of the amine group was observed. Similar head-to-tail dimeric structures are also reported for the other metal ions.[29,30] The proximity of adjacent phosphate groups in structure III makes it seem unlikely as a major species. On the basis of this discussion, structures I and II may be regarded as the most likely for the observed products.

Characterization of Intermediates. We have also attempted to characterize the kinetic intermediates by phosphorus-31 and proton NMR. Both the proton and phosphorus-31 NMR spectra were recorded at various time intervals during the reactions. The only peaks observed in these spectra are due to the formation of the products as evidenced by the continuous growth in intensity of the peaks until the end of the reaction. By using the rate constants for the ATP reaction as an example, the maximum concentration of the intermediate during the reaction can be calculated to be 15% of the initial platinum concentration. Since the concentration of the platinum complex used is limited by the solubility of the complex and was within the range 5–7 mM, it would be difficult to observe such an intermediate by NMR. Similarity of the spectra of products and intermediates would make the identification of intermediates more difficult. The mechanism proposed for nucleotide complexation suggests that the reactions of nucleotides with the chloroaquadiammineplatinum(II) are much faster than the direct reaction between the dichloroplatinum species and the nucleotides. Therefore, a preaquated species was used as the platinum reactant instead of the dichloro complex in an attempt to generate an intermediate of sufficient concentration to allow its detection and characterization by NMR. Both the proton and phosphorus-31 NMR spectra of the reaction of the preaquated species with ATP were recorded at regular time intervals. An initial product was observed within 2 h of reaction at room temperature for which the phosphorus-31 NMR spectrum exhibits a downfield coordination chemical shift of 0.1 to 0.2 ppm for the α- and γ-phosphate groups (as shoulders on the peaks for the free nucleotide). These peaks disappeared with the formation of the peaks due to the final products. In contrast, the proton NMR spectra recorded at the same time intervals did not exhibit any new signals until 7 h after initiation of the reaction, and these new signals were due to the formation of the final products. These results suggest that a phosphate bound intermediate could be formed initially. A possibility which cannot be ruled out is that the two initial shoulders in the phosphorus-31 NMR spectra arise from an outer-sphere complex formed by the deprotonated nucleotide and positively charged aquated platinum(II) species, where the phosphate group is in proximity of the platinum(II) ion. An argument in favor of initial phosphate coordination may be made on the basis of our recent kinetic study[11] of the rate of reaction of inorganic phosphates with *cis*-$PtCl_2(NH_3)_2$. The rate constants for the reaction of these phosphates at pH 6.0 suggest that phosphate coordination should be half completed within 3 h, well before the peaks for final products appear in the NMR spectrum.

Conclusion

The results of this study clearly show phosphate coordination in the products and are consistent with the possible existence of phosphate-coordinated initial species as well. Phosphate-coordinated adenine nucleotide products have not been previously reported. It may be that appropriate experimental techniques have not been utilized to search for phosphate coordination. A study comparing the reactivities of various nucleotides with *cis*-$PtCl_2(NH_3)_2$ on the basis of IR and Raman spectral measurements was reported by Tobias et al.[8] The reactivities were reported to be guanosine > adenosine > cytidine > uridine. Louie and Bau[10] have observed phosphate coordination in the case of cytidine monophosphate at neutral pH. By studying the coordination of various inorganic phosphate ligands near neutral pH, we have established that the phosphate complexation is favorable both thermodynamically and kinetically; the deprotonated phosphate ligands are good nucleophiles. A direct comparison of the rate constants of phosphate coordination with rate constants for N7 coordination is not possible because of the paucity of definitive kinetic data dealing with N7 coordination. Since both cytidine and adenine nucleotides form stable phosphato complexes, it remains to be seen whether complex formation through the phosphate group takes place in the case of guanine and uridine nucleotides. The crystal structure of a methyl phosphate ester of guanosine 5'-monophosphate exhibits coordination through N7, but the higher nucleophilicity of the guanine mononucleotide as compared to the phosphate ester might result in the formation of phosphate-coordinated species.

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Registry No. I, 102696-66-2; II, 102696-67-3; III, 102696-68-4; AMP, 61-19-8; ADP, 58-64-0; ATP, 56-65-5; Pt, 7440-06-4; *cis*-$PtCl_2(NH_3)_2$, 15663-27-1.

(29) Aoki, K.; Clark, G. R.; Orbell, J. D. *Biochem. Biophys. Acta* **1976**, *425*, 369–372. Sletten, E.; Lie, B. *Acta Crystallogr. Sect. B* **1976**, *32*, 3301–3304.

(30) Karlik, S. J.; Elgavish, G. A.; Eichhorn, G. L. *J. Am. Chem. Soc.* **1983**, *105*, 602–609.