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NMR Studies of the Interaction of *cis*-Diamminedichloro-platinum(II) and Corresponding Hydrolysis Products with Adenosine Phosphates

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Abstract

Complexes formed in aqueous solution between cisplatin or hydrolysis species and 5′ adenosine monophosphate (AMP) or 5′ adenosine triphosphate (ATP), the latter with and without chloride ions, have been determined using ^{195}Pt, ^{31}P, ^{13}C and ^{1}H NMR. The present results lead to the conclusion that the only monodentate complexes with AMP are *cis*-$Pt(NH_3)_2$(AMP-N7)Cl at acid pH and *cis*-$Pt(NH_3)_2$(AMP-N7)OH at neutral and basic pH. Other bidentate complexes were identified as *cis*-$Pt(NH_3)_2(AMP\text{-}N7)_2$ and *cis*-$Pt(NH_3)_2$(AMP-N7)(AMP-PO). Also discussed herein are the binding of platinum to the phosphate group Pγ with ATP and at acid pH, and the formation of the [*cis*-$Pt(NH_3)_2$(ATP-N7)$H_2O]^+$ complex. In neutral and basic pH ranges, the phosphate moiety of ATP is the most reactive site. In the presence of an excess of chloride ions, the complexation rates between the ATP and the cisplatin are decreased. Furthermore, in the experimental conditions used neither the ATP nor the AMP have shown binding to N1.

Introduction

cis-Diamminedichloroplatinum(II), *cis*-$PtCl_2(NH_3)_2$ (CDDP), a potent antitumor agent, is a square-planar coordination complex; DNA is thought to be the main target molecule [1, 2]. Some authors have suggested that minor interaction may occur with the phosphate group [3], while others found no association between platinum complexes and the nucleotide chain [4]. Moreover, many studies have been conducted on phosphate buffer [5–8], although Bose *et al.* have clearly shown that an interaction takes place between phosphate groups and platinum compounds [9]. In order to elucidate the impact of the phosphate moiety [10] and the influence of its length on the complexation of the adenosine nucleotides, we studied the binding of CDDP and its hydrolysis products with AMP and ATP at various pH values and NaCl concentrations. Proton, phosphorus, platinum and carbon NMR were used in order to observe the reactions at different binding sites.

Materials and Methods

Reagents

Pure cisplatinum was supplied by Roger Bellon Laboratories. Adenosine 5′-triphosphate and adenosine 5′-monophosphate were provided by Boehringer. All other chemicals used were from Merck. All these products were used without further purification. Cisplatinum (6 mg/ml) was hydrolysed in D_2O for 5 days at the appropriate pH without any buffer, and added at time zero to AMP or ATP (10^{-2} M) at the same pH.

Instruments and Techniques

NMR spectra at 200.133 MHz (^{1}H), 81.015 MHz (^{31}P), 43.022 MHz (^{195}Pt) and 50.323 MHz (^{13}C) were obtained with a Bruker AM 200 instrument, with a tunable 10 mm multinuclear probe at ambient temperature**. ^{1}H spectra were run with 3 μs pulses and a 1.638 s acquisition time. Shifts are given in relation to external TMS. ^{31}P spectra were carried out using 15 μs pulses and an acquisition time of 0.655 s. Chemical shifts were compared to 85% H_3PO_4 used as an external reference. ^{195}Pt spectra were recorded using 10 μs pulses and 0.131 s acquisition time. All chemical shifts were measured from $(PtCl_6)^{-2}$ as external standard. ^{13}C spectra were conducted with 8 μs pulses and 0.41 s acquisition time. Chemical shifts were related to TMS used as an external reference.

The pH measurements were performed with a Beckman Selection 5000 pH meter. Values were not corrected for D_2O isotopic effect.

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Results and Discussion

CDDP Hydrolysis

CDDP is hydrolysed in aqueous solution (Fig. 1). Using the values of constants published by Leroy *et al.* [11] we calculated the concentrations of the various complexes at different pH values without NaCl (Fig. 2) and with 0.1 M NaCl (Fig. 3) (high chloride concentrations are known to decrease hydrolysis [12]).

[*cis*-$Pt(NH_3)_2(H_2O)_2]^{++}$ and [*cis*-$Pt(NH_3)_2(H_2O)$-$(OH)]^+$ concentrations were found to be low and may be neglected with and without NaCl. In the acid pH range without NaCl, the formation of a high concentration of (*cis*-$Pt(NH_3)_2(H_2O)Cl)^+$ results from hydrolysis. 0.1 M NaCl concentration prohibits hydrolysis, but only in the acid and neutral pH range, as can be deduced by comparing Figs. 2 and 3. In the basic pH range, Figs. 2 and 3 show similar concentrations of *cis*-$Pt(NH_3)_2(OH)Cl$ and *cis*-$Pt(NH_3)_2$-$(OH)_2$ and show parallel variations in these concentrations.

The platinum spectra recorded with acid pH demonstrate that hydrolysis, considerable without salt (Fig. 4), is negligible in 0.1 M NaCl solutions. However, the CDDP and [*cis*-$Pt(NH_3)_2(H_2O)Cl]^+$ concentration ratio, deduced from the spectrum in Fig. 4, is not in good agreement with the theoretical ratio value. One reason may be the use of kinetic constants measured at a temperature of 25 °C whereas the probe temperature is higher because of the proton broad band decoupling; another reason may be the time delay before spectra acquisition. These curves (Figs. 2 and 3) nevertheless provide qualitative information about the concentrations of hydrolysis products.

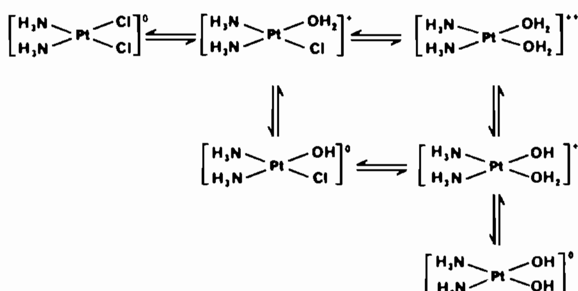


Fig. 1. Aquation reactions of CDDP (From ref. 11).

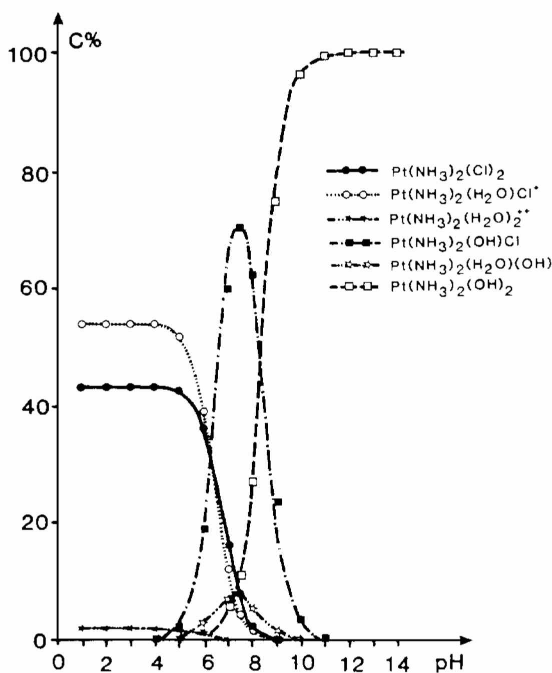


Fig. 2. Computed species distribution in aqueous solution.

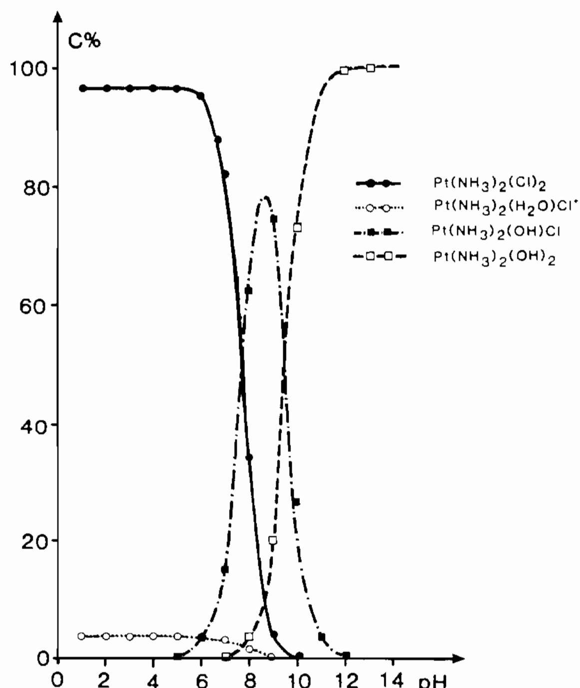


Fig. 3. Computed species distribution in aqueous solution with 0.1 M NaCl.

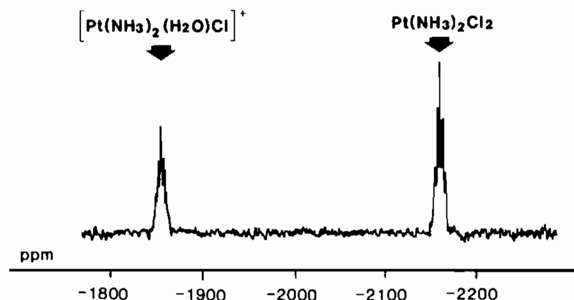


Fig. 4. ^{195}Pt NMR spectrum of CDDP aqueous solution at pH: 4.

AMP–CDDP Interaction without NaCl

Acid pH range (pH: 3.8)

The proton spectra began to change 3 h after CDDP was mixed with AMP. Two new singlets were then distinguishable at 8.84 and 8.20 ppm. The area of these peaks corresponds to 5% of the total AMP after 6 h and only 14% after 24 h (Fig. 5). The downfield singlet was attributed to proton H8 and the upfield singlet to proton H2 of the AMP–platine complex (complex **I**). Phosphorus spectra monitored during the same time-periods showed no change (Fig. 6).

Neutral pH range (pH: 7.9)

The proton spectrum changed more rapidly. After 1 h of reaction time, two singlets at 9.0 and 8.11 ppm indicated the formation of the first complex (complex **I**). Then after 3 h, two pairs of singlets appeared simultaneously at 9.45 and 7.95 ppm (complex **II**) and 9.30 and 7.93 ppm (complex **III**). After 24 h, the complex **I** concentration was only 5% of the total AMP, the complex **II** concentration predominated (44%) and the complex **III** concentration was about 27%. AMP was not totally complexed; the free AMP concentration measured on the spectrum was about 24% (Fig. 5).

The phosphorus spectrum obtained after a 5 h contact time shows that the signal at 4.28 ppm, due to free phosphate moiety, presents a shoulder at 4.47 ppm. The spectra obtained later show an increase in the peak at 4.47 ppm and a decrease in the peak at 4.28 ppm. However, due to their line widths, these signals could be well-resolved only by drastic apodization prohibiting integration (Fig. 6).

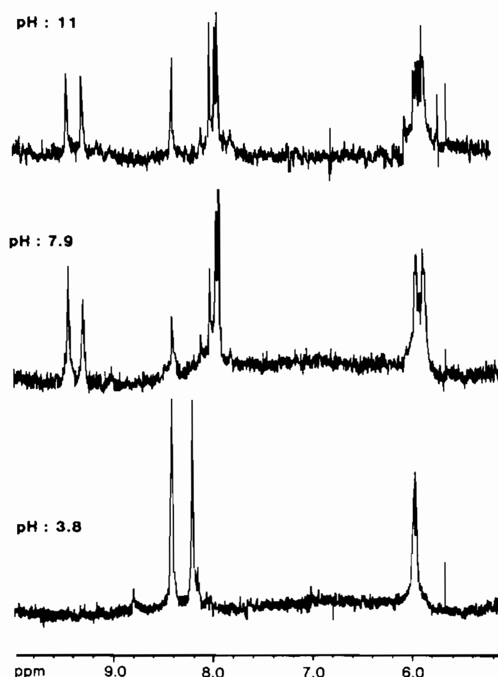


Fig. 5. ^1H NMR spectra of the reaction mixture of hydrolyzed CDDP and AMP after 24 h.

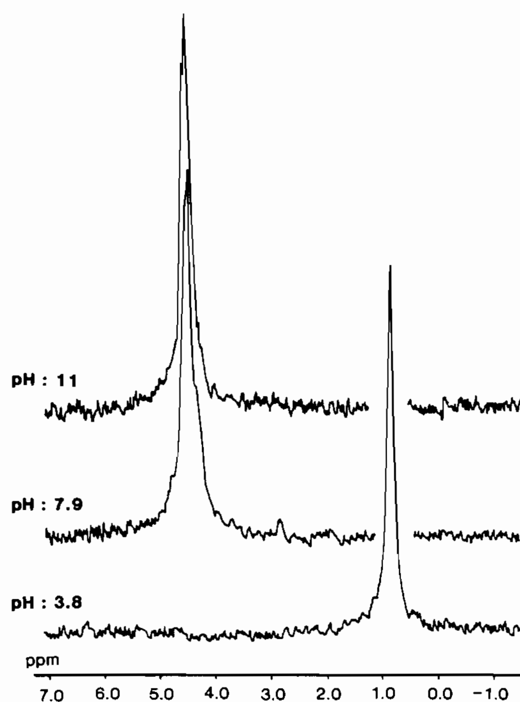


Fig. 6. ^{31}P NMR spectra of the reaction mixture of hydrolyzed CDDP and AMP after 24 h.

Basic pH range (pH: 11)

On the proton spectrum obtained 2 h after mixing, integration of the two singlets at 9.0 and 8.11 ppm permitted the calculation of the complex **I** concentration, found to be equal to 10%. One day later, this concentration was the same, whereas complex **II** (singlets at 9.45 and 7.94 ppm) and complex **III** concentration amounted to about 27% and 24% of total AMP, respectively (Fig. 5). At the same time the phosphorus spectrum showed two singlets at 4.53 and 4.59 ppm, both with the same integrated intensity (Fig. 6).

Ribose moiety

Figure 7 shows the evolution, as a function of time, of the ribose proton spectrum obtained in the neutral pH region. For 6 h the general appearance of the spectrum showed no change in spite of small chemical shifts and line width variations. The concentrations of AMP–CDDP complexes were then relatively weak, but considerable binding of platinum to phosphate took place. However, the spectrum monitored after 24 h contact time was greatly modified and showed four wide peaks corresponding to H5′ and H5″.

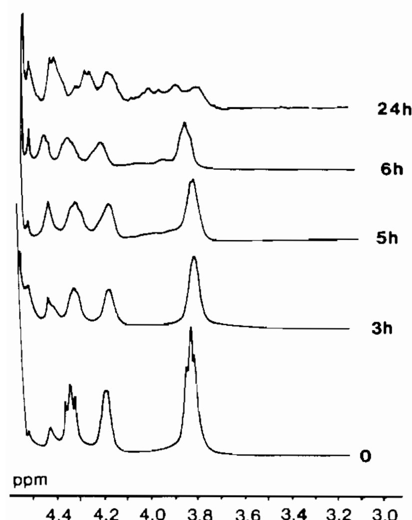


Fig. 7. Progress of the reaction of AMP with hydrolyzed CDDP in the neutral pH range monitored by ^1H NMR. Only the ribose portion of the spectrum is shown.

Discussion

At the acidic pH used, near the pK of N1, half of the AMP molecules bear a positive charge spread over the heterocycle. At the same pH, the cisplatinum to monoaquomonochloroplatinum concentration ratio was about 1:1. The phosphorus spectra show that the phosphate group does not react with platinum, whereas adenosine ring reactivity was weak, as was observed with proton spectra. The proton chemical shift variation was higher with proton H8 (+0.39 ppm) than with proton H2 (−0.05 ppm), and so we conclude in agreement with other authors [13–16] that nitrogen N7 was involved in the binding.

The binding of CDDP and/or monoaquomonochloroplatinum may lead to the formation of *cis*-$Pt(NH_3)_2$(AMP-N7)Cl or [*cis*-$Pt(NH_3)_2$(AMP-N7)$H_2O]^+$ species. Formation of $Pt(NH_3)_2$ClN7 is in agreement with the findings of Dijt [13] who reported that formation of this complex during the reaction of CDDP with 5′-GMP gives rise, in the proton NMR spectrum, to a peak shift of 0.43 ppm, a value which is very close to ours (0.39 ppm). The weak reactivity observed may be explained by the electrostatic repulsion between the monoaquomonochloroplatinum and the positively charged adenosine ring and by the fact that Cl is a poor leaving ligand compared to H_2O ligand [17–18].

Under the neutral pH conditions, four platinum species coexisted in solution, the monohydroxomonochloroplatinum complex being predominant. In the basic pH range the platinum complex was found to be mainly the dihydroxoplatinum complex. In these two pH ranges, the proton NMR spectra show the same chemical shift variation (0.6 ppm) for the proton H8 of complex **I**. It can be concluded that, in these two pH ranges, complex **I** is *cis*-$Pt(NH_3)_2$(AMP-N7)OH, which is consistent with the quicker increase of concentration of complex **I** in the basic pH range.

It should be noted that this complex concentration reached a maximum value and then decreased in favour of complexes **II** and **III**, as was observed with the ^1H NMR spectra under neutral and basic conditions.

The high downfield variations revealed by the signal H8 of complexes **II** and **III** (chemical shifts are 9.45 ppm and 9.30 ppm, respectively) reflect a considerable modification in the adenosine ring and a high polarisation of the C—H8 bond.

Binding to N1 or N1 and N7 leads to a downfield shift in the case of proton H2 [15], though this phenomenon was not observed here. Binding to N1 is thus inconsistent with our experimental results. According to Dijt and co-workers [13], formation of the bidentate complex *cis*-$Pt(NH_3)_2(AMP\text{-}N7)_2$ might introduce only small variations in the chemical shifts in comparison with complex **I**. In fact, an (N7—Pt—N7) angle approaching 90° would preclude the possibility of the two rings being parallel and thus weakens any ring current effect.

However, an identical complex has been described by Kistenmacher [20] using XRD, for inosine-5′-monophosphate. Peak shifts of about 1 ppm observed in the NMR spectrum during the reaction of platinum with AMP have been attributed to this complex [21].

Mansy [22] has suggested a binding of CDDP to both N7 and the 6-NH_2 group [22]; this is in agreement with the theoretical calculations of Fukui *et al.* [23], which show the stability of this complex.

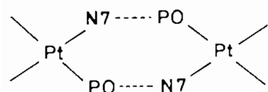
Moreover, a substitution by four nitrogen atoms is in agreement with the symbiotic effect described by Jørgensen [24], who reports that a complex with four inert ligands is more stable than a complex bearing two inert ligands (NH_3) and two leaving ligands (Cl). A platinum complex with four nitrogen ligands has been observed by Ismail [16] on a platinum NMR spectrum of CDDP fixation on AMP. But this latter author does not specify which nitrogen atoms are bound to platinum.

In the acid pH range, the phosphate group was only partially ionized and the interaction between the platinum species and the phosphate moiety did not occur as it does on the phosphorus NMR spectra. In the neutral and basic pH regions, phosphorus spectra showed that the interaction occurs after the binding of platinum species to the adenosine ring. Thus it seems that nitrogen N7 is the favoured fixation site and that the rate of binding is enhanced at neutral pH when monochloromonohydroxyplatinum is the main species.

The chemical shift of phosphate bound to platinum is about 0.2 ppm downfield relative to the

free phosphate (neutral pH), a value smaller than that observed by Bose using triphosphate [9]. An explanation might be the formation of the complex *cis*-$Pt(NH_3)_2$(AMP-PO)OH.

Since AMP presents two binding sites, first, the nitrogen atom N7 and second, the phosphate moiety, the following complex may be formed:



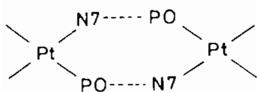
Similar complexes have been found in the case of different metal–AMP interactions [25]. On the other hand, a ^{15}N NMR spectrum was reported by Ismail [16] as showing a peak corresponding to a platinum bearing a nitrogen and an oxygen ligand. This signal appeared in the case of AMP–CDDP but not in that of inosine. The oxygen atoms bound with platinum might thus belong to the phosphate moiety.

Both the lack of complex **II** and complex **III** proton singlets and the lack of platinum phopshate complex signal on the NMR spectra monitored at acid pH are consistent with the possibility that either or both of the complexes **I** and **II** involve a Pt–O binding.

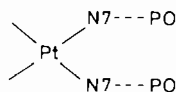
Binding effect on the ribose ring

We noted that during 6 h contact time proton ribose ring spectra showed no significant modification although binding on the phosphate moiety was high. This binding does not prohibit the rotation around C4′–C5′ and C5′–O5′ bonds, as can be seen from the fact that a single H5′ and H5″ signal was recorded [26].

Polissiou [14], studying $PtCl_4$ and guanosine-5′-monophosphate, has explained chemical shift variation by conformational changes and the increase in the ^3E conformer percentage. One of the most markedly shifted signals was the proton H3′ signal, a finding which has already been observed in the case of ATP–metal complexes [27] and which has been explained at the theoretical level by Prado [28]. The large number of proton 5′ and 5″ signals observed in the spectra monitored after 24 h of mixing is related to the increase of complex **II** and **III** concentrations. At the present time, we are not able to assign these peaks to any specific complex. However, the multiplicity and the line widths are consistent with the possibility that H5′ and H5″ may reflect a considerable interaction. This might have involved the following complexes:



or more probably:



because of steric hindrance.

Information concerning the conformation of the ribose ring can be obtained from the coupling constant $J_{H1'-H2'}$. With a coupling constant of 6 Hz determined for AMP, the Karplus relation [29] gives a mean result of 140° for the dihedral angle H1′–C1′–C2′–H2′. The same value was obtained by XRD study [30]. It thus emerges that the conformer ^2E is favoured. The coupling constants for platinum complexes are as follows:

$J_{H1'-H2'}$*cis*-$Pt(NH_3)_2$(AMP-N7)OH = 3.94 Hz

$J_{H1'-H2'}$ (complex **II** or **III**) = 3.73 Hz

$J_{H1'-H2'}$ (complex **III** or **II**) = 3.80 Hz

The dihedral angle H1′–C1′–C2′–H2′ with a coupling constant of 4 Hz works out at 130°. This decrease indicates that platinum binding produced a $^2E \rightarrow {}^3E$ ribose ring conformational change. A similar result has been described by Polissiou [14] with platinum–guanosine-5′-monophosphate interaction.

ATP–CDDP Interaction without NaCl

Acid pH region (pH: 2.8)

After a 2 h contact time the effects of platinum binding on the ^1H NMR spectrum are illustrated by the presence or the three complexes: complex **I** = 17%, complex **II** = 10%, complex **III** = 17%. Analysis of phosphorus-31 NMR shows one doublet for Pα and one for Pβ and a broad triplet for Pγ. After 24 h, the platinum causes broadening of all ^{31}P NMR signals. Binding of platinum to Pγ is assumed to have taken place by apodization (Fig. 9). The ^1H NMR spectra show the increase in the concentrations of complexes **I**, **II** and **III** (Fig. 8).

Neutral pH range (pH: 7.4)

After 3 h of contact time, only one species was formed during the course of the reaction. Integration of the complex **I** signal gives 17%. After 5 h the ^{31}P NMR spectrum contained two doublets for Pγ and two triplets for Pβ, while Pα was unchanged. After 24 h, we observed an increase in the complex **I** concentration. Complex **II** and complex **III** concentrations each amounted to about 20% (Fig. 8). The phosphorus-31 NMR spectrum contained a new doublet at −1.93 ppm. The corresponding triplet centered at −21.64 ppm was attributed to a bidentate complex [5] (Fig. 9): *cis*-$Pt(NH_3)_2$(ATP-PαO), (ATP–PγO) or Pt(PαO, PγO).

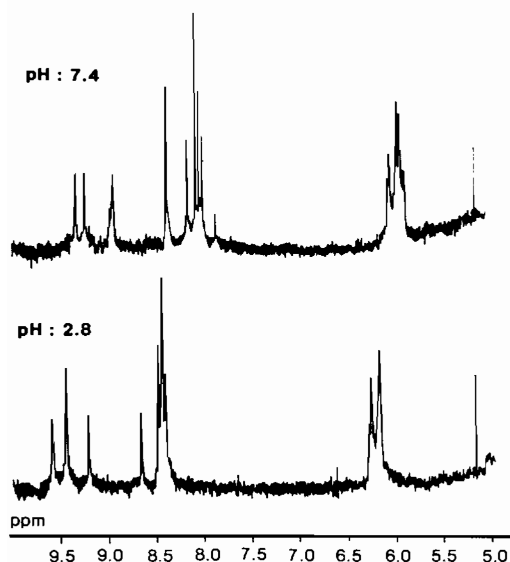


Fig. 8. ^1H NMR spectra of the reaction mixture of hydrolyzed CDDP and ATP after 24 h in aqueous solution without NaCl.

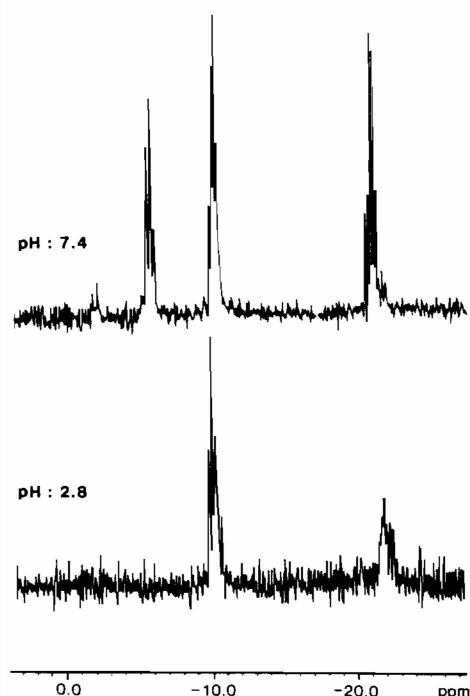


Fig. 9. ^{31}P NMR spectra of the reaction mixture of hydrolyzed CDDP and ATP after 24 h in aqueous solution without NaCl.

Basic pH region (pH: 10)

After 8 h ^1H NMR results indicate a weak interaction between platinum and ATP. After 2.5 h, the Pγ complex reached its highest percentage, 40%. One day later Pt(PαO, PγO) and Pt(PβO, PγO) the corresponding quadruplet was centered at ≃ −11 ppm) were present in solution.

^{13}C NMR properties

A solution of platinum and ATP was maintained at 80 °C for 30 min at pH 7 and then ^{13}C NMR spectrum recorded (Fig. 10). The chemical shifts of carbon resonances of ATP were based on the assignments of Dorman [31] and Mantsch [32]. It can be seen from the ^{13}C NMR spectrum that free ATP and bound ATP coexist, as was evidenced by the proton spectrum.

For carbons C2, C8, C1′ and C2′ additional peaks, downfield shifted, are observed. In the case of C3′ and C5′ the new signals shifted upfield. There was no change in C4′. C1′ and C8 are the atoms which were the most disturbed by the platinum binding.

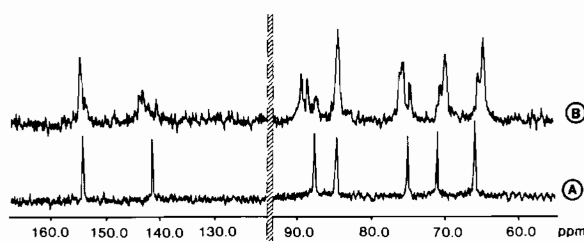


Fig. 10. ^{13}C NMR spectra of ATP in (A) and ATP with hydrolyzed CDDP after heating at 80 °C during 30 min in (B).

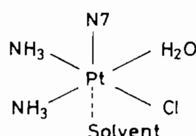
Discussion: phosphorus and proton NMR spectra

In the acidic pH range, the most noteworthy phenomenon is the different reactivity of the ATP and the AMP imidazole rings. Whereas AMP has weak reactivity and consequently a low percentage of *cis*-$Pt(NH_3)$(AMP-N7)Cl, ATP has high reactivity since complexes **I**, **II** and **III** appeared after 2 h of contact time. Moreover, and only for this pH, a larger line width was observed for Pβ whereas Pα and Pγ were unchanged. The H8 chemical shift variation in complex **I** was 0.54 ppm. This value is superior to that of the *cis*-$Pt(NH_3)_2$(AMP-N7)Cl complex at acid pH, and it is nearly the same as that observed for the *cis*-$Pt(NH)_3$(AMP-N7)OH complex in the neutral and basic pH ranges. Hence, we can conclude that complex **I** of ATP has the following structure: [*cis*-$Pt(NH_3)_2$(ATP-N7)$H_2O]^+$.

Several hypotheses can be advanced as to the rapid formation of complex **I**:

(i) Since non-hydrolyzed CDDP is not influenced by electrostatic repulsion, the reaction may begin by the formation of the *cis*-$Pt(NH_3)_2$(ATP-N7)Cl complex. This complex would then be rapidly hydrolyzed into [*cis*-$Pt(NH_3)_2$(ATP-N7)$H_2O]^+$, not in agreement with the low reactivity of AMP with respect to the CDDP.

(ii) The phosphate chain, larger than that of AMP, is negatively charged and is located on the imidazole ring [30]. The approach of the monochloromonoaquoplatinum complex would thereby be facilitated. Thus, on one hand, the formation of the following activated complex would be favoured:



and, on the other hand, the Pt–H_2O bond would be stabilized, leading to Cl ligand substitution. The larger line width of phosphate β would indicate its influence on the formation of both the activated complex and the [*cis*-Pt$(NH_3)_2$(ATP-N7)H_2O]$^+$ complex.

These observations are confirmed by the weak reactivity of AMP. Because of the conformation of AMP and the position of the phosphate group, the N7 atom would probably not be subject to the same interactions in the activated complex. In the neutral and basic pH ranges, there is competition between the heterocycle and Pγ. In the neutral pH range, the phosphate Pγ negative charge is higher than in the acid pH range, which then makes this group more reactive. This explains why complex Pt(PγO) is formed before [*cis*-Pt$(NH_3)_2$(ATP-N7)(OH)]$^+$ complex.

In the basic pH range, the greater reactivity of the phosphate chain explains why the bidentate complexes Pt(PαO, PγO) and Pt(PβO, PγO) are seen earlier.

Discussion: ^{13}C spectrum

The small variations in the chemical shifts of the carbons in the ribose ring and the lack of change in the carbon 4′ signal both show that this ring plays no part in platinum binding. The upfield and downfield shifts are due to modifications in the ribose ring conformation, as has been demonstrated previously with AMP. It is interesting to note that several peaks appeared for carbons 1′ and 8 whereas only one appeared for C2. The downfield shift values are 3.3, 2.7 and 1.5 ppm for C8, 1.1 ppm for C2, and 2.0 and 0.9 ppm for C1′. If fixation on a nitrogen atom is taken as disturbing the electronic density of all the atoms in the imidazole ring thereby leading to variations in the chemical shifts of these atoms, the complexation in N7, N1, N1 and N7, should give three peaks for both carbon 2 and carbon 8. Yet, these three peaks were not observed in the case of C2, which would confirm the absence of platinum binding in N1. However, the three peaks obtained for C8 show that, in each of the three complexes, the N7 atom binds with the platinum.

ATP–CDDP Interaction with NaCl

Acid pH (2.8)

Because of the slow reaction rate of platinum binding, the two singlets belonging to complex **I** appear only after 6 h of contact time. Phosphorus spectra show no new peaks.

After several days, the concentrations of complex **I**, **II** and **III**, calculated by integration, were 42%, 16% and 28%, respectively, and the concentration of free ATP was 14% (Fig. 11). The phosphorus spectrum exhibits line broadening, and apodization of this spectrum reveals a pair of doublets for Pγ, a single doublet for Pα, and two triplets for Pβ (Fig. 12). These results demonstrate the formation of the Pt(PγO) complex.

Neutral pH (7.2)

The rates of complex formation are similar to those observed at acid pH. The N7 complex on the proton spectrum appears only after 6 h of contact time and the phosphorus spectrum is unchanged.

After several days, the proton NMR spectrum shows the formation of complexes **II** and **III** whereas the peaks corresponding to both free ATP and complex **I** had broader line widths (Fig. 11). The phosphorus spectrum done concurrently contained three wide signals (Fig. 12).

Basic pH (10)

After 6 h of contact, the concentration of complex **I** equaled 18% of the total ATP, as can be seen in the proton spectra. After 1 h of contact time, the phosphorus spectra indicate that the concentra-

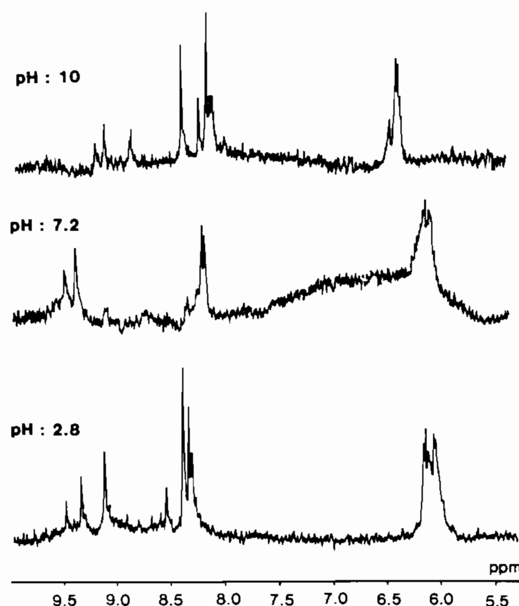


Fig. 11. ^{1}H NMR spectra of the reaction mixture of hydrolyzed CDDP and ATP in aqueous solution with NaCl 0.1 M.

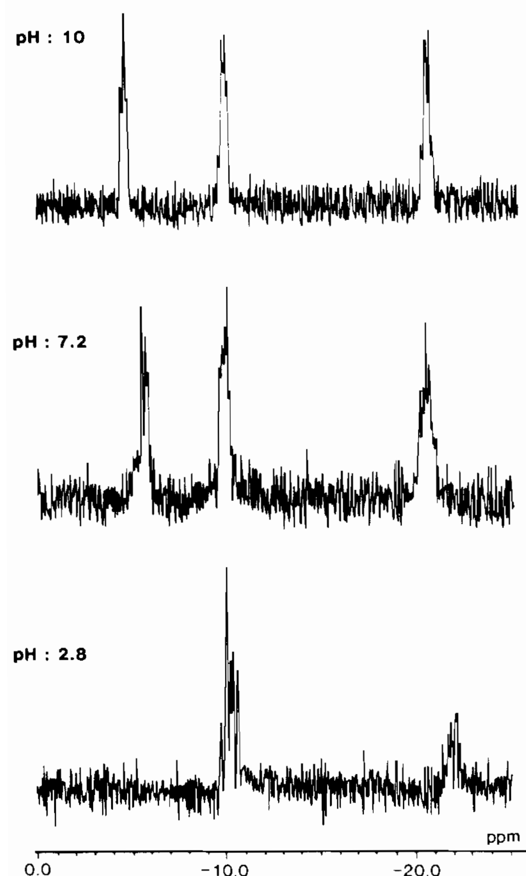


Fig. 12. ^{31}P NMR spectra of the reaction mixture of hydrolyzed CDDP and ATP in aqueous solution with NaCl 0.1 M.

tion of the Pt(PγO) complex was 40%. After 24 h, complexes **I**, **II** and **III** have concentrations of 22, 12 and 18% of total ATP, respectively (Fig. 11). The concentration of complex Pt(PγO) shows only a slight increase (50%). Bidentate complexes were not detected (Fig. 12).

At basic pH, the different species present have longer lifetimes; therefore the phosphorus spectra have become finer. The fixation rates on the imidzole ring and on the Pγ are faster than those at acid and neutral pH, and these rates are similar to those observed for salt-free solutions, as would be expected from solutions containing high percentages of dihydroxoplatinum species.

Discussion: ^{31}P and ^{1}H NMR spectra (with NaCl)

A high NaCl concentration decreases the hydrolysis of cisplatin. This leads to a high concentration of dichloroplatinum complex at acid pH and a lower concentration at neutral pH, whereas at a very basic pH NaCl has no effect on the concentration of $Pt(NH_3)_2(OH)_2$. However, a concentration high in sodium ions favours interactions between, on one hand, salt and the phosphate moiety and, on the other hand, interactions between salt and the N7 atom, as was shown by crystallography [30]. The low concentration of hydrolyzed complex and the above mentioned interactions with sodium might explain the weak reactivity of ATP in the acid and neutral pH range. Indeed, in both ranges, the formation of complex **I** by platinum binding to N7 is apparent only after about 6 h of contact time. At acid pH, the difference between the complex **I** H8 signal and the ATP H8 signal is 0.58 ppm, which is nearly the same value as that obtained from a salt-free solution with a similar pH. As concerns this salt-free solution, two hypotheses are advanced to explain the formation of the $[cis\text{-}Pt(NH)_3(ATP\text{-}N7)H_2O]^+$ complex: firstly, cisplatin binding followed by hydrolysis of the formed complex or, secondly, binding of the monoaquomonochloroplatinum complex followed by exchange of a Cl ion.

Under the first hypothesis, a high concentration of non-hydrolyzed cisplatin should provoke a faster binding to N7. However, a slow reaction rate is observed, demonstrating that N7 binding, at acid pH, would be due to the monoaquomonochloroplatinum compound. The competition between platinum and sodium, during their interaction on the phosphate chain, leads to line broadening on the phosphorus spectrum, corresponding to the mean value, in the frequency range used, of the lifetimes of the two forms in equilibrium $PO_4{-}Na \rightleftharpoons PO_4{-}Pt$.

Discussion

A ^{195}Pt NMR spectrum (Fig. 13) monitored at pH 2.5 after 4 days of mixing shows no peak corresponding to the monoaquomonochloroplatinum complex at about −1830 ppm, confirming its intervention in ATP complexation. Analysis of the chemical shift shows no peaks corresponding to platinum binding to the phosphate group [34], proving that the binding rate on the phosphate moiety is very slow at this pH and demonstrating that the favoured

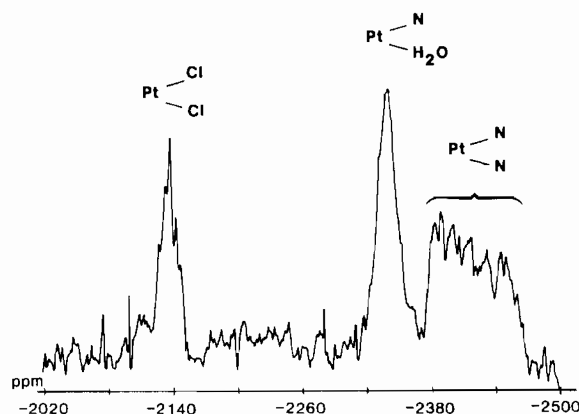


Fig. 13. ^{195}Pt NMR spectra of the ATP with CDDP in the presence of 0.1 M NaCl, 4 days after mixing (pH 2.5).

binding site is N7. According to Ismail [16] the peak at −2340 ppm can be attributed to [*cis*-Pt$(NH_3)_2$-(ATP-N7)$H_2O]^+$ complex, once again confirming the formation of this complex in the experimental conditions used. This same author, studying platinum binding to AMP, observed a singlet at −2420 ppm whereas our spectrum shows a broad signal ranging from −2390 to −2450 ppm. This region is characteristic of a platinum coordinated with four nitrogen atoms [15]. These findings corroborate the possibility, mentioned earlier, of *cis*-Pt(NH_3)(ATP-N7)$_2$ being formed. Marcelis *et al.* [35] have shown that, for molecules smaller than ATP, the rotation around the Pt–N7 bond is rapid. However, due to the steric hindrance provoked by the two ATP molecules and to the interaction between the phosphate chain and the sodium ions, the rotation rate around the Pt–N7 bond decreases, thereby broadening the line.

Conclusion

cis-Dichlorodiammineplatinum has a varying reactivity and binding rate depending upon the choice of nucleotide and upon the experimental conditions used, notably the pH and the presence or absence of NaCl. This may explain certain disagreements observed in the literature. Due to the presence of one or more phosphate groups, AMP and ATP do not have the same binding sites as adenosine. These groups can bind with the cisplatin or its hydrolyzed species; in fact, at basic pH, this binding is preferential to that of the N7 in the imidazole ring. Because of the possible fixation to the phosphate moiety, neither the AMP nor the ATP, in the concentration ratios used, showed binding to N1, which is not the case for adenosine [15]. The length of the phosphate chain also seems to influence binding to the heterocycle. We have observed that, at acid pH, binding to N7 is faster for ATP than for AMP. This led us to conclude that, through the formation of the activated complex resulting from the reaction between ATP and monohydroxomonochloroplatinum, the ATP phosphate chain plays an important part in the complexation of N7. This further confirms the role of hydrolyzed platinum compounds in the complexation of nucleotides and, in particular, that of DNA.

The NMR spectra show that the reaction of platinum species with AMP and ATP leads to the formation of bidentate complexes, implicating only the N7 atoms in the imidazole ring, *i.e.*, *cis*-Pt(NH_3)(AXP*-N7)$_2$. Indeed, though the formation of a *cis*-Pt-$(NH_3)_2$(AXP-N7)(AXP-N1) complex has been described by other authors [16, 20, 21], because the NMR spectra show no other bond with the imidazole ring and because the phosphate groups have a high reactivity, we have concluded that a *cis*-Pt$(NH_3)_2$-(AXP-N7)(AXP-PO) complex is formed.

In a later study, we will consider the two bidentate complexes which might play an important part in the cisplatin reaction with DNA.

*AXP: X = M for AMP, X = T for ATP.

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