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Cisplatin forms a 1:2 complex with ATP in aqueous solutions between pH 2 and 9

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Abstract

Interaction of ATP with cisplatin in the pH range 1.9–9.5 was studied by NMR. A new complex with the composition $Pt(NH_3)_2(ATP)_2$, which formed very slowly after cisplatin was added to ATP solution, was identified by DOSY and long range $^1H-^{15}N$ HMQC experiments. In the complex, ATP bound Pt at N7, and the N7–Pt bond showed the nature of kinetically inert interaction. At low pH, beside the 2:1 complex of N7 type, a 1:1 complex of N1 type co-existed in the solution but the binding interaction was ionic and the complex exchanged rapidly with free ATP on the NMR time scale.

Keywords: ATP–cisplatin complexes; pH variation; NMR DOSY; Long range $^1H-^{15}N$ HMQC

1. Introduction

The clinically used drug *cis*-$PtCl_2(NH_3)_2$ (cisplatin) is one of the most effective anticancer compounds, which is well known for its interaction with DNA [1–3]. The binding of Pt(II) to N7 of guanine residues in DNA is now generally accepted as the mechanism of its anticancer activity [4,5]. The major DNA adduct with cisplatin involves a N7–Pt–N7 intrastrand cross-link between adjacent guanine residues [6], predominantly assuming the head-to-head conformation, although the head-to-tail conformation has also been identified [7–9]. Sometimes cisplatin selects adenine instead of guanine to form the complex *cis*-$Pt(NH_3)_2d(ApG)$ [10], but the product is in low yield.

ATP is very common in living things. It can provide two possible binding sites, N1 and N7, when interacting with metal ions. In our series of studies of ATP–metal interactions, we found that lower pH favors N1 binding, while higher pH (up to 7) favors N7 binding [11–16]. The metal ions that we studied previously, including Fe^{3+} [11,13], Zn^{2+} [12], Mg^{2+} [14,15] and Cd^{2+} [16], are not capable of forming strong coordination bonds with ATP. In this study, we choose cisplatin as the reactant, which usually binds N7 of guanine through a kinetically inert coordination, to see if and how ATP can interact with Pt(II). The results show that at low pH, Pt binds at N1 but the binding is ionic. However, in the whole pH range (1.9–9.5), Pt forms a complex with ATP through N7 binding. A systematic NMR study indicates that the N7-type ATP–cisplatin complex assumes the composition $Pt(NH_3)_2(ATP)_2$ which, to our best knowledge, is a new compound.

2. Experimental

The sodium salt of ATP was purchased from Boehringer Mannheim, GmbH, Germany. Cisplatin were purchased from Acros Organics, USA. Other chemicals (NaOH and HCl) were of analytic purity from Shanghai Chemical Company, China. They were directly used without further purification. The pH values were determined with a Cole–Parmer pH meter equipped with a Cole–Parmer glass-body combination pH electrode (Chicago). Buffers of pH 4.01, 6.86 and 9.18 were used to calibrate the pH meter.

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ATP solutions were prepared by quantitatively dissolving ATP in H_2O containing 10% D_2O to form solutions with a concentration of 40 mM. The pH values were adjusted using tiny drops of NaOH and HCl. The solutions with different pH values were transferred to NMR tubes with and without cisplatin. For the sample containing cisplatin, the molar ratio of Pt(II)/ATP was 1:2. The samples were protected under N_2 and sealed.

NMR experiments were performed on a Bruker ARX-500 or a Varian INOVA-600 spectrometer at 298.2 K. Water signal serves as the reference (4.8 ppm) of ^{1}H spectra. ^{31}P NMR chemical shifts were referenced to 85% phosphoric acid (0 ppm).

^{15}N chemical shifts were measured at 50.68 MHz via ^{1}H–^{15}N HMBC experiments using the four-pulse HMQC sequence [17]. The data were acquired with 32 scans for pure ATP and 100 scans for cisplatin–ATP and with 150 t_1 increments. The digital resolution in the F1 dimension was 1 ppm. The evolution time (1/2J) was 35.7 ms, corresponding to $J = 14$ Hz. The interpulse delay was 2 s, during which the water resonance was suppressed by low-power irradiation. Formamide $HCONH_2$ (−258 ppm) served as a reference for ^{15}N chemical shifts, corresponding to 0 ppm for nitromethane.

The diffusion coefficients of the protons in ATP were detected by BPP-LED pulse sequence [18]. The eddy-current delay was 40 ms. The diffusion delay (300 ms) and the gradient pulse length (1 ms) were kept constant throughout the experiments, while the gradient strength g was varied in the range between 0.05 and 0.35 T m^{-1} with 16 random values.

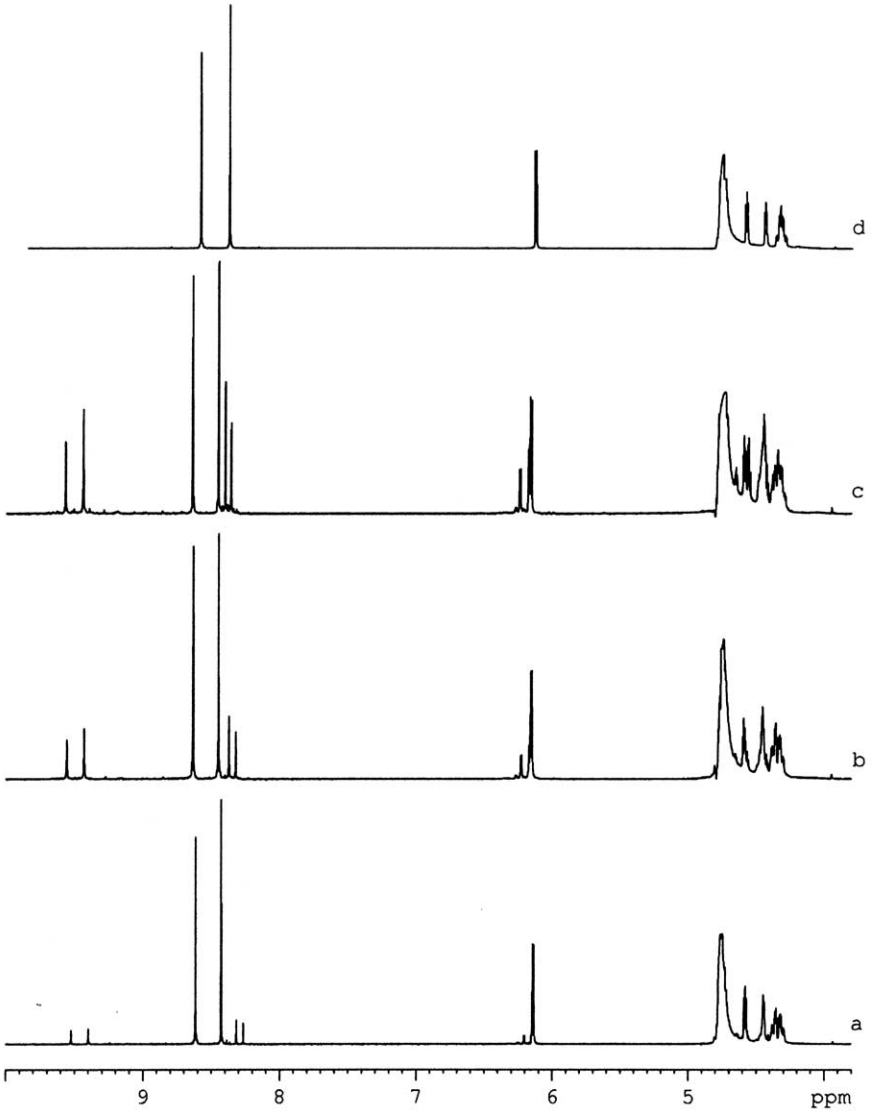

Fig. 1. ^{1}H NMR spectra of ATP solution with equimolar cisplatin at pH 3.73, 25 °C, stored in refrigerator for (a) 0 days, (b) 40 days, (c) 80 days; (d) is the ^{1}H NMR spectrum of pure ATP at pH 3.73.

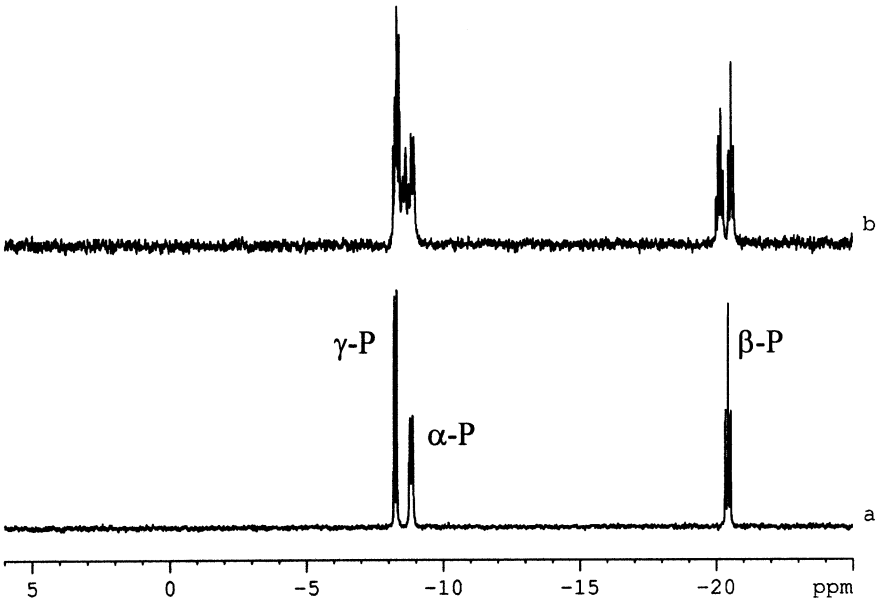

Fig. 2. ^{31}P NMR spectrum of ATP solution with equimolar cisplatin at pH 3.73, 25 °C. (a) ATP, (b) cisplatin–ATP.

3. Results and discussion

When the samples of ATP containing cisplatin were prepared, cisplatin was only partly dissolved, and a residue remained at the bottom of the NMR tube. The ATP NMR signals in the presence of cisplatin were shifted slightly downfield compared with the spectrum of pure ATP solution at the same pH. In addition, two sets of new peaks appeared, though rather weak. These weak peaks grew with time. After about 3 months, the solid residue disappeared, and the extra peaks reached their maximum. The ^{1}H NMR spectra of the solution of

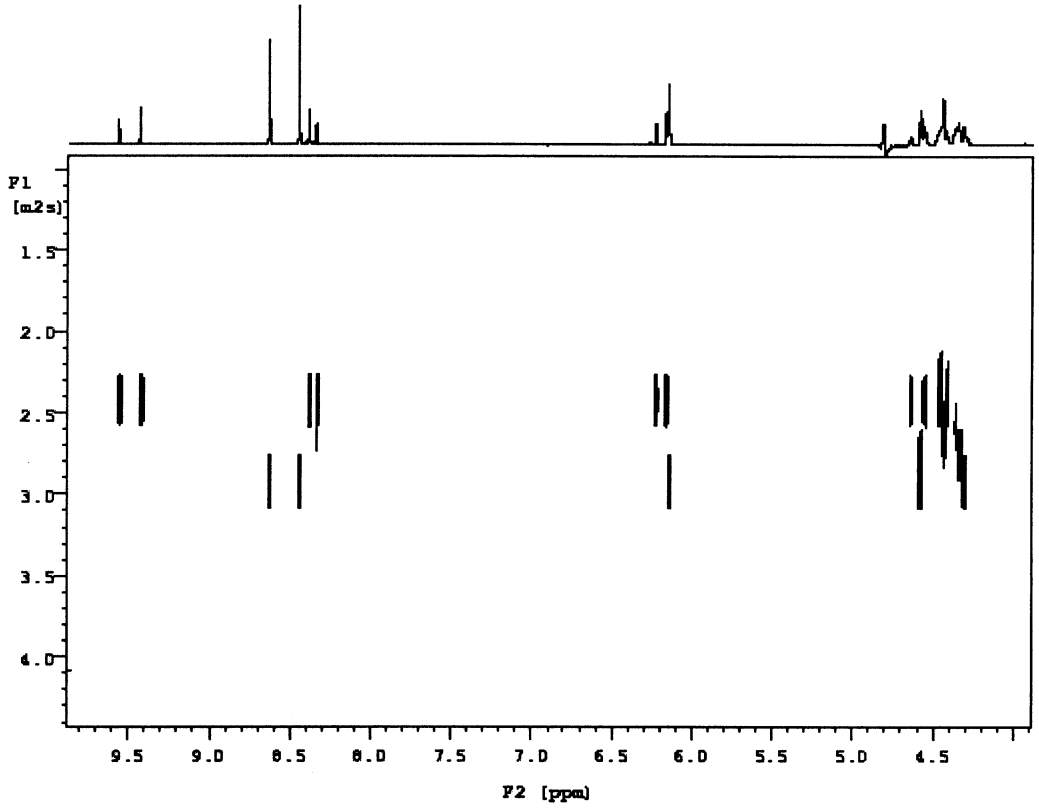

Fig. 3. Diffusion experiment of ATP solution with equimolar cisplatin at pH 3.73, 25 °C.

the ATP–cisplatin mixture at pH 3.73 are shown in Fig. 1, where (a) was recorded when the sample was freshly prepared, while (b) and (c) were recorded, respectively, 40 and 80 days later. For comparison, in Fig. 1(d) is shown the spectrum of a pure ATP solution. Integration indicated that the two sets of extra peaks had equal intensities and should be assigned to some new species in the solution. The ^{31}P spectrum (Fig. 2) does not suggest any decomposition of ATP, since there is no inorganic phosphate peak that, if present, should appear around 2–5 ppm, and the ^{31}P spectrum is in agreement with the ^{1}H spectra that a new substance or new substances must have been formed in the solution.

To find out what happened in the solution, we performed DOSY and two bond ^{1}H–^{15}N HMBC experiment. The DOSY spectrum of the mixture at pH 3.73 is shown in Fig. 3, where all the new peaks are associated with a single diffusion coefficient, which is 30% smaller than that of the strong peaks. This result suggests that these new peaks come from a single stable compound that is larger in size than the species that gave the strong ^{1}H peaks.

In the HMBC spectrum (Fig. 4, left side) of the same solution the natural abundant ^{15}N signals are clearly seen. For convenience the HMBC spectrum of the ATP solution in the absence of cisplatin at the same pH is displayed on the right side of Fig. 4. The assignment of pure ATP is straightforward based on the early work of Happe and Morales [19]. The assignment of the N1 and N3 peaks in the spectrum of the mixture can also be readily done. However, care should be taken for the assignment for N7 and N9. It can be seen that the two peaks at much higher field (−234 and −235 ppm in the ^{15}N dimension) are stronger than the two peaks at −195 and −194 ppm. In our experiment, the evolution period was 35.7 ms, corresponding to $^{2}J(^{1}\mathrm{H}-^{15}\mathrm{N}) = 14$ Hz which is a compromise among the four coupling constants: $^{2}J(\mathrm{H2/N1}) = 15.8$ Hz; $^{2}J(\mathrm{H2/N3}) = 17.1$ Hz; $^{2}J(\mathrm{H8/N7}) = 11.0$ Hz; $^{2}J(\mathrm{H8/N9}) = 5$ Hz [20]. The evolution period certainly favored the detection of N1,

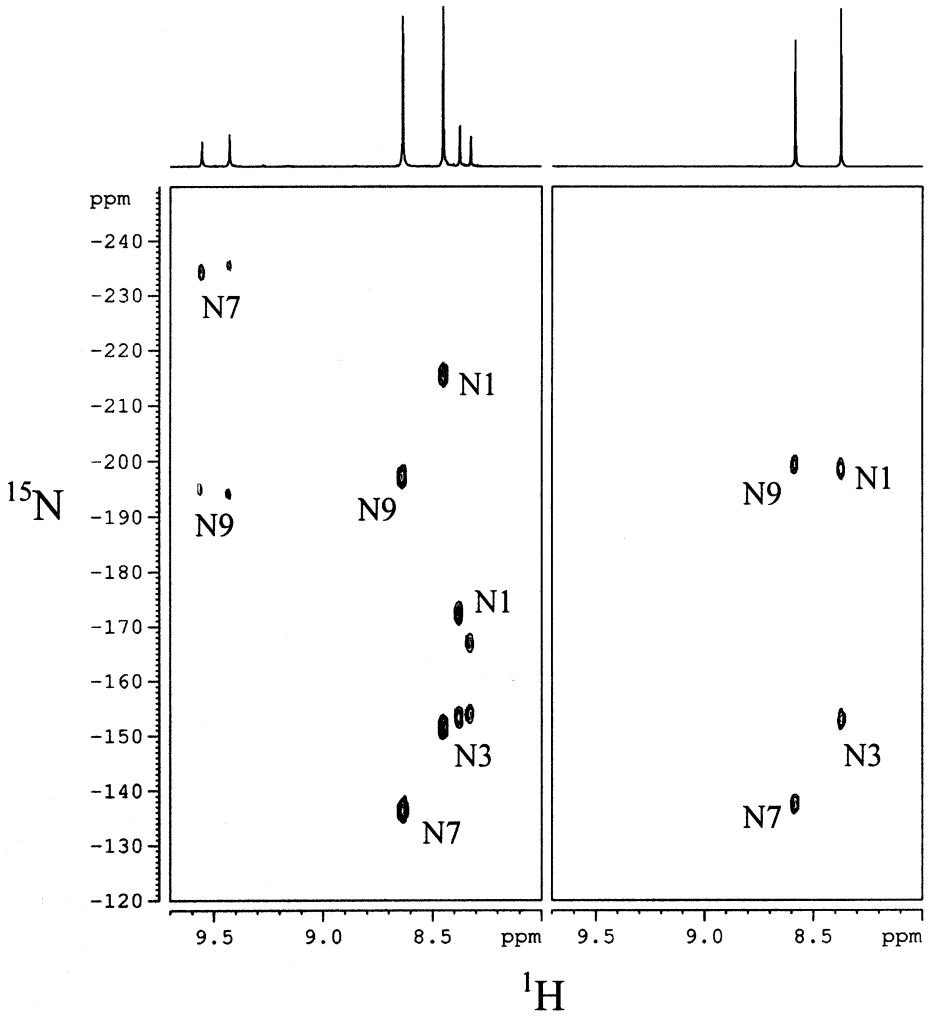

Fig. 4. Regional ^{1}H–^{15}N HMBC spectra of ATP solution with (left) and without (right) equimolar cisplatin at pH 3.73, 25 °C.

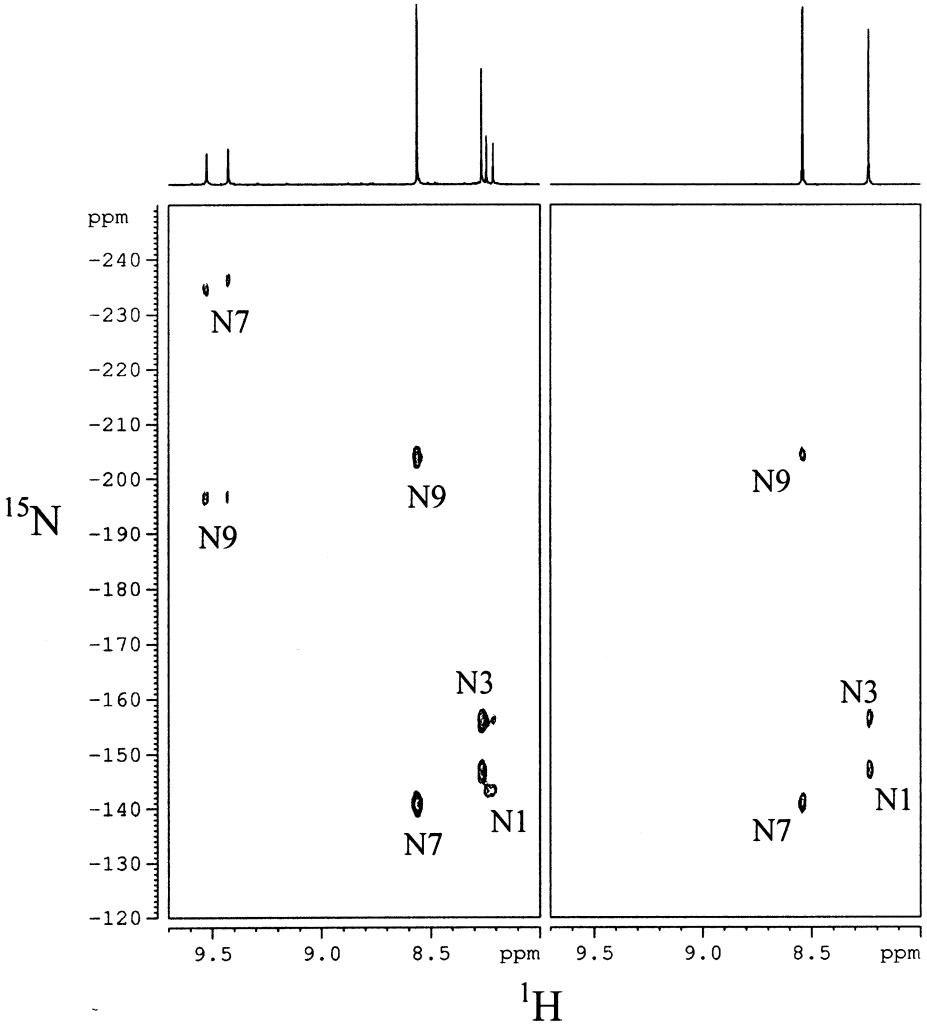

Fig. 5. Regional $^{1}H-^{15}N$ HMBC spectra of ATP solution with (left) and without (right) equimolar cisplatin at pH 7.10, 25 °C.

N3 and N7, but not so good for N9. Therefore, the N9 peak should be weaker than the other peaks. By comparison of the intensities of the four peaks just mentioned, we assign the two peaks at −195 and −194 ppm to N9, and the two peaks at −234 and −235 ppm to N7. Thus we can conclude that the two N7 peaks jumped nearly 100 ppm upfield, compared with N7 in pure ATP, while the N9 chemical shift did not change much upon N7 binding. We can also conclude that, in the solution, a N1 type complex, possibly with a composition of $PtCl(NH_3)_2(ATP)$ or $Pt(OH)(NH_3)_2(ATP)$, was formed, which gave the four strong ^{15}N peaks with N1 shifted upfield by 18 ppm and a relatively bigger diffusion coefficient. In addition, there was a N7 type complex with the composition of $Pt(NH_3)_2(ATP)_2$, to which the eight weak peaks in the HMBC spectrum are assigned with a smaller diffusion coefficient. This conclusion is in good agreement with the DOSY spectrum in which the two diffusion coefficients are related to the two types of complexes.

The N7 type complex was formed not only in acidic solutions. In the whole pH range (1.9–9.1) we studied, we constantly observed signals coming from the N7 type complex. However, the N1 type complex was not found when the pH was high. The HMBC spectra of the ATP and ATP–cisplatin mixed solutions at pH 7.1 is shown in Fig. 5, where only a very small change in the chemical shift of N1 was observed. The small change of the strong N1 peak implies that at higher pH N1 binding did not occur. The small change of the two weak N1 peaks implies that N1 chemical shift is not sensitive to N7 binding to cisplatin. Based on this fact, we can make a more reasonable explanation of the 25–30 ppm downfield shift of N1 in the N7 type complex at pH 3.73 (Fig. 4, left spectrum); this big change should not be necessarily due to the binding interaction. It is more

likely that, upon the dissolution of the cisplatin, the pH was increased, which caused the deprotonation of N1 and a corresponding large downfield N1 shift [12,19].

The ratios between the two diffusion coefficients in the DOSY spectra (see Fig. 3) under six different pH conditions were determined, which turned out to be within 1.28 ± 0.6. These data are very close to each other, but at lower pH values the ratios are smaller. Since Fig. 5 suggests that at high pH the N1 type complex did not exist in the solution, it is reasonable to deduce that at low pH free ATP also co-existed in the solution, which exchanged rapidly with the N1 binding complex on the NMR time scale. However, the free ATP molecules did not exchange with the N7 type complex, as has been evidenced by EXSY experiments (data not shown). There was a small difference in the diffusion data for the set of stronger peaks, which can be rationalized by the fact that the diffusion coefficient of the N1 type complex does not differ too much from that of free ATP.

The very large N7 shifts and the inert property in chemical exchange suggest that the N7–Pt–N7 linkage in the N7 type complex, like that in DNA–cisplatin adducts [1], is a kinetically inert coordination bond in nature. It is interesting to see that the two ATP molecules in the complex $Pt(NH_3)_2(ATP)_2$ were magnetically unequivalent. It is very likely that the complex adopts a head-to-tail conformation, as has been found in cisplatin complex with guanine derivatives [7–9]. Isolation of the complex from the solution is in progress and further structure information is expected in the near future.

4. Conclusion

Interaction of ATP and cisplatin in solutions at different pH has been investigated by NMR. The results showed that cisplatin bound strongly with N7 of ATP to form a complex with the composition $Pt(NH_3)_2(ATP)_2$, causing N7 to shift upfield by 100 ppm, The results also showed that at lower pH, N1 of ATP interacted with cisplatin, but the binding was intrinsically ionic and this N1 type complex exchanged rapidly with free ATP.

Acknowledgements

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