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REACTIONS OF CISPLATIN HYDROLYTES WITH THIOLS. 3: REACTIONS OF *cis*-$[Pt(^{15}NH_3)_2(H_2O)_2]^{2+}$ WITH GLUTATHIONE

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Abstract

The reaction of *cis*-$[Pt(^{15}NH_3)_2(H_2O)_2]^{2+}$ (3) with glutathione (GSH) was investigated in aqueous solution. In this reaction, the ammine in the platinum complex formed was liberated. Surprisingly two chelate rings were observed, six-membered-*S,O*-chelate ring complex *cis*-$[Pt(^{15}NH_3)_2(SG\text{-}S,O)$ (7) and five-membered-*S,N*-chelate ring complex *cis*-$[Pt(^{15}NH_3)_2(SG\text{-}S,N)]$ (8). The bis (thiolate) platinum(II) complex, *cis*-$[Pt(^{15}NH_3)_2(SG)_2]$ (9) was always present in this reaction in any mole ratio used. The dinuclear sulphur-bridged complex (10), giving a broad peak in ^{15}N NMR, was only present in very tiny amounts.

Keywords: ^{15}N NMR, glutathione, cisplatin hydrolytes

1. Introduction

The main emphasis in the study of cisplatin, *cis*-$[PtCl_2(NH_3)_2]$ (1), a metal based-drug, with biological system has centred on interactions with DNA, and it is widely accepted that the antitumour activity of cisplatin and related compounds is due to primarily to interactions with DNA, however, reactions with other molecules in biological fluids are likely to affect the efficiency with which platinum compounds reach that target, as well as being involved in drug toxicity. The most important appear to be those containing sulphur as a potential coordination site. These include cysteine, methionine, s-adenosy-L-homocysteine, glutathione and a variety of proteins [1].

Interactions between platinum and biomolecules containing sulphur have been implicated in the nephrotoxicity of cisplatin [2], antitumour resistance to cisplatin [3-5], cell repair mechanism [6] and in the formation of crosslinks with monofunctional DNA adduct to prevent formation of bifunctional lesions [6-8].

Thiols such as cysteine (H_2cys), N-acetylcysteine (H_3accys) and glutathione (GSH) are among the more reactive biological molecules towards the antitumour drug, cisplatin *cis*-$[PtCl_2(NH_3)_2]$ (1). These reactions play a significant role in the metabolism of cisplatin and its hydrolysis products *cis*-$[PtCl(NH_3)_2(H_2O)]^+$ (2) and *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ (3) [9].

It is established that the DNA and nucleotide reactions are primarily limited by the rate of aquations of cisplatin [9] and that the reactive species from cisplatin is diammineaquachloroplatinum(II)(2) [9,11-15]. Cisplatin can undergo hydrolysis reactions as shown in Figure 1.

It has previously been established [16–18] that thiolate tends to form a bridge between the two metal ions. It is therefore important to investigate the chemistry of the thiolate complexes which is relevant to the behaviour of cisplatin metabolites *in vivo* condition, where concentration of Pt-species is low, and there is no bridging.

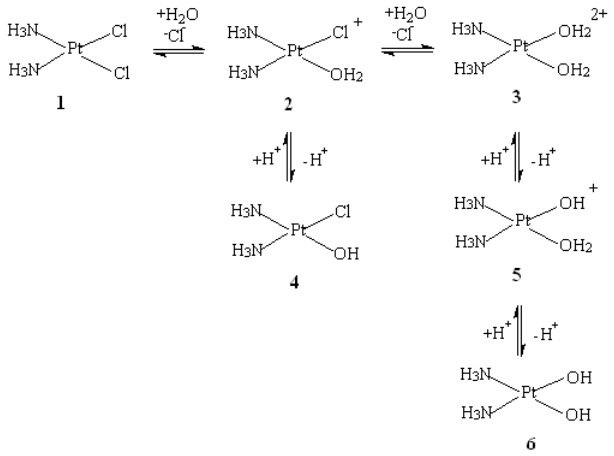

Figure 1. Hydrolysis of cisplatin

The series of reactions were therefore explored in solution with low concentrations of the hydrolytes (1 – 10 mM). ^{15}N NMR method may be used under these conditions if there is 100% ^{15}N in the ammine ligands.

This paper describes the reaction of cisplatin hydrolytes, *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ (3) with glutathione (GSH), a sulphur-containing ligand. The other papers related to this work which discuss the reaction of complexes 2 and 3 with different sulphur-containing ligands has been published [19]

2. Experimental

^{15}N-labelled $(NH_4)_2SO_4$ (> 98% ^{15}N) was obtained from Novachem (Melbourne Australia), and was used to prepare cisplatin which was prepared by the known procedure [20]. Glutathione (GSH) was purchased from Sigma Aldrich and used without further purification.

To a suspension of cisplatin, *cis*-$[PtCl_2(^{15}NH_3)_2]$ in water was added two mol equivalents $AgNO_3$. The mixture was heated at 60 – 70°C for 4 hours, and then left stirring in a flask protected from light with aluminum foil overnight. Silver chloride was removed by gravity filtration to give a solution containing *cis*-$[Pt(^{15}NH_3)_2(H_2O)_2]^{2+}$ (checked with ^{15}N NMR).

The reactions were carried out under Argon gas to minimise the oxidation of the GSH. No buffer was added as it reacts with the starting material used. The solid GSH were added to a small bottle containing a solution of 3 with pre-measured pH. For 1D ^{15}N DEPT NMR purposes a concentration of 10 mM was used. In 2D [^1H, ^{15}N] HSQC NMR a solution of 3 with concentration of 1 mm was used. Within 10 to 15 seconds the mixture reaction was transferred to a 5-mm NMR tube, then placed in the AV400 NMR spectrometer (already tuned for ^{15}N NMR) and accumulation of 40.54 MHz ^{15}N NMR spectra was commenced. For 1D ^{15}N DEPT NMR as reaction proceeded quite fast, one experiment was left it run for about 6-10 hours continuously (in multi experiment mode). The 2D [^1H, ^{15}N] HSQC NMR experiment normally took up to 48 hours.

The 1D 40.54 MHz ^{15}N NMR spectra were recorded using DEPT pulse sequence [21] to increase the sensitivity in a Bruker Avance 400 MHz spectrometer with a 5 mm broadband multinuclear probe. The number of scans used to obtain spectra was normally 250 - 500. A recycle time of 3.54 s was used with pulse width of 12.55 μs (tilt angle of 45 degrees). The number of data points used was 32 K. Chemical shifts are reported relative to 2.5 M $(^{15}NH_4)_2SO_4$ in 1 M H_2SO_4 (δ_N = 0.00) in coaxial capillary. The 2D [^1H, ^{15}N] heteronuclear single-quantum coherence (HSQC) NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer (^1H, 400.1 MHz; ^{15}N, 40.54 MHz) using the sequence of Stonehouse *et. al.* [22].

The ^{195}Pt spectra were recorded at 86.07 MHz on a Bruker AMX 400 spectrometer fitted with a 5 mm broadband probe. The magnetisation tilt angle was 90° A recycle time of 0.066 s was used with a pulse width of 23 μs. The spectra obtained were both proton coupled and proton decoupled. A spectral width used was 125,000 Hz. The number of scans used to obtain spectra varied from 1000 to 20,000. The number of data points obtained was 16K. ^{195}Pt shifts relative to a separate sample containing an aqueous solution of $Na_2[PtCl_6]$ (0.5 g ml^{-1}) ($\delta_{Pt} = 0$).

The following method was used to prepare sample for ES-MS: To a small bottle containing solid GSH (1.515 mg, 5 mmol) was added 0.5 mL 5 mM solution 3 (2.5 mmol) and pH was adjusted to ~2.0 under argon gas. The bottle was then sealed with parafilm to minimise the oxidation of GSH. The mole ratio of GSH and Platinum complex was 2 :1. The reaction mixture was left for 45 minutes to 1 hour then electrospray ionization mass spectrometry (ES-MS) was undertaken.

3. Results and Discussion

As indicated by ^{15}N NMR spectra, the reaction between *cis*-$[Pt(^{15}NH_3)_2(H_2O)_2]^{2+}$ and glutathione tends to mimic those of *cis*-$[Pt(^{15}NH_3)_2(H_2O)_2]^{2+}$ with H_3accys [19]. Based on the series of NMR spectra (Figure 1 and 2) the reactions that occurred are summarized in Figure 2 and the NMR parameters of the reaction products are presented in Table 1.

45 minutes after mixing the reaction mixture of 3 with GSH in 1 : 1 mole ratio of platinum complex to GSH, in acidic solution (pH ~ 2), the ^{15}N NMR spectra showed 6 new singlet peaks (apart from a peak due to unreacted 3) (Figure 3).

One of the 6 major peaks, $\delta_N = 0$ ppm is due to the released of ammine to form ammonium ion (similar case to those of H_3accys [19] and cysteine). The other five peaks were assigned with careful monitoring of the intensities of these peaks over time. It was clear that one of the peaks, δ_N at –41.59 ppm, was due to the formation of bis (thiolate) platinum(II), *cis*-$[Pt(^{15}NH_3)_2(SG)_2]$ (9).

This assignment was based on the observation that, when more GSH was used, it became a dominant product as its peak became much more intense in the ^{15}N NMR spectrum. It was not assigned as dinuclear sulphur-bridged (10), as the peak was very sharp, rather a broad peak as expected for the dinuclear species [17].

The other four peaks corresponded to two pairs of peaks from two different platinum(II) species. The peaks were at δ_N –42.97 ppm (corresponding to ammine *trans* to a sulphur donor [24–27]) and δ_N -81.96 ppm ppm (corresponding to ammine *trans* to an oxygen donor [23–26]) and were assigned in a similar way to that of complex *cis*-$[Pt(NH_3)_2(Haccys\text{-}S,O)]^+$with H_3accys [19], to a complex containing a six-membered *S,O*-chelate ring 7. The second pairs of peaks was at δ_N –46.03 ppm (corresponding to ammine *trans* to a sulphur donor [23–26]) and δ_N -68.92 ppm corresponding to ammine *trans* to a nitrogen donor [23–26] were assigned in a similar way to those of complex cis-$[Pt(^{15}NH_3)_2(H_2O)$ (cys-S,N)]with H_2cys, with a five-membered *S,N*-chelate ring 8.

The reaction was also monitored with 2D [^1H, ^{15}N] HSQC NMR (Figure 1). There is no difference in the products observed, so the assignments in 1D NMR were strengthened. The peaks observed were: δ_N/δ_H at –42.97/3.94 ppm (corresponding to ammine *trans* to a sulphur_donor [24–27]) and δ_N/δ_H at –81.96 / 4.14 ppm corresponding to ammine *trans* to an oxygen donor [23–26] were assigned to complex (7), δ_N/δ_H at –46.03 /3.83 ppm (corresponding to ammine *trans* to a sulphur donor [24–27]) and δ_N/δ_H at –68.92 / 3.71 ppm (corresponding to ammine *trans* to a nitrogen donor [24–27]) were assigned to complex (8). With this low concentration of 3 initially, bis (thiolate) complex (9) and sulphur-bridged complexes (10) were also present but in tiny amounts. But after 48 hours of reaction, these complexes (9) and (10) were present in more significant amounts.

Table 1. **^{1}H and ^{15}N NMR parameters of the complexes from reaction of *cis*-$[Pt(^{15}NH_3)_2(H_2O)_2]^{2+}$ and Glutathione (GSH)**

Compound	δ_H (ppm) *trans* to			δ_N (ppm) *trans* to		
	S	N	O	S	N	O
cis-$[Pt(NH_3)_2$(SG-*S,O*))] (**7**)	3.91		4.14	-42.97		-81.96
cis-$[Pt(NH_3)_2$(SG-*S,N*))] (**8**)	3.83	3.71		-46.03	-68.92	
cis-$[Pt(NH_3)_2(SG)_2]$ (**9**)	3.85			-41.59		
cis-$[Pt(NH_3)_2(\mu\text{-}SG)_2]$ (**10**)	3.77			-41.63		

All reactions were carried out in H_2O/5%D_2O

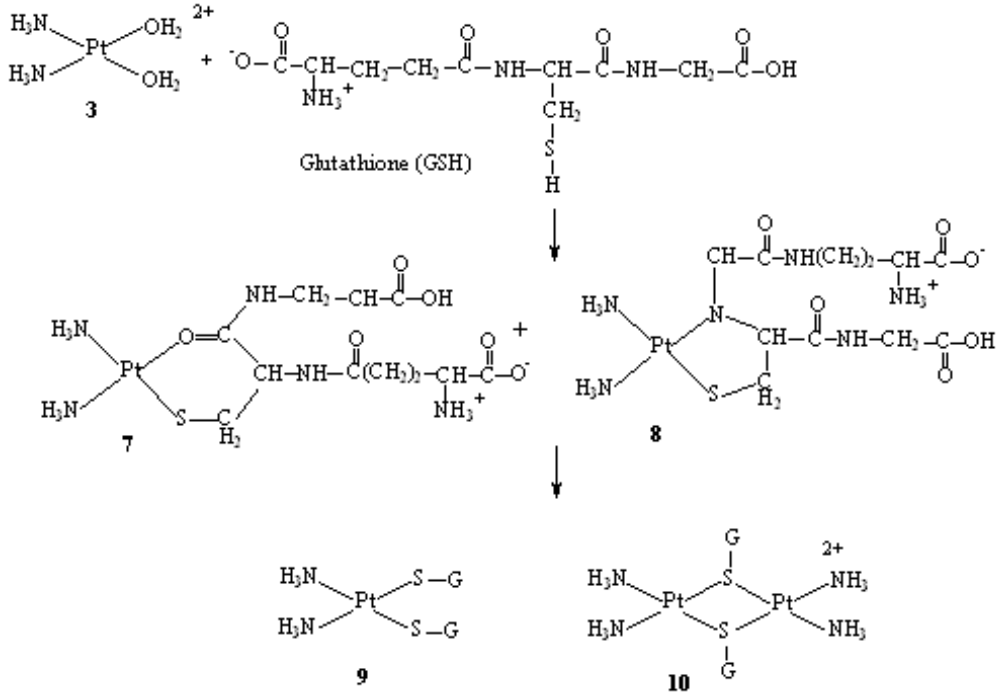

Figure 2. Reaction of 3 with GSH

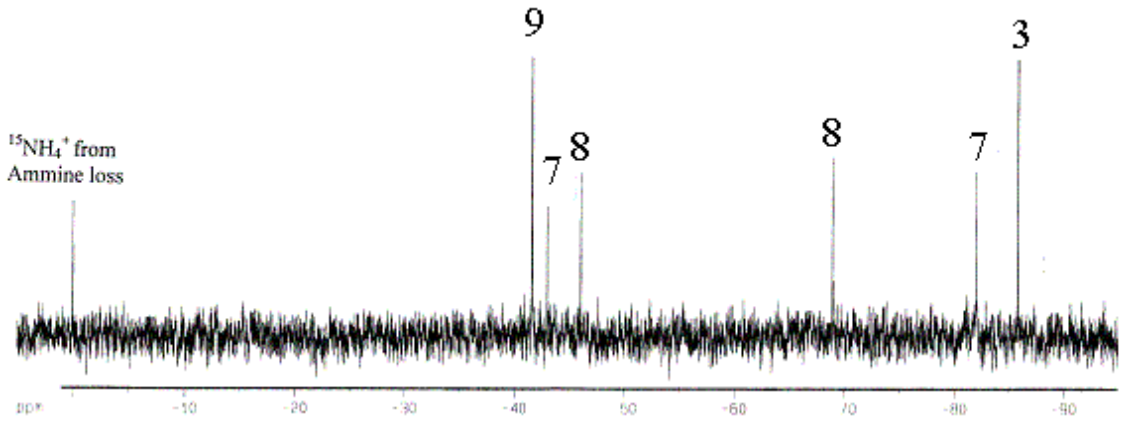

Figure 3. **40.54 MHz ^{15}N DEPT NMR spectrum of a solution obtained from the reaction of 10 mM (3) and GSH in 1:1 mol ratio, 45 minutes after mixing**

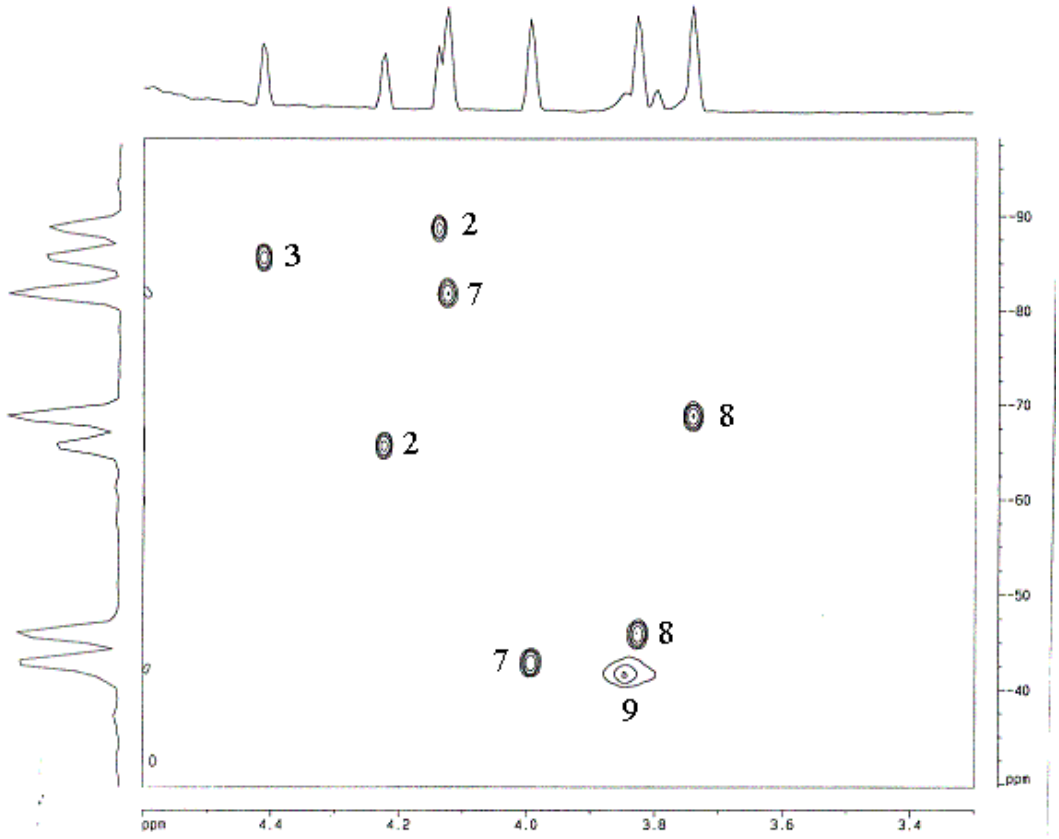

Figure 4. 2D [^{1}H, ^{15}N] HSQC NMR spectrum of a solution obtained from the reaction of 1 mM (3) and GSH in 1:1 mol ratio, 1 day after the reaction

All of these platinum species were very stable in the solution mixture under the conditions used for the 2D NMR experiment. After 24 hours of reaction, they were still present in the solution.

The ^{195}Pt NMR spectrum of a solution mixture 3 with glutathione in 1 : 1 mole ratio gave similar peaks to those observed with N-acetyl-L-cysteine [19]. The platinum chemical shifts observed were also at same region as with N-acetyl-L-cysteine.

Electrospray mass spectrum was obtained in a similar way to that used for the sample containing for H_3accys. The result obtained was also similar to that with H_3accys [19]. There were two platinum complexes detected with m/z = 1072 and 1054 amu. The peaks showed isotope pattern corresponding to dinuclear platinum. The peaks were assigned as dinuclear sulphur-bridged platinum (II) species with one of the carboxylic group in GSH was depronoted giving overall charge of +1. This complex would give m/z 1072 amu. Due to the *trans* effect of sulphur, then ammine ligand was cleaved off. With one ammine ligand lost ($^{15}NH_3$), the complex would give m/z 1054.

4. Conclusions

Compounds containing monodentate S-bound thiolate are moderately stable in solution at low concentration. Ammine loss *trans* to sulphur is rapid, especially in the chelate complex. The results presented here, are believed, represent the first adequate characterization of these species in such reaction. The other reactions of the compound 2 and 3 with other sulphur-containing ligand will be published elsewhere.

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