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

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ABSTRAK

Interaksi antara senyawa kompleks *cis*-[PtCl($^{15}NH_3$)$_2$(H_2O)]$^+$ (**2**) dengan glutation (GSH) telah dilakukan pada larutan berair. Pada reaksi ini, padatan yang berbentuk polimer tidak terbentuk dan pelepasan ligan amina lebih lambat dibanding reaksi sejenis untuk kompleks *cis*-[Pt($^{15}NH_3$)$_2$(H_2O)$_2$]$^{2+}$ (**3**). Dalam seri penelitian ini juga diamati bahwa walaupun reaksi yang terjadi antara kompleks **2** dengan berbagai tiol memberikan hasil yang akhir yang berbeda, akan tetapi pada tahap awal reaksi, senyawa yang terbentuk adalah sama yaitu terbentuknya spesi platina(II) dengan ligan klorida dan tiolat yang terikat secara mondentat melalui atom sulfur. Pada reaksi ini, setelah terbentuk hasil awal yaitu kompleks platina dengan glutation terikat secara monodentat *cis*-[PtCl($^{15}NH_3$)$_2$(SG)] (**7**), tidak ada senyawa komplek lain yang teramati. Seperti pada reaksi kompleks **3** dengan GSH, senyawa bistiolat *cis*-[Pt(NH_3)$_2$(SG)$_2$)](**8**) selalu teramati dalam reaksi ini. Sedangkan, kompleks sulfur dua inti berjembatan yang memberikan serapan lebar pada ^{15}N NMR hanya teramati dalam jumlah yang sangat sedikit.

Kata kuuci: ^{15}N NMR, glutation, hidrolisat cisplatin

ABSTRACT

The interactions of cis-[PtCl($^{15}NH_3$)$_2$(H_2O)]$^+$ (2) with glutathione (GSH) was investigated in aqueous solution. In this reaction, polymeric solid was not formed and the ammine liberation was slower than that for cis-[Pt($^{15}NH_3$)$_2$(H_2O)$_2$]$^{2+}$ (3). It is also observed that although in the reactions of 2 with a variety of thiols gave different ultimate product in each reaction, initially the only product observed was a platinum(II) species with chloride and the thiolate ligand bound monodentate through sulfur. In this reaction, after the initial formation of a complex with glutathione bound monodentate cis-[PtCl($^{15}NH_3$)$_2$(SG)] (7), no other product was observed. As with reaction of (3) the bis (thiolate) platinum(II) complex, cis-[Pt(NH_3)$_2$(SG)$_2$)](8) was always formed. The dinuclear sulphur-bridged complex, giving a broad peak in ^{15}N NMR, was only present in very tiny amounts in this reaction.

Key words: *^{15}N NMR, glutathione, cisplatin hydrolytes*

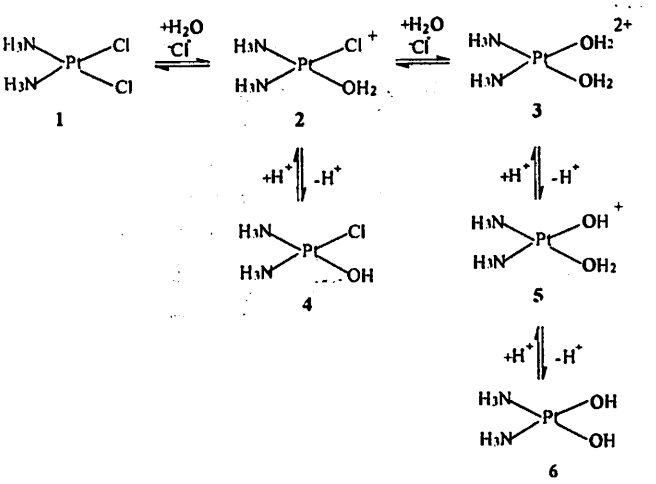
INTRODUCTION

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 (Lempers & Reedijk, 1991).

Interactions between platinum and biomolecules containing sulfur have been implicated in the nephrotoxicity of cisplatin (Borch & Pleasants, 1979), antitumour resistance to cisplatin (Fram *et al.*, 1985; Hamilton *et al., 1985*; Hospers *et al.*, 1985), cell repair mechanism (Eastman, 1987) and in the formation of crosslinks with monofunctional DNA adduct to prevent formation of bifunctional lesions (Eastman, 1987; Eastman & Barry, 1987; Bodener *et al.*, 1986).

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**) (Bancroft *et al.*, 1990).

It is established that the DNA and nucleotide reactions are primarily limited by the rate of aquations of cisplatin Bancroft *et al.*, 1990) and that the reactive species from the cisplatin is diammineaquachloroplatinum(II)(**2**) (Bancroft *et al.*, 1990; Barnham *et al.*, 1994; Bose *et al.*, 1997; Miller & House, 1989a,b, Miller & House, 1990, Miller & House, 1991). Cisplatin, *cis*-$[PtCl_2(NH_3)_2]$ (**1**) can undergo hydrolysis reactions as shown in Scheme 1.



Scheme 1. Hydrolysis of cisplatin

It has previously been established that thiolate tends to form a bridge between the two metal ions (Odenheimer & Wolf, 1982, Dedon & Borch, 1987, Appleton *et al.*, 1989c). It is 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.

These 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*-$[PtCl(^{15}NH_3)_2(H_2O)_2]^+$ (**2**) with glutathione (GSH), a sulphur-containing ligand. The other papers related to this work which discuss the reaction of complexes **2** with other thiols have also been submitted to be published elsewhere (Hadi, 2006a,b,c).and reactions of **3** with different sulphur-containing ligands has been published (Hadi, 2005).

EXPERIMENTAL

Starting Materials

^{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 (Dhara, 1970). Glutathione was purchased from Sigma Aldrich and used without further purification.

Preparation of *cis*-$[PtCl(^{15}NH_3)_2(H_2O)]^+$ (2)

The typical procedure used to prepare *cis*-$[PtCl(^{15}NH_3)_2(H_2O)]^+$ (**2**) was by converting *cis*-$[Pt(^{15}NH_3)_2(H_2O)_2]^{2+}$ (**3**) to *cis*-$[PtCl(^{15}NH_3)_2(H_2O)]^+$ (**2**) and was based on the procedure described by Appleton *et al.* (1985). This was done by the slow addition of one mol equivalent of a solution of NaCl, by using a small syringe to deliver one drop every 2 minutes and stirring the mixture reaction continuously. This is the most critical step, since this will affect the formation of *cis*-$[PtCl(^{15}NH_3)_2(H_2O)]^+$. Normally the solution will contain mostly *cis*-$[PtCl(^{15}NH_3)_2(H_2O)]^+$ (80 – 87%), *cis*-$[Pt(^{15}NH_3)_2(H_2O)_2]^{2+}$ (7 – 15 %) and cisplatin (5 – 10 %) (based on the peak heights in the ^{15}N NMR or integration in ^1H NMR). It is not possible to obtain a solution containing 100 % *cis*-$[PtCl(^{15}NH_3)_2(H_2O)]^+$.

Typical Reaction Condition

The reactions were carried out under Argon gas to minimise the oxidation of the thiols. No buffer was added as it reacts with the starting material used. The solid GSH was added to a small bottle containing a solution of **2** with pre-measured pH. 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. The reaetion proceeded quite fast, one experiment was left it run for about 6 – 10 hours continuously (in multi experiment mode).

NMR Speetra

The 1D 40.54 MHz ^{15}N NMR spectra were recorded using DEPT pulse sequence (Berners-Price & Kuchel, 1990) 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 ($\delta_N = 0.00$) in coaxial capillary.

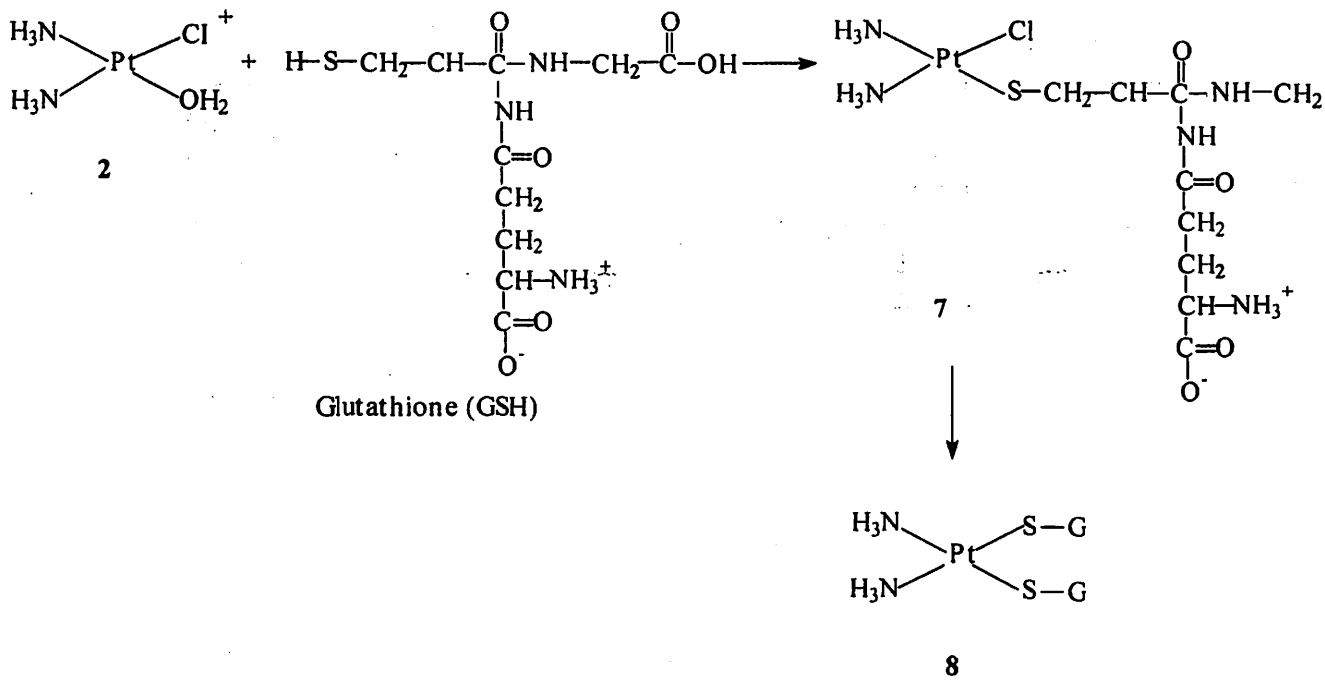
RESULTS AND DISCUSSION

The reaction of **2** with glutathione gave similar products to those with H_3accys, except that in this reaction the formation of (bis-thiolate) platinum products is more rapid. It was observed that the polymeric solid was not formed and the ammine liberation was slower compare to that for **3** (Hadi, 2005). Initially the only product observed was a platinum(II) species with chloride and the thiolate ligand bound monodentate through sulphur. The reactions that occurred are summarized in Scheme 2 and the NMR parameters for reaction products are presented in Table 1.

Table 1. ^1H and ^{15N} NMR parameters of the complexes from reaction of **2** and glutathione

Complex	δ_H (ppm)			δ_N (ppm)		
	trans to S	*trans* to Cl	*trans* to O	*trans* to S	*trans* to Cl	*trans* to O
cis-$[PtCl(^{15}NH_3)_2(H_2O)]$		4.22	4.17		-65.87	-89.22
cis-$[PtCl(^{15}NH_3)_2(SG)]$	3.83	4.08		-43.18	-62.45	
cis-$[Pt(NH_3)_2(SG)_2)]$(**8**)	3.77			-41.59		

All reaetions were earried out in $H_2O/5\%D_2O$.



Scheme 2. Reaction of (**2**) with GSH

Previous extensive studies (Appleton *et al.*, 1988) in related system have shown that the sulphydryl group of H_3accys is the preferred binding site of platinum. The presence of free ammonium ion was readily detected from the ^{15}N NMR spectrum of the solution if the reference capillary containing $^{15}NH_4^+$ was removed. The signal from free ammonium ion was detected after 30 minutes and continued to grow with time. This ammonium ion is from ammine loss which occured due to the high *trans* effect of thiolate sulphur.

When solid GSH was added to the solution of **2** in 1 : 1 mol ratio, the ^{15}N NMR spectrum 1 h after mixing (Fig. 1), showed one pair of new peaks with equal intensities (δ_N – 43.18. and –62.45 ppm) and one other peak at δ_N –41.59 ppm. The pair of peaks corresponded to a species with ammine *trans* to sulfur and chloride respectively (Appleton *et al.*, 1985, Appleton *et al.*, 1988, Appleton *et al.*, 1989a, Appleton *et al.*, 1989b) and was assigned to complex *cis*-[PtCl($^{15}NH_3$)$_2$(SG)](**7**) (Scheme 2). The other peak, δ_N –41.59 ppm, present must be due to the formation of bis (thiolate) platinum(II) *cis*-[Pt(NH_3)$_2$(SG)$_2$)] (**8**), this paltinum(II) species was also observed in the reaction of **3** with GSH (Hadi, 2006a).

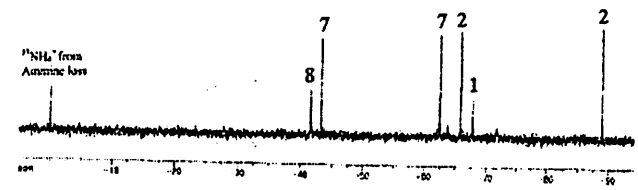


Fig. 1. 40.54 MHz ^{15}N DEPT NMR spectrum of a solution obtained from the reaction of 7.4 mM **2** and GSH in 1:1 mol ratio, 1 h after mixing

No further change with time was observed. After 12 hours, the peaks remaining were from unreacted (**1**), free ammonium ion released, and bis (thiolate) complex (**8**). When the reaction was carried out with 1 : 2 mol ratio (or GSH in excess), there was no differencc in the product formation, except the formation of complex **8** was much faster.

CONCLUSIONS

Compounds containing monodentate S-bound thiolate are moderately stable in solution at low concentration. Ammine loss *trans* to sulfur is rapid, especially in the chelate complex. The results presented here, is 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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