Interaction of Platinum(II) Amino Acid Complexes with Nucleosides

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We report the synthesis of mixed ligand complexes of platinum(II) with glycine or alanine as primary ligands and the nucleosides adenosine, guanosine, inosine, cytidine and uridine as secondary ligands. Interaction of platinum(II) amino acid complexes with nucleosides was carried out in a 1:1 ratio, resulting in the formation of cis dichloro complexes. It was found that adenosine and guanosine coordinate to the metal ion through $N_{(7)}$, whereas inosine binds through $N_{(1)}$. The pyrimidine nucleosides coordinate to the metal ion through N_3. In these complexes the amino acid behaves as a monodentate ligand coordinating through the amino nitrogen. These complexes are insoluble in aqueous medium but on addition of base they become soluble due to the neutralization of the proton located on the carboxylic group of the amino acid.

Introduction

After the discovery of the antitumour activity of cis-$Pt(NH_3)_2Cl_2$ by Rosenberg [1], there have been many attempts to synthesize more active cis compounds of platinum(II) [2, 3]. It has been reported that many active complexes react with DNA [4–10] and inhibit its synthesis. Cis-dichlorodiammineplatinum(II) is considerably toxic and the substitution of amine ligands with molecules that are found in biological systems may decrease its toxicity. Earlier we reported [11] the preparation and characterization of platinum(II) complexes having cis chlorides with glycine or alanine and purine and pyrimidine bases. We have also reported many complexes of platinum group metal ions with purines, pyrimidines and nucleosides [12–14]. In this paper we report the synthesis and characterization of platinum(II) complexes with the amino acids glycine or alanine and a nucleoside. It was observed that the metal ion coordinates to the nucleoside adenosine or guanosine through N_7 and to inosine through N_1. The metal binding site in the pyrimidine nucleoside is N_3.

Experimental

Hexachloroplatinic acid (H_2PtCl_6) was obtained from Alfa Ventron (U.S.A.) and was converted to potassium tetrachloroplatinate(II) by the published procedure [15]. The nucleosides glycine and α-alanine (DL) were purchased from Sigma Chemicals and used without further purification. Potassium dichloroglycinatoplatinate(II) [16] and potassium alaninatoplatinate(II) [17] were prepared according to published procedures.

Micro analyses of the samples were performed at CSIRO (Australia). The percentage of chlorine in the complexes was estimated by the Al Steyermark *et al.* method [18]. The IR and U.V. spectra were recorded on Perkin Elmer (337) and Beckman (Model DB) spectrophotometers, respectively. ^1H NMR spectra (in DMSO-d_6) were recorded on a Jeol 60 and 100 MHz spectrometers. Due to the insolubility of a few complexes electronic and ^1H NMR spectra are not reported.

Synthesis

Glycine Mixed Ligand Complexes. Cis-Dichloroadenosineglycineplatinum(II) Trihydrate, Cis-Dichloroglycineguanosineplatinum(II) Tetrahydrate, Cis-Dichloroglycineinosineplatinum(II) Tetrahydrate, Cis-Dichlorocytidineglycineplatinum(II) Monohydrate and Cis-Dichloroglycineuridineplatinum(II) Dihydrate

Potassium dichloroglycinatoplatinate(II) (0.20 g, 0.5 m*M*) was dissolved in 15 ml of acidified water (pH 6) and to this a solution of the ligands (0.5 m*M*) adenosine, guanosine, inosine, cytidine or uridine was added. Guanosine was dissolved in slightly basic medium (pH 8). The resulting solution was refluxed for two to four hours. A cream coloured precipitate was obtained with adenosine; inosine and

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TABLE I. Analytical Data.

No.	Complex	% Carbon*	% Hydrogen	% Chlorine
1.	Cis-Pt(GlyH)(Ado)$Cl_2 \cdot 3H_2O$	21.30 (21.71)	3.72 (3.65)	10.84 (10.70)
2.	Cis-Pt(GlyH)(Guo)$Cl_2 \cdot 4H_2O$	20.65 (20.61)	3.71 (3.76)	10.84 (10.18)
3.	Cis-Pt(GlyH)(Ino)$Cl_2 \cdot 4H_2O$	20.85 (20.11)	3.80 (3.69)	10.65 (10.41)
4.	Cis-Pt(GlyH)(Cyd)$Cl_2 \cdot H_2O$	21.68 (21.88)	3.25 (3.31)	11.38 (11.77)
5.	Cis-Pt(GlyH)(Urd)$Cl_2 \cdot 2H_2O$	20.72 (21.22)	3.47 (3.40)	10.65 (11.41)
6.	Cis-Pt(AlaH)(Ado)$Cl_2 \cdot 3H_2O$	22.45 (23.06)	3.87 (3.80)	10.37 (10.49)
7.	Cis-Pt(AlaH)(Guo)Cl_2	24.53 (24.43)	3.12 (3.15)	10.84 (11.12)
8.	Cis-Pt(AlaH)(Ino)$Cl_2 \cdot 4H_2O$	22.50 (22.42)	3.60 (3.90)	10.84 (10.21)
9.	Cis-Pt(AlaH)(Cyd)$Cl_2 \cdot H_2O$	23.01 (23.35)	3.91 (3.92)	12.07 (10.51)
10.	Cis-Pt(AlaH)(Urd)Cl_2	23.61 (24.02)	3.20 (3.91)	11.30 (11.84)

*Calculated values are in parenthesis.

uridine gave yellow precipitates. In the case of guanosine and cytidine brown coloured precipitates were obtained. The guanosine complex was obtained by lowering the pH of the solution to ~pH 5.

Alanine Mixed Ligand Complexes. Cis-Dichloroadenosinealanineplatinum(II) Trihydrate, Cis-Dichloroalanineguanosineplatinum(II), Cis-Dichloroalanineinosineplatinum(II) Tetrahydrate, Cis-Dichloroalaninecytidineplatinum(II) Hydrate and Cis-Dichloroalanineuridineplatinum(II)

Potassium dichloroalaninatoplatinate(II) (0.216 g, 0.5 m*M*) was dissolved in 15 ml of water and to this a solution of the ligand (0.5 m*M* adenosine, inosine, cytidine or uridine in acidified water, pH 6) was added. Guanosine was dissolved in slightly basic medium (pH 8) before addition to the above platinum compound. The resulting solution was refluxed for three to eight hours. Light yellow coloured precipitates were obtained for the adenosine and the inosine complexes and dark brown precipitates for the guanosine and uridine complexes. Cytidine gave a dark bluish purple precipitate. In the case of guanosine the complex was obtained by lowering the pH of the solution to ~pH 5.

Analytical data of the complexes are given in Table I.

Results and Discussion

The ligands guanosine, inosine, cytidine and uridine exist mostly in keto form [19, 20]. In the IR spectra the C=O stretching frequency is exhibited as a strong band at ~1700 cm^{-1}. The amino acids glycine and α-alanine exist as Zwitter ions both in solution and solid state, and as such glycine exhibits NH_3^+ deformation and COO^- symmetric stretching frequencies at 1585 and 1623 cm^{-1}, while alanine exhibits these peaks at 1610 and 1597 cm^{-1} [21, 22], respectively. The platinum–glycine complexes in which C=O of glycine is not coordinated, *e.g.* the trans-$[Pt(glyH)_2Cl_2]$ complex, [24] show a strong peak at ~1700 cm^{-1} in their IR spectra. In the mixed ligand complexes of glycine and nucleosides a strong band is observed in the region 1700–1690 cm^{-1} which can be assigned to the stretching frequency of the carbonyl group of the amino acid. Since the stretching frequencies of the carbonyl group are not shifted in the mixed ligand complexes, it may be inferred that the group is not involved in binding to the metal ion [23]. This strong peak also indicates that in the mixed ligand complexes the carboxylic group is not ionised [24]. All complexes exhibit a broad and strong band at around 3000–3300 cm^{-1} which is due to the OH stretching frequency of nucleoside and amino acid and also shows extensive hydrogen bonding of the hydrogen of the COOH of the amino acid with a basic centre N or OH on purine nucleoside or pyrimidine nucleoside. A strong peak due to the NH_2 deformation was observed in the IR spectra of adenosine, guanosine and cytidine at 1665, 1670 and 1620 cm^{-1}, respectively [25]. In the mixed ligand complexes of glycine this peak is not shifted to a noticeable extent

TABLE II. Electronic Spectra of Complexes.

Ligand/Complex	λ_{max} (nm)	ϵ_{max}	Transition
Adenosine	268	1.1×10^4	$\pi \rightarrow \pi^*$
1. Cis-Pt(GlyH)(Ado)$Cl_2 \cdot 3H_2O$	273	2.3×10^4	$\pi \rightarrow \pi^*$
	336	319.5	$^1A_1 \rightarrow {}^1E$
	350	302.5	$^1A_1 \rightarrow {}^1A_2$
2. Cis-Pt(GlyH)(Guo)$Cl_2 \cdot 4H_2O$	265	1.278×10^4	$\pi \rightarrow \pi^*$
	340	772	$^1A_1 \rightarrow {}^1E$
	353	752	$^1A_1 \rightarrow {}^1A_2$
Inosine	256	1.14×10^4	$\pi \rightarrow \pi^*$
3. Cis-Pt(GlyH)(Ino)$Cl_2 \cdot 4H_2O$	262	2.443×10^4	$\pi \rightarrow \pi^*$
	339	507	$^1A_1 \rightarrow {}^1E$
	345	500.5	$^1A_1 \rightarrow {}^1A_2$
Cytidine	266	8.0×10^3	$\pi \rightarrow \pi^*$
4. Cis-Pt(GlyH)(Cyd)$Cl_2 \cdot 2H_2O$	274	1.6×10^3	$\pi \rightarrow \pi^*$
	332	290	$^1A_1 \rightarrow {}^1E$
	364	145	$^1A_1 \rightarrow {}^1A_2$
Uridine	266	7.9×10^3	$\pi \rightarrow \pi^*$
5. Cis-Pt(GlyH)(Urd)$Cl_2 \cdot 2H_2O$	268	1.7×10^3	$\pi \rightarrow \pi^*$
8. Cis-Pt(AlaH)(Ino)$Cl_2 \cdot 4H_2O$	277	6.1×10^3	$\pi \rightarrow \pi^*$
	340.45	1159	$^1A_1 \rightarrow {}^1E$
	355–360	1173	$^1A_1 \rightarrow {}^1A_2$
9. Cis-Pt(AlaH)(Cyd)$Cl_2 \cdot H_2O$	260–290	4.31×10^3	$\pi \rightarrow \pi^*$
	330	323	$^1A_1 \rightarrow {}^1E$
	342	307	$^1A_1 \rightarrow {}^1A_2$

indicating that the NH_2 group is not involved in coordination [26] (only 5 to 10 cm^{-1}). This is because the lone pair of electrons present on the nitrogen of the NH_2 group are being attracted into the ring [27]. The mixed ligand complexes of alanine show a strong and broad band in their IR spectrum in the region 1660–1620 cm^{-1}. This may be due to the overlap of the antisymmetric stretching frequencies of the C=O and NH_2 deformation mode of the ligands and could not be assigned separately. In the uncoordinated nucleoside, the C=C and C=N stretching frequencies are observed at around 1380 and 1500 cm^{-1}, respectively. These peaks are shifted to a lower frequency by about 50–70 cm^{-1} in the mixed ligand complexes indicating the involvement of the ring nitrogen atom in coordination [28]. In the mixed ligand complexes the M–N stretching frequencies of the coordinated ring nitrogen of the nucleoside and the coordinated NH_2 of the amino acid overlap and give a broad band at around 530–570 cm^{-1}. The mixed ligand complexes exhibit two more peaks for M–Cl in the region 360–310 cm^{-1} corresponding to the presence of cis chlorides in the coordination sphere of the metal ion.

The electronic spectra of the nucleosides guanosine, inosine, cytidine and uridine exhibit a strong band at around 255–270 nm which is assigned to the $\pi \rightarrow \pi^*$ transitions of the ligand [28]. On complexation of the nucleosides with platinum(II) a slight shift occurs [29], and this band is observed in all the complexes at around 260–280 nm. Two more bands are exhibited in the electronic spectra of the mixed ligand complexes at around 330 and 365 nm assigned to the d–d transitions $^1A_1 \rightarrow {}^1E$ and $^1A_1 \rightarrow {}^1A_2$ of the platinum(II) square planar complex of a C_s symmetry. These bands have charge transfer character (Table II).

K[Pt(Gly)Cl_2] shows a signal in the NMR spectrum at 3.76 ppm which is assigned to the methyl-

TABLE III. ^{1}H NMR Spectra of the Complexes.[a]

Complex No.	Complex/Ligand	Purine nucleoside		Pyrimidine nucleoside		Amino acid	
		$C_{(2)}H$	$C_{(8)}H$	$C_{(5)}H$	$C_{(6)}H$	CH_2/CH	CH_3
	$K[Pt(Gly)Cl_2]$					3.76	
	Guanosine		7.85				
2.	$Cis\text{-}Pt(GlyH)(Guo)Cl_2 \cdot 4H_2O$		8.72			3.61	
	Inosine	8.0	7.6				
3.	$Cis\text{-}Pt(GlyH)(Ino)Cl_2 \cdot 4H_2O$	7.09	7.4			3.70	
	Cytidine			6.04	4.64		
4.	$Cis\text{-}Pt(GlyH)(Cyd)Cl_2 \cdot 2H_2O$			5.65	4.7	3.80	
	$K[Pt(Ala)Cl_2]$					3.83	1.48
9.	$Cis\text{-}Pt(AlaH)(Cyd)Cl_2 \cdot H_2O$			8.10	5.40	2.49	1.3

[a]Solvent: DMSO-d_6/D_2O (δ values are given in parts per million).

ene protons of the coordinated glycine [30]. In the mixed ligand complexes this peak is observed at around 3.6 to 3.8 ppm (Table III).

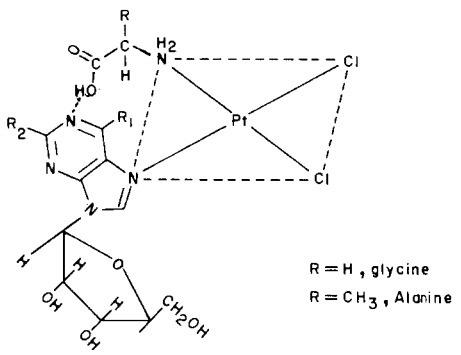
The NMR spectrum of guanosine exhibits a signal due to the C_8H proton at 7.85 ppm. The CH_2 and CH ribose protons are observed at around 2.6 to 6.5 ppm. In the mixed ligand complex *2* the C_8H proton of guanosine is shifted downfield by 0.87 ppm indicating that N_7 of guanosine is the site of coordination to the metal ion [23]. In the NMR spectrum of inosine signals due to the C_8H and C_2H protons are observed at 7.60 ppm and 8.00 ppm, respectively. Signals due to the ribose protons in the ligand and complex *3* are observed in the region 2.3 to 6.25 ppm. In complex *3*, the signals corresponding to the C_2H and C_8H protons are shifted upfield by 0.91 ppm and 0.2 ppm, respectively. Since the signal due to the C_2H proton is shifted to a greater extent, N_1 of the inosine is proposed as the binding site for the metal ion.

Cytidine exhibits signals due to C_5H and C_6H protons at 6.04 and 4.64 ppm, respectively. In complex *4*, The C_5H protons are observed at 5.65 ppm and C_6H protons are observed at 4.70 ppm. Since the signal due to the C_6H protons is shifted considerably, it is proposed that N_3 of cytidine is the binding site for the metal ion. The signals due to the ribose protons are observed in the region 3.82–6.0 ppm and are not shifted in the mixed ligand complex [31]. In the NMR spectrum of complex *9* a broad doublet is observed at around 8.10 ppm and a strong signal is observed at 5.40 ppm which can be assigned to the C_5 and C_6 protons of the pyrimidine ring, respectively. Since the signal due to the C_5H protons is shifted downfield to a greater extent (1.96 ppm) compared to the C_6 protons (0.76 ppm), N_3 of the ligand is proposed as the coordination site. Signals due to the ribose protons are observed at 6.1 ppm, 3.9 ppm and 3.6 ppm (broad). Two more signals at 1.3 ppm (doublet) and 2.49 ppm appear, which can be assigned to the methyl and methylene protons of the coordinated alanine, respectively.

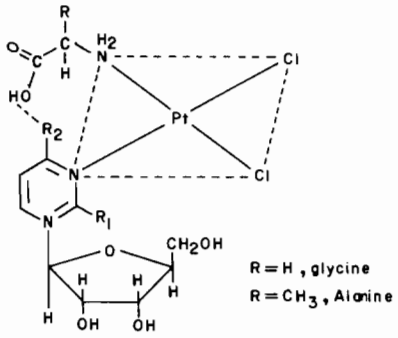
In the complexes *2* and *3* the signal due to the methylene protons of the glycine is shifted slightly upfield whereas in complex *4* it is shifted downfield indicating that these protons are sensitive to the changes in the basicities of the nucleosides. Greater shielding of the $-CH_2$ protons of glycine occurs in complexes *2* and *3*. The cytidine complex *4* shows a downfield shift corresponding to the deshielding of $-CH_2$ protons by removal of electron density from the $-NH_2$ group of glycine.

Based on the above data structures I and II are tentatively proposed for the complexes of adenosine, guanosine and the pyrimidine nucleosides, respectively.

The mixed ligand complexes are insoluble in aqueous medium, but the addition of an equivalent amount of base (0.05 N) to the complex removes the proton from the carboxylic group of the amino acid making it more polar so that the complex becomes soluble. The complex can be precipitated from the solution by the addition of an equivalent amount of acid.



Cis-Pt(AlaH)(Pur. nucleoside)$Cl_2 \cdot XH_2O$
a) $R_1 = NH_2$, $R_2 = H$, adenosine.
b) $R_1 = OH$, $R_2 = NH_2$, guanosine.
Structure I



Cis-Pt(AlaH)(Pyrimidine nucleoside)$Cl_2 \cdot XH_2O$
a) $R_2 = NH_2$, $R_1 = OH$, cytidine.
b) R_1 and $R_2 = OH$, uridine.
Structure II

$$Pt(AH)(L)Cl_2 \underset{\text{HCl eq.}}{\overset{\text{eq. KOH}}{\rightleftharpoons}} K[Pt(A^-)(L)Cl_2)]$$

AH = Glycine or alanine, L = Nucleoside.

The same reaction scheme is proposed for the mixed ligand complexes as reported earlier [11].

Mixed ligand platinum(II) complexes of alanine and nucleosides are less soluble than glycine complexes and some of them are even insoluble. The reason for this may be the presence of the hydrophobic methyl group.

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