

## Platinum Blues. Evidence of the Existence of Two Isomeric Platinum–Glutamine Complexes

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Interest in the platinum complexes has been raised in recent years by the discovery that *cis*-dichlorodiaminoplatinum(II) (*cis* DDP) has antitumor properties [1, 2]. It has been postulated that this activity could be related to specific interactions between the *cis*-DDP (or its aquated forms) and the purine and pyrimidine bases of DNA [3, 4]. An important result of the *in vitro* study of these interactions was the isolation of a new type of complexes, the pyrimidine blues [5].

Some of these products seem to be very promising with regard to their high index of antitumor activity and diminished nephrotoxicity in comparison to the *cis*-DDP itself [5–7]. However, other sites of action of the platinum drugs in the cell may be considered, e.g. aminoacids and proteins [8, 9]. Using an equilibrium dialysis technique, Morris and Gale obtained evidence that *cis*-dichloro(dipyridine)platinum(II) may associate with electron-rich areas of nucleic acids and proteins and also with free nucleo- and aminoacids [10]. Furthermore, it has been suggested that L-Asparagine or L-Glutamine may be used to direct complexed metal ions into tumor cells [11, 12].

In a recent paper we have reported on the reaction between  $K_2PtCl_4$  and L-Asparagine leading to a blue species [13]. Similarly, evidence of the formation of paramagnetic complexes has been gained from an EPR study of systems involving *cis*-DDP and tryptophan [14].

In the present work it will be shown that two species, a blue paramagnetic complex and a white-greenish dimagnetic one, may be obtained during the reaction of  $K_2PtCl_4$  with L-glutamine.

### Experimental

Infra-red spectra were recorded using a Perkin-Elmer Model 225 spectrometer in the 4000–200  $cm^{-1}$  range using CsBr disks.

The ESR spectra were obtained using a Bruker ER-200 D with a conventional X-band (9.63 GHz). pH values were monitored by an automatic Radiometer-Copenhagen titration chain.

### Preparation and Characterization of the Complexes

#### Platinum L-Glutamine Blue (P.G.B.)

To a solution of  $K_2PtCl_4$  (0.1 M) in water 10 equivalents of L-Glutamine were added. The resulting mixture (pH = 5.2) was stirred in the dark at 35 °C. After three days the pH was 3.2 and a blue species precipitated. The blue complex was separated by centrifugation from a brown supernatant. The crude product was repeatedly washed with hot water to remove any unreacted ligand, and finally dried *in vacuo*.

#### Platinum L-Glutamine Green (P.G.G.)

A solution of L-glutamine (1.5 g) in 25  $cm^3$  of water was adjusted to pH = 6.5 with KOH (0.1 M). After addition of  $K_2PtCl_4$  (0.4 g) the pH was brought again to 6.5 and the resulting stirred mixture kept in the dark for two days at the same constant pH. A white greenish precipitate was obtained and isolated by centrifugation. The compound was washed with lukewarm water and with methanol.

Analytical data of compounds are given in Table I. No chlorine or potassium contents were found.

TABLE I. Elemental Analysis.

	C	H	N	Pt
PtL <sub>2</sub> *	26.64	3.69	11.49	40.04
Platinum Glutamine Green (PGG)	24.68	3.68	11.56	39.43
Platinum Glutamine Blue (PGB)	24.19	4.00	11.29	39.63

\* L is mono-deprotonated glutamine.

Attempts to crystallize these two complexes have been largely unsuccessful and the X-ray powder diffraction patterns are characteristic of amorphous substances. Furthermore the two complexes are almost insoluble in common solvents.

### Results and Discussion

Judging from electron spin resonance spectroscopy, the blue complex is paramagnetic while the

green one is diamagnetic. In the first case, ESR signals are detected in the solid state (powder). The spectra may be interpreted in terms of an axially symmetrical ion with  $g_{\perp} = 2.44$ ,  $g_{\parallel} = 1.99$ . The large  $g$ -shift suggests considerable spin-orbit coupling, a behaviour typical of paramagnetic heavier transition metal ions. Very similar data have been obtained in the case of Pt(IV) doped Magnus' green salt [15], Krogman's salt [16], 'irradiated' Pt(II) complexes and the known platinum blues [7, 13, 17, 18] (Table II). They have been interpreted in terms of  $d_{z^2}$  hole state with an admixture of the degenerate  $d_{xz}, d_{yz}$  states due to spin-orbit interaction. An extended but ill-resolved hyperfine structure is observed and may be attributed to the interaction of the unpaired spin with  $^{195}\text{Pt}$  nuclei ( $I = \frac{1}{2}$ ).

TABLE II.  $g$  Values of One-dimensional Chain Compounds of Platinum and Platinum Blues.

	$g_{\parallel}$	$g_{\perp}$	Ref.
Magnus' green salt [Pt(NH <sub>3</sub> ) <sub>4</sub> ][PtCl <sub>4</sub> ]	1.939	2.504	15
K <sub>2</sub> Pt(CN) <sub>4</sub> B <sub>1/3</sub> ·3H <sub>2</sub> O	1.946	2.336	16
<i>Platinum Blues</i>			
$\alpha$ -pyridone Blue	1.976	2.380	17
uracil-blue	1.99	2.40	7
PDD-cytosine	1.970	2.394	18
PDD-thymine	1.995	2.378	18
Asparagine Blue	1.97	2.39	13
Glutamine Blue	1.99	2.44	This work

L-Glutamine has three sites suitable for bonding: on the one hand, the amino entity and, on the other hand, the amino and the carboxylate groups which have been found operative in the PtA<sub>2</sub> and Pt(AH)<sub>2</sub>-Cl<sub>2</sub> complexes of glycine and alanine [19].

Speer and collaborators noted that *trans*-Pt-(Glutamine)<sub>2</sub> does not have promise as an antitumor agent [20]. However, the preparative method for this bis-chelate was not given.

IR spectra are expected to afford some indications of the groups involved in coordination to the metal. Thus, it has been claimed from IR data that coordination of L-glutamine to copper and zinc is achieved through the primary amino group in the CuL<sub>2</sub> and ZnL<sub>2</sub> complexes [22].

The most surprising characteristic of the spectra of the blue and green species is their close resemblance, suggesting that a similar coordination mode is operative in both complexes. L-glutamine shows characteristic frequencies due to vibrations of NH<sub>3</sub><sup>+</sup> group at 3180, 1483 and 532 cm<sup>-1</sup> [21-24]. They are no longer present in the spectra of the metal complexes indicating that the amphionic form has

vanished and that either the nitrogen of the primary amino group, or the carboxylate group, participates in the coordination.

In the free ligand spectrum an absorption at 1685 cm<sup>-1</sup>, which disappears upon complexation, has been interpreted as being the amide I band [21]. However its intensity is low and we think that a better attribution would be either the 'amino-acid I band'—a NH<sub>3</sub><sup>+</sup> deformation mode (which is often weak and, in some cases, appears only as a shoulder [24]), or a carboxyl vibration with reduced intensity due to the importance of the amphionic form.

Two bands at 1600 and 1410 cm<sup>-1</sup> are attributable to respectively  $\nu_a \text{COO}^-$  and  $\nu_s \text{COO}^-$ . They are only slightly affected in the complexes where the acid function must consequently remain deprotonated. Keeping in mind the disappearance of the NH<sub>3</sub><sup>+</sup> absorptions one may conclude that the COO<sup>-</sup> group is undoubtedly involved in coordination.

The characteristic amide bands would be located at 1630 (amide I) and 1590 cm<sup>-1</sup> (amide II) in the free ligand spectrum but they are difficult to localize in the complexes since many absorptions are occurring in this spectral region.

However, from the crystallographic study of the  $\alpha$ -pyridone blue [25] and from various works carried out on platinum blue complexes [26, 27], it might be assumed that the main coordination site is the amino group. Thus, both spectral and analytical analogies between the two complexes show a close resemblance of the mode of coordination of the ligand. Such a behaviour has already been mentioned. For instance a blue paramagnetic species and several non-blue diamagnetic compounds have been isolated in the reaction of  $\alpha$ -pyridone with [(NH<sub>3</sub>)<sub>2</sub>Pt(H<sub>2</sub>O)<sub>2</sub>]<sup>2+</sup>. The geometry of the tetrameric blue complex is nearly identical to that of the geometry of its non-blue analog except that the platinum-platinum distance is shorter [28]. This Pt-Pt proximity is thought to stabilize the partially oxidized paramagnetic ion through delocalization of the free spin over several platinum atoms. The chromophore arises from a transition to or from the orbital sheltering the unpaired electron. On the contrary, in the greenish complex a longer Pt-Pt distance would exclude this type of stabilization yielding a diamagnetic platinum(II) complex.

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