One- and Two-dimensional ^{31}P NMR Characterization of Pure Phosphato Chelates in Cytidine-5′-di- and -tri-phosphatoplatinum(II) Complexes

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cis-Diamminedichloroplatinum(II) treated with CTP and CDP yields phosphato chelates as the major products; the chelates are diastereoisomers (Λ and Δ) formed by co-ordination through the two adjacent phosphate groups of the nucleotides, diplatinum complexes in which two nucleotide molecules bridge platinum centres through N(3) and terminal phosphate co-ordination in a 'head-to-tail' fashion are the minor products, these dinuclear complexes are the dominant products of the same reactions when *cis*-diamminediaquaplatinum(II) cation is used.

The platinum complex, *cis*-diamminedichloroplatinum(II) (*cis*-DDP) is the most widely used antitumour drug. A small fraction of the administered drug becomes available to DNA to exert antineoplastic activity;[1,2] the majority of the platinum complex reacts with other biomolecules in the cell. These undesired reactions perhaps inactivate the drug and induce toxic effects.[3] The direct irreversible phosphate co-ordination to platinum by various mononucleoside phosphates in the cell may influence metal-dication-dependent enzyme reactions that require binding to the phosphate for their activity. Indeed, *cis*-diamminediaquaplatinum(II) cation inhibits the activity of mitochondrial ATPase,[4] a metal-dication-dependent enzyme. Here we report phosphato chelates of CDP (cytidine-5′-diphosphate) and CTP (cytidine-5′-triphosphate) complexes of platinum(II). To the best of our knowledge, this is the first report that describes the formation of pure platinum(II) chelates with nucleoside-5′-phosphate ligands. The complex formation by both phosphate and nitrogen donor atoms of the purine and pyrimidine rings have been documented.[5—7] Louie and Bau[5] reported the *X*-ray crystal structure of the binuclear complex, $[\{Pt(en)(CMP)\}_2]$, (CMP = cytidine-5′-monophosphate; en = ethylenediamine) in which nucleotide molecules bridge two platinum atoms through the phosphate group and N-3 in a 'head-to-tail' fashion. Marzilli *et al.*[6] recently reported the NMR characterization of platinum(II) IMP (inosine-5′-monophosphate) and ITP (inosine-5′-triphosphate) macrochelates in which N-7 and phosphate of the same nucleotide molecule are covalently bonded to a single platinum. Earlier, we also suggested similar modes of binding by ATP to platinum(II).[7]

The proton decoupled ^{31}P NMR spectrum† of the reaction mixture containing *cis*-diamminedichloroplatinum(II) and CTP at pH 7.0 is shown in Figure 1(a). Two doublets (A and B) and the triplet (C) are resonances of the excess unreacted CTP molecules. Two smaller doublets (D and E) and the doublet of doublets (F) are due to the formation of platinum(II) phosphato chelates (see below). When $[cis\text{-}Pt(NH_3)_2(H_2O)_2]^{2+}$ was used as the platinum substrate, additional resonances (G and H), which dominate the product spectrum, were observed. Two dimensional COSY spectrum (Figure 2) (phosphorus–phosphorus correlation) establishes that the latter signals are coupled to each other and hence arise from a single product. These signals (G and H) are attributed to the bis(CTP)bridged diplatinum(II) complex analogous to the CMP-bridged dinuclear complex reported by Louie and Bau.[5]‡ Finally, when pre-equilibrated *cis*-DDP§ was used for the reaction, the ^{31}P spectrum exhibited peaks for both complexes (mononuclear phosphato chelates and the dinuclear N_3-γ-PO_4 bridged complex).

Figure 3 shows the COSY (phosphorus–phosphorus correlation) spectrum† of the reaction mixture containing pre-equilibrated *cis*-DDP and CTP in which we observe both the products.¶ The sets of peaks D, E, and F are assigned to the pure phosphato chelates[8] based on the following considerations: (i) phosphorus–phosphorus connectivity establishes that peaks D and E are coupled to F; (ii) the splitting pattern of the resonances is in keeping with the structures in that the β-phosphate group exhibits doublets of doublet (F) due to the coupling with two non-equivalent phosphate groups (α and γ), the γ-phosphate group shows doublets (D and E) due to the coupling with β-phosphate group, and the doublet of the α-phosphate group is masked under the doublet B for the free CTP molecule; (iii) the two doublets (D and E) of the γ-phosphorus atoms are for the two diastereoisomers (Λ and Δ) similar to those observed for the ATP complexes of Co^{III}[9] and Rh^{III};[10] (iv) resonances for the β and γ-phosphorus atoms appear at 10 and 8 ppm downfield as compared to the corresponding signals for the free ligand; (v) the co-ordination chemical shifts are within the values observed for the phosphato chelates of diamagnetic metal ions;[9—12] (vi) the ^{31}P NMR chemical shifts do not show significant change within pH range 5—8. The pH-dependent chemical-shift data suggest that the two co-ordinated phosphate groups are completely deprotonated above pH 5. The ionization of unco-ordinated α-phosphate group does not have much influence on the chemical shift of the bound phosphate group which has been observed recently in our laboratory in dealing with inorganic pyro- and tri-phosphato platinum(II) complexes.[12]

† ^{31}P NMR spectra were obtained on a GE 300 MHz instrument. Data acquisition parameters are: 25 μs pulse (90°), 1—2 s pulse delay, 16 K data point, 1—2 s acquisition time. The chemical shifts are with respect to 85% H_3PO_4. For the 2D-experiment, usual pulse sequences, $\pi/2\text{-}t_1\text{-}\pi/2\text{-}a_t$ (t_1 and a_t are the evolution and acquisition times), were followed utilizing 512 × 512 matrix and 2000 Hz sweep width. A gaussian window function of 3 Hz was used before generating the symmetrical contour plots. The total time elapsed for a typical 2D experiment was 18 h. Correlations between the less intense product peaks still exist in the non-symmetrised contours.

‡ The CMP-bridged diplatinum compound was isolated from the reaction mixture,[5] *cis*-$Pt(en)(H_2O)_2^{2+}$ and CMP at pH 6—7. Identical experimental conditions were chosen for the CTP-reaction. Furthermore, ^{31}P NMR spectra of the Bau's CMP-bridged compound exhibit 4—6 ppm co-ordination chemical shifts in the pH range 2—8. Similar co-ordination chemical shifts were observed for peaks G. Moreover, the HPLC separation on a reversed phase C-18 column is also consistent with a dimeric structure.

§ In this experiment, *cis*-platin was dissolved first. At equilibrium, the platinum substrate distributes into 66% $Pt(Cl)(NH_3)_2(H_2O)^+$, 12% $Pt(NH_3)_2(H_2O)_2^{2+}$ and *cis*-DDP.

¶ The ^1H NMR spectrum of the reaction mixture exhibits two doublets at 7.98 (*J* 7.4 Hz) and 6.15 ppm (*J* 7.5 Hz) which appeared as shoulders on the two doublets of H-6 (7.83 ppm, *J* 7.6 Hz) and H-5 (6.05 ppm, *J* 7.6 Hz) protons of free cytidine base protons. These base protons which are usually affected by nitrogen co-ordination are too far away to be influenced by the phosphate binding. No major differences in the sugar protons (H_1', H_2' *etc.*) for the complexed and uncomplexed nucleotide were observed.

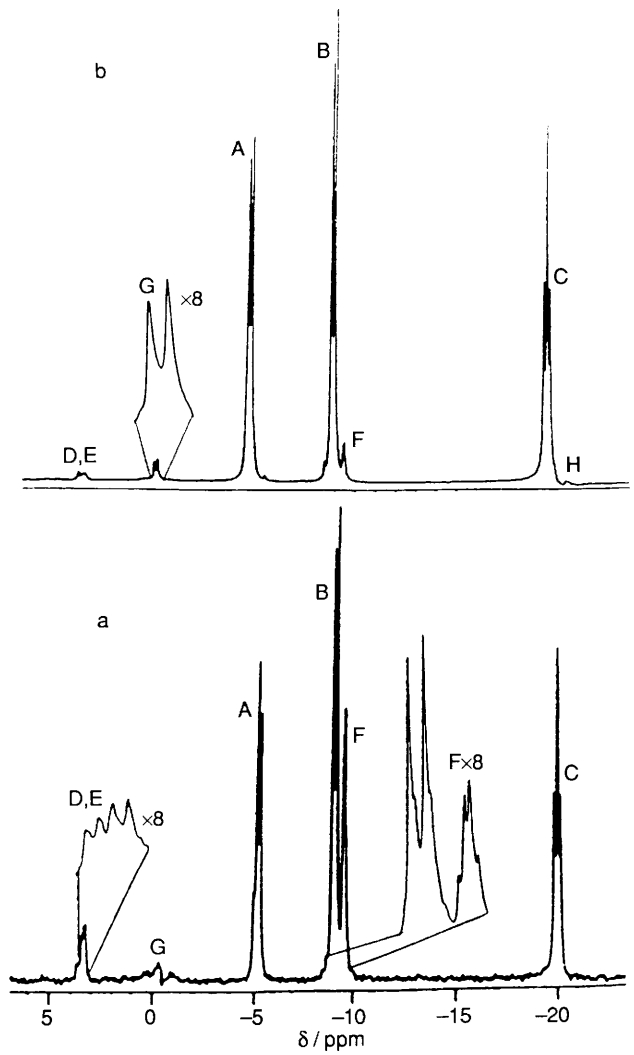


Figure 1. Proton decoupled 126.5 MHz ^{31}P NMR spectra of (a) CTP (20 mM) plus *cis*-DDP (5 mM) after 12 h of mixing at 40 °C (pD 7.0); (b) CTP (20 mM) plus pre-equilibrated *cis*-DDP. Peaks A, B, and C are the resonances of the excess free CTP molecules; D, E, and F are for the Λ and Δ phosphato chelates, G and H are for bis(CTP-bridged) diplatinum complex.

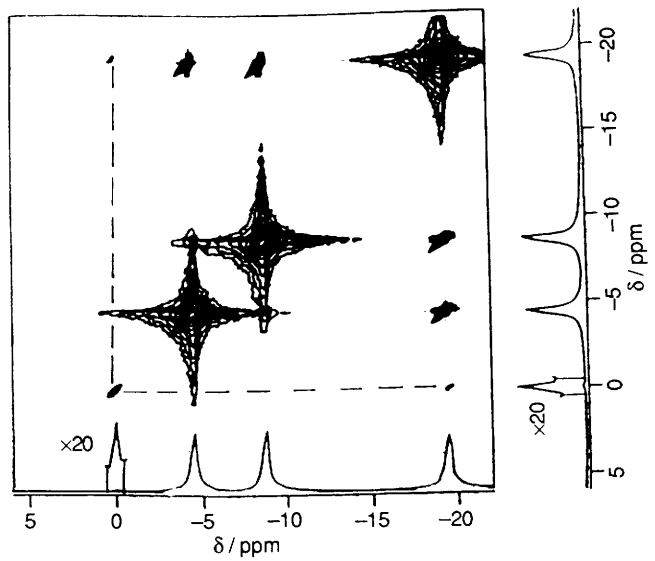


Figure 2. COSY spectra (phosphorus–phosphorus correlation) of CTP (20 mM) plus $[cis\text{-}Pt(NH_3)_2(H_2O)_2]^{2+}$ (5 mM). Other conditions are the same as in Figure 1. The major product peaks are G and H and they are coupled as indicated by the dotted line.

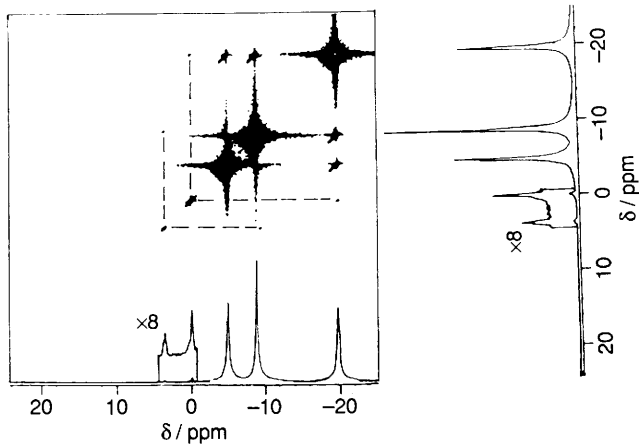
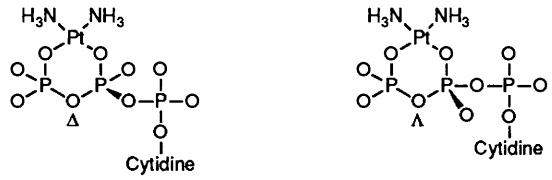


Figure 3. COSY spectrum (phosphorus–phosphorus correlation) of the reaction mixture consisting of CTP and pre-equilibrated *cis*-DDP as indicated in Figure 1(b). Connectivities of the product peaks are indicated by dotted lines.



The reaction of *cis*-DDP with CDP also yielded two phosphato chelates (diastereoisomers, Λ and Δ) as evidenced by the presence of two sets of doublets in the ^{31}P spectrum appearing *ca.* 10 ppm downfield from the corresponding resonances of the free ligand. Phosphato chelates and dimeric complexes can be separated from the reaction mixture utilizing a reversed-phase C-18 column with phosphate (pH 6.8) and formate buffers (pH 3.8). The complexes are stable in phosphate buffer but undergo relatively rapid aquation ($t_{1/2}$ *ca.* 10 min) in the lower pH (3—4 in the absence or presence of formate buffer). This aquation is in accordance with the acid-catalysed hydrolysis of platinum(II) phosphato complexes.

Results of this work may help us to gain an insight into the mechanism of inhibition of ATPase. The irreversible phosphate chelation by the nucleoside triphosphate at physiological pH perhaps blocks the dication binding on the substrate and inhibits the activities of this phosphorylation enzyme. Moreover, a slow but irreversible phosphate binding may also affect the fidelity of DNA synthesis since nucleoside-5′-triphosphates are primary substrates for DNA polymerases. In fact, Pt^{II} induced mutagenesis has been documented.[13] Finally, Λ and Δ phosphato chelates of some substitution inert metal ions such as Cr^{III}, Rh^{III}, and Co^{III} are used to elucidate the stereospecificity of certain phosphokinase enzyme activities.[14] Platinum(II) phosphato chelates offer advantages over the tripositive metals in that the platinum complexes are quite inert toward hydrolysis at physiological pH while base-catalysed aquations take place at appreciable rates for the tricationic metal complexes. Platinum(II) mimics the biolog-

ical dication metal requirements for enzyme activities, and NMR spectroscopy can be used to characterize the enzyme-catalysed products in solution (Pt is diamagnetic).

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