^{195}Pt and ^{31}P Nuclear Magnetic Resonance Studies of the Binding of the *cis*-$Pt(NH_3)_2^{2+}$ Moiety to Phosphate in Aqueous Solution

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In investigations of the origins of the antitumor activity of *cis*-$Pt(NH_3)_2Cl_2$, a clinically useful drug, the binding of the *cis*-$Pt(NH_3)_2^{2+}$ moiety to a variety of functional groups found in nucleic acids and proteins has been examined [1–4]. Little attention has been given to the binding of *cis*-$Pt(NH_3)_2^{2+}$ to the oxygen atoms of phosphate groups despite the fact that early experimental studies of the binding of platinum complexes to DNA and nucleosides produced evidence that suggested that platinum–phosphate binding was a significant factor in these systems [5, 6]. Here we present ^{195}Pt (spin ½, natural abundance 33.4%) and ^{31}P (spin ½, natural abundance 100%) NMR spectroscopic evidence for binding of phosphate by $Pt(NH_3)_2^{2+}$. In addition to its significance regarding the interaction of nucleic acids with platinum compounds, this work clearly demonstrates that phosphate buffers will have a definite affinity for these complexes and caution needs to be exercised in using phosphate buffers in the study of *cis*-$Pt(NH_3)_2(H_2O)_2^{2+}$.

Our data are shown in Figs. 1 and 2 where ^{195}Pt and ^{31}P NMR spectra obtained from mixtures of *cis*-$Pt(NH_3)_2(H_2O)_2^{2+}$ and sodium phosphate buffer at pH 3.22 are displayed. Both sets of spectra were acquired under similar conditions, and they are directly comparable. The ^{195}Pt-NMR spectra were obtained using ammonia which is enriched in ^{15}N (spin ½). This eliminates the line broadening due to the quadrupolar nucleus ^{14}N and enables direct observation of Pt–N coupling. As can be seen in Fig. 1 the triplet (labeled A) due to *cis*-$Pt(NH_3)_2(H_2O)_2^{2+}$ decreases in intensity over a period of 30 minutes in the presence of phosphate buffer while four new triplets (B, $^1J(Pt, N)$ 377 Hz; C, $^1J(Pt, N)$ 380 Hz; D, $^1J(Pt, N)$ 380 Hz; E, $^1J(Pt, N)$ 386 Hz) grow in intensity. The triplet nature of these resonances indicates that both amine ligands are retained within the coordination sphere of platinum, and the magnitudes of $^1J(Pt, N)$ for these species indicate that the amine ligands are opposite ligands of low *trans*-effect [7]. Since the condensation of *cis*-$Pt(NH_3)_2(H_2O)_2^{2+}$ to form hydroxy bridged dimers and trimers is repressed at pH 3.22, it is necessary to conclude that phosphate binding to the *cis*-$Pt(NH_3)_2^{2+}$ moiety is occurring.

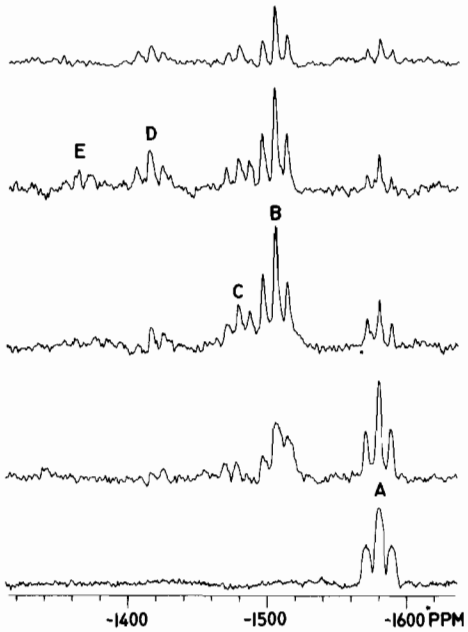

Fig. 1. The 42.9 MHz $^{195}Pt\{^1H\}$ NMR spectra of an aqueous solution (12 mm tube) containing 0.20 *M* *cis*-$Pt(^{15}NH_3)_2(H_2O)_2^{2+}$ and 0.60 *M* sodium phosphate buffer at pH 3.22 at 25 °C. The bottom trace shows the spectrum of the platinum complex alone in the absence of phosphate. The other traces were obtained at the times indicated after adding the phosphate to the platinum solution. The spectra were obtained using a 16 KHz spectral width using 5000 scans with a 20-μs pulse (tilt angle 60°) and an acquisition time of 125 millisecond and 250 millisecond delay time. The reference is an external, aqueous Na_2PtCl_6 solution.

The perturbation of phosphate is confirmed by the ^{31}P-NMR spectra shown in Fig. 2. In addition to the singlet due to uneffected phosphate at 0 ppm, four new resonances (labeled F, G, H, and I) are seen at lower field. These grow in rapidly and appear to have reached a steady state after about 30 min. The rate of growth of these signals indicates that they are intimately related to the changes seen in the ^{195}Pt-NMR spectra (Fig. 1). The lack of detectable phosphorus-platinum coupling in these spectra is not surprising.

Along with the spectral changes shown in the Figures, these solutions acquire a very pale blue coloration during the course of our observations and a small quantity of a blue/black, non-crystalline solid precipitates from solution. The quantity of

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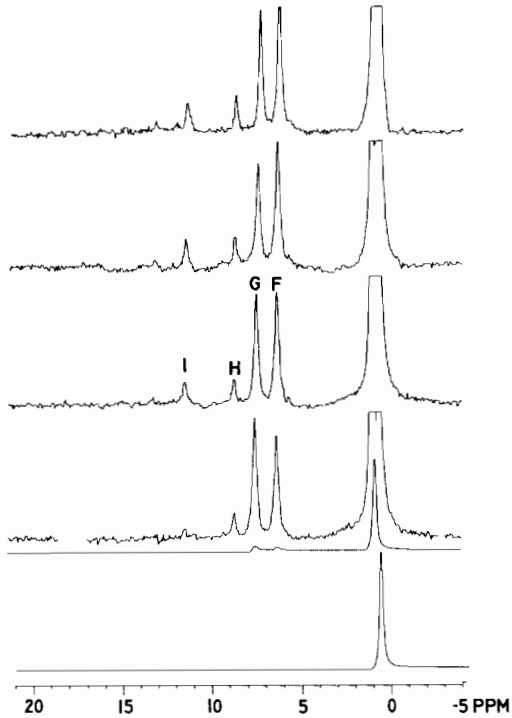

Fig. 2. The 81 MHz $^{31}P\{^1H\}$ NMR spectra of an aqueous solution (12 mm tube) containing 0.20 *M* *cis*-$Pt(NH_3)_2(H_2O)_2^{2+}$ and 0.60 *M* sodium phosphate buffer at pH 3.22 at 25 °C. The bottom trace shows the spectrum of the phosphate buffer alone before the addition of the platinum complex. The other traces were obtained at the times indicated after the addition of the platinum complex. The spectra were obtained using a 10 KHz spectral width using scans with a 7 μs pulse (tilt angle 60°) and an acquisition time of 819 millisecond and a 500 millisecond delay time. The reference 85% external phosphoric acid and the high frequency positive convention recommended by IUPAC is used in reporting chemical shifts.

solid formed continues to increase for many hours. This platinum phosphate blue has negligible solubility in water, and it appears to be similar to other platinum blues [3]. The infrared spectrum of the solid shows the following absorption bands 3415b, 3238s (νNH), 1705b, 1580s, 1126s, 1085s, 1045s, 894m, 864m, 718m, cm^{-1}.

The multiplicity of individual resonances seen in Figs. 1 and 2 suggest that several different complexes are formed between *cis*-$Pt(NH_3)_2^{2+}$ and phosphate. The obvious possibilities include monodentate, chelating, and bridging phosphate ligands. The formation of the solid platinum phosphate blue suggests that phosphate bridges are eventually formed. Further identification of the structural characteristics of these complexes would involve premature speculation.

These results clearly demonstrate that phosphate in acidic, chloride-free aqueous solution binds to $Pt(NH_3)_2(H_2O)_2^{2+}$. These observations from solution together with the X-ray crystal structure data on the complexes, $Pt_2(\mu\text{-}P_2O_7)(NH_3)_4$ [8], [Pt(5′-cytodine-monophosphate)(ethylenediamine)]$_2$ [9], and $Na_2[Pt_2(HPO_4)_4(H_2O)_2]$ [10], where platinum binding to phosphate oxygens in the solid state is shown, emphasize the importance of platinum phosphate interactions.

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