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ON THE NON-STOICHIOMETRY OF THE BINDING OF Pt(II) ANTI-NEOPLASTIC AGENTS TO INOSINE 5'-MONOPHOSPHATE#

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Summary. A non-stoichiometric material $[Na_4(5'\text{-IMP})_2 \cdot 15H_2O]_{0.2}[Na_2(Pt(5'\text{-IMP})_2(\text{trimethylenediamine})) \cdot 13.5H_2O]_{0.8}$ has been prepared and investigated by single-crystal X-ray methods and ^1H and ^{13}C nmr spectroscopy. The compound is isomorphous with the monosodium and disodium salts of 5'-IMP and two Pt(II)-5'-IMP compounds previously reported to be non-stoichiometric. However, the structural changes in the packing motif of the 5'-IMP molecules induced on Pt(II) coordination are uniform only if the 5'-IMP complex containing $(NH_3)_2Pt(II)$ is stoichiometric. Preliminary studies on the latter complex, synthesized in our laboratories, demonstrate that the complex is indeed stoichiometric.

Introduction. It was first observed by Rosenberg(1) that certain Pt(II) compounds are effective anti-cancer agents. Subsequent *in vivo* and *in vitro* studies implicate the binding of Pt(II) compounds to nucleic acids(2). Numerous solid state and solution studies aimed at elucidating the nature of the metal binding in these systems have been undertaken(2). Results obtained thus far clearly implicate metal binding at N(7) of guanosine or inosine as a primary mode of action. The exact nature of the binding is as yet unknown, however. Recently, intrastrand cross linking has been increasingly mentioned as a possible binding mode to explain the stoichiometry of the binding of Pt(II) compounds to polynucleotides(3). Such intrastrand cross linking could help to explain the anti-neoplastic activity of *cis*$[Pt(NH_3)_2Cl_2]$ and the inactivity of *trans*$[Pt(NH_3)_2Cl_2]$ (1,2).

Crystallographic studies on two Pt(II)-inosine 5'-monophosphate (5'-IMP)

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complexes bear directly on the intrastrand cross linking question. The cis[Pt(5'-IMP)$_2$(NH$_3$)$_2$]$^{2-}$(4) and cis[Pt(5'-IMP)$_2$(en)]$^{2-}$(5), where en = ethylenediamine, complex anions are formed by the reaction of cis[Pt(NH$_3$)$_2$(H$_2$O)$_2$]$^{2+}$ and cis[Pt(en)(H$_2$O)$_2$]$^{2+}$(6) with the disodium salt of 5'-IMP. In each case, the two water ligands of the diaquo complexes are displaced by the N(7) atoms of two symmetry-related 5'-IMP ligands. Two interesting and important features of these crystals were reported. First, the solids are non-stoichiometric (reported as 56% in the diammine complex and 38% in the en complex). Second, the crystal structures are isomorphous with those of the monosodium(7) and disodium(8) salts of 5'-IMP, with the Pt moiety occupying a water site in the structure of the sodium salts.

In a recent study on [Cu(5'-IMP)$_2$(diethylenetriamine)]$^{2-}$, we found that the 5'-IMP ligands in this stoichiometric Cu(II) complex occupied very similar sites to the four isomorphous 5'-IMP derivatives mentioned above(9). An analysis of these structures led us to propose that the non-stoichiometry of the Pt(II) compounds may result from a competition between the crystal packing forces operative in the sodium salts of 5'-IMP and the coordination-induced distortions in this basic 5'-IMP structural motif. Such distortions are expected to be greater on coordination of the 5'-IMP ligands by a Pt(II) than by a Cu(II) center.

We wish to report here briefly on the structural properties of the complex [Pt(5'-IMP)$_2$(tn)]$^{2-}$, where tn = trimethylenediamine. The addition of the structural information on the tn complex has allowed us to significantly extend our understanding of these systems and to call into question the reported non-stoichiometry of the diammine complex(4).

Experimental. Reaction of [Pt(tn)(H$_2$O)$_2$]$^{2+}$(6) with the disodium salt of 5'-IMP in neutral aqueous solution produces crystalline material with the following formulation: [Na$_4$(5'-IMP)$_2$·15H$_2$O]$_{0.2}$[Na$_2$(Pt(5'-IMP)$_2$(tn))·13.5H$_2$O]$_{0.8}$. Standard crystallographic data are as follows: a = 8.754(3)A, b = 22.956(7)A, c = 22.381(10)A, V = 4497.6A^3, D_{calcd} = 1.79 g cm^{-3}, D_{measd} = 1.79(2) g cm^{-3}, space group C222$_1$. Intensities for 3359 reflections were collected on a Syntex P-1 automated diffractometer employing graphite-monochromatized MoKα radiation.

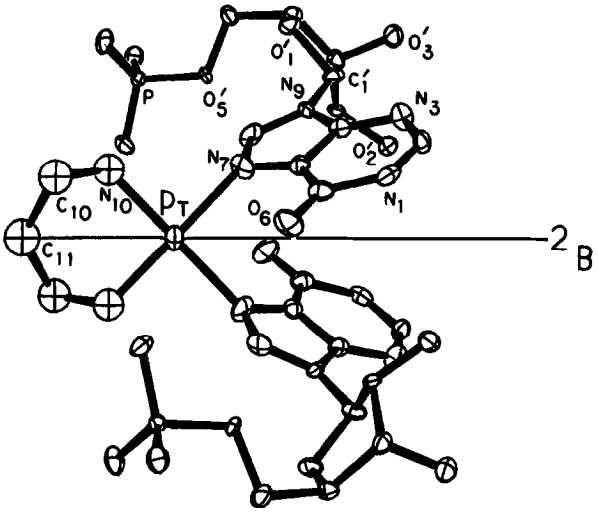

Fig. 1. A perspective view of the $[Pt(5'\text{-IMP})_2(tn)]^{2-}$ complex anion. The Pt-N(7) distance and the N(7)-Pt-N(7) bond angle are 2.08(1)A and 90.7(6)°, respectively.

A structural model based on the formulation given above has led to a final R value $[\Sigma||F_o|-|F_c||/\Sigma|F_o|]$ of 0.087.

Results and Discussion. The structure of $[Na_4(5'\text{-IMP})_2\cdot 15H_2O]_{0.2}[Na_2(Pt(5'\text{-IMP})_2(tn))\cdot 13.5H_2O]_{0.8}$, like the $(NH_3)_2Pt(4)$ and (en)Pt(5) complexes, bears a strong resemblance to the monosodium(7) and disodium(8) salts of 5'-IMP. In fact, all five structures are isomorphous. In the structure of the sodium salts of 5'-IMP, a water molecule lies on a crystallographic two-fold axis, 2_b, and links via hydrogen bonds of the type $OH_2\cdots N(7)$, two symmetry-related 5'-IMP anions. In the present system, this water molecule is partially substituted [approximately 80% as verified by nmr and the refinement of the Pt occupancy factor in the structural analysis] by the (tn)Pt moiety. The two hydrogen-bonds in the sodium salts(7,8) are replaced by cis coordination bonds between the (tn)Pt moiety and the N(7) atoms of the two-fold related 5'-IMP ligands, Fig. 1. Although the (tn)Pt compound studied here is similar to the previously reported $(NH_3)_2Pt$ and (en)Pt compounds, we have analyzed our data with a different model in mind.

TABLE I. Selected structural parameters in some $[Pt(5'\text{-IMP})_2(amine)]^{2-}$ complexes

Complex	N(7)···N(7)	Dihedral angle between purine rings	Ref.
$Na[5'\text{-IMPH}]\cdot 8H_2O$	3.48A	22°	7
$[Pt(5'\text{-IMP})_2(en)]^{2-}$	3.26	31	5
$[Pt(5'\text{-IMP})_2(tn)]^{2-}$	2.96(1)	37.9(8)	This work
$[Pt(5'\text{-IMP})_2(NH_3)_2]^{2-}$	2.83	43	4

In our analysis, crystals of the tn complex are made up of two types of unit cells, one which can be identified with the disodium salt of 5'-IMP and one which can be identified with the complex $Na_2[Pt(5'\text{-IMP})_2(tn)]\cdot 13.5H_2O$. We have been able, within the limits of the resolution of the atomic coordinates in these two unit cells, to nearly fully determine how the molecular constituents differ in location and occupancy in the two cells. In this regard, it is readily apparent that only the purine ring of the 5'-IMP ligand undergoes significant translational and rotational displacement on the substitution of the hydrogen-bonded water molecule by the (tn)Pt group. The adjustments in the purine ring parameters are insufficient, however, to allow us to refine separately the hydrogen-bonded and the coordinated 5'-IMP molecules. Thus, the selected parameters we report in Table I are a weighted average of the coordinated and hydrogen-bonded sites.

This structural information on the (tn)Pt complex has allowed us to clarify and correlate the structural data reported on the $(NH_3)_2Pt$ and (en)Pt complexes. In Table I, we present two of the most important structural parameters in these Pt complexes and compare them to those found in the monosodium salt. A perusal of Table I shows a smooth decrease in the N(7)···N(7) distance between the two-fold related 5'-IMP ligands in going from the monosodium salt to the diammine complex. A similar increase in the dihedral angle between the symmetry-related purine ring systems is also evident. Graphical representations of these parameters plotted

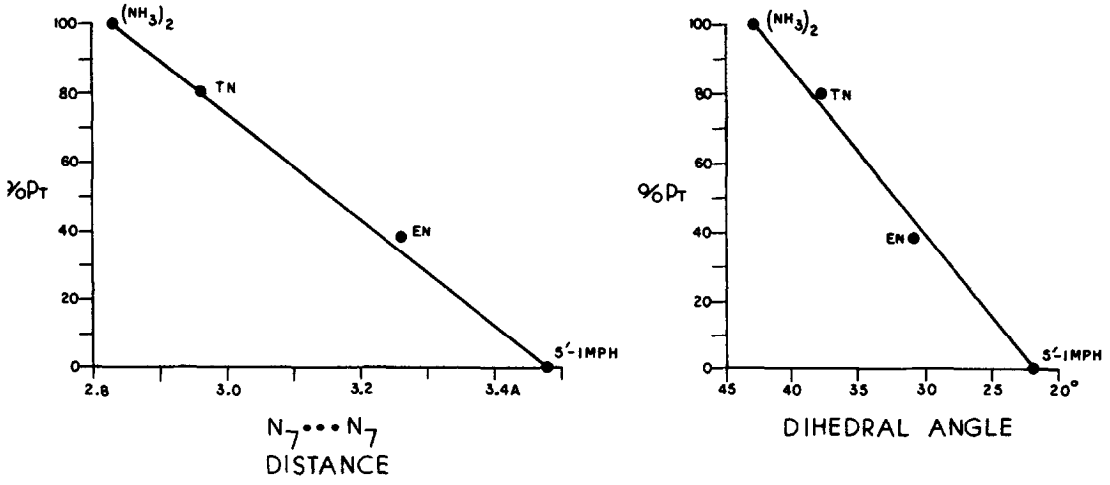

Fig. 2. Plots of % Pt vs the N(7)···N(7) distance and the dihedral angle between symmetry-related purine rings.

against the % Pt reported in the literature do not yield sensible relationships when the $(NH_3)_2Pt$ complex is included at the reported 56% occupancy. However, the data for the $(NH_3)_2Pt$ complex(4) fits the trends established by the monosodium salt and the tn and en complexes if we assume that the diammine complex is stoichiometric, Fig. 2. We, therefore, reprepared the diammine complex following the literature procedure and have been able to obtain crystalline samples of the diammine complex. On the basis of nmr data and density measurements on a large number of crystals, the diammine complex, indeed, appears to be stoichiometric with the following formulation: $Na_2[Pt(5'\text{-IMP})_2(NH_3)_2]\cdot 15.0H_2O[D_{calcd} = 1.823$ g cm^{-3}, $D_{obsd} = 1.815(6)$ g cm^{-3}; the D_{calcd} for the previously reported non-stoichiometric formulation is 1.731 g cm^{-3}]. We have at present reinvestigated some of the structural properties of the diammine complex and find cell constants virtually identical to those reported for the supposed non-stoichiometric compound(4).

The corrected formulation for the diammine complex and the smooth trends depicted in Fig. 2 reinforce our original argument that the crystal packing forces operative in the sodium salts are competitive with the distortions necessary to produce the ligand geometry displayed in the diammine complex. Apparently, the

en and tn complexes are less competitive (possibly owing to the increased steric factors associated with their chelate rings) with these packing forces than the diammine system. These crystal packing forces place restrictions on the geometry that the purine bases can adopt when complexed to the Pt(II). The ordered structure of double helical DNA would also place restrictions on the bonding. Reagents such as the Pt(II) anti-neoplastic agents may be able to induce greater distortions in the DNA structure than other metal species, such as Cu(II) complexes(9), which form weaker bonds. According to our analysis, the diammine complex is the first stoichiometric bis(Pt(II))nucleotide complex reported to date. The structure of this complex and the others in this series should be highly relevant to the interaction of cis$[Pt(NH_3)_2Cl_2]$ with DNA.

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6. We retain here the simplistic formulation of the reactive Pt species as the diaquo complex although the actual situation may in fact be much more complex; see for example, Faggiani, R., Lippert, B., Lock, C. J. L., and Rosenberg, B. (1977) J. Am. Chem. Soc. 99, 777.

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