DNA Binding of a Platinum(II) Complex Designed To Bind Interstrand but Not Intrastrand

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Pt(II)-based anticancer drugs such as *cis*-DDP (*cis*-[Pt(NH_3)$_2$$Cl_2$]) bind to DNA and form a number of bifunctional adducts.[1-3] Of these, one or more of the GG intrastrand, AG intrastrand, and GG interstrand adducts are believed to be responsible for the anticancer activity.[4,5] However, it is yet to be unequivocally established which of these adducts is responsible.[6-8] In order to shed further light on this question, we have modeled both intrastrand and interstrand *cis*-DDP adducts.[9-11] In both cases the NH_3 ligands of the drug form H-bonds with the DNA as shown in Figure 1. In the intrastrand adduct, one H-bond is to the phosphate backbone and the other is to the exocyclic oxygen of the 3′ guanine; in the interstrand adduct, both are to the phosphate backbone. The differences in the arrangements of these H-bonds suggested to us the design of a complex which should be readily able to form the interstrand adduct but less suited to forming the intrastrand adduct. The general form of such a complex is also shown in Figure 1, as is its proposed binding in intrastrand and interstrand binding sites. The two amine protons of such a complex are ideally disposed to form the H-bonds observed in the interstrand adduct but should not be able to form either of the H-bonds seen in the intrastrand adduct. Moreover, in the place of these H-bonds would be unfavorable interactions between the DNA and the aliphatic regions of the ligand. Whether these interactions would be sufficient to prevent intrastrand binding is unknown; however, our studies on the nonformation of GA adducts[9,10,12,13] indicate that an unfavorable interaction involving O6 of the 3′ G does mitigate against formation of the intrastrand bifunctional adduct.

The target complex was prepared by reaction of homopiperazine ($x = 2$, $y = 3$) with K_2PtCl_4, which yielded a pure, pale-yellow compound, [Pt(hpip)Cl_2] (hpip = homopiperazine = 1,4-diazacycloheptane). The structure of this compound was confirmed by X-ray crystallography.[14]

In order to establish whether this complex, [Pt(hpip)Cl_2], forms interstrand adducts, we carried out a simple DNA cross-linking assay using methods based upon those reported elsewhere.[8] Linearized plasmid DNA (pGEM3Zf(–)) was treated with [Pt(hpip)Cl_2] for 24 h and then denatured with 30 mM NaOH and

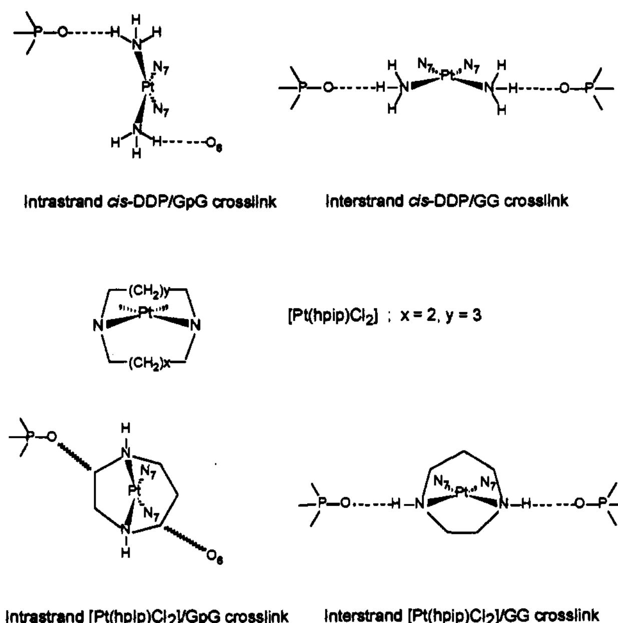

Figure 1. Schematic representations of the interactions between *cis*-DDP, [Pt(hpip)Cl_2], and DNA.

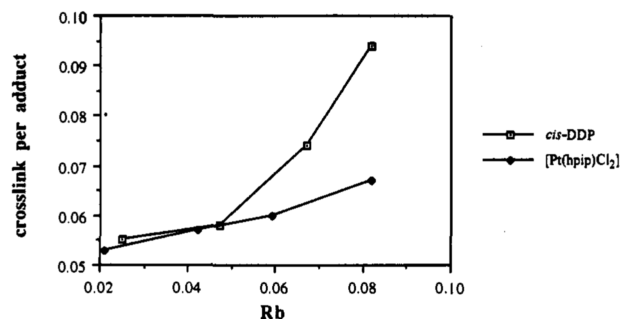

Figure 2. Interstrand cross-link adduct formation observed for *cis*-DDP and [Pt(hpip)Cl_2] binding to a synthetic 50 base-pair DNA oligomer.

renatured by the addition of 30 mM HCl. The Pt/DNA complex was eluted on an agarose gel with ethidium bromide as developing agent. *cis*-DDP, [Pt(dien)Cl]$^+$, and the bisplatinum complex [Cl(NH_3)$_2$PtNH_2CH_2CH_2NH_2Pt(NH_3)$_2$Cl]$^{2+}$ were run as comparisons and gave results similar to those obtained previously.[15] Since only cross-linked strands can re-form duplex DNA following renaturation, the presence of duplex DNA indicates that cross-linking has occurred. Visual estimation of the intensity of the duplex band, taken as an estimate of the degree of interstrand adduct formation, showed that [Pt(hpip)Cl_2] is as effective at forming interstrand adducts as *cis*-DDP. In order to better quantify cross-link formation, a radiolabeled 50 base-pair oligonucleotide was subjected to a similar denaturing/renaturing assay. Single and double-stranded DNA were separated on a denaturing polyacrylamide gel, and the amount of each was determined from the radioactivity of each band as described previously.[15] The results are summarized in Figure 2. At low R_b (moles of Pt bound per mole of nucleotide) values, [Pt(hpip)Cl_2] and *cis*-DDP were found to be equally effective at forming interstrand cross-links, and at higher R_b values, *cis*-DDP was more effective. The nature of the interstrand adducts formed by [Pt(hpip)Cl_2] cannot be deduced from these studies. Brabec and Leng have shown that *trans*-[Pt(NH_3)$_2$$Cl_2$] forms interstrand cross-links between guanine and the complementary cytotsine.[16]

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(14) [Pt(hpip)Cl_2] crystallizes in the orthorhombic space group *Pbcm* with $a = 7.7019(8)$, $b = 9.8080(12)$, and $c = 12.1944(14)$ Å. The structure was solved by heavy atom methods and refined using full-matrix least-squares methods to $R = 0.023$. Full details of the structure will be published elsewhere. An ORTEP diagram and tables of coordinates, bond lengths, and bond angles have been deposited as supplementary material.
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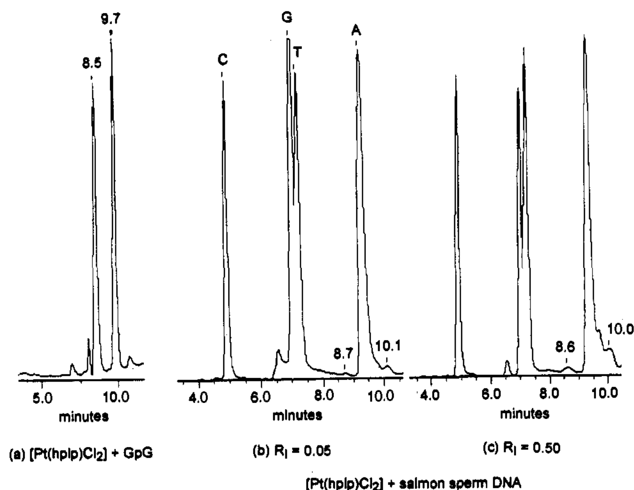

Figure 3. HPLC traces of the enzymatic digestion products resulting from reaction of (a) $[Pt(hpip)Cl_2]$ with GpG (1:1) and $[Pt(hpip)Cl_2]$ with salmon sperm DNA at (b) $R_t = 0.05$ and (c) $R_t = 0.50$.

A similar adduct seems unlikely in the case of $[Pt(hpip)Cl_2]$ because of its rigorously *cis* geometry and the gross deformation this would necessarily induce in the DNA structure.

The degree of intrastrand binding was assessed by reacting $[Pt(hpip)Cl_2]$ with salmon sperm DNA for 24 h, enzymatically digesting the products, and analyzing the mixture by HPLC. If $[Pt(hpip)Cl_2]$ formed significant levels of the intrastrand GG adduct, then the product which would result from digestion of this adduct would be $[Pt(hpip)GpG]^+$.[1-3] The chromatographic behavior of $[Pt(hpip)GpG]^+$ was first established by reacting $[Pt(hpip)Cl_2]$ with GpG followed by enzymatic digestion. This revealed two bands which we have shown by two-dimensional NMR spectroscopy to be isomers, probably arising from the asymmetry of the hpip ligand.[17] When DNA was treated with $[Pt(hpip)Cl_2]$ at an R_t (moles of Pt added per mole of nucleotide) value of 0.05 and digested, neither of the peaks corresponding to $[Pt(hpip)GpG]^+$ were observed in the chromatographic trace (Figure 3). At an R_t value of 0.5, weak bands corresponding to the two isomers of $[Pt(hpip)GpG]^+$ were observed. When the same procedure was repeated with $[Pt(en)Cl_2]$ at an R_t value of 0.05, a strong peak corresponding to $[Pt(en)GpG]^+$ was seen, as has been reported previously.[1,3] Intrastrand AG adducts were assessed in the same way; in this case, four HPLC peaks arose from the reaction between $[Pt(hpip)Cl_2]$ and ApG, probably because of the hpip asymmetry and the restricted rotation of the adenine about the Pt–N7(A) bond. No peaks corresponding to these adducts were seen in the digestion products of the reaction between $[Pt(hpip)Cl_2]$ and salmon sperm DNA. These results are consistent with $[Pt(hpip)Cl_2]$ not readily forming intrastrand crosslinks. $[Pt(hpip)(G)_2]$, the product expected from digestion of interstrand and intrastrand GpNpG adducts, elutes at 12.1 min. In the elution profile from the $[Pt(hpip)Cl_2]$/DNA reaction, large peaks were observed at this time point; however, these are believed to arise from monofunctional adducts (*vide infra*).

If $[Pt(hpip)Cl_2]$ does not form large numbers of either interstrand or intrastrand bifunctional adducts, then the question arises as to how the bulk of it does bind. Atomic absorption analysis of the chromatographed samples showed that the bulk of the Pt was present in a number of broad peaks eluting at 10–15 min. To determine whether these peaks corresponded to various monofunctional adducts of the type $[Pt(hpip)(X)G]^{x+}$, NH_4HCO_3 was added to the DNA/$[Pt(hpip)Cl_2]$ mixture 24 h after initiation of the reaction. Following a further 24 h and digestion, a broad band containing more than 50% of the Pt eluted at 9–11 min. $[Pt(hpip)(NH_3)G]^+$, the product expected from digestion of monofunctional adducts treated with NH_4HCO_3, eluted at 9–11 min. A second broad band containing the remaining platinum eluted at 12–14 min. Analysis of this fraction showed that it was not a Pt/nucleotide complex and therefore must arise from $[Pt(hpip)Cl_2]$ that has not reacted with the DNA. Thus, at least 90% of $[Pt(hpip)Cl_2]$ that does react with the DNA binds monofunctionally. The apparent decrease in the size of the peak corresponding to free guanine on going from $R_t = 0.05$ to $R_t = 0.5$ may be additional evidence for the formation of large numbers of monofunctional adducts.

Monofunctional *cis*-DDP/guanine adducts are precursors to bifunctional intrastrand adducts, and closure to the bifunctional adduct occurs on the time scale of hours.[18,19] $[Pt(hpip)Cl_2]$ is evidently able to form monofunctional adducts but is less able to form bifunctional intrastrand adducts than is *cis*-DDP.

In order to determine the cytotoxic activity of $[Pt(hpip)Cl_2]$, a series of *in vitro* assays was carried out using methods outlined in detail elsewhere.[20] An IC_{50} of greater than 50 μM was found against the L1210 mouse leukemia line in cell culture using both cell count and MTT assaying methods. Against the human tumor cell line UCRU BL13/0,[21] the IC_{50} after 72 h was found be 45 μM. Thus, compared to *cis*-DDP, which had IC_{50} values in the range 1–1.5 μM in these assays, $[Pt(hpip)Cl_2]$ is very inactive *in vitro*.

$[Pt(hpip)Cl_2]$ was designed to be able to bind interstrand but to be less able to bind intrastrand. Cross-linking and DNA binding assays give results consistent with our having achieved this design goal, and *in vitro* determinations of cytotoxicity show it to be inactive. This inactivity is unusual because the complex fulfills all of the requirements believed to be necessary for activity;[5] it has a square-planar geometry, *cis* leaving groups, *cis* amine groups, and each amine has at least one hydrogen attached to it. Also, it has a solubility similar to that of *cis*-DDP. Thus, the inactivity is probably a consequence of the stereochemistry of the complex and specifically the fact that it is unlikely to readily form bifunctional intrastrand adducts. The inactivity of $[Pt(hpip)Cl_2]$ taken with its ability to form interstrand adducts is potentially evidence for the intrastrand adduct *not* being a major cause of cell death, at least *in vitro*. However, other possible explanations for this inactivity, such as poor transport into the cell, need to be investigated.

In conclusion, we have successfully designed and prepared a complex that readily forms bifunctional interstrand adducts but not bifunctional intrastrand adducts. Contrary to expectations based on established structure–activity relationships, this compound is inactive *in vitro*.

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Supplementary Material Available: Tables of positional parameters, bond lengths and angles, and thermal and hydrogen positional parameters and an ORTEP plot of $[Pt(hpip)Cl_2]$ (2 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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