Bis(platinum) Complexes Containing Two Platinum *cis*-Diammine Units. Synthesis and Initial DNA-Binding Studies

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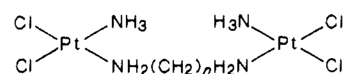
Abstract: The synthesis of bis(platinum) complexes of the general formula $[\{cis\text{-}PtCl_2(NH_3)\}_2NH_2(CH_2)_nNH_2]$ is reported. These complexes contain two units of the antitumor-active platinum *cis*-diammine entity tethered together by a variable-length diamine backbone. The DNA binding of these complexes has been examined by assessment of their inhibition of restriction endonuclease activity on linearized DNA. The complexes, especially the butanediamine ($n = 4$) derivative, inhibit restriction endonuclease activity significantly better than the parent $cis\text{-}[PtCl_2(NH_3)_2]$. Where antitumor complexes act by a DNA-binding mechanism, analogues with improved DNA binding may have increased chemotherapeutic potential.

The intracellular target of the anticancer drug $cis\text{-}[PtCl_2(NH_3)_2]$, cisplatin, is believed to be DNA.[2,3] The most favored explanation for the mechanism of action, and particularly the fact that the cis isomer is antitumor active whereas the trans isomer is not, is that the intrastrand link between two adjacent guanine (G) bases formed by the cis isomer, and sterically inaccessible to the trans, causes a localized conformational change such as a kink in DNA,[4-6] and models relating to the further effects of the kinking on the DNA helix have been proposed. The intrastrand linkage, or the overall conformational alteration resulting from that lesion, may be especially difficult to repair in comparison to the interstrand linkages.

A recent study has shown that a principle target for platinum in the tumor virus SV40 is the regulatory sequence GGGCGG, and the suggestion has been made that targeting this sequence may contribute to drug activity.[7] The results confirm earlier reports where a study using restriction enzymes indicated a "hyperreactive" site around the guanine-rich Bgl 1 cleavage site and within the regulatory region of the virus.[8] Although there is much further evidence that platinum complexes bind preferentially to guanine,[1,2] the binding is somewhat sequence and conformation specific, and one example noted is the absence of binding in a G_5CG_2 region in a 165-bp DNA studied by exonuclease III digestion.[9]

A pertinent question for any DNA-binding drug is whether increased DNA binding would result in more effective antitumor properties. This communication reports on the synthesis and DNA binding of bis(platinum) complexes containing two units of the *cis*-diammine complex linked by variable-length hydrocarbon chains of the general formula $[\{cis\text{-}PtCl_2(NH_3)\}_2NH_2(CH_2)_nNH_2]$.



The complexes are prepared by reaction of the monoanion $K[PtCl_3(NH_3)]$ with an appropriate diamine (1,4-diaminobutane etc.) in MeOH. Substitution occurs cis to the NH_3 ligand,[10] and the diamine thus also achieves the bridging. (There is precedence for the use of 1,4-butanediamine as a bridging rather than a chelating ligand in the reaction of the diamine with $cis\text{-}[PtCl_2(Me_2SO)_2]$, giving the analogous complex $[\{trans\text{-}PtCl_2(Me_2SO)\}_2NH_2(CH_2)_4NH_2]$.[11]

The complexes reported here have two platinum *cis*-diammine moieties functionalized in the same molecule separated by a variable-length backbone. The complexes are white to cream solids, only very sparingly soluble in water and alcoholic solvents, and soluble in polar solvents such as dimethyl sulfoxide (DMSO), dimethylformamide (DMF), and dimethylacetamide (DMA). Elemental analysis (C, H, N, Cl) confirms the stoichiometry.[12] Recrystallization is effected in DMA/1 N HCl.

The cis configuration of the chlorides on each platinum atom is confirmed by IR spectroscopy. Representative values of ν(Pt-Cl) are 326 and 318 (sh) cm^{-1} for the $n = 4$ derivative, 330 and 310 (vbr) cm^{-1} for $n = 5$, and 326 cm^{-1} for $n = 6$. Higher homologues ($n > 7$) show one broad band centered at 330 cm^{-1}. The presence of broad bands in the ^1H NMR spectrum corresponding to the various groups of CH_2 resonances is indicative of unhindered rotation around the diamine backbone. The geometry, and purity, is further confirmed by observation of single ^{195}Pt NMR resonances at −1930 ppm ($n = 6$), −2035 ppm ($n = 5$), and −2021 ppm ($n = 4$) relative to $PtCl_6^{2-}$ and indicative of a $cis\text{-}N_2Cl_2$ core (cf. $cis\text{-}[PtCl_2(NH_3)_2]$ at −2048 ppm[13]). Interaction of the $n = 5$ dimer with H_2O_2 gave an analytically pure yellow solid[11] characterized by a slight increase in frequency of ν(Pt-Cl), 336 cm^{-1}, and the presence of a broad band centered

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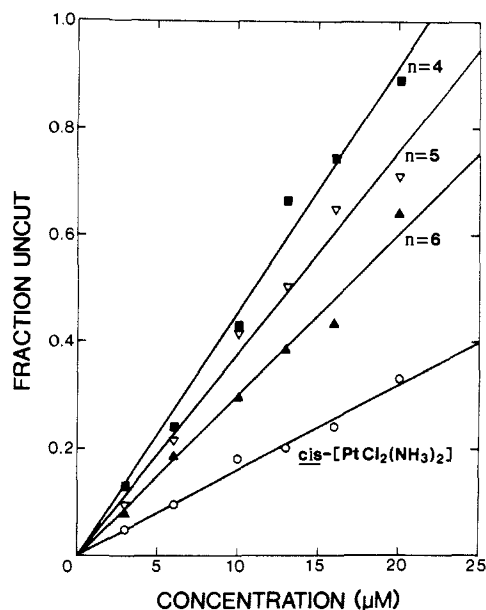

Figure 1. Inhibition of Eco R1 endonuclease activity on plasmid (pSV2-gpt) DNA after a 1-h incubation as a function of platinum complex concentration for $[\{cis\text{-}PtCl_2(NH_3)\}_2NH_2(CH_2)_nNH_2]$: ■, $n = 4$; ▽, $n = 5$; ▲, $n = 6$; ○, $cis\text{-}[PtCl_2(NH_3)_2]$.

at 540–560 cm^{-1}, attributed to ν(Pt–OH) and thus indicative of oxidation to Pt(IV).

The DNA binding of the bis(platinum) complexes has been studied with the inhibition of restriction endonuclease activity on plasmid DNA. The assay has been used by us previously to study binding of platinum-radiosensitizer complexes and has been described in detail.[14] Binding of a molecule at or near the restriction site inhibits the enzyme cleavage. Briefly, the plasmid DNA pSV2-gpt (5.2 kilobase pairs) is dissolved in Tris-Cl (100 mM)/EDTA (10 mM) buffer, pH 8, and linearized using PvuII. After exposure of the linear DNA to the Pt complex (dissolved in the same buffer) at 37 °C, at stated concentrations and given times (see figures), the complex is removed by gel filtration and treated with the restriction enzymes Bam H1 or Eco R1 (for 30 min at 37 °C, pH 7.9). These enzymes (Bam H1, recognition site G/GATCC, and Eco R1, recognition site G/AATTC) were chosen for study because there is only one such sequence per linearized DNA. The DNA is then electrophoresced in agarose gel, stained with aqueous ethidium bromide, and photographed. The density of the bands (cleaved vs uncleaved) is used to assess the degree of enzyme activity.

Figure 1 shows the degrees of inhibition of the bis(platinum) complexes compared to $cis\text{-}[PtCl_2(NH_3)_2]$ after a 1-h incubation at various concentrations. The concentration to achieve 20% inhibition of Eco R1 for the $n = 4$–6 bis(platinum) complexes is approximately 4.5, 5.3, and 7 μM, respectively, compared to a value of 12 μM for the monomeric complex (Figure 1). For all complexes Bam H1 is inhibited to a greater extent (2–3-fold) than Eco R1 in accordance with previous results,[8,14] emphasizing again the affinity of Pt for G-rich regions of DNA.

Figure 2 shows a time course study of the Eco R1 inhibition by the three bis(platinum) complexes and $cis\text{-}[PtCl_2(NH_3)_2]$ at 10 μM. The binding occurs relatively rapidly, especially for the $n = 4$ complex, where there is a 3–4-fold increase in inhibition over the monomeric complex after 1 h. The differences shown in Figure 1 therefore reflect this kinetic effect. The Bam H1 inhibition is also faster than that of Eco R1.

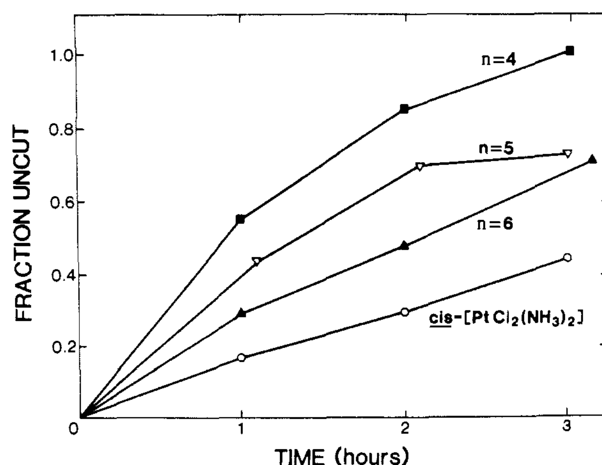

Figure 2. Inhibition of Eco R1 endonuclease activity as a function of time of incubation at 37 °C for the complexes $[\{cis\text{-}PtCl_2(NH_3)\}_2NH_2\text{-}(CH_2)_nNH_2]$: ■, $n = 4$; ▽, $n = 5$; ▲, $n = 6$; ○, $cis\text{-}[PtCl_2(NH_3)_2]$. All complexes were at 10 μM.

The inhibition of restriction enzyme cleavage is a reflection of platinum binding at that site. There is thus differential DNA binding with chain length variation, and, for the $n = 4$ dimer especially, this is not merely due to the presence of two Pt atoms capable of independent binding. When the potential for increased selectivity is assessed, other factors, such as comparative Pt/nucleotide ratios, must be taken into consideration. A further relevant point is that the relative inhibition by Pt complexes is not solely a function of the guanine content within the restriction sequence. Thus, in a detailed study of the action of restriction enzymes on SV40 DNA, the cleavage produced by $cis\text{-}[PtCl_2(NH_3)_2]$ binding is enhanced by the presence of guanines (especially adjacent guanines) close to the cutting site.[8,15] Similarly, preferential inhibition of a Pst 1 site in pSMI DNA next to a four-guanine sequence has been observed.[16]

The bis(platinum) complexes represent the first examples of Pt species (as their chloride complexes) with greater affinity for DNA in comparison to cisplatin. It is probable that the localized distortion produced on DNA by binding of these molecules will be much more pronounced than for monomeric $cis\text{-}[PtCl_2(NH_3)_2]$. Further, due to the kinetic preference of guanine binding to Pt and the importance of guanine sequences as noted above, sequences of more than two guanines may be more efficiently targeted by the bis(platinum) complexes in comparison to $cis\text{-}[PtCl_2(NH_3)_2]$. This may have consequences both for enhanced cytotoxicity and for repair of cytotoxic lesions. The cytotoxicity of these complexes does in fact follow the order of DNA binding (in both CHO and L1210 cell lines), and full results on the biological activity of these complexes will be presented in due course.

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