DNA Promotes the Reaction of *cis*-Diamminedichloroplatinum(II) with the Exocyclic Amino Groups of Ethidium Bromide

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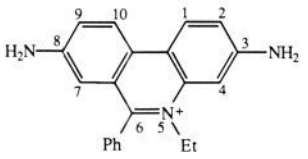
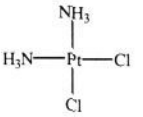
DNA functions as a template for replication and transcription of genetic information in cells. Duplex DNA can also provide a one-dimensional surface that modulates the reaction of small molecules[1] or directs the diffusion of macromolecules.[2] Here we report details of a chemical reaction between the antitumor drug *cis*-diamminedichloroplatinum(II) (*cis*-DDP) and the ethidium



Ethidium
Eth



cis-Diamminedichloroplatinum(II)
cis-DDP

cation (Eth) that is promoted by DNA. In this chemistry, a *cis*-$\{Pt(NH_3)_2Cl\}^+$ unit coordinated at a purine N7 site on DNA is ideally positioned to react with an ethidium molecule intercalated between the adjacent base pairs. The result is a metastable complex in which the *cis*-$\{Pt(NH_3)_2\}^{2+}$ moiety covalently links the 3- or 8-amino group of Eth and the DNA nucleobase.

Duplex DNA dramatically increases the extent of reaction between *cis*-DDP and ethidium bromide.[3] The nature of the Pt–Eth–DNA ternary complex so formed has been uncertain, however, since the complex is rather unstable, slowly losing ethidium upon incubation at 37 °C ($t_{1/2} \approx 10$ h). The recent synthesis and structural characterization of *cis*-$[Pt(NH_3)_2(N9\text{-}9\text{-AA})Cl](NO_3)$,[4] in which platinum is coordinated to the exocyclic amino group of 9-aminoacridine (9-AA), suggested that platinum might also bind to the exocyclic amino groups of ethidium to form complexes of the general formula *cis*-$[Pt(NH_3)_2(Eth)Cl]^{2+}$ that could be characterized chemically and subsequently bound to DNA. As anticipated, these platinum–ethidium precursor complexes form in the reaction of ethidium nitrate and $[Pt(NH_3)_2ClL]^n$, $L = NO_3^-$ ($n = 0$) or DMF ($n = 1+$), in DMF.[5] This reaction produces, after HPLC purification, the acetate salts *cis*-$[Pt(NH_3)_2(N3\text{-Eth})Cl](OAc)_2$ (**1**) and *cis*-$[Pt(NH_3)_2(N8\text{-Eth})Cl](OAc)_2$ (**2**).[6] The identity of the purified complexes has been unambiguously determined by ^1H NMR, ^{195}Pt NMR, and mass spectrometric studies.[6] The structure of the N8 linkage isomer has also been determined by X-ray diffraction (Figure 1).[6,7]

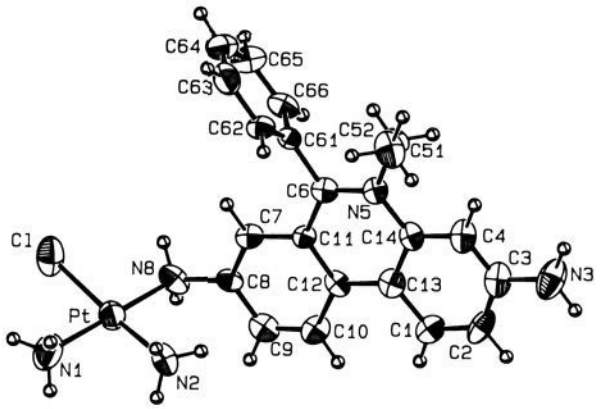


Figure 1. Structure of *cis*-$[Pt(NH_3)_2(N8\text{-Eth})Cl]^{2+}$ showing the 40% thermal ellipsoids (except for hydrogen) and atom labeling scheme. Selected bond distances (Å) and angles (deg) are as follows: Pt–Cl, 2.280 (9); Pt–N1, 2.03 (2); Pt–N2, 2.11 (2); Pt–N8, 2.05 (1); N3–C3, 1.39 (2); N8–C8; 1.46 (2); Pt–N8–C8, 118.0 (8). Only one of the two disordered positions for the platinum coordination plane is depicted (see Supplementary Material).

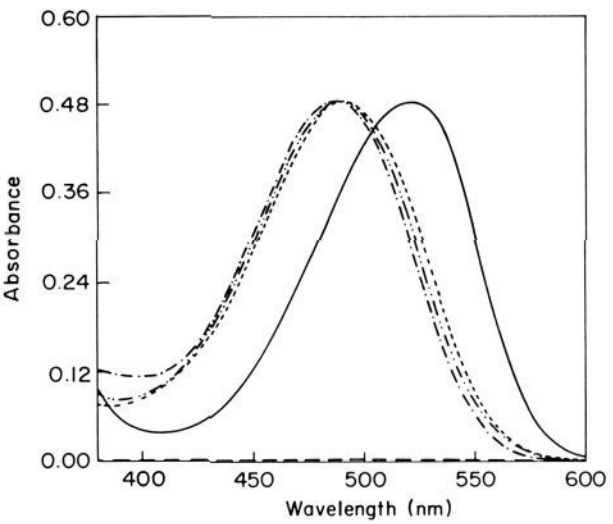


Figure 2. Optical absorption spectra of ethidium–DNA complexes: (—) ethidium + DNA before *n*-butanol extraction, (— —) ethidium + DNA after extraction, (–·–) *cis*-$[Pt(NH_3)_2(N3\text{-Eth})Cl](OAc)_2$ (**1**) + DNA after extraction, (- -) *cis*-$[Pt(NH_3)_2(N8\text{-Eth})Cl](OAc)_2$ (**2**) + DNA after extraction, (–···–) ethidium + *cis*-DDP + DNA after extraction. Experimental details are given in Supplementary Material.

The optical absorption spectra of the DNA adducts of **1** and **2** are shown in Figure 2. These spectra are quite distinct from that of intercalatively bound ethidium but almost exactly match the absorption spectrum of the ternary complex formed by *cis*-DDP and ethidium on DNA (Figure 2).[6] Furthermore, ethidium is slowly released from DNA complexes of **1** and **2** upon incubation at 37 °C. These data demonstrate that the precursor ethidium–platinum complexes **1** and **2** form the same ternary complexes on DNA as obtained in the reaction of *cis*-DDP with ethidium bromide in the presence of DNA. The ternary complexes therefore must consist of platinum coordinated by two cis ammines, an N3- or N8-bound ethidium, and a DNA ligand, presumably the N7 atom of either guanine or adenine.[8]

We assume that the Pt–Eth–DNA complex forms via ethidium intercalation, known to be rapid,[9] followed by monofunctional binding of *cis*-DDP to DNA. To model this intermediate, a molecular mechanics energy minimized structure[10] of the duplex DNA hexamer, d[C(1)pG(2)pC(3)pG(4)pC(5)pG(6)]·d[C(7)-

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(6) Details are given in Supplementary Material.

(7) Crystal data for *cis*-$[Pt(NH_3)_2(N8\text{-Eth})Cl](ClO_4)_2 \cdot MeOH \cdot 0.5H_2O$, $C_{22}H_{31}N_5O_{9.5}Cl_3Pt$, $M_r = 818.97$, triclinic, space group $P\bar{1}$, $a = 13.269$ (7) Å, $b = 16.125$ (3) Å, $c = 8.156$ (5) Å, $\alpha = 99.93$ (4)°, $\beta = 96.29$ (6)°, $\gamma = 112.29$ (3)°, $V = 1561$ (3) Å^3, $Z = 2$, $\rho_{calcd} = 1.741$ gm/cm^3. For 2697 observed reflections and 376 variables, the current discrepancy indices are $R = 0.052$, $R_w = 0.068$.

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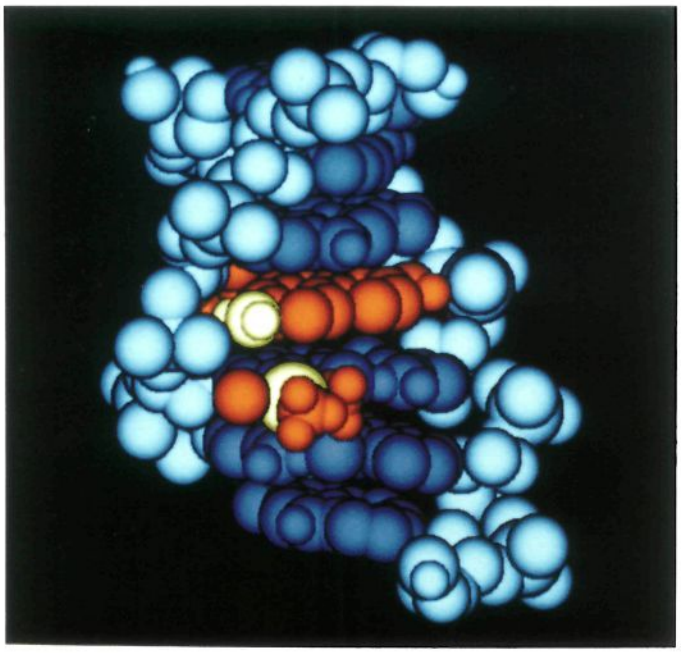

Figure 3. Proposed intermediate in the DNA-promoted reaction of *cis*-DDP with ethidium. This model is comprised of a duplex DNA hexamer, d[C(1)pG(2)pC(3)pG(4)pC(5)pG(6)]·d[C(7)pG(8)pC(9)pG(10)pC(11)pG(12)], in which ethidium is intercalated between the C(3)·G(10) and G(4)·C(9) base pairs, and an idealized *cis*-{$Pt(NH_3)_2Cl$}$^+$ fragment is coordinated to N7 of G(4) (see text for discussion). The two reacting groups, $-NH_2$ and Pt, are highlighted in yellow.

pG(8)pC(9)pG(10)pC(11)pG(12)], in which ethidium is intercalated between the C(3)·G(10) and G(4)·C(9) base pairs,[11] was linked to an idealized *cis*-[$Pt(NH_3)_2Cl$]$^+$ fragment at N7 of G(4).[12] The resulting structure (Figure 3) illustrates how DNA could serve as a template to orient the ethidium N8 amino group above the square planar platinum complex (Pt—*N8*-Eth = 3.9 Å, *N7*-G(4)—Pt—*N8*-Eth = 62°) in the major groove of the double helix to effect the nucleophilic displacement of the chloride ligand. A similar model in which platinum is coordinated to N7 of G(10) on the other DNA strand reveals a favorable orientation for reaction with the N3 amino group of ethidium, although the distance is somewhat greater (Pt—*N3*-Eth = 4.7 Å).

DNA does not promote the analogous reaction of *trans*-DDP with ethidium.[3] Model studies reveal that it is stereochemically impossible for the exocyclic amino groups of intercalated ethidium to bind trans to a coordinated nucleobase in duplex DNA. The reaction of *cis*-DDP with proflavin, however, is facilitated by DNA.[3] A model based upon the crystal structure of proflavin intercalated into the duplex dimer $(CpG)_2$[13] reveals the exocyclic amino groups of proflavin, like those of ethidium, to be in a favorable position to react with platinum bound to N7 of guanine in the major groove of DNA. Acridine,[3] which has no exocyclic amino group, and 9-aminoacridine,[14] which intercalates with the N9 amino group projecting into the minor groove,[15] do not react with *cis*-DDP in the presence of DNA. The model in which the *cis*-{$Pt(NH_3)_2Cl$}$^+$ unit is bound to N7 of a purine base in the major groove of DNA at a site adjacent to the exocyclic amino group of the intercalator explains all of these observations.

In the absence of DNA, 3.2 mM *cis*-DDP and ethidium bromide react to consume 21% of the intercalator in 8 h; at 50 μM concentrations, less than 2% of the ethidium reacts after 8 or 24 h.[6] In the presence of DNA, however, 19.9% of the ethidium reacts in 8 h at 50 μM concentrations of *cis*-DDP and ethidium bromide (r_f 0.25).[6] The reaction of *cis*-DDP with EthBr therefore proceeds to the same extent at 60-fold lower concentrations of both reactants when carried out in the presence of DNA.

In conclusion, the present results reveal details of how DNA can promote the reaction of a Lewis acid (platinum) with a Lewis base (Eth exocyclic amino group) under conditions where no significant reaction would occur in its absence. This acid–base chemistry[3] joins electron transfer and macromolecular diffusion as a general class of reactions facilitated by DNA.

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Supplementary Material Available: Experimental synthetic, mechanistic, spectroscopic, analytical, and reaction chemistry details as well as tables of atomic positional and thermal parameters for *cis*-[$Pt(NH_3)_2$(*N8*-Eth)Cl]$(ClO_4)_2$·MeOH·0.5H_2O (5 pages). Ordering information is given on any current masthead page.

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