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Interaction of the Antitumor Agents *cis,cis,trans*-$Pt^{IV}(NH_3)_2Cl_2(OH)_2$ and *cis,cis,trans*-$Pt^{IV}[(CH_3)_2CHNH_2]_2Cl_2(OH)_2$ and Their Reduction Products with PM2 DNA†

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ABSTRACT: The ability of two platinum(IV) antitumor agents, *cis,cis,trans*-$Pt^{IV}[(CH_3)_2CHNH_2]_2Cl_2(OH)_2$ (**2**) and *cis,cis,trans*-$Pt^{IV}(NH_3)_2Cl_2(OH)_2$ (**4**), to interact with PM2 DNA was examined. Analysis using gel electrophoresis showed that neither compound is able to alter the electrophoretic mobilities of the three forms of PM2 DNA in the gel. However, incubation of **2** and **4** with 2 equiv of $Fe(ClO_4)_2 \cdot 6H_2O$ or 1 equiv of ascorbic acid results in reduction to yield the divalent complexes *cis*-$Pt^{II}(NH_3)_2Cl_2$ (**1**) and *cis*-$Pt^{II}[(CH_3)_2CHNH_2]_2Cl_2$ (**3**). The structures of the reduction products were characterized by using elemental analysis as well as infrared and ^{195}Pt NMR spectroscopies. Both **1** and **3** were found to bind to and unwind supercoiled form I PM2 DNA. The aforementioned observations support the suggestion that reduction is a means of activating the antitumor properties of **2** and **4**.

A variety of platinum complexes are known to be active as anticancer agents (Prestayko et al., 1980; Rosenberg et al., 1969; Hacker et al., 1984; Bradner et al., 1980). Although the bulk of these materials are analogues of the square-planar platinum(II) complex *cis*-diamminedichloroplatinum(II) [CDDP (**1**)] (Figure 1), a number of octahedral platinum(IV) complexes have been found to be potent anticancer agents. One such compound, *cis,cis,trans*-$Pt^{IV}[NH_2CH(CH_3)_2]_2Cl_2(OH)_2$ [CHIP (**2**)] (Figure 1), is currently undergoing clinical trials in the United States as a potential second-generation analogue of **1**.

Since platinum(IV) complexes are exchange inert (Hartley, 1973), binding to important cellular components such as DNA in a manner similar to that of **1** is very likely not the basis for the cytotoxicity of **2**. However, as was suggested by Tobe & Khokhar (1977), and later by Cleare et al. (1980), platinum(IV) complexes may be activated in vivo by reduction to platinum(II) compounds which in turn exert their cytotoxic effects in a manner analogous to **1**. Significant in this regard is the observation that one of the metabolites of **2**, recovered from the plasma and urine of cancer patients receiving the compound (Pendyala et al., 1984), is the Pt(II) complex *cis*-dichlorobis(isopropylamine)platinum(II) (**3**) (Figure 1). Recently, Crooke and co-workers (Mong et al., 1979, 1980a, 1982) have focused attention away from the reduction mechanism by presenting evidence that **2** is capable of cleaving and cross-linking PM2 DNA. This latter observation raises the possibility that certain platinum antitumor agents possess mechanistic profiles similar to those of the natural product antitumor agents bleomycin and neocarzinostatin (Dabrowiak, 1982; Goldberg et al., 1981). In an effort to elucidate the chemical and biochemical events which underlie the cytotoxicity of platinum(IV) antitumor compounds, we have examined **2** and the hydrogen peroxide oxidation product of **1**, namely, *cis,cis,trans*-$Pt^{IV}(NH_3)_2Cl_2(OH)_2$ (**4**) (Figure 1), in light of the aforementioned mechanistic postulates. In previous reports, we demonstrated that neither **2** nor **4** is able to cleave PM2 DNA and they do not appear to be capable of producing radical species which can be detected by using a spin trap (Vollano et al., 1984; Brandon & Dabrowiak, 1984). In addition, incubation of PM2 DNA with the platinum(IV) compounds does not alter the electrophoretic mobilities of the various forms of PM2 DNA (Vollano et al., 1984). In this report, we demonstrate that compounds **2** and **4** undergo a facile reduction in the presence of $Fe(ClO_4)_2 \cdot 6H_2O$ or ascorbic acid to give compounds **3** and **1**, respectively. We also show that, similar to **1**, compound **3** can bind to and unwind supercoiled PM2 DNA. The aforementioned observations

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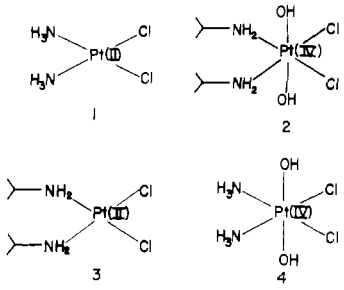

FIGURE 1: Structures of compounds used in this study.

strongly suggest that reduction to platinum(II) may be a means of activating the antitumor properties of these platinum(IV) compounds.

Materials and Methods

Materials

The ethidium bromide used in the study was purchased from Sigma Chemical Co. Ascorbic acid, purchased from Fisher, was recrystallized 3 times from methanol–water and finally passed over a Bio-Rad Chelex resin to remove metal ions. The $Fe(ClO_4)_2 \cdot 6H_2O$ was used as supplied from G. Fredrick Smith Co. The high-pressure liquid chromatography (HPLC) grade dimethylformamide (DMF) used in the NMR studies was dried over 4A molecular sieves. Lyophilized PM2 DNA, supplied by Boehringer Mannheim, was dissolved in 500 μL of H_2O and extensively dialyzed against the buffer used for the platinum–DNA studies, 20 mM tris(hydroxymethyl)-aminomethane (Tris)–nitrate (Sigma), pH 7.0. The compounds *cis*-diamminedichloroplatinum(II) [CDDP (**1**)], *cis,-cis,trans*-dichlorobis(isopropylamine)dihydroxoplatinum(IV) [CHIP, JM-9 (**2**)], and *cis*-dichlorobis(isopropylamine)platinum(II) [JM-6 (**3**)], were supplied by Bristol-Myers Co. Compound **4**, *cis,cis,trans*-dichlorodiamminedihydroxoplatinum(IV) (JM-93), was synthesized in the previously described manner (Vollano et al., 1984). Infrared, ^{195}Pt NMR, and elemental analyses (Galbraith Laboratories, Knoxville, TN) confirmed the structures of all of the aforementioned compounds.

Methods

Agarose gel electrophoresis was performed on a Model H5 horizontal slab apparatus (Bethesda Research Laboratories) using 0.8% agarose (Bio-Rad) in a Tris–acetate buffer system. DNA was stained with ethidium bromide and visualized under ultraviolet light. Photography was done by using a Polaroid MP-3 camera with Polaroid 55 or 57 film.

The IR data were collected by using Nujol mulls with a Beckman 4220 spectrometer. ^{195}Pt NMR spectra were obtained (22 ± 1 °C) by using a Bruker WM 360 spectrometer operating at 77.2 MHz. The spectral data were collected in either H_2O (**2** and **4**) or DMF (**1** and **3**) solution since **3** is only sparingly soluble in water. Solution concentrations varied from 1.5 mM for **4** to 24 mM for **2**. No evidence for reaction of the solutes with solvent or conversion to other species in solution during the data collection period (~400000 transients, ~10 h) was detected. All chemical shifts (δ) are referenced to K_2PtCl_6 in H_2O [δ $K_2PtCl_6(H_2O)$ = δ $K_2PtCl_6(DMF)$ − 350]. The ^{195} Pt NMR spectral data for **1** and **4** were obtained by using broad-band proton decoupling.

Reduction of **2** *with Ascorbic Acid.* One-tenth gram (0.239 mmol) of **2** was completely dissolved in 20 mL of distilled water. To the resulting yellow solution was added 0.0421 g (0.239 mmol) of ascorbic acid, and the reaction mixture was stirred for 1 h at room temperature. The precipitate which formed was removed by filtration and air-dried (yield 0.073 g, 79%): IR (Nujol) 3268 (s), 3210 (s), 3136 (s), 1592 (s), 1251 (s), 1158 (s), 1110 (s), 1070 (s), 939 (s), 438 (m), 314 (s) cm^{-1}. Anal. Calcd for $PtC_6N_2H_{18}Cl_2$ (**3**): C, 18.75; H, 4.68; N, 7.29; Cl, 18.46. Found: C, 18.94; H, 4.79; N, 7.23; Cl, 17.82.

Reduction of **2** *with* $Fe(ClO_4)_2 \cdot 6H_2O$. To a yellow solution of 0.100 g (0.239 mmol) of **2**, dissolved in 25 mL of water, was added 0.173 g (0.478 mmol) of $Fe(ClO_4)_2 \cdot 6H_2O$. After 1.5 h of stirring at room temperature, the crude light orange solid which formed was removed by filtration and air-dried (yield 0.082 g, 89%). No further purification was attempted. Since the color of **3** is light yellow, the orange color of the product recovered from the reduction appears to be due to a small amount of an unknown impurity: IR (Nujol) 3268 (s), 3212 (s), 3136 (s), 1592 (s), 1251 (s), 1158 (s), 1110 (s), 1070 (s), 939 (s), 438 (m), 314 (m) cm^{-1}.

Reduction of **4** *with Ascorbic Acid.* To 0.201 g (0.602 mmol) of **4** suspended in 15 mL of water was added 0.212 g (1.20 mmol) of ascorbic acid. After the mixture was stirred and heated (35 °C) for 15 min, compound **1** crystallized from solution. The reaction was allowed to proceed for 2 h after which time the bright yellow-orange powder was removed by filtration and air-dried (yield 0.140 g, 78%): IR (Nujol mull) 3200 (s), 3212 (m), 1615 (w, br), 1536 (w), 1310 (sh), 1298 (m), 800 (s), 550 (w), 328 (m) cm^{-1}. Anal. Calcd for $PtN_2H_6Cl_2$ (**1**): N, 9.33; H, 2.00; Cl, 23.63. Found: N, 9.21; H, 1.95; Cl, 24.15.

Reduction of **4** *with* $Fe(ClO_4)_2 \cdot 6H_2O$. One-tenth gram (0.301 mmol) of **4** was suspended in 15 mL of water. The $Fe(ClO_4)_2 \cdot 6H_2O$ (0.218 g; 0.602 mmol) was dissolved in 10 mL of water and added dropwise to the suspension with stirring. The solid dissolved completely to give a red solution. The solution was allowed to evaporate slowly at room temperature, and after 24 h, a crop of yellow-orange crystals was removed by filtration and air-dried (yield 0.084 g, 93%): IR (Nujol mull) 3280 (s), 3210 (m), 1618 (br, m), 1536 (m), 1310 (s), 1298 (s), 800 (s), 550 (vw), 319 (m) cm^{-1}. Anal. Calcd for $PtN_2H_6Cl_2$ (**1**): N, 9.33; H, 2.00; Cl, 23.63. Found: N, 9.30; H, 1.97; Cl, 24.76.

Results

Reaction of compounds **2** or **4** in aqueous media with either 1 equiv of ascorbic acid (a two-electron reducing agent) or 2 equiv of $Fe(ClO_4)_2 \cdot 6H_2O$ (a one-electron reducing agent) yields sparingly soluble products subsequently identified by elemental, infrared, and ^{195}Pt NMR analyses as compounds **1** and **3** (Figure 2). In both cases, the reduction products exhibited ^{195}Pt NMR resonance lines shifted ~3000 ppm to higher field relative to their Pt(IV) counterparts. Although the ^{195}Pt NMR spectra of the compounds containing $(CH_3)_2CHNH_2$ as a ligand are broad and featureless, the spectra of **4** and its reduction product **1** clearly show coupling, with relative intensity of 1:2:3:2:1, to two symmetry-equivalent nitrogen nuclei. The coupling constants, $J(^{195}Pt-^{14}N)$, for **1** and **4** are 205 and 194 Hz, respectively.

Incubation of the platinum(IV) compounds **2** or **4** with PM2 DNA in 20 mM Tris–nitrate, pH 7.0, buffer for 18 h (37 °C) at input drug to DNA base pair ratios, r_t, of $0 < r_t \leq 1.0$ did not cause a change in the electrophoretic mobilities of the various forms of PM2 DNA (data not shown). However, incubation of the platinum(II) compounds **1** and **3**, using conditions identical with those employed for the platinum(IV) compounds, gave evidence of DNA platination. As shown in Figure 3, both platinum(II) compounds *increase* the mobilities of forms II and III PM2 DNA but *decrease* the mobility of

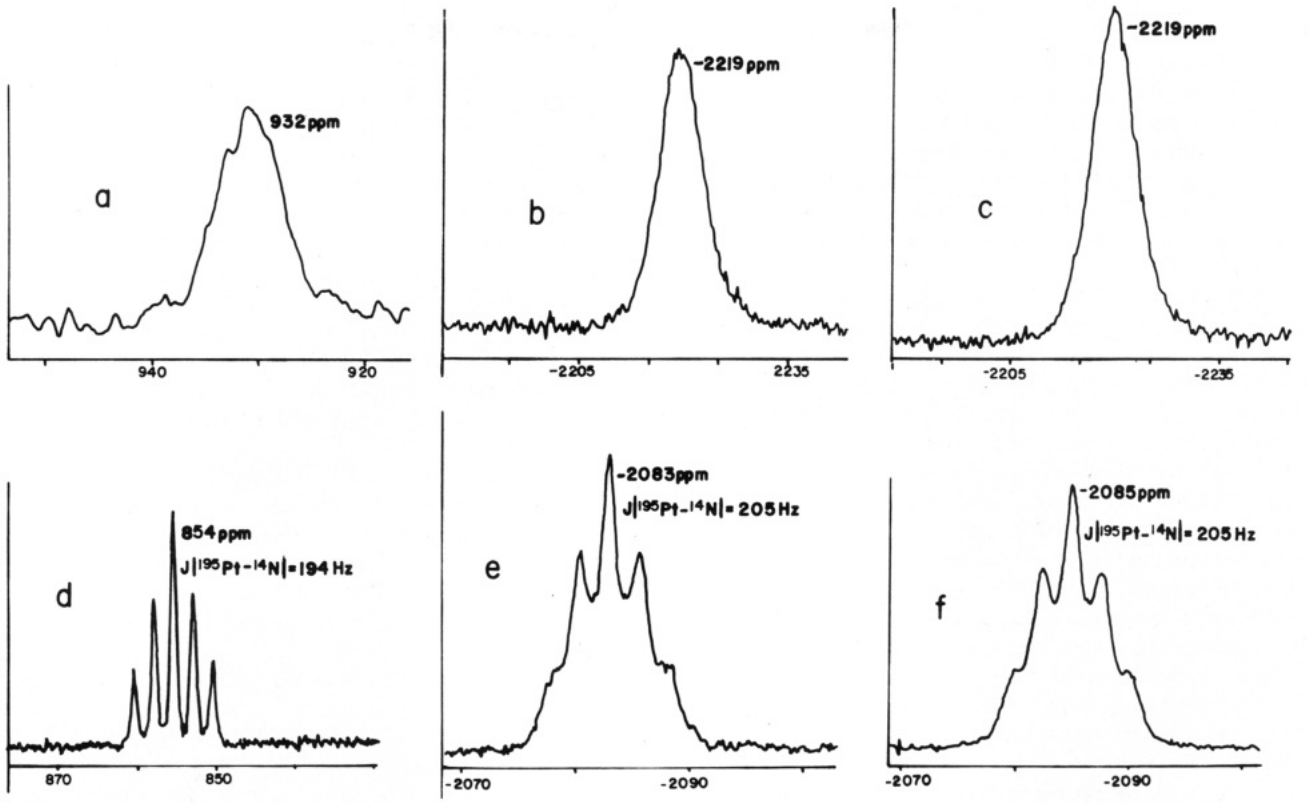

FIGURE 2: ^{195}Pt NMR spectra at 77.2 MHz: (a) compound **2**, solvent H_2O; (b) reduction product of compound **2**, solvent DMF; (c) compound **3**, solvent DMF; (d) compound **4**, solvent H_2O; (e) reduction product of compound **4**, solvent DMF; (f) compound **1**, solvent DMF. All chemical shifts are relative to external K_2PtCl_6 in H_2O.

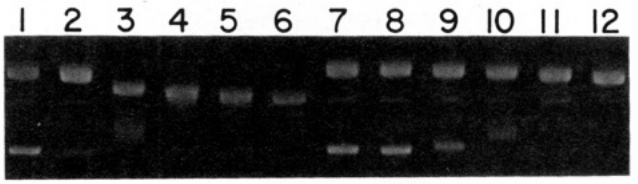

FIGURE 3: Agarose gel electrophoresis of 0.44 μg of PM2 DNA following incubation with compounds **1** and **3**. The reactions were carried out for 18 h at 37 °C in a solution containing 37 μM DNA base pairs. Control lanes are lanes 1 and 7. The lane number and the value of r_t for the two compounds are as follows: compound **1**, 2, 0.025; 3, 0.125; 4, 0.25; 5, 0.50; 6, 1.00; compound **3**, 8, 0.025; 9, 0.125; 10, 0.25; 11, 0.50; 12, 1.00.

form I DNA. Compound **3** was found to be less effective than **1** in decreasing the electrophoretic mobility of form I PM2 DNA in the gel matrix.

Discussion

Reaction of $Fe(ClO_4)_2 \cdot 6H_2O$ or ascorbic acid with the 6-coordinate platinum(IV) antitumor agents **2** and **4** results in reductive elimination to yield 4-coordinate platinum(II) compounds having a *cis*-nitrogen, *cis*-chloride geometry. It has been noted in the literature that the cytotoxicity of **2** toward Chinese hamster ovary cells is decreased in the presence of cysteine (Laverick et al., 1981). Kuroda et al. (1982) also suggest that cysteine can reduce **2** in tissue culture media. Therefore, the reaction of cysteine with **2** and **4** was examined in an attempt to clarify the interaction between this potential reducing agent and the two platinum(IV) antitumor drugs. Cysteine was found to react with the platinum(IV) compounds to yield highly insoluble (very likely polymeric) products. The insolubility of the purified products precluded their complete characterization; however, analysis of the products showed platinum and sulfur to be present. Although these results suggest that a direct interaction between **2** and **4** and cysteine occurs, neither the oxidation state of the metal ion nor the fate of the amino acid, cysteine or cystine, in the product could be ascertained. It is possible that the product could be a polynuclear species containing cysteine and platinum(II) (Pneumatikakis & Hadjiliadis, 1979).

As shown in Figure 2, the reduction products of **2** and **4** in DMF solution exhibit a single ^{195}Pt NMR resonance in the region expected for a platinum(II) ion ligated by two nitrogen and two chloride donors (Ismail & Sadler, 1983). Although the low symmetry of the electric field about the nitrogen atom in the isopropylamine analogues prevents observation of nitrogen quadrupolar coupling to the platinum nucleus, the coupling is clearly resolved in **4** and its reduction product. The value of $J(^{195}Pt-^{14}N)$ for the reduction product, 205 Hz, is consistent with the product being a Pt(II) complex (Ismail & Sadler, 1983). The structures of the reduction products of **2** and **4** were ultimately determined by comparison of infrared and ^{195}Pt NMR data (Figure 2) of the products with samples of **1** and **3** which were synthetically prepared.

The closed-circular genome of PM2 DNA is an ideal substrate for the study of drug–DNA interactions. While DNA nicking drugs can convert superhelical form I PM2 DNA into forms II and III, intercalating agents and covalent binding drugs such as **1** can bind to and reduce the superhelical density of the closed-circular form of the polymer (Dougherty, 1983; Mong et al., 1980b). The change in superhelical density can easily be detected by the reduction in electrophoretic mobility of drug-ligated DNA in an agarose gel matrix.

Studies with compounds **2** and **4** in a buffer medium containing chloride ion (Vollano et al., 1984) or in a Tris–nitrate buffer medium (this study) indicated that neither complex is capable of altering the electrophoretic mobilities of the various forms of PM2 DNA in the gel. This observation and the documented slow exchange kinetics of platinum(IV) com-

pounds (Hartley, 1973) strongly suggest that covalent attachment to DNA via ligand loss from **2** and **4** is not occurring. However, binding to form I of the polymer in a manner which does not alter its superhelical density cannot be unequivocally ruled out. Equilibrium binding to PM2 DNA without ligand loss but with rapid on–off rate kinetics typical of those of intercalating agents is also unlikely for **2** and **4** (Krugh et al., 1980). The platinum(IV) compounds are uncharged and possess geometries which appear to be unsuited for intercalation into DNA.

Figure 3 shows that the reduction products of **2** and **4**, compounds **3** and **1**, respectively, strongly influence the electrophoretic mobilities of the various forms of PM2 DNA in the gel. When conditions similar to those earlier reported for the binding of **1** to covalently closed circular DNA (Cohen et al., 1979; Scovell et al., 1982; Mong et al., 1980b) are used, both **1** and **3** bind to and reduce the superhelical density of form I PM2 DNA. Figure 3 also shows that compound **3** is less effective than **1** in reducing the mobility of form I of the polymer in the gel matrix. Since the levels of DNA platination are unknown, it is not possible to interpret the difference in mobilities in terms of compounds **1** and **3** having different DNA unwinding angles. Further studies are presently being undertaken by this group to examine the respective levels of DNA platination. Both **1** and **3** have similar effects (an increase is observed) on the mobilities of forms II and III PM2 DNA.

In summary, we present evidence that the platinum(IV) antitumor agents do not affect the mobilities of the various forms of PM2 DNA. However, the compounds can be reduced by Fe(II) or ascorbic acid to platinum(II) compounds that retain the *cis*-nitrogen, *cis*-chloride geometry. The reduction products can bind to and unwind closed-circular PM2 DNA. These observations support the suggestion (Tobe & Khokhar, 1977; Cleare et al., 1980; Pendyala et al., 1984) that reduction is a means of activating the antitumor properties of **2** and **4**.

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