^{31}P NMR Investigation of Fourteen Self-complementary Oligodeoxyribonucleotides (8-*mer* to 14-*mer*) Treated with Platinum Complexes: the Downfield ^{31}P NMR Signal Characteristic of Anti-cancer Drugs is Observed Only for Molecules with Adjacent G Residues*

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Abstract

The reaction products of fourteen self-complementary oligodeoxyribonucleotides (from 8 to 14 bases in length) treated with *cis*-PtA_2Cl_2 (A = amine) and ten treated with *trans*-$Pt(NH_3)_2Cl_2$ were studied by ^{31}P NMR spectroscopy. Treatments were carried out under high salt (0.1 M $NaNO_3$) and low temperature (12 °C) conditions which favor the duplex form. A non-transient downfield ^{31}P NMR signal was observed at *ca.* -3.0 ± 0.1 ppm (relative to trimethyl phosphate) for each *cis*-$Pt(NH_3)_2Cl_2$- and $Pt(en)Cl_2$-treated oligodeoxyribonucleotide which contained an NGG moiety. In some cases two downfield signals were observed. One downfield signal was attributed to the phosphate group in a platinated GpG moiety for the single-stranded oligomers. The additional downfield signals were attributed to phosphate groups in distorted unplatinated moieties in single-stranded forms. For the $Pt(en)Cl_2$-treated octadeoxyribonucleotide, $d(GGAATTCC)_2$, a ^{31}P NMR signal was observed at -3.41 ppm. The relatively high field position of this signal is most likely due to the absence of a nucleotide or phosphate group 5′ to the GG moiety. No signals were observed outside the normal shift region for any of the oligodeoxyribonucleotides treated with *trans*-$Pt(NH_3)_2Cl_2$. Non-transient downfield ^{31}P signals were not observed for any of the *cis*-PtA_2Cl_2-treated dodeca- and tetradecadeoxyribonucleotides which did not contain a GG sequence but did contain reactive A, GA, AG and GNG moieties. For the two tetradecadeoxyribonucleotides which contain AG sequences, $d(TATAGCTAGCTATA)_2$ and $d(ATAGCTTAAGCTAT)_2$, treatment with $Pt(en)Cl_2$ produced ^{31}P signals shifted upfield from the main signal by *ca.* 1.4 ppm. These signals were observed only at low temperature (15 °C). Therefore, for these oligodeoxyribonucleotides at low temperature, $Pt(en)Cl_2$ induced distortions severe enough to be observed by ^{31}P NMR spectroscopy. This survey provides additional evidence that Pt-crosslinked GG moieties are responsible for the occurrence of the downfield signal observed for DNA treated with anti-cancer active Pt drugs. The evidence is strong that such downfield signals must include the signal from the phosphate group in the platinated GpG moiety. However, it is possible that the signal also contains contributions from other phosphate groups in the vicinity of the platinated GpG moiety.

Introduction

Since it is widely believed that the molecular target of Pt anti-cancer drugs is DNA [1, 2], well defined spectroscopic and structural studies of adducts formed between these Pt agents and oligodeoxyribonucleotides are growing both in intensity and significance [3–8]. It is important to establish by several criteria that such adducts are directly relevant to the DNA adducts formed *in vivo* by anti-cancer drugs such as $Pt(en)Cl_2$ and *cis*-$Pt(NH_3)_2Cl_2$. The latter compound is now the most widely distributed and used anti-cancer drug in the U.S.A., primarily because of its effectiveness in combination chemotherapy against testicular and ovarian cancers [1, 9, 10].

Immunological procedures have established that antibodies to adducts of *cis*-$Pt(NH_3)_2Cl_2$ with DNA or with polydG-polydC react with DNA isolated from tumor cells which have been treated with *cis*-$Pt(NH_3)_2Cl_2$ [1]. It is difficult to apply many

*Paper presented at the Symposium on Cisplatin and Inorganic Anticancer Drugs, Bari, November 6–7, 1986.

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0020-1693/87/$3.50

spectroscopic techniques to DNA and larger molecular aggregates. However, since ^{31}P chemical shifts are dependent on ROPOR′ bond and torsional angles [11] ^{31}P NMR spectroscopy can be used to monitor changes in the secondary structure of DNA. We have found that ^{31}P NMR spectra of either DNA or nucleosomes treated with anti-cancer Pt compounds contain a new signal at −3 ppm, *ca.* 1.0 ppm downfield from the normal DNA signal (referenced to TMP*) [12–15]. DNA adducts of inactive Pt compounds such as *trans*-$Pt(NH_3)_2Cl_2$ or [Pt(dien)Cl]Cl do not produce this downfield signal [12, 13, 15]. The −3 ppm signal is clearly observed at low ratios of Pt to DNA (0.05 Pt/P). At low ratios, the major adduct formed is a GG intrastrand crosslink where the Gs are linked via N7 to Pt [3, 4, 16]. Although difficult to quantify, the downfield signal in the DNA spectra appears to reach a maximum value of *ca.* 5 to 10% of the total signal area [12, 15]. The complex d(GpG)*cis*-$Pt(NH_3)_2$ (which is an enzymatic degradation product of *cis*-$Pt(NH_3)_2Cl_2$-treated DNA [16]) has a downfield shifted ^{31}P NMR signal [4, 16]. However, the 'destructive' strategy of degrading the DNA by either chemical or enzymatic methods has limitations. These methods do not prove that the species with a downfield ^{31}P NMR signal in a DNA degradation fragment was derived from the lesion which caused the downfield signal in the DNA sample.

Two 'constructive' strategies have been employed to correlate the ^{31}P NMR spectral change with the adduct responsible for the downfield signal. These strategies, which also have limitations, are:

(1) We find that, of the several duplexed polynucleotides examined, only those with two adjacent 6-oxopurines (polydG-polydC [13] and polyI-polyC [17]) give this spectral change – again, *trans*-$Pt(NH_3)_2Cl_2$ does not induce such downfield signals on platination of any polynucleotide. However, the form of synthetic polymers may be other than the B form of DNA. Polymers of the type polyd(AT) may not give ^{31}P NMR spectral changes similar to DNA because the conformation of the bases in the synthetic polymers could be different from that in a similar short sequence in DNA.

(2) Treatment of non-self-complementary single-stranded oligodeoxyribonucleotides containing adjacent G residues with Pt anti-tumor agents gave a downfield ^{31}P NMR signal [5, 6, 14, 15, 19]. Addition of the complementary strand to such platinated single-stranded oligomers gave duplexes with a downfield ^{31}P NMR signal [14, 15, 19]. Thus, it would appear that synthetic oligodeoxyribonucleotides exhibit ^{31}P NMR spectral characteristics similar to those found in complex molecular aggregates such as nucleosomes. However, these ^{31}P NMR studies with oligodeoxyribonucleotides have limitations. First, the reactions have used single-stranded oligodeoxyribonucleotides. The reaction of denatured single-stranded DNA with Pt anti-tumor agents does not give the same ^{31}P NMR spectral changes as the reaction of duplexed DNA [15]. Similarly, polyI and polyI-polyC give different products as judged by ^{31}P NMR spectroscopy and by ^{1}H NMR spectroscopy [17, 20]. Second, the only reactive sites in the molecules are usually G residues, the remainder of the single-stranded oligodeoxyribonucleotides contain only C (which react poorly, especially at low pH) and T (which react very poorly) residues [11]. Significantly, except for d(TGGT) [5, 6] these oligodeoxyribonucleotides were not treated with inactive Pt complexes.

In this report, we present a survey of the ^{31}P NMR spectral changes on platination of fourteen self-complementary duplexed oligodeoxyribonucleotides in the 8 to 14-*mer* range under high salt and low temperature conditions. This is the first extensive study of oligodeoxyribonucleotide platination reactions using ^{31}P NMR spectroscopy. In this study, duplexed oligodeoxyribonucleotides with alternative binding sites (A residues) and inactive anti-tumor agents have been investigated. We are able to simulate 'reconstructively' DNA reactions carried out under conditions used by this laboratory previously (but at slightly higher temperature). We are also able to evaluate whether other types of platinated oligodeoxyribonucleotides exhibit the ^{31}P NMR spectral dependence which appears characteristic of platinated polymers [13, 17]. In some cases, the molecules have adjacent A and G residues, a situation which could not be investigated easily with synthetic polymers.

Experimental

Materials

Oligodeoxyribonucleotides, prepared as described previously [21] were precipitated twice from ethanol. The precipitate was dissolved in and dialyzed for several days against a 1:9 dilution of PIPES 10 buffer (0.010 M PIPES, 0.100 M $NaNO_3$, 0.001 M EDTA, pH 7):H_2O. After dialysis, the purity was evaluated by ^{1}H NMR spectroscopy in D_2O. Stock solutions were prepared by removing the solvent and adding the appropriate amount of deionized H_2O to reconstitute undiluted PIPES 10. Oligodeoxyribonucleotide concentrations were determined spectrophotometrically at 260 nm and $\epsilon = 6300\ M^{-1}\ cm^{-1}$, an average extinction coefficient, was used for all oligodeoxyribonucleotides.

*Abbreviations: TMP = trimethyl phosphate, G = guanine, A = adenine, T = thymine, C = cytosine, N = any of the 4 bases (G, A, T or C), dien = diethylenetriamine, en = 1,2-diaminoethane, tn = 1,3-diaminopropane, DACH = 1,2-diaminocyclohexane, PIPES = piperazine-*N,N′*-bis(2-ethane sulfonic acid).

Methods

HPLC analyses of samples to evaluate sample purity were performed on a Rainin Rabbit Gradient HPLC System with a Hitachi 100-30 spectrophotometer set at 260 nm. Reverse phase gradient elutions were employed with Rainin Microsorb C18 (4.6 mm X 25 cm) analytical and Alltech RSiL-C18-HL (10 mm X 25 cm) preparative columns. Eluent A, 0.1 M triethylammonium acetate, was prepared by dissolving the calculated amount of triethylamine and slightly less than the equivalent amount of acetic acid in distilled water. The solution was passed through a 0.45 μm Millipore filter and the pH adjusted to 7.0 with acetic acid. Eluent B for the analytical column was HPLC grade acetonitrile and, for the preparative column, HPLC grade methanol. Typical gradients were 5 to 25% B (acetonitrile) in 20 min at a flow rate of 1.3 ml/min and 20 to 50% B (methanol) in 25 min at a flow rate of 3 ml/min. For analysis of NMR samples, the solutions were diluted with distilled water (1:10) and 1 to 2 μl were injected.

NMR Sample Preparation

Stock solutions of oligomers were diluted with PIPES 10 buffer to make oligodeoxyribonucleotide concentrations of 0.010 M or 0.020 M in DNA bases. Enough PIPES 10 in D_2O was included to provide a lock signal (approximately 10 to 15% *v/v*). For ^1H NMR spectral studies, a small amount of TSP ($(CH_3)_3SiCH_2CH_2COONa$ from Aldrich) was usually added as an internal reference. ^{31}P NMR samples were contained in cylindrical bulb inserts or in 5 mm NMR tubes concentrically placed in 10 mm NMR tubes. The identical solvent surrounded the insert. A small amount of TMP ($(CH_3O)_3PO$ from Aldrich) was added as an internal reference.

NMR Spectroscopy

^1H NMR spectra were obtained at 361.065 MHz with a Nicolet 360-NB spectrometer. For H_2O/D_2O samples, a Nicolet-modified version of the Redfield [22] 21412 pulse sequence was employed. Typical conditions for recording the Redfield spectra include: 4000 scans; 1.0 Hz line broadening; 8000 data points; and 0.50 ml sample volume.

^{31}P NMR spectra were obtained at 81.01 MHz with an IBM WP-200SY spectrometer. Typical conditions for recording the spectra include 10000 scans, 45° pulse width, 4000 data points, 1.0 Hz line broadening and 0.5 ml sample volume. Spectra were obtained at 15 °C and in some cases at 30 °C. ^{31}P and ^1H NMR spectra were obtained on the same sample.

Platination Reactions

The *cis*-PtA_2Cl_2 compounds (A_2 = en, tn, $(CH_3NH_2)_2$) were prepared by the method of Dhara [23]. The compound Pt(*R,R*-DACH)SO_4 was a gift from Dr. J. Hoeschele. The isomers of $Pt(NH_3)_2Cl_2$ were purchased from Aldrich. Platinum compounds were added to the NMR samples either as a solid or as freshly prepared solutions. These stock solutions were prepared by adding 1 to 3 mg of the platinum compound to 1 ml PIPES 10 buffer and heating at 60 °C until all the solid dissolved. Samples were maintained at 12 °C for at least one week. Reactions were monitored by HPLC.

Atomic Absorption (AA) Spectroscopy

Platinum analyses of oligodeoxyribonucleotide solutions were performed on a Perkin-Elmer Model 306 spectrometer equipped with an HGA-2100 Controller, a graphite furnace, a Perkin-Elmer Model AS-1 automatic sampler and a platinum hollow cathode lamp. Peak heights were recorded at 265.9 nm on a Houston Instruments Omniscribe recorder. The optimum parameters for platinum determination were: drying cycle, 200 °C for 20 s; charring cycle, 1100 °C for 20 s; and atomization cycle, 2700 °C for 8 s.

Working standard solutions were prepared from a 1000 ppm Platinum Atomic Absorption Standard Solution purchased from Sigma. This solution was diluted volumetrically with dilute HCl to 50 ppm platinum and was prepared fresh each day. The 50 ppm platinum solution was then diluted with an appropriate amount of deionized water to give 10 solutions varying from 5 to 50 ppm platinum. 20 μl samples of each solution were injected with the automatic sampler set at 2. The absorbance readings were averaged for each concentration and a working curve of absorbance *versus* micrograms of platinum was plotted.

PIPES 10 buffer and excess Pt were removed from the NMR samples by HPLC (see HPLC section for eluents and conditions). The oligodeoxyribonucleotide concentrations of the collected solutions were determined spectrophotometrically at 260 nm. 20 μl samples of each solution were injected 3 times. The absorbance readings were averaged and the amount of platinum present determined from the standard curve. In some cases, if the concentration of platinum was less than 5 ppm platinum, larger volumes of the sample were manually injected and the drying time was increased to prevent boiling during the charring cycle.

Results

The self-complementary oligodeoxyribonucleotides reported here are categorized as belonging to one of two groups by an objective criterion: the non-transient appearance of a downfield ^{31}P NMR signal on treatment with Pt complexes with two *cis* Cl leaving groups. Those in group I exhibit this signal. Other oligomers are placed in group II.

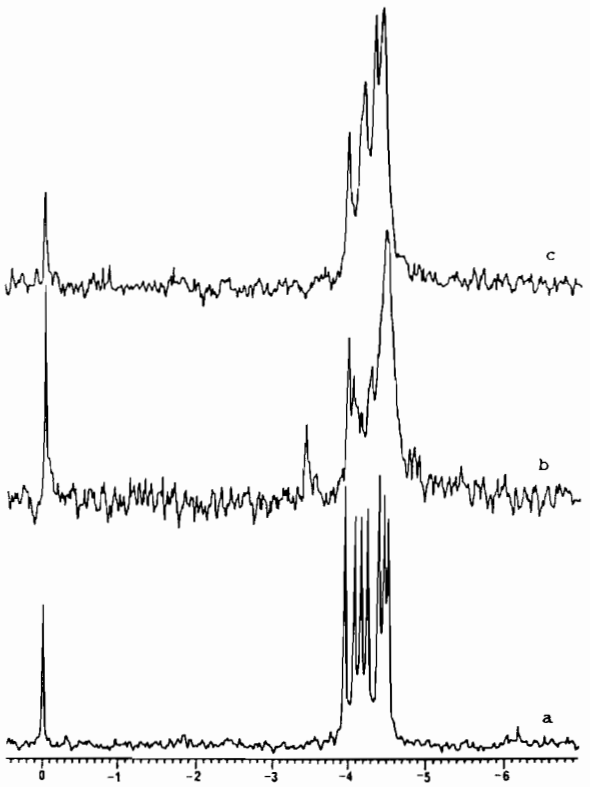

Fig. 1. The 81.01 MHz ^{31}P NMR spectra of d(GGAATTCC)$_2$ and various Pt adducts. (a) 15 °C, 0.020 M bases, no Pt, pH 7. (b) Same as in (a) but with 4 Pt(en)Cl$_2$ per duplex. (c) Same as in (a) but with 4 *trans*-Pt(NH$_3$)$_2$Cl$_2$ per duplex. Solution, reaction and spectral conditions are listed in the Experimental section.

^{31}P NMR

Group I

d(GGAATTCC)$_2$: The ^{31}P NMR spectrum at 15 °C consists of seven sharp peaks (Fig. 1) [24]. Addition of 2 Pt(en)Cl$_2$ per duplex (i.e., one equivalent of Pt per strand) gave rise to a strong new peak at −3.41 ppm and broadened the remaining essentially unshifted resonances, with some loss of resolution, Table I. Further addition of Pt(en)Cl$_2$, to a ratio of 4 Pt(en)Cl$_2$/duplex, did not appear to affect the intensity of the downfield signal, although there was some additional broadening of the other signals. Addition of 4 *trans*-Pt(NH$_3$)$_2$Cl$_2$ per duplex broadened the signals and led to overlapping peaks in the region of −3.8 to −4.8 ppm (Fig. 1). No downfield signal was observed.

d(ATTGGTTAACCAAT)$_2$: The ^{31}P NMR spectrum at 15 °C consists of several overlapping peaks between −3.8 and −4.6 ppm, Fig. 2. Addition of 2 Pt(en)Cl$_2$ per duplex gave rise to two new signals. One, at −2.7 ppm, was broad and barely detectable; the other, at −3.03 ppm, was sharp, Table I and Fig. 2. The spectrum of this oligomer at a ratio of 4 Pt(en)Cl$_2$ per duplex was virtually unchanged. At 25 °C, the broad signal had sharpened, was shifted slightly upfield to −2.8 ppm, and exhibited roughly the same intensity as the −3.03 ppm peak.

TABLE I. Summary of ^{31}P NMR Data on Group I Oligodeoxyribonucleotides Before and After Treatment with Pt Complexes[a]

Pt compound	Downfield signals (ppm)	Main signals (ppm)
d(GGAATTCC)$_2$		
Pt(en)Cl$_2$	−3.41	−3.8 to −4.8
trans-Pt(NH$_3$)$_2$Cl$_2$		−3.7 to −4.8
		−3.9 to −4.6
d(ATTGGTTAACCAAT)$_2$		
Pt(en)Cl$_2$	−2.7[b], −3.03	−3.5 to −5.0
trans-Pt(NH$_3$)$_2$Cl$_2$		−3.3 to −4.9
		−3.6 to −4.7
d(TATATGGCCATATA)$_2$		
Pt(en)Cl$_2$	−2.95, −3.21[c]	−3.6 to −5.2
cis-Pt(NH$_3$)$_2$Cl$_2$	−3.03, −3.29	−3.7 to −5.0
Pt(tn)Cl$_2$	−2.57, −3.45	−3.5 to −4.8
cis-Pt(MeNH$_2$)$_2$Cl$_2$	−2.58, −3.23	−3.7 to −5.0
trans-Pt(NH$_3$)$_2$Cl$_2$		−3.6 to −5.0
		−3.7 to −4.8
d(ATATGGTACCATAT)$_2$		
Pt(en)Cl$_2$	−2.95	−3.8 to −4.9
Pt(*R*,*R*-DACH)SO$_4$	−3.13, −3.64	−3.8 to −4.9

[a]15 °C, PIPES 10 buffer, pH 7.0. Data are given for the ratio of 4 Pt/duplex. [b]Broad. [c]Disappeared when pH was raised to 11.

d(TATATGGCCATATA)$_2$: The ^{31}P NMR spectrum at 15 °C has eight overlapping signals between −3.8 and −4.9 ppm, whereas eleven are expected. On addition of 2 Pt(en)Cl$_2$ per duplex, two downfield signals of equal intensity were observed, Table I. Addition of 2 more equivalents of Pt(en)Cl$_2$ per duplex caused the signal at −2.95 ppm to increase, without an apparent increase in the other downfield resonance, although spectral resolution did not permit an accurate area analysis. At 25 °C, the spectrum of the 4 Pt(en)Cl$_2$ per duplex sample exhibited only one sharp peak at −2.91 ppm. Increasing the pH to 11 sharpened all the signals; the one downfield signal remained at −2.91 ppm. Addition of other Pt complexes with *cis* leaving groups also resulted in the appearance of two new downfield peaks at 15 °C (Table I). The *cis*-Pt(NH$_3$)$_2$Cl$_2$ adduct (4 Pt/duplex) yielded sharp downfield signals at −3.03 and −3.29 ppm (Fig. 3). On addition of 50% more oligodeoxy-

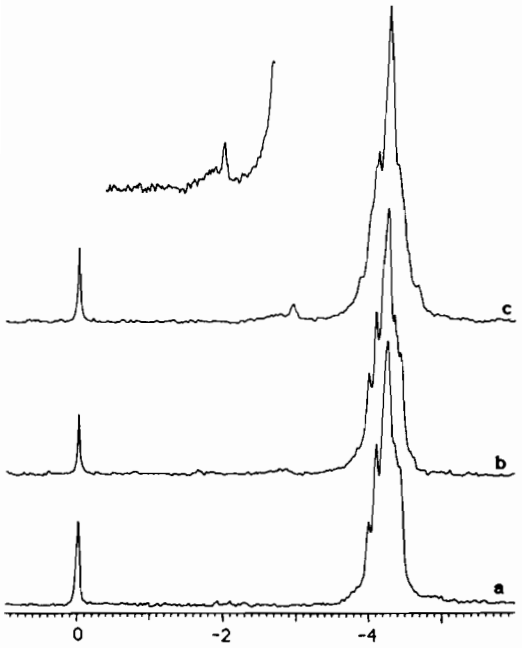

Fig. 2. The 81.01 MHz ^{31}P NMR spectra of d(ATTGGTTAA-CCAAT)$_2$ and its Pt(en) adduct. (a) 15 °C, 0.020 M bases, no Pt, pH 7. (b) Same as in (a) but with 1 Pt(en)Cl$_2$ per duplex. (c) Same as in (b) but with 2 Pt(en)Cl$_2$ per duplex. Inset shows the broad and sharp signals downfield of the main signal area. Solution, reaction and spectral conditions are listed in the Experimental section.

ribonucleotide, the signal at −3.29 ppm increased from 46% to 58% of the total downfield area.

d(ATATGGTACCATAT)$_2$: This oligomer was examined less extensively. Addition of 4 Pt(en)Cl$_2$ per duplex gave rise to a new peak at −2.95 ppm which constitutes approximately 3% of the total signal area. On addition of 4 Pt(*R,R*-DACH)SO$_4$ per duplex, two peaks (−3.13 and −3.64 ppm) are observed which constitute approximately 8% of the total signal area, Table I.

Group II

d(ATTCGTTAACGAAT)$_2$: The ^{31}P NMR spectrum at 15 °C consists of overlapping peaks between −3.7 to −4.7 ppm, Table II. Addition of 2 Pt(en)Cl$_2$ per duplex, after 2 weeks of reaction, resulted in two broad, very weak peaks at −3.3 and −3.6 ppm. These signals disappeared after 6 weeks. Further addition of reagent to 4 Pt/duplex also broadened the main ^{31}P NMR signal. No new downfield ^{31}P NMR signals were observed, either in this case or on addition of 4 *trans*-Pt(NH$_3$)$_2$Cl$_2$ per duplex, Table II.

d(TATATCGATATA)$_2$: The ^{31}P NMR spectrum at 15 °C consists of overlapping signals in the region of −3.8 to −4.9 ppm. Addition of 4 Pt(en)Cl$_2$ per

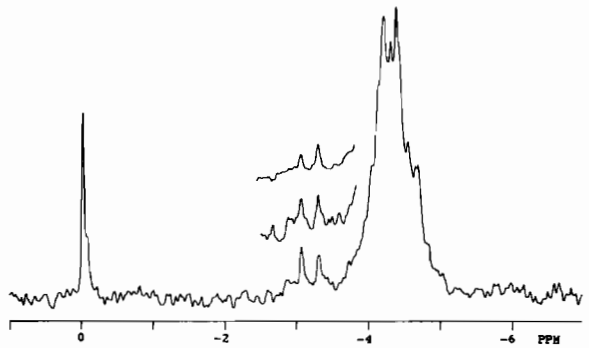

Fig. 3. The effect of addition of untreated d(TATATGGCC-ATATA)$_2$ on the ^{31}P NMR spectrum of the same oligomer treated with *cis*-Pt(NH$_3$)$_2$Cl$_2$ at 15 °C. The spectrum was recorded after treatment of 0.50 ml of 10 mM (bases) oligomer with 4 equivalents of *cis*-Pt(NH$_3$)$_2$Cl$_2$ per duplex ([Pt] = 1.4 mM) at 12 °C for two weeks. Lower inset: after addition of 0.0625 ml of 20 mM oligomer ([oligomer]$_{total}$ = 11 mM, [Pt]$_{total}$ = 1.3 mM, [oligomer]$_{untreated}$/[oligomer]$_{treated}$ = 0.25). Upper inset: after addition of another 0.0625 ml of 20 mM oligomer ([oligomer]$_{total}$ = 12 mM, [Pt]$_{total}$ = 1.1 mM, [oligomer]$_{untreated}$/[oligomer]$_{treated}$ = 0.50). See the Experimental section for solution and spectral conditions.

duplex broadened the main signals and no new downfield peaks were observed, Table II. Similarly, the addition of 4 *trans*-Pt(NH$_3$)$_2$Cl$_2$ per duplex broadened the main signals without inducing the appearance of downfield peaks.

d(ATACGTTAACGTAT)$_2$: The ^{31}P NMR spectrum at 15 °C consists of overlapping signals from −3.9 to −4.7 ppm. As for other oligodeoxyribonucleotides in group II, addition of 4 Pt(en)Cl$_2$ or 4 *trans*-Pt(NH$_3$)$_2$Cl$_2$ per duplex broadened the main signals (Table II). No new peaks were observed on addition of either Pt compound.

d(ATTTTTTAAAAAAT)$_2$: The ^{31}P NMR spectrum at 15 °C (Fig. 4) has closely spaced signals between −4.1 and −4.7 ppm. Addition of 4 Pt(en)Cl$_2$ per duplex caused a slight broadening of the signals; no new peaks were observed, Fig. 4 and Table II. The ^{31}P NMR spectrum of the *trans*-Pt(NH$_3$)$_2$Cl$_2$ adduct exhibited a broad tailing peak at −3.9 ppm.

d(ATATCGATCGATAT)$_2$ and d(ATACGAATT-CGTAT)$_2$: The ^{31}P NMR spectra at 15 °C consist of overlapping peaks (Table II). Addition of 4 Pt(en)Cl$_2$ or 4 *trans*-Pt(NH$_3$)$_2$Cl$_2$ per duplex broadened the main signals. No new signals outside the main cluster were observed. The results for d(ATACGAATTCG-TAT)$_2$ are given in Fig. 5.

d(TATATGCGCATATA)$_2$ and d(TATATCGCG-ATATA)$_2$: These oligomers were examined less

TABLE II. Summary of ^{31}P NMR Data on Group II Oligodeoxyribonucleotides Before and After Treatment with Pt Complexes[a]

Sequence	No Pt	$Pt(en)Cl_2$	*trans*-$Pt(NH_3)_2Cl_2$
$d(ATTCGTTAACGAAT)_2$ [b]	−3.8 to −4.7	−3.8 to −4.9	−3.7 to −4.8
$d(TATATGCATATA)_2$	−3.8 to −4.9	−3.7 to −5.1	−3.4 to −5.0
$d(ATACGTTAACGTAT)_2$	−3.9 to −4.7	−3.6 to −4.9	−3.5 to −4.9
$d(ATTTTTTAAAAAAT)_2$	−4.1 to −4.7	−3.9 to −4.9	−3.5 to −5.0
$d(ATATCGATCGATAT)_2$	−3.8 to −4.7	−3.8 to −4.9	−3.6 to −5.0
$d(ATACGAATTCGTAT)_2$	−3.9 to −4.6	−3.7 to −4.9	−3.4 to −4.9
$d(TATATGCGCATATA)_2$		−3.3 to −5.0	
$d(TATATCGCGATATA)_2$		−3.5 to −4.9	
$d(TATAGCTAGCTATA)_2$ [c, d]	−3.8 to −4.75	−3.2 to −4.9	−2.9 to −5.6
$d(ATAGCTTAAGCTAT)_2$ [e]		−3.9 to −4.9	

[a]15 °C, PIPES 10 buffer, pH 7.0. Data are given for the ratio of 4 Pt/duplex. [b]After incubation with 2 $Pt(en)Cl_2$ for 2 weeks, downfield peaks at −3.27 and −3.5 ppm were observed. These peaks were no longer apparent after an additional 4 weeks. [c]Upfield signals are observed at −5.55 and −5.65 ppm at low temperature only after incubation with $Pt(en)Cl_2$. [d]Broad (possibly 2 peaks) observed at −5.65 ppm at low temperature only after incubation with *cis*-$Pt(NH_3)_2Cl_2$. Main signal observed from −3.2 to −4.9 ppm. [e]Upfield signals are observed at −5.52 and −5.74 ppm only at low temperature.

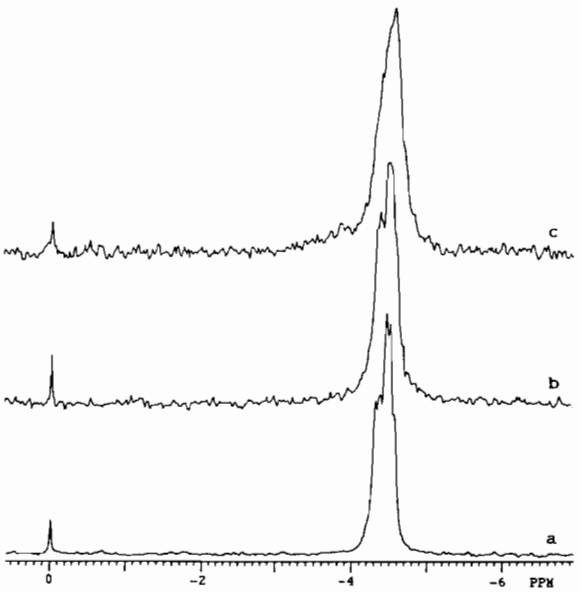

Fig. 4. The 81.01 MHz ^{31}P NMR spectra of $d(ATTTTTT-AAAAAAT)_2$ and various Pt adducts. (a) 15 °C, 0.020 M bases, no Pt, pH 7. (b) Same as in (a) but with 4 $Pt(en)Cl_2$ per duplex. (c) Same as in (a) but with 4 *trans*-$Pt(NH_3)_2Cl_2$ per duplex. Solution, reaction and spectral conditions are listed in the Experimental section.

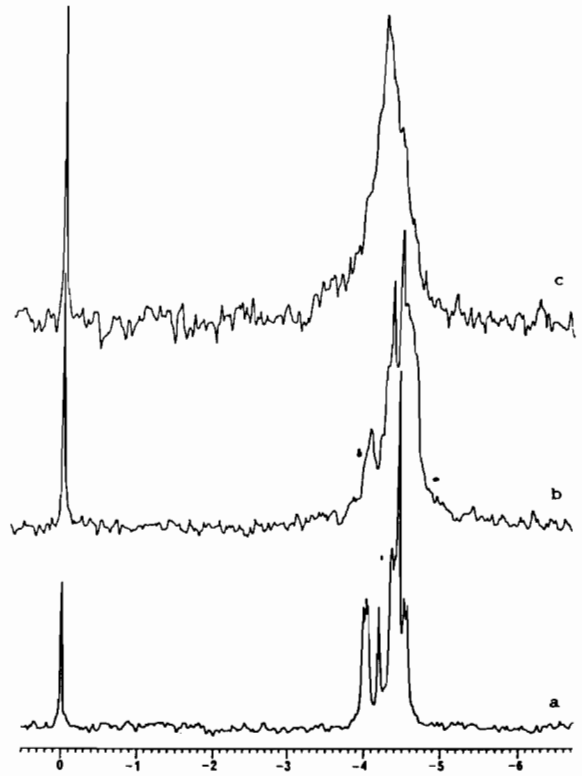

Fig. 5. The 81.01 MHz ^{31}P NMR spectra of $d(ATATCGAT-CGATAT)_2$ and various Pt adducts. (a) 15 °C, 0.020 M bases, no Pt, pH 7. (b) Same as in (a) but with 4 $Pt(en)Cl_2$ per duplex. (c) Same as in (a) but with 4 *trans*-$Pt(NH_3)_2Cl_2$ per duplex. Solution, reaction and spectral conditions are listed in the Experimental section.

extensively. Addition of 4 $Pt(en)Cl_2$ per duplex caused broadening, Table II. No downfield ^{31}P NMR signals were observed. These duplexes were not studied with *trans*-$Pt(NH_3)_2Cl_2$.

$d(TATAGCTAGCTATA)_2$: The ^{31}P NMR spectrum at 25 °C consists of 5 signals between −3.9 and −4.7 ppm with approximately a 2:2:4:2:1 intensity ratio (from downfield to upfield), Fig. 6a. Addition of either *cis*-$Pt(NH_3)_2Cl_2$ or $Pt(en)Cl_2$ to a ratio of 4 Pt/duplex resulted in the appearance of two upfield signals at −5.55 and −5.65 ppm at 15 °C, Table II and Fig. 6c. These signals were not transient (they were still apparent in a 3 month old sample) but they

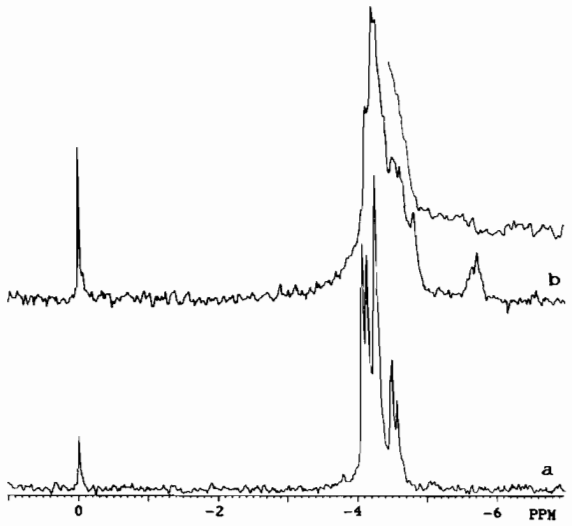

Fig. 6. The 81.01 MHz ^{31}P NMR spectra of d(TATAGCTAGCTATA)$_2$ before and after treatment with Pt(en)Cl$_2$. (a) 25 °C, 0.020 M bases, no Pt, pH 7.0. (b) 15 °C, treated with 4 Pt(en)Cl$_2$ per duplex. Inset shows the upfield region of the solution described in (b) but at 25 °C. Solution, reaction and spectral conditions are listed in the Experimental section.

were absent in spectra recorded at 25 °C, Fig. 6b. The spectrum of the adduct with *trans*-Pt(NH$_3$)$_2$Cl$_2$ (4 Pt/duplex) did not exhibit these unusual temperature-sensitive upfield signals.

d(ATAGCTTAAGCTAT)$_2$: Addition of 4 Pt(en)Cl$_2$ per duplex resulted in the appearance of two upfield peaks at −5.52 and −5.74 ppm. As in d(TATAGCTAGCTATA)$_2$, which also contains AG sequences, these upfield peaks are not observed in the 25 °C spectrum. This duplex was not studied with *trans*-Pt(NH$_3$)$_2$Cl$_2$.

^1H NMR

In self-complementary duplexes, each base pair is related by a twofold axis to a second equivalent base pair. For the purposes of our discussion of imino proton resonances in 10 to 15% D$_2$O recorded by the Redfield method [22], we will refer to only one member of the set. Base pairs are numbered from the end, such that the terminal base pair is given the value (1). For example,

5′d(G- G- A- A- T- T- C- C)

(1) (2) (3) (4)

3′d(C- C- T- T- A- A- G- G)

The imino ^1H NMR signals for the T N3 proton in an AT base pair or the G N1 proton in a GC base pair are typically observed from 15 to 13 ppm and from 13 to 12 ppm, respectively [25]. These signals can be observed only when the sample temperature is well below the temperature required for disrupting the particular base pair in a duplex [25–32]. Characteristically, the imino resonance decreases in intensity as the base pair becomes thermally disrupted since the NH can exchange rapidly with H$_2$O [26–28, 30, 31]. There is also an upfield shift of the imino resonances with increasing temperature. This shift has been attributed to disruption of base pairing without exchange of the imino H with H$_2$O and without formation of an H-bond to water [30, 31]. The relatively larger upfield shift observed for the AT imino resonances compared with the GC resonances is probably due to the differences in the stability of the base pairs [30]. These spectral changes are referred to as 'melting'. Since this melting proceeds from the end base pairs towards the central base pairs, an external base pair will melt before an internal base pair [28–31]. However, when only a few base pairs are left intact, the remaining signals 'melt' simultaneously. Thus, melting can be used for partial signal assignment, Table III. Details of the imino signal assignments and the melting temperatures have been reported [32].

Group I

For each oligomer in this group, imino signals were still observed after treatment with Pt(en)Cl$_2$. In every case, the signals were broader and not so well defined, compared to those for the unreacted species. In two cases, d(GGAATTCC)$_2$ and d(ATTGGTTAACCAAT)$_2$, treatment with 4 Pt(en)Cl$_2$ per duplex produced new imino signals. The signals of the untreated oligomers were no longer observed. For both d(TATATGGCCATATA)$_2$ and d(ATATGGTACCATAT)$_2$, the imino signals of the Pt-treated oligomers, although broadened and diminished, were similar to the signals of the unreacted species.

In most cases, after treatment with 4 *trans*-Pt(NH$_3$)$_2$Cl$_2$ per duplex, no imino signals were observed. For d(GGAATTCC)$_2$, where there is neither a phosphate group nor a nucleotide 5′ to the adjacent G residues, diminished imino signals were observed after treatment with *trans*-Pt(NH$_3$)$_2$Cl$_2$.

Group II

Treatment with 4 Pt(en)Cl$_2$ per duplex caused a loss of the imino signals of most of the oligomers of this group. However, for d(ATTCGTTAACGAAT)$_2$, d(TATATGCGCATATA)$_2$ and d(TATATCGCGATATA)$_2$, diminished signals similar to the signals of the unreacted duplexes were observed after treatment with Pt(en)Cl$_2$. The imino signals of d(ATTTTTTAAAAAAT)$_2$, which contains no G residues, were essentially unchanged after treatment of the duplex with Pt(en)Cl$_2$.

As with the oligodeoxyribonucleotides of Group I, no imino signals were observed for most of the oligomers of Group II after treatment with *trans*-

TABLE III. Summary of Imino ^{1}H NMR Data (ppm) at 0 °C Before Treatment with Pt Complexes[a]

Group I

d(GGAATTCC)$_2$		d(ATTGGTTAACCAAT)$_2$		d(TATATGGCCATATA)$_2$	
1	13.04	1	13.21	1	[b]
2	12.84	2	13.93	2	13.49
3, 4	13.94	3	13.93	3	13.33
4, 3	13.83	4, 5	12.72	4	13.33
		5, 4	12.67	5	13.57
		6, 7	13.93	6, 7	12.73
		7, 6	13.59	7, 6	12.91

Group II

d(ATTCGTTAACGAAT)$_2$		d(TATATGCATATA)$_2$		d(ATACGTTAACGTAT)$_2$		d(ATTTTTTAAAAAAT)$_2$	
1	13.36	1	12.1	1	[b]	1	13.64
2	13.95	2	13.54	2	[b]	2	14.22
3	13.95	3	13.35	3	13.63	3	14.35
4	12.72	4, 5	13.54	4	12.68	4	14.15
5	12.64	5, 4	13.38	5	12.73	5, 6, 7	14.01
6, 7	13.95	6	12.50	6, 7	13.60	6, 7, 5	13.86
7, 6	13.61			7, 6	13.97	7, 5, 6	13.54

d(ATATCGATCGATAT)$_2$		d(ATACGAATTCGTAT)$_2$		d(TATATGCGCATATA)$_2$		d(TATATCGCGATATA)$_2$		d(TATAGCTAGCTATA)$_2$	
1	[b]	1	13.1	1	11.99[c]	1	12.0[c]	1	[b]
2	13.54	2	13.61	2	13.48	2	13.51	2	13.45
3	13.40	3	13.61	3	13.34	3	13.31	3	13.45
4	13.64	4, 5	12.64	4	13.31	4	13.33	4, 7	13.79
5	12.58	5, 4	12.56	5	13.48	5	13.63	5, 6	12.80
6	12.58	6, 7	13.90	6, 7	12.90	6, 7	12.85	6, 5	12.77
7	13.64	7, 6	13.78	7, 6	12.52	7, 6	12.68	7, 4	13.70

[a]PIPES 10 buffer, pH 7.0, chemical shifts given in ppm relative to TSP. Numbers in the left-hand columns under each sequence indicate possible assignments based on methods described in the Results section. The numbering scheme employed assigns the terminal base pair to 1. In some cases, two or more alternative assignments are suggested. [b]Resonance not observed. [c]Broad signal.

TABLE IV. Summary of Pt Content of Oligodeoxyribonucleotide Samples as Determined by AA Spectroscopy[a]

Sequence	Pt compound	Pt/duplex added	Pt/duplex found
$d(ATATGGTACCATAT)_2$	$Pt(en)Cl_2$	4	3.6
$d(GGAATTCC)_2$	$Pt(en)Cl_2$	4	4.6
$d(ATTCGTTAACGAAT)_2$	$Pt(en)Cl_2$	4	3.5
$d(TATATGCATATA)_2$	$Pt(en)Cl_2$	4	3.9
$d(TATATGCGCATATA)_2$	$Pt(en)Cl_2$	4	5.6
$d(TATATCGCGATATA)_2$	$Pt(en)Cl_2$	4	4.0
$d(TAAAAAATTTTTTA)_2$	$Pt(en)Cl_2$	4	2.0
	trans-$Pt(NH_3)_2Cl_2$	4	2.6
$d(ATATCGATCGATAT)_2$	*cis*-$Pt(NH_3)_2Cl_2$	4	3.6
$d(TATGGGTACCCATA)_2$ [b]	$Pt(en)Cl_2$	1	1.1
	$Pt(en)Cl_2$	2	1.7
	$Pt(en)Cl_2$	4	4.6

[a]See the Experimental section for instrumental parameters and sample preparation. [b]From reference 19.

TABLE V. HPLC Data of Several Oligodeoxyribonucleotides and Their Pt Adducts[a]

Sequence	Untreated	$Pt(en)Cl_2$	*trans*-$Pt(NH_3)_2Cl_2$
$d(GGAATTCC)_2$	13.2	13.2	13 to 18[b]
$d(ATTCGTTAACGAAT)_2$	15.0	15.5	10 to 14[b]
$d(TATATGCATATA)_2$	14.5	14.5[c], 15.5[d]	13 to 14[b]
$d(ATACGTTAACGTAT)_2$	15.1	15.1, 15.5[d]	13 to 15[b]
$d(ATACGAATTCGTAT)_2$	14.5	15.0, 15.5	10 to 14[b]
$d(ATATCGATCGATAT)_2$	14.4[e]	15.2, 15.5[c]	15.3
$d(ATTTTTTAAAAAAT)_2$	20.2	19 to 23[b]	
$d(TATGGGTACCCATA)_2$ [f]	14.0	14.0	13 to 15[b]

[a]Retention times given in minutes. Samples were treated at a ratio of 4 Pt/duplex. See the Experimental section for solution and instrument parameters. [b]Numerous overlapping peaks observed in this range. [c]Broad peak. [d]Small overlapping peak. [e]Distorted peak. [f]From reference 19.

$Pt(NH_3)_2Cl_2$. However, for $d(ATTTTTTAAAAAAT)_2$ treated with 4 *trans*-$Pt(NH_3)_2Cl_2$ per duplex, signals similar to the unreacted species were observed. These signals were greatly diminished and broadened.

AA

Atomic absorption analysis of nearly all oligodeoxyribonucleotides tested revealed that the amount of Pt added to the sample, from 1 to 4 Pt per duplex, remained associated with the oligomer, Table IV. Of the adducts tested, only $d(ATTTTTTAAAAAAT)_2$ contained less Pt than originally added, Table IV.

HPLC

Retention times (t_R) of the unreacted oligodeoxyribonucleotides ranged from 13 to 15 min, Table V. In all cases, t_R values for oligodeoxyribonucleotides treated with $Pt(en)Cl_2$ were either unchanged or were greater than for the unreacted compound. The relative t_R of oligomers treated with *trans*-$Pt(NH_3)_2Cl_2$ varied, although in most cases the elution profiles obtained were very broad.

Discussion

The ^{31}P NMR signal in B-type DNA typically occurs within a few tenths of a ppm of −4.2 ppm. The native DNA signal usually does not shift outside this range on denaturation [33], treatment with H^+ [34] or addition of many metal ions [12]. However, for DNA treated with *cis*-PtA_2Cl_2 drugs, a new downfield ^{31}P NMR signal at *ca.* −3.0 ppm has been observed [12–15]. This signal is not observed for DNA treated with inactive Pt drugs such as *trans*-$Pt(NH_3)_2Cl_2$ or $[Pt(diethylenetriamine)Cl]^+$ [12, 13, 15].

Chromatographic analyses of the enzymatic degradation products of salmon sperm DNA treated with *cis*-$Pt(NH_3)_2Cl_2$ revealed that the d(GG)*cis*-$Pt(NH_3)_2$ reaction product (in which adjacent G residues are crosslinked by Pt via N7) accounts for *ca.* 50–60% of the adducts [3, 16]. This percentage, which is higher than the expected value (*ca.* 37%) if all the G residues were equally reactive [16] implies that sites containing adjacent G residues are more reactive than single G sites. The frequency of

TABLE VI. ^{31}P NMR Data (ppm) of Platinated Oligodeoxyribonucleotides (Single- and Double-stranded) Containing a GG Sequence[a]

Sequence	Pt compound	Downfield signal	Main signals
d(TCTCGGTCTC)-d(GAGACCGAGA)[b, c]	*cis*-$Pt(NH_3)_2Cl_2$	−3.1	−4.0 to −5.0 −3.8 to −4.5
d(TCTCGGTCTC)[b, c]	*cis*-$Pt(NH_3)_2Cl_2$	−2.60, −3.10	−4.0 to −4.8 −3.8 to −4.3
d(GG)[d]	*cis*-$Pt(NH_3)_2Cl_2$	−3.35	−4.1[e]
d(CGG)[f]	*cis*-$Pt(NH_3)_2Cl_2$	−2.6	−4.1
d(TGGT)[g, h]	$Pt(en)Cl_2$	−2.88	−4.17, −4.21
	cis-$Pt(NH_3)_2Cl_2$	−3.02	−4.19, −4.28
	$Pt(Me_2tn)Cl_2$	−3.08	−4.11, −4.29
	$Pt(tn)Cl_2$	−3.09	−4.09, −4.29
	cis-$Pt(MeNH_2)_2Cl_2$	−3.22	−4.04, −4.21
	trans-$Pt(NH_3)_2Cl_2$		−3.9 to −4.6 −4.1 to −4.3
d(TTGG)[h]	$Pt(en)Cl_2$	−3.03	−4.21, −4.44
	cis-$Pt(MeNH_2)_2Cl_2$	−3.38	−4.22, −4.35 −4.1 to −4.4
d(GGTT)[k]	$Pt(en)Cl_2$	−3.60	−4.21, −4.30
	cis-$Pt(MeNH_2)_2Cl_2$	−3.45	−4.18, −4.24 −4.1 to −4.3
d(pGGTT)[h, j]	$Pt(en)Cl_2$	−3.34	−4.23, −4.34 −4.1 to −4.3
d(pGGG)[k]	*cis*-$Pt(NH_3)_2Cl_2$	−3.3	−4.2 −4.1, −4.2

[a]Chemical shifts given in ppm and referenced to TMP. Data given for spectra at *ca.* 25 °C and pH 7, except when indicated. See reference cited for additional instrument and solution conditions. [b]From ref. 19. [c]From ref. 14. [d]From ref. 4. [e]From ref. 42. [f]From ref. 8; pH 6.5. [g]From ref. 5. [h]From ref. 6. Data for d(TGGT) collected at 15 °C and pH 5.5. Data for other oligomers collected at 25 °C and pH 6.0. [i]Me_2tn = 2,2-dimethyl-1,3-diaminopropane. [j]The ^{31}P signal of the 5′ phosphate group was observed at −2.56 ppm for the untreated oligomer and at −2.74 ppm after treatment with $Pt(en)Cl_2$. [k]From ref. 3b. Data are for the major product, [d(pGGG)-N7(1),N7(2)]*cis*-$Pt(NH_3)_2$. The ^{31}P signal for the 5′ phosphate group was observed at −2.4 ppm for the untreated oligomer and at −2.3 ppm for the Pt-treated oligomer.

d(AG)*cis*-$Pt(NH_3)_2$ (5′→3′) adducts (20–25%) corresponds to the expected theoretical percentage based on the AG frequency of the DNA [16]. As with the GG crosslink, only the *cis* isomer of $Pt(NH_3)_2Cl_2$ can form an intrastrand crosslink with an AG moiety. Finally, from 4 to 8% of the Pt reaction products was *cis*-$Pt(NH_3)_2d(GMP)_2$ [16]. It was suggested that, assuming a 1% frequency of interstrand crosslinks, most of this product originated from an intrastrand GNG platinated moiety [16].

The requirement for amines in a *cis* configuration for anti-cancer activity of the Pt drugs [1, 3] and the inability of inactive *trans*-$Pt(NH_3)_2Cl_2$ to form intrastrand GG crosslinks [35] have focused most studies of well defined DNA fragments on GG-containing oligomers treated with *cis*-PtA_2Cl_2 species [3–8, 14, 15, 19].

In order to carry out an extensive survey, we selected self-complementary oligodeoxyribonucleotides for study. Such species have a C_2 axis, and thus each GG-containing duplex has at least two reactive GG centers. For all species with a GG sequence studied here, treatment with $Pt(en)Cl_2$ led to the appearance of a non-transient downfield ^{31}P NMR signal. It is noteworthy that in each case (except for $d(GGAATTCC)_2$) the Pt(en) and *cis*-$Pt(NH_3)_2$ adducts gave a ^{31}P NMR signal at −3.0 ± 0.1 ppm. In all other ^{31}P NMR studies, downfield signals have been observed for *cis*-PtA_2Cl_2-treated single- and double-stranded oligonucleotides containing a GG moiety, Table VI.

In contrast, no downfield ^{31}P NMR signals were observed for any of the self-complementary oligomers which were treated with *trans*-$Pt(NH_3)_2Cl_2$, Tables I and II. Additionally, none of the ten self-complementary oligodeoxyribonucleotides lacking a GG sequence studied here gave any non-transient downfield ^{31}P NMR signals under identical reaction conditions.

Unlike most previous ^{31}P NMR studies carried out with single-stranded oligodeoxyribonucleotides containing a GG moiety [5, 6, 14, 15, 19], we have A bases present. Since A is reactive towards Pt, a distribution of products is expected. In fact, the HPLC

analysis of d(ATTTTTTAAAAAAT)$_2$ revealed a broad distribution of products. The presence of A moieties and of AG, GA, and GNG sequences in some of the Pt(en)Cl_2-treated oligodeoxyribonucleotides studied, combined with the high signal-to-noise ratio in the spectra obtained, demonstrates that platinated crosslinked GG sequences are required for the occurrence of downfield shifted ^{31}P NMR signals.

Interestingly, ^{31}P signals were observed upfield from the main signal at low temperature (15 °C) for the Pt(en) adducts of the two oligomers which contain AG sequences, d(TATAGCTAGCTATA)$_2$ (−5.55 and −5.65 ppm) and d(ATAGCTTAAGCTAT)$_2$ (−5.52 and −5.74 ppm), Table II. These signals were not observed at *ca.* 25 °C. In other studies, no upfield signals were observed for the Pt(en) adduct of d(TAGT), even at low temperature [6]. The temperature dependence of the upfield signals suggests a conformation (e.g., a duplex or hairpin-like form) which is favored at lower temperatures. Such structures may be necessary to induce distortions severe enough in the phosphodiester moieties to be observed by ^{31}P NMR spectroscopy (see below). Temperature-dependent upfield signals observed for oligomers containing an AG moiety have not been previously reported. Although these upfield signals do not account for the downfield signal area observed in DNA, they indicate distortions which are induced after Pt binding. Consequently, the platinated GG and AG adducts merit strong consideration as the adducts responsible for the activity of antitumor Pt drugs, if distortions are necessary for such activity.

NMR studies of oligodeoxyribonucleotides containing a GNG moiety have shown that *cis*-Pt(NH_3)$_2$-Cl_2 is capable of producing GNG intrastrand crosslinks [3, 7, 36]. Of the species we studied, d(TATATGCGCATATA)$_2$ and d(TATATCGCGATATA)$_2$ contain a GCG sequence. No ^{31}P signals were observed outside the main signal area for either oligomer after treatment with Pt(en)Cl_2, Table II. There is speculation that a GNG crosslink is not the adduct responsible for the activity of the Pt drugs since d(GNG)*cis*-Pt(NH_3)$_2$ and d(GNG)*trans*-Pt(NH_3)$_2$ could have grossly similar structures [1, 37].

It is interesting that none of the duplexes studied here are as reactive as d(TATGGGTACCCATA)$_2$ [19]. Although it is possible that the Pt reactions require 'breathing' of the oligomers (*i.e.* when the bases become unpaired), the self-complementary duplex d(TATGGGTACCCATA)$_2$, with its two (GGG)(CCC)$_2$ sequences, is expected to be the most stable oligomer studied by ^{31}P NMR spectroscopy [19]. In contrast, d(ATTGGTTAACCAAT)$_2$ has the GG moieties well separated from each other. This separation should lead to the greatest amount of breathing of the four GG-containing 14-*mers*. Treatment of this oligomer gave the least intense downfield ^{31}P NMR signals. The duplex formed by d(TATATGGCCATATA)$_2$ should have less breathing near the GG sites because of the central GGCC. Reaction of one GG sequence could alter the duplex stability and affect the ability of the adjacent CC sequence to base pair with the GG of the self-complementary strand. Two downfield ^{31}P signals of reasonable intensity were observed for each of the Pt adducts of this latter oligomer (Table I).

In seeking our objectives, we primarily studied duplexes in which the G moieties were at least two base pairs from the ends. However, an atypical duplex studied was d(GGAATTCC)$_2$. It has no flanking nucleotide or phosphate group 5′ to the GpG moiety. The ^{31}P NMR signal is observed at relatively high field, −3.41 ppm (Table I). This value is similar to the ^{31}P NMR signal of d(GG)*cis*-Pt(NH_3)$_2$ [4] and d(GGTT)Pt(en) [6] (Table VI) which also have no flanking 5′ nucleotides or phosphate groups. Molecular mechanics studies of the duplexes [d(TCTCGGTCTC)*cis*-Pt(NH_3)$_2$]-d(GAGACCGAGA) [38, 39] and [(GGCCGGCC)*cis*-Pt(NH_3)$_2$]-d(GGCCGGCC) [38] and X-ray structures of d(pGpG)*cis*-Pt(NH_3)$_2$ [40] and d(CpGpG)*cis*-Pt(NH_3)$_2$ [41] have implicated the 5′ phosphate group as being involved in H-bonding to the amine ligand. This H-bonding may fix the d(GG)*cis*-PtA_2 moiety in a configuration which induces a further downfield shift of the ^{31}P NMR signal of at least *ca.* 0.4 ppm. Additionally, for the simple species, d(CGG)*cis*-Pt(NH_3)$_2$, the (GpG)*cis*-Pt(NH_3)$_2$ ^{31}P NMR signal is at *ca.* −2.5 ppm at low temperature (5 °C) [8]. An upfield shift of this signal with increasing temperature was attributed to thermal disruption of base stacking interactions [8]. Accordingly, the unusually high field shift in d(GGAATTCC)-Pt(en) is probably a consequence of the absence of either a 5′ nucleotide or a 5′ phosphate group flanking the GG sequence.

It is interesting that the downfield signals are somewhat dependent on the non-leaving amine of the platinum complex. In a previous study of various Pt adducts of d(TGGT), we found that decreasing the bulk of the amine group on Pt shifted the GpG ^{31}P signal further downfield [6]. This finding suggested that H-bonding of the 5′-phosphate to the amine resulted in an opening of the ROPOR [1] bond or torsional angles. In selected cases, the *cis*-Pt(MeNH_2)$_2$, Pt(tn), and Pt(*R*,*R*-DACH) adducts of d(TATGGGTACCCATA)$_2$ [19], d(TATATGGCCATATA)$_2$, and d(ATATGGTACCATAT)$_2$ give chemical shifts different from the values observed for the Pt(en) or *cis*-Pt(NH_3)$_2$ crosslinked GpG adducts. Thus, the amine group of the Pt complex apparently influences the distortions induced by Pt binding.

For d(TCTCGGTCTC)*cis*-Pt(NH_3)$_2$ [14, 19] the two downfield signals, similar to those in Table I, were assigned to the (GpG)*cis*-PtA_2 moiety and to an unplatinated moiety, Table VI. d(TCTCGGTCTC)*cis*-

$Pt(NH_3)_2$ gave two downfield signals at −2.60 and −3.10 ppm, which were assigned to (GpG)*cis*-$Pt(NH_3)_2$ and the 5′ CpT moiety, respectively [14, 19]. Thus, a conformation in which the 5′ end of the oligomer folds back toward the Pt moiety is implied.

Based on previous [14] and recent [19] ^{31}P NMR data on [d(TCTCGGTCTC)*cis*-$Pt(NH_3)_2$]-d(GAGACCGAGA) and data on [d(CTTTTGGTTTTC)-Pt(en)]-d(GAAAACCAAAAG) [18] duplex formation causes an upfield shift to *ca.* −3.2 ppm of the platinated GpG ^{31}P NMR signal. Thus, downfield signals can be observed for the platinated GpG moiety in both single-stranded and duplexed oligomers and for moieties in single-stranded oligomers removed from the Pt binding site. The conformations leading to these signals are most likely sequence dependent.

Conclusions

In conclusion, this survey provides empirical evidence that the downfield signal observed for DNA treated with Pt drugs [12–15] is due in part to Pt crosslinked GG moieties. A maximum value of *ca.* 5 to 10% of the total ^{31}P signal area for this signal was found [15]. Since the GG frequency in mammalian DNAs is only *ca.* 5% [12, 15] this value seems high especially because a large percentage of the Pt adds to AG sites. Such adducts do not appear to give downfield signals. In studies with d(TATGGGTACCCATA)Pt(en) [19] and d(TCTCGGTCTC)*cis*-$Pt(NH_3)_2$ [14, 19] downfield signals were observed for moieties removed from the Pt binding site. These signals, which were temperature and pH sensitive, indicate that Pt binding to a GG sequence can induce additional distortions along the ribose-phosphate backbone. The high signal area observed in the DNA studies may be a reflection of such distortions. However, measurements of small signal areas adjacent to the large DNA signal may be inaccurate. Nevertheless, our survey suggests that the only adducts which can lead to the downfield signals are GpG adducts where the Gs are bound to *cis* positions on Pt. Whether these signals in DNA are exclusively due to the platinated GpG moiety or are due to this moiety and some adjacent ones is a question which remains to be resolved.

Acknowledgements

Support for this work by NIH grant GM 29222 and a loan of K_2PtCl_4 from Matthey-Bishop, Inc. are greatly appreciated. We also thank Kathleen A. Gallo and Carrie Samson for their assistance in the synthesis and isolation of the oligodeoxyribonucleotides.

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