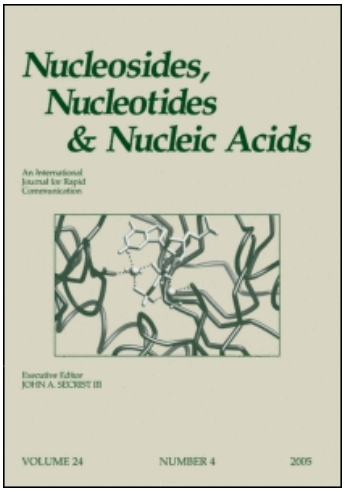

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Spectroscopic Characterization of the Decadeoxynucleotide Duplex Modified with Dichloroethylenediamineplatinum(II)

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SPECTROSCOPIC CHARACTERIZATION OF THE DECADEOXYNUCLEOTIDE DUPLEX MODIFIED WITH DICHLOROETHYLENEDIAMINEPLATINUM(II)#

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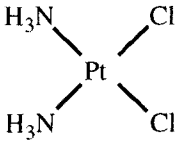
ABSTRACT: We investigated the structure of a decadeoxynucleotide duplex which was modified with Dichloroethylenediamineplatinum(II) ($PtCl_2(en)$) at the central GG site by using some spectroscopic techniques. The results suggest that the DNA structural changes induced by binding of $PtCl_2(en)$ are quite similar to those of cisplatin.

INTRODUCTION

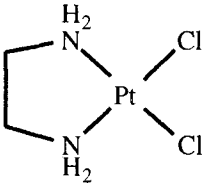
It is widely accepted that the major intracellular target of the anticancer drug, *cis*-diamminedichloroplatinum(II) (cisplatin or *cis*-$[Pt(NH_3)_2Cl_2]$) is DNA (3). This drug binds preferentially to purine N7 positions of DNA (4) and forms bifunctional adducts at GpG, ApG and GpNpG sequences, accounting respectively for about 65%, 25% and 6% with loss of two chloride ions (5,6). The bifunctional chelation of cisplatin at the GpG sequence considerably decreases the thermal stability of double-stranded oligonucleotides (7-9). Although X-ray crystallographic studies on *cis*-$Pt(NH_3)_2$-modified short single-stranded oligonucleotides were reported (10-12), there have been no such reports for a bifunctionally platinated double-stranded oligonucleotide. ^1H NMR studies revealed that hexanucleotides complexed with cisplatin at the GG site no longer form a duplex structure (13,14), but such complexes of octa- and decanucleotides can form base-pairing including

Dedicated to Professor Morio Ikehara on the occasion of his 70th birthday.

the coordinated guanines (7, 8, 15-17). These NMR data and molecular mechanics calculations (18,19) provide models of a kinked structure toward the major groove. As a



cisplatin



$PtCl_2(en)$

result of the bifunctional cross-linking of cisplatin at a GG site, the site-specifically incorporated adduct on template DNA inhibits replication and transcription elongation catalyzed by DNA and RNA polymerases (20,21). These evidences suggest the cross-links of this type to be responsible for anticancer activity of cisplatin.

Dichloroethylenediamineplatinum(II) ($PtCl_2(en)$), an analog of cisplatin possessing a two-carbon bridge between the two amines, has cytotoxic activity (22) and reactivity (23-26) toward DNA and its constituents comparable with those of cisplatin. Conformational alteration of dinucleotides induced by binding of $PtCl_2(en)$ is also similar to that of cisplatin (24). However, the influence of chelation of Pt(en) on the conformation of a double-stranded oligonucleotide has yet to be determined. This paper reports the identification of the chemical structure of the Pt(en)-decanucleotide complex, d(GCTCG*G*ACAC) (asterisks denote the Pt(en) chelation site) and the effects of Pt(en) chelation on the conformation of the double-stranded decadeoxynucleotide in the presence of the complementary strand.

RESULTS AND DISCUSSION

The Pt content of the complex was measured by atomic absorption spectroscopy. The molar extinction coefficient at 260 nm per mol of Pt was estimated to be 109900. This result suggests that one Pt molecule is complexed to the decanucleotide. The structure of the complex was further confirmed by an ion-spray mass spectrometry (27). The ion-spray ionization method produces multiple charged negative ions from solution *via* ion evaporation, giving mass spectra containing a series of quasimolecular ion species with different numbers of charges. Under the conditions employed here, the formation of sodium and ammonium adduct ions can be suppressed (27). The platinum(II)-free 10-mer showed $[M-2H]^{2-}$, $[M-3H]^{3-}$ and $[M-4H]^{4-}$ peaks at m/z 1505.35, 1003.30 and 752.38, respectively, and the average mass was determined to be 3013.06, which is in good agreement with the calculated molecular weight. For the Pt(en)-10-mer complex, [M-

$2H]^{2-}$, $[M-3H]^{3-}$ and $[M-4H]^{4-}$ peaks were observed at m/z 1631.82, 1087.94 and 815.61, respectively. Thus, 3266.32 was determined as the average mass. In this case, a calculated value of the average mass is 3268.20 (fully protonated form of the phosphates). The difference of 2 mass units between the experimental and calculated values should reflect +2 charges on the Pt(en) moiety and intra-molecular neutralization of a negative charge on two phosphate residues of the Pt(en)-decamer.

The coordination sites of platinum(II) can be determined by chemical shift differences of the base protons between a free oligonucleotide and its platinum(II) complex. The coordination of platinum(II) to a purine N7 position induces a relatively large downfield shift of the H8 resonance (28-30). In this case, the H8 resonances of G5* and G6* showed larger downfield shifts than the other base protons by complexing with Pt(en) (see Fig. 4). It thus very likely that the Pt(en) moiety coordinates bifunctionally at the N7 positions of the central guanine doublet site. This result was also supported by enzymatic digestion experiments of the free and platinated strands with nuclease P1. The HPLC analysis of the digestion mixture for the Pt(en)-10-mer showed no peak corresponding to two dGMPs but a new peak comigrating with Pt(en)-d(pGpG) was observed (data not shown).

In the presence of the complementary strand, temperature dependent UV and CD experiments of the free and platinated decanucleotides were performed. A number of investigators have reported the considerable decrease of thermal stability of oligonucleotide duplexes by bifunctional Pt chelation (7-9) and also even by monofunctional chelation (31). Figure 1 shows the melting profiles of the free and Pt(en)-duplexes, in which the thermal stability of the platinated duplex (Tm = 41.4 °C) is also significantly decreased when compared to the parental free duplex (Tm = 49.2 °C). The CD spectra at low temperature show both duplexes to retain a double-stranded B-form type structure, and the Pt(en)-duplex has more intense CD band at around 280 nm than the free duplex (Figure 2). A similar phenomenon is observed on reaction of cisplatin with double-stranded oligonucleotides (8) and DNA (23,32) at low drug/base pair ratios. However, the monofunctional platination of a double-stranded oligonucleotide slightly weakened its CD intensity (33). It is thus very likely that the increase of the CD intensity by platination is closely related with bifunctional modification of DNA. Since the monofunctional chelation of platinum(II) does not induce the kinked structure as shown by an NMR study of the $Pt(NH_3)_3$-nonanucleotide duplex (33), the increased CD intensity of platinated duplexes may reflect their kinked structure.

In addition to macroscopic characterization of the Pt(en)-duplex, two-dimensional NOESY and DQF-COSY experiments were carried out to obtain information on the local

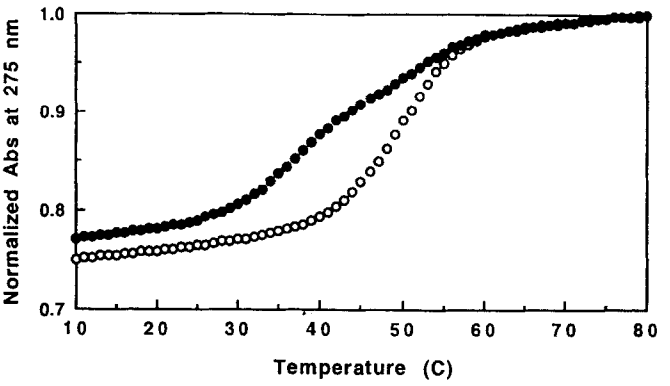


Figure 1 UV-melting profiles of the unmodified duplex (open circles) and the Pt(en)-modified complex (closed circles). Duplex concentration was 5 μM in 0.1 M NaCl, 10 mM sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA.

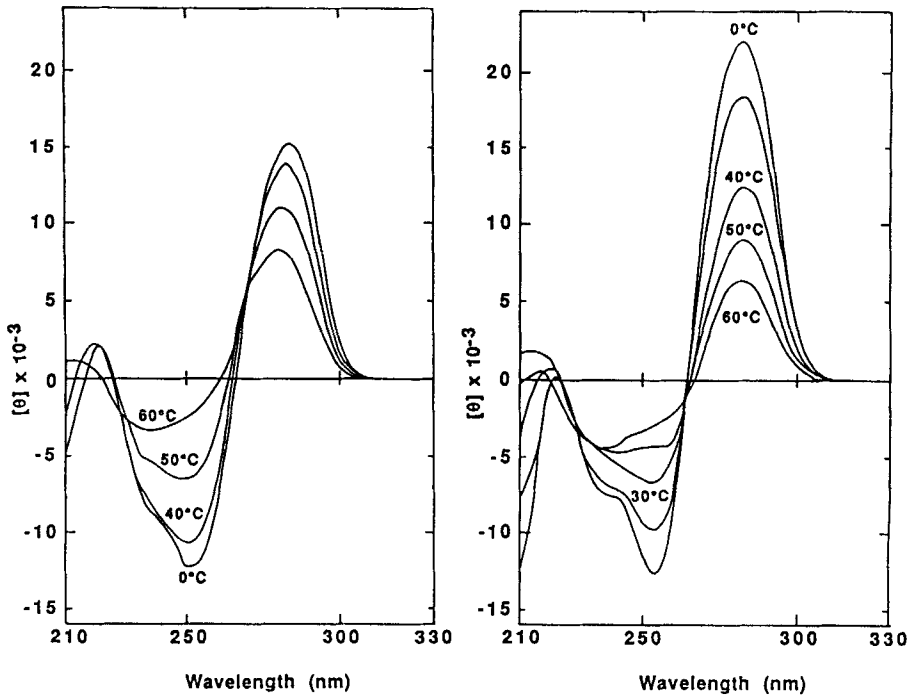


Figure 2 Temperature dependent CD spectra of the unmodified duplex (left) and the Pt(en)-modified duplex (right). The buffer conditions are identical to those described in Figure 1 legend.

structure. The numbering system of the duplex was shown below;

5'-G(1) C(2) T(3) C(4) G*(5) G*(6) A(7) C(8) A(9) C(10)

C(20) G(19) A(18) G(17) C(16) C(15) T(14) G(13) T(12) G(11)-5'

The signal assignment was carried out by using the sequential assignment method which is widely applied to B-form DNA fragments (34). Figure 3 shows the sequential NOE connectivities between the base and sugar H1' protons of the Pt(en)-duplex. The connectivities of the platinated strand were interrupted at the G5*-G6* step, which is the coordination site. However, the sequential NOE connectivities between H8/H6-H2'/H2" were completely continuous including the G5*-G6* step. This allowed the assignment of G6H8. The H2 resonances of the adenine residues were assigned by 400 ms NOESY, in which the H2 resonances exhibit NOEs to its own sugar H1', the adjacent sugar H1' in the 3' direction on the same strand, and the adjacent sugar H1' in the opposite direction on the complementary strand (35). Although the assignment of the signals derived from the ethylene group of the ligand were unsuccessful because of signal overlapping with the H2'/H2" resonances and no NOEs with other resonances, all the non-exchangeable protons other than H5'/H5" of both strands were assigned. This enables a comparison of the chemical shifts between the free and Pt(en)-duplexes. The chemical shift differences of each non-exchangeable proton between both duplexes are summarized in Figure 4. It can be readily seen that significant chemical shift changes lie in the coordination core sequence. The large downfield shifts of the G5*H8 and G6*H8 signals strongly suggest the bifunctional coordination of Pt(en) at the N7 position of these bases. The chemical shift values of G5*H8 (8.63 ppm) and G6*H8 (8.25 ppm), which are identical to those of the H8 signals of the coordinated two guanines in the *cis*-$Pt(NH_3)_2$-modified oligonucleotide duplexes (30), may suggest that the $[Pt(en)(Gua)_2]^{2+}$ moiety has interplanar angles ([α; β] defined by Kozelka *et al.* (30)) between the platinum coordination plane and two guanine planes of about [110° ; -60°]. The sugar geometry of the G5* residue in the Pt(en)-duplex was determined to be an unusual *N*-conformer by the following experimental evidence; the DQF-COSY spectrum of the duplex showed an intense G5*H3'-G5*H4' cross-peak, and a strong NOE between G5*H8 and G5*H3' was observed in the NOESY spectrum. This unusual *N*-sugar puckering of the coordinated 5'-G residue is also observed in *cis*-$Pt(NH_3)_2$-modified duplexes (16,17).

The base protons of C15 and C16 in the coordination core sequence and the sugar H1' protons of the C4 and G5* residues also exhibit a significant downfield shift. It seems thus very likely that the stacking interactions between these bases are weakened by chelation

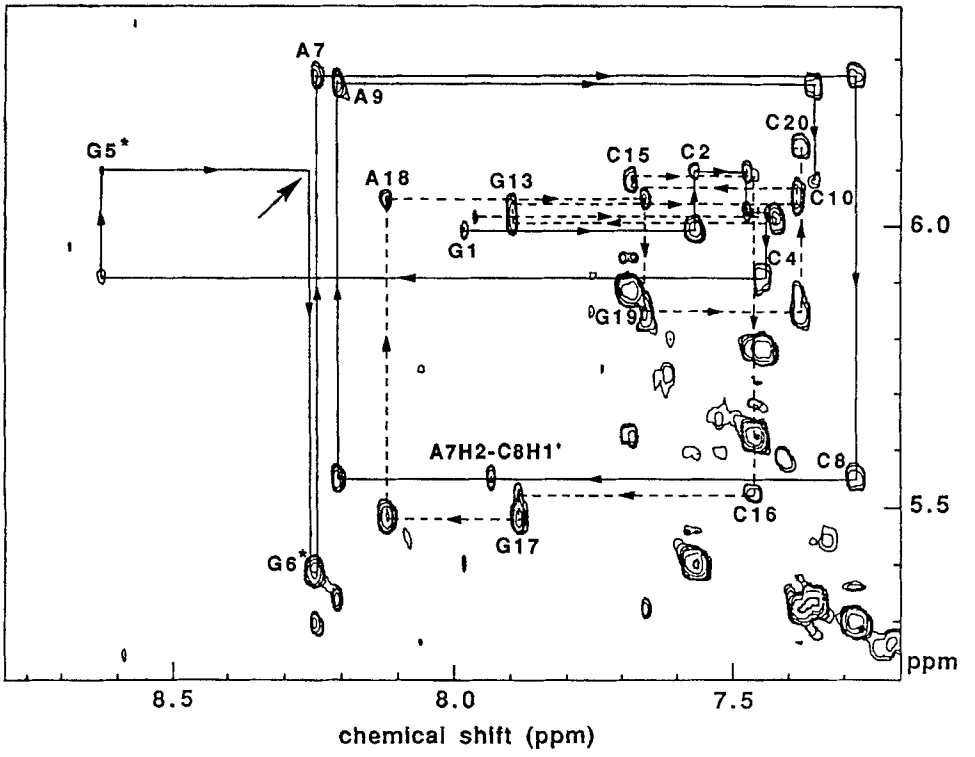


Figure 3 Expanded region of the NOESY spectrum (250 ms mixing time) of the Pt(en)-d(GCTCG*G*ACAC)•d(GTGTCCGAGC) complex in D_2O containing 0.1 M NaCl, 0.1 mM EDTA and 10 mM sodium phosphate (pD 7.5) at 25°C. The sequential NOE connectivities between the base and sugar H1' protons were linked by solid lines for the platinated strand and dashed lines for the complementary strand. The connectivities are broken at the G5*-G6* step as indicated by an arrow (G5*H1'-G6*H8).

of Pt(en). On the other hand, the C4H2' resonance is shifted 0.52 ppm upfield compared to the unmodified duplex, suggesting that the C4H2' proton is oriented onto the G5* aromatic ring and is shielded by the ring current effects. Such a characteristic upfield shift of H2' in the 5'-neighboring residue of the coordination site is also observed in *cis*-$Pt(NH_3)_2$-modified duplexes (16,17) and may suggest the structure to be kinked toward the major groove.

Thus, all the conformational features of the Pt(en)-modified duplex described here are almost the same as those seen in *cis*-$Pt(NH_3)_2$-modified duplexes. The similarity between the distorted DNA structures induced by cisplatin and $PtCl_2(en)$ should explain the similarity between their biological activities.

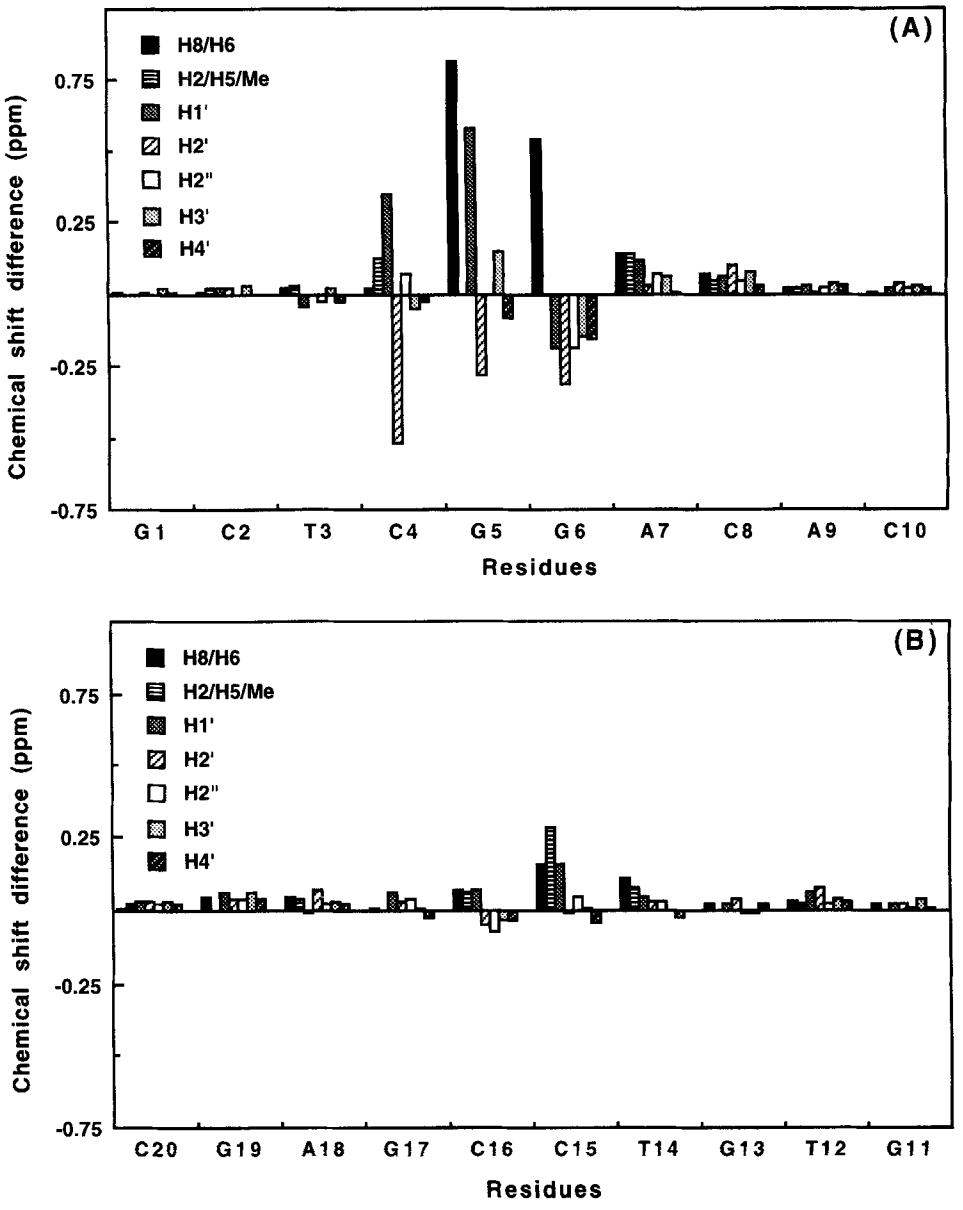


Figure 4 Chemical shift changes upon platination of the decanucleotide duplex at 25°C, (A) for the platination strand and (B) for the complementary strand. Positive values denote a downfield shift ($\Delta\delta = \delta$(platinated)–δ(free)).

EXPERIMENTAL

Materials and Methods

Dichloroethylenediamineplatinum(II) was synthesized according to a literature method (36). Decadeoxynucleotides, d(GCTCGGACAC) and d(GTGTCCGAGC) were synthesized by a β-cyanoethylphosphoramidite method (37). The extinction coefficients of 92700 $M^{-1}cm^{-1}$ and 93500 $M^{-1}cm^{-1}$ at 260 nm were calculated for d(GCTCGGACAC) and d(GTGTCCGAGC), respectively (38) and the oligonucleotide concentration was measured by UV absorbance at 260 nm.

Platinum atomic absorption analysis was performed with a Nippon Jarrell-Ash AA-880 spectrophotometer equipped with a FLA-1000 graphite atomizer. CD spectra and UV-melting curves were obtained by JASCO J-500 spectropolarimeter and JASCO Ubest-55 specrophotometer equipped with a programmable temperature controller, respectively. Ion-spray mass spectra were acquired using a Perkin-Elmer Sciex API III triple quadrupole tandem mass spectrometer equipped with an ion-spray atmospheric pressure ionization source. Samples were dissolved in 1 mM ammonium acetate in 50% acetonitrile-H_2O containing 0.1% formic acid, pH 3.5 (27). Mass spectra were obtained in the negative-ion mode scanning.

^1H NMR spectra were measured by JEOL GX500 spectrometer. Chemical shifts were measured relative to internal 2-methyl-2-propanol (1.23 ppm from DSS). Two-dimensional NOESY and DQF-COSY spectra in D_2O were recorded with 2048 points in t_2 and 256 points in t_1 (spectral width, 5000 Hz each). The NOESY and DQF-COSY data were collected in the phase-sensitive mode by the method of States *et al* (39). In the NOESY experiments, a mixing time of 100, 250 and 400 ms was used. The time domain data were zerofilled to 1024 points in the t_1 dimension before Fourier transformation.

Platination of d(GCTCGGACAC)

Stoichiometric amounts of $PtCl_2$(en) (27) and d(GCTCGGACAC) (4 x 10^{-5} M) in unbuffered solution (pH 4.0) were incubated in the dark for 10 h at 37°C. The reaction mixture was applied to a DEAE-cellulose column (11 x 210 mm) pre-equilibrated with 0.1 M NaCl, 20 mM Tris-HCl (pH 8.0) and The product was eluted with a linear gradient of NaCl (0.1-0.6 M) in 20 mM Tris-HCl (pH 8.0) and was concentrated, and then applied to a C18 column (11 x 150 mm) . Elution was performed with a linear gradient of acetonitrile (3-18%) containing 50 mM ammonium acetate. The fractions containing the chromatographically pure product were collected and diluted to a salt concentration of 0.05 M by distilled water. The solution was applied to a DEAE-cellulose column (HCO_3^- form). The column was washed with 50 mM triethylammonium bicarbonate (pH 7.5), and then the product was eluted by 1 M triethylammonium bicarbonate (pH 7.5). The volatile matters

were evaporated and the residue was co-evaporated by distilled water several times. Finally, the platinated decanucleotide was converted to the sodium form by passing it through a column of Dowex 50w (Na^+ form). The final isolated yield of the product was 53.5% and the recovery of the unreacted decamer was 19.2%. NMR samples for free and platinated duplexes were prepared by adding D_2O solution (0.4 mL) of 0.1 M NaCl, 0.1 mM EDTA and 10 mM sodium phosphate (pD 7.5) to the 1:1 mixture of the upper and the complementary strands (about 2 mM). After the pD was adjusted to 7.5, the mixtures were lyophilized. Each sample was further lyophilized three times from D_2O followed by dissolution in "100%" D_2O.

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