DNA Conformational Change Produced by the Site-Specific Interstrand Cross-Link of *trans*-Diamminedichloroplatinum(II)†

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ABSTRACT: The DNA distortion produced by the interstrand cross-link of *trans*-diamminedichloroplatinum-(II) has been described by means of gel electrophoresis, chemical probes, and molecular mechanics modeling. Synthetic double-stranded oligodeoxyribonucleotides of varying lengths (19–22 base pairs) were synthesized that contained a unique site-specific interstrand cross-link within their central sequence d(TGCT)/d(AGCT) between complementary guanine and cytosine residues. We find that the platinated deoxyriboguanosine residue adopts *syn* conformation. The duplex is distorted on both sides of the cross-link, but the bases are still paired. The distortion introduces some flexibility into the helix. In addition, the double helix is unwound and bent toward the major groove.

cis-Diamminedichloroplatinum(II) {*cis*-[$Pt(NH_3)_2Cl_2$]},[1] clinically referred to as cisplatin, is widely used for cancer chemotherapy alone and in combination with other drugs. The *trans* isomer of cisplatin {*trans*-[$Pt(NH_3)_2Cl_2$]} is clinically ineffective [for general reviews, see Brabec et al. (1990), Eastman (1987), Johnson et al. (1989), Lepre & Lippard (1990), and Reedijk (1987)].

Both *cis*- and *trans*-[$Pt(NH_3)_2Cl_2$] bind to DNA, preferentially to guanine residues at the N(7) position, producing monofunctional adducts that can subsequently close to bifunctional lesions (Bancroft et al., 1990; Eastman, 1986; Fichtinger-Schepman et al., 1985; Johnson et al., 1985). Most of the structural information currently available pertains to the intrastrand cross-links of both isomers. Intrastrand cross-links between adjacent purines represent major DNA adducts of *cis*-[$Pt(NH_3)_2Cl_2$]. This type of adducts cannot be formed by inactive *trans*-[$Pt(NH_3)_2Cl_2$] for sterical reasons. It has been, therefore, speculated that intrastrand adducts of *cis*-[$Pt(NH_3)_2Cl_2$] between two adjacent guanine residues are most likely responsible for the antitumor activity of this drug. Nevertheless, the validity of the DNA lesion(s) of *cis*-[$Pt(NH_3)_2Cl_2$] producing anticancer effects still remain(s) to be established conclusively.

cis- and *trans*-[$Pt(NH_3)_2Cl_2$] also form DNA interstrand cross-links. *In vitro trans*-[$Pt(NH_3)_2Cl_2$] forms approximately double the quantity (more than ca. 12%) of interstrand cross-links in linearized plasmid DNA than does *cis*-[$Pt(NH_3)_2Cl_2$] (Brabec & Leng, 1993). The kinetics of interstrand cross-linking by *trans*-[$Pt(NH_3)_2Cl_2$] is, however, markedly slower. In addition, while DNA interstrand cross-links of *cis*-[$Pt(NH_3)_2Cl_2$] are preferentially formed at the d(GC/GC) sites between guanine residues (Hopkins et al., 1991; Lemaire et al., 1991), the *trans* isomer preferentially forms these lesions between complementary guanine and cytosine residues (Brabec & Leng, 1993). *cis*-[$Pt(NH_3)_2Cl_2$] interstrand adduct bends the double helix by approximately 55° toward the major groove, and the distortion induced by the adduct is located at the platinated sequence d(GC/GC) (Síp et al., 1992). Information on the distortion induced in DNA by the *trans*-[$Pt(NH_3)_2Cl_2$] interstrand cross-link is, however, missing.

In this paper we describe the conformational change induced in a double-helical oligodeoxyribonucleotide [19–22 base pairs (bp)] by a unique interstrand adduct of *trans*-[$Pt(NH_3)_2Cl_2$]. We have applied electrophoresis to determine directly local duplex unwinding and bending. By means of chemical probes, we have determined the extent of the distortion around the adduct and generated a structural model using molecular mechanics modeling techniques.

MATERIALS AND METHODS

Chemicals. *trans*-[$Pt(NH_3)_2Cl_2$] was synthesized in Lachema. *trans*-[$Pt(NH_3)_2Cl(H_2O)]^+$ was generated from *trans*-[$Pt(NH_3)_2Cl_2$] (0.5 mM) by the addition of 0.9 mol equiv of $AgNO_3$ in 10 mM $NaClO_4$ at 37 °C for 24 h in the dark. The AgCl precipitate was removed by centrifugation. All enzymes used in this work were from Bethesda Research Laboratories. Osmium tetraoxide (OsO_4) and diethyl pyrocarbonate (DEPC) were from Sigma. Chloroacetaldehyde (CAA) from Fluka was doubly distilled before use. Acrylamide, bis(acrylamide), urea, and NaCN were from Merck.

Oligonucleotides and Their Platinations. The oligodeoxyribonucleotides (Figure 1) were synthesized, purified, allowed to react with the platinum compound, and repurified as described previously (Brabec et al., 1992). Briefly, the oligonucleotides synthesized on an Applied Biosystems solid-phase synthesizer were purified by ion-exchange FPLC. The single-stranded oligonucleotides (the top strands in Figure 1) were reacted with *trans*-[$Pt(NH_3)_2Cl(H_2O)]^+$. The platinated oligonucleotides were purified by FPLC. It was verified (Brabec et al., 1992; Lemaire et al., 1991) that the oligonu-

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[1] Abbreviations: bp, base pair; CAA, chloroacetaldehyde; *cis*-[$Pt(NH_3)_2Cl_2$], *cis*-diamminedichloroplatinum(II); d(TGCT)/d)AGCA) (19) etc. are names used to identify oligonucleotide duplexes, as defined in Figure 1; DEPC, diethyl pyrocarbonate; FPLC, fast protein liquid chromatography; *trans*-[$Pt(NH_3)_2Cl_2$], *trans*-diamminedichloroplatinum-(II); *trans*-[$Pt(NH_3)_2(H_2O)Cl]^+$, *trans*-diamminemonoaquamonochloroplatinum(II).

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                                        *
d(TGCT)/d(AGCA)(19):      5' CTTCTCCTTGCTCTCCTTC
                             AAGAGGAACGAGAGGAAGG  5'
                                        *

                                        *
d(TGCT)/d(AGCA)(20):      5' CTTCTCCTTGCTCTCCTTCT
                             AAGAGGAACGAGAGGAAGAG  5'
                                        *

                                        *
d(TGCT)/d(AGCA)(21):      5' CTTCTCCTTGCTCTCCTTCTC
                             AAGAGGAACGAGAGGAAGAGG  5'
                                        *

                                        •
d(TGCT)/d(AGCA)(22):      5' CTTCTCCTTGCTCTCCTTCTCT
                             AAGAGGAACGAGAGGAAGAGAG  5'
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FIGURE 1: Synthetic oligodeoxyribonucleotides used in the present study and their abbreviations. The top and bottom strands of each pair are designated top and bottom, respectively, in the text. The asterisks indicate the location of the interstrand cross-link after modification of the oligonucleotides by *trans*-$[Pt(NH_3)_2Cl(H_2O)]^+$ in the way described in the experimental section.

cleotides contained one platinum atom that was bound at the single guanine residue. The platinated strands were allowed to anneal with unplatinated complementary strands (the bottom strands in Figure 1). After dialysis against 0.1 M $NaClO_4$ at 4 °C, the samples were incubated for 24 h in the dark at 37 °C. The duplexes containing the interstrand cross-link were purified by FPLC in an alkaline gradient. The location of the interstrand cross-link between guanine residue in the top strands and the complementary cytosine residues (shown in Figure 1 by the asterisks) was verified as described in our previous paper (Brabec & Leng, 1993).

Ligation and Electrophoresis. Unplatinated single strands (top strands in Figure 1) and the duplexes (Figure 1) containing a unique interstrand cross-link were 5′-end-labeled with [γ-^{32}P]-ATP by using T4 polynucleotide kinase. Unplatinated single strands were annealed with their phosphorylated complementary strands. Unplatinated and interstrand cross-link-containing duplexes were allowed to react with T4 DNA ligase. Platinum was removed from the self-ligated cross-linked DNA by treatment with cyanide ion. Such reactions were performed on one half of the typical ligation mixture (10 μL) by the addition of 40 μL of 0.25 M NaCN (alkaline pH) for 10 h at 45 °C. The other half was stored at −20 °C, and the two along with ligated unplatinated duplexes were subsequently examined on 8% native polyacrylamide [mono:bis(acrylamide) ratio = 29:1] electrophoresis gels. Other details of these experiments were the same as those described in the previously published papers (Bellon & Lippard, 1990; Koo et al., 1986).

Chemical Modifications. The modifications were performed on single strands or duplexes 5′-end-labeled with [γ-^{32}P]ATP by using T4 polynucleotide kinase. The modifications of oligonucleotides by OsO_4, DEPC, and CAA were performed as described previously (Marrot & Leng, 1989), with minor alterations. The modification by OsO_4 was performed in the presence of 2,2′-bipyridine; both OsO_4 and 2,2′-bipyridyl were at the concentration of 0.75 mM, and the reaction was performed for 10 min at 20 °C. The modification by CAA was performed in 0.1 M sodium acetate, pH 6.4, for 1 h at 25 °C; the concentration of CAA was ca. 0.3 M. In the case of the platinated oligonucleotides, platinum was removed after reaction of the DNA with the probe by incubation with 0.2 M NaCN (alkaline pH) at 45 °C for 10 h in the dark.

Molecular Modeling Methods. The modeling procedure described previously (Síp et al., 1992) for DNA conformation modified by the *cis*-$[Pt(NH_3)_2Cl_2]$ interstrand adduct has been largely retained for computations performed in this work. Initial conformations of DNA modified by *trans*-$[Pt(NH_3)_2Cl_2]$ interstrand cross-link were generated by the JUMMA program (Lavery, 1988) and then minimized by means of the AMBER program (Weiner & Kollmann, 1986). Improper dihedral angles were used to define the geometry of the N(7)–Pt bond according to Herman et al. (1990), calculated so that the out-of-plane bending constant of this bond was approximately 24.1 kcal·mol^{-1}·rad^{-2} (J. Kozelka, private communication). The same value was attributed to the Pt–N(3) out-of-plane bending constant of cytosine.

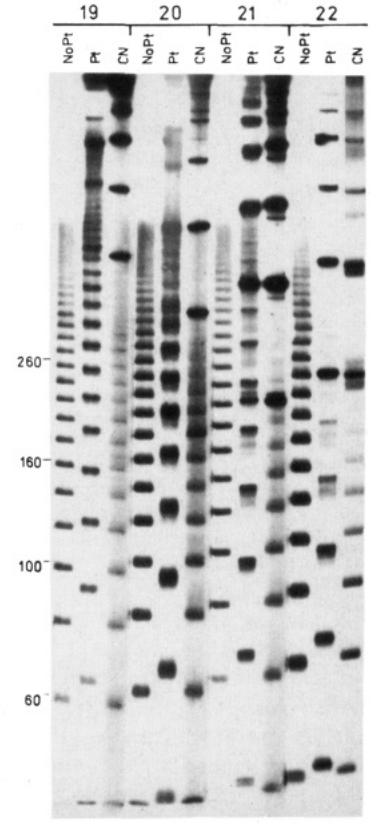

FIGURE 2: Autoradiograms of the ligation products of double-stranded oligonucleotides d(TGCT)/d(AGCA) (19–22) containing a unique *trans*-$[Pt(NH_3)_2Cl_2]$ interstrand cross-link separated on an 8% polyacrylamide gel. Unplatinated oligomers, lanes No Pt; platinated oligomers, lanes Pt; platinated oligomers treated with NaCN as described in the text prior to running of the gel.

RESULTS

Gel Electrophoresis. Bending of the longitudinal axis of the DNA double helix can be revealed by electrophoresis in native polyacrylamide gels (Koo et al., 1986). Autoradiograms of electrophoresis gels revealing resolution of the ligation products of 19–22-bp duplexes d(TGCT)/d(AGCA) (19–22) (Figure 1) containing a unique interstrand cross-link of *trans*-$[Pt(NH_3)_2Cl_2]$ are shown in Figure 2. Maximum retardation was observed for 21-bp multimers. The variations of the *K* factor (apparent length relative to the sequence length) versus sequence length are shown in Figure 3. The steepest slope was obtained for the 21-bp curve. As compared to the 21-bp curve, the 22-bp curve had only a slightly smaller slope, whereas the 20-bp curve differed more pronouncedly. This asymmetry suggests that the natural 10.5-bp repeat of B-DNA and that of DNA perturbed by the *trans*-$[Pt(NH_3)_2Cl_2]$ interstrand cross-link are different as a consequence of DNA unwinding (Bellon et al., 1991).

The exact helical repeat of the interstrand cross-linked duplex and from it the unwinding angle were calculated by interpolation with the use of the *K* versus interadduct distance

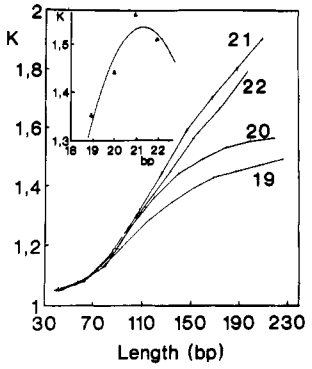

FIGURE 3: Plot showing the relative mobility K versus sequence length curves for the oligomers d(TGCT)/d(AGCA) (19–22), denoted respectively as 19, 20, 21, 22. Inset: Plot showing the relative mobility K versus interadduct distance d (bp) for the platinated oligomers with a total length of 140 bp. The triangles represent the average of three independent electrophoresis experiments. The curve represents the best fit of these experimental points to the equation $K = ad^2 + bd + c$ (Bellon et al., 1991).

curve as described in the paper of Bellon et al. (1991) for intrastrand adducts of *cis*-$[Pt(NH_3)_2Cl_2]$. The maximum of this curve constructed for the interstrand cross-linked duplexes with a total length of 140 bp (Figure 3, insert) was determined to be 21.35 ± 0.04 bp. Total sequence lengths other than 140 bp were examined and generally gave results within a few hundredths of a bp of the above value. To convert the interadduct distance in bp corresponding to the curve maximum into a duplex unwinding angle in degrees, the value is compared with that of the helical repeat of B-DNA, which is 10.5 ± 0.05 bp (Rhodes & Klug, 1980; Wang, 1979). The difference between the helical repeat of B-DNA and the DNA containing interstrand cross-link of *trans*-$[Pt(NH_3)_2Cl_2]$, therefore, is $[(21.35 \pm 0.04) - 2(10.5 \pm 0.05)] = 0.35 \pm 0.09$ bp. There are 360 deg/10.5 bp, so the DNA unwinding due to one interstrand adduct of *trans*-$[Pt(NH_3)_2Cl_2]$ is 12 ± 3°.

The appreciation of the relationship between interadduct distance and phasing for self-ligated multimers composed of the identical number of monomeric duplexes (bend units) resulted in a bell-shaped pattern (e.g., Figure 3, inset) characteristic for bending (Bellon & Lippard, 1990; Rice et al., 1988). The quantitation of the bend angle of the *trans*-$[Pt(NH_3)_2Cl_2]$ interstrand cross-link was performed in the way described previously (Bellon & Lippard, 1990; Koo & Crothers, 1988) utilizing the empirical equation

$$K - 1 = (9.6 \times 10^{-5}L^2 - 0.47)(RC)^2 \qquad (1)$$

where L represents the length of a particular oligomer with relative mobility K and RC the curvature relative to a DNA bending induced at the tract of six adenines (A_6 tract) (Rice et al., 1988). Application of eq 1 to the 105- and 126-bp multimers of the 21-bp oligomer leads to a curvature of 0.65 for d(TG*CT)/d(AGC*A) (21), relative to an A_6 tract. The average bend angle per helix turn can be calculated by multiplying the relative curvature by the absolute value of an A_6 tract bend [20° (Bellon & Lippard, 1990; Levene et al., 1986)]. The result indicates that the bend induced by the *trans*-$[Pt(NH_3)_2Cl_2]$ interstrand cross-link is about 26°. Other details of the calculations of the unwinding and bend angles are given in the papers of Bellon et al. (1991), Bellon and Lippard (1990), and Koo and Crothers (1988).

Also produced in ligations of all monomers investigated in this work were a separate series of bands arising from small DNA circles that migrate close to the top of the gel (Figure 2, lanes Pt). The occurrence of small DNA circles was best evident if the platinum was removed from the products of the ligation reaction by NaCN.

The highest tendency to yield DNA circles was observed for the 21- and 22-bp multimers (Figure 2, lanes Pt). Quite interestingly, the ligation products of the interstrand cross-linked duplexes contained several types of DNA circles (Figure 2, lanes Pt and CN^-). Moreover, the DNA circles were also observed in the ligation products of the interstrand cross-linked 19-bp duplexes (Figure 2), even when the tendency to form the circles was considerably lower in comparison with that of the products of the cross-linked 21-bp duplex.

Chemical Probes of DNA Conformation. To test whether the interstrand cross-link distorts or even denatures locally the double helix, the platinated d(TGCT)/d(AGCA) (22) was treated with several chemical agents that are used as tools for determining non-B-DNA. These agents include OsO_4, DEPC, and CAA, which react preferentially with single-stranded DNA and distorted double-stranded DNA (Nielsen, 1990).

OsO_4 is hyperreactive with thymine residues and slightly reactive with cytosines in single-stranded nucleic acids and in distorted DNA as compared to B-DNA (Palecek et al., 1990). OsO_4 reacted with no residue within the unplatinated duplex (Figure 4, lane ds). Mainly thymine residues were reactive strongly and a few cytosine residues weakly in the unplatinated single-stranded top oligonucleotide (Figure 4, lane ss). If the duplex contained an interstrand cross-link of *trans*-$[Pt(NH_3)_2Cl_2]$, then the first 5′ thymine residue in the top strand adjacent to the adduct was strongly reactive (lane Pt). A weak but significant reactivity was also observed for the first 3′ cytosine and thymine residues adjacent to the cross-link. No reactivity between OsO_4 and the residues in the bottom strand of the cross-linked duplex was noticed.

DEPC carbetoxylates purines at the N(7) position. It is hyperreactive with unpaired and distorted adenine residues in DNA and with left-handed Z-DNA (Herr, 1985; Johnston & Rich, 1985). Adenine and guanine residues within the unplatinated single-stranded oligonucleotides (top and bottom) readily reacted with DEPC (Figure 4, right, lanes ss top no Pt and ss bottom no Pt). However, within the single-stranded top oligomer modified monofunctionally by *trans*-$[Pt(NH_3)_2Cl(H_2O)]^+$ at the single guanine residue, this residue was no longer modified (lane ss top Pt). Similarly, no reactivity of adenine and guanine residues was observed within the unplatinated double-stranded oligonucleotide (lane ds no Pt). Within the interstrand cross-linked double-stranded oligonucleotide, two residues became reactive (lane ds Pt): adenine and slightly guanine residues complementary to the 5′ thymine and 3′ cytosine residues in the top strand which were reactive with OsO_4.

CAA reacts with N(1) and N(6) of adenine and N(3) and N(4) of cytosine residues. It is hyperreactive with these residues in single-stranded DNA, which makes this probe particularly suitable for observing base residues in denatured regions of DNA. CAA is also hyperreactive with adenine and cytosine residues in the junctions between B- and Z-DNA and with adenine residues in Z-DNA as compared with B-DNA (Kohwi-Shigematsu et al., 1987; Lilley, 1983; McLean at al., 1987). All adenine and cytosine residues within the unplatinated top and bottom single strands readily reacted with CAA (not shown). No reactivity of these residues with CAA was, however, observed within the unplatinated or interstrand cross-linked duplexes.

All results obtained with chemical probes are summarized in Figure 5.

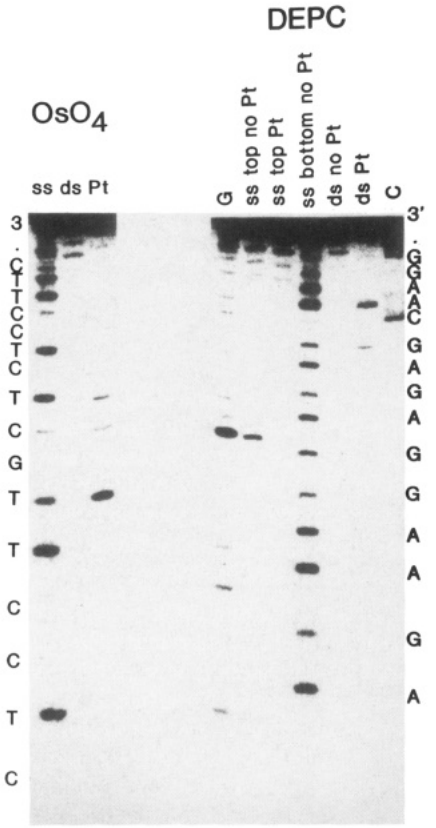

FIGURE 4: Piperidine-induced specific strand cleavage at OsO_4-modified (left) and diethyl pyrocarbonate-modified (right) bases in unplatinated and platinated d(TGCT)/d(AGCA) (22). All oligomers were 5′-end-labeled, and, if not stated otherwise, the duplexes were labeled at both strands. OsO_4: The lane ss is relative to the unplatinated top strand. The lane ds is relative to the unplatinated duplex. The lane Pt is relative to the duplex containing the *trans*-$[Pt(NH_3)_2Cl_2]$ interstrand cross-link. DEPC: The lane G is the Maxam–Gilbert-specific reaction for the unplatinated duplex that had only the top strand end-labeled. The lane ss top no Pt is relative to the unplatinated top strand. The lane ss top Pt is relative to the top strand modified monofunctionally by *trans*-$[Pt(NH_3)_2Cl(H_2O)]^+$ as described in the experimental section. The lane ss bottom no Pt is relative to the unplatinated bottom strand. The lane ds no Pt is relative to the unplatinated duplex. The lane ds Pt is relative to the duplex containing the *trans*-$[Pt(NH_3)_2Cl_2]$ interstrand cross-link. The lane C is the Maxam–Gilbert-specific reaction for the unplatinated duplex that had only the bottom strand end-labeled.

Molecular Mechanics Modeling. A molecular mechanics study was performed to assess possible conformations of a platinated octameric duplex. This duplex corresponds to the central part of the oligonucleotides studied by gel electrophoresis and chemical probes. The numbering for the residues in this duplex was as follows:

H1- C2- p3- T4- p5- T6- p7- G*8- p9- C10-p11-T12-p13-C14-p15-T16-H17

H34-G33-p32-A31-p30-A29-p28-C*27-p26-G25-p24-A23-p22-G21-p20-A19-H18

The asterisks show the location of the *trans*-$[Pt(NH_3)_2Cl_2]$ interstrand cross-link. A rotational degree of freedom around the axis N(7)(G8)–Pt–N(3)(C27) yielded, if some stacking should be preserved, two possible conformations denoted I and II.

The conformation I was obtained by rotating the guanine residue of a Watson–Crick G·C bp in the base plane so that N(7)(G*8) faced N(3)(C*27) and by minimizing the straight B-DNA fragment containing the platinated G8 (Figure 6, top). In this model, the platinated bp collided considerably with the regular double helix, bringing the two strands closer. The determining distance N(9)(G*8)–N(3)(C*27) was 0.8 nm, compared to about 1.0 nm in standard B-DNA. This shortening created a tension, which was expressed by a buckle of 46° of the adjacent thymine·adenine bp. There was no stabilizing hydrogen bonds within the modified bp. This geometry placed the platinated guanine residue partially into the minor groove, with a resulting loss of stacking. The energy of the molecule minimized by AMBER was –116.9 kcal·mol^{-1}.

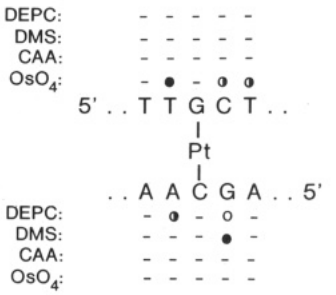

FIGURE 5: Summary of the reactivity of chemical probes. Data for DMS are from our previous paper (Brabec & Leng, 1993). ●, ◑, o and - designate strong, medium, weak, and no reactivity, respectively.

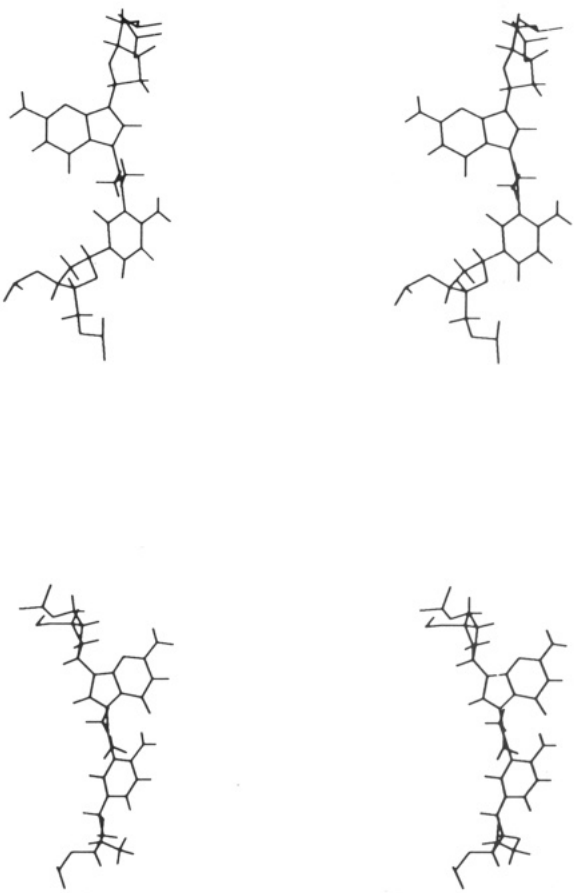

FIGURE 6: Stereoview of the central guanine·cytosine base pair in the octameric duplex having a central sequence d(TG*CT)/d(AGC*A). The duplex contained the interstrand cross-link of *trans*-$[Pt(NH_3)_2Cl_2]$ at the base residues denoted by the asterisks. The top and bottom rows are relative to the conformations I and II, respectively.

The conformation II was obtained by rotating the platinated guanine around the N(7)(G*8)–N(3)(C*27) axis by 180°. This attempt was inspired by the observation that this configuration of the guanine residue was found in the crystals of methylated guanine and cytosine residues cross-linked by *trans*-$[Pt(NH_3)_2Cl_2]$ (Dieter-Wurm et al., 1992). The conformation II (Figure 6, bottom) was generated starting with

Table I: Characteristics of the Calculated Conformations

	conformation					
	II(−25)	II(0)	II(10)	II(25)	II(30)	II(40)
energy (kcal/mol)	−117.6	−112.4	−123.8	−126.1	−125.6	−125.9
curvature (deg)	−5	3	12	27	34	41

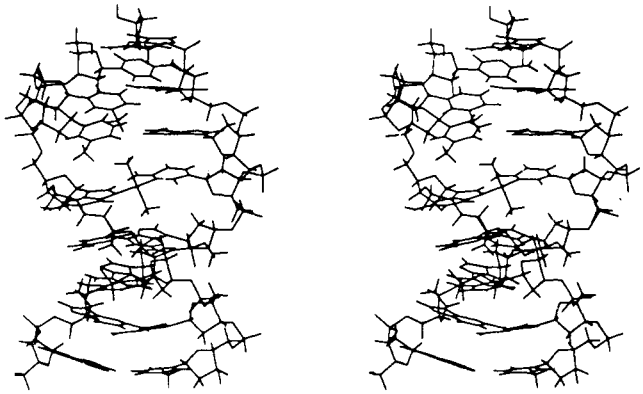

FIGURE 7: Stereoview of the octameric duplex d(TGCT)/d(AGCA) interstrand cross-linked by *trans*-[$Pt(NH_3)_2Cl_2$] (for details, see Figure 6). The model is relative to the conformation II(25).

the G* residue having a propeller twist of 180°. The rotation resulted in a metal-modified Hoogsteen base pairing. The N(7)(G*8)–N(3)(C*27) distance was 0.84 nm, and the bp was stabilized by a hydrogen bond [O(6)(G*8)–HN(4)(C*27) distance was 0.203 nm]. This geometry conserved stacking of the modified guanine residue, and the energy of this conformation was −122.4 kcal·mol^{-1}.

Comparison of the energies of both conformations favors the conformation II. This conclusion is also supported by an apparent tendency of the platinated guanine base residue in the helix adopting conformation I to rotate around the N(7)-(G*8)–N(3)(C*27) axis (Figure 6, top), which is a fundamental feature of the conformation II.

The conformation II was examined with respect to the curvature induced by the *trans*-[$Pt(NH_3)_2Cl_2$] interstrand cross-link. For this purpose, a series of conformations exhibiting a kink was generated by JUMMA and then minimized. The results are summarized in Table I. An initial conformation with the kink oriented toward the minor groove [II(−25)] yielded upon minimization an almost straight double helix whose energy was relatively high (Table I). Increasing further the value of the initial kink toward the minor groove resulted in a marked growth of the energy (for instance, the minimized conformation II(−40) had an energy of −93.8 kcal·mol^{-1}). On the other hand, the initial conformations with the kinks toward the major groove kept roughly their initial curvature and had energies lower than the conformations II with the kink oriented toward the major groove (Table I). Thus, the kink oriented toward the minor groove seems unlikely. The energy minimum was attained by the model with the initial kink of 25° [II(25)], yielding the curvature of 27°. However, the energy well was flat (Table I), which suggests some flexibility of the helix in the direction of the major groove.

The model exhibiting the lowest energy [II(25)] is shown in Figure 7. In this model, the basic features of B-DNA are conserved, with the exception of the region around the adduct. Apart from the inverted position of the platinated guanine (G*), the most striking feature of the structure is a lack of stacking of both the T6–A29 and G25–C10 bp with G*8–C*27 bp. This is apparently due to van der Waals contacts of *trans*-[$Pt(NH_3)_2Cl_2$] amino groups mainly with T6 and G26. These contacts increase the rise of the base residues to about 0.5 nm and their propeller twists to −43° and −39°, respectively. The increased propeller twists are still transmitted to the next base pairs in both directions. The value of the propeller twist for the T4–A31 and T12–A23 bp is about 20°. The initial C(2′)-endo-type sugars relax again into an *S* conformation. The largest deviations of this kind are observed again for the T6 and G25 sugar residues, which adopt the O(1′)-endo conformation.

DISCUSSION

This paper is aimed at describing the conformational distortion induced in DNA by the interstrand cross-link of *trans*-[$Pt(NH_3)_2Cl_2$] between complementary guanine and cytosine residues. The present experiments were carried out with the double-stranded oligodeoxyribonucleotides (19–22 bp) containing the unique interstrand adduct in their central sequence d(TGCT)/d(AGCA) (Figure 1).

The platinated and unplatinated duplexes were polymerized and the products analyzed by gel electrophoresis. The extent of DNA unwinding and bending produced by the interstrand cross-link was revealed by their gel mobility shifts relative to unplatinated controls. The extent of unwinding and bending was calculated according to the procedure used previously for *cis*-[$Pt(NH_3)_2Cl_2$] intrastrand adducts (Bellon & Lippard, 1990; Bellon et al., 1991; Rice et al., 1988). We find that the *trans*-[$Pt(NH_3)_2Cl_2$] interstrand cross-link unwinds DNA by approximately 12° and bends the double helix by about 26°.

The ligation products of the platinated duplexes examined in this work contained more than one type of the DNA circles (Figure 2). In addition, the 19-bp duplexes containing *trans*-[$Pt(NH_3)_2Cl_2$] interstrand cross-link (Figure 2) exhibit a low but still significant tendency to yield the DNA circles. These results can be interpreted to mean that the formation of the *trans*-[$Pt(NH_3)_2Cl_2$] interstrand cross-link results in the directional bending with some flexibility.

A limited flexibility introduced to the helix by the *trans*-[$Pt(NH_3)_2Cl_2$] interstrand cross-link is sustained by the observation that this lesion creates a local conformational distortion revealed by the chemical probes (Figures 4 and 5). This distortion extends mainly over 4 bp. The lack of reactivity of CAA with the platinated duplex indicates that the interstrand cross-link of *trans*-[$Pt(NH_3)_2Cl_2$] does not create single-stranded regions in DNA (Kohwi-Shigematsu et al., 1987; Lilley, 1983; McLean et al., 1987).

Molecular mechanics calculations yield the lowest energy structure, in which the platinated deoxyriboguanosine residue adopts a *syn* conformation. This structure also includes a kink toward the major groove of about 27°, in agreement with the results of the gel electrophoresis. The models calculated for various values of the initial kink reveal a shallow energy well around the minimum, which also agrees with a limited flexibility of the helix deduced from the gel electrophoresis experiments.

In conclusion, gel electrophoresis, chemical probes, and molecular mechanics modeling propose the following features of the conformational alterations induced in B-DNA by an interstrand cross-link of *trans*-[$Pt(NH_3)_2Cl_2$]. The platinated deoxyriboguanosine residue adopts *syn* conformation. The nondenaturational distortion occurs on both sides of the cross-link and introduces some flexibility into the helix. In addition, the helix is unwound and bent toward the major groove. Mean

values of the bending and unwinding angles are 26° and 12°, respectively.

DNA interstrand cross-links of antitumor *cis*-$[Pt(NH_3)_2Cl_2]$ are believed unlikely to be lesions responsible for the antitumor effect of this drug. This view is supported by the observation that clinically ineffective *trans*-$[Pt(NH_3)_2Cl_2]$ forms in DNA more interstrand cross-links than its *cis* isomer. However, acceptance of this view implies that the nature and character of the interstrand lesions induced in DNA by both isomers are identical. This and other papers indicate significant differences in the interstrand cross-links produced in DNA by *cis*- and *trans*-$[Pt(NH_3)_2Cl_2]$. Thus, the role of these lesions in the mechanism of antitumor effects of platinum drugs deserves further systematic examinations.

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