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Distortions Induced in DNA by *cis*-Platinum Interstrand Adducts†

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ABSTRACT: A 22 base pair double-stranded oligonucleotide containing a unique interstrand adduct resulting from chelation of the two guanine residues within the central sequence d(TGCT/AGCA) by a *cis*-platinum residue has been studied by means of gel electrophoresis, chemical probes, and molecular mechanics. The anomalously slow electrophoretic mobility of the multimers of the platinated and ligated oligomers suggests that the platinated oligonucleotide is bent. The two cytosine residues (complementary to the platinated guanines) are hyperreactive to hydroxylamine, indicating a large exposure of the two bases to the solvent. The adduct does not induce a local denaturation within the flanking sequences since the adenine residues are not reactive with diethyl pyrocarbonate. This is confirmed by the nonreactivity of the complementary T residues with osmium tetraoxide. These results and the molecular mechanics modeling suggest that the interstrand adduct bends the double helix by approximately 55° toward the major groove, that the double helix conserves its average twist angle, and that the distortion induced by the adduct is localized at the platinated sequence d(GC/CG).

cis-Diamminedichloroplatinum(II) (cis-DDP)[1] is widely used in the treatment of human tumors. Numerous results suggest that the cytotoxic action of *cis*-DDP is related to its ability to react with cellular DNA. Lesions produced in DNA have been characterized as bifunctional adducts including mainly intrastrand and interstrand cross-links [for general reviews, see Eastman (1987), Reedijk (1987), Johnson et al. (1989), and Lepre and Lippard (1990)]. The major adducts are DNA intrastrand cross-links. Although the interstrand cross-links represent a minor portion of the total lesions, they have often been implicated with cytotoxicity [for a general review, see Roberts et al. (1988)].

Structural knowledge of the lesions induced in DNA by the binding of *cis*-DDP is a necessary step to understand the antitumor activity of *cis*-DDP or at least to understand the processing of DNA lesions involved in mutagenesis and DNA repair. Several structural studies have been already devoted to the intrastrand adducts [for general reviews, see Eastman

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[1] Abbreviations: *cis*-DDP, *cis*-diamminedichloroplatinum(II); OsO_4, osmium tetraoxide; HA, hydroxylamine; DEPC, diethyl pyrocarbonate.

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(1987), Reedijk (1987), and Lepre and Lippard (1990)]. However, little is known about the structure of the interstrand cross-links.

DNA interstrand cross-linking occurs predominantly between two guanine N7 atoms on opposite strands (Eastman, 1985; Fichtinger-Schepman et al., 1986) at the d(GC) sequences (Lemaire et al., 1991; Hopkins et al., 1991). A distance of about 3 Å is required for the *cis*-DDP cross-linking reaction (Sherman et al., 1988; Lippert, 1989). In B-DNA the two guanine N7 atoms are separated by about 7 Å. We were interested to characterize the distortions induced in DNA by the interstrand adducts. We studied a double-stranded oligonucleotide (22-mer) containing a unique interstrand adduct. To describe the distortions, we used techniques giving either a global view of the double helix (electrophoresis) or a local view (chemical probes). Moreover, a structural model was generated using molecular mechanics modeling techniques.

Material and Methods

The oligonucleotides synthesized on an Applied Biosystems solid-phase synthesizer were purified by ion-exchange FPLC. Klenow polymerase, T4 polynucleotide kinase, T4 DNA ligase, and restriction enzymes were purchased from Boehringer-Mannheim and from Bethesda Research Laboratories. Enzyme buffers were those recommended by the suppliers. Electrophoresis-grade acrylamide, bis(acrylamide), and sodium cyanide were from Merck.

Synthesis of the Interstrand Adducts. The interstrand adducts represent a minor proportion of the total adducts formed in the reaction between DNA and *cis*-DDP. In order to prepare a double-stranded DNA containing a unique interstrand adduct within a given sequence with a reasonable yield, the following procedure has been used. The oligonucleotide d(CTTCTCCTTGCTCTCCTTCTTC) containing a unique G residue was first reacted with the platinum complex *cis*-[$Pt(NH_3)_2$ (*N7*-*N*-methyl-2,7-diazapyrenium) Cl]$^{2+}$. The reaction occurred mainly on the N7 of the G residue (Malinge et al., 1990). After purification by ion-exchange FPLC, the modified oligonucleotide was mixed with its complementary strand and incubated in 1 M NaCl during 20 h at 37 °C. A recent observation which will be published in detail elsewhere has shown that in these conditions about 50% of the adduct was transformed into a G residue bearing a monofunctional platinum adduct resulting from the release of *N*-methyl-2,7-diazapyrenium. After three precipitations with ethanol, the platinated double-stranded oligonucleotides were dissolved in 50 mM $NaClO_4$ and incubated overnight at 37 °C. The cross-linked oligonucleotide was purified by electrophoresis in a denaturing 12% polyacrylamide gel. The formation of an interstrand adduct between the two G residues within the sequence d(GC/CG) was verified by HPLC on a reverse-phase C18 column (Lichrosorb RP-8 from Merck) after digestion of the oligonucleotides with the endonuclease P1 and then treatment with alkaline phosphatase (Fichtinger-Schepman et al., 1985; Eastman, 1986). The location and nature of the adduct were confirmed by the chemical reactivity of the platinated bases with dimethyl sulfate and formic acid respectively, as previously described (Lemaire et al., 1991).

Chemical Modifications. The reaction with osmium tetraoxide, hydroxylamine, and diethyl pyrocarbonate were performed as previously described (Marrot & Leng, 1989). The concentration of the oligonucleotide was about 3×10^{-5} M (in nucleotide residues). The $NaClO_4$ concentration was adjusted to 100 mM. With all the chemical reagents, it has been verified that about one cleavage per single-stranded oligonucleotide occurred. The duplexes were labeled either at one 3′ end with Klenow polymerase and [α-^{32}P]dGTP or dCTP (from Amersham) or at one 5′ end with T4 polynucleotide kinase and [γ-^{32}P]ATP (Amersham) before the reaction with the chemical probes.

Cleavage by Restriction Endonucleases. Unplatinated and platinated samples were incubated with *Eco*RI or *Bam*HI endonucleases at 37 °C. The buffer was 100 mM NaCl, 10 mM $MgCl_2$, 50 mM Tris-HCl, pH 8. One endonuclease unit was added per microgram of DNA.

Ligation and Electrophoresis. These were performed as described by Koo et al. (1986).

Molecular Modeling Methods. Initial DNA conformations have been generated by the JUMNA program (Lavery, 1988). The terminal bases of the first strand have been locked to their local helical axis segments, and a central kink was introduced between the two GC base pairs. Six structures with axis curvature ranging from 0° to 90° have been generated and minimized by JUMNA. The straight structure corresponds to the regular B-DNA double helix.

The resulting coordinates were introduced into the EDIT module of AMBER (Weiner & Kollmann, 1986). The internal geometry read from the LINK module contained a platinated G residue, so that the *cis*-$Pt(NH_3)^{2+}$ moiety could be automatically positioned at half the distance between the two N7 atoms respecting approximately the valence bond angles. The atomic charges and the force field parameters used for nucleotides were taken from the AMBER data base. Parameters concerning the Pt residue were set accordingly to Herman et al. (1990) with the following modification (J. Kozelka, private communication). Instead of using the four Pt-X-X-X improper torsional angles, the equilibrium position of the N7–Pt bonds was defined by dummy atoms DU placed in the geometrical center of the G five-membered ring. The equilibrium bond angle DU–N7–Pt was set to 180°. Probably the most important parameter, which determines the structural changes of the adduct, is the out-of-plane bending constant for the N7–Pt bond. This constant has been recently evaluated using ab initio calculations. Its relatively low value 24.1 kcal·mol^{-1}·rad^{-2} (J. Kozelka, private communication) allows the Pt atom to deviate from the base plane, making possible a better base stacking. The structures were energy minimized in vacuo using a gas-phase potential and a distance-dependent dielectric constant $\epsilon_{ij} = cr_{ij}$ (c = 4 Å^{-1}). The high value of the dielectric constant prevents effectively the minimized molecule from forming additional intramolecular hydrogen bonds. Control refinements with c = 1 Å^{-1} resulted in hydrogen bonding between one amino group of the Pt residue and phosphate group oxygens in the kinked structures, which did not appear when c = 4 Å^{-1} was used for minimizing the same structure. The closest distance between a phosphate group oxygen and an amino group of the Pt residue is greater than 4.3 Å.

The resulting structures have been analyzed by means of the ANAL module of AMBER and the CURVES program (Lavery & Sklenar, 1988).

Results

Most of the experiments here reported were done with the following oligonucleotide (22-mer), which will be referred to by its central sequence d(TG*CT/ACG*A) (an asterisk denotes a base modified by a platinum residue).

```
5′ C T T C T C C T T G*C T C T C C T T C T T C
     A A G A G G A A C G*A G A G G A A G A A G C 5′
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Gel Electrophoresis. Several studies have shown that the distortions induced in DNA by the covalent binding of drugs

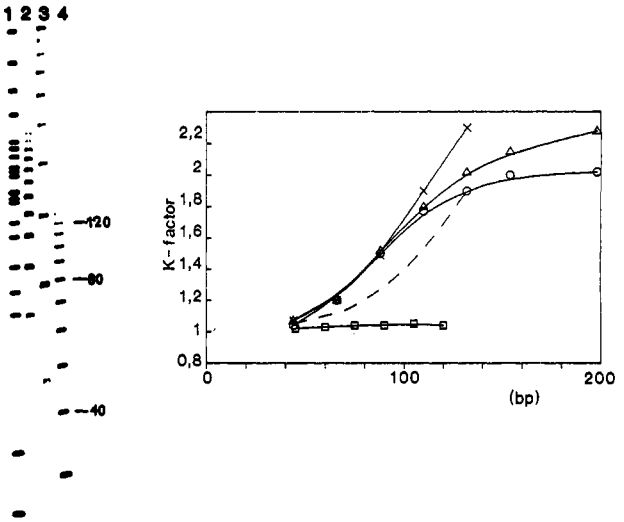

FIGURE 1: Comparison of the migration of platinated to unplatinated multimers on a nondenaturing 8% polyacrylamide gel. Left: Marker fragments (pBR322 DNA cleaved by the endonuclease *Hpa*II), lane 1; unplatinated d(TGCT/AGCA) (22-mer) ligated to multimers, lane 2; platinated d(TG*CT/AG*CA) (22-mer) ligated to multimers, lane 3; *Bam*HI linker (10-mer) ligated to multimers, lane 4. Right: K-factor (apparent length relative to sequence length) versus sequence length for the multimers of the 22-mer d(TG*CT/AG*CA) at 4 °C (×), room temperature (Δ), and 37 °C (○) and for the multimers of the 15-mer d(CTCCTTG*CTCCTCTC/GGAGAGGAG*CAAGGA) (□) at room temperature. The dashed line is relative to the multimers with intrastrand adducts at the d(GG) sites [taken from Rice et al. (1988)].

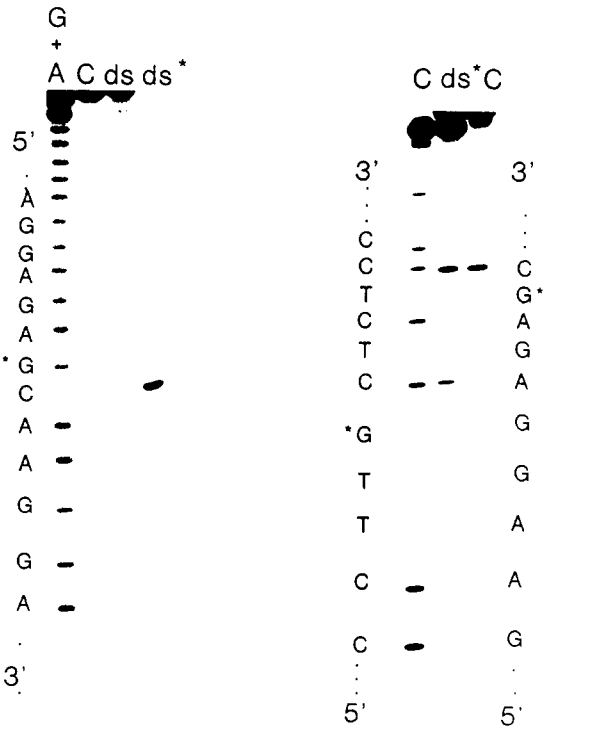

FIGURE 2: Piperidine-induced specific strand cleavage at hydroxylamine-modified bases in the 22-mer d(TG*CT/AG*CA). Left: The results are relative to the purine-rich strand labeled at the 3′ end. The lanes ds and ds* correspond respectively to the unplatinated and the platinated oligonucleotides. Lanes G + A and C are Maxam–Gilbert specific reactions for the unplatinated oligonucleotide. Right: The lane ds* is relative to the platinated oligonucleotide in which both the 5′ ends are labeled. The two lanes labeled C are Maxam–Gilbert specific reactions for the unplatinated oligonucleotide.

can be revealed by electrophoresis in nondenaturing polyacrylamide gels [for a general review, see Leng (1990)]. For example, *cis*-DDP bound either to d(GG) or d(AG) sites bends DNA similarly (Rice et al., 1988; Schwartz et al., 1989; Bellon & Lippard, 1990). We have compared the electrophoretic mobility of the multimers of the ligated 22-mer d(TGCT/ACGA) with and without interstrand adducts. A typical autoradiogram of a polyacrylamide gel is shown in Figure 1. While an anomalous gel migration occurs with DNA fragments containing interstrand adducts, there is only very weak evidence for formation of circles. Moreover, the K-factor (apparent length relative to sequence length) does not change in the temperature range 4–37 °C, at least for the first multimers (Figure 1). These results are more in favor of a bending of the double helix induced by the interstrand adducts rather than an increase in flexibility. The bending was further demonstrated by the study of the electrophoretic mobility of the multimers of the ligated 15-mer d-(CCTCCTTGCTCCTCT/GAGAGGAGCAAGGAG) with and without interstrand adducts. The corresponding multimers have the same normal mobility [in fact, the platinated samples present a slight constant retardation due to the two positive charges of the adduct in agreement with previous results on multimers modified by a monofunctional platinum derivative (Marrot & Leng, 1989)]. Thus, the K-factor is equal to 1 (Figure 1), which suggests that the helical repeat is not altered by the interstrand adduct.

For comparison, the values of the K-factor relative to the multimers of the ligand 22-mer d(TGGT/ACCA) modified at the d(GG) sites by *cis*-DDP are given in Figure 1 [dashed line taken from Rice et al. (1988)]. The adducts were in phase with the turn of the helix, and thus maximum retardation was observed. Since the K-factor for the 22-mer samples with out-of-phase interstrand adducts is larger than the K-factor for the 22-mer samples with in-phase intrastrand adducts, we conclude that the interstrand adducts at d(GC) sites bend DNA more than the intrastrand adducts at d(GG) or d(AG) sites.

Chemical Probes. Our purpose was to describe at the nucleotide level the conformational changes induced in DNA double helix by the interstrand adducts. In order to study these conformational changes, we took advantage of the fact that the rates of reactivity of several chemical reagents with individual bases are strongly dependent on the structure of DNA. After specific modification of nucleotide residues by the chemical reagents, the residues are directly sensitive to cleavage by piperidine, and the fragments generated in this way can be resolved as a ladder of bands on a denaturing gel [for general reviews, see Leng (1990) and Nielsen (1990)].

Hydroxylamine (HA) is hyperreactive with C residues in single-stranded DNA and in distorted DNA as compared to in B-DNA (Rubin & Schmid, 1980; Johnston & Rich, 1985). The platinated oligonucleotide labeled at one 3′ end was reacted with HA. Both the C residues in the sequence d-(G*C/CG*) are reactive, with the results relative to the purine-rich strand being shown in Figure 2 (left). Another experiment was done in order to determine whether one of the two strands of the helix was more distorted. The two 5′ ends of the platinated oligonucleotide were simultaneously labeled, and then the reaction with HA was done. Cleavage of the strands by piperidine (Maxam & Gilbert, 1980) and then removal of the adduct by cyanide ions at basic pH (Bauer et al., 1978) generate two fragments of different lengths. The intensities of the bands corresponding to these two fragments are of the same order of magnitude. This result is in favor of a symmetrical distortion but does not exclude the presence of several different conformations giving in sum the same amount of reaction with HA.

Table I: Summary of the Reactivity of Chemical Probes

probe	target	reactivity
HA	C	+
DMS	G	−
DEPC	A	−
OsO_4	T	−

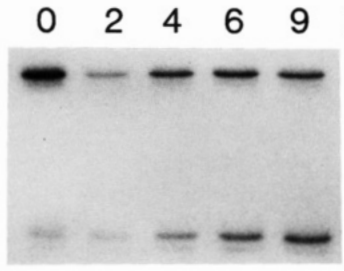

FIGURE 3: Autoradiogram of a denaturing 12% polyacrylamide gel of the products of the reaction between cyanide ions and the double-stranded oligonucleotide d(TG*CT/AG*CA) containing a single interstrand adduct. The platinated sample (3×10^{-5} M) was incubated at 37 °C and in 0.2 M NaCN, 20 mM Tris-HCl, adjusted to pH 8.3 by addition of HCl. The times (in hours) of incubation are given above the lanes.

Several other chemical probes were used and none of them were reactive with the platinated sample.

Osmium tetraoxide (OsO_4), in the presence of piperidine, is mainly hyperreactive with T residues in single-stranded DNA and in distorted DNA such as the B-DNA–Z-DNA junctions (Johnston & Rich, 1985; Lilley & Palecek, 1984; Palecek et al., 1987). No reactivity was detected even with the T residues adjacent to the adduct on the 5′ or 3′ side (not shown).

Diethyl pyrocarbonate (DEPC), which carbethoxylates purines at the N7 position, is hyperreactive with left-handed Z-DNA and with single-stranded DNA as compared to B-DNA (Herr, 1985; Johnston & Rich, 1985). None of the purine residues were reactive with DEPC (not shown).

Dimethyl sulfate (DMS) reacts with the G residues in native DNA (Maxam & Gilbert, 1980). The two G residues within the sequence d(G*C/CG*) were not reactive (not shown).

All of these results, summarized in Table I, suggest that the distortion of the double helix does not spread beyond the platinated sequence d(G*C/CG*).

Sodium Cyanide. The interstrand adducts in a natural DNA sequence are more stable to cyanide ions than the major intrastrand adducts. After 4 h of incubation in 0.2 M NaCN, pH 8.3, and at 37 °C, about 90% of the total adducts and only about 15% of the interstrand adducts are removed (Lemaire et al., 1991). The resistance of the interstrand adduct within the double-stranded oligonucleotide (dTG*CT/ACG*A) to the reaction with cyanide ions has been now studied. The reaction was followed by gel electrophoresis in denaturing conditions as previously described (Rahmouni & Leng, 1987; Schwartz et al., 1990). After a few hours of incubation, only two bands are visible on the autoradiogram of the gel (Figure 3) which correspond respectively to the platinated sample (upper band) and to the unplatinated sample (lower band). In 0.2 M NaCN, pH 8.3, and at 37 °C, the half-life of the interstrand adduct is about 14 h. It is worth noting that in the presence of NaCN the interstrand adduct does not behave as the intrastrand adduct at a d(AG) or at a d(GG) site (Schwartz et al., 1990). The analysis of the products of the reaction between NaCN and the intrastrand adducts by gel electrophoresis has revealed the formation of an intermediate species corresponding to the adduct *cis*-$[Pt(NH_3)_2(N7\text{-}dG)\text{-}CN]^+$.

Table II: Characteristics of the Calculated Conformations

conformation	B0	B1	B2	B3	B4	B5
energy (kcal·mol^{-1})	−140.7	−154.6	−148.6	−152.1	−142.2	−138.0
curvature (deg)	9	24	25	57	58	88

Sensitivity to Endonucleases. Recently, we have described the purification of a 43 base pair DNA fragment containing a unique interstrand adduct (Lemaire et al., 1991). The sequence of the central part of the fragment is

```
5′ ... G A A T T C G A G*C T C G G A T C C ...
   ... C T T A A G C T C G* A G C C T A G G ... 5′
       EcoRI                          BamHI
```

The interstrand adduct is flanked by the recognition sites of the endonucleases *Eco*RI and *Bam*HI. In conditions recommended by the manufacturers, the fragments with and without the interstrand adduct are equally cleaved by the two nucleases. This suggests that the distortion does not spread over several base pairs, in agreement with the results obtained with the chemical probes.

Molecular Mechanics Modeling. A molecular mechanics study has been performed in order to assess possible conformations of the interstrand adducts. A good agreement between molecular mechanics modeling and experimental results has been already obtained in the case of the intrastrand adducts at the d(GG) sites (Kozelka et al., 1987; Herman et al., 1990) and the d(GCG) sites (Mazeau et al., 1989). In our study, we have applied computational modeling methods to predict possible conformations of a platinated octanucleotide corresponding to the central part of the oligonucleotide studied by gel electrophoresis and chemical probes. The numbering of the residues is

```
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-C27-p26-G*25-p24-A23-p22-G21-p20-A19-H18
```

Platinum binding constrains the distance between the two N7 atoms of the G residues to about 2.8 Å and the orientation of the G residues so that the Pt–N7 bonds are directed towards the centers of the five-membered ring of the G residues. Moreover, all the initial structures have been generated by JUMNA, taking a regular B-DNA as a starting point. These facts considerably reduce the conformational space. Similar to the intrastrand DNA adduct (Kozelka et al., 1987; Mazeau et al., 1989; Herman et al., 1990), two sets of conformations can be considered. In the first case, guanine residues are tilted out of the stack without inducing a kink, whereas in the second case the helix follows the local geometry change forming a kink. Since the platinum binding geometry decreases the base pair spacing on the major groove side and increases the spacing (rise) of the base pair edges in the minor groove, the kink is directed toward the major groove.

The energy and curvature of the resulting conformations are listed in Table II. The two lowest energy conformations B3 and B1 are bent with angles of 57° and 24°, respectively. The straight conformation B0, which has converged to a curvature of 9°, and the B4 conformation are energetically less favorable. The B5 conformation energy is by 16.6 kcal·mol^{-1} higher than the minimum attained with the B1 conformation, which makes the B5 conformation unlikely.

The most striking feature of these minimized structures is a tilting of both G*8 and G*25 with respect to the helical axis. In terms of the helical parameters (Lavery, 1989) this is expressed by nonzero tilt and roll values concerning G*8 and

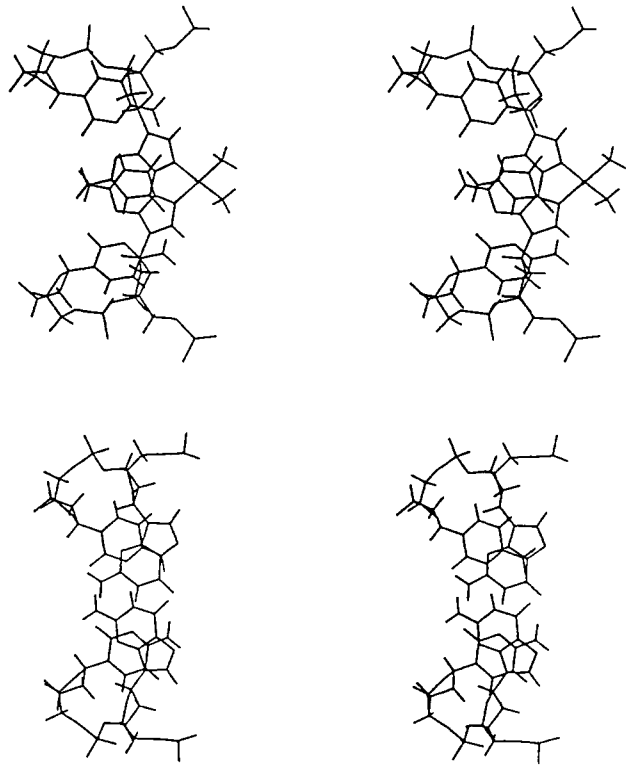

FIGURE 4: Stereoview of the central d(GC/CG) sequence along the helical axis: platinated sample (top) and unplatinated sample (bottom).

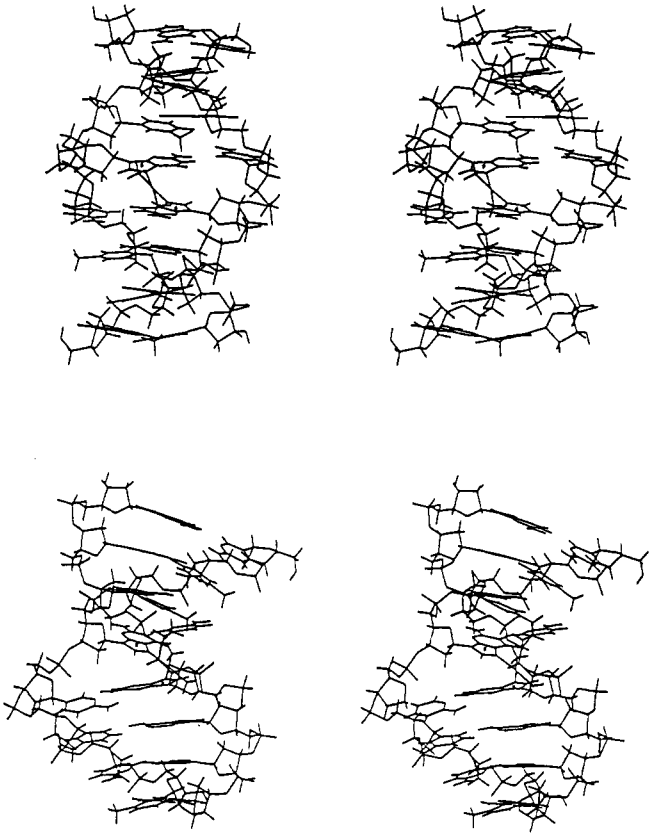

FIGURE 5: Stereoview of the platinated octanucleotide: model B1 (top) and model B3 (bottom).

G*25 and a subsequent increase of rise values of the two G*C base pairs, which are ranging from 4.5 Å for the B3 conformation to 5.3 Å for the B0 structure.

The geometrical distance constraints on the relative position of the N7 atoms of the G residues pull the two G toward the center of the double helix. This is expressed by a global slide of the two G*C pairs comprised in the range 3 Å (B0) to 4.5 Å (B1). The facing C bases are pushed in the opposite direction out of the stack. Their position is further destabilized by a propeller twist, which increases the G–C hydrogen bond distances up to 2.4 Å for the O6(G8)–HN4(C27) bond.

Simultaneously, the twist angle is increased from 36° (B-DNA) to about 55° between the two G*C base pairs. This increase is compensated for by a decrease of the inter base pair twist between the G*C pairs and the pairs T6-A29 and T12-A23, so that for all the model conformations considered, the interstrand adduct does not globally unwind the double helix. The geometry of the two G*C base pairs is depicted in a view along the helical axis in Figure 4 (top). For comparison, Figure 4 (bottom) shows the same view in a regular B-DNA.

Geometrical constraints on the position of DNA bases affect the conformation of the DNA backbone in the proximity of the two platinated base pairs. The major change is a stretching of the backbone to compensate for the large slide between the two G*C base pairs. The starting C2′-endo type sugars relax mostly again into an *S* conformation. The largest deviations are observed for the G*8 and G*25 sugars, which adopt the O1′-endo conformation. The facing cytidine sugars are in the C2′-conformation. Repuckering of the sugars of the nonmodified base pairs into the C2′-endo conformation does not bring a significant energy decrease. The stereoviews of the two lowest energy structures B1 and B3 are represented in figure 5.

Discussion

The purpose of this work was to characterize the distortions induced in DNA by the interstrand adducts formed by *cis*-DDP at the d(GC/CG) sequences.

The multimers of the platinated and ligated oligonucleotides (22-mer) show a largely retarded electrophoretic mobility through polyacrylamide gels while the multimers of the platinated and ligated oligonucleotides (15-mer) show a normal mobility. Taking into account the investigations on bent DNA [for general reviews, see Diekmann (1987), Hagerman (1988), and Crothers et al. (1990)], we conclude that the interstrand adducts (i) induce a systematic bending in which the direction of the helix is altered in a definitive way and (ii) do not alter the helix screw. Moreover, the multimers with interstrand adducts have a slower electrophoretic mobility than the corresponding multimers with intrastrand adducts at d(GG) sites. It is known that the intrastrand adduct at the d(GG) site bends DNA by about 30–40° (Rice et al., 1988; Bellon & Lippard, 1990). The conclusion is that the bending induced by the interstrand adduct is larger than 30–40°.

The conformation of the platinated oligonucleotide has been studied at the nucleotide level by means of chemical probes. The nonreactivity of DMS with the N7 atom of G residues within the sequence d(G*C/CG*) confirms the site of platination. The C residues complementary to the platinated bases become accessible to hydroxylamine. The out-of-plane attack by HA implies that the C residues are less stacked with adjacent bases in the platinated sample. The A residues on the 3′ and 5′ sides of the adduct are not reactive with DEPC and thus are paired with the T residues. The complementary T residues do not react with OsO_4. This observation supports the view that the T residues are in a conformation similar to the one in the unplatinated oligonucleotide. We recall that OsO_4 is a very sensitive probe which can detect small variations in the conformations of DNA. For example, T residues when mismatched with G residues react with OsO_4 (Cotton et al.,

1988; Schwartz et al., 1989). It is known from X-ray and NMR studies (Kennard, 1987; Patel et al., 1987) that the G-T mismatches induce a minimal distortion in the conformation of the DNA double helix, the mismatched T moving into the major groove.

From the gel electrophoresis and chemical probes experiments, we suggest that the interstrand adduct induces a kink in DNA and that the distorted region is limited to the d-(G*C/CG*) sequence.

Molecular mechanics calculations yield two different lowest energy structures. On the 3′ and 5′ sides of the adduct, the two structures are similar to unplatinated B-DNA, in agreement with the chemical probes. Both structures exhibit an almost complete lack of stacking interaction for the C residues complementary to the platinated G residues. The larger slide of the neighboring G residues facilitates the out-of-plane attack on the C5–C6 double bond of the C residues by HA. Nevertheless, these C residues are still paired. The two lowest energy structures differ by their angle of curvature. For model B1, this angle is much smaller than the one deduced from gel electrophoresis. Thus, only model B3 fits with the experimental data. It is interesting to point out that model B3 predicts that the helix is not unwound, in agreement with the experimental data. However, a large change in the local twist values at the adduct level is observed.

In conclusion, on the basis of electrophoresis, chemical probes, and molecular mechanics modeling data, we propose that the cross-linking of the two guanine N7 atoms on opposite strands within the sequence d(GC/CG) by *cis*-DDP induces a kink of about 55° of the helix axis toward the major grrove, that the double helix conserves its average twist angle, and that the distortion is localized at the platinated d(GC/CG) sequence.

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