Conformational Analysis of Site-Specific DNA Cross-Links of Cisplatin−Distamycin Conjugates†

Hana Kostrhunova and Viktor Brabec*

Institute of Biophysics, Academy of Sciences of the Czech Republic, CZ-61265 Brno, Czech Republic

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ABSTRACT: The requirement for novel platinum antitumor drugs led to the concept of synthesis of novel platinum drugs based on targeting cisplatin to various carrier molecules. We have shown [Loskotova, H., and Brabec, V. (1999) *Eur. J. Biochem. 266*, 392−402] that attachment of DNA minor-groove-binder distamycin to cisplatin changes several features of DNA-binding mode of the parent platinum drug. Major differences comprise different conformational changes in DNA and a considerably higher interstrand cross-linking efficiency. The studies of the present work have been directed to the analysis of oligodeoxyribonucleotide duplexes containing single, site-specific adducts of platinum-distamycin conjugates. These uniquely modified duplexes were analyzed by Maxam−Gilbert footprinting, phase-sensitive gel electrophoresis bending assay and chemical probes of DNA conformation. The results have indicated that the attachment of distamycin to cisplatin mainly affects the sites involved in the interstrand cross-links so that these adducts are preferentially formed between complementary guanine and cytosine residues. This interstrand cross-link bends the helix axis by ∼35° toward minor groove, unwinds DNA by approximately 95° and distorts DNA symmetrically around the adduct. In addition, CD spectra of restriction fragments modified by the cisplatin−distamycin conjugates have demonstrated that distamycin moiety in the interstrand cross-links of these compounds interacts with DNA. This interaction facilitates the formation of these adducts. Hence, the structural impact of the specific interstrand cross-link detected in this study deserves attention when biological behavior of cisplatin derivatives targeted by oligopeptide DNA minor-groove-binders is evaluated.

The clinical efficacy of the antitumor platinum drugs such as cisplatin[1] and carboplatin is diminished by side effects and acquired and intrinsic resistance. Efforts have therefore focused on searching for a new class of platinum antitumor compounds that would have better therapeutic efficacy than cisplatin or carboplatin. Several concepts on how to overcome these problems have been proposed. One is based on the targeting of cisplatin to DNA by attaching the platinum moiety to a suitable carrier. It is generally accepted that biological target of platinum anticancer drugs is DNA (*1*, *2*). It is, therefore, reasonable to expect that tethering cisplatin to various carrier molecules may lead to synthesis of a new class of anticancer agents distinct from the parent drug in DNA binding and consequently in antitumor activity. Attempts to use this strategy in antitumor platinum drug design have already led to the synthesis of new composite analogues of cisplatin. The molecules in which cisplatin was tethered to DNA intercalators (*3*, *4*), antiviral nucleoside analogues (*5*, *6*), and oligopeptide DNA minor groove binders (*7*) are examples of such compounds. Thus, the concept based on tethering various carrier molecules to cisplatin represents an interesting possibility for mechanistic studies aimed at improving knowledge needed for designing new platinum anticancer drugs.

Recently, we reported on model studies in which modifications of natural DNAs in a cell-free medium by cisplatin tethered to the AT specific, minor groove binder distamycin (Figure 1) were investigated using various biochemical and biophysical methods (*8*). The results have indicated that attachment of distamycin to cisplatin changes several features of the DNA-binding mode of the parent platinum drug. Major differences comprise a considerably higher efficiency of the conjugated drug to form interstrand cross-links and different conformational alterations in DNA. While nontargeted cisplatin forms ca. 6% of interstrand cross-links in linear DNA and even more in negatively supercoiled DNA (*9*, *10*), tethering this drug to distamycin increases its interstrand

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* To whom correspondence should be addressed. Phone: ++420-5-41517148. Fax: ++420-5-41211293. E-mail: brabec@ibp.cz.

[1] Abbreviations: A, adenine; bp, base pair; C, cytosine; carboplatin, *cis*-diammine(cyclobutanedicarboxylato)platinum(II); CD, circular dichroism; cisplatin, *cis*-diamminedichloroplatinum(II) {*cis*-[$PtCl_2(NH_3)_2$]}; CT, calf thymus; [PtCl(dien)]Cl, chlorodiethylenetriamineplatinum(II) chloride, dien = diethylenetriamine; DEPC, diethyl pyrocarbonate; DMS, dimethylsulphate; FAAS, flameless atomic absorption spectrophotometry; FPLC, fast-protein-liquid chromatography; G, guanine; Pt-DIST(R), R form of cisplatin−distamycin conjugate; Pt-DIST(S), S form of cisplatin−distamycin conjugate; oxaliplatin, (*trans-R,R*)-diaminocyclohexaneoxaloplatinum(II); r_b, the number of molecules of the platinum compound bound per nucleotide residue; T, thymine.

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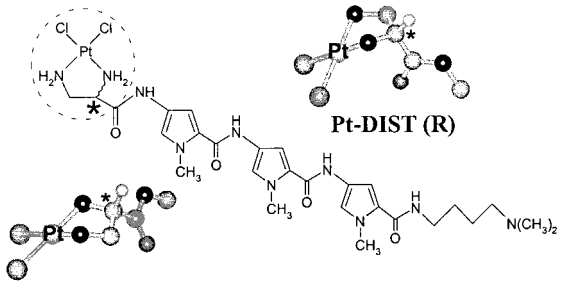


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d(TGCT)/d(AGCA)(22):
5'-CCTCTCCTTGCTCTCCTTCTCT-3'
3'- GAGAGGAACGAGAGGAAGAGAG-5'
      21 19 17 15 13 11  9  7  5  3  1

d(TGCT)/d(AGCA)(23):
5'-CCTCTCCTTGCTCTCCTTCTCTC-3'
3'- GAGAGGAACGAGAGGAAGAGAGG-5'

d(TGCT)/d(AGCA)(24):
5'-CCTCTCCTTGCTCTCCTTCTCTCT-3'
3'- GAGAGGAACGAGAGGAAGAGAGAG-5'

d(TGCT)/d(AGCA)(25):
5'-CCTCTCCTTGCTCTCCTTCTCTCTC-3'
3'- GAGAGGAACGAGAGGAAGAGAGAGG-5'

d(CGCT)/d(AGCG):
5'-CTCTCCTCTCGCTCTCCTTCTT-3'
3'- AGAGGAGAGCGAGAGGAAGAAG-5'
      21 19 17 15 13 11  9  7  5  3  1

d(TGCT)/d(AGCA)+d(A/T)5(36):
5'-CCTCTCCTTGCTCTCCTTCAAAAATCTCTCCTTCTC-3'
3'- GAGAGGAACGAGAGGAAGTTTTTAGAGAGGAAGAGG-5'

d(TGGT)/d(ACCA)(20):
5'-CCTCTCCTTGGTCTCCTTCT-3'
3'- GAGAGGAACCAGAGGAAGAG-5'

d(TGGT)/d(ACCA)(21):
5'-CCTCTCCTTGGTCTCCTTCTC-3'
3'- GAGAGGAACCAGAGGAAGAGG-5'

d(TGGT)/d(ACCA)(22):
5'-CCTCTCCTTGGTCTCCTTCTCT-3'
3'- GAGAGGAACCAGAGGAAGAGAG-5'

d(TGGT)/d(ACCA)(23):
5'-CCTCTCCTTGGTCTCCTTCTCTC-3'
3'- GAGAGGAACCAGAGGAAGAGAGG-5'
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FIGURE 1: Structures of Pt-DIST(R) and Pt-DIST(S), and sequences of the synthetic oligodeoxyribonucleotides used in the present study with their abbreviations. The top and bottom strands of each pair are designated top and bottom, respectively, in the text. The bold letter in the top strands of d(TGCT)/d(AGCG)(22−25) and d(CGCT)/d(AGCG) duplexes indicates the location of the monofunctional adduct of Pt-DIST complexes formed before interstrand cross-linking reaction in the way also described in the Experimental Section. For the duplexes d(TGCT)/d(AGCG)(22) and d(CGCT)/d(AGCG) the numbering of the nucleotide residues in their bottom strands is also shown. The bold letters in top strands of d(TGGT)/d(ACCA)(19−22) duplexes indicate the location of the intrastrand cross-link after modification of the oligonucleotides by Pt-DIST complexes in the way described in the Experimental Section.

cross-linking efficiency roughly three times (*8*).

To obtain a deeper insight into the character of the distinct modifications of DNA due to tethering cisplatin to carrier molecules, we directed our further studies on analysis of interstrand cross-links of two isomers of cisplatin−distamycin conjugates [Pt-DIST(R) and Pt-DIST(S), see Figure 1]. We have undertaken the studies aimed at finding whether the distamycin moiety in this conjugated compound affects bases involved in the interstrand cross-link and conformational alterations induced in DNA by this adduct. To address these points, we investigated oligodeoxyribonucleotide duplexes containing single, site-specific interstrand adducts of this composite molecule. Moreover, cisplatin and its analogues form besides interstrand cross-links also intrastrand adducts, with more of the latter. Although considerable evidence indicates that the biological efficacy of these drugs is the result of these DNA lesions (*1*, *2*, *4*, *11*) their relative efficacy remains unknown. Therefore, we also analyzed in the present work oligodeoxyribonucleotide duplexes containing single, site-specific 1,2 d(GpG) intrastrand adduct of the Pt-DIST complexes for comparative purposes.

MATERIALS AND METHODS

Chemicals. The platinated derivatives of distamycin Pt-DIST(R) and Pt-DIST(S) (Figure 1) were a gift from Moses Lee of Furman University, Greenville and were prepared, characterized and used as described previously (*7*, *8*). The mono- or diaqua species were generated from Pt-DIST complexes (0.5 mM) by the addition of 0.95 or 1.9 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. The synthetic oligodeoxyribonucleotides (Figure 1) were synthesized and purified as described previously (*12*). Plasmid pSP73 [2464 base pairs (bp)] was isolated according to standard procedures and banded twice in CsCl/EtBr equilibrium density gradients. T4 DNA ligase, T4 polynucleotide kinase, *Nde*I and *Ssp*I restriction endonucleases were purchased from New England Biolabs (Beverly, MA). Acrylamide, bis(acrylamide), urea, and NaCN were from Merck KgaA (Darmstadt, Germany). Dimethyl sulfate (DMS), $KMnO_4$, diethyl pyrocarbonate (DEPC), KBr and $KHSO_5$ were from Sigma. [γ-^{32}P]ATP was from Amersham (Arlington Heights, IL).

Platination Reactions. The single-stranded oligonucleotides [the top strands of d(TGCT)d(AGCA)(22−25), d(CGCT)/d(AGCG), and d(TGCT)/d(AGCA)+d(A/T)$_5$(36) duplexes in Figure 1] were reacted in stoichiometric amounts with monoaqua derivatives of Pt-DIST(R) or Pt-DIST(S) whereas the top strands of d(TGGT)/d(ACCA)(20−23) duplexes (Figure 1) were reacted in stoichiometric amounts with diaqua derivatives of either Pt-DIST isomer. The platinated oligonucleotides were repurified by ion-exchange fast protein liquid chromatography (FPLC). It was verified by platinum flameless atomic absorption spectrophotometry (FAAS) and by the measurements of the optical density that the modified oligonucleotides contained one platinum atom. It was also verified using DMS footprinting of platinum on DNA (*9*, *13*) that in the platinated top strands of d(TGCT)/d(AGCA)(22−25), d(CGCT)/d(AGCG), and d(TGCT)/d(AGCA)+d(A/T)$_5$(36) duplexes the N7 position of single G residue was not accessible for reaction with DMS as well as in the platinated top strands of d(TGGT)d(ACCA)-(20−23) the N7 position of both neighboring Gs. Briefly, platinated and nonmodified top strands (5′-end-labeled with ^{32}P) were reacted with DMS. DMS methylates the N7 position of G residues in DNA, producing alkali labile sites

(*14*). However, if N7 is coordinated to platinum, it cannot be methylated. The oligonucleotides were then treated with hot piperidine and analyzed by denaturing 24% polyacryalmide gel electrophoresis. For the nonmodified top strands, shortened fragments due to the cleavage of the strand at one or two methylated Gs were observed in the gel. However, no such bands were detected for the oligonucleotides modified by Pt−DIST complexes. These results indicate that one Pt-DIST molecule was coordinated either to single G in the top strands of d(TGCT)d(AGCA)(22−25), d(CGCT)/d(AGCG), and d(TGCT)/d(AGCA)+d(A/T)$_5$(36) duplexes or to neighboring G residues forming 1,2-d(GpG) intrastrand cross-link in the top strands of d(TGGT)/d(ACCA)(20−23). The top strands containing the monofunctional adduct were allowed to anneal with nonplatinated complementary strands in 0.4 M NaCl (pH 7.4) at 25 °C for 24 h. After dialysis against 0.1 M $NaClO_4$ for 4 h at 4 °C, the samples were incubated for 48 h in the dark at 37 °C. The resulting products were still purified by FPLC in an alkaline gradient. Using this denaturing gradient, noninterstrand cross-linked strands were eluted as single strands, whereas the interstrand cross-linked products were eluted later in a single peak as a higher-molecular-mass species. This single peak was only collected so that the samples of the interstrand cross-linked duplexes contained no single-stranded molecules. Alternatively, the duplexes containing the interstrand cross-links were separated on 12% polyacrylamide/8 M urea denaturing gel, and the bands corresponding to interstrand cross-linked duplexes were cut off from the gel, eluted, precipitated by ethanol and dissolved in 50 mM NaCl plus 10 mM Tris·HCl/EDTA buffer, pH 7.4. Both procedures of the purification of interstrand cross-linked duplexes provided the products whose subsequent analysis (vide infra) gave identical results. The yields of these interstrand cross-linking reactions were approximately 70%. The duplexes were still further analyzed for platinum content by FAAS. Additional quantitation of cross-linked duplex by UV absorption spectrophotometry was used to ascertain that 1:1 adducts (one Pt per duplex) had formed. The sites involved in interstrand cross-links were deduced from Maxam−Gilbert footprinting experiments (vide infra). The platinated top strands containing TGGT central sequences were allowed to anneal with nonplatinated complementary strands in 50 mM NaCl plus 10 mM Tris·HCl/EDTA buffer, pH 7.4, to prepare the duplexes containing the intrastrand cross-link.

*Nde*I/*Ssp*I fragment of plasmid pSP73 (367 bp) was incubated with Pt-DIST(R) or Pt-DIST(S) in 10 mM $NaClO_4$ at 37 °C for 24 h in the dark. The number of molecules of the platinum compound bound per nucleotide residue in this fragment (r_b value) was determined by FAAS. Two types of the platinated samples containing interstrand cross-links and without interstrand cross-links were separated by electrophoresis in 2% agarose gel under denaturing (strand separating) conditions. The gel was neutralized and the two types of fragments were eluted. After one heating/reannealing cycle, the fragments were analyzed by CD spectroscopy.

FPLC purification and FAAS measurements were carried out on a Pharmacia Biotech FPLC System with MonoQ HR 5/5 column and a Unicam 939 AA spectrometer equipped with a graphite furnace, respectively.

Chemical Modifications. The modification by $KMnO_4$, DEPC, and KBr/$KHSO_5$ were performed as described previously (*15*−*18*). The strands of the duplexes were 5′-end-labeled with [γ-^{32}P]ATP. In the case of the platinated oligonucleotides, the platinum complex 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.

Ligation and Electrophoresis of Oligonucleotides. Nonplatinated single strands (top strands in Figure 1), single-stranded oligonucleotides containing a unique intrastrand cross-link and the duplexes containing a unique interstrand cross-link were 5′-end-labeled with [γ-^{32}P]ATP by using T4 polynucleotide kinase. Then the single stranded oligonucleotides were annealed with their phosphorylated complementary strands [nonplatinated or containing 1,2-d(GpG) intrastrand cross-links of Pt-DIST compounds]. Nonplatinated, interstrand, or intrastrand cross-link containing duplexes were allowed to react with T4 DNA ligase. The resulting samples along with ligated nonplatinated duplexes were subsequently examined on 8% native polyacrylamide [mono:bis(acrylamide) ratio = 29:1] electrophoresis gels. Other details of these experiments were as described in previously published papers (*19*−*22*).

Circular Dichroism. CD spectra of DNA modified by Pt-DIST compounds were recorded at 25 °C in 10 mM $NaClO_4$ on a JASCO spectropolarimeter, model J720.

RESULTS

Interstrand Cross-Link

Nucleotides Preferentially Involved in Interstrand Cross-Linking. Dichloroplatinum(II) complexes react with DNA in a two step process (*23*). Monofunctional adducts are formed preferentially at N7 atoms of G residues. These lesions subsequently close to bifunctional cross-links (intrastrand and/or interstrand). Considering this fact, we have designed for this part of the present work a series of synthetic oligodeoxyribonucleotide duplexes [d(TGCT)/d(AGCA)(22−25), d(CGCT)/d(AGCG), and d(TGCT)/d(AGCA)+d(A/T)$_5$-(36)], their sequences are in Figure 1. The pyrimidine-rich top strands of these duplexes only contained one G (printed bold in the Figure 1). These top strands were modified by Pt-DIST(R) or Pt-DIST(S) complexes so that they contained a single monofunctional adduct at this G site (see Materials and Methods). Thus, the choice of this nucleotide allowed for a cross-linking study under competitive conditions, i.e., interstrand cross-links were in principle possible in the d(CGCT)/d(AGCG)(22) duplex between the central G in the top strand and either complementary C13 or adjacent 5′ G12 or 3′ G14 residues in the bottom strand and in the case of the d(TGCT)/d(AGCA)(22) duplex between the central G in the top strand and complementary C14 or adjacent 5′ G13 in the bottom strand. The monoadducted top strands of the duplexes d(TGCT)/d(AGCA)(22−25), d(CGCT)/d(AGCG), and d(TGCT)/d(AGCA)+d(A/T)$_5$(36) were hybridized with their complementary, 5′-end ^{32}P-labeled (bottom) strands. The mixtures were incubated in 0.1 M $NaClO_4$ at 37 °C. The aliquots were withdrawn at various time intervals and analyzed by gel electrophoresis or FPLC under denaturing conditions (not shown). Similarly, as in the same experiments described in recent reports on site-specific DNA interstrand cross-linking by other platinum complexes (*13*, *15*), one band on the autoradiogram was only observed for the non-cross-linked duplex. The subsequent incubation resulted in a new

single band migrating markedly more slowly (or in a single peak in the denaturing FPLC profile eluted markedly later than noninterstrand cross-linked single strands). Its intensity increased with the incubation time with a concomitant decrease in the intensity of the band corresponding to the non-cross-linked duplex. This observation can be interpreted to mean that interstrand cross-links were formed. From the ratio of the sum of intensities of all bands corresponding to cross-linked duplexes and the sum of intensities of all bands, the percentage of interstrand cross-links was calculated. The half-times of this interstrand cross-linking reaction by both Pt-DIST isomers in all duplexes examined in the present work (Figure 1) were identical and were about 7 h.

After 48 h of the cross-linking reaction by Pt-DIST(R) or Pt-DIST(S), the samples of the duplexes were eluted from the bands corresponding to the interstrand cross-linked fraction or were separated using FPLC (see Materials and Methods). These duplexes, in which either both strands or only the upper strand were 5′-end labeled with ^{32}P, were reacted with DMS [which readily methylates G residues at N7 position, but does not react with platinated G because the N7 position is no longer accessible (*9*, *13*, *14*)]. Then the adducts were removed by NaCN (*9*, *13*, *24*, *25*), and the samples were treated with piperidine. In the nonplatinated duplexes, the central G in the top strands was reactive with DMS [shown in lanes NoPt(top) in Figure 2, panels A and B, and NoPt(top + bottom) in Figure 2A]. It was no longer reactive in both cross-linked duplexes (shown in Figure 2, panels A and B, lanes R and S). This observation confirms that the unique G in the upper strands remained platinated and was involved in the adduct of the interstrand cross-linked duplexes (*9*, *14*).

In further studies the duplexes d(TGCT)/d(AGCA) or d(CGCT)/d(AGCG) interstrand cross-linked by Pt-DIST(R) or Pt-DIST(S) in which the both strands were 5′-end labeled with ^{32}P were examined. Results of a typical experiment, in which the location of nucleotides involved in the interstrand cross-link of Pt-DIST(R) or Pt-DIST(S) in the duplex d(TGCT)/d(AGCA)(22) was determined, are shown in Figure 2A, lanes R, R/CN$^-$, S, S/CN$^-$. The duplexes were reacted with DMS. One-half of these samples was still treated with NaCN to remove the adducts. Both samples (one treated with NaCN and the other not subjected to a cyanide reversal step) were subsequently treated with piperidine. The treatment with piperidine of the control, nonplatinated duplex resulted in the cleavage at all G sites in the bottom strand [lane NoPt-(bottom) in Figure 2A]. If the d(TGCT)/d(AGCA)(22) duplex containing the cross-link not treated with NaCN was cleaved, G residues in the bottom strand up to the position 13 from the 5′-end (see Figure 1 for nucleotide numbering) were only detected at the expected positions. G residues more distant from the 5′-end were not detected because they were cross-linked to the upper strand and migrated considerably more slowly (Figure 2A, lanes R and S). If NaCN was used to remove platinum from DMS-treated duplexes, all G residues were found at the expected positions (Figure 2A, lanes R/CN$^-$ and S/CN$^-$). This result proves that no G residue in the bottom strand is involved in the interstrand cross-link of Pt-DIST isomers. Moreover, the only reasonable explanation of this result is that the cross-linking site in the bottom strand is between G residues in the positions 13 and 17, i.e., cytosine (C) residue in the position 14 (complementary to the platinated G in the top strand) or one of the two adjacent 3′ adenine (A) residues (in the positions 15 and 16).

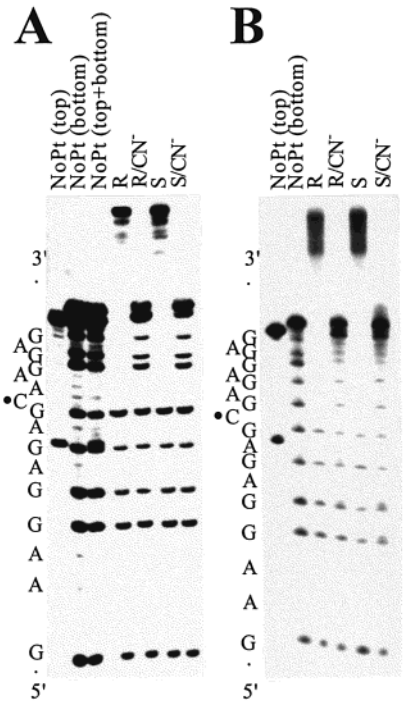

FIGURE 2: Maxam−Gilbert footprinting experiments. Autoradiograms of denaturing 24% polyacrylamide/8 M urea gels of the products of the reaction between DMS (G-specific reactions) and the duplexes d(TGCT)/d(AGCA)(22) (A) and d(CGCT)/d(AGCG) (B) containing an interstrand cross-link of Pt-DIST(R) or Pt-DIST-(S). Both strands [lanes NoPt (top + bottom), R, R/CN$^-$, S, and S/CN$^-$] or only top strand [lanes NoPt (top)] or bottom strand [lanes NoPt (bottom)] of the duplex were 5′-end labeled. Lanes: NoPt (top), NoPt (bottom), and NoPt (top + bottom), nonplatinated duplex; R and S, the duplex containing the interstrand cross-link of Pt-DIST(R) and Pt-DIST(S), respectively; R/CN$^-$ and S/CN$^-$, the same as in the lanes R and S, respectively, but with platinum removed by NaCN after modification by DMS. The nucleotide sequence of the bottom strand of the duplexes is shown on each panel; the closed circles designate C opposite to the platinated G in the top strand.

To distinguish among these eventualities, the same analysis based on DMS footprinting of platinated G residues was also performed with the interstrand cross-linked duplex d(CGCT)/d(AGCG) (Figure 2B). If this duplex containing the cross-link not treated with NaCN was cleaved, G residues in the bottom strand up to the position 12 from the 5′-end were only detected at the expected positions (Figure 2B, lanes R and S). If NaCN was used to remove platinum from DMS-treated duplexes, all G residues were found at the expected positions (Figure 2B, lanes R/CN$^-$ and S/CN$^-$). These results imply that similarly as in the case of the cross-link formed by the Pt-DIST isomers in the d(TGCT)/d(AGCA) duplex, no G residue in the bottom strand of the duplex d(CGCT)/d(AGCG) is involved in the interstrand cross-link of Pt-DIST isomers. In addition, these results indicate that the cross-linking site in the bottom strand is between G residues in the positions 12 and 14, which is the C residue complementary to the platinated G residue in the top strand. This is in contrast to nontargeted cisplatin, which preferentially forms interstrand cross-links between G residues at the 5′-(GC) sites.

Chemical Probes of DNA Conformation. The oligonucleotide duplex d(TGCT)/d(AGCA)(22) containing the single

interstrand cross-link of Pt-DIST(R) or Pt-DIST(S) between G in the top strand and the complementary C was treated with several chemical agents that are used as tools for monitoring the existence of conformations other than canonical B-DNA. These agents include $KMnO_4$, DEPC and bromine. They react preferentially with single-stranded DNA and distorted double-stranded DNA (*15*−*18*, *26*).

$KMnO_4$ is hyperreactive with thymine (T) residues in single-stranded nucleic acids and in distorted DNA as compared to B-DNA (*16*, *18*, *27*, *28*). $KMnO_4$ reacted with no residue within the nonplatinated duplex (Figure 3A, left panel, lane ds). All T residues were strongly reactive in the nonplatinated single-stranded top oligonucleotide (Figure 3A, left panel, lane ss). The duplex containing the interstrand cross-link of either Pt-DIST isomer showed strong reactivity of the 5′ T residue adjacent to the adduct (Figure 3A, left panel, lanes R and S). A somewhat weaker reactivity was also observed for the second 5′ T and the 3′ T across C residue adjacent to the platinated G involved in the cross-link.

DEPC carbetoxylates purines at the N(7) position. It is hyperreactive with unpaired and distorted A residues in DNA and with left-handed Z-DNA (*16*, *18*, *29*, *30*). A and G residues within the nonplatinated single-stranded oligonucleotide d(TGCT)/d(AGCA)(22) (top and bottom) readily reacted with DEPC (shown for the bottom strand in Figure 3A, central panel, lane ss). No reactivity of A and G residues was observed within the bottom strand of the nonplatinated double-stranded oligonucleotide (shown for the bottom strand in Figure 3A, central panel, lane ds). Within the double-stranded oligonucleotide containing the interstrand cross-link of Pt-DIST(R) or Pt-DIST(S), four base residues in the bottom strand became reactive [Figure 3A, central panel, lanes R and S]. These are readily identified as the three A residues complementary to the reactive T residues of the top strand and G residue 5′ to the C involved in the cross-link. Importantly, a strong reactivity with DEPC was only observed for the A residue complementary to strongly reactive T residue and for G residue complementary to C residue strongly reactive with $KBr/KHSO_5$ (vide infra, Figure 3A, right panel, lanes R/top and S/top). In contrast A residues complementary to somewhat less strongly reactive T residues also reacted with DEPC only weakly.

Bromination of C residues and formation of a piperidine-labile sites are observed when two simple salts, KBr and $KHSO_5$, are allowed to react with single-stranded or distorted double-stranded oligonucleotides (*17*). The reaction proceeds via generation of Br_2 in situ, which reacts selectively with the 5,6 double bond to add Br and OH, respectively. H_2O is then eliminated to give 5-bromodeoxycytidine, which is susceptible to depyrimidination under basic conditions. All C residues within the nonplatinated single-stranded top or bottom strands of d(TGCT)d(AGCA)(22) were strongly reactive (Figure 3A, right panel, lanes ss/top and ss/bot). No reactivity of these residues was observed within the nonplatinated duplex (Figure 3A, right panel, lanes ds/top and ds/bot). Within the double-stranded duplex containing the cross-link of either Pt-DIST isomer 3′ C residue in the top strand adjacent to the cross-link and C residue in the bottom strand involved in the cross-link were strongly reactive (Figure 3A, right panel, lanes R/top, S/top, R/bot, and S/bot).

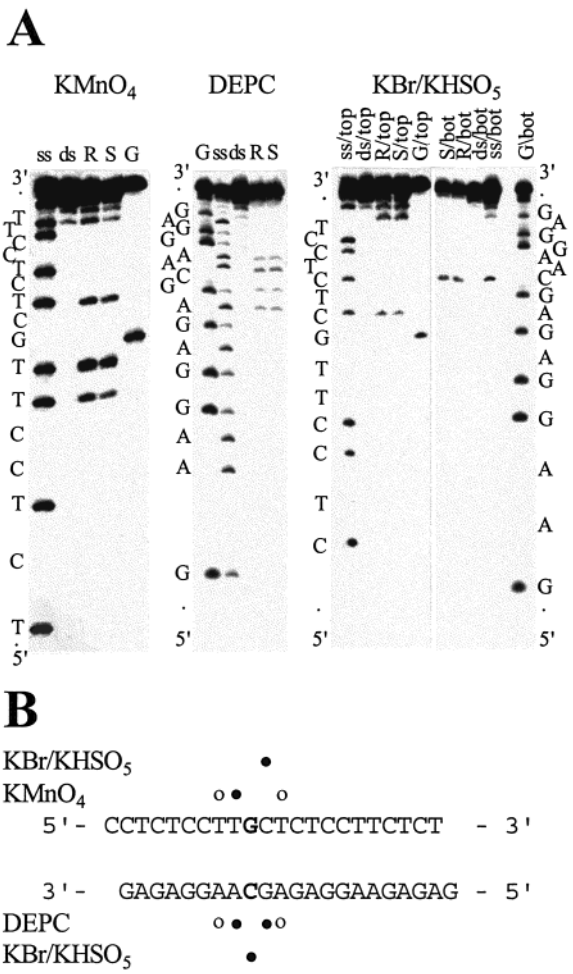

FIGURE 3: (A) Piperidine-induced specific strand cleavage at $KMnO_4$-modified (left), DEPC-modified (center), and $KBr/KHSO_5$-modified (right) bases in the duplex (TGCT)/d(AGCA)(22) nonplatinated or containing single interstrand cross-link of Pt-DIST(R) or Pt-DIST(S). The oligomers were 5′-end labeled at their top or bottom strands. Lanes in the left panel ($KMnO_4$, only top strand end labeled): ss, the nonplatinated top strand; ds, the nonplatinated duplex; R and S, duplex interstrand cross-linked by Pt-DIST(R) or Pt-DIST(S), respectively; G, a Maxam−Gilbert specific reaction for the nonplatinated duplex. Lanes in the central panel (DEPC, only bottom strand end labeled: ss, the nonplatinated top strand; ds, the nonplatinated duplex; R and S, duplex interstrand cross-linked by Pt-DIST(R) or Pt-DIST(S), respectively; G, a Maxam−Gilbert specific reaction for the nonplatinated duplex. Lanes in the right panel ($KBr/KHSO_5$): ss/top, the nonplatinated top strand (5′-end labeled); ds/top, the nonplatinated duplex (the top strand was 5′-end labeled); R/top and S/top, duplex interstrand cross-linked by Pt-DIST(R) or Pt-DIST(S), respectively (the top strand was 5′-end labeled); G/top, a Maxam−Gilbert specific reaction for the nonplatinated duplex (the top strand was 5′-end labeled); ss/bot, the nonplatinated bottom strand (5′-end labeled); ds/bot, the nonplatinated duplex (the bottom strand was 5′-end labeled); R/bot and S/bot, duplex interstrand cross-linked by Pt-DIST(R) or Pt-DIST(S), respectively (the bottom strand was 5′-end labeled); G/bot, a Maxam−Gilbert specific reaction for the nonplatinated duplex (the bottom strand was 5′-end labeled). (B) Summary of the reactivity of chemical probes. Closed and open circles designate strong or weak reactivity, respectively.

No reactivity of other C residues in the top strand was observed. The results obtained with chemical probes are summarized in Figure 3B.

DNA Unwinding and Bending. Among the alterations of secondary and tertiary structure of DNA to which it may be subject, the role of intrinsic bending and unwinding of DNA

is increasingly recognized as of potential importance in regulating replication and transcription functions through specific DNA−protein interactions. For nontargeted cisplatin adducts, the structural details responsible for bending and subsequent protein recognition have recently been elucidated (*31*, *32*). Given the recent advances in our understanding of the structural basis for the bending of DNA caused by nontargeted cisplatin cross-links, it is of considerable interest to examine how tethering cisplatin to distamycin in the interstrand adduct also affects conformational properties of DNA such as bending and unwinding. In this work we further performed studies on the bending and unwinding induced by single, site-specific interstrand cross-links of Pt-DIST isomers formed in oligodeoxyribonucleotide duplexes between complementary G and C residues using electrophoretic retardation as a quantitative measure of the extent of planar curvature.

The oligodeoxyribonucleotide duplexes d(TGCT)/d(AGCA)-(22−25) (22−25 bp, see Figure 1) used in the bending and unwinding studies of the present work contained an identical central base pair sequence at which an interstrand cross-link was formed between G residue in the top strand and its complementary C residue. All sequences were designed to leave a 1-nucleotide overhang at their 5′-ends in double-stranded form. These overhangs facilitate polymerization of the monomeric oligonucleotide duplexes by T4 DNA ligase in only one orientation and maintain a constant interadduct distance throughout the resulting multimer. Autoradiograms of electrophoresis gels revealing resolution of the ligation products of 22−25 bp duplexes nonplatinated or containing a unique interstrand cross-link of Pt-DIST(S) are shown in Figure 4. A significant retardation was observed for the multimers of all platinated duplexes. Decreased gel electrophoretic mobility may result from a decrease in the DNA end-to-end distance (*33*). Various platinum(II) complexes have been shown to form DNA adducts which decrease gel mobility of DNA fragments due to either stable curvature of the helix axis or increased isotropic flexibility (*15*, *22*, *34*−*38*). DNA multimers of identical length and number of stable bend units, but with differently phased bends, have different end-to-end distances. The DNA bends of a multimer must be, therefore, spaced evenly and phased with the DNA helical repeat in order to add constructively. Such constructively phased bends add in plane, yielding short end-to-end distances and the most retarded gel migration. In other words, gel electrophoresis of multimers of oligonucleotide duplexes which only differ in length and contain a stable curvature induced by the same platinum adduct should exhibit a phase effect, i.e., the maximum retardation should be observed for the multimers having the bends in phase with helix screw. In contrast, the normal electrophoretic mobility should be observed for the multimers having the bends separated by a half-integral number of DNA turns. The *K* factor is defined as the ratio of calculated to actual length. The calculated length is based on a multimer's mobility, and is obtained from a calibration curve constructed from the mobilities of nonplatinated multimers. The variation of the *K* factor versus sequence length obtained for multimers of the duplexes 22−25 bp long and containing the unique 1,1-d(G-Pt−C) interstrand cross-link of Pt-DIST(S) is shown in Figure 5A. Maximum retardation was observed for the 24-bp duplex containing the adduct of both Pt-DIST isomers [shown in Figure 5A for Pt-DIST(S)]. This observation suggests that the natural 10.5-bp repeat of B-DNA and that of DNA perturbed by the Pt-DIST(R) or Pt-DIST(S) interstrand cross-link are different as a consequence of DNA unwinding (*39*). Interestingly, the 23-bp curves had only a slightly smaller slope than 24-bp curves, whereas the 25-bp curves differed more pronouncedly (Figure 5A). This asymmetry is also consistent with a significant DNA unwinding due the formation of the interstrand cross-link by both isomers.

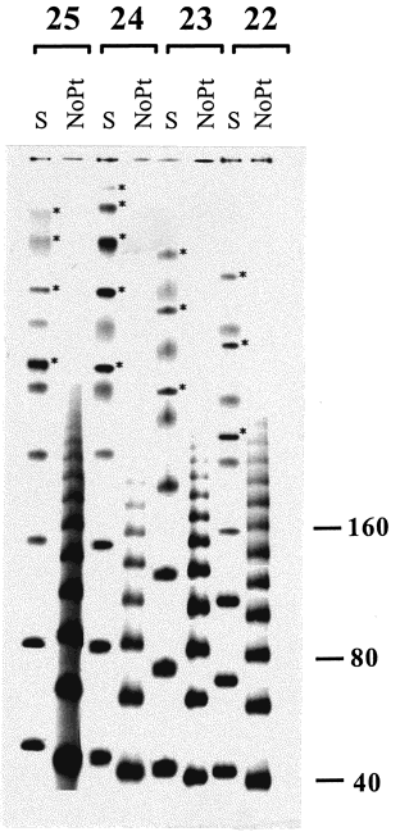

FIGURE 4: Autoradiogram of the ligation products of double-stranded oligonucleotides d(TGCT)/d(AGCA)(22−25) containing a unique interstrand cross-link formed by Pt-DIST(S) compound between central G in the top strand and complementary C. The ligation products were separated on an 8% polyacrylamide gel (lanes S). Nonplatinated oligomers, lanes NoPt.

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 curve as described in the previous paper for intrastrand adducts of cisplatin (*39*). The maximum of these curves constructed for the duplexes interstrand cross-linked by either Pt-DIST isomer with a total length of 130 bp [shown for Pt-DIST(S) in Figure 5B] were determined to be 23.76 ± 0.01 for both isomers. Total sequence lengths other than 130 bp were examined and gave identical results. 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 (*40*, *41*). The difference between the helical repeat of B-DNA and the DNA containing interstrand cross-link of Pt-DIST isomers, therefore, is $[(23.76 \pm 0.01) - 2(10.5 \pm 0.05)] = 2.76 \pm 0.06$ bp. There are 360°/10.5 bp, so the DNA unwinding due to one interstrand adduct of Pt-DIST isomers is 95 ± 2°. This unwinding angle is somewhat larger than that found for 1,2-

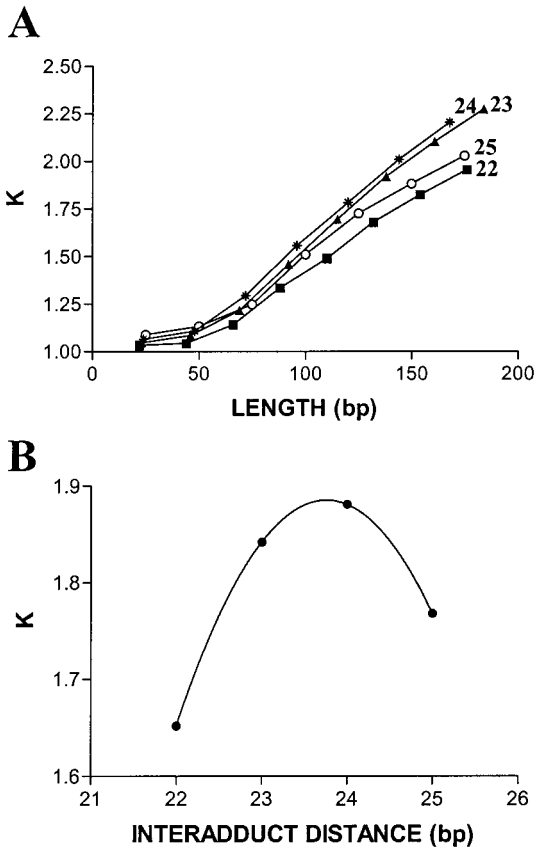

FIGURE 5: (A) Plots showing the relative mobility *K* versus sequence length curves for the oligomers d(TGCT)/d(AGCA)(22−25) containing single interstrand cross-link of Pt-DIST(S), denoted respectively as 22, 23, 24, and 25. (B) Plots showing the relative mobility *K* versus interadduct distance in bp for the oligomers d(TGCT)/d(AGCA)(22−25) interstrand cross-linked by Pt-DIST-(S) with a total length of 130 bp. The experimental points represent the average of three independent electrophoresis experiments. The curves represent the best fit of these experimental points to the equation $K = ad^2 + bd + c$ (*39*).

d(G-Pt-G) interstrand cross-link of nontargeted cisplatin using the same experimental procedure [79° (*37*) or ∼90° (*36*)]. Thus, tethering cisplatin to distamycin has slightly increased unwinding induced by the interstrand cross-link.

The evaluation 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 (Figure 5B) characteristic for bending (*15*, *22*, *34*−*38*). The quantitation of the bend angle of the interstrand cross-link of Pt-DIST isomers was performed in the way described previously (*15*, *22*, *34*−*38*) utilizing the empirical equation

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

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 A residues (A tract) (*34*, *42*). Application of eq 1 to the 120- or 138-bp multimers of the 24-bp oligomers containing the single interstrand cross-link of both Pt-DIST isomers leads to a mean curvature of 0.875, relative to the A tract. The average bend angle per helix turn can be calculated by multiplying the relative curvature by the absolute value of the A tract bend [20° (*22*, *34*, *42*, *43*)]. The results indicate that the bend induced by the interstrand cross-link of Pt-DIST(R) or Pt-DIST(S) is about 35°. We assigned the bend direction by reference to an A tract, which is bent by ∼20° toward the minor groove (*42*) using the same procedure as in the previously published paper (*36*). The duplex d(TGCT)/d(AGCA)+d(A/T)$_5$(36) (Figure 1) was used which also contained, besides the single interstrand cross-link of either Pt-DIST isomer (between G residue in the top strand and its complementary C residue), the A tract located "in phase" from the cross-link (the cross-linked base pair and the center of the A tract were separated by 12 bp) (Figure 1). In the cross-linked multimers, the cross-links or the A tracts were separated by 36 bp, corresponding to about three helical turns after the incorporation of the estimated 95° of unwinding at the lesion (vide supra). The cross-linked multimers of d(TGCT)/d(AGCA)+d(A/T)$_5$(36) were in all cases more retarded than their nonplatinated counterparts (not shown). Hence, the effective bend of the helix axis at the center of the Pt-DIST interstrand cross-link is in the same direction as that at the center of the A tract, i.e., toward the minor groove. Other details of the calculations of the unwinding and bending angles are given in the previously published papers (*15*, *22*, *34*−*38*).

Also produced in ligations of monomers investigated in this work were separate bands arising from small DNA circles that migrate close to the top of the gel (see the bands marked by asterisk in Figure 4, lanes R as example). The occurrence of small DNA circles was even better evident if the Pt-DIST complex was removed from the products of the ligation reaction by NaCN (not shown). The highest tendency to yield DNA circles was observed for the 23- and 24-bp interstrand cross-linked multimers confirming a close match between the 24-bp sequence repeat and the helix screw (*34*, *44*). Interestingly, the ligation products of the interstrand cross-linked duplexes contained several types of DNA circles and the DNA circles were also observed in the ligation products of all interstrand cross-linked duplexes tested in the present work including 22-bp duplexes. Importantly, formation of small DNA circles has not been noticed in ligation reactions of the duplexes containing unique interstrand cross-links of nontargeted cisplatin (*36*, *37*).

1,2-d(GpG) Intrastrand Cross-Link

Chemical Probes of DNA Conformation. To further characterize the distortion induced in DNA by intrastrand cross-links of Pt-DIST isomers, the d(TGGT)/d(ACCA)(21) containing the 1,2-d(GpG) intrastrand cross-link of Pt-DIST-(R) or Pt-DIST(S) was examined using chemical probes of DNA conformation. In these experiments $KMnO_4$, DEPC, and bromine were used in the same way as when we analyzed in the present work the duplexes containing interstrand cross-link (vide supra). The pattern and degree of reactivity toward the chemical probes was identical for the two Pt-DIST isomers (not shown) indicating similar character of the conformational distortion. In addition, these results were similar to those obtained earlier for the 1,2-(GpG) intrastrand cross-link of nontargeted cisplatin (cf. ref. *45*) suggesting that the local conformational distortion extends over at least five bp.

DNA Unwinding and Bending. In this work, we also performed studies on DNA bending and unwinding induced by single, site-specific intrastrand cross-links of Pt-DIST

isomers formed in oligodeoxyribonucleotide duplexes between neighboring G residues. These examinations were performed using a phase-sensitive gel electrophoresis bending assay (not shown) in the same way as in the bending and unwinding studies of the oligonucleotide duplexes containing interstrand cross-links (vide supra).

A duplex unwinding angle 14 ± 2 or 15 ± 3° for Pt-DIST(R) or Pt-DIST(S) intrastrand cross-links were calculated, respectively. The average bend angle per helix turn induced by the intrastrand cross-link of Pt-DIST(R) or Pt-DIST(S) was about 32 or 33° toward the major groove of DNA, respectively. These unwinding and bending angles are similar to those found for 1,2-d(GpG) intrastrand cross-link of nontargeted cisplatin (13°) using the same experimental procedure (*39*). Thus, tethering cisplatin to distamycin has no pronounced effect on unwinding and bending induced by the intrastrand cross-link formed by the platinum complex between neighboring G residues.

CD Spectroscopy

We have shown in our recent paper (*8*) that CD spectral characteristics of calf thymus (CT) DNA in the presence of increasing amounts of Pt-DIST compounds are considerably changed. The most striking alteration due to the covalent binding of the two isomers to CT DNA has been an appearance of the CD band centered around 322 nm. This observation has been interpreted to mean that DNA binding of Pt-DIST compounds involves a combined mode including both coordination of the platinum atom to base residues in DNA and a noncovalent type of fixation via van der Waals contacts of distamycin moiety with the groups in the minor groove of DNA. Interestingly, the intensity of the induced Cotton effect observed upon DNA binding of Pt-DIST compounds at around 322 nm due to noncovalent binding is markedly lower than that observed when the same amount of molecules of free distamycin (not linked to cisplatin) is bound to CT DNA. It has been, therefore, suggested (*8*) that distamycin moiety interacts with DNA in a way resulting in the induced Cotton effect only in a relatively small fraction of DNA adducts of Pt-DIST complexes. In addition, other characteristics of the CD spectra (isoelliptic point at 289 nm) have supported the view that there is only one type of DNA-binding site for distamycin moiety in those DNA adducts of Pt-DIST complexes that give rise to the induced CD at ~322 nm.

Cutting of pSP73 DNA by *Nde*I and *Ssp*I endonucleases yielded a 367-bp fragment. Two types of the fragment modified by Pt-DIST compounds were prepared and purified in the present work. The fragment was first platinated by either Pt-DIST compound at r_b of 0.012. Gel electrophoresis under denaturing conditions revealed that ca. 35% of these fragments contained mainly intrastrand cross-links, but no interstrand cross-link. In contrast, the remaining ca. 65% of these fragments contained at least one interstrand cross-link. The interstrand cross-linked fraction and the platinated fraction containing no interstrand cross-link were separated and purified. It was verified by FAAS that r_b values in these fractions remained after their separation unchanged. CD spectra of these fractions are shown in Figure 6 for the fragment modified by Pt-DIST(R) compound. While the fragments platinated at $r_b = 0.012$ containing no interstrand cross-link yielded no CD band centered around 322 nm, the fragments platinated to the same level but containing at least one interstrand cross-link of Pt-DIST(R) yielded this CD band. Similar results (not shown) were obtained with the fragment containing the adducts of Pt-DIST(S) compound. Thus, these results strongly support the view that the relatively small fraction of DNA adducts of Pt-DIST compounds in which the distamycin moiety in the Pt-DIST compounds interacts with DNA in a way that results in the induced Cotton effect are interstrand cross-links. In contrast, intrastrand cross-links give rise apparently to no induced CD at ~322 nm.

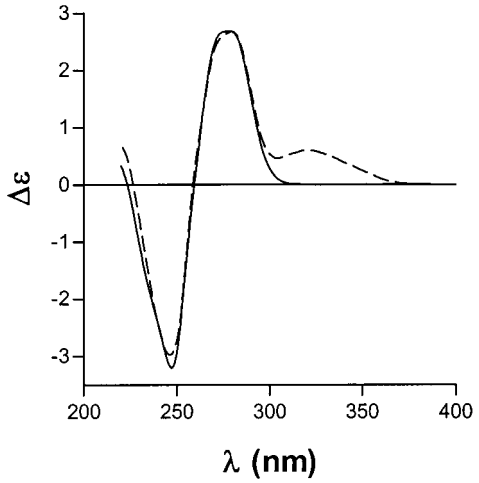

FIGURE 6: CD spectra of *Nde*I/*Ssp*I restriction fragment modified by Pt-DIST(R) complex at $r_b = 0.012$ in 10 mM $NaClO_4$. DNA was at the concentration of 0.072 mM (related to the monomeric nucleotide content). After reaction with the Pt-DIST(R) the fragments with and without interstrand cross-links were separated (dashed and full line, respectively). For other details, see the text.

DISCUSSION

The major differences induced in DNA by Pt-DIST compounds and by nontargeted cisplatin described in our preceding work (*8*) mainly comprised higher interstrand cross-linking by the former (although not so high that the interstrand cross-link would become the major adduct) and altered local conformation around the adducts, such as a higher extent of the mean DNA unwinding. Another important feature of the altered DNA-binding mode of Pt-DIST compounds was a fact that distamycin moiety in a small fraction of the adducts interacted with DNA in a similar way as does free distamycin (not attached to cisplatin), i.e., formed noncovalent bonds with the groups of the minor groove of DNA (*46*, *47*). On the other hand, the major platination sites were not distinctly different so that it was reasonable to conclude that Pt-DIST compounds preferentially coordinated to GG and AG sites similarly as nontargeted cisplatin (*8*). The latter observation was also interpreted to mean that attachment of distamycin to cisplatin had no fundamental effect on capability of cisplatin to form, as major adducts, intrastrand cross-links between neighboring purine residues.

The 1,2-intrastrand adducts of cisplatin are accommodated into the major groove of DNA with formation of coordination bonds to the N7 atoms of two adjacent purine residues. A phase-sensitive gel electrophoresis bending assay revealed that this intrastrand cross-link of nontargeted cisplatin bent

Table 1: Summary and Comparison of Basic Characteristics of Intrastrand and Interstand Cross-Links Formed in DNA by Pt-DIST Compounds and Cisplatin in Cell-free Media

	Pt-DIST(R)		Pt-DIST(S)		cisplatin	
	1,2-IACL[a]	XCL[b]	1,2-IACL[a]	XCL[b]	1,2-IACL[a]	XCL[b]
bases preferentially involved	GG, AG[c]	complementary G and C[c]	GG, AG[c]	complementary G and C[c]	GG, AG	Gs in the 5′-GC-3′ sequence[d]
bending	32° toward major groove[c,e]	35° toward minor groove[c,e]	33° toward major groove[c,e]	35° toward minor groove[c,e]	32−34° toward major groove[c,f]	20−40° toward minor groove[c,g]
unwinding	14° [c,e]	95° [c,e]	15° [c,e]	95° [c,e]	13° [c,h]	79−87° [c,g]

[a] 1,2-Intrastrand cross-link. [b] Interstrand cross-link. [c] This work. [d] Lemaire et al. (*13*). [e] Determined by gel electrophoresis. [f] Rice et al., 1988 (*34*), Bellon and Lippard, 1990 (*21*). [g] Huang et al., 1995 (*36*), Malinge et al., 1994 (*37*). [h] Bellon et al., 1991 (*39*).

helix toward its major groove and unwound the duplex by 32 and 13°, respectively (*22*, *39*). From the present results of the bending and unwinding measurements (Table 1) along with the conformational analysis based on using chemical probes, it is clear that tethering distamycin to cisplatin has no essential effect on the character of the local conformational alteration in DNA induced by the intrastrand 1,2-(GpG) adduct. In addition, the G-bridging cisplatin in these adducts lies in the major groove (*48*, *49*) so that it is reasonable to assume that the distamycin moiety in the 1,2-(GpG) intrastrand adducts of Pt-DIST also lies in the major groove. Free distamycin (not tethered to cisplatin) has no or very weak affinity to interact with the groups in the major groove of DNA (*46*, *47*, *50*−*52*). Hence, interaction of distamycin moiety in the 1,2-intrastrand cross-links of Pt-DIST compounds with DNA appears unlikely. As the intrastrand adduct between neighboring purine residues represents the major DNA lesion of Pt-DIST compounds, this suggestion is also consistent with the view (*8*) that one type of the adducts in which distamycin moiety interacts with DNA and produces the induced Cotton effect in CD spectra only represents a small fraction of all adducts. Thus, it is not surprising that restriction DNA fragments without interstrand cross-links mainly containing intrastrand cross-links of Pt-DIST compounds yield no CD band associated with the Cotton effect (Figure 6).

On the other hand, the occurrence of the CD band corresponding to this induced Cotton effect is apparently associated with the presence of minor interstrand cross-links formed in DNA by Pt-DIST compounds (Figure 6). Thus, this observation matches the requirement that the only type of the adduct of Pt-DIST compounds in which distamycin moiety binds noncovalently to the groups in the minor groove should be a less frequent lesion (*8*). Taken together, the results of the present work suggest that in particular the interstrand cross-links (and not intrastrand adducts) are lesions responsible for the different conformational alterations due to the global DNA modification by these composite compounds if they are compared with those due to the modification by nontargeted cisplatin (*8*).

The attachment of distamycin to cisplatin resulted in a higher yield of cross-links in which cisplatin coordinated two bases in different strands of double-helical DNA. The sites, which were preferentially coordinated by platinum when DNA was globally modified by Pt-DIST compounds, were determined by transcription mapping (*8*). Hence, the sites involved in the interstrand adducts could be obscured by those involved in the major intrastrand cross-links because even enhanced amount of interstrand cross-links observed as a consequence of tethering distamycin to cisplatin represented a minority among all adducts. In addition, in these transcription mapping experiments the strong bands were only evaluated (*8*). A careful examination of the autoradiograms (see Figure 3 in ref *8*) also revealed bases as minor DNA-binding sites other than G and A. Therefore, we determined in the present work the nature of bases involved in the interstrand cross-links with the aid of a more accurate experimental procedure (Figure 2). This approach revealed that G and complementary C were the preferential binding sites of Pt-DIST complexes in the interstrand cross-links (Table 1). This result represents a very striking effect of tethering distamycin to cisplatin because nontargeted parent platinum drug forms these interstrand adducts between two G residues in the 5′-GC/5′-GC sites (*13*). Interestingly, the interstrand cross-links between G and C residues were also formed by nontargeted cisplatin in the synthetic polydeoxyribonucleotide complex poly(dG)·poly(dC) (in this double-helical DNA one strand contains only G whereas the other strand C residues) (*50*). The preferential formation of the GG interstrand cross-links over the cross-links between complementary G and C residues by nontargeted cisplatin is attributable to incompatible topological requirements for the transformation of the monofunctional adduct [preferentially formed at G residues (*23*)] into interstrand lesion on double-helical B-DNA. From these considerations, we conclude that conversion of the GG interstrand adduct of nontargeted cisplatin into the cross-link between complementary G and C caused by the attachment of distamycin to this platinum drug may require additional mechanism that would facilitate the formation of this lesion.

The present work was also aimed at describing the conformational distortion induced in DNA by the cross-link formed by Pt-DIST compounds between complementary G and C residues (Table 1). The experiments were carried out with the oligodeoxyribonucleotide duplexes containing the unique, site-specific interstrand cross-link in their central sequence d(TGCT)/AGCA) (Figure 1). The extent of DNA unwinding by the interstrand cross-link of Pt-DIST compounds was approximately 95°. The cross-link bent the helix axis of DNA by ∼35° toward the minor groove. In addition, the ligation products of the duplexes interstrand cross-linked by Pt-DIST compounds contained more than one type of the DNA circles (Figure 4). Interestingly, a significant tendency to form the DNA circles was also observed for the ligation products of 22-bp cross-linked duplexes, i.e., for those that exhibited the minimum retardation in the gel. Thus, the cross-links in these ligation products were apparently located not in phase to produce planar curvature. From these results, it is clear that the local distortion of the duplex around the

cross-link also comprises an enhanced flexibility of the duplex not seen in the case of interstrand cross-links of nontargeted cisplatin.

Local conformational alterations induced in DNA by the interstrand cross-links of Pt-DIST compounds were also investigated using chemical probes of DNA conformation (Figure 3). These results revealed that this distortion was similar for both isomers, symmetrical around the cross-linked base pair and extended mainly over five bp.

An important feature of the structure of the interstrand cross-link of nontargeted cisplatin is that the platinum residue protruded in the minor groove of the DNA duplex and the N7 atoms of the cross-linked guanine residues (localized initially in the major groove of B-DNA) moved in the minor groove. The present work demonstrates that several conformational features of interstrand cross-links of nontargeted cisplatin and Pt-DIST compounds, such as bending and unwinding (Table 1), are not markedly different. It seems reasonable to suggest that this radical distortion of DNA conformation is associated with the presence of the platinum atom in the minor groove in the interstrand cross-links of Pt-DIST compounds as well. This suggestion is also corroborated by the fact that a very high unwinding induced by Pt-DIST interstrand cross-links cannot be rationalized by major groove models of this lesion (*36*). One possible explanation for moving the platinum atom to the minor groove more readily in the case of the interstrand cross-links formed by Pt-DIST compounds is that this process is facilitated by affinity of the distamycin moiety to bind preferentially in the minor groove of DNA (*46*, *47*) (vide supra).

No sufficient and systematic data on the effects of tethering the DNA minor groove binders to cisplatin on its biological activity are available. Thus, at present it would be too early to speculate how the distinct difference in DNA interstrand cross-linking by nontargeted cisplatin and Pt-DIST compounds is related to their biological effects. Nevertheless, the results of the present work suggest that the structural impact caused by the specific interstrand cross-link detected in this study deserves attention when biological behavior of cisplatin derivatives targeted by oligopeptide DNA minor groove binders and structurally related platinum drugs are examined and evaluated.

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