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Chemical Probes of the Conformation of DNA Modified by *cis*-Diamminedichloroplatinum(II)†

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ABSTRACT: The purpose of this work was to analyze at the nucleotide level the distortions induced by the binding of *cis*-diamminedichloroplatinum(II) (*cis*-DDP) to DNA by means of chemical probes. In order to test the chemical probes, experiments were first carried out on two platinated oligonucleotides. It has been verified by circular dichroism and gel electrophoresis that the binding of *cis*-DDP to an AG or to a GTG site within a double-stranded oligonucleotide distorts the double helix. The anomalously slow electrophoretic mobility of the multimers of the platinated and ligated oligomers strongly suggests that the platinated oligonucleotides are bent. The reactivity of the oligonucleotide platinated at the GTG site with chloroacetaldehyde, diethyl pyrocarbonate, and osmium tetraoxide, respectively, suggests a local denaturation of the double helix. The 5'G residue and the T residue within the adduct are no longer paired, while the 3'G residue is paired. The double helix is more distorted (but not denatured) at the 5' side of the adduct than at the 3' side. In the case of the oligonucleotide platinated at the AG site, the double helix is also more distorted at the 5' side of the adduct than at the 3' side. The G residue within the adduct is paired. The reactivities of the chemical probes with six platinated DNA restriction fragments show that even at a relatively high level of platination only a few base pairs are unpaired but the double helix is largely distorted. No local denaturation has been detected at the GG sites separated from the nearest GG or AG sites by at least three base pairs. The AG sites separated from the nearest AG or GG sites by at least three base pairs do not denature the double helix locally when they are in the sequences puAG/pyTC. When they are in the pyAG/puTC sequences, the reactivity of osmium tetraoxide with the T residues complementary to the platinated A residues indicates either a distortion or an unpairing of the bases. The T residues within the sequences (CGT/GCA) react strongly with osmium tetraoxide. It is suggested that the distortion within these sequences is induced by adducts located further away along the DNA fragments, these sequences not being the major sites for the binding of *cis*-DDP.

Many studies suggest that both the cytotoxic and the antitumor activities of *cis*-diamminedichloroplatinum(II) (*cis*-DDP)[1] are a consequence of its reaction with cellular DNA. Most of the adducts formed in the reaction of *cis*-DDP and DNA have been identified. Two major adducts arise from an intrastrand cross-link between two adjacent guanine residues and between the adjacent adenine and guanine residues. Minor adducts arise from intrastrand cross-links between two guanine residues separated by at least one nucleotide residue and from interstrand cross-links between two guanine residues (Roberts & Pera, 1983; Lippard, 1987; Reedijk, 1987; Eastman, 1987, and references cited therein).

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[1] Abbreviations: *cis*-DDP, *cis*-diamminedichloroplatinum(II); dien-Pt, chlorodiethylenetriamineplatinum(II); DEPC, diethyl pyrocarbonate; CAA, chloroacetaldehyde; HA, hydroxylamine; I, d(CTCTCTTCTGTGTCTTCTCT); I*, the same oligonucleotide modified by *cis*-DDP at the GTG site; I-dienPt, the same oligonucleotide modified by dien-Pt at one G residue; II, d(AGAGAGAAGACACAGAAGAG); III, d(CTTCTCTTCTAGTCTTCTCT); III*, the same oligonucleotide modified by *cis*-DDP at the AG site; IV, d(GAGAGAAGACTAGAAGAGAA); V, d(CTCATCAGTCACTCT); V*, the same oligonucleotide modified *cis*-DDP at the AG site; VI, d(GAGAGTGACTGATGA); r_b, molar ratio of platinum residues per nucleotide; bp, base pair. Equimolar mixtures of the complementary oligonucleotides I and II, III and IV, and V and VI are respectively I + II, III + IV, and V + VI.

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Several physicochemical, biochemical, and immunochemical studies have shown that the binding of *cis*-DDP to nucleic acids induces some distortion of the double helix (Lippard, 1987; Reedijk, 1987, and references cited therein). Recently, structural knowledge of the distortions has been deduced from X-ray studies of platinated oligonucleotides (Sherman et al., 1985; Admiraal et al., 1987), from NMR studies of platinated double-stranded oligonucleotides (den Hartog et al., 1984, 1985a,b; van Hemelryck et al., 1984, 1986), and from molecular mechanics calculations (Kozelka et al., 1986, 1987).

Our purpose was to describe at the nucleotide level the conformational changes induced by the adducts that are formed in the reaction of *cis*-DDP with native DNA. 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. For example, diethyl pyrocarbonate (DEPC), which carbethoxylates purines at the N7 position, is hyperreactive with left-handed Z-DNA and with denatured DNA as compared to B-DNA (Herr, 1985; Johnston & Rich, 1985; Runkel & Nordheim, 1986). Chloroacetaldehyde (CAA), which reacts with N-1 and N^6 of adenosine and N-3 and N^4 of cytidine, is hyperreactive with denatured DNA and Z-DNA as compared to B-DNA (Lilley, 1983; McLean et al., 1987; Kohwi-Shigematsu et al., 1987; Vogt et al., 1988). Osmium tetraoxide (OsO_4) in the presence of pyridine binds through addition across the 5,6 double bond of the pyrimidine rings in single-stranded nucleic acids and in distorted DNA such as the B-DNA–Z-DNA junctions (Johnston & Rich, 1985; Lilley & Palecek, 1984; Palecek et al., 1987). Hydroxylamine (HA) reacts with cytosine residues at the C-4 and C-6 positions in single-stranded DNA and distorted DNA (Johnston & Rich, 1985). 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 (Johnston & Rich, 1985; Herr, 1985; Runkel & Nordheim, 1986; Vogt et al., 1988).

In this paper, we first show by circular dichroism and electrophoretic mobility that the binding of *cis*-DDP either to an AG or to a GTG site within a double-stranded oligonucleotide distorts the double helix. Subsequently, by means of chemical probes, we characterize at the nucleotide level these structural changes and also those that are induced by *cis*-platinum bound to native DNA.

Materials and Methods

Oligonucleotides were synthesized on a Applied Biosystem solid-phase synthesizer. They were purified in two steps by ion-exchange and reverse-phase HPLC (Hitachi Model 655 HPLC). The plasmids pUC18 and pBR322 were isolated according to standard procedures (Birnboim & Doly, 1979) and banded twice in CsCl/ethidium bromide equilibrium density gradients. The restriction enzymes, alkaline phosphatase, P1 nuclease, Klenow polymerase, and T4 polynucleotide kinase were purchased from Boehringer-Mannheim and Bethesda Research Laboratory. Ultrapure agarose was from Bethesda Research Laboratory and electrophoresis-grade acrylamide and bis(acrylamide) were from Merck.

DNA Restriction Fragments. DNAs were digested with the appropriate restriction endonuclease and labeled at either the 5′ end (T4 polynucleotide kinase) or the 3′ end (Klenow polymerase). After secondary restriction enzyme digestion, uniquely end-labeled DNA fragments were separated on 1% agarose gels and then isolated on DEAE membranes (Schleicher and Schuell). The following 3′ or 5′ ^{32}P end labeled DNA fragments were prepared: *Eco*RI–*Bgl*I fragment (202 bp) from pUC18; *Sal*I–*Bgl*I fragment (278 bp) and *Bam*HI–*Sal*I fragment (276 bp) from pBR322. The *Hin*dII–*Bam*HI fragment (346 bp) and the *Eco*RI–*Dra*I fragment (418 bp), 5′ ^{32}P end labeled respectively at the *Hin*dIII site or at the *Eco*RI site, were isolated from pBR322.

Chemical Modifications. Reactions between platinated (or unplatinated) nucleic acids and the chemical probes were performed as described by Herr (1985) for diethyl pyrocarbonate, by Johnston and Rich (1985) for hydroxylamine, and by Nejedly et al. (1985) for osmium tetraoxide. In the case of chloroacetaldehyde, the procedure of Vogt et al. (1988) was used with minor modifications (temperature 20 °C; time of reaction 1 h). Piperidine treatment and preparation of the samples were identical as for sequencing reaction (Maxam & Gilbert, 1980). Equivalent amounts of radioactivity for each sample were loaded on a 6% polyacrylamide buffer gradient sequencing gel (Biggin et al., 1983). For each gel, about 150–200 bases were easily analyzed. Chemical degradation sequencing reactions were as described (Maxam & Gilbert, 1980).

Reaction of Platination. The reaction between platinum derivatives (a gift of Dr. J. L. Butour, Toulouse) and single-stranded oligonucleotides (concentration 2 mM) was done in 5 mM $NaClO_4$ at 37 °C for 4 days (input molar ratio, 1 platinum residue/oligonucleotide). The platinated oligonucleotides were purified in two steps by ion-exchange and reverse-phase HPLC. The major products (I* and III*, respectively) were shown by atomic absorption spectroscopy to have about 1 platinum atom/oligonucleotide. The sites of platination were verified by HPLC analysis of the digests after incubation of the platinated oligonucleotides with P1 nuclease (Fichtinger-Schepman et al., 1985) and then alkaline phosphatase (Eastman, 1986). The yields of I* and III* were about 70% and 15%, respectively.

The reaction between 5′ or 3′ ^{32}P end labeled DNA restriction fragments and platinum derivatives was done as previously described (Malinge et al., 1987).

Kinasing, Ligation, and Electrophoresis. The oligonucleotides, each hybridized to its complementary strand, were kinased with [γ-^{32}P]ATP, and the hybridized duplexes were self-ligated to make multimers of the oligonucleotide according to the procedure previously described (Koo et al., 1986). The ligated products were run on 8% polyacrylamide gels [mono:bis(acrylamide) ratio = 29:1; 90 mM Tris–borate, pH 8, 2.5 mM EDTA]. The applied voltage was 7 V cm^{-1}, and electrophoresis was performed at room temperature.

Absorption and circular dichroism spectra were recorded with a Kontron Uvikon 810 spectrophotometer and with a Jobin-Yvon III dichrograph, respectively.

Results

Before the reaction between platinated DNA and the chemical probes was performed, it seemed necessary to test the chemical probes with well-defined adducts. In other words, we wanted first to verify by means of techniques sensitive to the geometry of the double helix that the adducts induce a distortion of the double helix and then to analyze the distortion by means of chemical probes. For this purpose, two platinated oligonucleotides were compared to the corresponding unplatinated oligonucleotides by ultraviolet absorption, circular dichroism, electrophoretic mobility, and reactivity with chemical probes.

(A) Platinated Oligonucleotides. (1) Ultraviolet Absorption and Circular Dichroism. The thermal stability of the double-stranded oligonucleotides with cohesive ends I + II, I* +

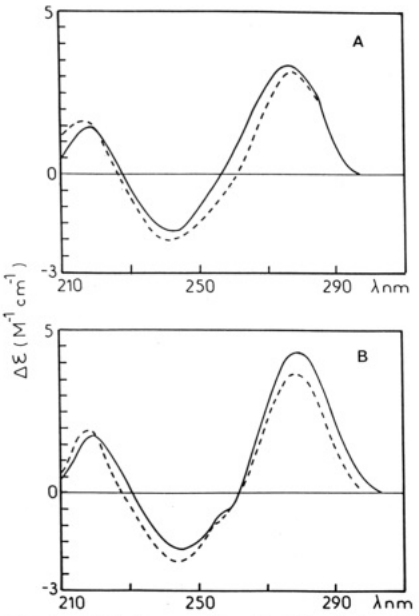

FIGURE 1: Circular dichroism spectra of platinated and unplatinated oligonucleotides: (A) (---) I + II, (—) I* + II, GTG adduct; (B) (---) III + IV, (—) III* + IV, AG adduct. Solvent is 15 mM $NaClO_4$, 5 mM Tris-HCl, 0.1 mM EDTA, pH 7.5. Concentration of oligonucleotides is 1.25 10^{-4} M, in nucleotide residues.

II, III + IV, and III* + IV has been determined by following the absorption at 260 nm as a function of temperature. The platinated and unplatinated oligonucleotides melted cooperatively (results not shown). In the experimental conditions given in Figure 1, the T_m and hyperchromicity are 43 °C and 24%, respectively, for I + II, 36 °C and 21% for I* + II, 38 °C and 23% for III + IV, and 33 °C and 22% for III* + IV. The binding of *cis*-DDP to a GTG site or to an AG site decreases the thermal stability of the double helix.

Both AG and GTG adducts distort the double helix, but differently, as shown by circular dichroism (Figure 1). In first approximation, the spectra of I + II and III + IV are similar, with a first positive band centered at 278 nm and a negative band centered at 242 nm. The main changes induced by the GTG adduct in I* + II are a shift of the first positive band toward the short wavelengths and a decrease of the intensity of the negative band, while the main changes induced by the AG adduct in III* + IV are an increase of the intensity of the first positive band and a decrease of the intensity of the negative band.

(*2*) *Gel Mobility*. Intrinsic bending of DNA molecules is generally thought to be the origin of the abnormal electrophoretic behavior of some DNA fragments (Diekmann, 1987; Hagerman, 1988; Trifonov & Ulanovsky, 1988, and references cited therein). A gel migration anomaly has been found for DNA fragments containing GG adducts (Rice et al., 1988). As shown in Figure 2, retardation in gel mobility occurs with DNA fragments containing AG or GTG adducts. Lane 1 corresponds to the multimers of V + VI, lane 2 to the multimers of I + II, and lane 6 to the multimers of III + IV. All these multimers present a normal mobility. On the other hand, the platinated multimers I* + II (lane 4) and III* + IV (lane 5) display a reduced mobility, yielding an increased apparent length. This is illustrated by the variation of the *K* factor (apparent length to sequence length), which is larger than one. The reduced mobility is not due to a charge effect (a platinum residue bears two positive charges) since the mobility of the multimers (I-dienPt + II) is slightly but constantly reduced (lane 3). Finally, the *K* factors relative to the fragments platinated at the AG sites show that the multimers of the 15-mer V* + VI are less anomalous than the multimers of the 20-mer III* + IV. In conclusion, both circular dichroism and gel electrophoresis show that AG and GTG adducts distort the double helix.

(*3*) *Chemical Reactivity*. To characterize the distortions of the double helix induced by the adducts, several chemical probes (OsO_4, CAA, DEPC) were used. After reaction with the chemical probes, uniquely end-labeled platinated or un-

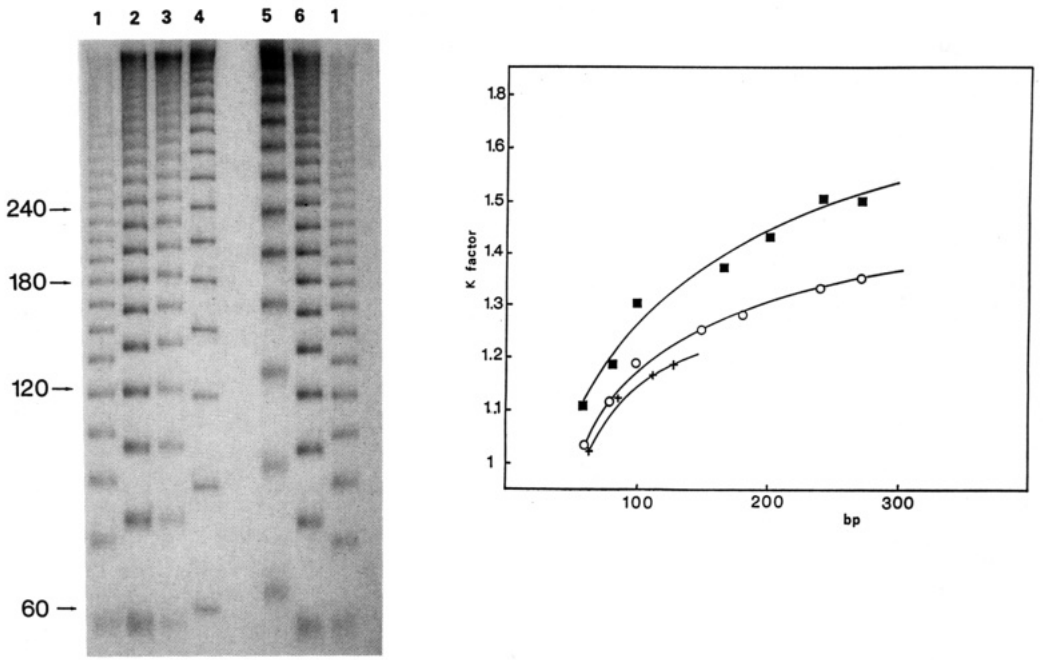

FIGURE 2: Comparison of the migration of platinated to unplatinated multimers on a nondenaturing 8% polyacrylamide gel. (Left) (Lane 1) Unplatinated 15 bp oligomers (V + VI) ligated to multimers; (lane 2) unplatinated 20 bp oligomers (I + II) ligated to multimers; (lane 3) platinated 20 bp oligomers (I-dienPt + II) ligated to multimers; (lane 4) platinated 20 bp oligomers (I* + II) ligated to multimers; (lane 5) platinated 20 bp oligomers (III* + IV) ligated to multimers; (lane 6) unplatinated 20 bp oligomers (III + IV) ligated to multimers. (Right) *K* factor (apparent length relative to sequence length) versus actual chain length: (○) multimers (I* + II); (■) multimers (III* + IV); (+) multimers (V* + VI).

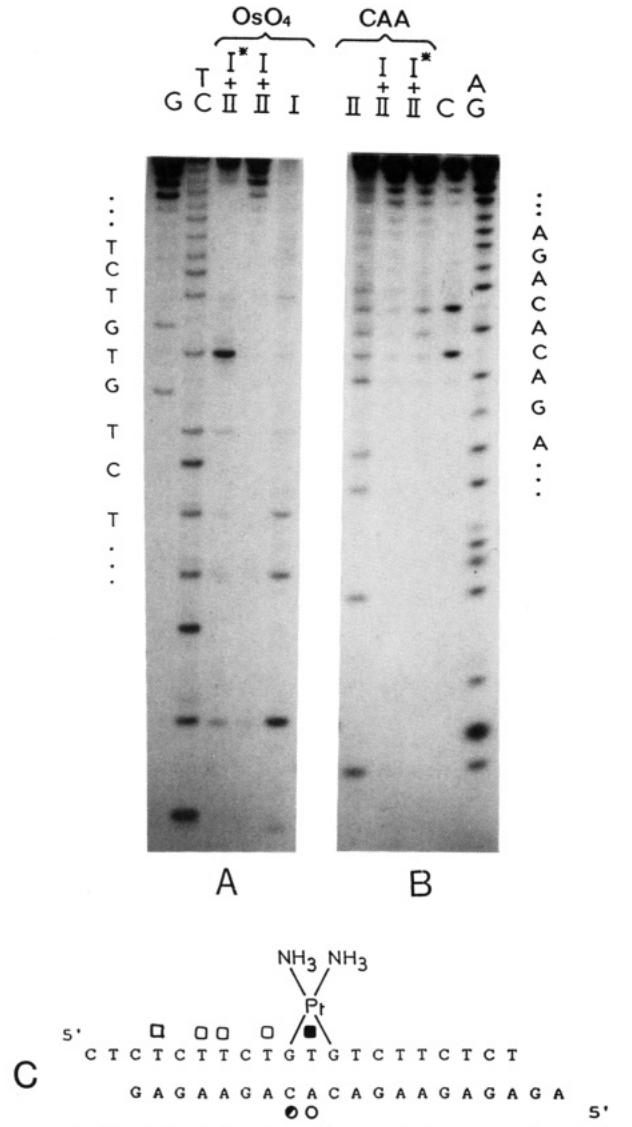

FIGURE 3: Piperidine-induced specific strand cleavage at chemically modified bases in platinated I* + II or in unplatinated I + II oligonucleotides: (A) Reaction of OsO_4 with I* in I* + II, with I in I + II, and with I, respectively; (B) reaction of CAA with II in I* + II, with II in I + II, and with II, respectively. In panels A and B are given Maxam–Gilbert specific reactions for the unplatinated oligonucleotides. A summary of the changes in chemical reactivity is given in panel C. Filled, half-filled, and open symbols indicate strong, intermediate, and low hyperreactivity, respectively: (□) OsO_4; (○) CAA.

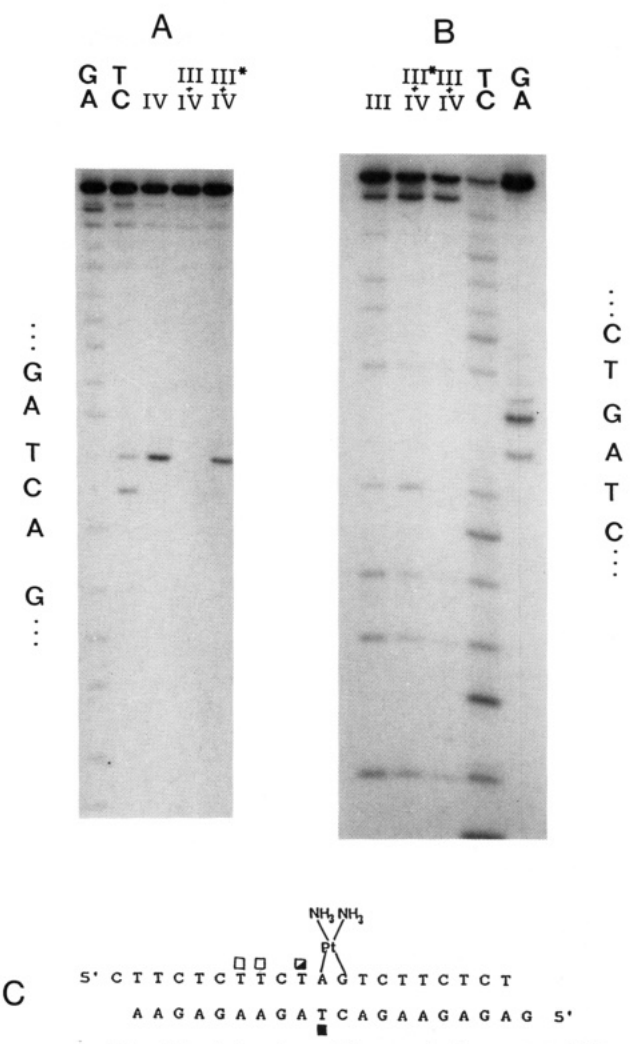

FIGURE 4: Piperidine-induced specific strand cleavage at OsO_4-modified bases in platinated III* + IV or in unplatinated III + IV oligonucleotides: (Left) Reaction of the probe with IV, with IV in III + IV, and with IV in III* + IV, respectively; (right) reaction of the probe with III, with III in III* + IV, and with III in III + IV, respectively. A summary of the changes in chemical reactivity is given under the panels. The meaning of the symbols (□) is as in the legend of Figure 3.

platinated oligonucleotides were incubated with NaCN [NaCN is known to remove bound platinum (Bauer et al., 1978)] and were then treated with piperidine. The resulting fragments were resolved on a sequencing gel along with the Maxam–Gilbert sequencing ladders of the same unplatinated oligonucleotides.

Results relative to I + II and I* + II are shown in Figure 3. The T residue between the two cross-linked G residues is strongly reactive with OsO_4, while the other T residues in the same strand are either weakly reactive (5′ side) or not reactive (3′ side). The C residues in the same strand were not modified by CAA (results not shown). CAA reacts with the C residue complementary to the 5′ G residue in the adduct but does not react with the C residue complementary to the 3′ G residue. The A residue complementary to the T residue in the adduct reacts slightly with CAA (Figure 3) and does not react with DEPC (results not shown).

The reactivity of OsO_4 and III* + IV is shown in Figure 4. Within the III* strand, the T residues at the 5′ side of the adduct but not those at the 3′ side are weakly reactive with OsO_4. The C residues were not reactive with CAA (results not shown). Within the IV strand, the T residue complementary to the A residue in the AG adduct is reactive with OsO_4 (Figure 4), while the other residues are not modified by CAA or DEPC (not shown).

It has been verified that the single-stranded oligonucleotides are reactive with the chemical probes, while the unplatinated double-stranded oligonucleotides are not reactive (Figures 3 and 4).

In conclusion, these experiments show that in the platinated oligonucleotides some base residues are modified by chemical probes. As discussed later, the reactivity and nonreactivity of the base residues provide some insights into the local structure of the double helix.

(*B*) *Platinated DNA.* It is known that native DNA is distorted by the binding of *cis*-DDP [for general reviews see Lippard (1987) and Reedijk (1987)]. The characterization of the distortions by means of chemical probes was as follows. A uniquely end-labeled DNA restriction fragment was first reacted with *cis*-DDP and then with a chemical probe (DEPC, OsO_4, HA, or CAA). After removal of the bound platinum

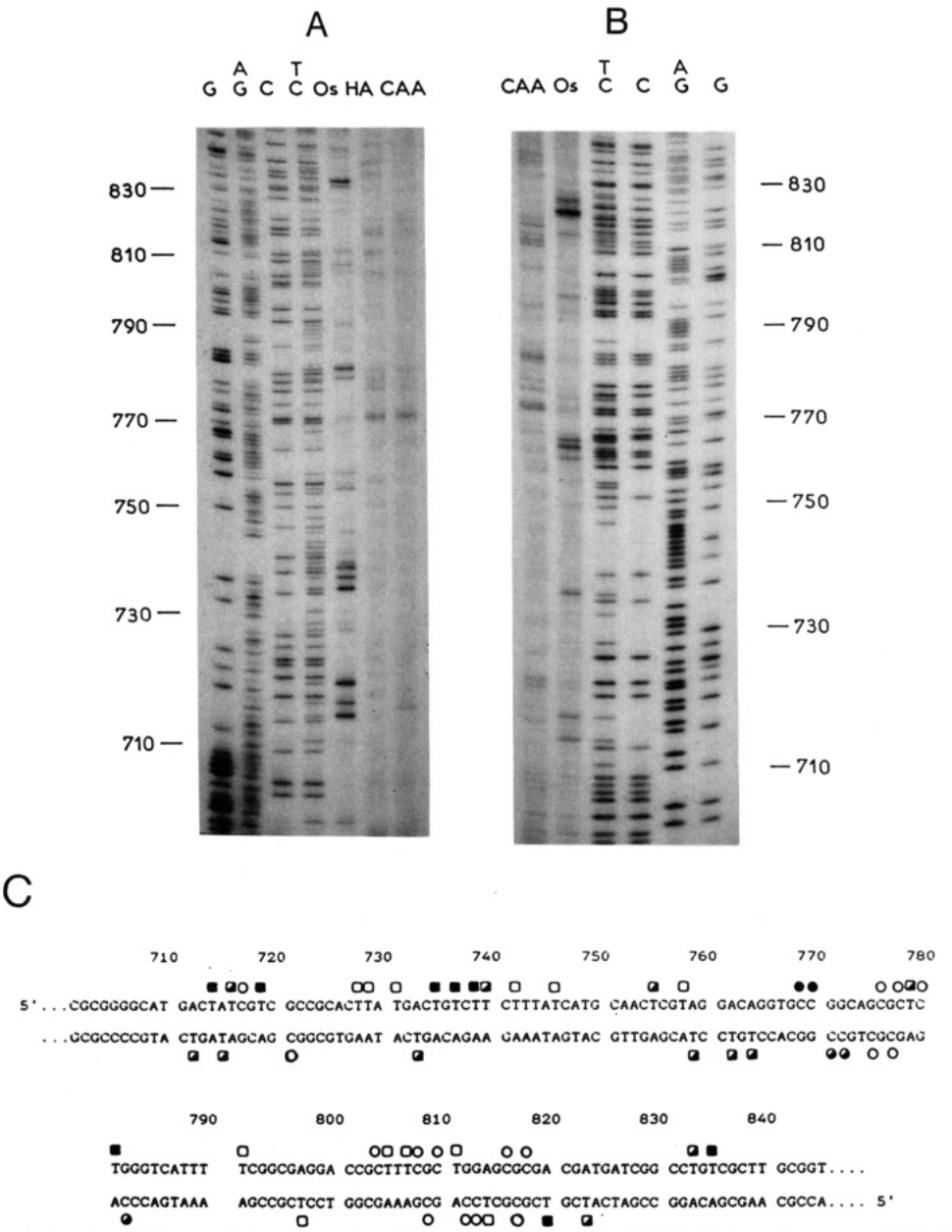

FIGURE 5: Piperidine-induced specific strand cleavage at chemically modified bases in pBR322 platinated at an $r_b = 0.05$. The pBR322 DNA was cleaved by *Sal*I, labeled in 5′ or 3′ ends, and recut by *Bgl*I: (A) 5′ end labeled fragments; (B) 3′ end labeled fragments. G, AG, C, and CT are Maxam–Gilbert specific fragments for the unplatinated samples. Panel C is a summary of the changes in chemically reactivity with OsO_4 (□) and with CAA (○). The meaning of filled, half-filled, and open symbols is as in the legend of Figure 3.

by NaCN, the chemically modified restriction fragment was treated with piperidine. The resulting fragments were resolved on a buffer gradient sequencing gel along with the Maxam–Gilbert sequencing ladders of the same unplatinated restriction fragment.

In Figure 5 are shown the results relative to the platinated *Sal*I–*Bgl*I restriction fragment from pBR322 after reaction with OsO_4, CAA, and HA. HA and CAA cleavage patterns are similar. Only a few DNA sites are reactive with both probes. Relatively more sites are reactive with OsO_4. No cleavages were observed after DEPC treatment or after reaction of the four chemical probes with the unplatinated *Sal*I–*Bgl*I restriction fragment (results not shown). A summary of the reactive bases with OsO_4 and CAA is given in Figure 5.

Analysis of the reactivity of platinated DNA with the chemical probes is complex even if we consider only the major potential binding sites. The GG or AG sites are not equally reactive with *cis*-DDP, as shown by digestion of platinated DNA restriction fragments either with exonuclease III (Royer-Pekora et al., 1981; Tullius & Lippard, 1982; Bowler & Lippard, 1986) or with T4 DNA polymerase (Malinge et al., 1987; Rahmouni et al., 1988). To minimize this distribution effect, the experiments here have been done with samples platinated at $r_b = 0.05$. At this r_b, most of the GG and AG sites are platinated (Eastman, 1986).

(*1*) *GG Sites.* Several GG sites are present in the *Sal*I–*Bgl*I restriction fragment. The reactivity of the complementary C residues with CAA (or HA) is not uniform. Some residues are not reactive, such as C residues 722–723, 760–761, 793–794, 801–802, and 829–830 or even the four C residues in a row (704–707). On the other hand, the four C residues 769–772 in the sequence CCGG/GGCC and C residue 782 in the sequence CTGG/GACC are reactive.

We define three kinds of sites: an isolated site, a site in a row, and a cross-site. In an isolated GG site, the sequences

Table I: Isolated GG and AG Adducts in *Sal*I–*Bgl*I Restriction Fragment

GG sites	
722–723	5′ T C G C C G C A A G C G G C G T 5′
793–794	5′ T T C G G C G A A A G C C G C T 5′
AG sites	
713–714	5′ T G A C T A T C A C T G A T A G 5′
734–735	5′ T G A C T G T C A C T G A C A G 5′
727–728	5′ G C A C T T A T C G T G A A T A 5′
838–839	5′ T C G C T T G C A G C G A A C G 5′
754–755′	5′ C A A C T C G T G T T G A G C A 5′

GG and AG are not found within the three 3′ and 5′ bases adjacent to GG, on the same strand as well as on the opposite strand. Examples are GG sites 722–723 and 793–794. In both the cases, the complementary C residues are not reactive with CAA (or HA) (Figure 5 and Table I). In a GG site in a row, GG is within a $(G/C)_n$ sequence, n being equal to or larger than 4. An example is the $(G/C)_4$ sequence 704–707, in which none of the complementary C residues react with CAA (or HA). In a GG cross-site, the two 3′ or 5′ bases adjacent to GG on the opposite strand are two G residues. Examples are GG site 769–772 within the sequence CCGGCA/GGCCGT and GG site 829–832 within the sequence TCGGCC/AGCCGG. In the former site, the four C residues (769, 770, 771, and 772) are reactive with CAA (or HA), while in the latter site none of the C residues were modified.

Similar results were obtained with the five other platinated restriction fragments: *Eco*RI–*Bgl*I and *Nar*I–*Eco*RI fragments from pUC18; *Bam*HI–*Sal*I, *Hin*dIII–*Bam*HI, and *Eco*RI–*Dra*I fragments from pBR322 (results not shown). The results can be summarized as follows. There are 17 isolated GG sites, and none of the complementary C residues are modified by CAA (or HA). There are one $(G/C)_5$ and two $(G/C)_4$ sequences. In two sequences, a very faint reaction occurs between CAA and the two C residues at the 3′ side. There are seven cross-sites. CAA does not react with the complementary C residues within the three sequences GGCC/CCGG. It reacts with the C residues within the four sequences CCGG/GGCC, but the four C residues are not equally reactive.

(*2*) *AG Sites*. The reactivity of the T residues complementary to the A residues in the AG isolated sites depends upon the nature of the 5′ residue adjacent to the AG site. As shown in Figure 5 and Table I, the complementary T residues to AG sites 713–714 and 734–735 are sensitive to the probe, while the complementary T residues to AG sites 727–728 and 838–839 are weakly or not sensitive to the probe. More generally, within the six restriction fragments, we find that the complementary T residues are poorly reactive or not reactive at all when the 5′ residue adjacent to the AG site is a purine residue, while they are reactive when the 5′ residue adjacent to the site is a pyrimidine residue. In addition, if the 5′ adjacent pyrimidine is a thymine, it reacts poorly (or not) with OsO_4, and if it is a cytosine, it does not react with CAA. This rule is not followed for AG sites in the sequences pyGAGpy, the AG site 754–755 in Figure 5 being an example. This can be explained by the fact that these sites are much less reactive with *cis*-DDP than the other AG sites (Rahmouni et al., 1988).

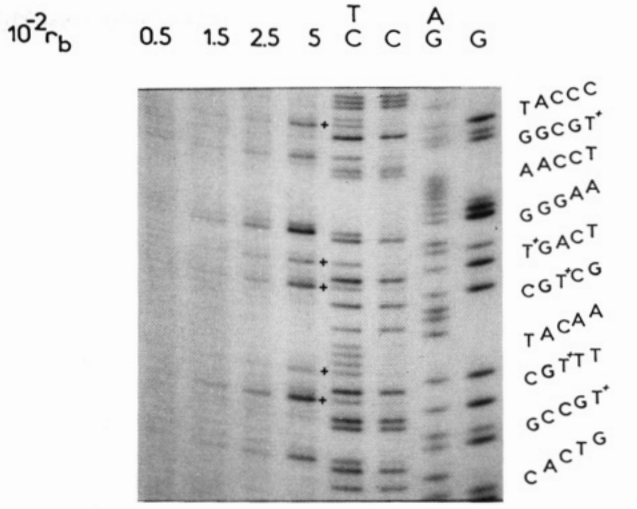

FIGURE 6: Piperidine-induced specific strand cleavage at OsO_4-modified bases in pUC18. The *Eco*RI–*Bgl*I restriction fragment, 5′ end labeled at *Eco*RI site, was platinated at various r_b. The reactive T residues in the sequences CGT/GCA are indicated by +. G, AG, C, and TC are Maxam–Gilbert specific fragments for the unplatinated sample.

Among the 20 AG isolated sites within the six restriction fragments, none of the C residues complementary to the G react with HA or CAA.

Only one $(AG)_2$ sequence is present within the six restriction fragments. The two complementary T residues were modified by OsO_4, and the two complementary C residues were not modified by HA or CAA.

There are several cross-sites implying AG and GG sites. An example is AG site 780–781 within the sequence CTGG/GACC. Both the complementary C and T residues react with the corresponding chemical probes. However, no simple rule can be deduced as in the case of GG cross-sites.

(*3*) *Reactivity of the T Residues in the Sequences CGT/GCA*. We observed that the T residues in the sequences CGT/GCA are reactive with OsO_4 (see the T residues 719 and 820 in Figure 5). In the six restriction fragments, 13 sequences (CGT/GCA) are present, and the 13 T residues are reactive. This reactivity can be detected at low r_b as shown by the study of the platinated *Eco*RI–*Bgl*I restriction fragment that contains five sequences (CGT/GCA). Modification of the T residues is not detected at $r_b = 0.005$ (Figure 6). On the other hand, the cleavage patterns at $r_b = 0.015$ and 0.05 are similar, but the intensities of the bands are different.

The reactivity of these T residues could reveal the presence of intrastrand, interstrand, or monofunctional adducts. Intrastrand adducts seem unlikely because in several cases G residues within CGT/GCA sequences are separated from the nearest G residues by at least two base pairs. The presence of monofunctional adducts seems also unlikely, because the analysis of the fragments obtained after reaction of OsO_4 with the restriction fragment platinated with the monofunctional compound dien-Pt did not show any modification of the T residues in the sequences CGT/GCA (results not shown).

To determine the presence of interstrand cross-links, the following experiments were done. The *Eco*RI–*Bgl*I restriction fragment was platinated at $r_b = 0.025$ and then reacted with OsO_4. At this r_b, the amount of interstrand cross-link is less than one per restriction fragment. The fragments with at least one interstrand cross-link and those with no interstrand cross-link were separated by gel electrophoresis in denaturing conditions (Rahmouni & Leng, 1987). It has been verified

that DNA strands were not cleaved. The fragments were eluted from the gel at neutral pH and then incubated in the presence of piperidine. The same cleavage patterns were obtained for the fragments with and without interstrand adducts (results not shown). The reactivity of the T residues in the sequences CGT/GCA cannot be explained by interstrand cross-links between the two neighboring G residues.

Discussion

In this paper, we show that some structural knowledge on the distortions of the double helix induced by the binding of *cis*-DDP to oligonucleotides and native DNA can be obtained by means of chemical probes.

Let us first consider the platinated double-stranded oligonucleotide (a 20-mer with cohesive ends) in which *cis*-platinum chelates the two guanine residues in the sequence GTG. This is a minor adduct in platinated DNA, but it appeared useful to test the chemical probes. A circular dichroism and NMR study of a double-stranded undecamer platinated at a GTG site has shown that the T residue between the two cross-linked G residues is bulged out and thus is no longer paired with the complementary A residue and that the 3′ G residue of the adduct is no longer paired with the complementary C residue (den Hartog et al., 1985a). In agreement with this study, we find that the GTG adduct destabilizes the double helix (the T_m is decreased by about 9 °C compared to the unplatinated oligonucleotide). The GTG adduct alters the shape of the molecule as shown by circular dichroism and by an abnormal electrophoretic mobility of the multimers of the ligated platinated oligomers. The abnormal mobility is not due to a charge effect since such a behavior is not observed with the multimers of the same oligomer modified by the monofunctional platinum derivative dien-Pt.

The strong reactivity of OsO_4 with the T residue in the GTG adduct suggests that the T residue is largely exposed to the solvent. It is no longer paired with the complementary A residue since the A residue reacts with CAA. The nonreactivity of this A residue with DEPC suggests that the N7 is not exposed to the solvent. The double helix is more distorted at the 5′ side of the adduct than at the 3′ side. The C residue complementary to the 5′ G residue of the adduct is slightly reactive with CAA, while the C residue complementary to the 3′ G residue is not reactive. The distortion spreads over several bases at the 5′ side of the adduct, as shown by the slight reactivity of the T residues with OsO_4. The double helix is distorted but not denatured since the complementary A residues do not react with CAA.

Let us now consider the double-stranded oligonucleotide (a 20-mer with cohesive ends) in which *cis*-platinum chelates an A residue adjacent to a G residue. The AG adduct destabilizes the double helix (the T_m is decreased by about 5 °C as compared with the unplatinated oligonucleotide). It distorts the double helix as shown by circular dichroism. It is worth noting that the spectral changes induced by the AG adduct look like those induced by the GG adduct in double-stranded oligonucleotides (den Hartog et al., 1985; van Hemelryck et al., 1986).

The multimers of the platinated and ligated oligonucleotides show retarded electrophoretic mobility through polyacrylamide gels. This anomalous mobility reflecting a decrease in multimer end-to-end distance (Lerman & Frisch, 1982; Lumpkin & Zimm, 1982) can arise from an increased flexibility or from systematic bending in which the direction of the helix axis is altered in a definite way (Diekmann, 1987, and references cited therein). The latter assumption seems more likely since the K factor is larger for the multimers of the platinated 20-mer than for the multimers of the platinated 15-mer. An increased flexibility is not expected to display a phase dependence. On the other hand, the K factor for the platinated $(15\text{-mer})_n$ is larger than one. If the adducts were separated by a half-integral number of turns, the K factor should be equal to one (Koo et al., 1986). We assume that the larger value of the K factor is mainly due to the fact that the helical repeat of the platinated samples is no longer 10–11 bp/turn. For the same reason, one cannot conclude from the values of the K factor that the AG adducts bend the DNA fragments more than GTG adducts.

In the platinated double-stranded oligonucleotide (AG adduct), the T residue complementary to the cross-linked A residue reacts with OsO_4. This confirms a local distortion of the double helix, but it is not known whether the T residue is still paired. The distortion spreads over a few bases at the 5′ side of the adduct but not at the 3′ side, as shown by the reactivity of the T residues with OsO_4. The reactive T residues at the 5′ side are probably paired since the complementary A residues do not react with CAA. The C residue complementary to the cross-linked G residue is not modified by CAA, and thus the two base residues are paired.

Six platinated DNA restriction fragments have been studied. A striking result illustrated in Figure 5 is that even at a relatively high r_b, a few C and A residues are reactive with CAA, which suggests that only a few bases are unpaired. On the other hand, the double helix is largely distorted since several T residues are reactive with OsO_4.

Seventeen isolated GG sites are present in the six restriction fragments. None of the complementary C residues are modified by CAA. In agreement with the NMR studies on platinated oligonucleotides (den Hartog et al., 1984, 1986; van Hemelryck et al., 1984, 1986), these results suggest that the C residues are paired with the cross-linked G residues. Since even in $(G/C)_5$ and $(G/C)_4$ sequences CAA does not react strongly with the complementary C residues, we conclude that at room temperature isolated GG adducts do not denature the double helix locally.

A sequence effect is revealed when two adjacent GG sites are located on the opposite strands (cross-site). In the sequences GGCC/CCGG, the complementary C residues do not react with CAA, while in the sequences CCGG/GGCC, they do react. This can be explained by assuming that the distortion of the double helix is more affected at the 5′ side of the adduct than at the 3′ side. This assumption, supported by studies of the GG adducts (Kozelka et al., 1987; van Hemelryck et al., 1986), seems valid for AG and GTG adducts, as shown by the reactivity of the chemical probes with the platinated oligonucleotides and by the spectrum of *cis*-platinum-induced mutations in *Escherichia coli* (Burnouf et al., 1987).

In the six restriction fragments, 20 isolated AG sites are present. None of the complementary C residues react with CAA. The reactivity of the complementary T residues depends upon the nature of the 5′ base adjacent to the site. They react with OsO_4 when the 5′ base is a pyrimidine. They do not react when the 5′ base is a purine. We conclude that the AG adducts in the sequences puAG/pyTC do not denature the double helix locally. As for the adducts in the sequences pyAG/puTC, if the double helix is denatured, only one base pair is disrupted.

A few cross-sites (the adjacent sites on the opposite strand being GG or AG sequences) are present in the six restriction fragments. A local denaturation of the double helix occurs in the sequences CCAG/GGTC as shown by the reactivity of C and T residues, respectively, with CAA and OsO_4.

However, no general rule can be deduced because of the small number of cross-sites.

Distortions and even local denaturations of the restriction fragments have been observed when two or more sites on the same strand and/or on the opposite strand are separated by one or more base pairs. Again, more data are necessary to form general rules except for the sequences CGT/GCA. Even at an r_b lower than 0.05, the 13 T residues within the 13 sequences CGT/GCA in the six restriction fragments are reactive with OsO_4. The other residues within these sequences are not reactive with CAA or DEPC, and thus the OsO_4 sensitivity indicates a distortion of the double helix. This distortion does not seem due to an interstrand adduct, to an intrastrand adduct, or to a monodentate adduct within the sequence CGT/GCA. Thus, we assume that the geometry of these sequences is influenced by the platination of other sequences located further away. Work is in progress to determine how far these structural effects can be transmitted along platinated DNA.

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