Sequence-Dependent Distortions Induced in DNA by Monofunctional Platinum(II) Binding†

Viktor Brabec,‡ Jan Reedijk,§ and Marc Leng*

Centre de Biophysique Moléculaire, CNRS, 1A, Avenue de la Recherche Scientifique, 45071 Orléans Cedex 2, France

Received June 5, 1992; Revised Manuscript Received September 24, 1992

ABSTRACT: The effects on thermal stability and conformation of DNA produced by the monofunctional adducts of chlorodiethylenetriamineplatinum(II) chloride {[Pt(dien)Cl]Cl} have been investigated. Oligodeoxyribonucleotide duplexes of varying lengths (9–20 base pairs) and of varying central trinucleotide sequences were prepared and characterized that contained site-specific and unique N(7)-guanine adducts. Included are adducts at the sequences of d(AGC), d(AGT), d(CGA), d(TGA), d(TGC), and d(TGT). All these monofunctional adducts decrease the melting temperature (T_m) of the duplexes. This destabilization effect exhibits a sequence-dependent variability. The highest lowering of T_m is observed for the modified duplexes containing the central sequence of pyrimidine–guanine–pyrimidine. The destabilization effect is reduced with decreasing concentrations of Na^+. Polarography, circular dichroism, phenanthroline–copper, and chemical probes reveal conformational distortions spreading over several base pairs around the adduct. The effects of monofunctional platinum(II) adducts on conformational distortions in DNA exhibit a sequence-dependent variability similar to those on thermal stability of DNA. The influence of the monofunctional adduct formed by *cis*-diamminemonoaquamonochloroplatinum(II) on the stability of the oligonucleotide duplex has been also studied. This lesion decreases thermal stability of DNA in the same way as does the adduct of [Pt(dien)Cl]Cl.

A probable mode of antitumor activity of *cis*-diamminedichloroplatinum(II) {*cis*-$[Pt(NH_3)_2Cl_2]$}[1] involves formation of platinum–DNA adducts that are capable of disrupting replication and transcription [for general reviews, see Eastman (1987), Johnson et al. (1989), Lepre and Lippard (1990), and Reedijk (1987)]. The processing of these adducts by cellular proteins has been proposed to be an important aspect of the molecular mechanism of antitumor activity of this drug (Bellon et al., 1991; Pil & Lippard, 1992). The relative amounts of the different adducts formed upon binding of *cis*-$[Pt(NH_3)_2Cl_2]$ to natural double-stranded DNA are 90% of intrastrand cross-links between adjacent purines and approximately 10% of monofunctional adducts, interstrand cross-links, and intrastrand cross-links between two nonadjacent guanines (Eastman, 1986; Fichtinger-Schepman et al., 1985).

cis-$[Pt(NH_3)_2Cl_2]$ binds to DNA in a two-step process, forming first monofunctional adducts that subsequently close to bifunctional lesions (Bancroft et al., 1990; Eastman, 1986; Fichtinger-Schepman et al., 1985; Johnson et al., 1985). The bifunctional DNA adducts of *cis*-$[Pt(NH_3)_2Cl_2]$ have been studied extensively, but only little attention has been so far paid to monofunctional adducts.

Chlorodiethylenetriamineplatinum(II) chloride {[Pt(dien)Cl]Cl} is the monodentate complex, used to afford DNA monofunctional adducts to simulate the first step of the bifunctional reaction of *cis*-$[Pt(NH_3)_2Cl_2]$ with DNA or minor monofunctional adducts of this drug. [Pt(dien)Cl]Cl binds preferentially at the the N(7) position of guanine residues (Johnson et al., 1982). This binding introduces a substituent which carries a +2 charge. It has been almost axiomatic that the monofunctional DNA adducts including those of [Pt(dien)Cl]Cl do not affect DNA conformation. This view has been related to antitumor inefficiency of [Pt(dien)Cl]Cl and persists in spite of some results indicating that the monofunctional platinum(II) adducts change the conformation of DNA. For instance, [Pt(dien)Cl]Cl binding to double-helical DNA increases the polarographic current of this biomacromolecule (Brabec et al., 1990; Vrana et al., 1986). Circular dichroism (CD) spectra of double-helical mammalian DNA are changed upon monofunctional binding of [Pt(dien)Cl]Cl and *cis*-$[Pt(NH_3)_2Cl_2]$ (Brabec et al., 1990; Kleinwachter et al., 1988). [Pt(dien)Cl]Cl promotes B → Z transition in poly(dG-dC)·poly(dG-dC) (Malfoy et al., 1981; Ushay et al., 1982). The melting temperature of the 9 base pairs (bp) duplex is markedly decreased due to a unique [Pt(dien)Cl]Cl binding (van Garderen et al., 1989). ^1H NMR and ^{31}P NMR spectra of this platinated duplex have revealed changes in the helix geometry limited to the platinated base pair (van Garderen et al., 1990). [Pt(dien)Cl]Cl unwinds superhelical DNA by 6–8° (Keck & Lippard, 1992).

These effects on DNA may have biological relevance. Monofunctional [Pt(dien)Cl]Cl adducts of double-helical DNA are recognized and repaired in assays with (A)BC excinuclease even with 2–3 times greater efficiency than the bifunctional and major adducts of *cis*-$[Pt(NH_3)_2Cl_2]$ (Page et al., 1990). Since DNA containing vastly different types of damage is recognized by the (A)BC excinuclease, it has been

† This work was supported in part by la Ligue Nationale Contre le Cancer, la Fondation de la Recherche Médicale, le Ministère de la Recherche et de la Technologie (contrat 91 T 0451), and the Internal Grant of Czechoslovak Academy of Sciences.

‡ On sabbatical leave from the Institute of Biophysics, Czechoslovak Academy of Sciences, Královopolská 135, 61265 Brno, Czechoslovakia (permanent address).

§ Present address: Department of Chemistry, Gorlaeus Laboratories, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands.

[1] Abbreviations: bp, base pair; CD, circular dichroism; *cis*-$[Pt(NH_3)_2Cl_2]$, *cis*-diamminedichloroplatinum(II); *cis*-$[Pt(NH_3)_2(H_2O)Cl]^+$, *cis*-diamminemonoaquamonochloroplatinum(II); DEPC, diethyl pyrocarbonate; EDTA, ethylenediaminetetraacetate dianion; FPLC, fast protein liquid chromatography; OP–Cu, 1,10-phenanthroline–copper complex; [Pt(dien)Cl]Cl, chlorodiethylenetriamineplatinum(II) chloride; TE buffer, 10 mM Tris-HCl, pH 7.4, with 1 mM EDTA; T_m, melting temperature. AGC/GCT(20), AGC/GCT(9), AGT/ACT(19), etc. are names used to identify oligonucleotide duplexes, as defined in Figure 1.

0006-2960/92/0431-12397$03.00/0

suggested (Page et al., 1990) that some distortion of the DNA, and not the [Pt(dien)Cl]Cl–DNA adduct itself, is recognized by this enzyme.

In the present work, we have extended methodologies based on melting temperature measurements, polarography, circular dichroism, the chemical nuclease, and chemical probes of nucleic acid conformations to evaluate stability and distortions of several oligomers sequence specifically modified by [Pt(dien)Cl]Cl and the monoaqua complex *cis*-diamminemonoaquamonochloroplatinum(II) {*cis*-$[Pt(NH_3)_2(H_2O)Cl]^+$}. In particular, the monofunctional platinum–DNA adducts have been uniquely incorporated into double-stranded 9–20-bp oligodeoxyribonucleotides (Figure 1) which differ in the central sequence involving the platination site.

MATERIAL AND METHODS

Chemicals. [Pt(dien)Cl]Cl and *cis*-$[Pt(NH_3)_2Cl_2]$ were synthesized in Lachema (Brno, Czechoslovakia). The *cis*-$[Pt(NH_3)_2(H_2O)Cl]^+$ cation was generated by allowing *cis*-$[Pt(NH_3)_2Cl_2]$ (1 mM) to react with 0.9 mol equiv of $AgNO_3$ in 10 mM $NaClO_4$ for 24 h at 37 °C in the dark. The AgCl precipitate was removed by centrifugation. All enzymes used in this work were from Boehringer-Mannheim and Bethesda Research Laboratories. Ultrapure agarose was from Bethesda Research Laboratories, and acrylamide, bis(acrylamide), urea, and NaCN were from Merck.

Oligodeoxyribonucleotides. The oligodeoxyribonucleotides synthesized on an Applied Biosystem solid-phase synthesizer were purified by ion-exchange FPLC with a linear gradient of 0.1–0.8 M NaCl with 10 mM NaOH. In this paper, the concentrations of oligonucleotides are related to the mononucleotide content.

Platination Reactions. The single-stranded oligonucleotides containing a unique G residue (the top strands of the duplexes listed in Figure 1) at the concentration of 6.8×10^{-4} M were reacted with [Pt(dien)Cl]Cl (the input molar ratio was 1 Pt per oligonucleotide) in 10 mM $NaClO_4$ at 37 °C for 24 h and again purified by ion-exchange FPLC with a linear gradient of 0.2–0.7 M NaCl with 10 mM Tris-HCl, pH 7.4. The platinum content of the product was determined by using a pulse polarographic assay (Brabec et al., 1983; Kim et al., 1990). Comparisons of the polarographic and UV spectroscopic results revealed the stoichiometry of the product to be one platinum atom per pyrimidine-rich oligonucleotide strand. The unique platination at the d(G) sites was verified by nonreactivity of dimethyl sulfate at these sites (Lemaire et al., 1991). Final yields of the platinations after purification averaged 70–90%.

The single-stranded 20-mer d(CTCTCCTCTGTCTCTCT-TCCTC) at the concentration of 6.8×10^{-4} M was also reacted with *cis*-$[Pt(NH_3)_2(H_2O)Cl]^+$ at an input platinum/strand ratio of 10 at 37 °C for 3 min. Then the concentration of NaCl was adjusted to 0.1 M and the platinated oligonucleotide was purified by ion-exchange FPLC. A product was collected (in ca. 0.4 M NaCl) which corresponded to a single principal peak in the elution profile. This peak appeared immediately before the elution peak of unplatinated oligonucleotide. The unique platination at the guanine site was verified as described above for the unique modification with [Pt(dien)Cl]Cl. The final yield of this platination reaction after purification was 20%.

The platinated top strands (or their unplatinated analogues) were allowed to anneal with unplatinated complementary strands in 50 mM NaCl, 10 mM Tris-HCl, pH 7.4 with 1 mM EDTA (TE buffer). The mixture of complementary oligonucleotides was heated at 60 °C for 5 min and subsequently slowly cooled (within 1 h) to 25 °C. In the case of the strand modified with *cis*-$[Pt(NH_3)_2(H_2O)Cl]^+$ or its unplatinated analogue, the hybridization was performed in 500 mM NaCl with TE buffer at 25 °C for 2 h.

```
AGC/GCT(20)   5' TCTCTCCTCTAGCTCTCCTTCT
                 AGAGGAGATCGAGAGGAAGAGA 5'

AGC/GCT(9)    5' CACCAGCCAC
                 TGGTCGGTGG 5'

AGT/ACT(19)   5' CTCTCCTCAGTCTCCTCTCT
                 AGAGGAGTCAGAGGAGAGAG 5'

AGT/ACT(14)   5' CTCATCAGTCACTCT
                 AGTAGTCAGTGAGAG 5'

CGA/TCG(19)   5' CTCCTCTCTCGATCTCCTCT
                 AGGAGAGAGCTAGAGGAGAG 5'

CGT/ACG(19)   5' CTTCCTCTCGTCTCCTTCTT
                 AAGGAGAGCAGAGGAAGAAG 5'

CGT/ACG(14)   5' CTCCTCCGTCTCTTC
                 AGGAGGCAGAGAAGG 5'

TGA/TCA(20)   5' CTCCTCTCTTGATCTCCTCT
                 GAGGAGAGAACTAGAGGAGA 5'

TGC/GCA(20)   5' CTTCTCCTTGCTCTCCTTCTCT
                 AGAGGAACGAGAGGAAGAGAGA 5'

TGC/GCA(10)   5' CCATGCTCAC
                 GGTACGAGTG 5'

TGT/ACA(19)   5' CTCTCCTCTGTCTCTTCCTC
                 AGAGGAGACAGAGAAGGAGG 5'
```

FIGURE 1: Synthetic oligodeoxyribonucleotides used in the present study and their abbreviations. The top and bottom strands of each pair are designated top and bottom, respectively, in the text.

Melting Curves. Melting curves of DNAs were recorded by measuring the absorbance at 260 nm by means of a Kontron Uvikon 810 spectrophotometer. The melting temperature (T_m) was determined as the temperature corresponding to a maximum on the first-derivative profile of the melting curves. The T_m values could be thus determined with an accuracy of ±0.3 °C.

Electrochemistry. Differential pulse polarographic curves were recorded on an EG&G PARC electrochemical analyzer, Model 384B, using the following apparatus settings: voltage scan rate of 2 mV/s, pulse amplitude of 50 mV, drop time of 1.0 s. The potentials are against the saturated calomel reference electrode.

Circular Dichroism. CD spectra were recorded on a Jasco spectropolarimeter, Model J720.

Ligations and Electrophoresis, Phenanthroline–Copper (OP–Cu) Digestion, and Chemical Modifications. All experiments were performed in 50 mM NaCl and at 20 °C. The single-stranded oligonucleotides were 5'-end-labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase. Ligations and electrophoresis were performed as described by Koo et al. (1986). OP–Cu digestions were performed according to the procedure described previously (Yoon et al., 1988) with minor modifications (Schwartz et al., 1989). Chemical modifications were also performed as previously described (Marrot & Leng, 1989). When the reactivity of OsO_4 with the platinated top strands was investigated, [Pt(dien)Cl]Cl was removed after reaction of the duplex with the probe by incubation with 0.2 M NaCN at 45 °C overnight.

RESULTS

A list of all sequences examined is given in Figure 1; the accompanying names refer to the unplatinated oligonucleotide duplexes and were chosen according to the central trinucleotide

Table I: Melting Temperatures of the Unplatinated Oligonucleotide Duplexes (T_m) and Their Changes Due to the Unique Modification of the Duplexes with [Pt(dien)Cl]Cl at the d(G) Sites of the Top Strands (ΔT_m)

	50 mM NaCl[b]		500 mM NaCl[b]	
duplex[a]	T_m (°C)	ΔT_m (°C)	T_m (°C)	ΔT_m[c] (°C)
AGT/ACT(19)	55.4	−1.1	67.9	−2.0 (−1.9)
AGT/ACT(14)	46.4	−1.2	58.7	−2.8 (−1.9)
AGC/GCT(20)	55.2	−1.0	68.3	−1.8 (−1.8)
AGC/GCT(9)			48.7	−4.2 (−1.9)
CGA/TCG(19)	55.6	−3.0	68.5	−4.1 (−3.9)
TGA/TCA(20)	55.8	−2.7	68.4	−4.2 (−4.2)
CGT/ACG(19)	55.4	−4.9	67.9	−5.7 (−5.4)
CGT/ACG(14)	48.0	−6.1	59.0	−8.0 (−6.0)
TGT/ACA(19)	55.7	−4.2	67.8	−6.3 (−6.0)
TGC/GCA(20)	55.6	−4.6	68.0	−5.1 (−5.1)
TGC/GCA(10)	41.3	−10.6	50.2	−13.2 (−6.6)
TGT/AGA(19)–cisPt[d]			67.8	−5.8 (−5.5)

[a] The concentration of DNA was 4×10^{-5} M. [b] Melting curves were measured in a medium containing NaCl in concentrations indicated in the table and TE buffer. [c] The numbers in parentheses are the ΔT_m values recalculated for an equal level of the platination corresponding to 1 platinum atom fixed per 20 bp of the duplex. [d] The duplex containing a unique monofunctional adduct of *cis*-$[Pt(NH_3)_2(H_2O)Cl]^+$ at the central d(G) site of the upper strand.

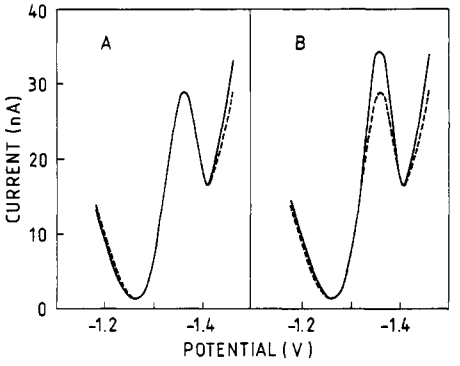

FIGURE 2: Differential pulse polarogram of AGC/GCT(20) (A) and TGC/GCA(20) (B) duplexes modified with [Pt(dien)Cl]Cl. Full lines are relative to the platinated duplexes. The duplexes were at the concentration of 5×10^{-5} M in 1.0 M NaCl with 0.2 M sodium acetate, pH 6.0, at 25 °C.

sequence in the top strands; all these oligonucleotides have only one guanine residue in the middle. The figure in parentheses behind the names indicates the number of base pairs formed after the annealing with the complementary bottom strand. Once complexed with [Pt(dien)Cl]Cl, the single strand or duplex has been asterisked at the G.

Melting of Oligonucleotide Duplexes. The character of melting profiles of oligonucleotide duplexes is not markedly changed as a result of their modification with [Pt(dien)Cl]Cl. The cooperative transitions occur over temperature intervals of 10 °C, and hyperchromicity at 260 nm is about 25% (not shown). Melting temperatures (T_m) of [Pt(dien)Cl]Cl-modified and unplatinated duplexes in 50 mM and 500 mM NaCl are given in Table I. The single modification with [Pt(dien)Cl]Cl lowers T_m values of all duplexes investigated in this study. In order to make a comparison of the platination effects on T_m of the duplexes more covenient, ΔT_m values have been also recalculated by relating them to the same level of platination (1 platinum atom/20 bp). This step has been carried out on the basis of only a rough assumption that the T_m values of oligonucleotide duplexes used throughout this work are approximately proportional to the oligonucleotide length expressed in base pairs (Table I). The recalculated T_m values are given in Table I in parentheses. Maximum destabilization effects occur within the central trinucleotide sequences pyrimidine–guanine–pyrimidine. If a pyrimidine residue in these sequences is replaced by the adenine residue, the destabilization effect is reduced. The replacement on the 5′ side of the central platinated guanine residue is more effective than that on the 3′ side. The reduction of the T_m values is more pronounced in 500 mM NaCl than in 50 mM NaCl (Table I).

The effect of ionic strength on the melting behavior of the duplexes has been investigated in detail in the case of TGC/GCA(10) and TG*C/GCA(10). The melting curves were recorded in TE buffer, pH 7.4, plus various concentrations of NaCl [NaCl] in the range of 0.02–0.5 M. The T_m values are changed according to the following equations:

$$T_m = 8.87 \log [NaCl] + 52.87$$

$$T_m(Pt) = 6.29 \log [NaCl] + 38.89$$

where T_m and $T_m(Pt)$ are melting temperatures of the unplatinated and [Pt(dien)Cl]Cl-modified duplexes, respectively. The straight line of the platinated duplex displays a smaller slope. This observation is consistent with the fact that [Pt(dien)Cl]Cl-modified duplexes have a part of their negative charge of phosphate groups neutralized by the platinum(II) moiety (carrying +2 charge).

The above-given equations yield the ΔT_m value [$T_m(Pt) - T_m$] of −14.0 °C in 1.0 M NaCl. If this value is recalculated for a 9-bp duplex, a value of −15.6 °C is obtained. This value agrees well with the ΔT_m obtained by van Garderen et al. (1989) for the 9-bp duplex having a central trinucleotide sequence CGT, which was modified at the d(G) site with [Pt(dien)Cl]Cl (−16 °C).

The results described above clearly indicate that the binding of [Pt(dien)Cl]Cl destabilizes short duplexes in a sequence-dependent manner. It could be, however, argued that this conclusion can be applied to the monofunctional binding of [Pt(dien)Cl]Cl and not to the monofunctional binding of *cis*-$[Pt(NH_3)_2Cl_2]$. In order to clarify this problem, we have investigated TGT/ACA(19) modified monofunctionally with *cis*-$[Pt(NH_3)_2(H_2O)Cl]^+$. This modification decreases the T_m value of this duplex in a similar manner as the modification with [Pt(dien)Cl]Cl (Table I). It is, therefore, reasonable to suggest that the effects of [Pt(dien)Cl]Cl and of monofunctional adducts of *cis*-$[Pt(NH_3)_2Cl_2$ on DNA are similar.

Electrochemistry. The first results indicating that a monofunctional binding of platinum complexes induces conformational distortions in DNA have been obtained with the aid of techniques of electroanalytical chemistry (Vrana et al., 1986). Figure 2 shows the results of electrochemical analysis of AG*C/GCT(20) and TG*C/GCA(20). The modification with [Pt(dien)Cl]Cl results in a significant increase of the electroreduction current of DNA only in the case of TG*C/GCA(20).

Circular Dichroism. CD spectroscopy is another physical technique suitable for detection of local conformational alterations induced in DNA by the binding of platinum compounds (Brabec et al., 1990; Macquet & Butour, 1978). CD spectra of the AGT/ACT(15), AG*T/ACT(15), CGT/ACG(15), and CG*T/ACG(15) duplexes are shown in Figure 3. Similarly to effects observed upon monofunctional platinations of high-molecular mass DNA (Brabec et al., 1990; Kleinwachter et al., 1988), amplitudes of the positive band at 280 nm are decreased as a consequence of [Pt(dien)Cl]Cl binding to both duplexes. The effect is again more pronounced in the duplex having the central trinucleotide sequence pyrimidine–guanine–pyrimidine.

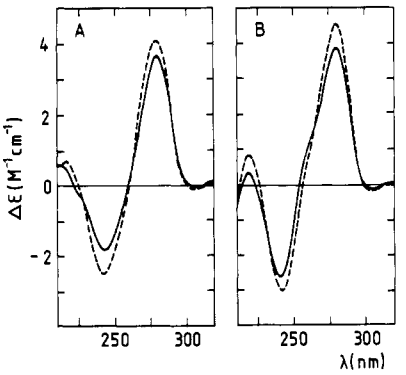

FIGURE 3: CD spectra of AGT/ACT(14) (A) and CGT/ACG(14) (B) duplexes modified with [Pt(dien)Cl]Cl. Full lines are relative to the platinated duplexes. Medium: 0.5 M NaCl with TE buffer at 25 °C.

Electrophoretic Mobility of Multimers of 20-bp Oligonucleotides. Intrinsic bending of DNA duplexes results in the abnormal electrophoretic mobility of DNA fragments. A gel migration anomaly has been found for DNA fragments containing bidentate adducts formed by *cis*-DDP at the d(GG), d(AG), and d(GTG) sites (Bellon & Lippard, 1990; Bellon et al., 1991; Marrot & Leng, 1989; Rice et al., 1988; Schwartz et al., 1989). On the other hand, the monofunctional binding of *cis*-[Pt(NH_3)$_2$(N(3)-cytosine)]Cl at the d(G) site in the CGT sequence (Bellon & Lippard, 1990), or of [Pt(dien)Cl]Cl at the d(G) site in the TGTGT sequence (Marrot & Leng, 1989), keeps the helix rodlike. We have compared the electrophoretic mobility of the multimers of the ligated 20-mers AGT/ACT, TGA/TCA, and TGT/ACA with and without monofunctional adducts of [Pt(dien)Cl]Cl. The corresponding multimers exhibit virtually no gel mobility shifts, migrating at almost exactly the same positions as the unplatinated ladder of multimers (results not shown). We can, therefore, conclude that no bending is induced in DNA containing monofunctional platinum adducts in any sequence, including those exhibiting the strong effect on the stability of the duplex.

Chemical Nuclease. Electrochemical and CD analyses have confirmed the justification of the assumption that monofunctional platinum binding distorts DNA. These results, however, do not yield information about the localization of these distortions in the duplex. In order to characterize these distortions better, we took advantage of the fact that the reaction rates of several chemical reagents with individual sugar–phosphate or base residues are strongly dependent on the conformation of DNA.

The 1,10-phenanthroline–copper complex (OP–Cu) is a chemical nuclease that nicks nucleic acids by oxidation attack on the sugar moiety (Sigman, 1990; Sigman & Chen, 1990). The cleavage efficiency of Op–Cu is dependent on the secondary structure of DNA. The cleavage patterns of the unplatinated TGT/ACA(19) and AGC/GCT(19) duplexes do not differ significantly (Figure 4A,B). A comparison of the cleavage patterns of unplatinated duplexes and those modified with [Pt(dien)Cl]Cl reveals a markedly lower reactivity of the central trinucleotide sequence containing the d(G*) site. Thus, the reduced reactivity of OP–Cu at the nucleotides around the [Pt(dien)Cl]Cl adduct suggests conformational distortions at these sites.

The effect is more expressed in the case of TG*T/ACA(19) duplex (Figure 4). In this case, the highest decrease in the reactivity of OP–Cu is observed for 5′ thymine residues adjacent to the adduct. The reactivity of nucleotide residues

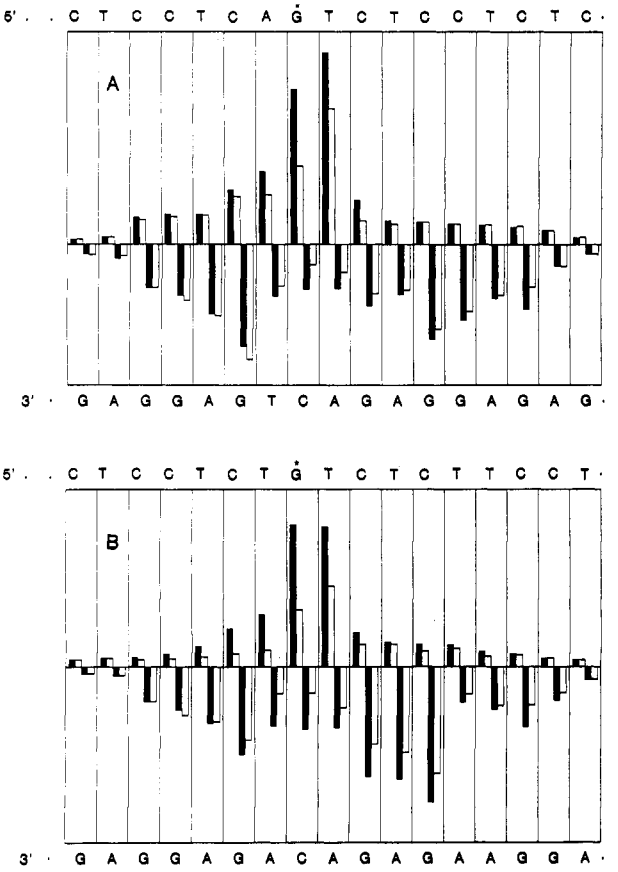

FIGURE 4: Quantitation of Op–Cu digestion of AGT/ACT(19), TGT/ACA(19), and their [Pt(dien)Cl]Cl-modified analogues. The autoradiograms were quantitated by microdensitometry. The black bars represent digestion of the unplatinated duplexes, and the light bars represent digestions of the platinated duplexes. The heights of the bars are proportional to the band intensities on the autoradiograms.

in bottom strands complementary to central trinucleotide sequences containing the adduct is also reduced, but less than the reactivity of nucleotide residues in the top strands (Figure 4A,B). The effects observed with the bottom strands are again more evident in the case of TG*T/ACA duplex and are highest at the cytosine residue complementary to the platinated guanine residue and at the next 3′ adenine residue (Figure 4).

It might be speculated that the reduced reactivity of OP–Cu (Figure 4) is also connected with the local change of electrostatic potential due to the +2 charge of the platinum moiety. However, this effect, if any, should not be very different in AG*T/ACT and TG*T/ACA, which is obviously not the situation observed in the present experiments (Figure 4).

We interpret the results obtained with OP–Cu nuclease to mean that (i) the geometry of the double helix is altered over several base pairs around the monofunctional platinum adducts and in particular on its 5′ side in the top strand and (ii) the conformational changes induced by this adduct are more extensive in the TG*T/ACA sequence as compared with AG*T/ACT sequence.

Chemical Probes of DNA Conformation. Osmium tetraoxide (OsO_4) is hyperreactive with thymine residues in single-stranded nucleic acids and in distorted DNA as compared to B-DNA (Palecek et al., 1990). The reactivities between OsO_4 and the top strands in AGT/ACT(19), TGA/TCA(20), and TGT/ACA(19) have been studied. Mainly thymine residues are reactive in unplatinated single-stranded oligonucleotides. OsO_4 does not react with any thymine residue within the unplatinated duplexes. If the duplexes

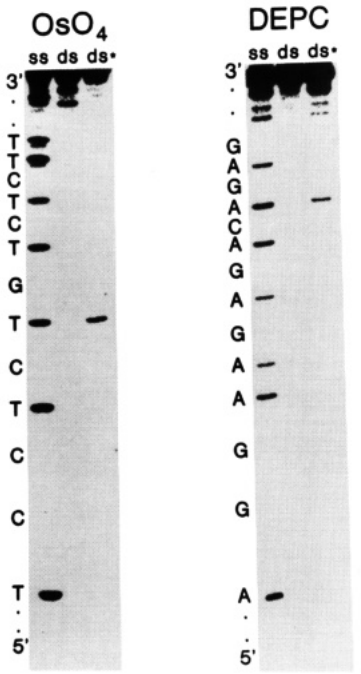

FIGURE 5: Piperidine-induced specific strand cleavage at OsO_4-modified (left) and diethyl pyrocarbonate-modified (right) bases in unplatinated and in [Pt(dien)Cl]Cl-modified TGT/ACA(19). The cleavage patterns of the upper strand in the duplex reacted with OsO_4 and of the lower strand in the duplex reacted with diethyl pyrocarbonate are shown. Lanes ss represent unplatinated single strands; lanes ds represent unplatinated duplex; lanes ds represent platinated duplex.

modified with [Pt(dien)Cl]Cl are investigated, only the first 5′ thymine residue adjacent to the adduct in TG*T/ACA(19) duplex is reactive (Figure 5). The reactivities between OsO_4 and the bottom strands in AG*T/ACT(19) and TG*A/TCA(20) duplexes have been also investigated under conditions when this probe reacts readily with thymine residues in the single-stranded unplatinated oligonucleotides. OsO_4 does not react with any of the thymine residues complementary to adenine residues adjacent to the adducts (not shown).

Diethyl pyrocarbonate (DEPC) is hyperreactive with unpaired adenine residues in DNA and with left-handed Z-DNA (Herr, 1981; Johnston & Rich, 1985). The reactivities between DEPC and the bottom strands in AGT/ACT(19), TGA/TCA(20), and TGT/ACA(19) have been studied. Adenine residues within the unplatinated single-stranded oligonucleotides readily react with DEPC. Within the unplatinated double-stranded oligonucleotides, adenine residues in the bottom strands are not modified. Within the platinated double-stranded oligonucleotides, only the adenine residue in the TG*T/ACA duplex complementary to the 5′ thymine residue in the top strand reactive with OsO_4 is modified (Figure 5).

DISCUSSION

This work describes the effect of monofunctional platinum(II) adducts on stability and distortions in double-helical oligonucleotides. On one hand, the monofunctional adducts stabilize the double helix via two positive charges of the platinum(II) moiety, but on the other hand they destabilize DNA via conformational distortions. The destabilization effects of the monofunctional platinum(II) adducts exhibit a sequence-dependent variability. The highest effects are observed if the adduct is flanked by single pyrimidines on both 3′ and 5′ sides. The replacement of one pyrimidine residue in such sequences by adenine results in lower destabilization effects; the replacement on 5′ side of the adducts is more efficient.

Experiments with chemical nuclease and probes indicate that the monofunctional adducts alter conformation of DNA over several base pairs around the adduct. The distortion is more pronounced in the strand containing the adduct. The most extensive distortions are observed if the adduct is flanked by two pyrimidines. Thus, the effects of monofunctional platinum adducts on stability and conformation of DNA exhibit similar sequence-dependent variabilities.

Conformational distortions induced by intrastrand bifunctional adducts at the d(GG) and d(AG) sites have been already investigated by the techniques (Schwartz et al., 1989) used in the present study. The reactivity of OP–Cu at the nucleotides around the platinum adduct is lowered more in the case of the monofunctional adducts (Figure 4) than in the case of the bifunctional adducts. OsO_4 reacts well with the 5′ thymine residue adjacent to the monofunctioal adduct at the d(TGT) site, and DEPC reacts with the adenine residue complementary to this thymine residue (Figure 5). On the other hand, none of the adjacent or other thymine residues and their complementary adenine residues react with these chemical probes in DNAs containing the bifunctional adduct of *cis*-$[Pt(NH_3)_2Cl_2]$ at the d(TGGT) site (Schwartz et al., 1989).

Double-stranded oligonucleotides containing a unique monofunctional platinum adduct were prepared by reacting single-stranded oligonucleotide with the platinum complex and by subsequent annealing the modified strand with its complementary counterpart. The results of this work exhibited no time dependence so that our platinated duplexes could be considered as being in thermodynamic equilibrium.

The results of this work along with those previously published of polarographic, CD, and NMR analyses of platinum(II)-modified DNA (Brabec et al., 1990; van Garderen, 1990) indicate that the monofunctional platinum(II) binding induces extensive conformational distortions in double-helical DNA, which are, however, qualitatively different from those induced by bifunctional platinum(II) intrastrand cross-links between adjacent purine residues.

The intrastrand cross-links of *cis*-$[Pt(NH_3)_2Cl_2]$ bend DNA by about 35° and unwind the duplex by 13° (Bellon et al., 1991). On the other hand, the monofunctional platinum(II) adducts unwind the duplex only by 6–8° (Keck & Lippard, 1992) and do not bend DNA (this work). The positive CD band of DNA at around 280 nm is increased as a result of *cis*-$[Pt(NH_3)_2Cl_2]$ binding but decreased due to the monofunctional platinum binding (Brabec et al., 1990; Kleinwachter et al., 1988). We have observed (Figure 3) that the lowering of the CD band is more pronounced if the duplex contains the platinated sequence for which more extensive distortions are found with the aid of polarography, OP–Cu nuclease, and chemical probes. These results establish a connection between the ability of the monofunctional adduct to distort DNA and disturbances of stacking interactions in double-helical DNA.

The results of this work support the view that the effects of the monofunctional binding of *cis*-$[Pt(NH_3)_2Cl_2]$ and [Pt(dien)Cl]Cl on DNA are similar. The present study provides additional information on the structure of monofunctional DNA adducts of platinum(II) complexes. This information may therefore help to better understand how antitumor *cis*-$[Pt(NH_3)_2Cl_2]$ forms the bifunctional lesions. In addition, a novel class of trisubstituted platinum(II) antitumor agents has been described in which platinum has

only one labile ligand (Hollis et al., 1991), thus violating the traditional rules used to predict the antitumor activity of platinum complexes. Therefore, a knowledge of alterations induced in DNA by monofunctional binding of platinum complexes may also be important for the development of new platinum antitumor drugs that may display an activity profile different from that of *cis*-$[Pt(NH_3)_2Cl_2]$.

REFERENCES

Bancroft, P. D., Lepre, C. A., & Lippard, S. J. (1990) *J. Am. Chem. Soc. 112*, 6860–6871.

Bellon, S. F., & Lippard, S. J. (1990) *Biophys. Chem. 35*, 179–188.

Bellon, S. F., Coleman, J. H., & Lippard, S. J. (1991) *Biochemistry 30*, 8026–8035.

Brabec, V., Vrána, O., & Kleinwachter, V. (1983) *Collect. Czech. Chem. Commun. 48*, 2903–2908.

Brabec, V., Kleinwachter, V., Butour, J.-L., & Johnson, N. P. (1990) *Biophys. Chem. 35*, 129–141.

Eastman, A. (1986) *Biochemistry 25*, 3912–3915.

Eastman, A. (1987) *Pharmacol. Ther. 34*, 155–166.

Fichtinger-Schepman, A. M. J., van der Veer, J. L., den Hartog, J. H. J., Lohman, P. H. M., & Reedijk, J. (1986) *Biochemistry, 28*, 7975–7989.

Herr, W. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 8009–8013.

Hollis, L. S., Sundquist, W. I., Burstyn, J. N., Heiger-Bernays, W. J., Bellon, S. F., Ahmed, K. J., Amundsen, A. R., Stern, E. W., & Lippard, S. J. (1991) *Cancer Res. 51*, 1866–1875.

Johnson, N. P., Macquet, J. P., Wiebers, J. L., & Monsarrat, B. (1982) *Nucleic Acids Res. 10*, 5255–5271.

Johnson, N. P., Mazard, J., Escalier, J., & Macquet, J. P. (1985) *J. Am. Chem. Soc. 107*, 6376–6380.

Johnson, N. P., Butour, J. L., Villani, G., Wimmer, F. L., Defais, M., Pierson, V., & Brabec, V. (1989) in *Progress in Clinical Biochemistry and Medecine*, Vol. 10, pp 1–24, Springer, Berlin.

Johnston, B. H., & Rich, A. (1985) *Cell (Cambridge, Mass.) 42*, 713–724.

Keck, M. V., & Lippard, S. J. (1992) *J. Am. Chem. Soc. 114*, 3386–3390.

Kim. S. D., Vrâna, O., Kleinwachter, V., Niki, K., & Brabec, V. (1990) *Anal. Lett. 23*, 1505–1518.

Kleinwachter, V., Vrâna, O., Brabec, V., & Johnson, N. P. (1988) *Studia Biophys. 123*, 85–89.

Koo, H., Wu, H. M., & Crothers, D. M. (1986) *Nature (London) 320*, 501–506.

Lemaire, M. A., Schwartz, A., Rahmouni, R. A., & Leng, M. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 1982–1985.

Lepre, C. A., & Lippard, S. J. (1990) in *Nucleic Acids and Molecular Biology* (Eckstein, F., & Lilley, D. M. J., Eds.) Vol. 4, pp 3–9, Springer, Berlin.

Macquet, J. P., & Butour, J. L. (1978) *Biochimie 60*, 901–914.

Malfoy, B., Hartmann, B., & Leng, M. (1981) *Nucleic Acids Res. 9*, 5659–5669.

Marrot, L., & Leng, M. (1989) *Biochemistry 28*, 1454–1461.

Page, J. D., Husain, I., Sancar, A., & Chaney, S. G. (1990) *Biochemistry 29*, 1016–1024.

Palecek, E., Boublikova, P., Jelen, F., Krejcova, P., Makaturova, E., Nejedly, K., Pecinka, P., & Vojtiskova, M. (1990) in *Structure and Methods* (Sarma, R. H., & Sarma, M. H., Eds.) Vol. 3, pp 237–253, Adenine Press, Schenectady.

Pil, P. M., & Lippard, S. J. (1992) *Science 256*, 234–237.

Reedijk, J. (1987) *Pure Appl. Chem. 59*, 181–192.

Rice, J. A., Crothers, D. M., Pinto, A. L., & Lippard, S. J. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 4158–4161.

Schwartz, A., Marrot, L., & Leng, M. (1989) *Biochemistry 28*, 7975–7979.

Sigman, D. S. (1990) *Biochemistry 29*, 9097–9105.

Sigman, D. S., & Chen, C-h. B. (1990) *Annu. Rev. Biochem. 59*, 207–236.

Ushay, H. M., Santella, R. M., Grunberger, D., & Lippard, S. J. (1982) *Nucleic Acids Res. 10*, 3573–3588.

van Garderen, C. J., van den Elst, H., van Boom, J. H., & Reedijk, J. (1989) *J. Am. Chem. Soc. 111*, 4123–4125.

van Garderen, C. J., Altona, C., & Reedijk, J. (1990) *Inorg. Chem. 29*, 1481–1487.

Vrâna, O., Brabec, V., & Kleinwachter, V. (1986) *Anti-Cancer Drug Des. 1*, 95–109.

Yoon, C., Kuwabara, M. D., Law, R., & Sigman, D. S. (1988) *J. Biol. Chem. 263*, 8458–8463.