Distortions induced in double-stranded oligonucleotides by the binding of *cis*- or *trans*-diammine-dichloroplatinum(II) to the d(GTG) sequence

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ABSTRACT

Conformational changes induced in double-stranded oligonucleotides by the binding of *trans*- or *cis*-diamminedichloro platinum(II) to the d(GTG) sequence have been characterized by means of melting temperatures, electrophoretic migrations in non-denaturing polyacrylamide gels, reactivities with the artificial nuclease Phenanthroline-copper and with chemical probes. The *cis*-platinum adduct behaves more as a centre of directed bend than as a hinge joint, the induced bend angle being of the order of 25 – 30°. The double helix is locally denatured over 2 base pairs (corresponding to the platinated 5'G residue and the central T residue) and is distorted over 4 – 5 base pairs. The *trans*-platinum adduct behaves also more as a centre of directed bend than as a hinge joint, the induced bend angle being of the order of 60°. The double helix is locally denatured over 4 base pairs (corresponding to the immediately 5'T residue adjacent to the adduct and to the three base residues of the adduct).Both the *cis*- and *trans*-platinum adducts decrease the thermal stability of the double helix.

INTRODUCTION

DNA is generally accepted as the critical target for the toxic action of the antitumor drug *cis*-diamminedichloro platinum (II)(*cis*-DDP)(1). Most of the lesions produced in the *in vivo* and *in vitro* reaction with DNA have been characterized. The two major adducts result from intrastrand cross-links between two adjacent guanine residues and between adenine residues adjacent to guanine residues. The minor adducts result from intrastrand cross-links between two guanine residues separated by at least one nucleotide residue, from interstrand cross-links between two guanine residues and from DNA-protein cross-links, respectively (general reviews 2 – 5).

The minor intrastrand adducts play an important role in the genetic alterations induced by *cis*-DDP. Using the *lacI* mutation assay, Brouwer et al (6) concluded that the d(GAG) and d(GCG) sequences are hot spots for *cis*-DDP-induced base-substitution mutations. A study of the mutational specificity of *cis*-DDP in the endogenous gene for adenine phosphoribosyltransferase of CHO cells has revealed that many mutations occur at or proximal to the d(GAG) sequences (7). On the other hand, using a forward mutation assay based on the inactivation of the tetracycline-resistant gene located on plasmid pBR322, Burnouf et al (8) found that more than 90% of the mutations are single-base-pair substitutions occuring at the d(AG) and d(GG) sequences.

Several physico-chemical studies of platinated double-stranded oligonucleotides in which *cis*-platinum chelates the two guanine residues in the d(GTG) sequence have been reported. For example, NMR studies (9) and chemical probes (10) have shown that the T residue between the two cross-linked guanine residues is bulged out and thus is no longer paired with the complementary adenine residue. The anomalous slow electrophoretic mobility of the multimers of the platinated and ligated oligomers suggest that the platinated oligonucleotides are bent (10). NMR and molecular mechanics modeling studies of platinated double-stranded oligonucleotides in which *cis*-platinum chelates the two guanine residues in the d(GCG) sequence suggest that the d(GTG) and d(GCG) adducts induce similar distortions (11 – 12). We here report some more results on the distortions induced in double-stranded oligonucleotides by the binding of *cis*-DDP to the d(GTG) sequences. To describe these distortions, we used several techniques giving either a global view of the double helix (gel electrophoresis) or a local view (chemical probes and artificial nuclease). In parallel, we have studied the distortions induced in the same double-stranded oligonucleotides by the binding of *trans*-diamminedichloroplatinum(II) (*trans*-DDP), the clinically ineffective isomer of cis-DDP.

MATERIALS AND METHODS

The oligonucleotides synthesized on a Applied Biosystems solid-phase synthesizer were purified by ion-exchange on a Pharmacia FPLC system. The sequences of the oligonucleotides were: d(CTCTCTTCTGTGTCTTCTCT), d(AGAGAGAAGACAC-AGAAGAG), d(CTTCCTCTTCTGTGTCTTCTCT), d(GAGA-GAAGACACAGAAGAGGAA), d(CTCTCTTCTGTGTCTT CTTCTTC), d(AGGAAGAAGAAGACACAGAAGAG), d(CTTCTCTTCTGTGTCTTCTCTCTC) and d(GGAGAGA-GAAGACACAGAAGAGAA). T4 polynucleotide kinase, T4 DNA ligase, nuclease P1 and alkaline phosphatase were purchased from Boehringer-Mannheim. Electrophoresis-grade acrylamide, bis- acrylamide, and sodium cyanide were from Merck. *Cis*-DDP and *trans*-DDP were from Johnson Matthey or were kindly provided by Dr J.L. Butour (Toulouse) and by Dr B. Lippert (Dortmund)

Reaction of platination

The reactions between *cis*-DDP or *trans*-DDP and single-stranded oligonucleotides were performed as previously described (10). The platinated oligonucleotides were purified by ion-exchange FPLC. The sites of platination (at the N7 position of guanine residues in the d(GTG) sequence) were verified by reverse-phase HPLC analysis of the digests after incubation of the platinated oligonucleotides with nuclease P1 (13) and then with alkaline phosphatase (14). They were also confirmed by the reaction between chemical probes and the platinated oligonucleotides (see results).

Chemical modifications

They were performed as previously described (10,15) on uniquely 5'end-labeled (22-mer) samples. All the experiments were performed at 20°C and the Na^+ concentration was 50 mM.

Phenanthroline-copper digestion

Digestions of the uniquely 5' end-labeled (22-mer) duplexes were performed according to the procedure described (16) with minor modifications (15).

Ligations and electrophoresis

They were performed as described by Koo et al (17). The apparent length of each platinated multimer is defined as being equal to the length of the unplatinated multimer having the same mobility (17).

Materials

A Camag microdensitometer was used to collect the data from sequence gels. Absorption spectra were recorded on a Kontron Uvikon 810 spectrophotometer.

RESULTS

The sequence of the duplex (20-mer) used in this work is

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5'  CTCCTCTTCTGTGTCTTCTC
      AGGAGAAGACACAGAAGAGG   5'
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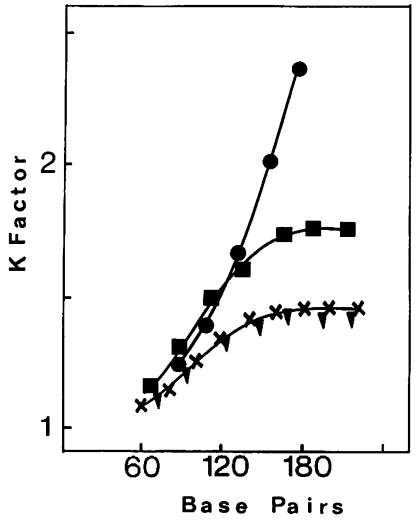

Fig.1 K-factor versus actual chain length for duplexes modified by *cis*-platinum. The multimers are composed of platinated 20-mer (▼), 22-mer (●), 23-mer (■) and 24-mer (X).

The other duplexes (22-mer, 23-mer and 24-mer) have the same general formula, the residues flanking the central sequence d(GTG) within the upper strand being exclusively pyrimidine residues and the corresponding residues within the lower strand being exclusively purine residues. After reaction with the single-stranded oligonucleotide (upper strand), *cis*-DDP or *trans*-DDP were bound to the G residues of the d(GTG) sequence. The double-stranded oligonucleotides were obtained by mixing the platinated or the unplatinated oligomers with the complementary strands, adjusted so that the resulting duplexes have cohesive ends.

I) Oligonucleotides modified by *cis*-DDP at the d(GTG) site

Electrophoretic mobility

A previous study (10) had shown that the multimers of the platinated and ligated (20-mer) oligomers presented an anomalous slow electrophoretic mobility in polyacrylamide gel which suggested that the platinated oligomers were bent. A similar study has been carried out with the multimers of the platinated duplexes (20-mer, 22-mer, 23-mer and 24-mer, respectively). Normal mobility was observed for the unplatinated duplexes (results not shown) which excluded any effect due to the particular sequences of the duplexes. The variation of the K-factor (apparent length to sequence length) versus sequence length shows the largest effect for the platinated (22-mer) duplexes (Fig. 1). This result suggests that a consequence of the platination is more a directed bend of the double helix axis than a locally increased isotropic flexibility (hinge joint) because an increased isotropic flexibility is not expected to display a phase dependence (17–19). In order to get an estimate of the *cis*-platinum-induced bent angle (20–21), the circles produced by ligation were detected and quantitated by two-dimensional gel electrophoresis as described by Ulanovsky

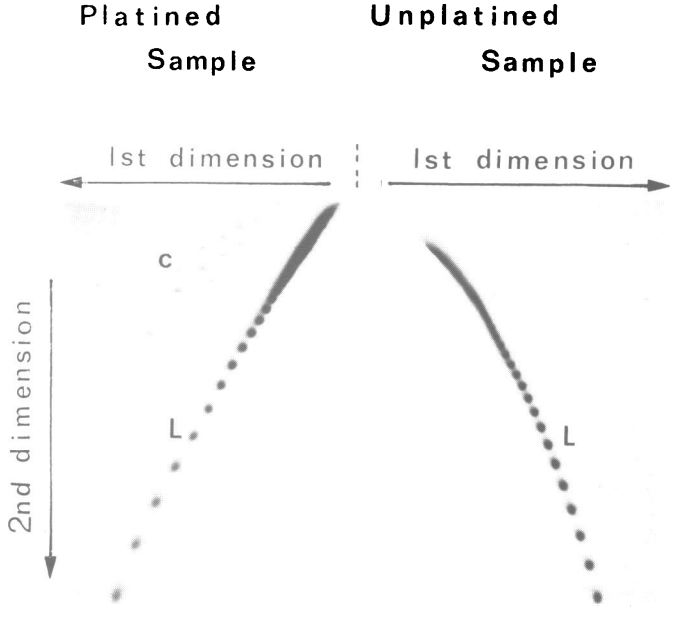

Fig.2 Analysis of ligation products of unmodified and *cis*-platinum modified DNA by two-dimensional gel electrophoresis. The products were separated first on a 4% polyacrylamide gel (Ist dimension) and then on a 8% polyacrylamide gel containing chloroquine (50 μg/ml) (2nd dimension). The linear and circular DNAs are indicated by the letters (L) and (C), respectively.

et al (21) (Fig. 2). The spots in the gel were cut out and the length of DNA was identified by analysis on denaturing gel. The first two spots are 6-mer and 7-mer of the 22-mer precursor which leads to a value of the bend of 31° and 24°, respectively.

Osmium tetraoxide (OsO_4)

To characterize whether the distortions induced by the adducts spread over several bases, we have used chemical probes and artificial nuclease which are able to detect, at the nucleotide level, local conformational changes of the double helix.

OsO_4, in the presence of pyridine, is hyperreactive with the 5,6 double bound of pyrimidine rings in single-stranded nucleic acids and in distorted DNA as compared to B-DNA (22−24). We previously reported (10) that the T residues at the 3′ side of the d(GTG) adduct were not reactive with OsO_4 while the T residues at the 5′ side were slightly reactive as compared to the central T residue. As shown in Fig.3, we confirm the high reactivity of the central T residue and the non-reactivity of the T residues at the 3′ side of the adduct. However, we find that only the immediately 5′T residue adjacent to the adduct is slightly reactive. We assume that the use of longer and thus more stable oligonucleotides can explain this discrepancy.

Phenanthroline-copper digestion

The nucleolytic agent 1,10-phenanthroline-copper (OP-Cu) attacks DNA from a binding site within the minor groove and cleaves DNA in a sequence dependent manner but not in a base-specific manner (16,25). The cleavage patterns relative to the unplatinated and platinated duplexes are shown in Fig.4. Within the upper strands, the larger differences are observed at the level of the d(GTGT) sequences, the platinated strand being much less reactive. Within the lower strand, mainly the cleavages at the level of the C residue complementary to the platinated 5′G residue and the A residue complementary to the central T residue are decreased. Thus, as judged by OP-Cu, the double helix is mainly distorted at the level of the platination site.

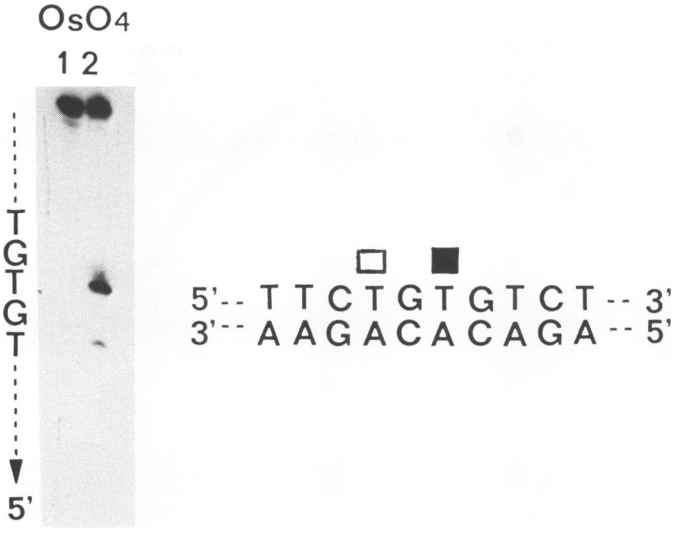

Fig.3 Piperidine-induced specific strand cleavage at OsO_4-modified bases in unplatinated and platinated duplexes. Left: lanes 1 and 2 are relative to the upper strand in the unplatinated and in the platinated samples, respectively. Right: summary of changes in chemical reactivity. Filled and open symbols indicate strong and low hyperreactivity, respectively.

Sodium cyanide

It is known that the adducts formed in the reaction between *cis*-DDP and DNA are stable over a long period of time in physiological conditions while in the presence of cyanide ions most of the bound platinum residues, but not all of them are rapidly removed (26−27). Immunological analysis of the platinated DNA after treatment with cyanide ions suggests a preferential removal of d(GG) and d(AG) adducts (28). Recently, we reported that the kinetics of removal of both the major adducts by cyanide ions were sensitive to the conformation of the oligonucleotides, the adducts within double-stranded oligonucleotides being less stable than the same adducts within single-stranded oligonucleotides (29).

The reactivity of cyanide ions with the d(GTG) adduct within single-stranded or double-stranded oligonucleotide has been studied. The platinated single-stranded (22-mer) oligonucleotide was ^{32}P labeled at the 5′ end. The corresponding double-stranded oligonucleotide was obtained by mixing the ^{32}P labeled oligonucleotide with the complementary unlabeled oligonucleotide. Both the platinated samples were treated with a large excess of cyanide ions (0.2 M, pH 8.3) at 37°C. At various times, aliquots were withdrawn and analyzed by gel electrophoresis under denaturing conditions. As shown in Fig. 5, the adducts are slightly more stable within the single-stranded oligonucleotide than within the double-stranded oligonucleotide. After 19 hours, about 50% and 70% of the platinum residues are still bound to the double-stranded and to the single-stranded oligonucleotide, respectively.

Thermal stability

The thermal stability of the unplatinated and the platinated 22-mer duplexes has been studied at two ionic strengths (10 and 100 mM Na^+, pH 7.5). In both cases, the duplexes melted cooperatively but the platination decreased the thermal stability by about 7°C and the hyperchromicity at 260 nm by about 20% in agreement with previous results (9−10).

II) Oligonucleotides modified by *trans*-DDP at the d(GTG) site

Although *trans*-DDP is clinically inactive, the characterization of the distortions induced in DNA by *trans*-DDP can be useful to explain the antitumor activity of *cis*-DDP. The reaction between *trans*-DDP and the single-stranded oligonucleotide (upper strand) leads to the formation of the d(GTG) adduct (30,32). This has been confirmed by reverse-phase HPLC analysis of the digests after enzymatic hydrolysis of the platinated

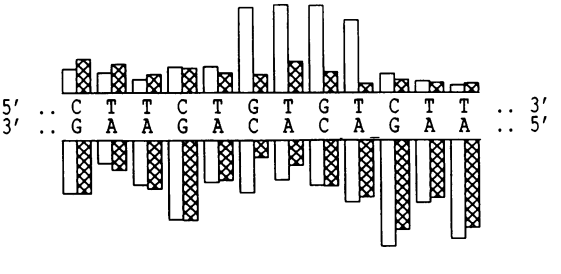

Fig.4 Quantitation of OP-Cu cleavage. The autoradiograms (not shown) were quantitated by microdensitometry. The white columns represent digestion of the unmodified duplex and the shaded columns represent digestion of the *cis*-platinum-modified duplex. The surfaces of the columns are proportional to the band intensities on the autoradiograms.

oligonucleotides and by chemical probes (as shown later). The behavior of the platinated duplexes has been studied by gel electrophoresis, chemical probes and thermal stability.

Electrophoretic mobility

The multimers of the platinated and ligated duplexes presented an anomalous slow electrophoretic mobility which depended upon the length of the oligomers (not shown). The K-factor for the *trans*-platinum 20-mer duplexes was larger than the corresponding K-factor for the *cis*-platinum duplexes (for example, 1.62 and 1.35 for the 120 base pairs duplexes, respectively). For longer oligonucleotides (22-mer, 23-mer and 24-mer), the proportion of circles increased while the proportion of linear duplexes decreased. The effect was the largest with the 23-mer oligonucleotides. As illustrated in Fig.6, the linear duplexes are hardly detectable. One can deduce an estimate of 60° for the *trans*-platinum-induced bend angle.

Chemical probes

The chemical probes OsO_4, chloroacetaldehyde (CAA) and diethylpyrocarbonate (DEPC) were used. The reactivity between OsO_4 and the upper strand is shown in Fig.7. Lane 1 is relative to the cleavage of the unplatinated single-stranded oligonucleotide. Mainly the T residues are reactive. OsO_4 does not react with any T residue within the unplatinated duplex (lane 2). Lane 3 is relative to the platinated duplex (the *trans*-platinum residues have not been removed after the reaction with OsO_4). Only the first 5'T residue adjacent to the adduct is reactive. After the reaction with OsO_4 and than removal of the bound *trans*-platinum residue by NaCN (the oligonucleotide was incubated in 0.2 M NaCN, at basic pH, overnight and at 40°C), the T residue within the sequence d(GTG) is detected (lane 4). Thus, only two T residues, the central T and the immediately 5'T are accessible to OsO_4. It can be noticed that the comparison between lanes 3 and 4 argues for a cross-link of the two G residues by *trans*-platinum.

CAA and DEPC were reacted with the lower strand. 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 (33−36). Lane 1 in Fig.7 represents the reactivity of the A and C residues within the unplatinated single-stranded oligonucleotide with CAA. Within the unplatinated double-stranded oligonucleotide, the A and C residues are not modified (lane 2). Within the platinated oligonucleotide, the two A and the two C residues complementary to the d(TGTG) sequence are modified, the central A and C residues being more reactive (lane 3). Thus, the binding of *trans*-platinum residues to the d(GTG) sequence within the duplex denatures locally 4 base residues.

DEPC, which carbethoxylates purines at the N7 position is hyperreactive with Z-DNA and with denatured DNA as compared to B-DNA (22,37−38). Lane 1 in Fig.7 shows the cleavage pattern of the unplatinated single-stranded oligonucleotide and confirms the reactivity of A and G residues. Within the unplatinated double-stranded oligonucleotide, none of these residues is modified by DEPC (lane 2). Within the platinated oligonucleotide, the A residue complementary to the 5'T residue adjacent to the adduct and the A residue complementary to the central T residue are reactive (lane 3). Thus, these results confirm that the double helix is denatured over 4 base pairs (corresponding

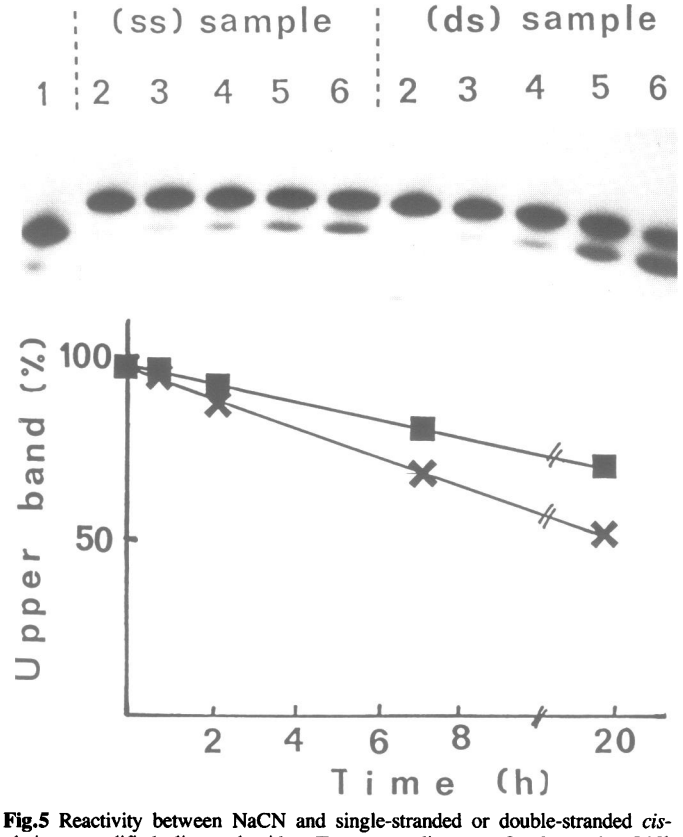

Fig.5 Reactivity between NaCN and single-stranded or double-stranded *cis*-platinum-modified oligonucleotides. Top: autoradiogram of a denaturing 24% polyacrylamide gel of the products of the reaction. The platinated sample (3. 10^{-6} M in platinum residues) were incubated at 37°C and in 0.2 M NaCN, 20 mM Tris-HCl adjusted to pH 8.3 by addition of HCl. Lane 1 correspond to the unplatinated oligonucleotide, lanes 2−6 correspond to the platinated oligonucleotides at times 0, 1, 2, 7 and 19 h. Bottom: relative concentrations of the platinated samples versus time; (X) double-stranded platinated oligonucleotide, (■) single-stranded oligonucleotide.

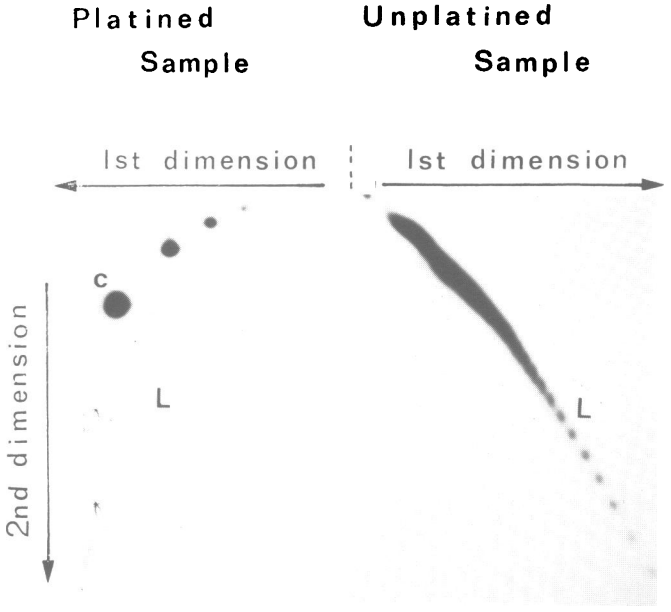

Fig.6 Analysis of ligation products of unplatinated and *trans*-platinum-modified DNA by two-dimensional gel electrophoresis. The products were separated first on a 4% polyacrylamide gel (Ist dimension) and then on a 10% polyacrylamide gel containing chloroquine (50μg/ml) (2nd dimension). The autoradiogram was overexposed to reveal the linear duplexes. The linear and circular DNAs are indicated by the letters (L) and (C), respectively.

to the d(TGTG) sequence) and that the immediately 3'T residue adjacent to the adduct is paired with the complementary A residue. A summary of the reactivity of OsO_4, CAA and DEPC with the platinated double-stranded oligonucleotide is given in Fig.7.

Thermal stability

The thermal stability of the unplatinated and platinated 22-mer duplexes has been studied at two ionic strengths (10 mM and 100 mM Na^+, pH 7.5). In both cases, the duplexes melted cooperatively but the platination decreased the Tm by about 17°C and the hyperchromicity at 260nm by about 40%.

DISCUSSION

In this work, we compare the distortions induced in double-stranded oligonucleotides by the binding of *cis*-DDP or *trans*-DDP to the d(GTG) sequence.

Let first consider the oligonucleotide modified by *cis*-platinum. The d(GTG) adduct alters the shape of the oligonucleotide as shown by the abnormal electrophoretic mobility of the multimers of the ligated oligomers. The largest retardation is observed with the 22-mer oligonucleotide which argues strongly that the direction of the helix axis is altered in a definite way . The *cis*-platinum-induced bent angle is in the range 25−30°. This angle is smaller than the one induced by the binding of *cis*-platinum to the d(GG) sequence (39).

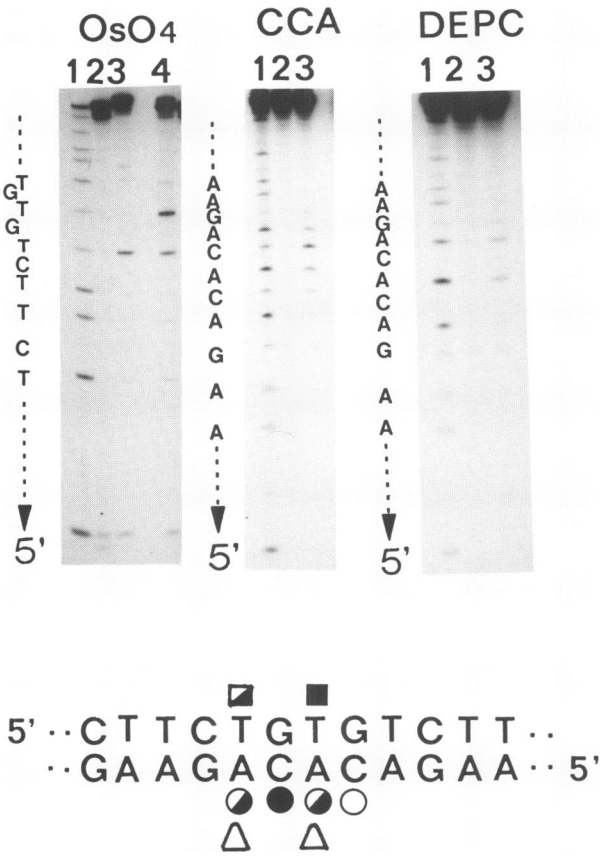

Fig.7 Piperidine-induced specific strand cleavage at chemically modified bases in unmodified and in *trans*-platinum modified oligonucleotides. Left: cleavage pattern of the reaction between OsO_4 and the upper strand; unplatined single strand (lane 1); unplatinated duplex (lane 2); platinated duplex (lane 3) (the bound platinum has not been removed); platinated duplex (lane 4), (the bound platinum has been removed after the reaction with OsO_4). Middle and right: cleavage patterns of the reaction between CAA or DEPC and the lower strand, respectively. Lanes 1 are relative to the unplatinated single strand, lanes 2 to the unplatinated duplex and lanes 3 to the platinated duplex. Bottom: summary of the changes in chemical; Filled, half-filled and open symbols indicates strong, intermediate and low hyperreactivity, respectively: (■) OsO4, (●) CCA and (▲) DEPC.

The distortion spreads over a few bases. The central T residue is largely accesssible to OsO_4 which supports the proposal (9) that this residue is bulged out. On the other hand, only the first 5'T residue adjacent to the adduct is reactive with OsO_4. Since the 3'T residue is not reactive, the double helix is more distorted at the 5' side of the adduct than at the 3'side. The cleavage pattern of the platinated oligonucleotide by the artificial nuclease OP-Cu is mainly modified at the level of the adduct as compared to the cleavage pattern of the unplatinated oligonucleotide. These results, the decrease of thermal stability and the previously reported reactivity (10) of the chemical probes (CAA, DEPC) suggest that platination at the d(GTG) sequence denatures locally the double helix over two base pairs (corresponding to the 5'G and T residues of the adduct) and that no more than two base pairs are distorted at the 5' side of the adduct. (We are aware that kinetics of reaction between base residues and chemical probes are necessary to better characterize the local denaturation).

In normal conditions, the bifunctional adducts are kinetically inert. In the presence of cyanide ions, most of the adducts but not all of them are rapidly removed (26−27). At 37°C and in 0.2 M NaCN, pH 8.3, the stability of the d(GG) and d(AG) adducts depends strongly upon the conformation of the oligonucleotide (29). The half-lives of the d(GG) and d(AG) adducts are respectively 20 min and less than 10 min within double-stranded oligonucleotides, 720 min and 120 min within single-stranded oligonucleotides. The d(GTG) adducts behave differently. They are more resistant to cyanide ions and less sensitive to the conformation of the oligonucleotides. The half-lives within the double-stranded and the single-stranded oligonucleotides are about 1140 min and 1800 min, respectively. This is likely to reflect a strong protective effect due to both the central and adjacent T residues (40−41).

The binding of *trans*-platinum to the d(GTG) sequence does not prevent the formation of a duplex. The base residues at the 3' side of the adduct and those at the 5' side (with the exception of the immediate adjacent base) are paired since they do not react with the chemical probes OsO_4, CAA and DEPC. The base pairing is disrupted over 4 base pairs within the sequence d(TGTG). This local denaturation destabilizes the thermal stability of the double helix, the Tm being decreased by 17°C. Nevertheless, the modified region behaves more as the centre of a directed bend than as a hinge joint as shown by the abnormal electrophoretic mobility. The *trans*-platinum-induced bent angle is of the order of 60°.

Very recently, the conformation of a double-stranded oligonucleotide modified by *trans*-platinum at the d(GAG) sequence has been characterized by spectroscopic and molecular mechanics modeling studies (42). In agreement with our findings, the platinated 5'G residue and the central A residue are unpaired. On the other hand, the *trans*-platinum-induced bent angle is 18° and the unplatinated and the platinated duplexes present the same thermal stability. Thus, the distortions induced by the binding of *trans*-platinum to a d(GXG) sequence depend upon the nature of the central X residue. In fact, Lepre et al (42) have shown that the central A residue is located in the minor groove and forms two hydrogen bonds through the amino group, hydrogen bonds which cannot be formed by the central T residue.

In conclusion, the double-stranded oligonucleotides modified at the d(GTG) sequence either by *cis*-DDP or by *trans*-DDP are structurally different. Several studies have already shown that *cis*- or *trans*-platinum-modified DNAs interact differently with various proteins (see general reviews 2–5). In order to get more detailed informations, we have undertaken a systematic study of the action of enzymes such as polymerases and repair enzymes on platinated oligonucleotides modified at an unique site.

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REFERENCES

1. Roberts,J.J. and Pera,M.F. (1983) Mol. Aspects of Anti-Cancer Drug Action, Neidle,S. and Waring,M.J. Eds, Macmillan, London, p 183–231.
2. Eastman,A. (1987) Pharmac. Ther. **34,** 155–166.
3. Reedijk,J. (1987) Pure and Appl Chem. **59,** 181–192.
4. Lippard,S.J. (1987) Pure and Appl Chem. **59,** 731–742.
5. Johnson,N.P.,Butour,J.L.,Villani,G.,Wimmer,F.L.,Defais,M., Pierson,V. and Brabec.V. (1989) Prog.Clinical Biochem. Med., Springer-Verlag, Berlin, **10,** p 1–24.
6. Brouwer,J.,van der Putte,P.,Fichtinger-Schepman,A.M.J. and Reedijk,J. (1981) Proc. Natl. Acad. Sci. USA **78,** 7010–7014.
7. de Boer,J.G. and Glickman,B.W. (1989) Carcinogenesis **10,** 1363–1367.
8. Burnouf,D.,Daune,M. and Fuchs,R.P.P. (1987) Proc. Natl. Acad. Sci. USA **84,** 3758–3762.
9. den Hartog,J.H.J.,Altona,C.,van del Elst,H.,van der Marel,G.A. and Reedijk,J. (1985) Inorg. Chem. **24,** 983–986.
10. Marrot,L. and Leng,M. (1989) Biochemistry **28,** 1454–1461.
11. Ptak,M.,Rahmouni,A.,Mazeau,K.,Thuong,N.T. and Leng,M. (1989) Anti-Cancer Drug Design **4,** 53–61.
12. Mazeau,K.,Vovelle,F.,Rahmouni,A.,Leng,M. and Ptak,M. (1989) Anti-Cancer Drug Design **4,** 63–78.
13. Fichtinger-Schepman,A.M.J.,van der Veer,J.L.,den Hartog, J.H.J.,Lohman,P.H.M. and Reedijk,J. (1986) Biochemistry **24,** 707–713.
14. Eastman,A. (1986) Biochemistry **5,** 3912–915.
15. Schwartz,A.,Marrot,L. and Leng,M. (1989) Biochemistry **28,** 7975–7989.
16. Yoon,C.,Kuwabara,M.D.Law,R. and Sigman,D.S. (1988) J. Biol. Chem. **263,** 8458–8463.
17. Koo,H.,Wu,H.M. and Crothers,D.M. (1986) Nature (London) **321,** 501–506.
18. Hagerman,P.J. (1988) Unusual DNA Structures, Wells,R.D. and Harvey,S.C., Eds, Springer, Berlin, p 225–236.
19. Diekman,S.V. (1987) Nucleic Acids and Molecular Biology, Eckstein,F. and Lilley,D.M.J.,Eds, Springer, Berlin, **1,** p 138–156.
20. Shore,D.,Langowski,J. and Baldwin,R. (1981) Proc. Natl. Acad. Sci. USA **78,** 4833–4837.
21. Ulanosky,L.,Bodner,M.,Trifonov,E.N. and Choder,M. (1986) Proc. Natl. Acad. Sci. USA **83,** 862–866.
22. Johnston,B.H. and Rich,A. (1985) Cell (Cambridge, Mass.) **42,** 713–724.
23. Lilley,D.M.J. and Palecek,E. (1984) EMBO J. **3,** 1187–1192.
24. Palecek,E.,Boublikova,P.,Galazka,G. and Klysik,J. (1987) Gen. Physiol. Biophys. **6,** 327–341.
25. Sigman,D.S. and Spassky,A. (1989) Nucleic Acids and Molecular Biology, Eckstein,F. and Lilley,D.M.J., Eds, Springer, Berlin **3,** p 13–27.
26. Bauer,W.,Gonias,S.L.,Kam,S.K.,Wu,K.C. and Lippard,S.J. (1978) Biochemistry **17,** 1060–1068.
27. Lippard,S.J. and Hoeschele,J.D. (1979) Proc. Natl. Acad. Sci. USA **76,** 6091–6095.
28. Lippard,S.J.,Ushay,H.M.,Merkel,C.M.and Poirier,M.C. (1983) Biochemistry **22,** 5165–5168.
29. Schwartz,A.,Sip,M. and Leng,M. (1990) J. Am. Chem. Soc. **112,** 3673–3674.
30. van der Veer,J.L.,Ligvoet,G.J.,van den Elst,H. and Reedijk,J. (1986) J. Am. Chem. Soc. **108,** 3860–3862.
31. Gibson,D. and Lippard,S.J. (1987) Inorg. Chem. **26,** 2275–2279.
32. Eastman,A.,Jennerwein,M.M. and Nagel,D.L. (1988) Chem. Biol. Interations. **67,** 71–80.
33. Lilley,D.M.J. (1983) Nucleic Acids Res. **11,** 3097–3113.
34. McLean,M.J.,Larson,J.E.,Wohlrab,F. and Wells,R.D. (1987) Nucleic Acids Res. **15,** 6917–6935.
35. Kohwi-Shigematsu,T.,Manes,T. and Kowi,Y. (1987) Proc. Natl Acad. Sci. USA **84,** 2223–2227.
36. Vogt,N.,Marrot,L.,Rousseau,N.,Malfoy,B. and Leng,M. (1988) J. Mol. Biol. **201,** 773–776.
37. Herr,W. (1985) Proc. Natl. Acad. Sci. USA **82,** 8009–8013.
38. Runkel,L. and Nordheim,A. (1986) Nucleic Acids Res. **14,** 7143–7158.
39. Rice,J.A.,Crothers,D.M.,Pinto,A.L. and Lippard,S.J. (1988) Proc. Natl. Acad. Sci. USA **85,** 4158–4161.
40. Raudaschl-Sieber,G. and Lippert,B. (1985) Inorg. Chem. **24,** 2426–2432.
41. Frommer,G. and Lippert,B. Inorg. Chem. (in press).
42. Lepre,C.A.,Chassot,L.,Castello,C.E. and Lippard,S.J. (1990) Biochemistry **29,** 811–823.