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Chemical Versatility of Transplatin Monofunctional Adducts within Multiple Site-Specifically Platinated DNA†

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ABSTRACT: The first step of the reaction between DNA and the antitumor drug cisplatin or its clinically inactive isomer transplatin yields monofunctional adducts. Most of the cisplatin monofunctional adducts further react and rather rapidly ($t_{1/2}$ smaller than a few hours) to form intrastrand and interstrand cross-links. It is generally accepted that the clinical activity of cisplatin is related to the formation of bifunctional lesions. As concerns transplatin, several studies disagree on the rate of closure of the monofunctional adducts and the nature of the bifunctional lesions. In order to explain these discrepancies, we have prepared several duplexes containing a single monofunctional *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ adduct and zero to two monofunctional $[Pt(dien)(dG)]^{2+}$ adducts at defined positions. In these duplexes, the inert $[Pt(dien)(dG)]^{2+}$ adducts mimic the presence of transplatin monofunctional adducts. We show that the closure of the transplatin monofunctional adducts is strongly affected by the presence of other adducts and by the length of the duplexes. These findings suggest that the discrepancies in the literature originate from the nature of the platinated samples (molar ratio of bound platinum per nucleotide, length of the DNA fragments). Our general conclusion is that within transplatin-modified DNA, at a low level of platination, the monofunctional adducts evolve slowly ($t_{1/2} > 24$ h) into bifunctional lesions and that these bifunctional lesions are mainly interstrand cross-links. This could explain, at least in part, the clinical inefficiency of transplatin.

It is now widely accepted that the antitumor activity of cisplatin [*cis*-diamminedichloroplatinum(II), *cis*-DDP][1] is related to its ability to form bifunctional lesions within DNA, the major ones being N7,N7-bidentate cross-links between adjacent purine residues (Lepre & Lippard, 1990; Reedijk, 1992; Sip & Leng, 1993). A striking feature of the DDP derivatives is that the *cis* isomer is an antitumor drug while the *trans* isomer (*trans*-DDP, transplatin) is devoid of biological activity even though it reacts with DNA (Lepre & Lippard, 1990; Lippert, 1996). Both isomers bind to DNA in a two-step process, forming first monofunctional adducts (preferentially at the N7 of guanine residues) that may subsequently evolve into bifunctional lesions. The nature and the kinetics of formation of the bifunctional lesions produced by each derivative are thought to be related to their

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[1] Abbreviations: bp, base pair; cisplatin and *cis*-DDP, *cis*-diamminedichloroplatinum(II); transplatin, and *trans*-DDP, *trans*-diamminedichloroplatinum(II); DDP, diamminedichloroplatinum(II); DEPC, diethyl pyrocarbonate; OsO_4, osmium tetroxide; FPLC, fast protein liquid chromatography.

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differential biological activity (Lepre & Lippard, 1990; Dalbiès et al., 1996; Lippert, 1996). Alternatively, selective trapping and removal of the *trans*-DDP monofunctional adducts by glutathione may account for the inefficiency of *trans*-DDP, consistent with the observation that depletion of glutathione increases the sensitivity of ovarian carcinoma cells to *trans*- but not to *cis*-DDP (Richon et al., 1987). Differential repair or effects on replication or transcription of the *cis*- and *trans*-DDP lesions, in relation with their ability to distort the DNA double helix or to promote the interaction of platinated DNA with proteins (Lepre & Lippard, 1990; McA'Nulty & Lippard, 1995), may also be implicated.

Whatever the explanation, careful analysis of the factors responsible for the biological effects relative to each isomer requires a better knowledge of their mode of interaction with DNA. This is emphasized by the fact that the spectrum of the DNA lesions produced *in vivo* by *trans*-DDP remains unknown and that divergent conclusions were drawn from *in vitro* experiments on the nature and kinetics of formation of the adducts (Eastman & Barry, 1987; Eastman et al., 1988; Bancroft et al., 1990; Boudvillain et al., 1995). Recently, novel platinum complexes showing antitumor activity have been discovered (Hollis et al., 1989; Farrell et al., 1992; Coluccia et al., 1993; Farrell, 1996). Some of them have structural features (*trans* geometry, for example) that violate the classical structure−activity relationships of platinum(II) complexes. Although it is not yet firmly established that DNA is the biological target for all these compounds, their antitumor activity might originate from a different mode of interaction with DNA and/or in extended structural alterations of the DNA double helix. It is therefore important to identify the adducts resulting from DNA platination and the factors governing their formation and/or removal, in order to elucidate the way(s) by which platinum(II) complexes can interfere with specific biological activities. In this paper, we show that the platination of discrete sites within a DNA double helix can mediate the closure of *trans*-DDP monofunctional adducts into bifunctional cross-links. The results shed light on the origins of the discrepancies between previous studies devoted to the study of the nature and kinetics of formation of *trans*-DDP adducts (Eastman & Barry, 1987; Eastman et al., 1988; Bancroft et al., 1990; Boudvillain et al., 1995).

MATERIALS AND METHODS

Materials. The oligodeoxyribonucleotides from Institut Pasteur (France) were purified as previously described (Boudvillain et al., 1995). The sequences of the oligonucleotides are given Figure 1. T4 polynucleotide kinase and T4 DNA ligase were purchased from New England BioLabs. The radioactive products were from Amersham. Iododiethylenetriammineplatinum(II) iodide (dien-Pt) was kindly provided by Pr. Lippert (Dortmund, Germany). Other chemicals were from Merck except *trans*-DDP which was from Johnson Matthey (U.K.).

Platination Reaction. *trans*-$[Pt(NH_3)_2(H_2O)Cl]^+$ and $[Pt(dien)(H_2O)]^{2+}$ species were generated by allowing *trans*-DDP or dien-Pt to react with 0.9 equiv of $AgNO_3$ at 37 °C overnight in the dark. The oligonucleotide containing a single *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ monofunctional adduct was obtained by reacting oligonucleotide A (the sequence of oligonucleotide A is given in Figure 1) (27 μM) with *trans*-$[Pt(NH_3)_2(H_2O)Cl]^+$ at a platinum to oligonucleotide ratio of 4.4 for 10 min at 37 °C in 10 mM $NaClO_4$ and 10 mM acetate buffer at pH 3.6 (Brabec & Leng, 1993). The platinated oligonucleotide was purified by strong anion exchange FPLC (Mono Q HR5/5 column, Pharmacia) with a 10 mM NaOH, 0.2 to 0.8 M NaCl gradient. The collected fraction was dialyzed for 2 h, at 4 °C, against 3 × 500 mL of 100 mM NaCl and 5 mM phosphate buffer at pH 7.5 and then stored at −20 °C. Oligonucleotide B (24 μM) was reacted with 2.5 equiv of $[Pt(dien)(H_2O)]^{2+}$ for 10 min at 37 °C in 10 mM $NaClO_4$ and 10 mM acetate buffer at pH 3.6. The crude product was loaded on a Mono Q column and eluted with a gradient of 20 to 80% buffer B over 40 min, where buffer B is 10 mM NaOH and 1 M NaCl and buffer A is 10 mM NaOH and 10 mM NaCl (flow rate, 1 mL/min). Four fractions were collected corresponding to the oligonucleotides containing two $[Pt(dien)(dG)]^{2+}$ monofunctional adducts or a single monofunctional adduct at either of the two guanine residues or no adducts, respectively. The fractions were further neutralized with acetic acid and Tris-HCl and desalted on Sep-Pak C18 cartridges (Waters). The nature and location of the adduct(s) within the platinated oligonucleotides were verified as previously described (Lemaire et al., 1991; Brabec & Leng, 1993).

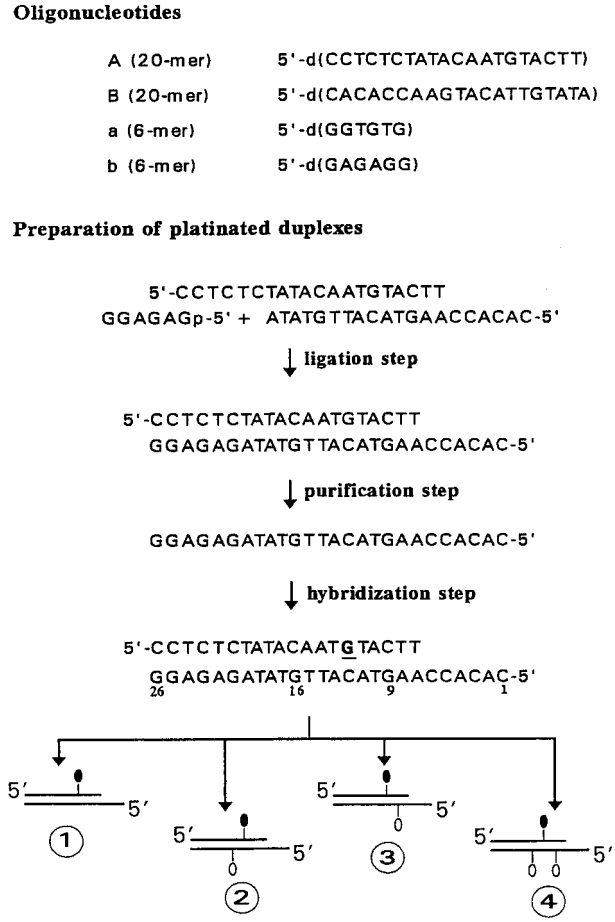

FIGURE 1: Sequences of the oligodeoxyribonucleotides (top) and scheme of the procedure used for the synthesis of the duplexes (bottom). For the sake of clarity, the different steps for the synthesis of only one duplex are shown. The four platinated duplexes are schematically represented, open and filled symbols corresponding to the monofunctional adducts $[Pt(dien)(dG)]^{2+}$ and *trans*-$[Pt(NH_3)_2(dG)Cl]^+$, respectively. The underlined G in the upper strand indicates the location of *trans*-$[Pt(NH_3)_2(dG)Cl]^+$.

Ligation. 26-mer platinated (or unplatinated) oligonucleotides were prepared by template-directed ligation of the 20-mer platinated (or unplatinated) oligonucleotides A or B with the corresponding 5′-end-phosphorylated 6-mer oligonucleotides a or b (see Figure 1) using T4 DNA ligase and standard procedures (Maniatis et al., 1982). The ligated products were further purified by gel electrophoresis (24% polyacrylamide) under denaturing conditions.

Conversion of trans-DDP Monofunctional Adducts. Oligonucleotide A (2.7 μM) containing a single *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ monofunctional adduct was annealed with the platinated (or unplatinated) 26-mer complementary strand and incubated for 5 min at room temperature and then for 60 min at 0 °C. The four duplexes (55 pmol) labeled at the 5′-end of the upper strand or the bottom strand were precipitated with ethanol and resuspended in 20 μL of 100 mM $NaClO_4$, 0.2 mM EDTA, and 3 mM phosphate buffer at pH 7.5. The mixtures were then incubated at 37 °C. At various times, aliquots were withdrawn, treated with thiourea (10 mM) for 15 min at 37 °C to remove the monofunctional adducts (Eastman & Barry, 1987), and analyzed by 24% polyacrylamide gel electrophoresis under denaturing conditions. Half-reaction times were deduced from the quantitation of the band intensities with a Molecular Dynamics Phosphorimager (using ImageQuant software version 3.3 for data processing) assuming pseudo-first-order kinetics (Bancroft et al., 1990). The duplexes containing an interstrand cross-link resulting from the closure of the *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ monofunctional adduct were purified by gel electrophoresis (24% polyacrylamide) under denaturing conditions. The bases involved in the interstrand cross-link were identified by Maxam−Gilbert sequencing of the purified duplexes containing a 5′-end-labeled top or bottom strand (Lemaire et al., 1991; Dalbiès et al., 1994).

Chemical Modifications. The modifications were performed on 5′-end-labeled single-stranded oligonucleotides or duplexes containing either a 5′-end-labeled top or bottom strand. The modifications of the oligonucleotides by osmium tetroxide (OsO_4) or diethyl pyrocarbonate (DEPC) were performed as previously described (Marrot & Leng, 1989), with minor modifications. Salt, concentration, and temperature conditions were identical to those for the kinetic experiments (see above), and the reaction time was reduced to 8 min. The reaction products were treated with piperidine under the usual conditions and resolved using a 24% polyacrylamide sequencing gel.

RESULTS

Synthesis of the Platinated Duplexes. Previous studies have shown that either a single *cis*-$[Pt(NH_3)_2(dG)Cl]^+$ or $[Pt(dien)(dG)]^{2+}$ monofunctional adduct distorts the DNA double helix but the distortions are located in the vicinity of the adducts (Brabec et al., 1992, 1994). Our aim was to determine whether several monofunctional platinum(II) adducts in close proximity interfere with the closure of these adducts into bifunctional cross-links. In order to explore these aspects, we have prepared four platinated duplexes containing a single *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ adduct within the upper strand and within the lower strand either no adduct or one or two $[Pt(dien)(dG)]^{2+}$ adducts, these inert adducts mimicking to some extent the transplatin monofunctional adducts. A schematic representation of the platinated duplexes is given in the bottom of Figure 1.

The synthesis of the platinated duplexes was done according to the following scheme. The two 20-mer oligonucleotides A and B complementary over 14 residues (Figure 1) were designed to contain only one and two guanine residues, respectively. These residues can be selectively modified by platinum(II) complexes at acidic pH. Oligonucleotide B was reacted with $[Pt(dien)(H_2O)]^{2+}$ and the different oligonucleotides within the reaction mixture [bearing either two $[Pt(dien)(dG)]^{2+}$ adducts or a single monoadduct at the 5′- or 3′-G residue or no adduct] were purified by strong anion exchange FPLC under denaturing conditions. Each of the purified oligonucleotides was hybridized to oligonucleotide A, and their 3′-ends were extended by ligation with 6-mer oligonucleotide b [see Figure 1 for the synthesis of the duplex containing a single *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ adduct]. The four oligonucleotides (26-mers) were purified by gel electrophoresis under denaturing conditions and then paired with oligonucleotide A bearing a single *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ adduct. The four platinated duplexes were named **1**−**4**, respectively.

Chemical Probing of the Platinated Duplexes. Prior to the measurement of the rate of closure of the *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ monofunctional adduct into the bifunctional adduct within the four duplexes, the conformation of the duplexes was tested by means of the chemical probes osmium tetroxide (OsO_4) or diethyl pyrocarbonate (DEPC). These two probes are hyperreactive with distorted DNA as compared to their activities with B-DNA [general reviews in Palececk et al. (1990), Leng (1990), and Nielsen (1990)]. After the duplexes were treated with the chemical probes, the fragments resulting from piperidine cleavage were analyzed by gel sequencing electrophoresis. As an illustration, the results relative to the reaction between OsO_4 and the lower strand of the four duplexes, as well as the Maxam−Gilbert sequencing reactions, are shown in Figure 2. The lanes G+A confirm the location of the adducts [it is known that platination at the N7 of G residues decreases the acid-catalyzed depurination (Johnson et al., 1985), and thus, the bands corresponding to the platinated G residues are missing]. The summary of the results with OsO_4 and DEPC (Figure 2, right) shows that the adducts induce distortions in the duplexes but the duplexes are not denatured. In duplex **4** which contains three adducts, the nonreactivity of T10, the weak reactivity of A11 (lower strand), and the nonreactivity of the complementary residues (upper strand) support the pairing of the bases. Within the lower strands of duplexes **1** and **2** and even of the unplatinated duplex (not shown), the reactivity of DEPC with A7 and to a lesser extent with A8 does not argue for a local denaturation. As compared to the size of the B-DNA major groove, DEPC is too large to reach the A residues (Nielsen, 1990). This does not hold for the two A residues at the end of the duplexes.

Closure of trans-DDP Monofunctional Adducts. The platinated duplexes (**1**−**4**) labeled at the 5′-end of the upper strand were incubated at 37 °C. At various times, aliquots were withdrawn, incubated in thiourea to remove the monofunctional adducts (Eastman & Barry, 1987), and then analyzed by gel electrophoresis under denaturing conditions. As shown previously, oligonucleotides containing intrastrand or interstrand cross-links are easily separated by this technique (Gaucheron et al., 1991; Payet et al., 1993). As a function of time, the intensity of the initial band (upper strand of the duplexes) decreases as new bands appear

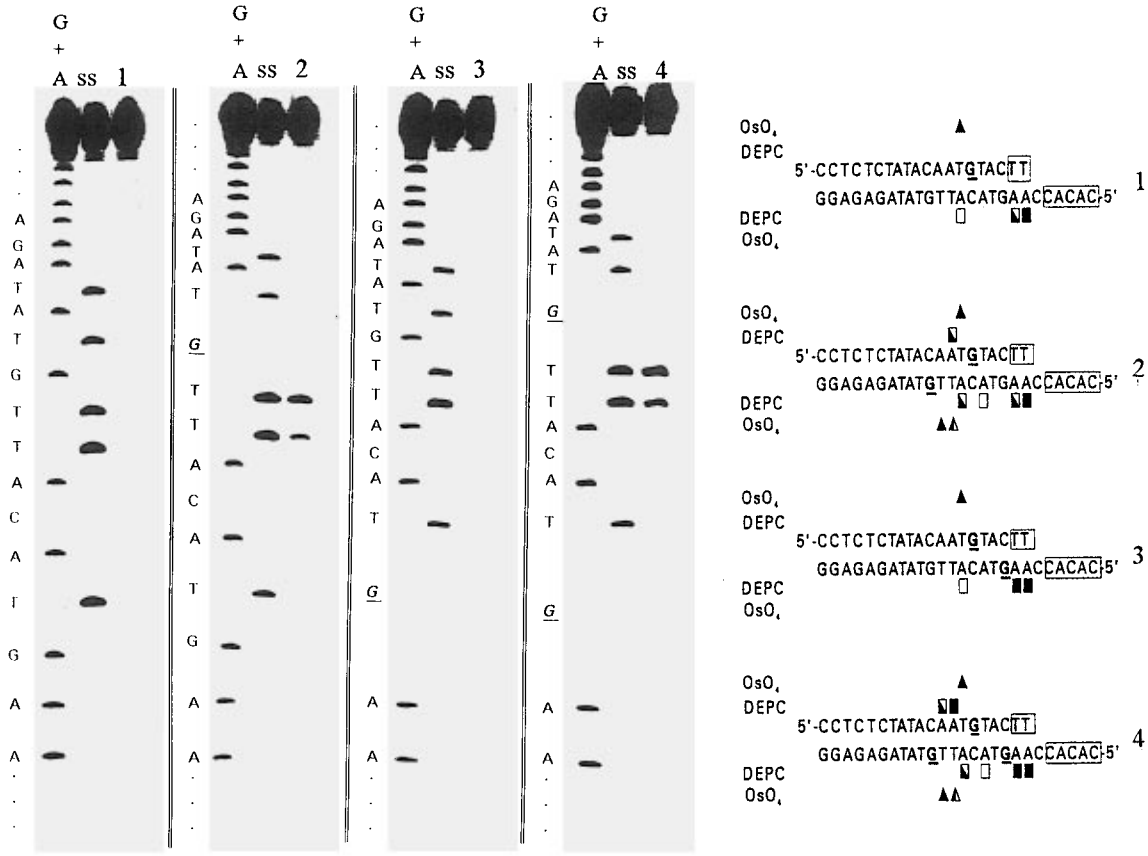

FIGURE 2: Reactivity of the duplexes (**1**−**4**) with the chemical probes OsO_4 or DEPC. (Left) Piperidine-induced specific strand cleavage at OsO_4-modified bases in the platinated duplexes. Lanes G+A are relative to the Maxam−Gilbert sequencing reactions of the 5′-end-labeled bottom strands. Lanes ss are relative to the platinated single-stranded bottom strands. On the side of the lanes are given the sequences of the oligonucleotides, the platinated G residues being underlined. Note that the platinum residues were not removed before the electrophoresis and thus the migration of the fragments containing the adducts were slowed as compared to that of the unplatinated ones. (Right) Summary of the hyperreactivity of OsO_4 and DEPC. Filled, half-filled, and open symbols indicate strong, medium, and low hyperreactivity, respectively. Rectangles enclose the residues for which it was not possible to evaluate the reactivity with OsO_4 or DEPC because of the resolution of the gel (the last two T residues on the 3′-side of the upper strand and the last four residues on the 5′-side of the bottom strand).

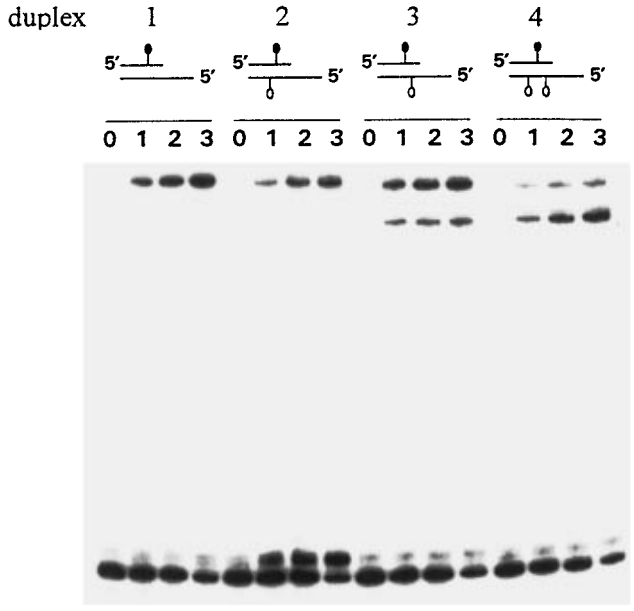

FIGURE 3: Kinetics of closure of the monofunctional adduct *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ within the four duplexes (20 bp). Autoradiogram of a denaturing 24% polyacrylamide gel. Lanes 0−3 refer to incubation times of 0, 9, 20, and 50 h, respectively.

(Figure 3). Depending upon the duplexes, the results are different. Concerning duplex **1**, there is only one new band which migrates much slower than the initial band. This indicates the formation of at least one kind of interstrand cross-link. Concerning duplex **2**, there are two new bands. One migrates slightly slower than the initial band, indicating the formation of one (or several) intrastrand cross-link(s), while the other migrates much slower, indicating the formation of an interstrand cross-link. With duplexes **3** and **4**, there are two new slowly migrating bands and thus at least two kinds of interstrand cross-links (Millard et al., 1991; Dalbies et al., 1994).

The rates of the disappearance of the *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ monofunctional adduct were deduced from the roughly linear plots of the logarithm of *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ percentages (calculated from the ratio of the intensity of the corresponding band to the sum of the intensity of all the bands) vs the reaction time (not shown). The $t_{1/2}$'s of the adduct within the four duplexes are on the same order of magnitude (24−43 h, see Table 1).

As a control, the same experiment was done with the four duplexes labeled at the 5′-end of the lower strands. Only slowly migrating bands indicating the formation of interstrand cross-links were detected (not shown).

Identification of the Interstrand Cross-Links. The nature of the interstrand cross-links within the four duplexes was determined by Maxam−Gilbert sequencing reactions. The piperidine treatment subsequent to the hydrazine attack results in the cleavage of the phosphodiester backbone at the level of the Py residues, including the platinated C

Table 1: Closure of the *trans*-DDP Monofunctional Adducts within the Platinated Duplexes[a]

duplex	$t_{1/2}$ (monoadduct)[b]	duplex	$t_{1/2}$ (monoadduct)[b]
1	43	**3**	32
1′	44	**3′**	51
2	26	**4**	24
2′	46	**4′**	81

[a] The kinetics were measured at 37 °C in 100 mM $NaClO_4$, phosphate buffer at pH 7.5 [b] The $t_{1/2}$ values (in hours) correspond to the times at which half of the monofunctional adducts have disappeared. Errors in measures are within 10%. The duplexes (**1**−**4** and **1′**−**4′**) contain 20 and 26 bp, respectively.

residues (Comess et al., 1990; Dalbiès et al., 1994). Thus, from the electrophoretic mobility of the fragments, all the Py residues up to the platinated residue should be detected at the expected positions, whereas those on the other side should not be detected because the interstrand cross-links were not removed. For the sake of clarity, only the results relative to duplex **4** (labeled at the 5′-end of the lower strand) are shown but the experiments have been done with all four duplexes. After incubation of duplex **4**, two slowly migrating products are formed (Figure 3), one (named ICL_1) migrating slower than the other (named ICL_2). The two products were eluted from the gel and treated with hydrazine or formic acid. As shown in Figure 4 (lane C+T left), all the bands up to the C12 residue are detected. Thus, within ICL_1, the G residue of the former *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ monofunctional adduct (upper strand) and the complementary C residue are cross-linked. This result is confirmed by the treatment with formic acid (lane G+A). A similar analysis of ICL_2 shows that the cross-link is between the G residue of the former *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ and the A8 residue of the lower strand (assuming the platination on the N7 and subsequently an increased stability of the residue in acidic medium). The fact that the platination occurs on the N1 of the A7 residue (which might make the residue sensitive to the acidic treatment) cannot be excluded. Analysis of the other slowly migrating products formed in duplexes **1**−**3** confirms that there are only two kinds of interstrand cross-links.

The identification of the cross-linked residues in the intrastrand cross-links formed within duplex **2** was not attempted.

Closure of trans-DDP Monofunctional Adducts within Longer Duplexes. The location of the adducts close to the 3′-end of the duplexes could amplify the distortions induced by the adducts and consequently interfere with the rate of the cross-linking reaction and the nature of the cross-links. The formation of the cross-links was studied within the same platinated duplexes but after elongation of the upper strand (this was achieved by template-directed ligation of the upper strand with ^{32}P-phosphorylated oligonucleotide a, see Figure 1). The four 26 bp duplexes (named **1′**−**4′**) were incubated at 37 °C. At various times, aliquots were withdrawn and analyzed by gel electrophoresis under denaturing conditions (Figure 5). As compared to those of the shorter duplexes, the rate of disappearance of *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ is not significantly changed except for duplex **4′** which contains three adducts (Table 1). In addition, there are two major differences. (i) Only one kind of interstrand cross-link is detected, and it results from the reaction of *trans*-$[Pt(NH_3)_2$-

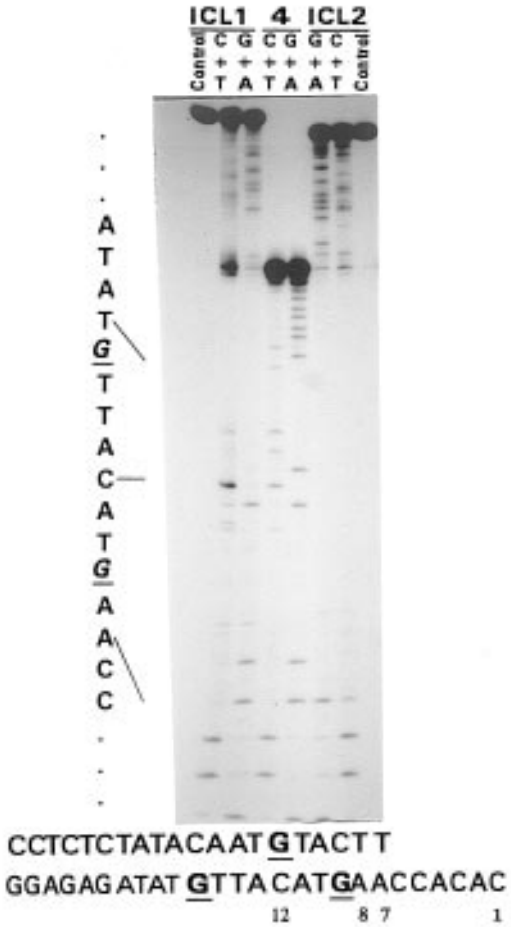

FIGURE 4: Identification of the interstrand cross-links resulting from the closure of the *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ monofunctional adducts within duplex **4**. The Maxam−Gilbert sequencing was done on the purified duplexes containing a single interstrand cross-link and 5′-end-labeled at the bottom strand. ICL_1 and ICL_2 refer to the upper and bottom interstrand cross-links shown in Figure 3, respectively. Four refers to the non-cross-linked duplex. Lanes C+T and G+A refer to the reactions with hydrazine and formic acid, respectively. Lanes Control refer to the untreated duplexes.

$(dG)Cl]^+$ with its complementary C residue. (ii) In duplex **4′**, intrastrand cross-links are formed, but slower than the interstrand cross-links.

DISCUSSION

The aim of this work was to understand the discrepancies relative to the formation of the bifunctional adducts in the reaction between *trans*-DDP and DNA (Eastman & Barry, 1987; Eastman et al., 1988; Bancroft et al., 1990; Boudvillain et al., 1995). They do not concern the first step of the reaction [formation of the monofunctional adduct *trans*-$[Pt(NH_3)_2(dG)Cl]^+$] but concern the second step (the rate of closure of the monofunctional adducts to bifunctional adducts and the nature of the adducts).

In one study (Eastman & Barry, 1987; Eastman et al., 1988), salmon testes DNA was platinated at an r_b of 0.01 (r_b is the molar ratio of bound platinum per nucleotide). The sample was enzymatically digested, and after HPLC separation, the products were analyzed by atomic absorption and NMR. The rearrangement of the monofunctional adducts to bifunctional adducts was only 80% complete in 48 h. The cross-links were between G and C (50%), G and G (40%), and G and A (10%). In another study (Boudvillain et al., 1995), a DNA restriction fragment (164 bp) platinated at an

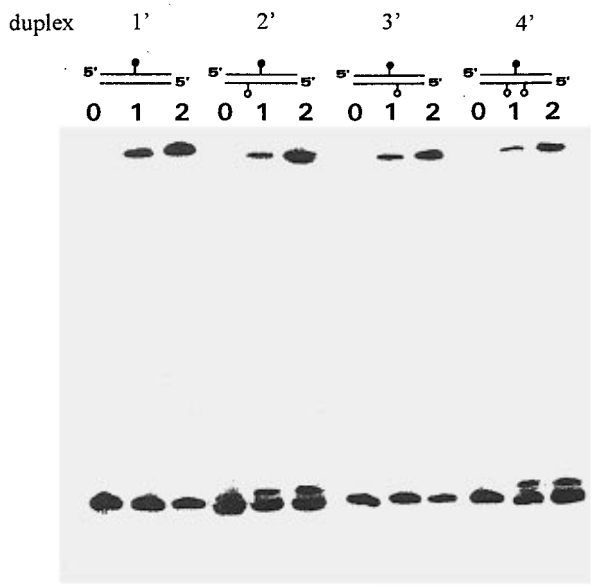

FIGURE 5: Kinetics of closure of the monofunctional adduct *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ within the four 26 bp duplexes. Autoradiogram of a denaturing 24% polyacrylamide gel. Lanes 0−2 refer to incubation times of 0, 24, and 48 h, respectively.

r_b of 0.005 was analyzed by atomic absorption and biochemical techniques. After 24 h of reaction, about 80% of the adducts were monofunctional and the cross-links were interstrand cross-links. In a third study (Bancroft et al., 1990), chicken erythrocyte DNA was enzymatically digested into double-stranded fragments (20−60 bp in length) which were platinated at an r_b of 0.07. The kinetics were investigated by ^{195}Pt NMR. The monofunctional adduct closure rate was fast ($t_{1/2}$ = 3.1 h). The results were in agreement with formation of 1,3- and longer range intrastrand cross-links. We expected that the differences in the cross-linking reactions originated in the DNA size and the level of platination rather than in the techniques. To test this, we have undertaken a study of the closure of a single *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ adduct within several double-stranded oligonucleotides. These duplexes differ in the length (20 or 26 bp), the location of the *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ adduct within the upper strand, and the nature of the lower strand which either is unplatinated or contains one or two inert monofunctional $[Pt(dien)(dG)]^{2+}$ adducts to mimic to some extent the presence of transplatin monofunctional adducts.

The conformation of the shorter duplexes (20 bp) has been studied by means of OsO_4 and DEPC. These chemical probes are very sensitive tools for detecting non-B conformation in DNA. Duplex **1** contains a single *trans*-$[Pt(NH_3)_2(dG)Cl]^+$. The 5′- and 3′-residues adjacent to the adduct are T residues. OsO_4 reacts with the 5′-T but not with the 3′-T. Similar results were obtained with duplexes containing a single *cis*-$[Pt(NH_3)_2(dG)Cl]^+$ or $[Pt(dien)(dG)]^{2+}$ monofunctional adduct (Brabec et al., 1992). This justifies, a posteriori, the use of $[Pt(dien)(dG)]^{2+}$ in mimicking the transplatin monofunctional adduct. When the duplexes contain the adducts [*trans*-$[Pt(NH_3)_2(dG)Cl]^+$ or $[Pt(dien)(dG)]^{2+}$], the double helices are more distorted but the distortions are different according to the relative locations of the adducts. However, in the four duplexes, the nonreactivity of the A·T bp at position 10 and the weak reactivity of the T·A bp at position 11 argue against a separation of the strands.

We now consider the kinetics relative to the two duplexes (20 and 26 bp) which contain a single *trans*-$[Pt(NH_3)_2(dG)Cl]^+$. The rate of closure of the transplatin monofunctional adduct and the nature of the bifunctional lesions are independent of the duplex length. The closure is slow ($t_{1/2}$ = 43 h, see Table 1) and yields one kind of bifunctional adduct which is an interstrand cross-link between the complementary G and C residues, confirming previous results (Brabec & Leng, 1993). The information gained from the present study and the previous results (Boudvillain et al., 1995) lead us to conclude that after 24 h of reaction between transplatin and double-stranded DNA, at low r_b, the major adducts are monofunctional adducts and the minor adducts are interstrand cross-links. Under the same experimental conditions, the closure of *cis*-$[Pt(NH_3)_2(dG)Cl]^+$ is fast ($t_{1/2}$ = 2 h) and yields mainly N7,N7-bidentate cross-links between adjacent purine residues [general reviews in Lepre and Lippard (1990), Reedijk (1992), and Sip and Leng (1993)]. This is a major difference in the behavior of the two isomers with respect to DNA. Taking into account the antitumor inactivity of dien-Pt, the results on the antitumor activity of cisplatin (Lepre & Lippard, 1990; McA'Nulty & Lippard, 1995), and the correlation between the successful response of patients to cisplatin chemotherapy and the formation of cisplatin bifunctional adducts (Fichtinger-Schepman et al., 1990, 1995), we propose in agreement with Eastman (1987) that the clinical inefficiency of transplatin originates at least in part from the slow closure of transplatin monofunctional adducts. In addition, *in vivo*, the efficiency of the interstrand cross-linking reaction is probably reduced because of the presence of glutathione which is known to react rapidly with transplatin monofunctional adducts (Eastman & Barry, 1987; Eastman et al., 1988; Bancroft et al., 1990).

We now consider the experiments on the same duplexes but containing in addition within the lower strands one $[Pt(dien)(dG)]^{2+}$ adduct located either 3 bp away from the *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ adduct and on its 5′-side or 2 bp away and on its 3′-side. The results differ according to the location of the adduct.

In the first case, whatever the length of the duplexes (20 or 26 bp), intrastrand and interstrand cross-links are formed. However, the relative yields of the cross-linking reactions depend upon the length of the duplexes. The interstrand cross-linking reaction is favored in the longer duplex, but the nature of the interstrand cross-links is independent of the length of the duplex (they are formed between the complementary G and C residues).

In the second case, whatever the length of the duplexes (20 or 26 bp), no intrastrand cross-links were detected. The distortions induced in the duplexes by the two adducts are such that the interstrand cross-linking reaction becomes preponderant. Surprisingly, in the shorter duplex, two kinds of interstrand cross-links are formed between the former platinated G residue (upper strand) and either its complementary C residue or a A residue located 3 or 4 bp away. The long range cross-link reflects a transient opening of the double helix and/or a bent structure which brought the reactive monofunctional adduct and the A residue in the right orientation and distance to react. Since in the longer duplex the long range cross-link is no longer formed, a transient opening of the double helix seems more likely.

From these results on the duplexes containing two adducts [*trans*-$[Pt(NH_3)_2(dG)Cl]^+$ and $[Pt(dien)(dG)]^{2+}$], we conclude that the two adducts distort the double helix sufficiently to allow the formation of several kinds of cross-links. Thus, in any attempt to compare the *in vitro* platination of DNA to the *in vivo* platination, one should work at a low r_b to obviate significant interaction between neighboring adducts. We think that this holds also for cisplatin but is masked by the preferential binding of cisplatin to G runs and the ability of the monofunctional adducts to react rapidly with adjacent purine residues. By contrast, in a monoadducted duplex, the rate of closure of the cisplatin monofunctional adduct into an intrastrand cross-link at the d(GTG) site is very slow ($t_{1/2}$ > 20 h) (Payet et al., 1993).

The last set of experiments concerning the duplexes containing two $[Pt(dien)(dG)]^{2+}$ adducts within the lower strand emphasizes the conjugate effects of neighboring adducts within short oligonucleotides. In the shorter duplex (20 bp), only long range interstrand cross-links are formed, and preferentially between the G and A residues separated by 3 (or 4) bp. In the longer duplex, intrastrand cross-links and interstrand cross-links (between the complementary G and C residues) are detected.

In conclusion, although a quantitative interpretation is still missing, these results show that the chemical reactivity of platinum(II) adducts can be modulated by factors acting on the local conformation and the dynamic structure of the DNA double helix. Insights into these aspects may facilitate the rational design of new platinum(II) derivatives possessing therapeutic properties. Among the new classes of platinum antitumor agents, dinuclear platinum complexes are of special interest because they show high activity *in vitro* and *in vivo* against various tumor cell lines (Farrell, 1993, 1996; Farrell et al., 1995). In the dinuclear complexes, the platinum residues are linked by a diamine chain. This chain, in close contact with the double helix, can modify locally the DNA dynamic structure (Sip & Leng, 1993). Changes in the nature of the dinuclear platinum complexes should interfere with the formation of the bifunctional adducts in the reaction between DNA and these complexes.

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