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DNA interstrand cross-links of *trans*-diamminedichloroplatinum(II) are preferentially formed between guanine and complementary cytosine residues

(SP6 and T7 RNA polymerases/transcription mapping/dimethyl sulfate)

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ABSTRACT **Bases in the opposite strands of DNA cross-linked by clinically ineffective *trans*-diamminedichloroplatinum(II) {*trans*-[Pt(NH$_3$)$_2$Cl$_2$]} have been identified by means of three experimental approaches. These include HPLC analysis of enzymatic digests of synthetic oligonucleotide duplexes containing the interstrand cross-link, footprinting experiments on the interstrand cross-linked oligonucleotide duplexes, and termination of the duplex transcription on *trans*-[Pt(NH$_3$)$_2$Cl$_2$]-treated fragments of plasmid DNA. The results reveal that deoxyguanine and complementary deoxycytosine residues are preferential binding sites of *trans*-[Pt(NH$_3$)$_2$Cl$_2$] in the interstrand adducts. The interstrand cross-linking reaction was studied by means of gel electrophoresis for the cis and trans isomers. The rate of formation of interstrand cross-links was lower for the trans isomer; however, *trans*-[Pt(NH$_3$)$_2$Cl$_2$] formed about twice the amount of interstrand cross-links as compared with the cis isomer after 48 hr. The present results are suggested to be relevant to differences in clinical activity of the two platinum(II) isomers.**

cis-Diamminedichloroplatinum(II) {*cis*-[Pt(NH$_3$)$_2$Cl$_2$]} is one of the most effective anticancer drugs. Numerous studies suggest that the cytotoxic action of *cis*-[Pt(NH$_3$)$_2$Cl$_2$] is related to its ability to react with cellular DNA (for general reviews, see refs. 1–4). Lesions produced in DNA have been characterized as bifunctional adducts, including mainly intrastrand and interstrand cross-links. Although the major lesion is an intrastrand cross-link between two adjacent dG residues, it is not yet established that this lesion is responsible for the antitumor activity of *cis*-[Pt(NH$_3$)$_2$Cl$_2$] (1–4).

Several studies have been devoted to the trans isomer, *trans*-[Pt(NH$_3$)$_2$Cl$_2$]. This compound is clinically ineffective. However, it binds to DNA and forms intrastrand and interstrand cross-links (1–4). Stereochemical limitations preclude *trans*-[Pt(NH$_3$)$_2$Cl$_2$] from forming intrastrand cross-links between adjacent base residues. Therefore, it has been speculated that the differences in antitumor activity of the two platinum(II) isomers may arise from the different nature of distortions induced in DNA by the intrastrand lesions.

Both isomers form DNA interstrand cross-links (ICLs). Although these lesions make up only a small fraction of all adducts, correlation between interstrand cross-linking by *cis*-[Pt(NH$_3$)$_2$Cl$_2$] and its cytotoxicity has been reported (5, 6). Recently, it was shown (7) that after *cis*-[Pt(NH$_3$)$_2$Cl$_2$] treatment several genes in the cells resistant to this drug and parental cells had similar initial contents of intrastrand and interstrand cross-links. However, the ICLs were removed more efficiently in the resistant than in the parental cell lines. These results suggest that acquired cellular resistance to *cis*-[Pt(NH$_3$)$_2$Cl$_2$] may be associated with an increased DNA repair efficiency of the ICLs (7). The ICLs of *cis*-[Pt(NH$_3$)$_2$Cl$_2$] are preferentially formed between guanine residues at the 5′-GC-3′ sites (8, 9). They bend the double helix by ≈55° toward the major groove, and the distortion is localized at the platinated sequence d(GC/GC) (10). The ICLs formed on DNA by *trans*-[Pt(NH$_3$)$_2$Cl$_2$] have been investigated less thoroughly. It is not yet even known which bases are involved in this adduct.

To address this point, we have undertaken the study of oligonucleotides and natural DNA containing an ICL of *trans*-[Pt(NH$_3$)$_2$Cl$_2$] by means of several techniques. The investigations reported here demonstrate that *trans*-[Pt(NH$_3$)$_2$Cl$_2$] cross-links complementary guanine and cytosine residues.

MATERIALS AND METHODS

The synthesis and purification of the single-stranded oligodeoxyribonucleotides (the top and bottom strands in the duplexes shown in Fig. 1) have already been described (11). Plasmid pSP73KB was prepared as described (9). SP6 and T7 RNA polymerases, ribonucleotide triphosphates, and RNasin ribonuclease inhibitor were from Promega. 3′-Deoxynucleotide triphosphates were purchased from Pharma–Waldhof (Düsseldorf, Germany). Restriction enzymes, Klenow fragment of DNA polymerase I, T4 DNA polymerase, and T4 polynucleotide kinase were from Boehringer Mannheim and Bethesda Research Laboratories. All radioactive products were from Amersham. Electrophoresis-grade acrylamide, *N,N′*-methylenebisacrylamide, agarose, sodium cyanide, thiourea, and dimethyl sulfate (DMS) were from Merck. *cis*- and *trans*-[Pt(NH$_3$)$_2$Cl$_2$] were from Lachema (Brno, Czech Republic). Monoaquamonochloro derivatives were generated by allowing *cis*- and *trans*-[Pt(NH$_3$)$_2$Cl$_2$] to react with AgNO$_3$ (12).

The oligonucleotide duplexes containing the ICL were prepared as follows. The single-stranded oligonucleotides (the top strands in Fig. 1) at a concentration of 0.12 mM were incubated with *trans*-[Pt(NH$_3$)$_2$Cl(H$_2$O)]$^+$ at an input platinum-to-strand molar ratio of 3.9 in 10 mM NaClO$_4$ (pH 5.2) at 37°C for 15 min. Then the NaCl concentration was adjusted to 0.1 M, and the platinated oligonucleotide was purified by ion-exchange chromatography (FPLC) in a gradient of 0.1–0.8 M NaCl (pH 7.4). The oligonucleotide contained one platinum atom as deduced from UV spectroscopic and polarographic measurements (13). The platinum atom was bound at the dG residue as verified by nonreactivity of DMS at this site (9). The platinated strands were allowed to anneal with unplatinated complementary strands in 0.4 M NaCl (pH

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Abbreviations: DMS, dimethyl sulfate; ICL, interstrand cross-link; r_i, formal drug-to-nucleotide ratio.

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d(TCGC)/d(GCGA): 5′ TCTCTCCTCTCGCTCTCCTTCT
AGAGGAGAGCGAGAGGAAGAAG 5′

d(TGCT)/d(AGCA): 5′ CTTCTCCTTGCTCTCCTTCTCT
AAGAGGAACGAGAGGAAGAGAG 5′

d(TCGT)/d(ACGA): 5′ CTTCCTCTCGTCTCCTTCTT
AAGGAGAGCAGAGGAAGAAG 5′

FIG. 1. Sequences of oligodeoxyribonucleotide duplexes investigated in the present study and their abbreviations.

7.4) at 25°C for 2 hr. After dialysis against 0.1 M $NaClO_4$ for 4 hr at 4°C, the samples were incubated for 20 hr in the dark at 37°C. The duplexes containing the ICL were purified by FPLC in a gradient of 0.1–0.8 M NaCl with 10 mM NaOH. The yield of this cross-linking reaction was ≈30%.

Maxam–Gilbert footprinting experiments were performed as described (9, 14).

For HPLC analysis, the duplexes containing the ICL were enzymatically digested. They were first cleaved by using 3′ → 5′ exonuclease activity of T4 DNA polymerase (1 unit per 40 nmol of the oligonucleotide under conditions specified by the manufacturer) and then treated with alkaline phosphatase. It was verified by gel electrophoresis that digestion to nucleosides was complete. The digests were analyzed by reversed-phase HPLC with a gradient of acetonitrile in ammonium acetate (15). The standard *trans*-$[Pt(NH_3)_2(dG)(dC)]^{2+}$ was prepared as described (16, 17).

Transcription with SP6 and T7 RNA polymerases and electrophoretic analysis of transcripts were performed according to the protocols recommended by Promega and in ref. 18. The (*Nde* I/*Hpa* I) restriction fragment (212 bp) from plasmid pSP73KB, which was used as a template for RNA polymerases, was modified with *cis*- or *trans*-$[Pt(NH_3)_2Cl_2]$ in 10 mM $NaClO_4$ at 37°C for 24 hr in the dark. The level of platination of DNA is described by means of a formal drug-to-nucleotide ratio (r_i).

RESULTS

trans-$[Pt(NH_3)_2Cl_2]$ binds to DNA in a two-step process, forming first a monofunctional adduct preferentially at the N-7 position of dG that subsequently closes to a bifunctional lesion (15–17, 19). To determine the nature of the second nucleoside involved in the ICL, two series of experiments were designed. In one series, single-stranded oligonucleotides containing a unique *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ monofunctional adduct were used as a starting material. These monoadducted oligonucleotides were hybridized with their complementary strands, and formation of the ICLs was examined. In another series of experiments, the ICLs were formed in the reaction of a fragment of natural double-stranded DNA with *trans*-$[Pt(NH_3)_2Cl_2]$ and then mapped by means of RNA polymerases.

Kinetics of Interstrand Cross-Linking in Oligonucleotide Duplexes. The single-stranded oligonucleotides referred to as d(TCGC), d(TGCT), and d(TCGT) (containing only one dG; see Fig. 1 for their complete sequences) were modified with *trans*-$[Pt(NH_3)_2Cl(H_2O)]^+$ so that the platinum moiety was attached monofunctionally to the dG residue. The monoadducted strands were hybridized with the corresponding bottom strands referred to as d(GCGA), d(AGCA), and d(ACGA), respectively, and the hybrids were incubated in 0.1 M $NaClO_4$ at 37°C. The aliquots were withdrawn at various time intervals and analyzed by gel electrophoresis under denaturing conditions. As shown in Fig. 2 for d(TGCT)/d(AGCA), only one band was observed for the non-cross-linked duplex. The subsequent incubation resulted in a new band migrating markedly more slowly. Its intensity increased with the incubation time with a concomitant decrease in the intensity of the band corresponding to the non-cross-linked duplex. An explanation for this observation is formation of the ICL. From the ratio of intensities of the two bands, the percentage of interstrand cross-linked duplexes was calculated. The $t_{1/2}$ of this interstrand cross-linking reaction is about 17 hr. Similar results (not shown) were obtained for d(TCGC)/d(GCGA) and d(TCGT)/d(ACGA).

Characterization of the Interstrand Cross-Linked Oligonucleotide Duplexes by HPLC. After a 20-hr reaction period, the d(TGCT)/d(AGCA), d(TCGT)/d(ACGA), and d(TCGC)/d(GCGA) duplexes containing the ICLs were purified by FPLC. The nature of the cross-linked residues was determined by HPLC analysis after complete enzymatic digestion of the oligonucleotides.

Examination of elution peaks in the HPLC profiles, identified by coinjection of standards (14, 16, 17), revealed the loss of one dC and one dG from the products and the presence of a new species. This species coeluted with the model compound *trans*-$[Pt(NH_3)_2(dG)(dC)]^{2+}$. These results indicate that the ICLs in the duplexes contained one dG (in the top strand) and one dC (in the bottom strand). Thus, the ICLs in the three duplexes (Fig. 1) are formed between dG and complementary dC residues.

Characterization of the Interstrand Cross-Linked Oligonucleotide Duplexes by Maxam–Gilbert Footprinting Experiments. To support further the latter conclusion, Maxam–Gilbert footprinting experiments were carried out on the three platinated duplexes. DMS is not expected to react with

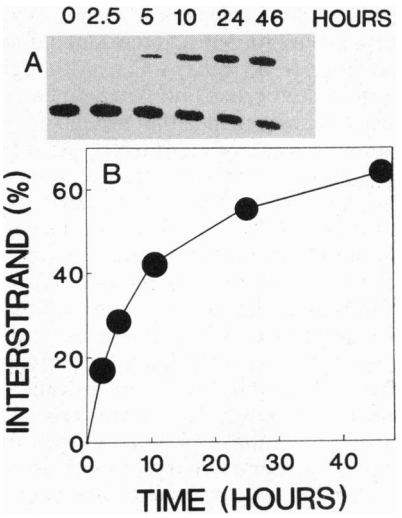

FIG. 2. Kinetics of the ICL formation in the duplex formed by mixing d(TGCT) uniquely monoadducted by *trans*-$[Pt(NH_3)_2Cl(H_2O)]^+$ at the dG residue and d(AGCA) at 37°C. (*A*) Autoradiogram of a 12% polyacrylamide/8 M urea denaturing gel of the duplex, whose bottom strand was 5′ end-labeled. The cross-linking reaction was stopped by adjusting the NaOH concentration to 10 mM and cooling the samples to 0°C. (*B*) The percentage of interstrand cross-linking was calculated from the ratio of the intensity of the band corresponding to the fragment containing the ICL (upper band in *A*) to the sum of the intensities of the two bands corresponding to the non-cross-linked and the cross-linked complexes.

platinated dG because the N-7 position is no longer accessible (20). Moreover, platination decreases the formic acid-catalyzed depurination (21) and thus the subsequent cleavage by piperidine.

The duplexes containing the ICL were ^{32}P-labeled at both 5′ ends and treated with DMS. The labeling of the two strands represented no disadvantage because the top strand contained only one dG, which was platinated so that it was resistant to the DMS attack. Consequently, the results shown in Fig. 3 are relative to the cleavage of the bottom strands. After subsequent treatment with piperidine, all dG residues on the 5′ side of the dC* (dC* indicates the single dC complementary to the platinated dG) were detected (Fig. 3, lanes Pt). In contrast, the fragments formed by the cleavage at the dG residues on the 3′ side of the dC* were not detected at their expected positions (the fragments generated by cleavage at the dG residues on the 3′ side of the ICL in the bottom strand migrated slower than the uncleaved bottom strand because they were cross-linked to the upper strand). If the platinum was removed from DMS-treated duplexes by NaCN (before the treatment with piperidine), all dG residues were revealed at the expected positions (Fig. 3, lanes Pt/NaCN). These results prove that no guanine residue is involved in the closure of the monofunctional adduct to the ICL. Similar experiments were also performed with the duplexes treated with formic acid (data not shown). They demonstrated that the ICLs contained no purine residues on the bottom strand. Thus, the analysis by Maxam–Gilbert sequencing procedures has confirmed that platinum in ICLs of *trans*-$[Pt(NH_3)_2]^{2+}$ is coordinated to complementary dG and dC residues.

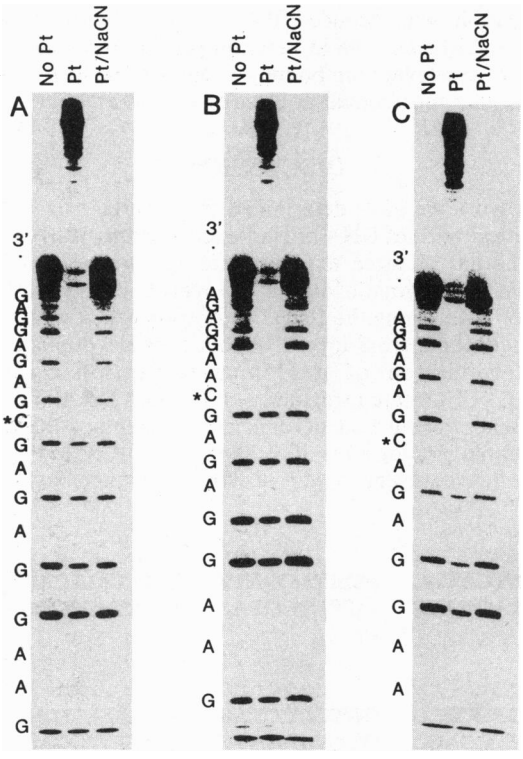

FIG. 3. Piperidine-induced specific strand cleavage at DMS-modified bases in the duplexes containing an ICL formed by mixing d(TCGC) (*A*), d(TGCT) (*B*), and d(TCGT) (*C*) uniquely monoadducted by *trans*-$[Pt(NH_3)_2Cl(H_2O)]^+$ at the dG with unplatinated d(GCGA), d(AGCA), and d(ACGA), respectively. The bottom strands in unplatinated duplexes (lanes No Pt) were 5′ end-labeled in contrast to both 5′ end-labeled strands in the cross-linked samples (lanes Pt and Pt/NaCN). The samples shown in lanes Pt/NaCN were, after modification by DMS, treated with 0.2 M NaCN at 45°C overnight. The base sequence of the bottom strand is shown on the left side of each panel; the star designates dC opposite to the platinated dG in the top strand.

Stability of the ICL in Oligonucleotide Duplexes. The ICL in the duplexes is stable under normal conditions. No disappearance of the ICLs was observed after incubation of the duplexes at 25°C in 0.5 M NaCl for at least 48 hr and in 10 mM thiourea (pH 7) for 10 min (16). They were, however, quantitatively removed by incubating platinated oligonucleotides in 0.1 M thiourea for 10 hr at 25°C in 0.2 M NaCN (basic pH) for 10 hr at 45°C.

Mapping of the Interstrand Cross-Links in the 212-bp DNA Fragments. Further investigations were aimed at finding the sites in natural DNA in which ICLs were formed during the reaction with *trans*-$[Pt(NH_3)_2Cl_2]$. Recent work has shown that the *in vitro* RNA synthesis by RNA polymerases on *cis*-$[Pt(NH_3)_2Cl_2]$-modified DNA template is terminated at the level of the adducts (9, 22, 23). A similar approach was employed in the present work.

Cutting of pSP73KB DNA by *Nde* I and *Hpa* I endonucleases yielded a 212-bp fragment containing SP6 and T7 RNA polymerase promotors directed toward each other from opposite ends of the fragment. Preliminary experiments were carried out using this fragment for RNA synthesis by SP6 or T7 RNA polymerases. RNA synthesis on the fragment modified by *trans*-$[Pt(NH_3)_2Cl_2]$ at $r_i = 0.01$ and treated with 10 mM thiourea for 10 min at 25°C yielded RNA fragments of defined sizes (Fig. 4 *Left*, lane Trans-Pt). This result indicates

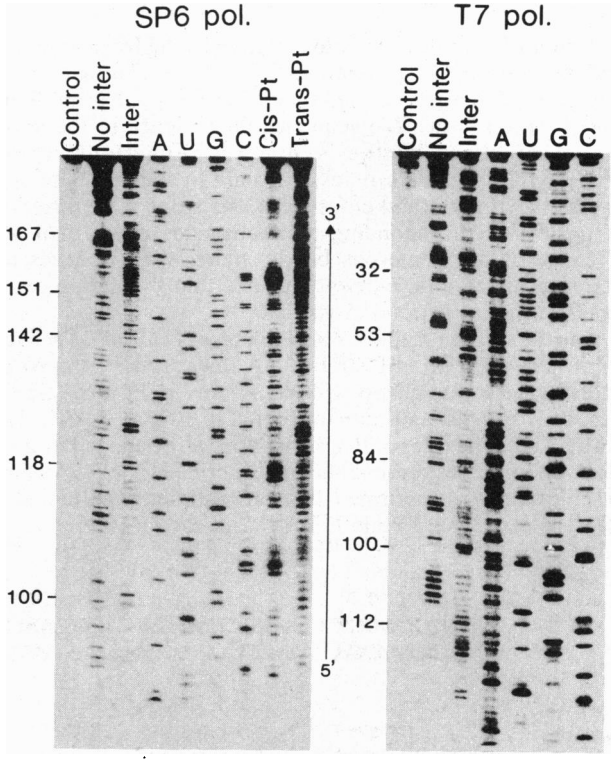

FIG. 4. Autoradiograms of 6% polyacrylamide/8 M urea sequencing gels showing inhibition of RNA synthesis by SP6 (*Left*) and T7 (*Right*) RNA polymerases on *trans*-$[Pt(NH_3)_2Cl_2]$-modified (*Nde* I/*Hpa* I) fragments. After reaction with *trans*-$[Pt(NH_3)_2Cl_2]$ at $r_i = 0.01$, the fragments with and without ICLs were separated. Lanes: Control, relative to the unplatinated template; No inter, relative to the DNA without interstrand adducts; Inter, relative to the DNA with interstrand adducts; A, U, G, and C are relative to chain-terminated marker RNAs; Cis-Pt, relative to the fragment modified by *cis*-$[Pt(NH_3)_2Cl_2]$ at $r_i = 0.01$; Trans-Pt (only in the left figure), relative to the fragment modified by *trans*-$[Pt(NH_3)_2Cl_2]$ at $r_i = 0.01$ and subsequently treated with 10 mM thiourea at 25°C for 10 min (in the latter two samples, no separation of the fragments with and without ICLs was performed).

that T7 (data not shown) and SP6 RNA polymerases are sensitive to the bifunctional adducts of *trans*-$[Pt(NH_3)_2Cl_2]$. The patterns of stops were independent of the values of r_i in the range of 0.002–0.02.

To determine the location of the ICLs of *trans*-$[Pt(NH_3)_2Cl_2]$, transcription mapping studies were carried out by using two types of the platinated 212-bp fragment as the template. One type of fragment contained exclusively intrastrand cross-links; the other contained principally ICLs.

In a preliminary experiment, the ICLs formed during 24 hr at 37°C as a function of r_i were quantitated by gel electrophoresis under denaturing conditions (9). About 50% of the fragments modified at $r_i = 0.01$ contained at least one ICL.

The 212-bp fragment was modified at $r_i = 0.01$ and then treated with 10 mM thiourea for 10 min at 25°C, and the fragments with and without ICLs were separated on a denaturing 2% agarose gel. The gel was neutralized and the two types of fragments were eluted. After one heating/reannealing cycle, the fragments were used as the templates for RNA synthesis by SP6 or T7 RNA polymerases.

Strong and medium intensity bands observed specifically for the template with ICLs (Fig. 4, lanes Inter) were taken to indicate the sites of preferential formation of the ICL. They all occurred at the level of dG and dC. This was confirmed by the analysis of the potential binding sites located between the two promoters. Bands corresponding to the positions 100, 112, 117, 118, 123, and 124 were found with the two RNA polymerases. These positions correspond to the dG·dC base pair.

A summary of the stop sites relative to the fragments with and without ICLs is given in Fig. 5. In contrast to the bifunctional adducts formed by *cis*-$[Pt(NH_3)_2Cl_2]$ (ref. 9 and Fig. 4, lane Cis-Pt), the sequence dependence of the termination of RNA synthesis due to bifunctional *trans*-$[Pt(NH_3)_2Cl_2]$ adducts is less regular. In the template with exclusively intrastrand cross-links, the stops were observed at positions corresponding to nucleosides other than dG. Because of the numerous bands, more work is necessary before drawing a definitive conclusion on the nature of the bases in the intrastrand cross-links.

Kinetics of Interstrand Cross-Linking of Natural DNA Fragment. The 2455-bp pSP73KB DNA linearized by the *Nde* I endonuclease was mixed with *trans*- or *cis*-$[Pt(NH_3)_2(H_2O)Cl]^+$ at $r_i = 0.001$ and then incubated in 10 mM $NaClO_4$ (pH 5.4) at 37°C. The rates of ICL formation were deduced from gel electrophoresis experiments as described above. Polarographic analysis confirmed that all platinum was bound to DNA in less than 30 min (13, 19). As shown in Fig. 6, the

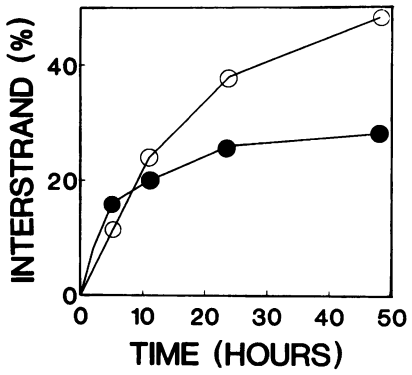

FIG. 6. Kinetics of the interstrand cross-linking in the 2455-bp pSP73KB DNA linearized by the *Nde* I restriction enzyme by *cis*- (●) and *trans*- (○) $[Pt(NH_3)_2Cl(H_2O)]^+$ in 10 mM $NaClO_4$ at 37°C at $r_i = 0.001$. The samples were analyzed on a denaturing 1% agarose gel. After a few hours of reaction, two bands were visible on the autoradiogram corresponding, respectively, to the fragments with and without ICLs. The percentage of interstrand cross-linking is as defined in the legend of Fig. 2.

interstrand cross-linking by the trans isomer was not completed even after 48 hr. The $t_{1/2}$ of this reaction is >11 hr. Identical results were obtained if the samples were treated with 10 mM thiourea (pH 7) for 10 min at 25°C before the gel electrophoresis. In contrast to *trans*-$[Pt(NH_3)_2Cl_2]$, the kinetics of interstrand cross-linking by the cis isomer was markedly faster ($t_{1/2}$ was about 4 hr). Assuming one ICL per DNA molecule, it was deduced that the ICLs formed by *cis*-$[Pt(NH_3)_2Cl_2]$ after a 48-hr reaction period represented about 6% of the total platinum bound, whereas ICLs formed by the trans isomer represented at least twice that amount.

DISCUSSION

In this work we have determined the nature of bases in the opposite strands of DNA cross-linked by *trans*-$[Pt(NH_3)_2Cl_2]$ with the aid of three experimental approaches: (*i*) HPLC analysis of enzymatic digests of synthetic oligonucleotide duplexes containing the ICL, (*ii*) footprinting of platinum on the interstrand cross-linked oligonucleotide duplexes, and (*iii*) termination of duplex transcription on *trans*-$[Pt(NH_3)_2Cl_2]$-treated fragments of plasmid DNA. The three approaches reveal that dG and complementary dC are the preferential binding sites of *trans*-$[Pt(NH_3)_2Cl_2]$ in the ICLs. This result represents a very striking difference between the

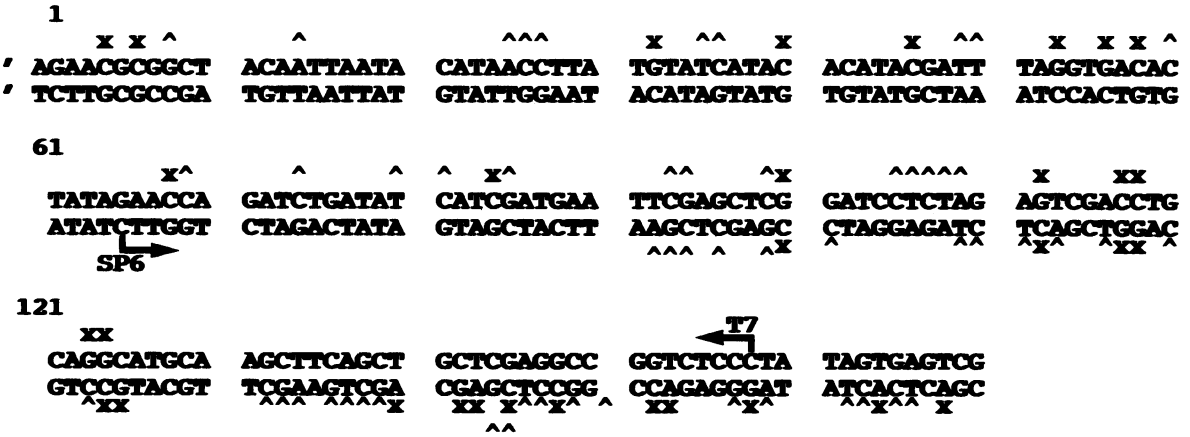

FIG. 5. Schematic diagram showing a portion of the sequence used to monitor inhibition of RNA synthesis by *trans*-$[Pt(NH_3)_2Cl_2]$. The arrows indicate the start sites of the two polymerases. x and ∧, Stop signals from Fig. 4 for T7 RNA polymerase (upper lines) and SP6 RNA polymerase (lower lines). x, Interstrand adducts; ∧, intrastrand adducts. Nucleotides 1 and 65 correspond, respectively, to nucleotides 2492 and 1 on the pSP73KB nucleotide sequence map.

effects of the two isomers; the ICLs of *cis*-$[Pt(NH_3)_2Cl_2]$ occur between two opposite dG residues within d(GC)/d(GC) sites (8, 9).

Recently, it was concluded from the NMR studies that the rate constants for closure of monofunctional adducts to bifunctional cross-links were of the same order of magnitude for both the cis and trans isomer (19). The reaction was nearly complete within 12 hr. Our results agree with this conclusion for the cis isomer. Interestingly, the kinetics of the reaction is markedly slower in the case of interstrand cross-linking by the trans isomer. The reaction has not yet been completed even after 48 hr. This observation could explain why, in the *in vivo* reaction, less ICLs are formed by *trans*-$[Pt(NH_3)_2Cl_2]$ than by its cis isomer. In cells, compounds such as glutathione are good candidates to trap monofunctional platinum adducts.

In spite of the greater $t_{1/2}$ observed *in vitro*, *trans*-$[Pt(NH_3)_2Cl_2]$ forms about double the quantity of ICLs in comparison with the cis isomer. Nevertheless, the ICLs are not the prominent lesions since they represent 10–20% of the total platinum bound to DNA. In the first step of its reaction with DNA, *trans*-$[Pt(NH_3)_2Cl_2]$ binds preferentially to dG (15–17). Subsequently, the monofunctional adduct could always react with the complementary dC and form the ICL. In reality, it also reacts with another residue in the same strand, and it is likely (1, 3) that cross-links are formed (16, 17).

In B-DNA, the distance between the two N-7 positions of purine (Pu) residues within (PuXPu) sequence (X is any base) is about 0.83 nm, which seems unfavorable for the cross-linking reaction. Thus, the amount of ICLs of *trans*-$[Pt(NH_3)_2Cl_2]$ could mean that they are formed only within defined sequences. Analysis of the results summarized in Fig. 5 reveals no simple rule for this preference. Interestingly, molecular mechanics modeling (V.B., M. Sip, and M.L., unpublished data) reveals that the platinated dG in the ICL of *trans*-$[Pt(NH_3)_2Cl_2]$ adopts a syn conformation. In left-handed Z-DNA, dG adopts a syn conformation (24). Thus, sequences known to favor the B-DNA → Z-DNA transition could be preferred for the formation of ICLs by *trans*-$[Pt(NH_3)_2Cl_2]$. Although several d(GC) and d(TG) sites are involved in this cross-linking reaction, not all of these sequences are reactive. Moreover, d(GG) sequences, which are unfavorable for the B-DNA → Z-DNA transition, are also involved in the ICLs. It is, therefore, likely that factors other than only a tendency of a DNA segment to adopt a Z-conformation are involved in interstrand cross-linking of DNA by *trans*-$[Pt(NH_3)_2Cl_2]$.

In conclusion, *cis*- and *trans*-$[Pt(NH_3)_2Cl_2]$ behave quite differently with respect to the nature of the cross-linked bases and the rates of closure of the monofunctional adducts to the ICLs. These results may have relevance to the differences in the clinical activity of the two platinum(II) isomers.

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