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Biochemistry

DNA double helix promotes a linkage isomerization reaction in *trans*-diamminedichloroplatinum(II)-modified DNA

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ABSTRACT **In the reaction between *trans*-diamminedichloroplatinum(II) and a single-stranded pyrimidine-rich oligodeoxyribonucleotide (22-mer) containing the central sequence TGAGT, the 1,3-*trans*-{Pt(NH$_3$)$_2$[d(GAG)]} cross-link is formed. The 1,3-intrastrand cross-link is inert within the single-stranded oligonucleotide. In contrast, it rearranges to an interstrand cross-link when the platinated oligonucleotide is paired with its complementary deoxyribo- or ribonucleotide strand. The half-life of the 1,3-intrastrand cross-link, ≈6 h at 37°C, is independent of the nature and concentration of the salt (NaCl or NaClO$_4$). It is not dramatically affected when the intervening adenine residue between the chelated guanine residues is replaced by a cytosine or a thymine residue or when the T·A base pair adjacent to the 5′ or 3′ side of the adduct is replaced by a C·G base pair. On the other hand, a mismatch on the 3′ or 5′ side of the adduct prevents the rearrangement. We propose that the linkage isomerization reaction results from a direct nucleophilic attack of the cytosine residue complementary to the platinated 5′ guanine residue on the platinum residue. Among others, the potential use of the DNA·RNA-promoted reaction is discussed in the context of the antisense strategy to irreversibly cross-link the antisense oligonucleotides to their targets.**

cis-Diamminedichloroplatinum(II) (*cis*-DDP) is a chemotherapeutic agent used largely in the clinical treatment of tumors. It is generally accepted that the cytotoxic action of *cis*-DDP is related to its ability to bind to cellular DNA and form covalent adducts. Although the major lesion is an intrastrand cross-link between two guanine (G) residues, it is not yet established that this lesion is responsible for the antitumor activity of *cis*-DDP (for general reviews, see refs. 1–4). Acquired resistance to *cis*-DDP may be associated with the increased gene-specific DNA repair efficiency of the interstrand adducts (5), a minor lesion in which the two G residues in the d(GC)·d(GC) site are cross-linked (6, 7).

A question still under debate concerns *trans*-dichlorodiammineplatinum(II) (*trans*-DDP), the stereoisomer of *cis*-DDP. This compound is less mutagenic and less cytotoxic than *cis*-DDP and is ineffective as an antitumor drug (1–4). In their reaction with DNA, the two isomers present similarities and differences (for general reviews, see refs. 8 and 9). The DNA modification by *cis*- or *trans*-DDP is controlled kinetically and the adducts are formed in two solvent-assisted reactions. The exchange of the chloro groups of both isomers is rate-limiting in both the initial attack of DNA and the closure of monofunctional adducts to bifunctional cross-links. The preferred site of initial binding of the isomers is the N7 atom of G residues. Subsequently, the monofunctional adducts react with the neighboring bases to form intrastrand and interstrand cross-links. Among the differences between the two isomers, one is that stereochemical limitations preclude *trans*-DDP from forming intrastrand cross-links between adjacent base residues (10). The most prevalent adducts are intrastrand cross-links between two G residues and between G and cytosine (C) residues separated by at least one residue (11–13). Another difference is that *trans*-DDP forms the interstrand cross-links preferentially between complementary G and C residues (14).

Initially, our purpose was to compare systematically the distortions induced in DNA by the binding of the two isomers and the interactions between *cis*- or *trans*-DDP-modified DNA and proteins to understand the biological processing of platinum-DNA adducts. In the course of the work, we discovered a linkage isomerization reaction promoted by the DNA double helix in *trans*-DDP-modified DNA. We now report that the 1,3-intrastrand cross-links between *trans*-DDP and two G residues separated by one intermediate base are stable within single-stranded (ss) oligonucleotides and become unstable as soon as the platinated oligonucleotides are paired with their complementary strands. The 1,3-intrastrand cross-links rearrange to form interstrand cross-links. This reaction might be useful for several purposes such as the design of *trans*-platinum derivatives forming preferentially intrastrand or interstrand cross-links or an antisense strategy to irreversibly cross-link the antisense oligonucleotides to their targets.

MATERIALS AND METHODS

The oligodeoxyribonucleotides (22-mer) were synthesized and purified as described (15). They were either pyrimidine-rich (top strands) or purine-rich (bottom strands). All the top strands had the same sequence except for the 5 central bases. The central sequence was PyGXGPy in which X is T, C, or A and Py is T or C. The bottom strands were complementary to the top strands. The sequence of one duplex is given in Fig. 1. The oligoribonucleotide r(AAGGAGACACAGAGGAGA) was from Genset (Paris); purity was >95% as assessed by gel electrophoresis. Endonuclease P1, T4 DNA polymerase, alkaline phosphatase, and T4 polynucleotide kinase were from Boehringer Mannheim. The radioactive products were from Amersham. All chemicals were from Merck except *trans*-DDP, which was from J. Matthey (Reading, U.K.) or Sigma.

The oligonucleotides containing the 1,3-*trans*-{Pt(NH$_3$)$_2$[d(GXG)]} cross-links were prepared as described (16, 17) with minor modifications. The ss oligonucleotides at 30 μM were incubated with *trans*-DDP at platinum/oligonucleotide molar ratio of 1 in 10 mM NaClO$_4$/4 mM acetate, pH 3.6, at 37°C for 24 h. Then, they were incubated in 10 mM thiourea at 37°C for 10 min to remove the monofunctional adducts (16, 18). The oligonucleotides containing the 1,3-intrastrand cross-links were purified by FPLC with a 0.2–0.7 M NaCl gradient. The platinum was bound at the G residues as verified by nonreactivity of dimethyl sulfate (DMS) at these sites (7). The nature of the adducts was verified by reverse-

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Abbreviations: DDP, diamminedichloroplatinum(II); ss, single stranded; ds, double stranded; DMS, dimethyl sulfate.

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          11 13
5' TCTCTCCTCTGAGTCTCCTTCT
   AGAGGAGACTCAGAGGAAGAAG 5'

d(TGAGT)·d(ACTCA)
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FIG. 1. Sequence and abbreviation (boldface type) of one of the oligodeoxyribonucleotide duplexes investigated in this study.

phase HPLC analysis of the digests after enzymatic hydrolysis with endonuclease P1 and then with alkaline phosphatase (19–21). The standard *trans*-$[Pt(NH_3)_2(dG)_2]^{2+}$ and *trans*-$[Pt(NH_3)_2(dG)(dC)]^{2+}$ were prepared as described (22). Oligonucleotides d(TGTGT) and d(TGAGT) containing the monofunctional adduct *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ were obtained by reacting the oligonucleotides at 30 μM with the monoaquomonochloro species [generated by allowing *trans*-DDP to react with $AgNO_3$ (9)] at a platinum/oligonucleotide molar ratio of 5 in 10 mM $NaClO_4$/4 mM acetate, pH 3.6, at 37°C for 0.5 h (14). The platinated oligonucleotides were purified by FPLC. As assessed by gel electrophoresis, purity was ≈90 and 85% for the platinated d(TGAGT) and d(TGTGT), respectively. (The unplatinated oligonucleotides were not completely removed.) The two G residues were approximately equally modified by the monoaquomonochloro species as verified by the reactivity of DMS at these sites.

To study the stability of the 1,3-*trans*-{$Pt(NH_3)_2$[d(GXG)]} cross-links within double-stranded (ds) oligonucleotides, the platinated strands at 20 μM were allowed to anneal with the unplatinated complementary strands in 100 mM $NaClO_4$/5 mM Tris·HCl, pH 7.5/0.1 mM EDTA at 20°C for 0.5 h and then for 2 h at 4°C. The duplexes at 2 μM (or higher) were incubated at 37°C. At various times, aliquots were withdrawn and analyzed by gel electrophoresis (denaturing 24% polyacrylamide/8 M urea gel). The nature of the bases in the interstrand cross-links was determined by reverse-phase HPLC analysis after digestion of the sample with T4 DNA polymerase (14) or endonuclease P1 (19–21) and then with alkaline phosphatase. The location of the interstrand cross-link were deduced from Maxam–Gilbert footprinting experiments (7, 14, 23, 24).

Quantitation of the gel bands was done on a Molecular Dynamics PhosphorImager using IMAGEQUANT software version 3.3 for data processing.

RESULTS

Recent studies have shown that the intrastrand chelates of two G residues separated by 1 base are formed in the reaction between *trans*-DDP and ss oligonucleotides containing the GAG or GTG sequences and that these chelates do not prevent the pairing of the platinated oligonucleotides with their complementary strands (16, 17). Thus, it is possible to compare the instability of the 1,3-intrastrand chelates within a ss or a ds oligonucleotide.

Instability of the 1,3-*trans*-{$Pt(NH_3)_2$[d(GAG)]} Cross-Link. The ss oligonucleotide d(TGAGT) (a 22-mer referred by its central sequence, Fig. 1) containing the single 1,3-*trans*-{$Pt(NH_3)_2$[d(GAG)]} cross-link and the corresponding duplex were incubated in 200 mM $NaClO_4$ at 37°C. At various times, aliquots were withdrawn and analyzed by gel electrophoresis under denaturing conditions.

The adduct within the ss oligonucleotide was inert over a long period of time; as shown in Fig. 2, only one band is present that migrates more slowly than the unplatinated oligonucleotide. As verified by complementary experiments (chemical probes and HPLC analysis), no rearrangement of the 1,3-intrastrand cross-link occurred even after a 24-h incubation. In contrast, the adduct within the duplex was

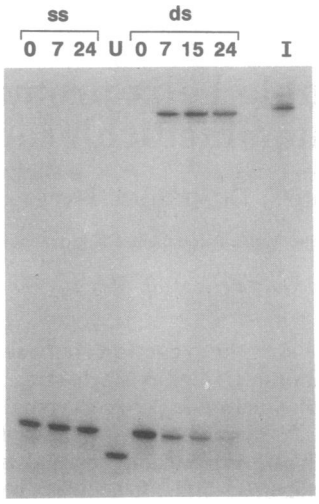

FIG. 2. Instability of the 1,3-*trans*-{$Pt(NH_3)_2$[d(GAG)]} cross-link within ss and ds oligonucleotides. Autoradiogram of a denaturing 24% polyacrylamide gel of the 5′-end-labeled d(TGAGT) containing the 1,3-*trans*-{$Pt(NH_3)_2$[d(GAG)]} cross-link (ss) and of the same oligonucleotide plus its complementary strand (ds). The samples (2–20 μM) were incubated at 37°C in 200 mM $NaClO_4$/10 mM Tris·HCl, pH 7.5/0.1 mM EDTA. Incubation times in hours are indicated above the lanes. The lane U corresponds to the unplatinated d(TGAGT). The lane I refers to d(TCTCTCCTCTCGCTCTCCTTCT)·d(GAAGAAGGAGAGCGAGAGGAGA) containing an interstrand cross-link in its central sequence (14). The lanes do not necessarily contain the same amount of labeled compounds.

labile. As a function of time, the intensity of the band corresponding to the 1,3-intrastrand cross-link decreased as a new band appeared (Fig. 2). This new band migrated much more slowly than the starting material but at approximately the same rate as the 22-bp duplex d(TCGC)·d(GCGA) containing a single interstrand cross-link [this cross-link resulted from the reaction between the oligonucleotide and *trans*-DDP carried out as described (14)]. No other bands were detected. Similar results were obtained at higher concentrations of the platinated duplex, which excludes an interduplex reaction. A reasonable explanation is that the 1,3-intrastrand cross-link is transformed into an interstrand cross-link. The plot of the 1,3-intrastrand cross-link percentages (logarithmic scale) as a function of time is roughly linear (Fig. 3). The $t_{1/2}$ of the 1,3-intrastrand cross-link within d(TGAGT)·d(ACTCA) was ≈6 h.

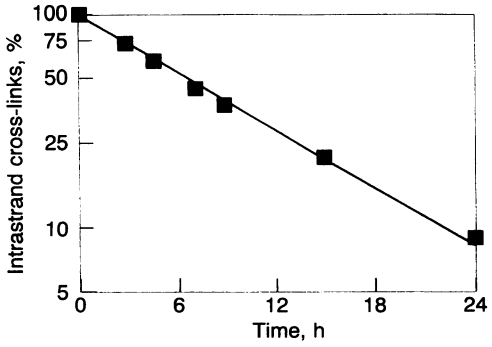

FIG. 3. Rearrangement of the 1,3-*trans*-{$Pt(NH_3)_2$[d(GAG)]} cross-link to an interstrand cross-link. Plot of the 1,3-*trans*-{$Pt(NH_3)_2$[d(GAG)]} cross-link percentages (logarithmic scale) vs. time. The ds oligonucleotide d(TGAGT)·d(ACTCA) containing the 1,3-*trans*-{$Pt(NH_3)_2$[d(GAG)]} cross-link in the top strand was incubated in conditions given in Fig. 2. The percentage of the 1,3-intrastrand cross-link was calculated from the ratio of the intensity of the lower band (Fig. 2) to the sum of the intensity of the two bands.

Identification of the Interstrand Cross-Link. To identify the bases in the interstrand cross-link resulting from the rearrangement of the 1,3-intrastrand cross-link, two sets of experiments were carried out on the product eluted from the slower migrating band (Fig. 2).

HPLC analysis of the enzymatic digestion products of the oligonucleotide revealed the presence of a product that cochromatographed with the standard *trans*-$[Pt(NH_3)_2(dG)(dC)]^{2+}$, indicating that the platinum residue cross-links G and C residues.

The location of the interstrand cross-link was deduced from Maxam–Gilbert footprinting (7, 16, 23). The sample in which the upper strand was 5′-end-labeled with ^{32}P was reacted with DMS. The adducts were removed by NaCN (24, 25) and then the sample was treated with piperidine. The upper strand contains two G residues at positions 11 and 13, respectively. In the unplatinated d(TGAGT), the two G residues were reactive with DMS (Fig. 4*A*, lane 1). They were no longer reactive in d(TGAGT) containing the 1,3-intrastrand cross-link (Fig. 4*A*, lane 2). After the linkage isomerization reaction, G11 but not the G13 was protected from reaction with DMS (Fig. 4*A*, lane 3).

The samples in which the lower strand was 3′-end- or 5′-end-labeled with ^{32}P were reacted with hydrazine or formic acid. (The samples were not subjected to a cyanide reversal step before piperidine treatment.) 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 residues (24). Thus, from the electrophoretic mobility of the fragments, all the Py residues up to the platinated one, on the ^{32}P-labeled side of the interstrand cross-link, 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. Similarly, formic acid and piperidine were used to cleave at the level of the nonplatinated Pu (purine) residues [platinated Pu residues are more resistant to the formic acid-catalyzed depurination (26)]. Analysis of the results in Fig. 4 *B* and *C* shows that the complementary G11 and C residues are cross-linked.

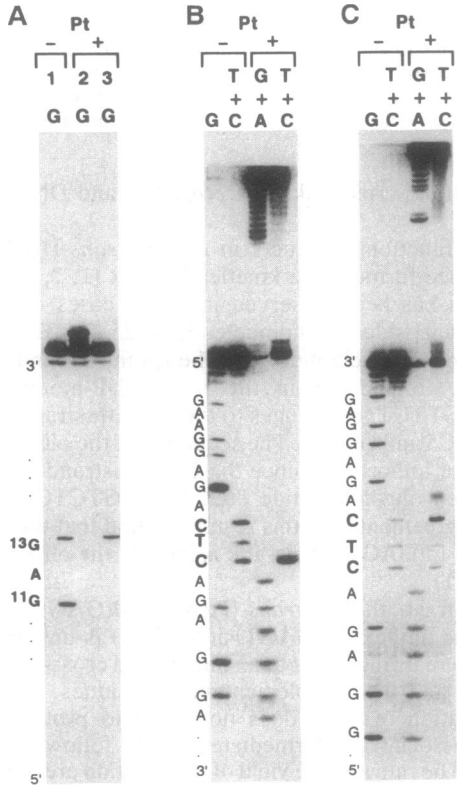

FIG. 4. Maxam–Gilbert footprinting experiments. Autoradiograms of denaturing 24% polyacrylamide gels of the products of the reaction between DMS, formic acid, or hydrazine and d(TGAGT)·d(ACTCA) containing an interstrand cross-link. Lanes G, G+A, and T+C are Maxam–Gilbert-specific reaction products for d(TGAGT)·d(ACTCA). (*A*) Reaction with DMS. The d(TGAGT) strand is 5′-end-labeled. Lanes: 1, unplatinated d(TGAGT); 2, d(TGAGT) containing the 1,3-intrastrand cross-link; 3, d(TGAGT)·d(ACTCA) containing the interstrand cross-link. Prior to the piperidine cleavage step, the samples were incubated in 0.2 M NaCN (basic pH) at 37°C overnight. (*B* and *C*) Reaction with formic acid or hydrazine. The d(ACTCA) strand was 3′-end-labeled (*B*) or 5′-end-labeled (*C*). In *B* and *C*, the samples were not treated with cyanide ions.

Factors Interfering with the Linkage Isomerization Reaction. *Salt.* The results presented in Fig. 2 were obtained in 200 mM $NaClO_4$/5 mM Tris·HCl, pH 7.5. The isomerization reaction was studied as a function of pH and of the salt concentration. In a first approximation, the reaction rate was independent of the pH of the solution, in 5 mM phosphate or Tris buffer, between pH 6.5 and pH 7.5. The reaction rate was also independent of the salt concentration from 50 to 500 mM and was the same in NaCl and $NaClO_4$. The conclusion is that during the rearrangement of the 1,3-*trans*-{$Pt(NH_3)_2$[d(GAG)]} cross-link, no intermediate species were trapped by chloride ions.

Neighboring bases. Several duplexes containing a single 1,3-intrastrand cross-link were studied. The general formula of the duplexes were d(PyGXGPy)·d(PuCYCPu) in which X and Y stand for A, T, and C. The duplexes were incubated in 200 mM $NaClO_4$ and at 37°C. The results (data not shown) resembled those presented in Fig. 2. As a function of time, the intensity of the band corresponding to the 1,3-intrastrand cross-link decreased while a new band (or two new close bands) of much slower mobility appeared. There were two new bands when the lower strand was d(ACACA). In all the interstrand cross-links, the platinum residue was linked to G11. For the duplexes d(TGXGT)·d(ACACA), two interstrand cross-links were formed. In the major one, G11 was linked to the complementary C. In the minor one, there is evidence that G11 is linked to the adenine (A) residue opposite to X but this has to be confirmed.

The plots of the 1,3-intrastrand cross-link percentages (logarithmic scale) vs. time were roughly linear. The $t_{1/2}$ values of the 1,3-intrastrand cross-links are between 2 and 6 h when Y is T or C and between 12 and 24 h when Y is A and X is A, T, or C (Table 1). The replacement of the 5′ or the 3′ T·A base pair adjacent to the adduct with a G·C base pair modifies the reaction rate. However, none of these changes

Table 1. $t_{1/2}$ of the 1,3-intrastrand cross-links in duplexes

Bottom strand (3′ → 5′)	Top strand (5′ → 3′)	$t_{1/2}$, h
d(ACTCA)	d(TGAGT)	6
	d(TGCGT)	3
	d(TGTGT)	3
d(ACCCA)	d(TGAGT)	5
	d(TGCGT)	2.5
	d(TGTGT)	2
d(ACACA)	d(TGAGT)	24
	d(TGCGT)	14
	d(TGTGT)	12
	d(CGTGT)	ND
	d(TGTGC)	ND
d(GCACA)	d(CGTGT)	6
d(ACACG)	d(TGTGC)	18

Samples were incubated in 200 mM $NaClO_4$ at 37°C. ND, no interstrand cross-links were detected.

are dramatic, which suggests that the neighboring bases are not directly involved in the isomerization reaction. On the other hand, the 1,3-intrastrand adduct is stable when the bases adjacent to the adduct are mispaired (Table 1).

DNA·RNA duplexes. Three duplexes d(TGTGT)·r(ACACA), d(TGCGT)·r(ACACA), and d(TGAGT)·r(ACACA) in which the deoxyribonucleotide strand contained a single 1,3-intrastrand cross-link were incubated in 200 mM $NaClO_4$. The results (not shown) resembled those shown in Fig. 2, indicating that the linkage isomerization reaction occurred. The $t_{1/2}$ values of the 1,3-intrastrand cross-links within the three duplexes are 20, 14, and ≈50 h, respectively. Thus, both the DNA·RNA and the DNA·DNA duplexes promote the rearrangement of the 1,3-intrastrand adduct to an interstrand cross-link.

Recently, we reported that the interstrand cross-linking reaction by *cis*-DDP at the GC·GC sites was much slower within the DNA·RNA duplexes than within the DNA·DNA duplexes (27). To know whether *trans*-DDP could form interstrand cross-links within a DNA·RNA duplex, the ss oligonucleotide d(TCGCT) was modified with *trans*-$[Pt(NH_3)_2Cl(H_2O)]^+$ so that the platinum moiety was attached monofunctionally to the G residue. The monoadducted strand was hybridized, respectively, with the complementary ribonucleotide strand or deoxyribonucleotide strand and then incubated in 200 mM $NaClO_4$ and at 37°C. Aliquots were withdrawn at various time intervals and analyzed by gel electrophoresis under denaturing conditions. The closure of the monofunctional adducts to interstrand cross-links was much faster within the DNA·DNA duplex than within the DNA·RNA duplex, the percentages of interstrand cross-links being, respectively, 60 and 10% after a 24-h incubation. These results and those obtained in NaCl (see above) suggest that during the rearrangement of the 1,3-intrastrand cross-links, the monofunctional adduct *trans*-$[Pt(NH_3)_2(dG)(H_2O)]^{2+}$ is not formed.

Intrastrand Cross-Linking Reaction vs. Interstrand Cross-Linking Reaction. It is generally accepted that in the reaction between *trans*-DDP and DNA, the 1,3-*trans*-{$Pt(NH_3)_2$[d(GNG)]} cross-links (where N is any base) (11, 12, 16) are preferentially formed and that the $t_{1/2}$ for closure of the monofunctional adducts to intrastrand cross-links is ≈3 h (28). Since we find that the 1,3-intrastrand cross-links are unstable, the following experiment was done to determine whether the 1,3-intrastrand cross-links were formed first and then rearranged to interstrand cross-links. The two ss oligonucleotides d(TGAGT) and d(TGTGT) were reacted with *trans*-$[Pt(NH_3)_2(H_2O)Cl]^+$ so that each oligonucleotide contained a single adduct. In a first approximation, the two G residues were equally reactive. The platinated oligonucleotides were hybridized with their complementary strands and then incubated in 100 mM $NaClO_4$ at 37°C. As shown in Fig. 5, no 1,3-intrastrand cross-links are detected. After a 24-h incubation, the percentage of interstrand cross-links is ≈35 and 15% within d(TGTGT)·d(ACACA) and d(TGAGT)·d(ACTCA), respectively. A quantitative analysis of these kinetics is premature because several parameters are still unknown. The interstrand cross-linking rate might vary as a function of the duplex sequence; the intrastrand cross-linking rate might depend on the location of the monofunctional adduct (on the 5′ G or 3′ G). Since no intrastrand cross-links were detected, we can only say that the intrastrand cross-linking reaction is not fast, $t_{1/2}$ being >10–15 h.

DISCUSSION

Oligonucleotides containing the sequences d(GXG) react with *trans*-DDP to yield 1,3-*trans*-{$Pt(NH_3)_2$[d(GXG)]} cross-links. We show that the 1,3-intrastrand cross-links rearrange

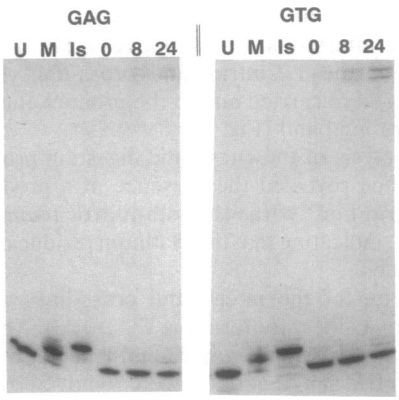

FIG. 5. Intrastrand vs. interstrand cross-linking reaction. Autoradiogram of a denaturing 24% polyacrylamide gel of the platinated d(TGAGT)·d(ACTCA) (*Left*) and d(TGTGT)·d(ACACA) (*Right*). In the duplexes, the d(TGAGT) and d(TGTGT) strands were monoadducted by *trans*-$[Pt(NH_3)_2(H_2O)Cl]^+$ and then 5′-end-labeled. The samples were incubated at 37°C in 100 mM $NaClO_4$. The incubation times in hours are indicated above the lanes. Before the electrophoresis, the samples were incubated at 37°C in 10 mM thiourea for 10 min to remove the monofunctional adducts (16, 18), because during the electrophoresis, the monofunctional adducts (aquated species) within single-stranded DNA react with the neighboring bases and form intrastrand cross-links. The lanes U correspond to the unmodified oligonucleotides. The lanes M and Is correspond to d(TGAGT) or d(TGTGT) containing a single monofunctional *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ adduct and the bifunctional 1,3-*trans*-$[Pt(NH_3)_2(dG)_2]^{2+}$ cross-link, respectively. In lanes M, the samples were not treated with thiourea because the monofunctional adducts (chloro species) are stable enough during the time of the electrophoresis.

to interstrand cross-links in DNA·DNA and DNA·RNA duplexes.

The bifunctional adducts in *cis*- or *trans*-DDP-modified DNA, once formed, are kinetically inert (1, 2, 8, 9). Metal migration has been observed in a few cases of platinated bases, nucleosides, and dinucleotides (25, 29–31) and in one platinated oligonucleotide (24). The 1,3-intrastrand cross-link between G6 and G8 in the *trans*-DDP-modified d(TCTACGCGTTCT) rearranges to the 1,4-intrastrand cross-link between C5 and G8 (24). The sequence of the oligonucleotide is of major importance since the 1,3-intrastrand cross-link in the related oligonucleotide d(CCTCGAGTCTCC) is stable (16). In agreement with this result, we find that the 1,3-*trans*-{$Pt(NH_3)_2$[d(GAG)]} cross-link is inert in the oligonucleotide d(TGAGT).

In contrast, the 1,3-*trans*-{$Pt(NH_3)_2$[d(GAG)]} cross-link within the duplex d(TGAGT)·d(ACTCA) is unstable. It undergoes a rearrangement to an interstrand cross-link between the 5′ G and the complementary C residues. This linkage isomerization reaction does not seem to proceed through solvent-associated intermediate for the following two reasons. (*i*) The rate and the yield of the reaction are independent of the nature of the salt (NaCl or $NaClO_4$), in a large concentration range (50–500 mM). In 500 mM NaCl, the monofunctional adduct *trans*-$[Pt(NH_3)_2(dG)(H_2O)]^{2+}$, if formed, should be trapped (1–4). (*ii*) Within the platinated DNA·RNA duplexes, the interstrand cross-links are formed much faster by rearrangement of the 1,3-intrastrand cross-links than by closure of the monofunctional adduct *trans*-$[Pt(NH_3)_2(dG)Cl]^+$ {we assumed that the hydrolysis rate *trans*-$[Pt(NH_3)_2(dG)Cl]^+ \rightarrow$ *trans*-$[Pt(NH_3)_2(dG)H_2O]^{2+}$ is the same in DNA·DNA and DNA·RNA duplexes and, consequently, is not the limiting step in the closure of the monofunctional adduct}. On the other hand, considering the related duplexes d(PyGXGPy)·d(PuCYCPu) where Y is T or C, it can be stated that X, Y, and the base pairs adjacent to

the adduct are not directly involved in the reaction. Thus we favor a direct nucleophilic attack of the C residue complementary to the 5′ G residue on the platinum residue. Such a mechanism requires a well-defined structure. It is known from the study of two related duplexes containing the 1,3-*trans*-{$Pt(NH_3)_2$[d(GAG)]} or the 1,3-*trans*-{$Pt(NH_3)_2$[d(GTG)]} cross-link (16, 17) that the distortion of the double helix is located at the level of the adduct and the adjacent 5′ base pair and that the torsional angles of the phosphodiester backbone are not largely changed, which is not opposed to the required well-defined structure. A mismatch on the 5′ or 3′ side of the 1,3-intrastrand cross-link is likely to alter the required structure and, subsequently, the linkage isomerization reaction does not occur.

In *trans*-DDP-modified DNA, the interstrand cross-links are not the prominent lesions since they represent 10–20% of the total platinum bound to DNA (11, 14). The 1,3-intrastrand cross-links between two G residues are one of the major adducts (11, 12, 16). These results imply that all the 1,3-intrastrand cross-links do not undergo the rearrangement to interstrand cross-links. We tentatively propose that the 1,3-intrastrand cross-links at the GNG sites flanked by Pu-rich sequences are unfavorable for the rearrangement reaction. Recently, it was concluded from NMR studies (28) that the closure of the monofunctional adducts *trans*-[$Pt(NH_3)_2$(dG)Cl]$^+$ to 1,3-intrastrand cross-links was fast ($t_{1/2}$ = 3.1 h at 37°C). It appears that even this reaction depends upon the DNA sequence, being slow in the two duplexes d(TGTGT)·d(ACACA) and d(TGAGT)·d(ACTCA). Thus, these results underline the importance of the DNA sequence in the reaction with *trans*-DDP. A better knowledge of the different steps of the reaction (initial attack of the DNA, closure of the monofunctional adducts to the bifunctional adducts, and linkage isomerization) should be valuable in the design of *trans*-platinum complexes optimizing these sequence effects. In agreement with this view, recent findings show that subsequent to the chemical modification of the nonleaving groups of *trans*-DDP, the relative percentages of intrastrand and interstrand cross-links in platinated DNA are different (32–34). Interestingly, some of these *trans*-platinum complexes are more cytotoxic than the *cis* congeners and are even endowed with significant antitumor activity (32–34).

Another potential use of the linkage isomerization reaction concerns the antisense strategy. Several studies have shown that antisense oligonucleotides can control the stability and the function of mRNA (for general reviews, see refs. 35 and 36). However, the binding of the oligonucleotides to their target sequences is reversible, and thus, it is difficult to completely block biological functions. To make the process irreversible, chemically and photoactivatable reagents have been covalently linked to the oligonucleotides (35, 36). Non-sequence-specific reactions have often been observed for chemically induced cross-links. Photochemical activation is difficult in the *in vivo* experiments. An advantage of most of the oligonucleotides containing 1,3-*trans*-{$Pt(NH_3)_2$[d(GXG)]} cross-links is that the cross-links are stable as long as the oligonucleotides are single-stranded. The rearrangement occurs only when the oligonucleotides bind to their targets.

Finally, it is worth comparing the key role of DNA double helix both in the rearrangement of the 1,3-*trans*-{$Pt(NH_3)_2$[d(GXG)]} cross-links and in the cleavage of the monofunctional adducts of the form *cis*-[$Pt(NH_3)_2$(Am)(Cl)]$^{n+}$ (26, 37). (Am is an heterocyclic amine such as pyridine, 5-nitroquinoline, *N*-methyl-2,7-diazapyrenium, ellipticine, etc.) These monofunctional adducts are stable within ss DNA and become unstable as soon as the duplexes are formed. Two independent reactions occur corresponding to the cleavage of the Pt-dG and Pt-Am bonds. It was argued that the DNA double helix behaves as an enzyme, the substrate being the adducts within the ss DNA (21, 26, 37). To conclude, it is tempting to speculate these DNA-promoted or even DNA-catalyzed reactions might serve as models for more elaborated systems such as the complexes of DNA, metal, and proteins.

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