Rearrangement of interstrand cross-links into intrastrand cross-links in cis-diamminedichloroplatinum(II)-modified DNA

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ABSTRACT

In the reaction of the anticancer drug cis-diamminedichloroplatinum(II) (cis-DDP) with DNA, bifunctional intrastrand and interstrand cross-links are formed. In this work, we show that at 37°C interstrand cross-links (ICL) are labile and rearrange into intrastrand cross-links. The ICL instability was first studied with a 10 base pairs (bp) double-stranded oligonucleotide containing a unique site-specific ICL resulting from chelation of the N7 position of two guanine residues on the opposite strands of DNA at the d(GC/GC) site by a cis-diammineplatinum(II) residue. The bonds between the platinum and the N7 of guanine residues within the interstrand adduct are cleaved. In 50 mM NaCl or NaClO₄, this cleavage results in the formation of monofunctional adducts which subsequently form intrastrand cross-links. One cleavage reaction takes place per cross-linked duplex in either of both DNA strands. Whereas the starting cross-linked 10 bp duplex is hydrogen bonded, the two complementary DNA strands separate after the cleavage of the ICL. Under these conditions, the cleavage reaction is irreversible allowing its rate measurement (t₁/₂ = 29 ± 2 h) and closure of monofunctional adducts to ICL (18). The recent knowledge of the nature of cross-linked sites in DNA has been the starting point to ICL-induced DNA distortions studies. At the nucleotide level, chemical probes indicated that the distortions are mainly located at the platinated d(GC/GC) site and that the cytosines are largely exposed to the solvent (19). Based on electrophoresis experiments, we have shown that ICL bend and largely unwind DNA (20). The cis-DDP ICL-modified DNA structure has been achieved recently in solution by nuclear magnetic resonance (NMR) (21,22). The double helix is locally reversed to a left-handed form, is unwound and bent toward the minor groove and the complementary cytosines to the cross-linked G residues are unpaired.

INTRODUCTION

cis-Diamminedichloroplatinum(II) (cis-DDP) is among the most widely used anticancer chemotherapeutic agents. It is generally accepted that the cytotoxic effects of cis-DDP are due to the formation of DNA adducts capable of blocking replication and/or transcription (1-5). cis-DDP forms several types of lesions in DNA, including mainly intrastrand and interstrand cross-links (ICL) (6,7). The respective role of these lesions in the antitumor activity of the drug is not yet established. Several results suggest that the biological properties of the major intrastrand adducts arise from the specific recognition of adducts-induced DNA structural distortions by proteins that contain high mobility group boxes (8,9). Several studies have suggested that cis-DDP ICL which represent a small portion (5-10%) of the total lesions (10,11) could be responsible for the cytotoxicity of the drug (11,12). The ICL strongly inhibit DNA transcription elongation by procaryotic and eucaryotic RNA polymerases (13). Resistance of cells to cis-DDP has been reported to be associated with increased gene-specific DNA repair efficiency of ICL (14).

ICL have been the first cis-DDP bifunctional adducts detected in vitro and in vivo ~25 years ago (15) but the identification of this adduct has been achieved only recently. ICL are formed between cis-DDP and the N7 of two guanine (G) residues at d(GC/GC) sites (16,17). In first approximation, all the d(GC/GC) sites are equally reactive with cis-DDP (17) although the neighboring bases interfere with the rate of cis-[Pt(NH₃)₂(N7-G)Cl]* monofunctional adducts closure to ICL (18). The recent knowledge of the nature of cross-linked sites in DNA has been the start point to ICL-induced DNA distortions studies. At the nucleotide level, chemical probes indicated that the distortions are mainly located at the platinated d(GC/GC) site and that the cytosines are largely exposed to the solvent (19). Based on electrophoresis experiments, we have shown that ICL bend and largely unwind DNA (20). The cis-DDP ICL-modified DNA structure has been achieved recently in solution by nuclear magnetic resonance (NMR) (21,22). The double helix is locally reversed to a left-handed form, is unwound and bent toward the minor groove and the complementary cytosines to the cross-linked G residues are unpaired.

In the course of a physico-chemical study of ICL-modified DNA, we noticed that the interstrand adducts were not stable. This result prompted us to undertake a systematic study of the ICL instability. In this paper, we show that under physiological conditions the bonds between platinum and N7 of G residues at the d(GC/GC) site are cleaved leading to the formation of monofunctional adducts. These adducts can further react to form bifunctional lesions (interstrand or intrastrand cross-links). The ICL lability is discussed in the context of adduct-induced structural features and of biological implications.

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FIGURE 1. Sequences and abbreviations of the oligodeoxyribonucleotide duplexes. The chelated bases in cis-DDP and in trans-DDP interstrand cross-links are indicated by asterisks and triangles, respectively. The top and bottom strands of each duplex are designated top and bottom, respectively, in the text.

MATERIALS AND METHODS

Materials

The oligodeoxyribonucleotides from Institut Pasteur, were purified by anion-exchange chromatography (Pharmacia monoQ column) on a FPLC Waters 600E Multisolvent Delivery System. Their sequences and abbreviations are given in Figure 1. The oligonucleotides concentration is expressed in moles of nucleotide residues. Endonuclease P1, alkaline phosphatase and endo nuclease Pl (6,18) and alkaline phosphatase (7) digestion procedures: (i) Maxam-Gilbert-specific reactions (26) (ii) endo nuclease Pl (6,18) and alkaline phosphatase (7) digestion followed by C18 reverse-phase HPLC (27). The adducts eluted from HPLC were treated by NaCN to remove platinum (20). The resulting deoxyribonucleosides were separated by C18 reverse phase HPLC and peaks identity was achieved by coinjection with standard deoxyribonucleosides (27).

Circular dichroism and absorption spectra were recorded with a Jobin-Yvon III dichrograph and a Kontron Uvikon 810 spectrophotometer, respectively.

Platination of oligonucleotides

The 20mer d(CG*CT/AG*CG) site-specifically modified by a single cis-DDP ICL, was prepared as described previously (20). Another method was used to prepare the 10mer I and II d(CG*CT/AG*CG) modified by a unique interstrand adduct. First, the oligonucleotides d(CCTCGCTTC) and d(TCTCTGCTTC) containing a monofunctional cis-[Pt(NH3)2(dG)Cl]+ adduct were prepared, purified and characterized as described before (23). Then, the platinated oligonucleotides were annealed with their complementary strands in 0.3 M NaCl, 10 mM Tris-HCl, pH 7.5 at 4°C. The resulting platinated duplexes were subsequently dialyzed against 500 mM NaCl04, 10 mM Tris- HCl, pH 7.5 at 4°C and then incubated at 37°C for 15 h to yield the lOmer

ICL lability and characterization of the resulting products

The lability of ICL within the 10mer I d(CG*CT/AG*CG) labeled at both 5'-ends was followed by gel electrophoresis under denaturing conditions.

The instability of ICL within the 10mer I d(CG*CT/AG*CG) was also investigated by anion-exchange FPLC with a 30 min linear gradient of 0.1–0.8 M NaCl, 10 mM NaOH, pH 10. Products were collected, neutralized, dialyzed against 0.1 M NaCl, 10 mM Tris- HCl, pH 7.5 at 4°C and then 32P-end-labeled with T4 polynucleotide kinase and [γ-32P]ATP.

Products eluted from gel electrophoresis and ion-exchange FPLC were further characterized by the following experimenta procedures: (i) Maxam–Gilbert-specific reactions (26) (ii) endo nuclease Pl (6,18) and alkaline phosphatase (7) digestion followed by C18 reverse-phase HPLC (27). The adducts eluted from HPLC were treated by NaCN to remove platinum (20). The resulting deoxyribonucleosides were separated by C18 reverse phase HPLC and peaks identity was achieved by coinjection with standard deoxyribonucleosides (27).

The reaction between OsO4 and the 10mer II d(CG*CT/AG*CG) labeled at both 5'-ends was done as previously described (28).

Kinetics measurements

The relative concentrations of the starting cross-linked duplexes and of the products released during incubation of the 10mer d(CG*CT/AG*CG) were quantitated with (i) a Molecular Dynamics Phosphorimager using ImageQuant software version 3.3 for the electrophoresis experiment; (ii) a Waters 746 Dat Module integrator for the FPLC experiment, each peak area being calculated according to the extinction coefficients of each product in the eluant buffer. Kinetic parameters were fitted to the rate data using the Apple Macintosh package Kaleidagraph (Sinerg Software, Reading, PA).

RESULTS

ICL lability

The 10mer I d(CG*CT/AG*CG) was 5'-end-labeled on both strands, and then incubated at 37°C in 50 mM NaCl04, 10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA. Aliquots were withdrawn at various times and analyzed by gel electrophoresis under denaturing conditions (29). As shown in Figure 2, at time zero, a single band is present which corresponds to the starting cross-link.
A quantitative analysis of the intensities of the bands b, c, d and e shows that the products b and d and the products c and e are in the same amounts. From this quantitative analysis and since there was no significant release of free cis-platinum during the incubation of the cross-linked duplex (the amounts released platinated and unplatinated strands are nearly the same in the presence of trapping DNA), we conclude that the two Pt-N7 bonds are cleaved but that the cleavages do not occur simultaneously.

A likely explanation of our data is that the ICL lability yields monofunctional adducts which further react to form intrastrand cross-links. They cannot form back interstrand cross-links because the duplexes (10 bp) containing a single monofunctional adduct melt (23). This was verified by UV absorbance, on the 10mer I d(CGCT/AGCG) modified by dien-Pt at the unique G residue of the top strand. In 50 mM NaClO4 and at an oligonucleotide concentration of 4 x 10^-5 M, the melting temperature is 31°C (not shown). To verify our proposal, it was first necessary to prove the formation of monofunctional adducts during the incubation of the 10mer I cross-linked duplex.

**ICL lability in trapping conditions**

To show the formation of monofunctional adducts, the 10mer I d(CGCT/AGCG) was incubated in 500 mM NaCl which are trapping conditions of these adducts as previously reported (30). Aliquots were withdrawn as a function of time and analyzed by ion-exchange chromatography to separate single-stranded oligonucleotides unplatinated and monofunctionally or bifunctionally platinated. In Figure 3A is shown the FPLC profile of the cross-linked 10mer I after incubation at 37°C during 30 h in 500 mM NaCl, 10 mM Tris-HCl pH 7.5, 0.1 mM EDTA. In addition to the starting material (peak 1), four new major products labeled as peaks 2, 3, 4 and 5 are present; minor peaks are labeled by brackets X and Y.

From elution times and Maxam–Gilbert-specific reactions, we found that products from peaks 2 and 3 are the unplatinated top and bottom strands, respectively, which confirms our data from the electrophoresis experiment.

The elution times suggested that products 4 and 5 were respectively the top and the bottom strands modified by a monofunctional adduct (Fig. 3A and C). This was verified by the following experiment: products 4 and 5 were purified and then annealed respectively with 32P-end-labeled top and bottom strands under conditions used to form cis-DDP ICL and the resulting platinated duplexes were incubated at 37°C in high ionic strength (500 mM NaClO4) to stabilize monofunctionally platinated duplexes. Then, the reaction products were analyzed by gel electrophoresis under denaturing conditions (not shown).

The appearance of a band migrating at the same rate as the cross-linked 10mer I suggested that the complementary labeled strand was cross-linked by an interstrand adduct. As demonstrated by means of technical procedures already described (17,20), this adduct was a cis-DDP ICL (not shown). The formation of a cis-DDP ICL at the d(GC/GC) site within the cross-linked duplexes means that the products 4 and 5 are the top and the bottom strands bearing a cis-[Pt(NH3)2(dGCl)] mono-functional adduct, respectively. Our conclusion is that the ICL cleavage results in monofunctional adducts formation.

**Closure of monofunctional adducts formed in the ICL instability to intrastrand cross-links**

To study the closure of monofunctional adducts trapped in 500 mM NaCl, the 10mer I d(CGCT/AGCG) after incubation in 500 mM NaCl during 30 h, was dialyzed against 50 mM NaClO4 and further incubated at 37°C during 4 h. Comparison of Figure 3A and B indicates that monofunctionally modified top and bottom strands (peaks 4 and 5) have disappeared and that products X and Y are formed.

Products X and Y were characterized by Maxam–Gilbert sequencing reactions and by HPLC analysis after enzymatic digestion (not shown). This study showed that products X and Y are respectively the top and the bottom strands containing a bifunctional intrastrand cross-link. The G residue at the d(GC) site was not reactive with DMS within the top and bottom strands.
NaClO4 (Fig. 3B). Thus, we conclude that the intrastrand electrophoresis experiment (band e in Fig. 2).

Agreement with the presence of a smear also observed in the within the strand except that at the d(GC) site. This data is in agreement with the presence of the complementary bottom strand showing that the duplex was not formed at 37°C in 50 mM NaClO4 (lane no Pt). The cross-linked 10mer II d(CG*CT/AG*CG) was labeled at both 5’ ends and then incubated at 37°C in 50 mM NaClO4. At various time intervals, aliquots were withdrawn and then reacted with OsO4. At time zero (lane 0), OsO4 reacts mainly with thymine residues located within the single-stranded 5’ and 3’ ends of the top strand but the thymine residues located within the cross-linked duplex are not reactive. The non reactivity of thymine residues with OsO4 has been already reported with cross-linked 20mer duplex at 20°C (19) which is in agreement with the recently NMR-derived structure of cis-DDP ICL (21,22). Thus, the starting cross-linked duplex has no unexpected distortions. As a function of incubation time of the cross-linked duplex (lanes 7–120), the three thymine residues located within the starting duplex part become reactive. After 120 h, all the thymine residues are equally reactive and thus the two complementary strands are no longer paired after ICL cleavage. We conclude that at 37°C, the starting ICL is contained within a hydrogen bonded double-stranded helix and that the two DNA strands are separated after the ICL cleavage.

Kinetic analysis of lability

The ICL decrease (%) as a function of incubation time of the 10mer I d(CG*CT/AG*CG) in 50 mM NaClO4 is shown in Figure 5. This time dependent decrease was analyzed in terms of a first-order decay process since the ICL percentages (logarithmic scale) as a function of time is roughly linear (Fig. 5, inset). The rate constant for ICL lability is 24 ± 1.7 x 10⁻³ h⁻¹ (t₁/₂ = 29 ± 2 h). Figure 5 exhibits also the relative concentrations of the sum of products arising from the same cleavage reaction as a function of time (sum of unplatinated top strand and platinated bottom strand and of platinated top strand and unplatinated bottom strand). The rate constants for the ICL decrease and for the appearance of the showing that it was still platinated. HPLC experiments indicated that the second cross-linked base is mainly a cytosine residue within the top strand and a guanine residue within the bottom strand. This platinated G residue was located at different positions within the bottom strand since DMS reacts with all the G residues within the strand except that at the d(GC) site. This data is in agreement with the presence of a smear also observed in the electrophoresis experiment (band e in Fig. 2).

These experiments show that the intrastrand cross-links are the same when they arise either from monofunctional adducts closure or from incubation of cross-linked duplex for 30 h, in 50 mM NaClO4 (Fig. 3B). Thus, we conclude that the intrastrand cross-links result from the closure of monofunctional adducts trapped in 500 mM NaCl. The presence of bifunctional intrastrand cross-links in trapping conditions with chloride ions (monofunctional to bifunctional adducts ratio is ~5:1) was rationalized by assuming that a part of the reaction goes through the hydrolysis path, even in 500 mM NaClO4 (31).

In agreement with our hypothesis, we have shown that the ICL lability yields monofunctional adducts that can further react to form intrastrand cross-links. Now, it seems important to us to verify that in our experimental conditions the starting cross-linked duplex presents no unexpected distortions. For this purpose, we have studied at the nucleotide level, the formation of the 10mer d(CG*CT/AG*CG) by means of a chemical probe. This study allowed us also to show the separation of the DNA strands after the ICL cleavage. In the next experiment, we have used the chemical probe OsO4 which is mainly hyperreactive with thymine residues in single-stranded nucleic acid as compared to B-DNA (32).
cleavage products are the same. The experimental results shown in Figure 5 fit well with the calculated relative concentrations deduced from the minimal reactional scheme (Scheme 1).

Within the interstrand adduct, the rate of cleavage of the two Pt-N7 bonds is the same \( k_1 = k_2 = 12 \pm 0.84 \times 10^{-3} \text{ h}^{-1} \), \( k_3 \) is large since the kinetic of conversion of monofunctional cis-[Pt(NH}_3)_2(dG)(H_2O)]^{2+} adds to bifunctional intrastrand cross-links is fast within single-stranded DNA \( (t_{1/2} < 30 \text{ min}) \) (30) and thus the kinetic of the ICL cleavage is the rate limiting step.

The amounts of unplatinated and platinated products are nearly the same in the presence of an excess of trapping DNA (not shown) indicating that mainly one cleavage reaction of the Pt-N7 bonds occurs per cross-linked duplex in either of both DNA strands. The presence of slightly more unplatinated products (15 ± 7%) suggests that two cleavage reactions per platinated oligonucleotide are a rare event.

The results are the same if (i) the initial concentration of the 10mer I d(CG*CT/AG*CG) varied in the range 2 × 10^{-5} to 8 × 10^{-5} M; (ii) the nature of the salt is changed (50 mM NaClO_4 or NaCl); (iii) the pH is varied in the range 5–8. On the contrary to cis-DDP ICL, the Pt-N7 bond is kinetically inert in the product cis-[Pt(NH}_3)_2(dGMP)]^{2+} and in ICL of the biological inactive isomer trans-DDP formed between a guanine to a complementary cytosine within the 12mer d(CG^A/AG^C) \( (t_{1/2} \approx 300 \text{ h}) \) (not shown).

**Influence of oligonucleotide length on ICL lability**

We have studied the ICL instability under conditions in which the ICL cleavage is reversible in contrast to the experiments carried out above with oligonucleotides of 10 bp in length. In order to have monofunctional adducts contained within double-stranded DNA, we have used longer oligonucleotides (20 bp in length) with the same central sequence d(CGCT/AGCG) (Fig. 1). Monofuctionally platinated 20mer duplexes are thermally stable at 37°C. This was verified with the 20mer d(CGCT/AGCG) modified by a monofunctional adduct of dien-Pt at the unique G residue of the top strand (not shown).

The ICL lability within the cross-linked 20mer d(CG*CT/AG*CG) followed by gel electrophoresis under denaturing conditions as performed with the cross-linked 10mer I (not shown). In 50 mM NaClO_4, the results resembled those obtained with the cross-linked 10mer I but ICL were apparently more stable within the 20mer \( (t_{1/2} = 120 \pm 12 \text{ h}) \) than within the 10mer \( (t_{1/2} = 29 \pm 2 \text{ h}) \). The starting ICL were completely degraded during the incubation yielding intrastrand cross-linked single-stranded oligonucleotides as identified on the basis of their electrophoretic mobility. We have verified that when incubation of cross-linked 20mer was performed in trapping conditions, the ICL half-life decreased \( (t_{1/2} = 35 \pm 3.5 \text{ h}) \) which is in agreement with monofunctional adducts formation. The results with the 20mer cross-linked duplexes are consistent with the reactional Scheme I if the ICL cleavage is now reversible, the closure of monofunctional adducts to ICL leading to slow down apparently the ICL instability.

To make sure that the starting 10- and 20mer cross-linked oligonucleotides have the same conformation, we have compared both platinated oligonucleotides by circular dichroism which is a suitable technique to study the DNA conformation. The changes induced by ICL for the two oligonucleotides (Fig. 6) are similar.

**Figure 4.** Piperidine-induced specific strand cleavage at OsO_4-modified bases in cross-linked 10mer II d(CG*CT/AG*CG) incubated at 37°C as a function of time. Lanes G and T+C correspond respectively to the 10mer II d(CGCT/AGCG) Maxam-Gilbert-specific reactions with only the top strand end-labeled. Lane no Pt is relative to the 5'-end-labeled top strand with or without its complementary strand. Lanes 0-120 are relative to the 10mer II d(CG*CT/AG*CG) labeled at both 5' ends incubated at 37°C in 50 mM NaClO_4, 10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA for 0, 7, 27, 52 and 120 h. The arrow indicates the purine-rich bottom strand labeled in the cross-linked duplex. Within the 10mer II d(CG*CT/AG*CG), the thymines located within the duplex part and within the single-stranded 5' and 3' ends of the top strand are indicated by (■) and (○), respectively. OsO_4 reaction was carried out on unplatinated and platinated oligonucleotides \( (c = 6 \times 10^{-6} \text{ M}) \) for 5 min at 37°C in 50 mM NaClO_4, 10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA. Quantitation of the gel bands was done with a phosphorimager. The increasing percentage of reactivity of thymines as a function of time was deduced from the ratio between the reactivity of thymines respectively indicated by ■ and ○. The kinetic of the cross-linked 10mer I d(CG*CT/AG*CG) Maxam-Gilbert-specific reactions with only the top strand end-labeled. Lane no Pt is relative to the 5' 32P-end-labeled top strand with or without its complementary strand. Lanes 0-120 are relative to the 10mer I d(CG*CT/AG*CG) incubated at 37°C as a function of time. Lanes G and T+C correspond respectively to the 10mer I d(CGCT/AGCG) Maxam-Gilbert-specific reactions with only the top strand end-labeled. Lane no Pt is relative to the 5'-end-labeled top strand with or without its complementary strand. Lanes 0-120 are relative to the 10mer I d(CG*CT/AG*CG) labeled at both 5' ends incubated at 37°C in 50 mM NaClO_4, 10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA for 0, 7, 27, 52 and 120 h. The arrow indicates the purine-rich bottom strand labeled in the cross-linked duplex. Within the 10mer I d(CG*CT/AG*CG), the thymines located within the duplex part and within the single-stranded 5' and 3' ends of the top strand are indicated by (■) and (○), respectively. OsO_4 reaction was carried out on unplatinated and platinated oligonucleotides \( (c = 6 \times 10^{-6} \text{ M}) \) for 5 min at 37°C in 50 mM NaClO_4, 10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA. Quantitation of the gel bands was done with a phosphorimager. The increasing percentage of reactivity of thymines as a function of time was deduced from the ratio between the reactivity of thymines respectively indicated by ■ and ○.
to those already reported for a cross-linked 20mer duplex with the central sequence d(TG\*CT/AG*CA) (20). The main point is that calculated spectra corresponding to the difference between the ICL within the 10mer and the 20mer duplexes are identical (see insets of Fig. 6A and B). The result was unchanged at higher temperature (37°C) or at higher salt concentration 500 mM NaClO4. Thus, we exclude that the different ICL half-life values are relative to the conformation of starting cross-linked duplexes.

DISCUSSION

In the reaction between cis-DDP and DNA, covalent adducts (intrastrand and interstrand cross-links) are formed. It is thought that these bifunctional adducts are stable. In this paper, we show that the interstrand cross-links are unstable and rearrange into intrastrand cross-links.

Upon incubation at 37°C of the 10mer I d(CG*CT/AG*CG) containing a unique ICL at the d(GC/GC) site, we have shown the following main points: (i) the bonds between Pt and the N7 of G residues within the interstrand adduct are cleaved, with mainly one cleavage reaction per cross-linked duplex in either of both strands; (ii) the ICL cleavage leads to the formation of monofunctional cis-[Pt(NH3)2(dG)(H2O)]++] adducts; (iii) the monofunctional adducts react further to form intrastrand cross-links.

The conversion of cis-DDP ICL to intrastrand cross-links occurs via monofunctional intermediates which excludes a direct rearrangement of a bifunctional adduct into another one as previously reported for a few cases (10) and more recently for a cross-linked 20mer d(CG*CT/AG*CG) (t1/2 120 h) is in agreement with a reversibility of the ICL cleavage. We have verified that in high NaCl concentration, the ICL cleavage is no longer reversible (the ICL half-life values are close for the 10mer and the 20mer cross-linked duplexes).

ICL instability results in intrastrand cross-links formation and thus a rearrangement of ICL into intrastrand cross-links occurs within double-stranded DNA. We propose that the ICL instability can be described by the minimal reaction scheme (Scheme 2). The half-life for the closure of cis-[Pt(NH3)2(dG)(H2O)]++] monofunctional adducts to ICL has been reported to be short (<30 min) (33). Assuming that this value is independent of the nature of the bases flanking the cross-linkable d(GC/GC) site, we deduce that intrastrand cross-linking reaction has a half-life of ~5 h within double-stranded DNA. This value is larger than those reported for the closure of monofunctional adducts at the d(GpG) site (t1/2 = 20 h) or d(GpA) (t1/2 = 8 h) (18). Within the sequence d(AGCT)/d(AGCT), the monofunctionally platinated G residue

Figure 5. Relative concentrations of products as a function of time in the reaction of ICL lability. (●) 10mer I d(CG*CT/AG*CG); (□) sum of unplatinated top strand and bottom strand modified by a bifunctional intrastrand adduct; (Δ) sum of top strand modified by a bifunctional intrastrand adduct; (★) sum of bottom strand modified by a bifunctional intrastrand adduct; (•) 10mer I d(CG*CT/AG*CG) at 37°C in 50 mM NaClO4, 10 mM Tris–HCl, pH 7.5, 0.1 mM EDTA. Temperature 20°C.

Figure 6. Circular dichroism spectra of unplatinated and cis-DDP ICL modified 20mer d(CGCT/AGCG) (A) and 10mer I d(CGCT/AGCG) (B) unplatinated (---) and platinated (——) duplexes. Insets: calculated spectra corresponding to the difference between the spectra of platinated and unplatinated oligonucleotides (concentrations expressed in mol of oligonucleotide duplexes) for the 20mer (A) and the 10mer (B) duplexes. Solvent: 50 mM NaClO4, 10 mM Tris–HCl, pH 7.5, 0.1 mM EDTA. Temperature 20°C.
reacts with the G residue on the opposite strand and not with the adjacent A residue. Within our investigated duplex, the presence of sites such as d(ApG) within the sequence d(CGCT/AGCG), d(GpCpG), d(GpC) and d(CpG) is consistent with a slow intrastrand cross-linking rate. Another important point is that the local conformation of the double helix containing the monofunctional adduct resulting from the cleavage of the ICL is not known (the formation of the ICL induces a local left-handed form with an inversion of the sugar geometry).

We do not know the driving forces that lead to the lability of ICL but it could be possible that this reaction is promoted by the unusual structure induced by this adduct in DNA (21,22). The double helix is symmetrically distorted with pseudo-octahedral geometry around the metal atom stabilized by electrostatic interactions of both phosphate residues oxyanions of the G residues at the d(GC/GC) site with the square-planar platinum(II). The axial Pt-O interaction may be similar to the attack by water molecules in solvolysis reaction. It has been shown that phosphate anions can displace chloride to form phosphato-platinum complexes (35). The destabilization of the ICL or even the cleavage of Pt-N7 bonds might result from an attack of the Pt residue by an oxyanion of phosphate residues. This mechanism explains the same rate of cleavage of the two Pt-N7 bonds.

The importance of the DNA double-stranded helix in the instability of platinum adducts has been previously reported: the lability of a monofunctional cis-platinum triamine complex (29) and the rearrangement of the trans-DDP 1,3-intrastrand cross-link into an ICL (24) are promoted by the DNA double helix (the adducts are kinetically inert within single-stranded DNA). The slow conversion of the major d(GpG) cis-DDP intrastrand adduct in the presence of the nucleophilic chloride ion was also reported recently (36). All these results suggest that unexpected chemical reactivities of platinum complexes can occur depending on structural features of the double helix imposed by the adducts.

In conclusion, the ICL instability leads to the formation of intrastrand cross-links when the monofunctional adducts arising from the ICL cleavage are not trapped. This process could involve underestimation of ICL number in experiments such as the ethidium binding fluorescence assay (37) in which the platinated DNA is heated up to 100°C. We have shown that the monofunctional adducts formed in the ICL lability can be trapped in vitro by nucleophiles such as chloride ions. If these adducts react in vivo with nucleophilic biological ligands (thiol or amine groups from proteins), the monoadducts might cross-link irreversibly. As reported for some platinum derivative complexes (38,39), repair proteins can be cross-linked by monoadducts and these adducts might become lethal lesions. On the other hand, our data can be relevant to previous cis-DDP ICL repair experiments. Cells from xeroderma pigmentosum complementation group A are normally thought to be without any nucleotide excision repair capacity but it was observed an 'ICL unhooking activity' (40). The rate of ICL cleavage (t1/2 = 29 h) is in agreement with the kinetics of cis-DDP ICL removal in these cells (30% of ICL removal after 24 h). It might be possible that the level of ICL repair has been overestimated in vivo because of the adduct lability. Further studies are necessary to assess that this lability can occur in vivo. For this purpose, it would be of interest to study whether strains induced in DNA by supercoiling or by proteins implicated in the process of cis-DDP ICL may interfere with the rate of such a reaction.

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