Effects of Coordination of Diammineplatinum(II) with DNA on the Activities of *Escherichia coli* DNA Polymerase I†

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ABSTRACT: The effects of the reaction of *cis*- and *trans*-diamminedichloroplatinum(II) with DNA have been measured with regard to DNA synthesis, 3′–5′ exonuclease (proofreading), and 5′–3′ exonuclease (repair) activities of *Escherichia coli* DNA polymerase I. Both isomers inhibit DNA synthetic activity of the polymerase through an increase in K_m values and a decrease in V_{max} values for platinated DNA but not for the nucleoside 5′-triphosphates as the varied substrates. The inhibition is a consequence of lowered binding affinity between platinated DNA and DNA polymerase, and of a platination-induced separation of template and primer strands. Strand separation enhances initial rates of 3′–5′ excision of [^{3}H]dCMP from platinated DNA (proofreading), while total excision levels of nucleotides are decreased. In contrast to proofreading activity, the 5′–3′ exonuclease activity (repair) discriminates between DNA which had reacted with *cis*- and with *trans*-diamminedichloroplatinum(II). While both initial rates and total excision are inhibited for the cis isomer, they are almost not affected for the trans isomer. This differential effect could explain why bacterial growth inhibition requires much higher concentrations of *trans*- than *cis*-diamminedichloroplatinum(II).

Of the stereoisomers of diamminedichloroplatinum(II), the cis isomer has been introduced as a potent therapeutical drug against several human cancers (Loeherer & Einhorn, 1984), while the trans isomer is ineffective. Similarly, much higher doses of the trans isomer than of the cis isomer are required in order to yield comparable biological effects in bacteria (Johnson et al., 1985).

The mechanism of action of platinum(II) complexes has been sought at the level of DNA. The therapeutical potency of *cis*-platinum(II)[1] drugs has been attributed, in part, to intramolecular cross-linking between adjacent guanine residues which are resistant to DNA repair (Alazard et al., 1982; Plooy et al., 1984; Ciccarelli et al., 1985; Johnson et al., 1985; Roberts & Friedlos, 1987). This product cannot be formed with the trans isomer for stereochemical reasons; only interstrand cross-linking between opposite DNA strands can be formed, and this is less accessible to repair. However, these lesions occur with low frequency (Pascoe & Roberts, 1974).

Although a differential repair of *cis*- versus *trans*-platinum DNA lesions has been postulated, biochemical system(s) involved in this repair has (have) not been elucidated. We report here results on DNA polymerase I of *Escherichia coli* as an example of a repair enzyme. Besides functioning as a 5′–3′ repair exonuclease, the enzyme also contains a 3′–5′ proofreading exonuclease and a DNA synthesis activity (Kornberg, 1980). This enzyme, therefore, allows us to study different parameters involved in DNA repair in a single test system.

Materials and Methods

Materials. *cis*-Platinum and *trans*-platinum were gifts of Degussa, Frankfurt. Nucleotides and nucleic acids were purchased from Boehringer/Ingelheim and Sigma/Munich, respectively. Radioactively labeled nucleotides were from Amersham/Braunschweig. *Escherichia coli* TB2 F$^+$ infected with fd 109 was a gift of Dr. Oertel, Regensburg. Phage fd was grown and DNA isolated according to Maniatis et al. (1982). *Escherichia coli* DNA polymerase I was prepared as described (Muise & Holler, 1985) and was homogeneous by electrophoretic analysis. Klenow fragment was purchased from Boehringer/Ingelheim. One unit of DNA polymerase activity refers to the incorporation of 10 nmol of nucleotides into acid-precipitable material after 30-min incubation in the presence of 66 mM glycine/NaOH buffer (pH 9.2) and the other conditions as in the standard DNA polymerase assay described previously by Schaller et al. (1987).

Solutions of *cis*- and *trans*-platinum were prepared in the presence of 10 mM KNO_3 as described (Schaller et al., 1987). Solutions of monoaqua complexes were prepared by several hours incubation of equimolar concentrations of diamminedichloroplatinum(II) and $AgNO_3$. Precipitating AgCl was removed by centrifugation. Salmon testis DNA was activated by limited digestion with DNase I according to Loeb (1969) to an extent of 10–13% acid-soluble nucleotides in A_{260} units with reference to the total initial DNA. For the measurement of 3′–5′ exonuclease activity, activated salmon testis DNA was 3′ end labeled with [^{3}H]nucleoside 5′-triphosphates (14 Ci/mmol) according to Richardson et al. (1964). These DNA segments had specific activities of (2–9) × 10^4 dpm/nmol nucleotides.

Nicked 5′ end-labeled fd double-stranded DNA was synthesized as follows: Double-stranded DNA of phage fd 109 (12 μg) was cut with *Hpa*II (12 units) in 80 μL of buffer (10 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, and 1 mM dithioerythritol) during 3 h at 37 °C. Half of the digest (6 μL) was coprecipitated with 30 μg of single-stranded phage fd 109 DNA after addition of ethanol. The pellet was redissolved in 100 μL of 0.5 M NaOH. After 15 min, the following solutions were added in the sequence indicated: 50 μL of 1 M Tris-HCl (pH 7.5), 0.4 μL of 0.1 M EDTA (pH 7.5), and 100 μL of 0.5 M HCl. The mixture was kept overnight at 60 °C for renaturation. It was then employed as template-primer

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[1] Abbreviations: *cis*-platinum, *cis*-diamminedichloroplatinum(II); *trans*-platinum, *trans*-diamminedichloroplatinum(II).

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for strand elongation in the presence of Klenow fragment (45 units/mL) and [^{32}P]dATP (100 Ci/mmol) during an incubation time of 160 min at 20 °C. The labeled DNA was separated from nucleotides by passage over Sephadex G75 (0.5 × 20 cm) and precipitated in the presence of LiCl/ethanol. The pellet was again digested with *Hpa*II, giving an average fragment size of 330 bp. The digest was then denatured and renatured in the presence of 4 μg of single-stranded fd 109 DNA. This new template-primer system was subjected to DNA synthesis in the presence of Klenow fragment and unlabeled dNTPs. After separation from mononucleotides and ethanol precipitation, the DNA had a specific radioactivity of 1400 dpm/pmol of nucleotides. It consisted mainly of circular double-stranded DNA with one strand nicked and the other intact. The 5′ end contained the ^{32}P-labeled segment. Acid-soluble radioactivity was not released in the presence of Klenow fragment, indicating that the 3′ ends were unlabeled.

Methods. Platination of DNA was carried out in the dark during 48 h at room temperature in the presence of 10 mM KNO_3 (pH 5.0–5.5) and appropriate amounts of stock solutions of platinum(II) complexes and chloride-free solutions of DNA. In kinetic experiments, the platination reaction was terminated by the addition of a quenching buffer as previously described (Schaller et al., 1987). It was observed that cyanide was not essential in the quenching mixtures, which otherwise resembled the reaction buffers (without other reactants) for DNA synthesis or excision of nucleotides. In controls, it was verified that after addition of such quenching mixtures platination was immediately terminated. DNA preparations which contained a high proportion of *cis*-platinum in the form of monoadducts were obtained by limiting the time of incubation to 40 min as has been described previously (Schaller et al., 1987). We shall refer to it as "*cis*-platinum/DNA monoadduct".

The synthesis of DNA was measured as the incorporation of tritiated dTMP into growing activated salmon testis DNA as previously described (Schaller et al., 1987). Amounts of DNA polymerase I or Klenow fragment used per assay were 0.01 unit in the presence of platinum-free DNA and 0.03–0.06 unit in the presence of platinated DNA. Concentrations of dNTPs varied between 0.05 and 22 μM and those of activated DNA between 30 and 800 μg/mL. Concentrations of either ^{3}H-labeled or unlabeled nucleotides were varied. It was verified that the kinetics of nucleotide incorporation were linear during the time of the measurement of initial velocities. Control incubations indicated that DNA polymerases themselves were not inactivated in the presence of platinum(II) complexes.

In the 3′–5′ exonuclease assay, 75 μL of a mixture containing 66 mM glycine/NaOH (pH 9.2), 6 mM $MgCl_2$, 0.1 mM EDTA, 2 mM 2-mercaptoethanol, 0.8 nM bovine serum albumin, 3.3 mM KNO_3, 6.6 μM (in terms of nucleotides) radioactively labeled DNA, and 1.6 units/mL DNA polymerase I (or Klenow fragment) was incubated for variable times at 37 °C. The ^{3}H-labeled DNA was precipitated in the presence of 100–500 nmol of salmon testis DNA as carrier and 10% trichloroactic acid. The filtered and washed precipitate was counted for radioactivity as described (Schaller et al., 1987). Control incubations without DNA verified that 3′–5′ exonuclease activity was itself not sensitivity to platinum(II) complexes. Furthermore, results obtained at pH 9.2 were qualitatively identical with those at pH 7.5; however, the reaction was much slower at the lower pH.

5′–3′ exonuclease activity was assayed in a 100-μL reaction mixture containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 7 mM $MgCl_2$, 2 mM KNO_3, 1 mM 2-mercaptoethanol, 0.57 μM 5′ end-labeled fd 109 DNA (8 × 10^4 dpm, 100 Ci/mmol [^{32}P]dAMP in the labeled segment), and 2.5 units/mL DNA polymerase I (or Klenow fragment). After variable reaction times at 37 °C, aliquots were drawn and added to carrier DNA. After precipitation as above and centrifugation, the acid-soluble radioactivity in the supernatant was measured. It was verified that the presence of platinated DNA did not change the intrinsic 5′–3′ exonuclease property of the polymerase against added nonplatinated DNA.

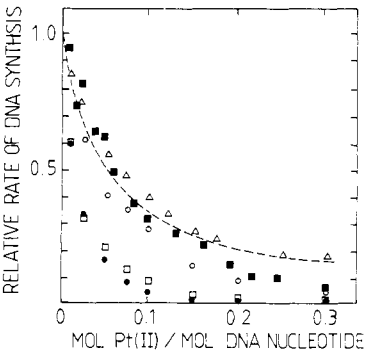

FIGURE 1: Relative rates of DNA synthesis as a function of the molar ratio of platinum to nucleotides in the substrate DNA. DNA was from salmon testis. Activation, platination, and measurement of DNA synthesis were described under Materials and Methods. (●) *cis*-Platinum/DNA (=DNA after reaction with *cis*-platinum to equilibrium), 0.04 unit of DNA polymerase I, [^{3}H]dCTP instead of [^{3}H]dTTP as the labeled nucleotide. (□) *cis*-Platinum/DNA, 0.12 unit of DNA polymerase I, standard conditions of DNA synthesis, however, with 66 mM glycine/NaOH buffer (pH 9.2) as the buffer. (△) *cis*-Platinum/DNA monoadduct (=DNA after reaction with *cis*-platinum for 40 min), 0.04 unit of DNA polymerase I. (■) *trans*-Platinum DNA, 0.04 unit of DNA polymerase I. (○) *trans*-Platinum DNA, 0.07 unit of Klenow fragment, standard conditions of DNA synthesis, however, with 66 mM glycine/NaOH (pH 9.2) as buffer. (---) Data from Schaller et al. (1987).

The procedures for DNA binding to nitrocellulose filters were carried out at 4 °C. The reaction mixture (50 μL) containing 80 μM ^{3}H-3′ end-labeled DNA (3.9 nmol of nucleotides, 10^5 dpm), 1.3–27.5 pmol of DNA polymerase, 20 mM potassium phosphate (pH 7.4), 0.8 mM EDTA, and 25 μM dithioerythritol was slowly filtered through washed and presoaked nitrocellulose filters (Schleicher & Schuell, 0.45 μm). The loaded filters were then washed with 5 mL of buffer (20 mM potassium phosphate, pH 7.4, 0.8 mM EDTA, and 20 μM dithioerythritol) at a speed of 0.1 mL/s, dried, and counted for radioactivity. Unspecific binding to heat-denatured DNA polymerase as a control was negligible. The amounts of filter-bound radioactivity were constant from a few minutes up to 1.5-h incubation of the reaction mixture. Binding of single-stranded DNA was carried out with the same assay but in the absence of DNA polymerase. To test whether *cis*-platinum adsorbed to the filter material and thereby led to the retention of DNA, a mixture of 0.5 mM *cis*-platinum and 0.5 mM K^{14}CN (after 2-h preincubation of the mixture at 0 °C) was passed through the filter as described above. No radioactivity was retained.

RESULTS

DNA Synthesis. The DNA synthetic activity of DNA polymerase I was tested as a function of increasing levels of *cis*-platinum coordinated to DNA (Figure 1). At a molar ratio of platinum to DNA nucleotides of 0.1, more than 90% of the original activity was inhibited in the case of DNA polymerase I and 70% in the case of Klenow fragment, which lacks the 5′–3′ exonuclease function. An inhibition of only 60% was observed for *cis*-platinum/DNA monoadduct. Ap-

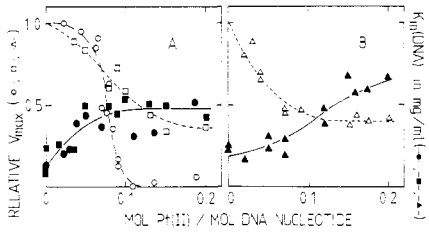

FIGURE 2: Michaelis–Menten parameters for platinated DNA as the varied substrate. DNA synthesis was measured with DNA polymerase I and platinated salmon testis DNA as described under Methods. The parameters are given as a function of the molar ratio of platinum to DNA nucleotides in the platination mixture. Panel A: (○, ●) DNA after reaction with *cis*-platinum to equilibrium; (□, ■) *cis*-platinum/DNA monoadduct (=DNA after reaction with *cis*-platinum for 40 min; see Methods). Panel B: (△, ▲) DNA after reaction with *trans*-platinum to equilibrium.

proximately the same level of inhibition was observed for DNA, which had reacted to equilibrium with *trans*-platinum, and results were the same for DNA polymerase I and Klenow fragment.

The Michaelis–Menten parameters K_m and V_{max} for the dNTPs as the varied substrates were not affected. However, those for platinated DNA reflected a strong dependence on the ratio of platinum to DNA (Figure 2). The main effect of *cis*-platinum at low molar ratios (below 0.08) was to increase the K_m value by a factor of 4–5, whereas at higher ratios the more severe inhibition was due to a steep decline in the V_{max} value between molar ratios of 0.08 and 0.1. In these experiments, DNA was used which had been reacted with *cis*-platinum at equilibrium, providing a high degree of platinum(II) cross-links (Pinto & Lippard, 1985a; Reedijk, 1987; Schaller et al., 1987). For *cis*-platinum/DNA monoadduct, the decline in V_{max} values is less dramatic (Figure 2A).

In contrast to the results for *cis*-platinum, values of K_m remained constant at low molar ratios of *trans*-platinum to DNA nucleotides, and the observed inhibition of DNA synthesis was mainly due to a comparably modest (50%) decrease in V_{max}. Its dependence resembles that of *cis*-platin/DNA monoadduct (Figure 2).

Affinity of Platinated DNA for DNA Polymerase. When radioactively labeled, nonplatinated DNA was applied to nitrocellulose filters, an equivalent of 1.8 nmol of nucleotides of DNA per 5 pmol of bound DNA polymerase I was retained as judged by the tangent method (Bartmann et al., 1975). This corresponded to an average of 300 nucleotides per molecules of enzyme, which is known to bind at the 3′ termini of primers (Kornberg, 1980). When DNA *cis*-platinum adduct was applied, binding was markedly reduced (Figure 3). This is consistent with the observed increase in the K_m value in Figure 2.

Single-Stranded DNA as a Result of Platination. With elevated molar ratios of *cis*-platinum to DNA nucleotides, increasing amounts of radioactivity were retained by the filters in the absence of DNA polymerase (Figure 3, inset). It is a common phenomenon that single-stranded but not double-stranded DNA binds to nitrocellulose. The observed retention of radioactivity, therefore, suggests that increasing levels of *cis*-platinum bound to DNA lead to progressive strand separation. According to Figure 3 (inset), this separation is cooperative with regard to the ratio of platinum bound to DNA. The dependence follows an inflection point of 0.08 mol of platinum/mol of DNA nucleotides and resembles in this respect that of V_{max} in Figure 2A.

3′–5′ Exonuclease Activity. Excision of [^{3}H]dNMPs from the 3′ terminus of activated salmon testis DNA was increasingly inhibited as the molar ratio of *cis*-platinum per DNA nucleotides was raised (Figure 4). Plateau values of excision followed the order dCMP > dAMP = dTMP > dGMP. Initial rates were independent or slightly decreased as a function of the ratio of platinum/DNA for dGMP and increased for dCMP as the excised nucleotide (Figure 4B, inset). Results were similar with DNA coordinated to *trans*-platinum. However, plateau values for the excision of different dNTPs were not distinguishable from each other (results not shown).

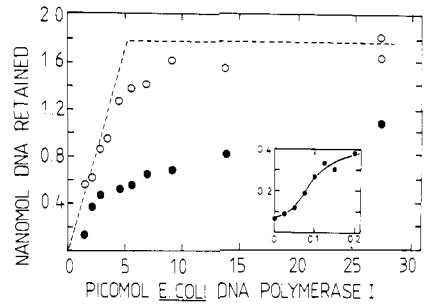

FIGURE 3: Effect of platination on the binding of ^{3}H-3′ end-labeled, activated salmon testis DNA to nitrocellulose in the presence (main figure) and in the absence (inset) of DNA polymerase I. For experimental details, see Methods. The molar ratio of *cis*-platinum to DNA nucleotides was 0.1. Dashed lines refer to tangents in the determination of the stoichiometry. (Inset) The ordinate refers to the fraction of total DNA retained on the filters and the abscissa to the molar ratio of *cis*-platinum to DNA nucleotides in the platination mixture.

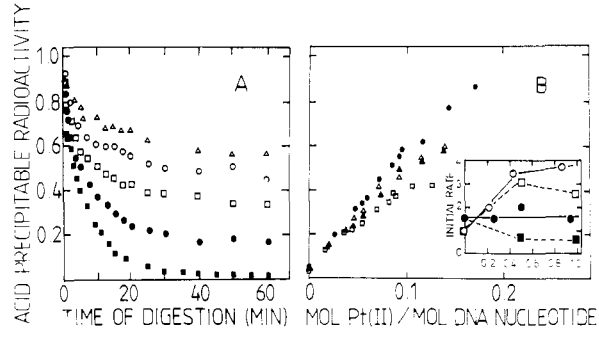

FIGURE 4: 3′–5′ exonuclease activity of DNA polymerase I on platinated, ^{3}H-3′ end-labeled salmon testis DNA. (Panel A) Kinetics of the digestion. Results are presented as the fraction of DNA remaining after various incubation times. Symbols refer to the molar ratio of *cis*-platinum to DNA nucleotides before digestion: (■) no *cis*-platinum; (●) 0.025, (□) 0.051, (○) 0.083, and (△) 0.093 *cis*-platinum. (Panel B) Plateau values (from experiments as in panel A) as a function of the molar ratio of platinum to DNA nucleotides. The symbols refer to the [^{3}H]nucleotides used in the assay: (△) dAMP; (□) dCMP; (●) dGMP; (▲) dTMP. Inset: initial rate (dpm of acid-soluble radioactivity per minute) as a function of the molar ratio of platinum to DNA nucleotides. Open symbols refer to [^{3}H]dCMP and closed symbols to [^{3}H]dGMP as the labeled nucleotide: (○, ●) *cis*-platinum; (□, ■) *trans*-platinum.

5′–3′ Exonuclease Activity. The excision of radioactively labeled nucleotides from 5′ ends of fd 109 DNA depended on the presence of complete DNA polymerase I; the Klenow fragment was inactive (Figure 5). The reaction of the DNA with *cis*-platinum led to a progressive inhibition of initial rates (Figure 5A) and of the maximal level of excision. Both parameters showed a similar dependence on the amount of platinum bound to DNA. Much less inhibition was found with *trans*-platinum (Figure 5A). These results demonstrate that 5′–3′ exonuclease (repair) activity can differentiate between adducts of the two isomers.

Both isomers are incorporated into DNA with almost the same rates (Schaller et al., 1987). Under the present conditions, the concentrations of fd 109 DNA and of platinum

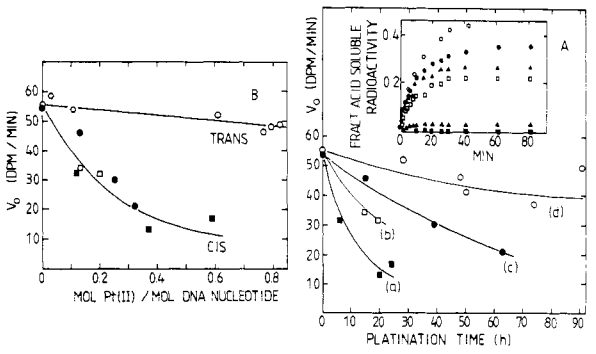

FIGURE 5: 5′–3′ exonuclease (repair) activity of DNA polymerase I on nicked phage fd 109 double-stranded DNA, which had been labeled with [^{32}P]nucleotides in segments at 5′ ends. Inset: the fraction of radioactivity that was excised from DNA as a function of time. Symbols refer to calculated molar ratios of incorporated platinum to DNA nucleotides: (○) no platinum; (●) 0.03, (△) 0.013, (■) 0.019, and (▲) 0.037; (■) no platinum, and Klenow fragment instead of DNA polymerase I. (Panel A) Initial rates of [^{32}P]dAMP excision as a function of platination time. Initial rates (v_0) were calculated from the tangents to curves in the figure inset at zero time for acid-soluble radioactivity released during 1 min. Platination of the labeled fd 109 DNA was carried out with platinum(II) complexes in their monoaqua form and the following concentrations: (a) 0.3 μM *cis*-platinum and 3.6 μM DNA (in terms of nucleotides); (b) 0.15 μM *cis*-platinum and 4.4 μM DNA; (c) 0.13 μM *cis*-platinum and 3.5 μM DNA; (d) 0.3 μM *trans*-platinum and 3.5 μM DNA. (Panel B) Initial rates as a function of the calculated molar ratio of incorporated platinum to DNA nucleotides. This was calculated on the basis of known rate constants (Schaller et al., 1987). Symbols are the same as in panel A.

complexes are so low that the bimolecular formation of primary adducts is rate limiting; i.e., the subsequent cross-linking reactions follow immediately. The known reaction rates (Schaller et al., 1987) were used to calculate the amount of platinum incorporated into DNA. The data points in Figure 5A were then plotted as a function of the molar ratio of platinum/DNA nucleotides. Again, little inhibition is seen for *trans*- in contrast to *cis*-platinum.

Discussion

Three different enzymatic activities are intrinsic to DNA polymerase I of *Escherichia coli*: DNA synthesis, 3′–5′ exonuclease (proofreading), and 5′–3′ exonuclease (repair) (Kornberg, 1980). *cis*-Platinum inhibits each of these activities, not by poisoning the enzyme but by its reaction with the substrate DNA. Similar results were obtained with *trans*-platinum with the important difference that it does not inhibit repair 5′–3′ exonuclease. This finding may explain why, at least in bacteria, the trans isomer is considerably less toxic and mutagenic than its cis analogue, and may bear on the question why *trans*-platinum(II) complexes are ineffective as antitumor drugs.

The mechanistic aspects drawn from the presented results are as follows: (1) The inhibition of DNA synthesis is expressed in terms of an increase in K_m values and a decrease in V_{max} values for platinated DNA as the substrate. The kinetic parameters for the dNTPs are not affected. This can be explained by postulating that DNA synthesis stops before each nucleotide–platinum(II) adduct on the DNA template strand. Indeed, such termination in front of DNA–platinum-(II) cross-links has been reported (Pinto & Lippard, 1985a). (2) At low molar ratios of the cis isomer to DNA nucleotides, inhibition is correlated with an increase in the K_m value and may, therefore, be referred to a decreased binding affinity of cis-platinated DNA for DNA polymerase I. In this range of molar ratios of platinum/DNA nucleotides, the K_m value is constant for DNA coordinated with *trans*-platinum. A possible explanation is that *cis*-platinum, but not *trans*-platinum, cross-links between adjacent guanine nucleotides and thereby induces a kink at this point of the DNA [see references in Reedijk (1987)], which would render it less accessible to the DNA polymerase binding cleft. (3) At higher molar ratios (0.08–0.1) of *cis*-platinum/DNA nucleotides, the steep decrease in V_{max} (Figure 2A) can be explained by separation of template and primer DNA strands. This "melting" is attributed to the cooperative action of cross-links as they populate DNA more densely. The induction of single-stranded regions has been previously suggested (Macquet & Butour, 1978; Mong et al., 1981) and is also evident in the inset of Figure 3. Because of the loss of primers, DNA synthesis is abolished. The decrease in V_{max} is much less with the trans isomer and commensurable with that of the cis isomer/DNA monoadduct (Figure 2). Single coordination does not induce significant DNA structural changes but, nevertheless, causes termination of DNA chain elongation (Schaller et al., 1987). We conclude that DNA synthesis is inhibited at low molar ratios of platinum/DNA mainly by termination at sites of platinated nucleotides and at high molar ratios also by the prevention of reinitiation of synthesis.

(4) Initial rates of 3′–5′ exonuclease (proofreading) activity increase with regard to dCMP as the excised nucleotide (Figure 4). This is in aggreement with the mentioned strand separation at the position of platinated guanine in the template strand and with the known specificity of this activity for single-stranded DNA (Kornberg, 1980). Such rapid excision has been proposed in order to explain the termination of DNA synthesis along platinated M13 single-stranded DNA (Pinto & Lippard, 1985a). (5) Contrary to the initial rates, plateau levels of 3′-excised nucleotides decrease for both the cis and the trans isomers as a function of the molar ratio of platinum to DNA nucleotides (Figure 4). A reasonable explanation for this observation is that 3′–5′ excision is terminated in front of every platinated nucleotide in the primer strand. Work is in progress in order to elucidate this point.

(6) DNase I is known to insert nicks into double-stranded DNA (Kornberg, 1980). DNA polymerase I binds to these nicks and polymerizes nucleotides to the 3′ termini of primers. During DNA synthesis, the nucleotides on the 5′ side of adjacent primers are excised (nick translation). As the reaction of DNA with *cis*-platinum leads to inhibition of both strand elongation and 5′–3′ nucleotide excision, the expected overall effect should be larger for the complete enzyme than for Klenow fragment, which is known to catalyze DNA polymerization without concomitant hydrolysis of 5′ termini (Kornberg, 1980). This prediction is supported by the results in Figure 1. Furthermore, since DNA-bound *trans*-platinum does not appreciably inhibit 5′–3′ exonuclease, this isomer should have less effect on DNA synthesis than its cis counterpart. Again, this prediction is verified (Figure 1). If the differential inhibition for the isomers is only due to their effects on the 5′–3′ exonuclease activity, then this difference should vanish for Klenow fragment. This is indeed observed (Figure 1). Using *cis*-platinum/DNA monoadduct (40-min platination) results in the same synthetic activity as DNA with *trans*-platinum adducts (Figure 1). This similarity suggests that nucleotide monoadducts of the cis isomer can be excised by the 5′–3′ exonucleolytic activity. Work is in progress to clarify this possibility.

Platinum(II) complexes form N7 coordination products especially with guanine and, to a lesser extent, with adenine nucleotides [see Pinto and Lippard (1985b) and Reedijk

(1987) and references cited therein]. A major difference between the isomers is that only *cis*-platinum(II) complexes can cross-link adjacent purine nucleotides in DNA. It is believed that this lesion escapes repair enzymes (Reedijk, 1987). The present results suggest that inhibition of excision could be one of the reasons. Another explanation could be the relatively small structural perturbation of this type of cross-linking, which would escape recognition by excision enzymes (Reedijk, 1987).

The results presented demonstrate how platinum(II) complexes affect properties of DNA polymerase I, which is a repair DNA polymerase. One has to be careful in extrapolation of the results for the replicative DNA polymerase (DNA polymerase III in *Escherichia coli*) and for the mechanism of cytotoxicity, which are considered more complicated.

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Registry No. DNA polymerase, 9012-90-2; *cis*-diamminedichloroplatinum(II), 15663-27-1; *trans*-diamminedichloroplatinum(II), 14913-33-8.

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