RNA Polymerases React Differently at d(ApG) and d(GpG) Adducts in DNA Modified by *cis*-Diamminedichloroplatinum(II)†

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ABSTRACT: Two duplexes (20-mers) were constructed containing either a single *cis*-[$Pt(NH_3)_2$[d(GpG)]] or *cis*-[$Pt(NH_3)_2$[d(ApG)]] intrastrand cross-link, the major DNA adducts of the antitumor drug *cis*-diamminedichloroplatinum(II). These synthetic duplexes were multimerized and the resultant polymers used as templates in single-step addition reactions of condensation of a single nucleoside triphosphate substrate to a dinucleotide primer (abortive elongation reaction) catalyzed by prokaryotic or eukaryotic RNA polymerases. Primer–substrate combinations were selected so as to direct trinucleotide product formation within the platinated bases of the templates. Transcription experiments established that *cis*-DDP–DNA adducts formed at d(ApG) or d(GpG) sites are not an absolute block to formation of a single phosphodiester bond by either *Escherichia coli* RNA polymerase or wheat germ RNA polymerase II. Furthermore, the kinetic data indicate that single-step addition reactions are much more impeded at the platinated d(GpG) than at the platinated d(ApG) site and that the mechanisms of inhibition of RNA polymerase activity are different at the two platinated sites. In particular, binding affinity between *E. coli* RNA polymerase and the d(GpG)-containing platinated template is lowered, as the apparent K_m of enzyme for the platinated polymer is increased by a factor of 4–5. In contrast, binding affinity between the RNA polymerase and the d(ApG)-containing template is not affected by modification of the d(ApG) site by *cis*-diamminedichloroplatinum(II). Similar experiments were carried out with synthetic templates containing the adducts at the d(GpG) sites, in which one of the two platinated dG residues is paired with a dT residue. With respect to overall enzymic activity and to enzyme–DNA interactions, the mismatch-containing and d(ApG)-containing platinated polymers behave similarly. These results are discussed in relation to the distortions induced in DNA by the two adducts.

It is now generally accepted that the curative effect of the antitumor drug *cis*-diamminedichloroplatinum(II) (*cis*-DDP)[1] results from the attack of cellular DNA and to the formation of several types of adducts with DNA bases [reviewed by Lepre and Lippard (1990)]. We previously reported that platinum–DNA adducts formed by *cis*-DDP and two adjacent purine bases, such as d(GpG) or d(ApG), provide a strong elongation block during in vitro RNA synthesis by prokaryotic and eukaryotic RNA polymerases (Corda et al., 1991; Lemaire et al., 1991). In agreement with the proposal of Srivastava et al. (1978) and of Sorenson and Eastman (1988a,b), we suggested the possibility that the antitumor drug may exert its effect in vivo, at least in part, at the level of DNA transcription. Several studies showed that both d(G*pG*) and d(A*pG*) adducts have similar structural properties (van der Veer et al., 1986; van Hemelryck et al., 1987; Rice et al., 1988; Schwartz et al., 1989), but at the nucleotide level they distort the double helix differently (Marrot & Leng, 1989; Schwartz et al., 1989). The purpose of the present work was to investigate whether such different distortions can be sensed by DNA-dependent RNA polymerases. To pursue this point, we studied the influence of platination of the DNA template on single-step addition reactions of a ribonucleotide to a dinucleotide primer catalyzed by these enzymes (abortive elongation reaction) (Oen & Wu, 1978), as this assay revealed that the rate of trinucleotide product formation depends on the sequence (Sylvester & Cashel, 1980; Dietrich et al., 1985) or conformation (Job et al., 1988, 1991) of the DNA template. By this method, we show herein that under these in vitro conditions *Escherichia coli* RNA polymerase and wheat germ RNA polymerase II can differentiate between the d(G*pG*) and d(A*pG*) adducts.

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[1] Abbreviations and Nomenclature: bp, base pair(s); DDP, diamminedichloroplatinum(II); EDTA, disodium salt of ethylenediaminetetraacetic acid; TLC, thin-layer chromatography; Tris, tris(hydroxymethyl)aminomethane. An asterisk denotes a deoxyribonucleotide modified by *cis*-diamminedichloroplatinum(II). d(GG/CC) is a double-stranded oligodeoxyribonucleotide of 20 bp, containing 18 pyrimidine bases and 2 central dG bases on one strand and 18 purine bases and 2 central dC bases on the complementary strand. d(G*G*/CC) is the corresponding double-stranded sequence in which the sequence d(GpG) is modified by *cis*-DDP. $d(GG/CC)_m$ and $d(G*G*/CC)_m$ are multimers of the ligated d(GG/CC) and d(G*G*/CC) motifs, respectively. d(AG/TC), d(A*G*/TC), $d(AG/TC)_m$, and $d(A*G*/TC)_m$ correspond to d(GG/CC), d(G*G*/CC), $d(GG/CC)_m$, and $d(G*G*/CC)_m$ in which one of the central dG·dC bp is replaced by a dA·dT bp, respectively. $d(GG/TC)_m$, $d(GG/CT)_m$, $d(G*G*/TC)_m$, and $d(G*G*/CT)_m$ correspond to $d(GG/CC)_m$ and $d(G*G*/CC)_m$ in which one of the central dG·dC bp is replaced by a dG·dT bp, respectively. Dinucleoside monophosphate primers, trinucleoside diphosphate, and tetranucleoside triphosphate products are referred to as dinucleotides, trinucleotides, and tetranucleotides, respectively.

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Materials and Methods

Oligodeoxyribonucleotide Synthesis, Platination, and Ligations. The sequences of the repeating units of the duplexes used in this work are

d(AG/TC), d(A*G*/TC) : 5' CTTCTCTTCTAGTCTTCTCT
AAGAGAAGATCAGAAGAGAG 5'

d(GG/CC), d(G*G*/CC) : 5' CTTCTCTTCTGGTCTTCTCT
AAGAGAAGACCAGAAGAGAG 5'

The synthesis, the platination, and the ligation of the oligonucleotides were performed as previously described (Marrot & Leng, 1989; Schwartz et al., 1989). Briefly, the two single-stranded oligonucleotides (upper strands) containing a unique d(ApG) or d(GpG) site were reacted with *cis*-DDP. The platinated oligonucleotides were purified by ion-exchange FPLC. The sites of platination were verified by reverse-phase HPLC analysis of the digests after incubation of the platinated oligonucleotides with P1 nuclease (Fichtinger-Schepman et al., 1985) and then with alkaline phosphatase (Eastman, 1986). It as also been verified by atomic absorption spectroscopy and ultraviolet absorption spectroscopy that there was about one platinum atom/oligonucleotide. The duplexes were obtained by mixing either the platinated oligonucleotides containing an unique adduct at the d(GpG) or d(ApG) site or the unplatinated oligonucleotides with the complementary strands, adjusted so that the duplexes have cohesive ends. We shall name the duplexes by their two central d(GG/CC) or d(AG/TC) bp. Since wheat germ RNA polymerase II requires a stretch of about 40 bp to bind to DNA (Chandler & Gralla, 1980), multimers of the 20-mer duplexes, referred to as $d(GG/CC)_m$, $d(AG/TC)_m$ (unplatinated DNA), and $d(G^*G^*/CC)_m$, $d(A^*G^*/TC)_m$ (platinated DNA), were prepared with T4 DNA ligase (Koo et al., 1986); it was verified by gel electrophoresis that the size of the major part of the multimers was greater than 100 bp.

DNA-Dependent RNA Polymerases. Wheat germ RNA polymerase II was purified as described by Jendrisak and Burgess (1975) and by Corda et al. (1991). *E. coli* RNA polymerase holoenzyme was a generous gift from Henri Buc (Institut Pasteur, Paris). The specific activity of the eukaryotic and prokaryotic enzymes was of the order of 2000 units mg^{-1} and of 65 000 units mg^{-1}, respectively (Corda et al., 1991).

Activity Measurements. The reaction mixtures contained, in a total volume of 20 μL, 2.4 nM *E. coli* RNA polymerase or 9.8 nM wheat germ RNA polymerase II, 2.4 μM DNA (bp units), 1.5 mM $MnCl_2$ or 10 mM $MgCl_2$, 330 μM GpA or ApC, and 1 μM [α-^{32}P]CTP or 1 μM [α-^{32}P]UTP (Amersham; 410 Ci $mmol^{-1}$), in transcription buffer as described before (Corda et al., 1991). In the case of wheat germ RNA polymerase II, measurements were restricted to the use of $MnCl_2$ as the metal cofactor, as the eukaryotic enzyme requires Mn^{2+} to catalyze the single-step addition reactions (Dietrich et al., 1986). Assays were incubated for 1 h (wheat germ enzyme) or 45 min (*E. coli* enzyme), at 35 °C. Reactions were stopped by adding two volumes of stop solution containing 1 mM EDTA, 80% formamide, and 0.1% xylene cyanol.

Syntheses of trinucleotides and tetranucleotides were detected after ascending TLC on poly(ethyleneimine)cellulose sheets of 4 μL (reaction mixtures + stop solution), using LiCl (0.05 M)/HCOOH (0.5 M) (syntheses of GpApC, GpApCpC, and ApCpU) or 1 M CH_3COOH (synthesis of ApCpC) as the solvent system. This provided a clear separation between the unused triphosphate substrate (which remained bound at the origin) and the reaction products. Following radioautography, all spots containing radioactivity were cut out, and the radioactivity was measured in a liquid scintillation counter.

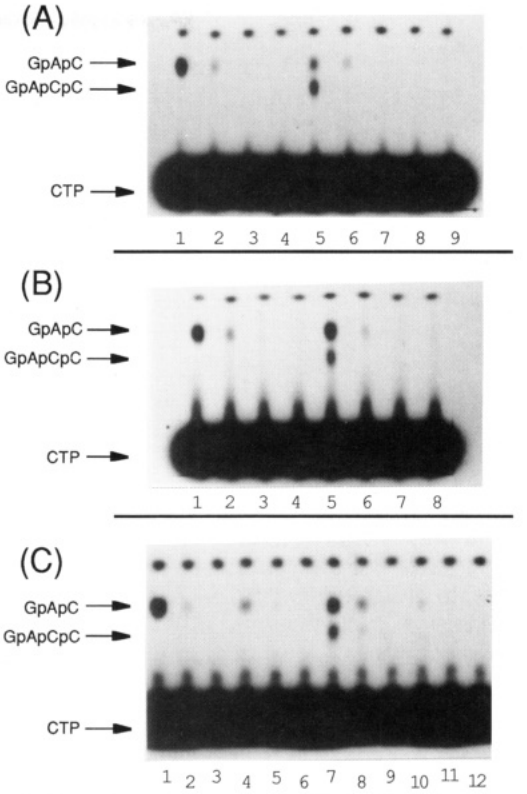

FIGURE 1: Reaction requirements for GpA-primed reactions. Single-step addition reactions were carried out, as described, with 330 μM GpA, 1 μM [α-^{32}P]CTP, 2.4 μM (bp units) DNA template, 2.4 nM *E. coli* RNA polymerase (A, B) or 9.8 nM wheat germ RNA polymerase II (C), and 1.5 mM $MnCl_2$ (A, C) or 10 mM $MgCl_2$ (B). Shown are TLC and radioautographic analyses of the products synthesized from incubations in the presence of (A) *E. coli* RNA polymerase and Mn^{2+}. Template: (lanes 1, 2, 5, and 6) $d(AG/TC)_m$, $d(A^*G^*/TC)_m$, $d(GG/CC)_m$, and $d(G^*G^*/CC)_m$, respectively. The concentration of CTP substrate converted into GpApC was 2.6 nM (lane 1), 0.5 nM (lane 2), 1.1 nM (lane 5), and 0.3 nM (lane 6); the concentration of CTP substrate converted into GpApCpC was 1.6 nM (lane 5). (Lanes 3, 4, 7, and 8) As in lanes 1, 2, 5 and 6, respectively, minus GpA primer. (Lane 9) Complete reaction mixture minus template. (B) *E. coli* RNA polymerase and Mg^{2+}. Conditions for incubations: (lanes 1–8) As for lanes 1–8 in panel A. The concentration of CTP substrate converted into GpApC was 2.4 nM (lane 1), 0.6 nM (lane 2), 2.5 nM (lane 5), and 0.3 nM (lane 6); the concentration of CTP substrate converted into GpApCpC was 1.2 nM (lane 5). (C) Wheat germ RNA polymerase II. Template: (lanes 1, 4, 7, and 10) $d(AG/TC)_m$, $d(A^*G^*/TC)_m$, $d(GG/CC)_m$, and $d(G^*G^*/CC)_m$, respectively. The concentration of CTP substrate converted into GpApC was 5.8 nM (lane 1), 1.2 nM (lane 4), 3.6 nM (lane 7), and 0.5 nM (lane 10); the concentration of CTP substrate converted into GpApCpC was 2.4 nM (lane 7). (Lanes 2, 5, 8, and 11) As in lanes 1, 4, 7, and 10, respectively, plus 50 μg mL^{-1} α-amanitin. (Lanes 3, 6, 9, and 12) As in lanes 1, 4, 7, and 10, respectively, minus GpA primer.

Results and Discussion

Requirements for GpApC Synthesis. Both repeating units of $d(GG/CC)_m$, $d(AG/TC)_m$, and their platinated derivatives contain a d(TpC) sequence on the 3′ side of the two central purine bases, in position adjacent to the d(GpG) or d(ApG) sequences. By using GpA as primer and CTP as substrate, the duplexes should permit, therefore, the direction of enzymic synthesis of GpApC. Additionally, the synthesis of GpApCpC should be detected with the d(GpG)-containing polymers. Although several d(TpC) sites are present in the repeating units of the duplexes, only one can support single-step addition reactions with the CTP substrate.

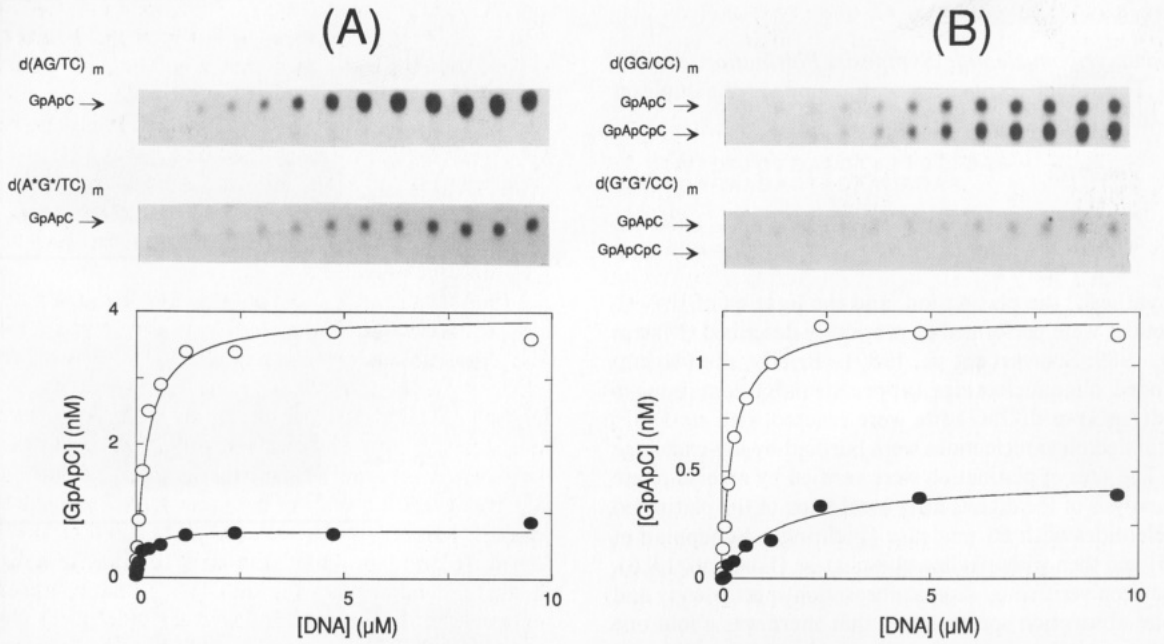

FIGURE 2: Michaelis–Menten parameters for template in GpApC synthesis. Incubations were carried out in the presence of 2.4 nM *E. coli* RNA polymerase, 1.5 mM Mn^{2+}, 1 μM [α-^{32}P]CTP, 330 μM GpA, and varied amounts of DNA templates. GpApC synthesis was measured as described under Materials and Methods. Product concentrations are expressed for the 45-min incubations at 35 °C. Radioautographic analyses of the data are shown on the top of the graphs; from left to right [DNA] = 0, 0.0093, 0.0185, 0.037, 0.074, 0.148, 0.296, 0.594, 1.19, 2.375, 4.75, and 9.5 μM. (A) $d(AG/TC)_m$ (○), $d(A^*G^*/TC)_m$ (●). (B) $d(GG/CC)_m$ (○), $d(G^*G^*/CC)_m$ (●). The kinetic data were analyzed by using the Gauss–Newton algorithm (Cleland, 1979). Smooth lines are computer fits to the rate equation (GpApC synthesis/45 min) = V_m[DNA]/(K_m + [DNA]), for the following best-fit values of the kinetic parameters: $d(AG/TC)_m$ (○), K_m = 0.24 μM, V_m = 4.0 nM; $d(A^*G^*/TC)_m$ (●), K_m = 0.17 μM, V_m = 0.72 nM; $d(GG/CC)_m$ (○), K_m = 0.26 μM, V_m = 1.23 nM; $d(G^*G^*/CC)_m$ (●), K_m = 1.4 μM, V_m = 0.47 nM; GpApCpC synthesis with $d(GG/CC)_m$, K_m = 0.36 μM, V_m = 2.3 nM.

Figure 1A shows that the synthesis of GpApC occurred after incubation for 45 min of reaction mixtures containing *E. coli* RNA polymerase, GpA, CTP, Mn^{2+}, and $d(AG/TC)_m$ as a template. When analyzed by TLC, GpApC migrated with a R_f value of 0.61, whereas unreacted CTP remained bound at the origin (lane 1). The formation of this product was absolutely dependent on the presence of the GpA primer (lane 3) and DNA template (lane 9).

GpApC synthesis was also evident with unplatinated $d(GG/CC)_m$ (lane 5). The reaction was strictly dependent on the presence of primer (lane 7) and template (lane 9). Furthermore, an additional product of lower mobility than that of GpApC (R_f of 0.48) was synthesized only from complete assays (lane 5). We assume, therefore, that this product corresponds to the tetranucleotide GpApCpC, as expected from the sequence of $d(GG/CC)_m$. The migration rates for the two products are compatible with their different charge densities (Randerath & Randerath, 1967). We conclude that the GpA-primed reactions fulfill the requirements for initiation of transcription at the level of the two dC and dT bases, adjacent to the central purine bases within the repeating 20-mer units of $d(AG/TC)_m$ and $d(GG/CC)_m$.

The synthesis of GpApC was also detected with platinated $d(A^*G^*/TC)_m$ and $d(G^*G^*/CC)_m$ (lanes 2 and 6), exhibiting the characteristic features observed by using the corresponding unplatinated $d(AG/TC)_m$ and $d(GG/CC)_m$ sequences. However, the level of trinucleotide formation was smaller. Moreover, the formation of GpApCpC was abolished with $d(G^*G^*/CC)_m$ (lane 6), as opposed to the greater extent of GpApCpC synthesis relative to that of GpApC directed by $d(GG/CC)_m$ (lane 5). This finding renders unlikely that the observed single step addition reaction with $d(G^*G^*/CC)_m$ is simply due to a small amount of residual contaminating unplatinated polymer in the platinated template. From these data, we calculate that overall enzymic activity was depressed by a factor of 5 in transcription of $d(A^*G^*/TC)_m$ and by a factor of 9 with $d(G^*G^*/CC)_m$. Essentially the same results were obtained by substituting Mn^{2+} for Mg^{2+} (Figure 1B). The incomplete inhibition of GpApC synthesis with the platinated templates is reminiscent of recent observations that thymine photodimers provide a strong block to transcription in vitro by *E. coli* RNA polymerase and that the enzyme transcribes up to and including the 3′ T of the dimer (Selby & Sancar, 1990).

Figure 1C shows that the extent of GpA-primed GpApC synthesis by wheat germ RNA polymerase II was reduced on platinated DNA, but not to a zero value. Much more pronounced inhibition was found for the reaction of formation of GpApCpC with $d(G^*G^*/CC)_m$ (lane 10). All reactions directed by either unplatinated or platinated templates were strongly inhibited by α-amanitin, as expected for class II RNA polymerases (Sawadogo & Sentenac, 1990). Moreover, overall enzyme activity was reduced by a factor of 5 with $d(A^*G^*/TC)_m$ and by a factor of 11 with $d(G^*G^*/CC)_m$. Clearly, the eukaryotic and prokaryotic enzymes exhibited the same behavior during GpApC synthesis.

Kinetic Analysis of GpApC Synthesis. The incomplete inhibition of the synthesis of GpApC provided a tool for a quantitative description of the effect of *cis*-DDP adducts on transcriptional activity in formation of a single phosphodiester bond. The influence of DNA template, GpA primer, and CTP substrate concentrations on GpApC synthesis was assessed by determination of apparent K_m values.

In the case of $d(AG/TC)_m$ and $d(A^*G^*/TC)_m$, Figure 2A shows that saturation with respect to template could be achieved with both polymers. With the data plotted in the form [observed GpApC synthesis] vs [DNA], hyperbolic relationships were obtained, yielding apparent K_m values for the templates that are nearly identical for the platinated (K_m = 0.17 μM) and unplatinated (K_m = 0.24 μM) polymers.

Therefore, platination of the d(ApG) sequence does not affect the apparent binding affinity of enzyme for the template. However, consistent with the data in Figure 1, the comparative V_m values [4 and 0.72 nM in 45-min incubations for the d-(AG/TC)$_m$ and d(A*G*/TC)$_m$ polymers, respectively] reflected the higher template efficiency of unplatinated d-(AG/TC)$_m$.

Plots of the data in the form [observed GpApC synthesis] vs [GpA primer] could also be fitted with hyperbolic relationships, providing apparent K_m values for the primer that are nearly identical for the two templates [390 μM for d-(AG/TC)$_m$ and 310 μM for d(A*G*/TC)$_m$]. With CTP as the varied substrate, Michaelis–Menten kinetic behavior was also observed: here inhibition of GpApC synthesis with platinated d(A*G*/TC)$_m$ was primarily accounted for by a 6-fold increase in the apparent K_m for CTP. Thus, this value increased from 2.4 μM with d(AG/TC)$_m$ to 15 μM with d-(A*G*/TC)$_m$, a feature which is not consistent with the possibility that the platinated template was contaminated by residual amounts of the corresponding unplatinated sequence. At saturating CTP, the apparent V_m values for GpApC synthesis were nearly the same with d(AG/TC)$_m$ and d-(A*G*/TC)$_m$: both values were in the range of 12–15 nM for 45-min incubations.

These results show that the accessibility of the enzyme molecules to the template and the number of available sites for GpApC synthesis do not differ appreciably upon platination of the d(ApG) sequence. We conclude that the polymerase is able to bind to d(A*G*/TC)$_m$ in a position where it can use the GpA primer and that the most reasonable explanation for the slower rate of GpApC synthesis with the platinated polymer is that the enzyme active site is not positioned properly in order to bind the CTP substrate.

With regard to d(GG/CC)$_m$ and d(G*G*/CC)$_m$, Michaelis–Menten behavior was also observed by varying the template, primer, or CTP concentration. However, platination of DNA at the d(GpG) site was associated with an increase in the K_m for template by a factor of 5 (Figure 2B; unplatinated template, K_m = 0.26 μM; platinated template K_m = 1.4 μM) and with an increase in the K_m for GpA primer by a factor of 4–5 (unplatinated template, K_m = 486 μM; platinated template, K_m = 1714 μM). In contrast, modification of the d(GpG) sequence by *cis*-DDP changed only slightly the apparent K_m for CTP substrate in GpApC synthesis (unplatinated template, K_m = 9 μM; platinated template, K_m = 14 μM). In all these experiments, the extent of formation of GpApCpC with d(G*G*/CC)$_m$ remained below the limit of detection. Clearly, the single-step addition reactions with the d(G*pG*)-containing polymer exhibit distinctive features compared to those with the template modified at the d(ApG) site. Thus, contrarily to what is observed with d(A*G*/TC)$_m$, in the case of d(G*G*/CC)$_m$ the RNA polymerase binds to the platinated template with reduced apparent affinity and in a position where utilization of the dinucleotide primer is strongly impeded.

ApC-Primed Trinucleotide Syntheses. The above data indicate that single-step addition reactions are more impeded at d(G*pG*) than at d(A*pG*) adducts. To further substantiate this fact, transcription experiments were conducted in the presence of ApC as primer and either CTP or UTP as substrate. Under these conditions, one expects the formation of ApCpC with d(GG/CC)$_m$ and d(G*G*/CC)$_m$, and ApCpU with d(AG/TC)$_m$ and d(A*G*/TC)$_m$. It was observed that, although both the syntheses of ApCpC and ApCpU by *E. coli* RNA polymerase were depressed with d(G*G*/CC)$_m$ and d(A*G*/TC)$_m$, respectively, inhibition of ApCpC synthesis was 7-fold greater than that of ApCpU, confirming a marked difference in reactivity of the RNA polymerase at the d-(A*pG*) and d(G*pG*) adducts. Similar behavior was observed with the wheat germ enzyme. It is noticeable that d(A*pG*) adducts are repaired better than d(G*pG*) by the ABC excision nuclease in vitro (Page et al., 1988) and that d(G*pG*) adducts appear to inhibit DNA replication by prokaryotic and eukaryotic DNA polymerases more than d(A*pG*) (Hoffmann et al., 1989).

Role of Distortions of the DNA Double Helix Induced by cis-DDP Binding. Taken together, the results indicate that the RNA polymerases can differentiate between the two d-(G*pG*) and d(A*pG*) adducts in synthesis of the first phosphodiester bond directed by the platinated bases. What might be the basis of the different inhibition patterns? Studies with structure-specific chemical probes and same DNA sequences as those used here have shown that the conformational changes induced by the two d(A*pG*) and d(G*pG*) adducts are different: they bind similarly the double helix but the distortion induced by the d(A*pG*) adduct appears more asymmetrical than that induced by d(G*pG*) (Marrot & Leng, 1989; Schwartz et al., 1989). We suggest that the different inhibition patterns observed in single-step addition reactions with d(A*G*/TC)$_m$ and d(G*G*/CC)$_m$ reflect the distinctive features of the platinated polymer conformations.

To substantiate this possibility, we studied the template properties of derivatives of d(GG/CC)$_m$ and d(G*G*/CC)$_m$ containing a base pair mismatch (dG·dT), i.e., d(GG/TC)$_m$, d(G*G*/TC)$_m$, d(GG/CT)$_m$, and d(G*G*/CT)$_m$. The rationale for using these polymers relies upon the observation that, within the d(G*G*/CC) duplex, substitution of a dG*·dC bp by the less stable dG*·dT bp also transforms the apparent symmetrical distortion into an asymmetrical one (Schwartz et al., 1989). Therefore, if the conformation of the platinated DNA plays a role in the single-step addition reactions, the kinetic behavior of RNA polymerase should differ with the mismatch-containing templates from that observed with d-(G*G*/CC)$_m$. We find that this is indeed the case, since the apparent K_m for template in GpA-primed GpApC synthesis was not significantly different when transcription experiments were conducted in the presence of d(G*G*/TC)$_m$ (K_m = 0.12 μM), d(G*G*/CT)$_m$ (K_m = 0.16 μM), d(GG/TC)$_m$ (K_m = 0.16 μM), and d(GG/CT)$_m$ (K_m = 0.15 μM), contrarily to the results for d(G*G*/CC)$_m$ and d(GG/CC)$_m$.

To further substantiate the importance of localized distortions of the double helix on transcription, we studied the template properties of unplatinated polymers containing mismatches. Within d(GG/TC) and d(GG/CT) double helices, the mismatched dT residues move into the major groove but with minimal distortions in the global conformation (Kennard, 1987; Patel et al., 1987). We have determined the ApC-primed synthesis of ApCpC with CTP as substrate. Under the conditions of Figure 1A, the extents of synthesis by *E. coli* RNA polymerase were 2.1, 9.4, and 14.4 nM for the d(GG/CC)$_m$, d(GG/TC)$_m$, and d(GG/CT)$_m$ templates, respectively. For the wheat germ enzyme, the extent of ApC-primed ApCpC synthesis was increased by a factor of 3.3 with d(GG/TC)$_m$ and by a factor of 7 with d(GG/CT)$_m$, relative to that with d(GG/CC)$_m$. Thus, the two enzymes are sensitive to the presence of a mismatch located on the nontranscribed strand of the template.

Conclusion

The present work was undertaken to better define the interaction between RNA polymerases and platinated DNA.

We previously reported that adducts formed at the d(GpG) and d(ApG) site provide a strong elongation block during in vitro RNA synthesis by prokaryotic and eukaryotic RNA polymerases (Corda et al., 1991; Lemaire et al., 1991). By using an abortive elongation assay, we now show that these adducts also interfere with the formation of phosphodiester bonds by both of the enzymes. We suggest that the observed behavior of the enzymes depends mainly upon distortions in helix geometry induced by the binding of *cis*-DDP at these sites. This suggestion is supported by the results obtained with the unplatinated templates containing mismatches.

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