Spectrum of DNA–Platinum Adduct Recognition by Prokaryotic and Eukaryotic DNA-Dependent RNA Polymerases†

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ABSTRACT: Double-stranded DNA oligomers were constructed to evaluate the effect of bifunctional and monofunctional platinum(II) complexes at the level of DNA transcription. They contained a single lesion, which is either a *cis*-[$Pt(NH_3)_2${d(GpTpG)}] intrastrand cross-link, a *trans*-[$Pt(NH_3)_2${d(GpTpG)}] intrastrand cross-link, a *cis*-[$Pt(NH_3)_2${d(GpC/GpC)}] interstrand cross-link, or a (diethylenetriamine)-platinum(II)–dG adduct. The synthetic duplexes were multimerized and then used as templates in dinucleotide-primed reactions catalyzed by prokaryotic or eukaryotic RNA polymerases. Reactions were conducted in the presence of a single triphosphate substrate (single-step addition reaction) or of a combination of triphosphate substrates, permitting elongation of the trinucleotide products to longer RNA chains (productive elongation reaction), respectively. In transcription of the platinated strands, none of the DNA adducts provided an absolute block to formation of a single phosphodiester bond by either *Escherichia coli* RNA polymerase or wheat germ RNA polymerase II. However, the single-step addition reactions were much more impeded from transcription of bifunctional adduct-containing templates as compared to those containing monofunctional lesions. Productive elongation was irreversibly blocked in transcription of the platinated strand of templates containing a *cis*-d(G*pTpG*) intrastrand cross-link or a *cis*-d(G*pC/G*pC) interstrand cross-link. In both cases transcription stopped at the level of the lesion. Termination occurred also several nucleotides before the elongation complexes reached the interstrand cross-link. A substantial amount of the RNA polymerase molecules was able of bypassing the *trans*-d(G*pTpG*) cross-links. In all the cases single-step addition reactions were enhanced on the template strand complementary to that containing the intrastrand cross-links. The enhancement was dependent upon the nature of the lesions, being much larger in the case of *trans*- than in the case of *cis*-d(G*pTpG*)-containing polymer template. In transcription of the platinated strand of the template containing the monofunctional adduct, considerable bypass of the adduct was observed.

cis-Diamminedichloroplatinum(II) (*cis*-DDP)[1] is a powerful antitumor agent used in the chemotherapy of several human cancers. It is generally accepted that the curative effect of the drug is related to the attack of cellular DNA and to the formation of several types of adducts with DNA bases. In vivo and in vitro, *cis*-DDP reacts preferentially with adjacent purine residues, yielding two major adducts, *cis*-[$Pt(NH_3)_2${d(GpG)-*N*7,-*N*7}] and *cis*-$Pt(NH_3)_2${d(ApG)-*N*7,-*N*7}], representing 80–90% of the bound platinum (Fichtinger-Schepman et al., 1985, 1987; Eastman, 1986). Besides the two major lesions, the occurrence of several other adducts of platinum with the nucleobases of DNA has been well documented. *cis*-DDP is able to form bifunctional intrastrand cross-links between two guanines separated by one or several nucleotides and interstrand cross-links between two guanines located on opposite DNA strands [for general reviews, see Eastman (1987), Reedijk (1987), and Lepre and Lippard (1990)]. Very little is known about the contribution of each of these adducts to the biological effectiveness of *cis*-DDP. Of interest in this context is the fact that the isomer *trans*-diamminedichloroplatinum(II) (*trans*-DDP) also can form bifunctional adducts with the nucleobases of DNA, but is clinically inactive.

Several studies have documented the potential role of the platinum–DNA adducts on DNA replication [for a general review, see Lepre and Lippard (1990)]. Srivastava et al. (1978) and Sorenson and Eastman (1988a,b) suggested, however, the possibility that *cis*-DDP may exert its effect in vivo, at least in part, at the level of DNA transcription. Recently, we showed that *Escherichia coli* RNA polymerase and wheat germ RNA polymerase II reacted differently at d(ApG) and d(GpG) adducts in *cis*-DDP-modified DNA (Corda et al., 1991, 1992). In the present paper, we assess the ability of some minor adducts in *cis*-DDP-modified DNA to interfere with DNA transcription by the two enzymes. We report the template properties of double-stranded polymers containing the site-specifically-placed platinum adducts *cis*-[$Pt(NH_3)_2${d(GpTpG)-*N*7(1),-*N*7(3)}] and *cis*-[$Pt(NH_3)_2${d(GpC/GpC)-*N*7,-*N*7)}]. We also extend this study to double-stranded polymers containing a site-specifically-placed adduct formed by chloro(diethylenetriamine)platinum(II) or by *trans*-DDP.

MATERIALS AND METHODS

Reagents

Ultrapure ribonucleoside triphosphates and the dinucleoside monophosphate primers were purchased from Pharmacia and

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Boehringer Mannheim. α-^{32}P-labeled ribonucleoside triphosphates (410 Ci mmol^{-1}) were from Amersham.

Oligodeoxyribonucleotide Synthesis, Ligations, and Platination

The sequences of the repeating units of the duplexes used in this work are the following:

for the *cis* and *trans* intrastrand cross-links

d(GTG/CAC), *cis*-d(G*TG*/CAC),

trans-d(G*TG*/CAC)

5' CTTCCTCTTCT***G*T*G***TCTTCTCT
AAGGAGAAGACACAGAAGAGAG 5'

for the *cis* interstrand cross-links

d(GC/GC), *cis*-d(G*C/G*C)

5' CTTCTCCTT***G***CTCTCCTTCTCT
AAGAGGAAC***G***AGAGGAAGAGAG 5'

for the monofunctional monoadducts

d(G/C), d(G*/C)

5' CTCTCCTCT***G***TCTCTTCCTC
AGAGGAGACAGAGAAGGAGG 5'

The target dG are shown in italic bold. Oligodeoxyribonucleotides were synthesized, platinated, and ligated as described elsewhere (Schwartz et al., 1989; Corda et al., 1991, 1992; Brabec et al., 1992; Sip et al., 1992). We shall name the duplexes by their central sequences. The pyrimidine-rich and the purine-rich strands of the duplexes are referred to as the upper and the lower strands respectively. Wheat germ RNA polymerase II requires a stretch of about 40 bp to bind to DNA (Chandler & Gralla, 1980). Therefore, multimers of the above duplexes, referred to as d(GTG/CAC)$_m$, d(GC/GC)$_m$, d(G/C)$_m$ (unplatinated DNA), *cis*- and *trans*-d(G*TG*/CAC)$_m$ (platinum intrastrand adduct), d(G*C/G*C)$_m$ (platinum interstrand adduct), and d(G*/C)$_m$ (platinum monoadduct), 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 enzyme preparations was of the order of 2000 units mg^{-1} (wheat germ) and 65 000 units mg^{-1} (*E. coli*) on poly[d(A-T)] as template and with 20 μM ATP, 5 μM UTP, and 1.5 mM $MnCl_2$, 1 unit being equivalent to the incorporation of 10 pmol of UMP into poly[r(A-U)] in 15 min at 35 °C.

Transcription Reactions Primed by Dinucleotides

Since the synthetic DNA templates used in this work do not contain a natural promoter site, and because of the complexity of eukaryotic transcription initiation (Sawadogo & Sentenac, 1990), selective initiation of transcription was achieved by the use of dinucleotide primers. Both *E. coli* RNA polymerase [Oen & Wu, 1978; reviewed in McClure (1985)] and wheat germ RNA polymerase II (Dietrich et al., 1985; Job et al., 1988) have been shown to use such primers to initiate transcription at sequences complementary to those of the primers. Two types of reactions were performed, differing on whether the assays contained a single triphosphate substrate or a set (either full or incomplete) of ribonucleotides. The former conditions led to abortive synthesis of trinucleotides (single-step addition reactions). The latter allowed elongation of the trinucleotides to longer RNA chains (productive elongation assays).

Reaction Assays and Activity Measurements

(*i*) *Single-Step Addition Reactions.* Except otherwise noted, assays contained, in a total volume of 20 μL, 2.4 nM *E. coli* RNA polymerase or 10 nM wheat germ RNA polymerase II, 2.4 μM DNA (bp units), 1.5 mM $MnCl_2$ or 10 mM $MgCl_2$, 330 μM dinucleotide primer, and 1 μM appropriate α-^{32}P-labeled ribonucleoside triphosphate, in transcription buffer as described before (Corda et al., 1991). They were incubated for 1 h (wheat germ enzyme) or 45 min (*E. coli* enzyme), at 35 °C. Reactions were stopped by mixing the 20-μL reaction mixtures with 20 μL of stop solution containing 1 mM EDTA, 80% formamide, and 0.1% xylene cyanol. Syntheses of trinucleotides were detected after ascending TLC on poly(ethyleneimine)–cellulose sheets of 4 μL (reaction mixtures plus stop solution), using as the solvent system LiCl (0.05 M)/HCOOH (0.5 M) for the syntheses of GpApC and UpGpC, or LiCl (0.8 M)/HCOOH (1 M) for the synthesis of UpGpU. This provided a clear separation between the unused substrate (which remained bound at the

[1] Abbreviations and Nomenclature: bp, base pair(s); DDP, diamminedichloroplatinum(II); TLC, thin-layer chromatography. An asterisk denotes a deoxyribonucleotide modified by *cis*- or *trans*-diamminedichloroplatinum(II), or chloro(diethylenetriamine)platinum(II). d(GTG/CAC) is a double-stranded oligodeoxyribonucleotide of 21 bp, containing 19 pyrimidine bases and a central d(GpTpG) sequence on one strand, and 19 purine bases and a central d(CpApC) sequence on the complementary strand. *cis*-d(G*TG*/CAC) and *trans*-d(G*TG*/CAC) are the corresponding double-stranded sequences in which the sequence d(GpTpG) is modified by *cis*-DDP or *trans*-DDP, respectively. d(GTG/CAC)$_m$, *cis*-d(G*TG*/CAC)$_m$, and *trans*-d(G*TG*/CAC)$_m$ are multimers of the ligated d(GTG/CAC), *cis*-d(G*TG*/CAC), and *trans*-d(G*TG*/CAC) motifs, respectively. d(AG/CT) is a double-stranded oligodeoxyribonucleotide of 20 bp, containing 18 pyrimidine bases and a central d(ApG) sequence on one strand, and 18 purine bases and a central d(TpC) sequence on the complementary strand. d(A*G*/CT) is the corresponding double-stranded sequence in which the sequence d(ApG) is modified by *cis*-DDP. d(AG/CT)$_m$ and d(A*G*/CT)$_m$ are multimers of the ligated d(AG/CT) and d(A*G*/CT), respectively. d(GC/GC) is a double-stranded oligodeoxyribonucleotide of 21 bp, containing 21 pyrimidine bases and a central dG on one strand (upper strand), and 21 purine bases and a central dC on the complementary strand. d(G*C/G*C) is the corresponding double-stranded sequence modified by *cis*-DDP so as to contain a single interstrand platinum adduct including the unique dG on the upper strand. d(GC/GC)$_m$ and d(G*C/G*C)$_m$ are multimers of the ligated d(GC/GC) and d(G*C/G*C) motifs, respectively. d(G/C) is a double-stranded oligodeoxyribonucleotide of 19 bp, containing 19 pyrimidine bases and a central dG on one strand (upper strand), and 19 purine bases and a central dC on the complementary strand. d(G*/C) is the corresponding double-stranded sequence modified by chloro(diethylenetriamine)platinum(II) so as to contain a single monofunctional platinum adduct at the level of the unique dG on the upper strand. d(G/C)$_m$ and d(G*/C)$_m$ are multimers of the ligated d(G/C) and d(G*/C) motifs, respectively. Dinucleoside monophosphate primers and trinucleoside diphosphate products are referred to as dinucleotides and trinucleotides, respectively.

origin) and the reaction product. Following radioautography at −80 °C, all spots containing radioactivity were cut and the radioactivity was measured in a liquid scintillation counter. Control experiments lacking DNA template or dinucleotide primer were performed routinely, and all the rate measurements shown in the figures and tables were corrected by such blanks.

(ii) Productive Elongation Assays. Reactions were conducted as above, except that the assays also received various combinations of elongating nucleotides. The distribution of RNA chain lengths was analyzed by high-resolution electrophoresis on 20% polyacrylamide gels (0.03 cm × 30 cm × 40 cm), with an 8 M urea, 50 mM Tris–borate, pH 8.0 buffer. Gels were run at a constant 2000 V, until the xylene cyanol dye marker had migrated 10 cm. The gels were then covered with Saran wrap, and exposed to Fuji RX films at −80 °C with a Cronex Li-Plus intensifying screen from DuPont.

RESULTS AND DISCUSSION

*Dinucleotide-Primed Reactions Directed by d(GTG/CAC)$_m$, cis-d(G*TG*/CAC)$_m$, and trans-d(G*TG*/CAC)$_m$ Template Polymers.* The repeating units of d(GTG/CAC)$_m$ and of its platinated derivatives, *cis*-d(G*TG*/CAC)$_m$ and *trans*-d(G*TG*/CAC)$_m$, contain a unique d(TpC) sequence flanking the central sequence on the 3′ side. By using GpA as primer and CTP as substrate, the duplexes should permit, therefore, the direction of enzymic synthesis of GpApC. Although several d(TpC) sites are present in the repeating units, only one can support the single-step addition reaction with the CTP substrate. Figure 1A shows that *E. coli* RNA polymerase catalyzed the synthesis of GpApC in the presence of Mn^{2+} and d(GTG/CAC)$_m$ as template (lane 3). This synthesis was strongly depressed, by a factor of 7 with *cis*-d(G*TG*/CAC)$_m$ (lane 5) and by a factor of 4 with *trans*-d(G*TG*/CAC)$_m$ (lane 7). It was verified that the kinetics of GpApC synthesis were linear during a period of 70 min with d(GTG/CAC)$_m$, *cis*-d(G*TG*/CAC)$_m$, and *trans*-d(G*TG*/CAC)$_m$ as template. In addition, functional saturation of a given amount of enzyme was achieved by varying the concentration of each of the three polymers. These experiments revealed that the template activity ratio (GpApC synthesis with the platinated templates)/(GpApC synthesis with the unplatinated template) was independent of the polymer concentration in the assay (not shown). Essentially the same results as those presented in Figure 1A were obtained by substituting Mn^{2+} for Mg^{2+} (not shown). The incomplete inhibition of GpApC synthesis with the platinated templates modified by *cis*- and *trans*-DDP at a d(GpTpG) site is reminiscent of previous results showing 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 *E. coli* RNA polymerase (Corda et al., 1991, 1992).

d(GTG/CAC)$_m$, *cis*-d(G*TG*/CAC)$_m$, and *trans*-d(G*TG*/CAC)$_m$ were used as templates in the presence of wheat germ RNA polymerase II (Figure 1B). This comparative study was restricted to the use of $MnCl_2$ as the metal cofactor because the eukaryotic enzyme requires Mn^{2+} to catalyze the single-step addition reactions (Dietrich et al., 1986). As for the prokaryotic RNA polymerase, the extent of GpA-primed GpApG synthesis was strongly reduced on platinated DNA, by a factor of 14 with *cis*-d(G*TG*/CAC)$_m$ (lane 4) and by a factor of 9 with *trans*-d(G*TG*/CAC)$_m$ (lane 7). All reactions were strongly inhibited by α-amanitin (lanes 2, 5, and 8), a potent inhibitor of class II RNA polymerases (Sawadogo & Sentenac, 1990; Job et al., 1992). Clearly, the eukaryotic and prokaryotic enzymes exhibit the same behavior during catalysis of phosphodiester bond formation at the level of the adducts between *cis*- or *trans*-DDP and DNA.

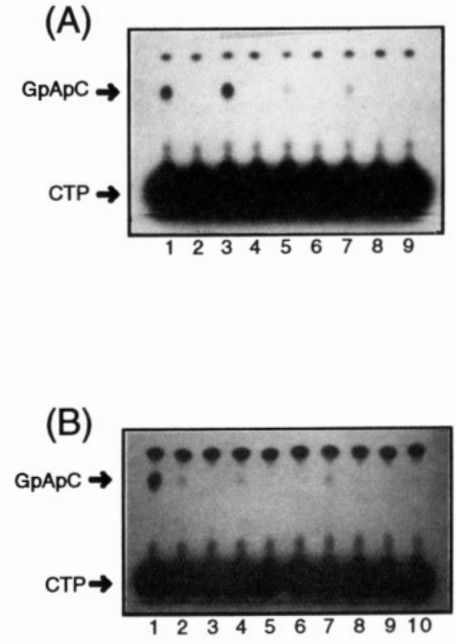

FIGURE 1: GpA-primed GpApC synthesis on the upper strand of d(GTG/CAC)$_m$, *cis*-d(G*TG*/CAC)$_m$, and *trans*-d(G*TG*/CAC)$_m$ with *E. coli* RNA polymerase and wheat germ RNA polymerase II. Single-step addition reactions were carried out, as described, with 330 μM GpA, 1 μM [α-^{32}P]CTP, 2.4 μM (bp units) DNA template, 1.5 mM $MnCl_2$, and 2.4 nM *E. coli* RNA polymerase (A) or 10 nM wheat germ RNA polymerase II (B). TLC and radioautographic analyses of the products synthesized from incubations in the presence of the following: (A) *E. coli* RNA polymerase. Template: (lanes 3, 5, and 7) d(GTG/CAC)$_m$, *cis*-d(G*TG*/CAC)$_m$, and *trans*-d(G*TG*/CAC)$_m$, respectively [the concentration of CTP substrate converted into GpApC was 2.6 nM (lane 3), 0.4 nM (lane 5), and 0.6 nM (lane 7)]; (lane 1) control showing the GpA-primed synthesis of GpApC on the upper strand of d(AG/CT)$_m$ (Corda et al., 1992); (lanes 2, 4, 6, and 8) as in lanes 1, 3, 5, and 7, respectively, minus GpA primer; (lane 9) complete reaction mixture minus template. (B) Wheat germ RNA polymerase II. Template: (lanes 1, 4, and 7) d(GTG/CAC)$_m$, *cis*-d(G*TG*/CAC)$_m$, and *trans*-d(G*TG*/CAC)$_m$, respectively [the concentration of CTP substrate converted into GpApC was 6.7 nM (lane 1), 0.5 nM (lane 4), and 0.7 nM (lane 7)]; (lanes 2, 5, and 8) as in lanes 1, 4, and 7, respectively, plus 50 μg mL^{-1} α-amanitin; (lanes 3, 6, and 9) as in lanes 1, 4, and 7, respectively, minus GpA primer; (lane 10) complete reaction mixture minus template.

d(GTG/CAC)$_m$, *cis*-d(G*TG*/CAC)$_m$, and *trans*-d(G*TG*/CAC)$_m$ were used as templates under conditions allowing productive RNA chain elongation reactions directed by the upper platinated strands. RNA synthesis was analyzed for product-length distribution by high-resolution gel electrophoresis. From the sequence of the upper strands, one should expect the synthesis of a discrete-length (21-mer) transcript in the presence of ApC as primer and of a limited set of triphosphate substrates (composed of ATP and GTP; the nucleotide substrate required for incorporation at position 22 is CTP). Under these conditions, the dinucleotide permits us to direct transcription at a unique d(TpG) site within the repeating units. [Note also that the length of the expected transcript corresponds to the distance between two adjacent platination sites within the d(GpTpG)-containing multimers]. Figure 2A shows the synthesis of a such discrete-length product in transcription of unplatinated d(GTG/CAC)$_m$ by *E. coli* RNA polymerase (lane 1). The product was elongated to

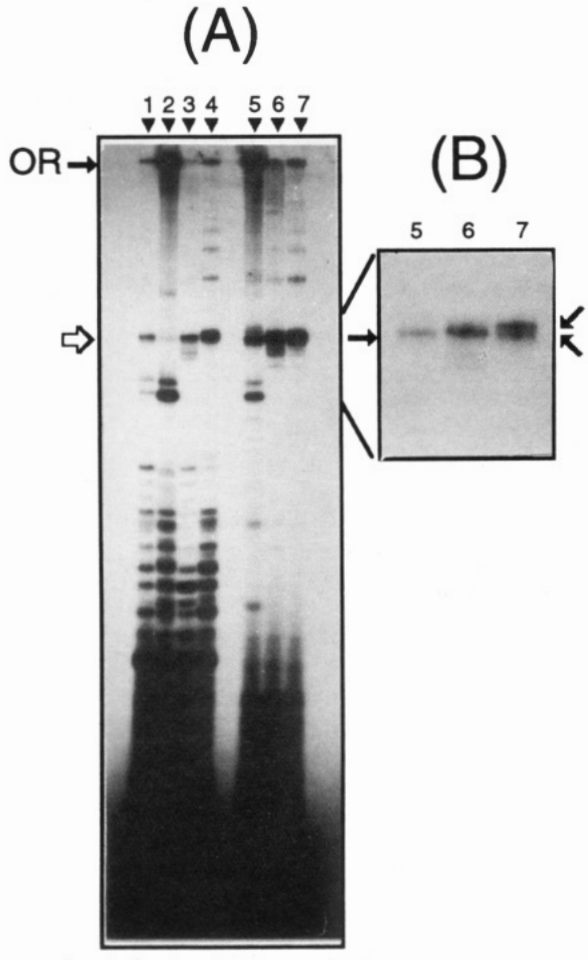

FIGURE 2: ApC-primed RNA synthesis on the upper strand of $d(GTG/CAC)_m$, *cis*-$d(G^*TG^*/CAC)_m$, and *trans*-$d(G^*TG^*/CAC)_m$ with *E. coli* RNA polymerase and wheat germ RNA polymerase II. (A) Transcription reactions were carried out, as described, with 330 μM ApC, 1 μM [α-^{32}P]ATP, 1 μM GTP, 2.4 μM (bp units) DNA template, 2.4 nM *E. coli* RNA polymerase or 10 nM wheat germ RNA polymerase II, and 1.5 mM $MnCl_2$. Aliquots of 10 μL (reaction mixture plus stop solution) were analyzed by polyacrylamide–urea gel electrophoresis. Incubations with *E. coli* RNA polymerase: (lane 1) $d(GTG/CAC)_m$ as template (the white arrow shows the longest oligonucleotide synthesized; the conditions allow the formation of a 21-mer transcript); (lane 2) as for lane 1 plus 1 μM CTP; (lanes 3 and 4) as for lane 2, with *cis*-$d(G^*TG^*/CAC)_m$ or *trans*-$d(G^*TG^*/CAC)_m$ as template, respectively. Incubations with wheat germ RNA polymerase II: (lanes 5, 6 and 7) as forlanes 2, 3, and 4, respectively, but in the presence of wheat germ RNA polymerase II. (B) is an enlargement of part of the data shown in (A), except that the radioautography was conducted for a shorter period of time. Black arrows show a single band in lane 6, and doubled bands in lane 7. OR stands for origin.

longer RNA chains in the additional presence of CTP (lane 2).

In the case of *cis*-$d(G^*TG^*/CAC)_m$ and the full set of triphosphates required for full copying of the repeating units (ATP + GTP + CTP), polymerization of long RNA chains was abolished (lane 3). The longest oligonucleotide synthesized under these conditions comigrated with the ApC-primed oligomer obtained from transcription of unplatinated $d(GTG/CAC)_m$ in the absence of CTP (compare lanes 1 and 3). This result indicates that the 3′ end of the transcript directed by *cis*-$d(G^*TG^*/CAC)_m$ extends up to the platinum adduct. Similar results were obtained in the presence of wheat germ RNA polymerase II (lanes 5 and 6), and for both eukaryotic and prokaryotic enzymes by substituting Mn^{2+} for Mg^{2+} (not shown). We conclude that RNA polymerases were able to use dinucleotide primers to initiate RNA synthesis within the *cis*-$d(G^*pTpG^*)$ cross-link and that transcription elongation was blocked on the platinum-adduct-containing strand, which indicates that the enzymes cannot translocate past the next platination sites. A similar behavior was previously observed in transcription of templates containing the intrastrand cross-links of *cis*-DDP formed at d(GpG) and d(ApG) sites (Corda et al., 1991).

Figure 2A (lane 4) shows the results of transcription elongation experiments directed by the platinated strand of *trans*-$d(G^*TG^*/CAC)_m$ in the presence of *E. coli* RNA polymerase, ApC primer, and the set of triphosphate substrates (ATP + GTP + CTP) required for full copying of this template strand. The enzyme behavior exhibited both similarities and differences to that encountered in transcription of *cis*-$d(G^*TG^*/CAC)_m$. First, for *cis*- and *trans*-DDP-modified templates the synthesis of long RNA chains was strongly blocked at the level of the first platinum adduct encountered by the elongation complex following initiation of RNA synthesis (compare lanes 4 and 3). Second, in contrast to the behavior observed with *cis*-$d(G^*TG^*/CAC)_m$ (lane 3), in the case of *trans*-$d(G^*TG^*/CAC)_m$ electrophoretic analysis of the transcripts showed the presence of doubled bands at the level of the major stop site (lane 4), indicating that the enzyme transcribed up to and including the first modified dG within the platinated $d(G^*pTpG^*)$ sequence. Third, in the case of *trans*-$d(G^*TG^*/CAC)_m$ a finite proportion of the enzyme molecules appeared capable to translocate past the platination sites (lane 4). It should be noted that the pattern of RNA chain length distribution from transcription of *trans*-$d(G^*TG^*/CAC)_m$ (lane 4) was completely different from that observed with unplatinated $d(GTG/CAC)_m$ (lane 2), which renders unlikely that the bypass of a *trans*-DDP adduct was simply due to a small amount of residual contaminating unplatinated polymer in the platinated template. We examined the possibility that the bypass was due to a decreased accuracy of RNA polymerases during insertion of nucleotide substrates complementary to the platinated DNA bases. Such a mechanism has been proposed to account for the bypass of interstrand DNA lesions formed by a psoralen derivative, 4′-(hydroxymethyl)-4,5′,8-trimethylpsoralen, in transcription catalyzed by T7 RNA polymerase (Shi et al., 1988b; Sastry & Hearst, 1991a). However, we did not observe the readthrough from transcription assays carried out with *trans*-$d(G^*TG^*/CAC)_m$ and an incomplete set of triphosphate substrates lacking CTP. In this case, the longest transcript corresponded to the expected 21-mer product (gel no shown). This experiment indicates that bypassing cannot be attributed to misinsertion effects in our present investigation. Results similar to those shown in Figure 2A were obtained in the presence of wheat germ RNA polymerase II (Figure 2, lanes 5, 6, and 7), and for both the prokaryotic and eukaryotic enzymes by substituting Mn^{2+} for Mg^{2+} (not shown). We conclude that the *trans*-$d(G^*pTpG^*)$ intrastrand adduct is not an absolute block to transcription elongation by the RNA polymerases, in contrast to the intrastrand adducts formed by *cis*-DDP at d(GpG) and d(ApG) sites (Corda et al., 1991) or at a d(GpTpG) site (Figure 2). Recent results by Comess et al. (1992) are interesting in connection with the observed bypassing phenomenon. These researchers showed that a site-specifically-placed adduct of *trans*-DDP, *trans*-$[Pt(NH_3)_2$-{d(CpGpCpG)-*N*3(1),-*N*7(4)}], was a poor block to DNA polymerases. Taking into account the fact that monofunctional adducts formed by chloro(diethylenetriamine)platinum(II) do not impede replication by DNA polymerase I (Pinto & Lippard, 1985), Comess et al. (1992) suggested that

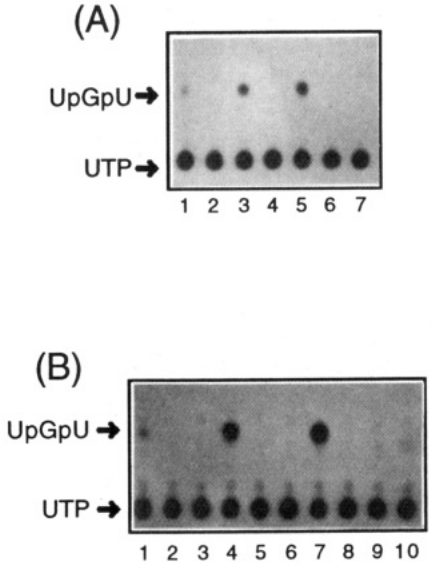

FIGURE 3: UpG-primed UpGpU synthesis on the lower strand of d(GTG/CAC)$_m$, *cis*-d(G*TG*/CAC)$_m$, and *trans*-d(G*TG*/CAC)$_m$ with *E. coli* RNA polymerase and wheat germ RNA polymerase II. Single-step addition reactions were carried out, as described, with 330 μM UpG, 1 μM [α-^{32}P]UTP, 1.5 mM $MnCl_2$, 2.4 μM (bp units) DNA template, and 2.4 nM *E. coli* RNA polymerase (A) or 10 nM wheat germ RNA polymerase II (B). TLC and radioautographic analyses of the products synthesized from incubations in the presence of the following: (A) *E. coli* RNA polymerase. Template: (lanes 1, 3, and 5) d(GTG/CAC)$_m$, *cis*-dG*TG*/CAC)$_m$, and *trans*-d(G*TG*/CAC)$_m$, respectively [the concentration of UTP substrate converted into UpGpG was 23.4 nM (lane 1), 73 nM (lane 3), and 106 nM (lane 5)]; (lanes 2, 4, and 6) as in lanes 1, 3, and 5, respectively, minus UpG primer; (lane 7) complete reaction mixture minus template. (B) Wheat germ RNA polymerase II. Template: (lanes 1, 4, and 7) d(GTG/CAC)$_m$, *cis*-d(G*TG*/CAC)$_m$, and *trans*-d(G*TG*/CAC)$_m$, respectively [the concentration of UTP substrate converted into UpGpU was 28 nM (lane 1), 243 nM (lane 4), and 337 nM (lane 7)]; (lanes 2, 5, and 8) as in lanes 1, 4, and 7, respectively, plus 50 μg mL^{-1} α-amanitin; (lanes 3, 6, and 9) as in lanes 1, 4, and 7, respectively, minus UpG primer; (lane 10) complete reaction mixture minus template.

replication bypass of bifunctional adducts might occur if the polymerases were to encounter an adduct in a transient monofunctional state. Although we do not have any evidence to support this type of mechanism for the observed transcriptional readthrough, it is noteworthy that, for RNA polymerases (see Figure 5) and DNA polymerase I, monofunctional platinum adducts do not appear to impede the elongation phase of the polymerization reaction.

To substantiate further the fact that the RNA polymerases reacted differently at the two *cis*- and *trans*-d(G*pTpG*) adducts, single-step addition reactions were conducted in the presence of UpG as primer and UTP as substrate. These conditions permitted direction of the synthesis of UpGpU at the level of the central dA-dC within the repeating units of the lower strands of the multimers, complementary to the dG involved in *cis*- and *trans*-DDP binding on the upper strands. Figure 3A shows that both platinated templates supported the synthesis of UpGpU with *E. coli* RNA polymerase. It is noticeable that the level of trinucleotide formation was higher with both *cis*-d(G*TG*/CAC)$_m$ and *trans*-d(G*TG*/CAC)$_m$ (lanes 3 and 5) as opposed to that with d(GTG/CAC)$_m$ (lane 1). The enhancement was dependent upon the nature of the lesions, being much larger in the case of *trans*- than in the case of *cis*-d(G*pTpG*)-containing polymer template (Table I). The same behavior was observed with *E. coli* RNA polymerase in the presence of $MgCl_2$ (not shown) and with wheat germ RNA polymerase II (Figure 3B, Table I). It is

Table I: Template Activity Ratio for UpG-Primed UpGpU Synthesis with Platinated and Unplatinated Polymers[a]

enzyme source	polymer templates (platinated versus unplatinated)	activity ratio[a]
E. coli	d(G*/C)$_m$/d(G/C)$_m$	2.4
E. coli	*cis*-d(G*TG*/CAC)$_m$/d(GTG/CAC)$_m$	3.1
E. coli	*trans*-d(G*TG*/CAC)$_m$/d(GTG/CAC)$_m$	4.5
wheat germ	d(G*/C)$_m$/d(G/C)$_m$	1.3
wheat germ	*cis*-d(G*TG*/CAC)$_m$/d(GTG/CAC)$_m$	8.2
wheat germ	*trans*-d(G*TG*/CAC)$_m$/d(GTG/CAC)$_m$	10.7

[a] (UpGpU synthesis with the indicated platinated polymer template)/(UpGpU synthesis with the corresponding unplatinated template). Single-step addition reactions were carried out, as described, with 330 μM UpG, 1 μM [α-^{32}P]UTP, 2.4 μM (bp units) DNA template, 2.4 nM *E. coli* RNA polymerase or 10 nM wheat germ RNA polymerase II, and 1.5 mM $MnCl_2$. Aliquots of 4 μL (reaction mixture plus stop solution) were analyzed by TLC.

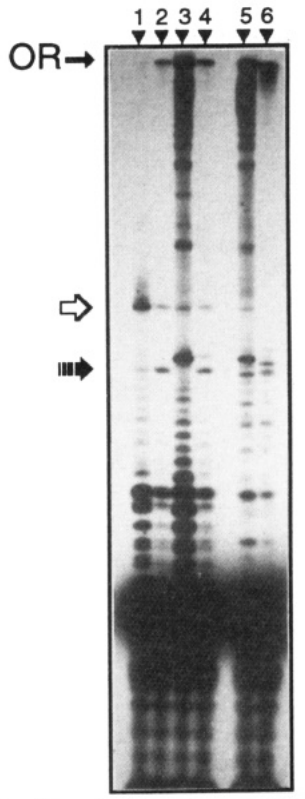

FIGURE 4: Dinucleotide-primed reactions of RNA synthesis on the upper strands of d(GC/GC)$_m$ and *cis*-d(G*C/G*C)$_m$ templates with *E. coli* RNA polymerase and wheat germ RNA polymerase II. Transcription reactions were carried out as described in the Materials and Methods. The dinucleotide primer was GpG. The divalent metal cofactor was Mn^{2+}. Aliquots of 10 μL (reaction mixture plus stop solution) were analyzed by polyacrylamide–urea gel electrophoresis. Key: (lanes 1 and 2), d(GC/GC)$_m$ or d(G*C/G*C)$_m$ as template, respectively, 330 μM GpG as primer, 1 μM [α-^{32}P]ATP and 1 μM GTP as substrates, and 2.4 nM *E. coli* RNA polymerase (the white and black arrows show the longest oligonucleotides synthesized with the unplatinated and platinated templates, respectively; the conditions allow the formation of a 19-mer transcript); (lanes 3 and 4) d(GC/GC)$_m$ or d(G*C/G*C)$_m$ as template, respectively, 330 μM GpG primer, 1 μM [α-^{32}P]ATP, 1 μM GTP, and 1 μM CTP as substrates, and 2.4 nM *E. coli* RNA polymerase; (lanes 5 and 6) as for lanes 3 and 4, respectively, but in the presence of 10 nM wheat germ RNA polymerase II. OR stands for origin.

known that the conformational changes (bending, unwinding, extent of local denaturation) induced by *cis*-d(G*pTpG*) and *trans*-d(G*pTpG*) adducts are different (Anin & Leng, 1990;

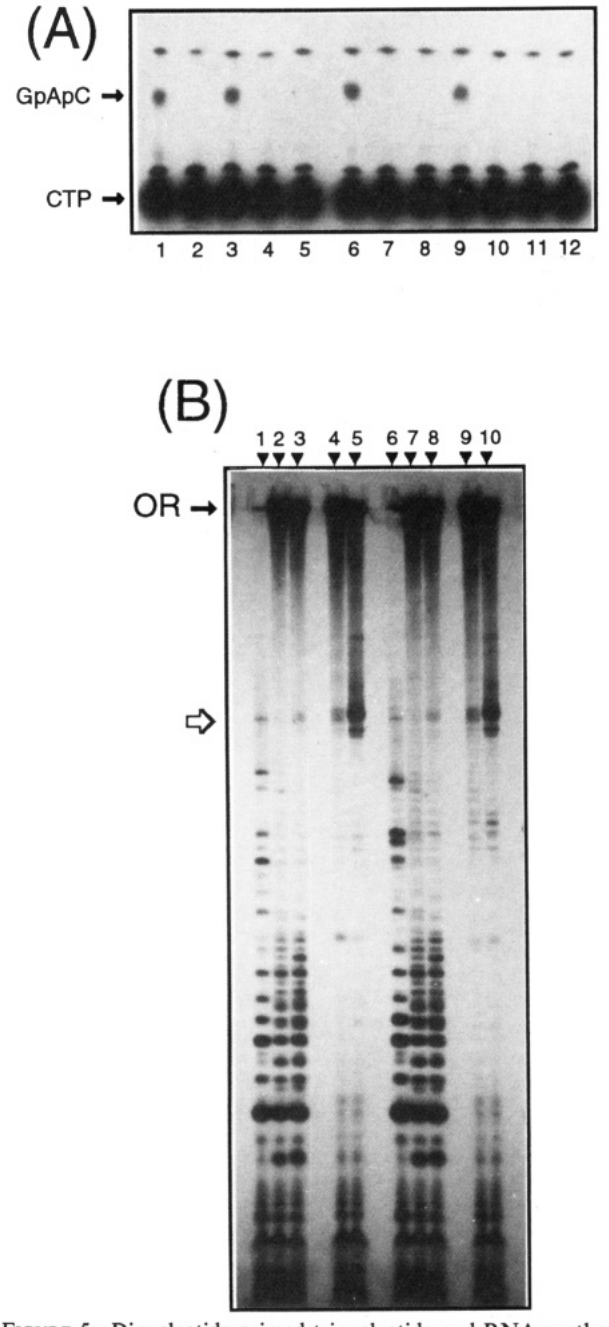

FIGURE 5: Dinucleotide-primed trinucleotide and RNA syntheses on the upper strand of $d(G/C)_m$ and *cis*-$d(G^*/C)_m$ with *E. coli* RNA polymerase and wheat germ RNA polymerase II. (A) GpA-primed GpApC synthesis. Single-step addition reactions were carried out, as described, with 330 μM GpA primer, 1 μM [α-^{32}P]CTP, 2.4 μM (bp units) DNA template, 2.4 nM *E. coli* RNA polymerase (lanes 1–5) or 10 nM wheat germ RNA polymerase II (lanes 6–12), and 1.5 mM $MnCl_2$. Aliquots of 4 μL (reaction mixtures plus stop solution) were analyzed by TLC. Key: (lanes 1 and 6) complete assays with $d(G/C)_m$ as template [the concentration of CTP substrate converted into GpApC was 5.5 nM (lane 1) and 8 nM (lane 6)]; (lanes 2 and 8) as for lanes 1 and 6, respectively, in the absence of GpA primer; (lanes 3 and 9) complete assays with $d(G^*/C)_m$ as template [the concentration of CTP substrate converted into GpApC was 6.2 nM (lane 3) and 6 nM (lane 9)]; (lanes 4 and 11) as for lanes 3 and 9, respectively, in the absence of GpA primer; (lanes 7 and 10) as for lanes 6 and 9, respectively, in the presence of 50 μg mL^{-1} α-amanitin; (lanes 5 and 12) as for lanes 1 and 6, respectively, in the absence of DNA template. (B) Dinucleotide-primed RNA synthesis. The dinucleotide primer was ApC or CpA. Aliquots of 10 μL (reaction mixture plus stop solution) were analyzed by polyacrylamide–urea gel electrophoresis. (Lane 1) 330 μM ApC as primer, 2.4 μM $d(G/C)_m$ (bp units) as template, 2.4 nM *E. coli* RNA polymerase, 1 μM [α-^{32}P]ATP and 1 μM GTP as substrates, and 1.5 mM Mn^{2+} as divalent metal cofactor (the white arrow shows the longest oligonucleotide synthesized; the conditions allow the formation of a 21-mer transcript); (lane 2) as for lane 1 plus 1 μM CTP; (lane 3) as for lane 2, but in the presence of 2.4 μM $d(G^*/C)_m$ (bp units) as template; (lanes 4 and 5) as for lanes 2 and 3, respectively, but in the presence of 10 nM wheat germ RNA polymerase II; (lane 6) 330 μM CpA as primer, 2.4 μM $d(G/C)_m$ (bp units) as template, 2.4 nM *E. coli* RNA polymerase, 1 μM [α-^{32}P]ATP and 1 μM GTP as substrates, and 1.5 mM Mn^{2+} as divalent metal cofactor (the conditions allow the formation of a 21-mer transcript, white arrow); (lane 7) as for lane 6 plus 1 μM CTP; (lane 8) as for lane 7, but in the presence of 2.4 μM $d(G^*/C)_m$ (bp units) as template; (lanes 9 and 10) as for lanes 7 and 8, respectively, but in the presence of 10 nM wheat germ RNA polymerase II. OR stands for origin.

Bellon et al., 1991; Boogaard et al., 1993). The present results support the notion (Corda et al., 1991, 1992) that RNA polymerases are sensitive to the distinctive features of the platinated polymer conformations.

Dinucleotide-Primed Reactions Directed by Unplatinated $d(GC/GC)_m$ and Platinated $d(G^*C/G^*C)_m$ Containing Site-Specifically-Placed Interstrand Bifunctional Adducts. Transcription elongation experiments were carried out by using as templates unplatinated $d(GC/GC)_m$ and its platinated derivative $d(G^*C/G^*C)_m$. In the presence of GpG primer and a set of triphosphate substrates composed of ATP and GTP, the maximum transcript length expected from the template sequence corresponds to a 19-mer (full copying of the strand requires the additional presence of CTP). Figure 4 shows the longest transcription product (white arrow) synthesized under these conditions by *E. coli* RNA polymerase with $d(GC/GC)_m$ (lane 1). As expected the formation of this transcript was abolished in the additional presence of CTP, and this was accompanied by a concomitant synthesis of longer RNA chains (lane 3). Under the same experimental conditions transcription of the upper strand of platinated $d(G^*C/G^*C)_m$ was strongly impeded and yielded RNA species of different lengths (lane 4). The longest of these oligonucleotides comigrated with the longest transcript detected in lane 1, and corresponds presumably to a block in transcription elongation at the level of the platinated bases. It is known that *E. coli* RNA polymerase (von Hippel et al., 1984) and RNA polymerase II (Goldring et al., 1992) have both unwindase and rewindase activities that define the limits of the DNA unwound region and catalyze its propagation through the double helix. Results showing that RNA polymerases are blocked specifically at the level of DNA–drug adducts such as those formed by actinomycin D (Phillips & Crothers, 1986) or psoralen (Shi et al., 1988a,b; Sastry & Hearst, 1991a,b) have suggested a close proximity between the catalytic site, where phosphodiester bonds are synthesized, and the unwinding activity on the enzyme molecule. The present results showing that the 3′ end of the nascent RNA extends up to the interstrand platinum adduct are consistent with this model.

During transcription of the upper strand of $d(G^*C/G^*C)_m$ the enzyme was also arrested with considerable efficiency several bases before the platination site (lane 4, black arrow). Similar results were obtained with wheat germ RNA polymerase II (lanes 5 and 6). It has been proposed, on the basis of gel electrophoresis, chemical probes, and molecular mechanics modeling data, that *cis*-DDP interstrand adducts bend the double helix by approximately 55° toward the major groove (Sip et al., 1992). In contrast, interstrand psoralen cross-links do not introduce appreciable bends in DNA (Sinden & Hagerman, 1984; Haran & Crothers, 1988). Possibly, premature termination of transcription may derive from alterations in DNA structure induced by the *cis*-DDP interstrand adduct.

A similar study using the platinated lower strand as a template confirmed that the prokaryotic and eukaryotic enzymes are stopped either at the level of the interstrand cross-link or a few nucleotides before the cross-link (not shown).

Dinucleotide-Primed Reactions Directed by Unplatinated $d(G/C)_m$ and Platinated $d(G^*/C)_m$ Containing Site-Specifically-Placed Monofunctional Adducts. The influence of platinum(II) monofunctional adducts on transcription was evaluated by using as templates unplatinated $d(G/C)_m$ and its derivative $d(G^*/C)_m$ modified by chloro(diethylenetriamine)platinum(II). Single-step addition reactions and productive elongation reactions were only slightly affected by the presence of the monofunctional adducts on the template strand (Figure 5). Both the prokaryotic and eukaryotic RNA polymerases were able to bypass the monofunctional platinum adducts to a considerable extent (Figure 5B). In single-step addition reactions conducted in the presence of UpG as primer and UTP as substrate, the synthesis of UpGpU directed by the central dA-dC-dA, complementary to the platination site on the upper strands, was enhanced with $d(G^*/C)_m$ compared with that directed by $d(G/C)_m$ (Table I), but this enhancement was much smaller than that observed with the templates containing bifunctional cross-links (Table I). Although the monofunctional adducts induce a distortion of the double helix (van Garderen et al., 1989; Brabec et al., 1992), the distortion by itself is not sufficient to arrest the *E. coli* and wheat germ RNA polymerases. This is in agreement with results showing that T7 RNA polymerase was not stopped by bulky monofunctional adducts of *cis*-DDP (Anin et al., 1992).

CONCLUSION

In the present study synthetic double-stranded DNA polymers containing site-specifically-placed platinum adducts of *cis*-DDP, *trans*-DDP, or chloro(diethylenetriamine)platinum(II) were used as templates in transcription experiments using purified prokaryotic and eukaryotic RNA polymerases. The results presented here, together with those of previous studies (Corda et al., 1991,1992), allow an analysis of the effect of the DNA–platinum adducts formed in vitro and in vivo at the level of DNA transcription in vitro and to define better the interaction between RNA polymerases and platinated DNA. The intrastrand *cis*-DDP adducts formed at d(GpG), d(ApG), or d(GpTpG) sites as well as the interstrand cross-links between two dG residues were found to provide a strong elongation block for the RNA polymerases. In contrast, neither the *trans*-DDP adduct nor the monofunctional platinum adduct presently studied provided an absolute block for the enzymes. We have presented evidence showing that the RNA polymerases react differently at the various platinum adducts. The results suggest that these adducts may not only constitute a physical barrier to the progress of RNA polymerase on the template but also alter specifically the properties of transcription complexes, owing to the specific conformational changes that they induce in the double helix. Future experiments will help to decipher whether the behavior observed in vitro is relevant for in vivo DNA transcription.

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