DNA Synthesis Arrest at C4′-Modified Deoxyribose Residues†

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ABSTRACT: Many genotoxic agents form base lesions that inhibit DNA polymerases. To study the mechanism underlying termination of DNA synthesis on defective templates, we tested the capacity of a model enzyme (Klenow fragment of *Escherichia coli* DNA polymerase I) to catalyze primer elongation across a series of C4′ deoxyribose derivatives. A site with inverted C4′ configuration or two different C4′ deoxyribose adducts were introduced into the backbone of synthetic templates without modifying the chemistry of the corresponding bases. Inverted deoxyribose moieties may arise in cellular DNA as a product of C4′ radical attack. We found that DNA synthesis by the Klenow polymerase was arrested transiently at the C4′ inversion and was essentially blocked at C4′ deoxyribose adducts. Major termination sites were located one position downstream of a C4′ selenophenyl adduct and immediately 3′ to or opposite a C4′ pivaloyl adduct. Primer extension studies in the presence of single deoxyribonucleotides showed intact base pairing fidelity opposite all three C4′ variants regardless of whether the Klenow fragment or its proofreading-deficient mutant was tested. These results imply that the coding ability of template bases is maintained at altered C4′ deoxyribose moieties. However, their capacity to impede DNA polymerase progression indicates that backbone distortion and steric hindrance are important determinants of DNA synthesis arrest on damaged templates. The strong inhibition by C4′ adducts suggests a potential target for new chemotherapeutic strategies.

Inhibition of DNA replication represents a major mechanism of cytotoxicity exerted by many DNA-damaging agents (Strauss, 1985; Hruszkewycz et al., 1992; Shibutani et al., 1993; Voigt & Topal, 1995), but the molecular basis of DNA synthesis arrest at sites of damage is not completely understood. Studies with purified DNA polymerases have shown that primer elongation stops either 3′ to or opposite a lesion on the template strand (Larson et al., 1985). Even those types of damage that disrupt the coding ability of template bases are compatible with the occasional addition of a deoxyribonucleotide opposite the lesion (Rabkin & Strauss, 1984; Strauss, 1991). After nucleotide addition across this critical site, however, further elongation of the growing polynucleotide is in most cases impaired. The extent of residual replicative bypass is modulated by the proofreading exonuclease activity of certain DNA polymerases (Strauss & Wang, 1990), but complete loss of 3′−5′ exonuclease activity is frequently not sufficient to promote primer elongation over sites of damage (Strauss & Wang, 1990; Moore et al., 1981; Shwartz et al., 1988; Woodgate et al., 1987). To account for this observation, it has been proposed that DNA polymerases suffer from the reduced stability of base pair interactions downstream of noninstructional lesions (Strauss, 1991). This view is challenged by the observation that polynucleotide chain elongation may not be possible even when correct deoxyribonucleotides are inserted opposite a damaged residue (Moore et al., 1982; Clark & Beardsley, 1986; Lindsley & Fuchs, 1994). Thus, it appears that additional factors contribute to the poor capacity of most DNA polymerases to perform translesion synthesis.

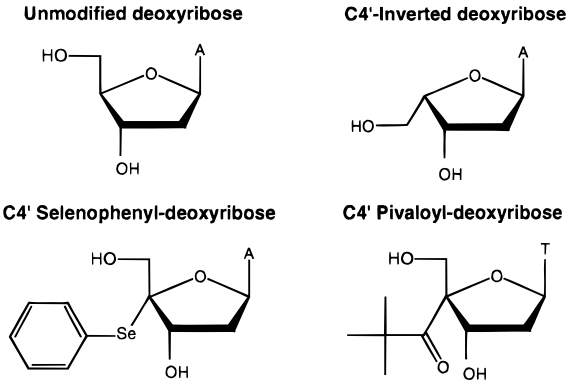

FIGURE 1: Structure of C4′ deoxyribose modifications. Deoxyribose derivatives were obtained by inversion of the configuration at carbon C4′ or by C4′ substitutions with a selenophenyl or a pivaloyl group.

To identify structural determinants of DNA synthesis inhibition, we tested the ability of a model DNA polymerase to replicate templates containing C4′-altered deoxyribose moieties. These DNA backbone modifications differ from normal deoxyribose residues by inversion of the molecular configuration at the stereogenic C4′ residue or by formation of C4′ deoxyribose adducts with either a selenophenyl or a pivaloyl group (Figure 1). The Klenow fragment of *Escherichia coli* DNA polymerase I was chosen for these studies because this enzyme has been characterized in great detail both at the functional and at the structural level (Ollis et al., 1985; Beese et al., 1993; Sousa, 1996; Bambara et al., 1976; Eger & Benkovic, 1992). In addition, DNA

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polymerase I or its Klenow fragment has been widely employed in previous studies to test the cytotoxic and mutagenic effects of a variety of base lesions [see for example Michaels et al. (1987), Taylor and O'Day (1990), Shibutani et al. (1991), Comess et al. (1992), Sun and Hurley (1992), Basu et al. (1993), Sagher et al. (1994), and Doisy and Tang (1995)]. Thus, the use of DNA polymerase I Klenow fragment allows us to compare the results of the present study on deoxyribose modifications with a large number of previous reports concerned with base modifications.

We observed that the C4′ inversion induced a transient block, while single C4′ selenophenyl- or pivaloyldeoxyribose adducts constituted nearly absolute barriers to completion of DNA synthesis by the tested polymerase. In parallel, we found that the inherent base pairing fidelity of the DNA polymerase was not detectably disturbed at C4′ deoxyribose variants. These results confirm that disruption of base pairing capacity is not the sole mechanism of damage-induced blocking of DNA polymerase progression. Instead, backbone distortion and steric hindrance constitute primary determinants of DNA synthesis arrest at DNA adducts or inappropriate nucleotides. The nonmutagenic properties displayed by the C4′ inversion *in vitro* may be relevant because of the possible formation of this lesion in cellular DNA (Cadet & Berger, 1985; Akhlaq et al., 1987). The strong block to DNA synthesis in response to C4′ deoxyribose adducts is a novel observation that may be exploited to develop nonmutagenic anti-cancer chemotherapeutics.

EXPERIMENTAL PROCEDURES

Chemicals and Enzymes. Deoxyribonucleotide 5′-triphosphates were purchased from Sigma. [γ-^{32}P]ATP (3000 Ci/mmol) was obtained from DuPont-New England Nuclear. DNA polymerase I Klenow fragment (6000 units/mg) was from Boehringer, and Klenow fragment lacking 3′−5′ exonuclease activity was from Amersham. T4 polynucleotide kinase was from Gibco BRL.

Synthesis of C4′-Altered Phosphoramidite Building Blocks. C4′ deoxyribose adducts were obtained as described previously (Giese et al., 1994a,b). Briefly, the selenophenyl adduct was synthesized starting from a substituted deoxyadenosine derivative. Swern oxidation yielded a 5′-aldehyde that was susceptible to reaction with triethylamine-activated phenylselenyl chloride. After reduction with tetrabutylammonium cyanoborohydride, the desired isomer of (selenophenyl)deoxyribose was isolated by flash chromatography (Giese et al., 1994a). This synthetic procedure also resulted in the formation of (selenophenyl)deoxyribose residues with inverted C4′ configuration, which were used to generate C4′-inverted deoxyadenosine derivatives. For that purpose, the inverted (selenophenyl)deoxyribose byproducts were exposed to UV irradiation (250 W, Hg lamp) for 2.5 h at room temperature. Irradiation was performed in the presence of tributyltin hydride, and the desired reaction product was isolated by silica gel chromatography. To obtain the pivaloyl adduct, an appropriate deoxythymidine precursor was converted to its aldehyde derivative, reacted with *tert*-butyllithium, reoxidized to the ketone, and purified by flash chromatography (Giese et al., 1994b). All three C4′-modified nucleosides were tritylated at their primary and phosphitylated at their secondary alcohol functions to produce building blocks for automated oligonucleotide synthesis.

TEMPLATES:

3'-CACCACGCTTAAGACACCTA-5'	Unmodified
3'-CACCACGCTTAAG**A**CACCTA-5'	C4'-Inverted deoxyribose
3'-CACCACGCTTAAG**A**CACCTA-5'	Selenophenyl-deoxyribose
3'-CACCACGCTTAAG**T**CACCTA-5'	Pivaloyl-deoxyribose

PRIMERS:

5'-GTGGTGCG-3'	8-mer
5'-GTGGTGCGAATTC-3'	13-mer
5'-GTGGTGCGAATTCT-3'	14-mer

FIGURE 2: Templates and primers used to assess the effects of C4′ deoxyribose modification on DNA synthesis. Oligonucleotide templates containing an altered backbone residue at position 14 were synthesized by the cyanoethyl phosphoramidite method using appropriate building blocks. The sites of template modification are shown in bold letters.

DNA Substrates. Templates and primers were synthesized using cyanoethyl phosphoramidite chemistry. DNA oligomers containing the C4′ deoxyribose variants were analyzed by sequential digestion with snake venom phosphodiesterase followed by matrix-assisted laser desorption/ionization−time of flight mass spectroscopy to confirm complete modification at the expected position. The primers were phosphorylated at their 5′ ends by incubation with T4 polynucleotide kinase and [γ-^{32}P]ATP (Sambrook et al., 1989) and purified by gel filtration using Quick Spin columns (Boehringer).

DNA Polymerase Reactions. 20-mer templates (150 pmol/mL) and 5′ end-labeled 8-mer primers (50 pmol/mL) were mixed in 62.5 mM Tris-HCl (pH 7.4), 12.5 mM $MgCl_2$, and 0.12 mM dithiothreitol, heated to 90 °C for 5 min, and cooled slowly to room temperature. After annealing, template/primers (2 pmol as the primer) were incubated at 25 °C in reaction mixtures of 50 μL containing 50 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, 0.1 mM dithiothreitol, 50 μg/mL BSA, 25 μM dATP, 25 μM dCTP, 25 μM dGTP, 25 μM TTP, and 0.5 unit (approximately 1 pmol) of DNA polymerase (Klenow fragment of DNA polymerase I or its exonuclease-deficient mutant). To determine which nucleotide is preferentially incorporated opposite C4′-modified substrates, 20-mer templates were annealed with 13-mer or 14-mer primers as indicated before, but the subsequent polymerase reaction mixtures contained only one deoxyribonucleotide 5′-triphosphate at a concentration of 25 μM. After the indicated time periods, incubations were stopped by the addition of EDTA to 20 mM and boiling for 30 s. Reaction products were analyzed by 20% polyacrylamide gel electrophoresis under denaturing conditions (Sambrook et al., 1989).

RESULTS

Inhibition of DNA Synthesis. To study the effect of C4′ deoxyribose variants on DNA synthesis, we constructed 20-mer oligonucleotides containing a single backbone modification at the 14th position from the 3′ end. These templates were annealed with radiolabeled 8-mer oligonucleotide primers as outlined in Figure 2 and incubated with DNA polymerase I Klenow fragment in the presence of all four deoxyribonucleotide 5′-triphosphates. Reaction products were resolved by denaturing polyacrylamide gel electrophoresis and visualized by autoradiography (Figure 3).

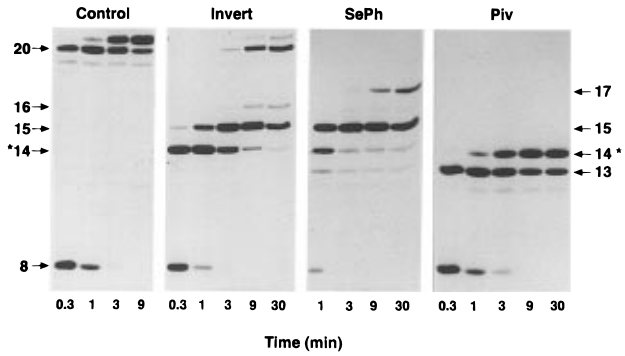

FIGURE 3: DNA synthesis arrest on templates containing C4′-modified deoxyribose residues. Twenty-nucleotide templates were annealed with a 5′ end-labeled 8-mer primer and incubated with Klenow polymerase for the indicated time periods. The templates either were unmodified (Control) or contained a C4′ inversion (Invert), a (selenophenyl)deoxyribose (SePh), or a pivaloyldeoxyribose modification (Piv) at position 14 from the 3′ end. Depending on the particular substrate, major DNA synthesis stop sites were found at template positions 13, 14, 15, or 17. The 21-mer product results from an inherent ability of the enzyme to extend blunt ends. The asterisk denotes the site of deoxyribose modification on the template.

When the unmodified control substrate was tested, DNA synthesis to the full length of the template was detected after only 20 s of incubation. The band corresponding to the original 8-mer primer disappeared completely after less than 3 min of incubation, and all radioactivity was transferred to the position of full length products, indicating complete and efficient replication of the unmodified template (Figure 3).

When we examined the templates containing single C4′ deoxyribose modifications, we found the same initial rate of primer extension as previously observed with the unmodified substrate. In all cases, the band corresponding to the radiolabeled 8-mer oligonucleotide primer essentially disappeared after 3 min of incubation (Figure 3). These initially identical reaction rates indicate that the enzyme was able to bind equally well to modified and unmodified substrate and start primer elongation with similar efficiencies. However, each C4′ deoxyribose variant induced a distinct pattern of DNA synthesis termination sites that were not observed with unmodified substrate (Figure 3). By comparison with the electrophoretic positions of appropriate markers, we found that a C4′-inverted deoxyribose residue located at template position 14 stopped primer elongation at positions 14 and 15. After 9 min of incubation, some full length product was obtained, indicating considerable bypass of the C4′ inversion. The (selenophenyl)deoxyribose adduct induced DNA synthesis stop sites mainly downstream of the modified residue, i.e. at positions 15 and 17, whereas the pivaloyldeoxyribose adduct (at the same nucleotide position, 14) caused stop sites either one nucleotide before or opposite the abnormal deoxyribose moiety. In contrast to the reactions with C4′-inverted substrate, no full length products were formed on templates containing a selenophenyl- or pivaloyl-modified deoxyribose (Figure 3). Notably, the two deoxyribose adducts were not bypassed even after 30 min of incubation, although the reaction conditions supported complete replication of the control substrate in less than 3 min.

The rate of DNA synthesis by Klenow fragment results from its two enzymatic functions, i.e. the 5′-3′ polymerase activity and the competing 3′−5′ exonuclease activity (Strauss, 1991; Echols & Goodman, 1991). To determine whether the 3′−5′ proofreading function favors DNA synthesis arrest at C4′ deoxyribose adducts, we repeated the same reactions using DNA polymerase I Klenow fragment devoid of 3′−5′ exonuclease activity. We found that the loss of exonuclease function did not markedly increase the capacity of the enzyme to bypass selenophenyl- or pivaloyldeoxyribose adducts and generate full length replication products (data not shown). These observations indicate that C4′ deoxyribose adducts constitute strong blocks to DNA polymerase progression independent of 3′−5′ exonuclease activity.

Base Pairing Fidelity. Due to their particular localization in the sugar-phosphate backbone (Figure 1), C4′ deoxyribose derivatives were expected to exert minimal effects on the hydrogen-bonding geometry of the respective bases. To test this hypothesis, unmodified DNA templates, or templates containing a modified backbone residue at the 14th position from the 3′ end, were primed with radiolabeled 13-mer oligonucleotides as indicated in Figure 2. The resulting substrates were incubated with Klenow polymerase in the presence of a single deoxyribonucleotide triphosphate. After reactions of 3 min, primer extension was analyzed by denaturing polyacrylamide gel electrophoresis. Elongation of 13-primers by one residue to form 14-mer products is observed if the particular deoxyribonucleotide added to the reaction mixture becomes incorporated opposite the modified site. This assay has been successfully used in previous studies, for example to demonstrate the mutagenic properties of templates carrying 8-hydroxyguanine (Shibutani et al., 1991) or α-deoxyadenosine residues (Ide et al., 1994).

The autoradiograph of Figure 4A shows that 13-mer primer extension on unmodified control substrate was by far most effective in the presence of TTP (lane 7), consistent with correct A·T base pairing. We noted that these steady state reactions with a single deoxyribonucleotide also yielded some 13-mer primer extension in the presence dATP (Figure 4A, lane 1), dCTP (lane 3), or dGTP (lane 5). However, nucleotide misincorporation was considerably reduced when the reactions were stopped before reaching steady state levels. After 20 s, for example, we detected only marginal primer elongation (<2% of primers) in the presence of dATP but essentially complete elongation (>95% of primers) in the presence of the correct deoxyribonucleotide. These amounts of misincorporation reflect *in vitro* polymerization error frequencies at the 10^{-3}−10^{-5} level [reviewed by Echols and Goodman (1991), Kunkel (1992), and Feig and Loeb (1995)].

As was found with unmodified substrate, 13-mer primer extension on the substrate containing a C4′ inversion was by far most effective when the incubation mixtures contained TTP (Figure 4A, lane 8). Minor incorporation of dAMP (Figure 4A, lane 2) or dCMP (lane 4) was observed opposite the C4′-inverted nucleotide on the template, but the observed level of misincorporation was rather decreased relative to that of the control reactions with unmodified template. Similarly, when the substrate containing a (selenophenyl)-deoxyribose adduct was tested, we found preferential 13-mer primer extension in the presence of TTP (Figure 4B, lane 5), indicating selective incorporation of TMP across dAMP residues. A minor level of primer extension was observed with dATP (lane 3), but dAMP misincorporation across the selenophenyl adduct was again not increased when compared to the control reaction with unmodified substrate (Figure 4A, lane 1).

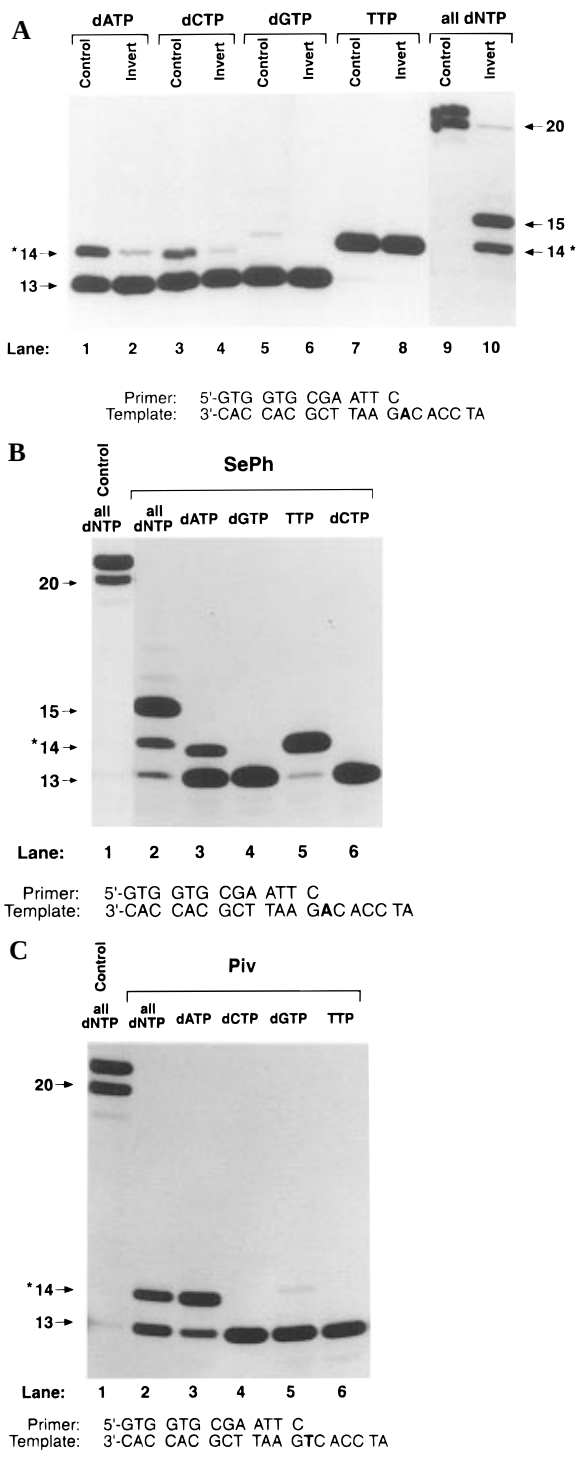

FIGURE 4: Replication fidelity of proofreading-proficient Klenow polymerase. Template DNA of 20 residues in length either was unmodified or contained a deoxyribose modification at position 14 from the 3′ end. After annealing with a 13-nucleotide radiolabeled primer, these substrates were incubated with Klenow polymerase in the presence of a single deoxyribonucleotide triphosphate. For comparison, parallel incubations were performed with all four deoxyribonucleotide triphosphates. Primer extension reactions were analyzed after 3 min of incubation, and the asterisk denotes the position of 14-mer extension products. (A) Unmodified template (Control) and template containing a C4′-inverted deoxyribose (Invert). (B) Selenophenyl-modified template (SePh); lane 1 shows a control reaction with unmodified substrate. (C) Pivaloyl-modified template (Piv); lane 1 shows again a reaction with unmodified substrate. The sequences of primed templates are shown at the bottom of each gel, with the site of deoxyribose modification indicated by bold letters.

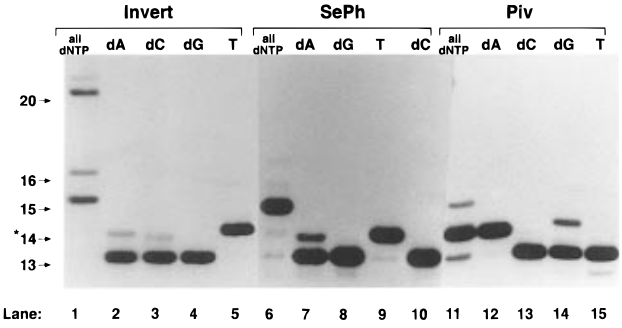

FIGURE 5: Replication fidelity of proofreading-deficient Klenow polymerase. Templates of 20 residues and 13-mer primers were incubated with a single deoxyribonucleotide triphosphate as in the experiments of Figure 4, but in the presence of a 3′−5′ exonuclease-deficient Klenow polymerase. Lanes 1, 6, and 11 show reactions performed with all four deoxyribonucleotide triphosphates. Nucleotide addition was analyzed after 3 min of incubation. The asterisk denotes the position of 14-mer extension products. Templates contained the following deoxyribose modifications at position 14 from the 3′ end: (Invert) C4′ inversion, (SePh) selenophenyl, and (Piv) pivaloyl.

The pivaloyldeoxyribose modification was inserted into the synthetic template as a component of TMP (Figure 2). In this case, correct base pairing should lead to dAMP incorporation across these sites. As expected, Figure 4C shows that 13-mer primers were preferentially extended on the pivaloyl-modified substrate when the reaction mixtures contained dATP (Figure 4C, lane 3). Only marginal primer extension was observed in the presence of dGTP (Figure 4C, lane 5). As was found in the experiments with C4′-inverted or selenophenyl-modified deoxyribose residues, these results indicate a strong preference for A·T base pairing at sites containing a pivaloyl substitution.

The time course of Figure 3 demonstrated that C4′-inverted and (selenophenyl)deoxyribose variants (located at template position 14) also induced DNA synthesis stop sites one position downstream of the modification. We therefore analyzed nucleotide incorporation across the dCMP residue located at position 15 using an appropriate 14-mer primer (Figure 2). These experiments showed that the highly preferential addition of dGMP opposite template position 15 was maintained in the presence of altered deoxyribose residues. In fact, misincorporation of dAMP, dCMP, or TMP at position 15 was either equal or, in the case of the C4′ inversion, decreased relative to the corresponding control reactions performed with unmodified template (gels not shown). Taken together, we concluded that neither the C4′ inversion nor the two deoxyribose adducts were able to disturb the inherent selectivity for correct Watson−Crick base pairing.

Base Pairing Fidelity in the Absence of Proofreading. To test whether the observed base pairing fidelity is dependent on 3′−5′ proofreading activity, we performed 13-mer primer extension experiments using the mutant DNA polymerase lacking 3′−5′ exonuclease activity (Figure 5). We observed that the proofreading-deficient enzyme maintained a strong preference for correct base pairing despite the presence of C4′ deoxyribose variants. On templates containing a C4′ inversion or (selenophenyl)deoxyribose modification, 13-mer primer extension was observed preferentially in the presence of TTP (Figure 5, lanes 5 and 9), as was found previouly with the proofreading-proficient enzyme (Figure 4A,B). Similarly, on pivaloyl-containing templates, the 13-mer

primer was preferentially extended in the presence of dATP (Figure 5, lane 12), and this result was again comparable to that obtained with the proofreading-proficient polymerase (Figure 4C). Using the 3′−5′ exonuclease-deficient enzyme, we also performed 14-mer primer extension on templates containing either the C4′ inversion or the selenophenyl modification to test nucleotide incorporation across the dCMP residue at position 15. The resulting pattern of nucleotide addition was nearly identical to that obtained with the nonmutated enzyme (gels not shown). Thus, discrimination against incorrect nucleotides across C4′ variants was not eliminated in the absence of 3′−5′ exonuclease activity, indicating that base pairing fidelity at C4′ deoxyribose modifications does not depend solely on the proofreading capacity of the DNA polymerase.

DISCUSSION

Most cytotoxic base lesions disrupt the natural hydrogen-bonding pattern required for normal base pairing but also induce conformational or sterical effects that may potentially interfere with DNA-tracking enzymes. To uncouple these different structural consequences of DNA damage, we have tested the response of a model DNA polymerase to templates containing C4′-modified sites in their backbone (Figure 1). The tested DNA polymerase was arrested transiently at a C4′-inverted deoxyribose residue but was completely blocked at two different C4′ deoxyribose adducts (Figure 3). In the sequence context examined, inhibition of DNA polymerase was observed despite the absence of any detectable loss of template-coding ability. In fact, primer extension experiments performed in the presence of single deoxyribonucleotide substrates showed that C4′ deoxyribose modifications were unable to reduce the selectivity of the DNA polymerase for correct nucleotide pairing (Figures 4 and 5).

The present findings have several implications. First, our results indicate that blocking of DNA polymerases on damaged templates may occur at sites that remain fully instructional with respect to their base pairing capability; i.e. DNA lesions may stop DNA synthesis without substantially altering the hydrogen-bonding geometry of template bases. This finding implies that additional molecular factors such as backbone distortion or steric hindrance are important parameters that determine the capacity of a particular DNA lesion to stop DNA synthesis. Evaluation of these molecular determinants of DNA synthesis arrest should be useful for predicting the cytotoxic potential of DNA adducts in pharmacological drug design or toxicological risk assessment.

Second, the present study suggests that C4′ deoxyribose inversion in DNA, a potential cellular product of C4′ radical attack, displays nonmutagenic properties. DNA containing such inversions can be generated *in vitro* by induction of C4′ deoxyribose radicals in the presence of hydrogen donors. The subsequent hydrogen transfer is accompanied, in part, by inversion of the normal configuration at the stereogenic C4′ (Cadet & Berger, 1985; Akhlaq et al., 1987). In mammalian cells, glutathione and other intracellular thiols may act as hydrogen donors to mediate a similar mechanism following exposure to ionizing radiation or other sources of oxygen radicals, including byproducts of normal aerobic metabolism and inflammatory processes. C4′ radicals are also a common intermediate in the induction of strand breaks by the antitumor antibiotics bleomycin, neocarzinostatin, or other members of the enediyne family (Dedon & Goldberg, 1992; Stubbe et al., 1996). Although quantitative information on the possible frequency of C4′ inversions following exposure of DNA to ionizing radiation or oxidative damage is not available, it is expected that these lesions arise predominantly in single-stranded and less frequently in double-stranded DNA (Akhlaq et al., 1987), suggesting that inverted C4′ deoxyriboses may occur preferentially in transcriptionally active sequences or during DNA replication.

Third, the strong inhibition of DNA polymerization by C4′ deoxyribose adducts is a novel observation that may be further exploited for chemotherapeutic purposes. It has been reported that the Klenow fragment shares with eukaryotic DNA polymerase β and reverse transcriptase an increased capacity to overcome damage-induced blocks to DNA replication (Hoffmann et al., 1995). However, in the present study, this enzyme was exquisitely sensitive to C4′ deoxyribose adducts, particularly to the C4′ pivaloyl substitution (Figure 3). Also, using two different assays based on DNA repair synthesis and oligonucleotide excision, we have recently reported that these C4′ deoxyribose adducts are largely refractory to human excision repair processes (Hess et al., 1996).

DNA is a major pharmacological target of antitumor therapy, but several problems are associated with conventional approaches such as treatment with ionizing radiation or DNA-reactive drugs. In fact, radiotherapy and many types of chemotherapy induce highly mutagenic DNA lesions that may accelerate further genetic changes in cancer cells, thereby enhancing progression to tumor malignancy or therapy resistance. Also, drug-induced mutations are thought to be responsible for the development of secondary tumors in cancer patients (Bradley et al., 1993). Thus, the search for highly cytotoxic DNA lesions that display low mutagenicity is critical to the development of new and more effective anti-cancer strategies. The apparently error-free response to C4′ deoxyribose modifications observed in this study provides a conceptual basis for such a new strategy. In particular, the combination of DNA synthesis arrest (indicating cytotoxicity), low mutagenicity, and poor repairability (indicating biological persistence; Hess et al., 1996) associated with C4′ deoxyribose substitutions suggests that DNA backbone analogs may be further developed for chemotherapeutic purposes. The crystallographic structure of mammalian polymerases bound to DNA template−primer (Pelletier et al., 1994) should facilitate the design of appropriate deoxyribose analogs. In addition, the observation that certain C4′-modified TTP residues are used as a substrate for strand elongation by several DNA polymerases (B. Giese & A. Marx, unpublished results) suggests a possible pathway for targeting such novel deoxyribose modifications to the DNA of cancer cells.

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