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Sequence dependence for bypass of thymine glycols in DNA by DNA polymerase I

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ABSTRACT

Single-stranded phage DNAs containing thymine glycols were prepared by oxidation with osmium tetroxide (OsO_4) and were used as templates for DNA synthesis by E. coli DNA polymerase I. The induction of thymine glycol lesions in DNA, as measured by immunoassay, quantitatively accounted for an inhibition of in vitro DNA synthesis on modified templates. Analysis of termination sites for synthesis by DNA polymerase I (Klenow fragment) showed that DNA synthesis terminated at most template thymine sites in OsO_4-treated DNA, indicating that incorporation occurred opposite putative thymine glycols in DNA. Nucleotides 5' and 3' to putative thymine glycol sites affect the reaction, however, since termination was not observed at thymines in the sequence 5'-CTPur-3'. Conversion of thymine glycols to urea residues in DNA by alkali treatment caused termination of DNA synthesis one nucleotide 3' to template thymine sites, including thymines in the 5'-CTPur-3' sequence, showing that the effect of surrounding sequence is on the elongation reaction by DNA polymerase rather than differential damage induction by OsO_4.

INTRODUCTION

Studies on the in vitro activity of DNA polymerases during synthesis on lesion-containing DNAs have increased our understanding of the possible mechanisms for induced mutagenesis (1,2). The important finding that the DNA damage caused by several mutagens blocks purified DNA polymerase at the lesion site suggests that other proteins are utilized for continued synthesis. Specific proteins synthesized after SOS induction of cells may fulfill this requirement for mutagenesis (3).

Using nucleotide sequencing technology, Moore and Strauss (4) showed that synthesis by DNA polymerase I stopped on single-stranded ϕX174 DNA one nucleotide before template lesions caused by ultraviolet irradiation, N-acetoxy-2-acetylaminofluorine and benzo[a]pyrene diolepoxide. Subsequent studies have demonstrated termination after nucleotide insertion opposite modified bases, or continued synthesis past lesion sites, dependent upon the presence of Mn^{++} and biased nucleotide pools in the reaction (5,2,6), particular lesion and polymerase interactions (6-8), or DNA conformation

effects (9). In perhaps the simplest model system for the study of replication blocks at non-instructive DNA lesions, DNA polymerase termination at abasic sites in M13 DNA showed complex nucleotide sequence effects that were not easily explained by local base-pairing or base-stacking interactions (10,11). The sequence surrounding lesion sites also affects insertion opposite bulky lesions, and different DNA polymerases direct that interaction in different ways that are not understood (2,6).

The *in vitro* studies cited here have been concerned with replicational blocks by SOS-dependent mutagens that cause major modifications of template structure, such as pyrimidine dimers or AAF adducts, or that remove template coding in the case of abasic sites. We have studied the interaction of DNA polymerase I and DNA templates containing *cis*-thymine glycol (5,6-dihydroxy-5,6-dihydrothymine), a minor base damage (12,13) that is nevertheless a substrate for SOS processing (14). In this report, we show that most thymine glycol sites block *in vitro* DNA synthesis on single-stranded phage DNA; in addition, specific nucleotide sequences allow progression of DNA polymerase I past thymine glycol sites.

MATERIALS AND METHODS

Chemicals and Enzymes

Osmium tetroxide (OsO_4) was purchased from Sigma and stored frozen as a 4 percent solution in water. Deoxy- and dideoxyribonucleoside triphosphates were purchased from Pharmacia-P.L. and [α-^{32}P]dATP (800 Ci/mmol) was from New England Nuclear. Oligonucleotide primers were from Pharmacia-P.L. or were synthesized using an Applied Biosystems Oligonucleotide Synthesizer and purified on 20 percent polyacrylamide/7M urea gels according to the protocol supplied by Applied Biosystems, Inc.

DNA polymerase I (Klenow fragment) was purchased from BRL or New England Biolabs and DNA polymerase I was the endonuclease-free grade from Boehringer Mannheim. *Taq*I restriction endonuclease was purchased from New England Biolabs and used as instructed by the manufacturer.

Phage and DNA Preparation

Phage M13mp2 and isolation of mutant derivatives were described (15). Phages f1-K12 and f1-RH10 are derivatives of phage f1 that contain inserts of *E. coli lac* DNA in opposite orientations. Their construction will be described elsewhere. Phage were grown as previously described (15) and purified as described (16), except for mutant derivatives of M13mp2, which were washed and concentrated by sedimentation through buffer (16 hrs at 30,000

rpm) instead of banding in CsCl gradients. Single-stranded DNA was purified as described (16).

OsO_4 and OsO_4/Alkali Treatment of DNA

cis-thymine glycols were selectively induced in single-stranded phage DNA by oxidation of thymine residues with OsO_4 (17-19). 125 ng phage DNA per µl in 10mM Tris-HCl (pH 8.0) was incubated with 0.08 percent or 0.16 percent OsO_4 at 30°C. Aliquots were removed, extracted three times with cold ether to stop the reaction (20) and centrifuged through 1-ml Sephadex G-50 columns.

For subsequent alkali treatment of OsO_4-reacted DNA, equal volumes (20 µl) of DNA and 0.2N NaOH were incubated at 37°C for 20 min. Solutions were neutralized by adding 4 µl 1N HCl and spun through 0.7-ml Sephadex G-25 columns.

After each treatment, recovery of DNA was determined by A_{260} absorbance, using $E^{M}_{260} = 7.35 \times 10^3$ (21).

Determination of Thymine Glycol Content in Phage DNA

The fraction of thymine residues converted to thymine glycols in phage DNA was determined by an immunochemical assay (ELISA) using anti-thymine glycol antibody as described by Rajagopalan et al. (22) and generously carried out by the laboratory of Dr. S. Wallace (New York Medical College). Standardization for the ELISA used [^{3}H]dThd-labeled M13 phage DNA, similarly treated with OsO_4 and analyzed for thymine glycol content by the acetol fragment assay (23). The results of these assays will be reported in detail elsewhere (Hayes, R.C., Wallace, S.S., and LeClerc, J.E., manuscript in preparation).

Conditions for DNA Polymerase I Reactions

Primer-templates were prepared by annealing 0.4 µg single-stranded phage DNA and 1-6 ng oligonucleotide primers, depending upon the primer used. Annealing was in 50 mM Tris-HCl, pH 7.5, and 0.1M NaCl for 15 min at 55°C, followed by gradual cooling to room temperature.

For determining inhibition of DNA synthesis by OsO_4 treatment of template DNA, primed synthesis was carried out on control or OsO_4-treated DNA in reaction mixtures (25 µl) containing 50 mM Tris-HCl, pH 7.5, 7.5 mM $MgCl_2$, 1 mM DTT, 50 µM each of the four dNTPs with [α-^{32}P]dATP labeled at 5590 cpm/pmol, and 1 unit DNA polymerase I. Incubation was for 60 min at 30°C. Incorporation was measured by precipitating portions (5 µl) of the reaction mixtures with 1N HCl-0.1M NaPPi, collecting on GF/C filters, washing with acid followed by cold ethanol, and liquid scintillation counting. Another portion of the reaction mixtures was heated at 65°C for 5 min to inactivate DNA polymerase and synthesis products were subjected to restriction

enzyme digestion.

For analyzing termination sites for DNA synthesis on OsO_4-treated DNA, similar reactions were carried out in 5 µl volumes with 10 µCi [α-^{32}P]dATP (800 Ci/mmole) as the labeled nucleotide. Incubation with DNA polymerase I (Klenow fragment) was at 30°C for 10 min, followed by a chase period of 20 min (unless otherwise indicated) in the presence of 80 µM unlabeled dATP. Reaction mixtures were processed as for nucleotide sequencing reactions.

Other Methods

Nucleotide sequencing reactions, electrophoresis on 8 percent polyacrylamide/8M urea gels and autoradiography were carried out as previously described (15). Native polyacrylamide gel electrophoresis, autoradiography and densitometric tracing of films were as previously described (16).

RESULTS

DNA Polymerase I-catalyzed Synthesis on OsO_4-treated DNA

Single-stranded DNA from f1-K12 hybrid phage was reacted with OsO_4 for 5, 10, 20, 40 and 60 minutes as described in Materials and Methods. The

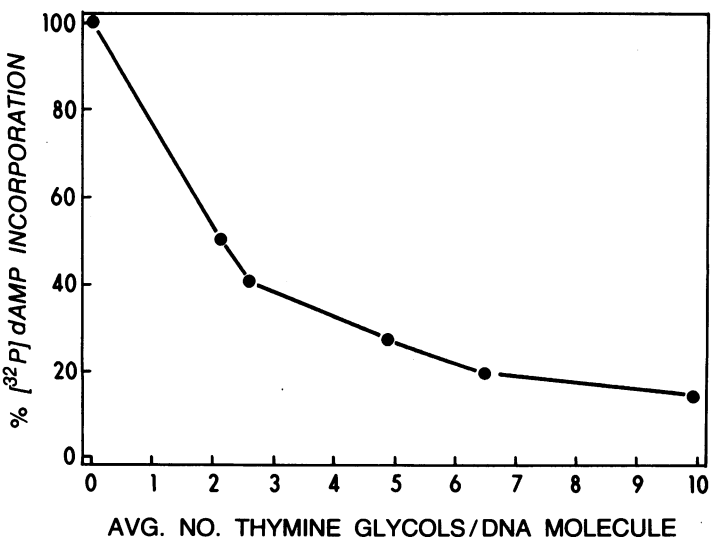

Figure 1. Effect of OsO_4 treatment of f1-K12 DNA on synthesis by DNA polymerase I. Single-stranded DNA treated for 0, 5, 10, 20, 40, and 60 min with 0.08 percent OsO_4 was used as template DNA for synthesis as described under Materials and Methods. Incubation was at 30°C for 1 hr. 100 percent relative synthesis corresponds to 157 nmoles [α-^{32}P]dAMP incorporated on untreated DNA.

average numbers of thymine glycols induced per molecule in these preparations were 2.3, 2.7, 5.1, 6.7 and 10.1, respectively, as determined by ELISA. Untreated or reacted DNAs annealed to a unique 15-nucleotide primer were used as templates in DNA polymerase I reactions and maximal incorporation of α-^{32}P-labeled dAMP was determined. Fig. 1 shows that the extent of

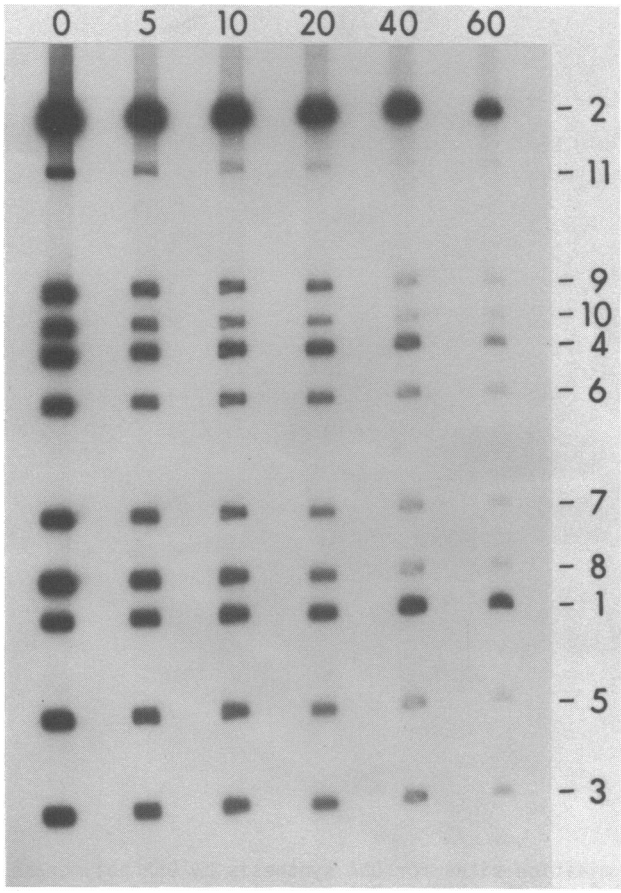

Figure 2. Gel electrophoresis of TaqI-digested products from DNA polymerase I reactions on OsO_4-treated DNA. Aliquots (50 ng) from the experiment of Fig. 1 were used. Restriction enzyme digestion, electrophoresis and autoradiography are described in Materials and Methods. Time of treatment (min) with OsO_4 is given at top of figure. Fragment numbers (right margin) are given according to the expected order of synthesis from a 15-nucleotide M13 primer (P.L.-Pharmacia) that anneals at positions 76-90 in the lacZ' gene of f1-K12 DNA (see Fig. 6A). A 22-nucleotide fragment 5' to the primer site is not observed.

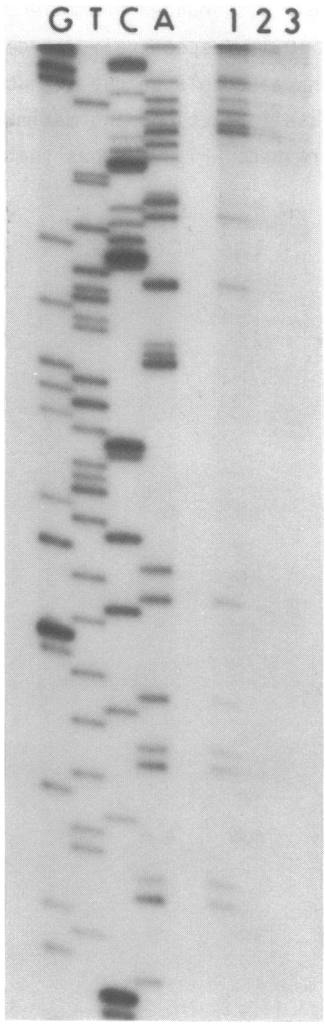

Figure 3. Termination sites for DNA synthesis by DNA polymerase I (Klenow fragment) on OsO_4-treated M13mp2 DNA. Single-stranded DNA was treated with 0.08 percent OsO_4 for 60 min (lane 1), 10 min (lane 2) or was untreated (lane 3) and incubated in DNA polymerase I (K.f.) reactions with [α-^{32}P]dATP for 15 min at 30°C, followed by chase with unlabeled dATP for 15 min at 30°C. G, T, C, A refer to dideoxynucleotide reactions in the standard sequencing procedure and correspond to the newly synthesized complementary strand. Synthesis was from a 15-nucleotide M13 primer (P.L.-Pharmacia) that anneals at positions 76-90 in lacZ' DNA (see Fig. 6A).

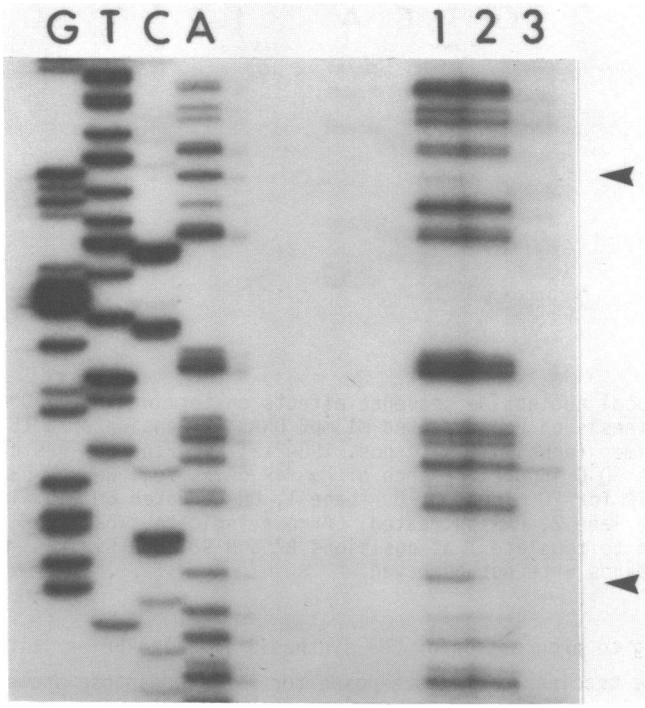

Figure 4. Bypass of putative thymine glycol sites during prolonged incubation of OsO_4-treated M13mp2 DNA with DNA polymerase I (K.f.). Single-stranded DNA was treated 60 min with 0.08 percent OsO_4 (lanes 1 and 2) or was untreated (lane 3). Synthesis was carried out in the presence of [α-^{32}P]dATP for 15 min at 30°C, followed by chase with unlabeled dATP for 15 min (lanes 1 and 3) or 60 min (lane 2) at 30°C. Arrows indicate elongation at template T sites corresponding to positions -58 and -15 (see Fig. 6A).

incorporation decreased with increasing thymine glycol content in template DNA. Determination of the average number of replication blocks per molecule (24) in these template preparations and comparison with measured thymine glycols gives an average of 1.3 thymine glycols per replication block. Although these data suggest that a fraction of thymine glycols are bypassed in the conditions of the *in vitro* reaction, the significant conclusion is that thymine glycols cause the inhibition of DNA synthesis, because of the selectivity of OsO_4 reaction (17-19) and the near unity of measured thymine glycols and replication blocks per DNA molecule.

The reaction products from the experiment of Fig. 1 were cleaved with *TaqI* restriction endonuclease and analyzed by polyacrylamide gel electrophoresis. An autoradiograph of the gel is shown in Fig. 2, with band numbers

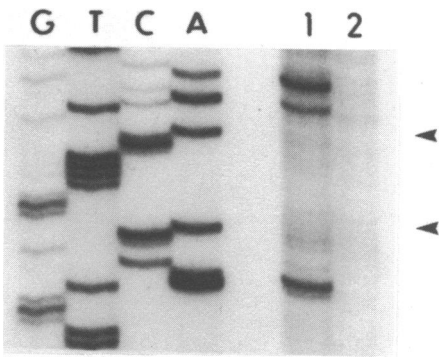

Figure 5. Local nucleotide sequence effects on termination of DNA polymerase I (K.f.) synthesis on OsO_4-treated M13mp2 DNA. Extension of a 15-nucleotide synthetic primer (annealing site pos. 198-212; cf. Fig. 6A) was carried out for 10 min at 30°C in the presence of [α-^{32}P]dATP, followed by chase with unlabeled dATP for 20 min at 30°C. Lane 1, DNA treated 60 min with 0.16 percent OsO_4; lane 2, DNA untreated. Arrows indicate two sites corresponding to template T at positions 87 and 98 (see Fig. 6A) where termination bands were not observed.

corresponding to progression of DNA synthesis from the primer site. Densitometric tracings of films exposed for linear response showed that double-stranded DNA fragments generated from the unique primer site quantitatively decreased with increased thymine glycol content. The data indicate that thymine glycols progressively block DNA synthesis and imply that damage is distributed throughout the phage DNA molecules.

Termination of In Vitro DNA Synthesis at Thymine Glycol Sites

In order to determine the exact positions for termination of DNA polymerase I-catalyzed synthesis on OsO_4-treated DNA, ^{32}P-labeled reaction products were analyzed on nucleotide sequencing gels. Comparison of lengths of fragments generated from a unique primer with those from dideoxy sequencing reactions (25) yields the nucleotide position for termination (4). Such an analysis is shown in Fig. 3 for OsO_4-treated M13mp2 DNA used in a reaction with DNA polymerase I (Klenow fragment). All termination sites occurred directly at A positions in the dideoxy sequence ladder, indicating the incorporation occurred opposite thymine residues in template DNA and terminated. Fig. 3 shows a dose dependence for termination of synthesis opposite template T sites, with progressively stronger termination bands for template DNA treatments of 10 and 60 minutes with OsO_4. Determination of thymine glycol content by ELISA gave averages of 3.1 and 25.1 thymine glycols per molecule in these template preparations, respectively. These data show

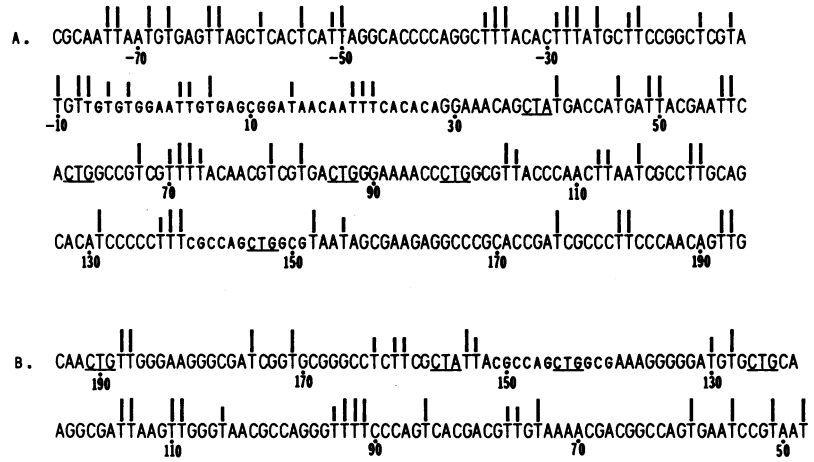

Figure 6. Summary of data on termination sites for DNA synthesis by DNA polymerase I (k.f.) on OsO_4-treated template DNAs. (A) shows the template sequence of the promoter/operator region and structural gene for lacZ' in M13mp2 and f1-K12 DNAs. (B) shows the template sequence for the lacZ' structural gene in f1-RH10 DNA, in opposite orientation to (A). Bars above T sites indicate relative intensities of termination bands on sequencing gels, observed as less (|) or more (‖) intense in multiple experiments. Underlined sequences indicate sites where termination bands were not observed under the reaction conditions used. Small letters represent regions of potential secondary structure in single-stranded DNA.

that thymine glycols are likely the blocking lesions for DNA synthesis and, furthermore, that the OsO_4 treatment is specific for damage induction at thymine sites.

The analysis given in Fig. 3 shows template thymine sites in the lac operator region of M13mp2 where termination bands do not appear, while experiments with other treated DNA preparations showed termination at T sites in the lac operator (data not shown). The specificity of the OsO_4 reaction for single-stranded DNA may cause variable reactivity with thymines in a region of potential double-stranded character, such as the lac operator. Other sequence-specific sites that do not show termination are analyzed below.

Bypass of Thymine Glycol Sites

Since the intensity of termination bands at putative thymine glycol sites varied at different DNA sequences, the length of the chase period in the presence of unlabeled dATP was altered to determine if certain sites could be bypassed by DNA polymerase I (Klenow fragment). Fig. 4 shows an experiment in which the chase period was carried out for 15 or 60 minutes. Two sites

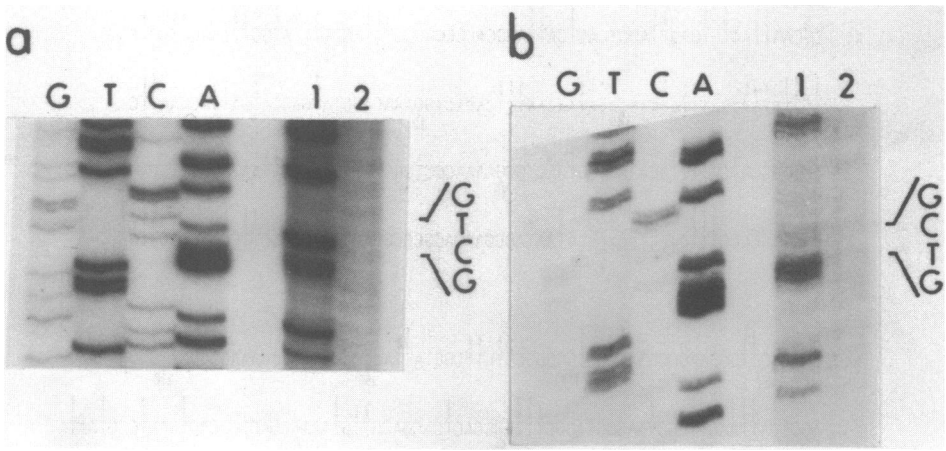

Figure 7. Effect of mutated sequences in OsO_4-treated M13mp2 DNA on termination of DNA polymerase I (K.f.) synthesis. DNA treatment, reaction conditions and figure numbering are as described in Fig. 5. (a) M13mp2 DNA: (b) M13mp2-138, in which the wild-type template sequence 5'-GTCG-3' (pos. 66-69) is changed to 5'-GCTG-3'.

corresponding to T at 5'-CTC-3' sequences (positions -58 and -15, Fig. 6a) were bypassed with prolonged incubation; other sites remained unchanged, including a CTC site at position -55 to -53. Carrying out the polymerase reaction in the presence of Mn^{++} also allowed bypass at some putative thymine glycol sites (data not shown). In both experiments, termination sites that could be chased were the less intense bands in the sequence ladder using standard reaction conditions.

Local Sequence Specificity for Termination at Thymine Glycol Sites

In order to determine the nucleotide sequence dependence for termination or pausing of DNA polymerase I-catalyzed synthesis on OsO_4-treated DNA, analyses similar to that in Fig. 3 were carried out using different template and primer combinations to generate more termination data. Termination bands only occurred at putative thymine glycol sites, but there was variability in their intensities and some thymine sites showed no termination. Fig. 5 shows an example of a local nucleotide sequence effect on termination, since no bands are seen at 5'-CTG-3' sequences but are intense at 5'-GTC-3' and 5'-GTT-3' sequences. The absence of termination bands 3' to template thymine sites at 5'-CTG-3' sequences indicates that elongation occurred past the lesion, rather than hydrolytic removal of the nucleotide inserted opposite the lesion. All of the data are summarized in Fig. 6, in which the relative intensity, or lack of termination bands at putative thymine glycol sites is

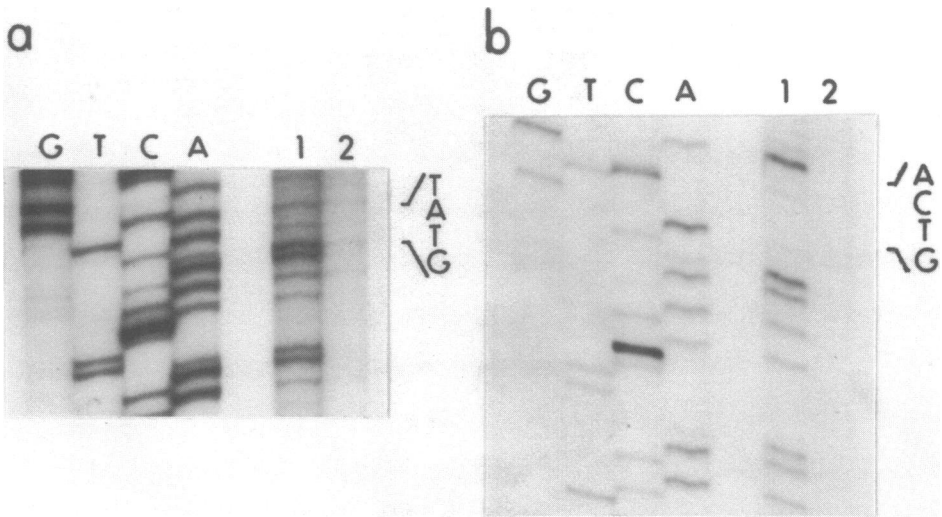

Figure 8. Effect of mutated sequences on termination. DNA treatment, reaction conditions and figure numbering are as described in Fig. 5, except that the M13 sequencing primer (P.L. - Pharmacia) was used. (a) M13mp2 DNA; (b) M13mp2-147 DNA, in which the wild-type template sequence 5'-TATG-3' (pos. -12 to -9) is changed to 5'-ACTG-3'.

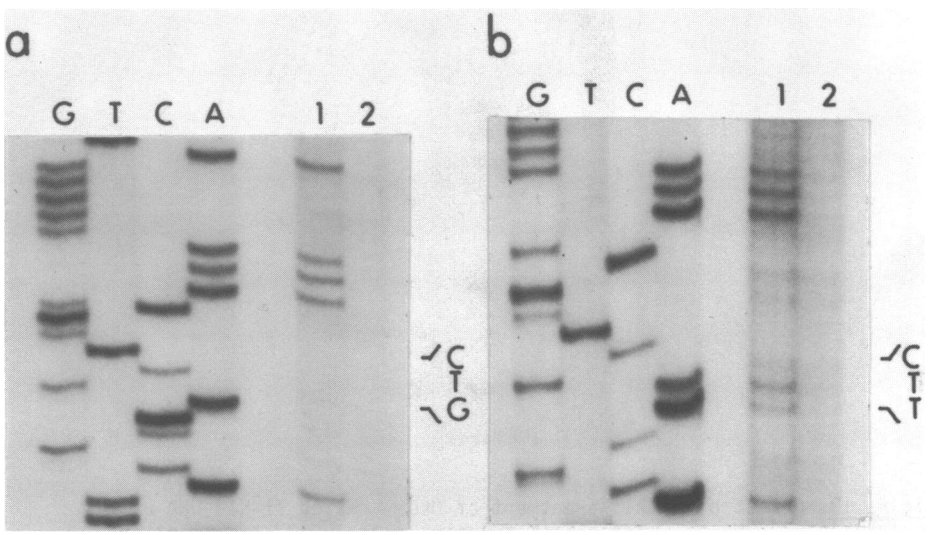

Figure 9. Effect of mutated sequences on termination. DNA treatment, reaction conditions and figure numbering are as described in Fig. 5. (a) M13mp2 DNA; (b) M13mp2-74 DNA, in which the wild-type template sequence 5'-CTG-3' (pos. 146-148) is changed to 5'-CTT-3'.

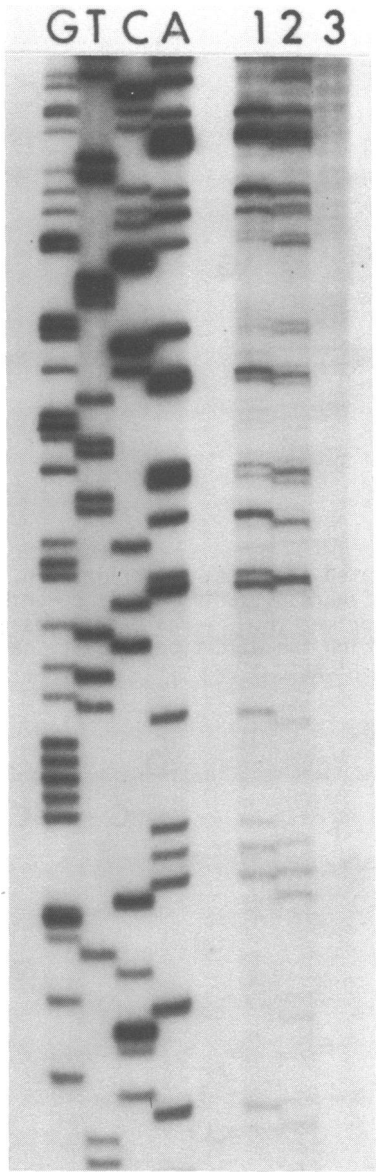

Figure 10. Effect of alkali treatment of OsO_4-treated f1-K12 DNA on termination of DNA polymerase I (K.f.) synthesis. OsO_4 treatment and reaction conditions were as described in Fig. 5. Lane 1, OsO_4-treated f1-K12 DNA; lane 2, OsO_4-treated DNA reacted with alkali as described in Materials and Methods; lane 3-untreated DNA.

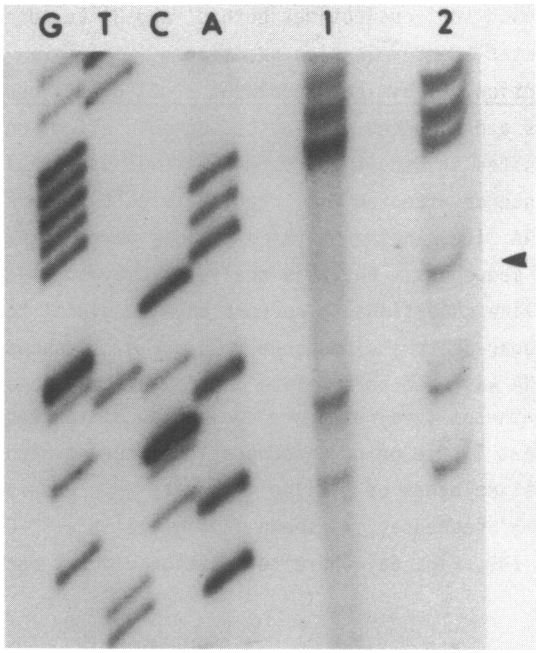

Figure 11. Termination of DNA polymerase I (K.f.) synthesis on f1-K12 DNA treated with OsO_4 and OsO_4/alkali. DNA treatments and reaction conditions were as described in Fig. 10. Lane 1, OsO_4-treated f1-K12 DNA; lane 2, OsO_4-treated DNA reacted with alkali. Arrow indicates T site in the template sequence 5'-CTG-3' (pos. 146-148, Fig. 6A).

indicated. All sequence data are consistent with the lack of termination sites at the thymine residue in the sequence 5'-CTPur-3'.

We investigated the effect of local nucleotide sequence on termination by carrying out experiments using mutated M13mp2 DNAs in which only the nucleotides surrounding putative thymine glycol sites are altered. Wild-type and mutant DNAs were reacted with OsO_4 (0.16 percent for 60 min at 30°), used for *in vitro* synthesis by DNA polymerase I (Klenow fragment), and the reaction products were analyzed on sequencing gels. Fig. 7 shows the result of a change in the template sequence from 5'-GTCG-3' (positions 66 to 69, Fig. 6a) to 5'-GCTG-3' and Fig. 8 shows a change from 5'-TATG-3' (positions -12 to -9, Fig. 7a) to 5'-ACTG-3'. In both cases, sequence changes to create 5'-CTG-3' sites resulted in loss of observable termination bands at putative thymine glycol sites. Conversely, as shown in Fig. 9, changing the template sequence 5'-CTG-3' (positions 146 to 148, Fig. 6a) to 5'-CTT-3' created a termination site and bands were observed opposite both thymine residues.

These analyses showed that nucleotides both 5' and 3' to putative thymine glycol sites affected the termination reaction.

Evidence for Induction of Thymine Glycols at 5'-CTPur-3' Sequences

Since there is a pronounced nucleotide sequence effect on termination opposite thymine sites in OsO_4-treated DNAs, it was essential to determine if the primary sequence affected OsO_4 reactivity at thymine sites in single-stranded DNA, i.e. whether thymine glycols were formed at thymine sites in all nucleotide sequences. For this analysis, OsO_4-treated f1-K12 DNA was incubated in alkaline conditions to convert thymine glycol to a urea residue attached to the sugar-phosphate backbone (26). Fig. 10 shows that when urea-containing DNA was used as template DNA for DNA polymerase I (Klenow fragment), DNA synthesis terminated one nucleotide 3' to reacted thymines in DNA, indicating that little or no incorporation occurred opposite urea residues. Termination bands of similar intensity were observed 3' to thymine sites at 5'-CTPur-3' sequences, as shown in Fig. 11 at a 5'-CTG-3' sequence (positions 146 to 149, Fig. 6a) where termination did not occur on solely OsO_4-treated DNA.

DISCUSSION

The results reported here demonstrate that thymine glycols in single-stranded DNA can terminate DNA synthesis catalyzed by DNA polymerase I. Drawing this conclusion depends on the selectivity of the OsO_4 reaction for oxidizing thymine residues in DNA (17-19) and is based on the evidence that thymine glycols measured by specific immunoassay account for the replication blocks in DNA, synthesis is halted progressively throughout thymine glycol-containing template DNA, and termination observed at the nucleotide level occurs opposite template thymine sites. Finally, conversion of thymine glycols to urea residues in DNA shifts the termination pattern to one nucleotide preceding template thymine sites. The cause of the replication block by thymine glycol is unknown. Saturation of the 5,6-double bond causes loss of the planar and aromatic ring structure of thymine (13), thus weakening normal base pairing with adenine and interrupting stacking interactions with adjacent bases. Nevertheless, DNA polymerase I inserts a nucleotide opposite the thymine glycol lesion before halting synthesis, so that some base-pairing capacity must be retained by oxidized thymine residues. In this respect, thymine glycols constitute a class of DNA damage unlike bulky adducts, baseless sites, or degraded base residues such as urea, which cause termination of DNA synthesis at least one nucleotide preceding template damage

sites using standard reaction conditions. In the latter cases, either nucleotide incorporation by DNA polymerase is blocked, or inserted nucleotides are removed by DNA polymerase-associated 3'-5' exonuclease activity (5,11). In the case of thymine glycols, the lack of bands on sequencing gels corresponding to termination 3' to template thymine sites indicates that the paired thymine glycol-primer terminus is not a substrate for the 3'-5' hydrolytic activity of DNA polymerase I, as well as not serving as a primer for elongation at most nucleotide sequences.

A biochemical determination of the nucleotide incorporated opposite thymine glycols has not been made. Experiments (not shown) were carried out using each of the dideoxynucleoside triphosphates to generate sequence ladders on OsO_4-treated template DNA; there was no indication of termination caused by incorporation of ddNMPs other than ddAMP at putative thymine glycol sites, including chase and bypass sites, suggesting that the correct nucleotide (dAMP or ddAMP) was incorporated. In separate experiments, we have shown by transfection studies that OsO_4 treatment of hybrid phage DNA causes a dose-dependent increase in mutations, but most mutation sites are not associated with nucleotide changes at potential thymine glycol sites (manuscript in preparation). Correct incorporation apparently occurs in the in vivo situation.

Weakened interactions at the template thymine glycol-primer terminus may prevent continued elongation by DNA polymerase I at most thymine glycol sites. However, a local nucleotide sequence dependence for polymerase bypass was observed, as evidenced by two criteria. First, DNA polymerase proceeded through thymine glycol at two 5'-CTC-3' sites upon prolonged incubation of the polymerase reaction mixture, although other CTC sites remained unchanged. Second, termination bands were not observed at thymine in 5'-CTPur-3' sequences, while termination 3' to urea residues in these sequences demonstrated the presence of thymine glycols in OsO_4-treated DNA. Therefore, there are effects of both nucleotides, 5' and 3' to thymine glycol, on the ability of DNA polymerase to bypass the lesion. These effects of adjacent nucleotides point to the likely contribution of base stacking to stabilize the primer-template structure. The order of stability from stacking interactions follows 5'pur-pur3' > 5'pyr-pur3' > 5'pur-pyr3' > 5'pyr-pyr3' (27). Given these general sequence dependences, it is likely that stacking interaction between incorporated (5') pyrimidines and adenine opposite the thymine glycol account for the effect of purines 3' to thymine glycols in 5'-CTPur-3' sequences. More importantly, the incorporation of dGMP opposite

the 5' template site may stabilize the thymine glycol-adenine base pair, both by favorable adenine-guanine stacking and by strong G:C base pairing. Viewed in this way, the termination of DNA synthesis at other nucleotide sequences is more a consequence of interrupted stacking interactions than loss of base-pairing capability at thymine glycol sites. Factors in addition to favorable stacking and pairing interactions with immediately local bases undoubtedly operate to override the block, as evidenced by the variable intensities of termination bands at similar nucleotide sequences, most notably at CTC sites. Furthermore, Wallace and colleagues have carried out similar analyses and find termination of DNA synthesis opposite all template thymine sites, although putative thymine glycols at 5'-CTPur-3' sequences comprise the least intense arrest sites (S.S. Wallace, personal communication). Hence, differences in the *in vitro* reaction conditions also contribute to the efficiency of the bypass reaction.

Both *cis*- and *trans*-thymine glycols in DNA are major base damage products (19 and refs. therein) and are partly responsible for the lethal effect caused by ionizing radiation (28,29). From the results reported here on the OsO_4-induced *cis* isomers, it is likely that the inactivation of single-stranded phage DNA by thymine glycols (20, unpublished results) is due to blockage of the DNA replication apparatus. The efficient reactivation of thymine glycol-containing DNA in SOS-induced cells (14, unpublished results) indicates that the replication block is relieved by components of the SOS system that operate on more severe lesions in template DNA. Studying the interaction of DNA replication proteins with thymine glycol-containing DNA should be a useful system for studying how DNA damage is bypassed during replication and particularly how neighboring nucleotide sequences modulate the challenge to the DNA replication apparatus.

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