Template Length, Sequence Context, and 3′–5′ Exonuclease Activity Modulate Replicative Bypass of Thymine Glycol Lesions in Vitro†

James M. Clark‡ and G. Peter Beardsley*
Departments of Pediatrics and Pharmacology, Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510

Received April 6, 1988; Revised Manuscript Received August 19, 1988

ABSTRACT: *cis*-Thymine glycol, a product of ionizing radiation damage to DNA, has been introduced quantitatively at a single site into oligonucleotide templates. The ability of DNA polymerases to replicate templates containing thymine glycol was studied by a primer extension assay, and three factors that influence replicative bypass of this lesion in vitro have been identified. These factors include template length, sequence context, and 3′–5′ exonuclease activity. Synthesis by the large fragment of DNA polymerase I (Klenow fragment) terminates quantitatively opposite thymine glycol when the template strand extends only two nucleotides beyond the lesion. Significant bypass is observed when the length of the template beyond the lesion is increased to six nucleotides. On the longer templates, the frequency of bypass of the Klenow fragment depends upon the identity of the base immediately 5′ to thymine glycol. The extent of bypass is greatest with cytosine and least with adenine at this position. Bypass of thymine glycol lesions by DNA polymerase α_2 from HeLa cells shows a qualitatively similar dependence upon local sequence context. In contrast, synthesis by T4 DNA polymerase is quantitatively blocked opposite the lesion regardless of template length or DNA sequence context. Synthesis by a mutant Klenow fragment that is deficient in 3′–5′ exonuclease activity, or by AMV reverse transcriptase, results in a significant increase in the frequency of bypass. Thus, increased nucleotide turnover at, or beyond, the site of the lesion is likely to contribute significantly to the arrest of synthesis provoked by *cis*-thymine glycol in vitro.

The ability of the cellular replication machinery to replicate DNA containing structural abnormalities introduced by radiation or chemical carcinogens plays a critical role in determining the overall biological responses induced by such agents. Lesions that completely block the polymerization step of DNA synthesis will be lethal unless they can be handled by alternative damage processing pathways either before (e.g., excision repair) or after replication [e.g., daughter strand gap repair; for reviews, see Hanawalt et al. (1979), Lindahl (1982), and Hall and Mount (1981)]. Direct synthesis past sites of damage in the DNA template is thought to be a major contributor to the mutagenic effects induced by DNA damaging agents (Strauss, 1985).

The use of in vitro polymerization assays involving primed synthesis on single-stranded, circular bacteriophage DNA modified by radiation or reactive chemicals has contributed greatly to our knowledge of the effects of DNA damage on DNA replication. Studies from a number of laboratories using this methodology have shown that a variety of structural lesions constitute significant blocks to synthesis by DNA polymerases (Moore & Strauss, 1979; Moore et al., 1981, 1982; Sagher & Strauss, 1983; Piette & Hearst, 1983; Pinto & Lippard, 1985).

We (Clark & Beardsley, 1986) and others (Rouet & Essigman, 1985; Ide et al., 1985; Hayes & LeClerc, 1986) have shown that *cis*-thymine glycol lesions, a significant component of oxidative DNA damage induced by ionizing radiation (Teoule & Cadet, 1979), block synthesis by procaryotic DNA polymerases. Although *cis*-thymine glycol lesions at most sites in M13 DNA resulted in termintion of DNA synthesis at the site of the lesion, Hayes and LeClerc (1986) and ourselves (Clark & Beardsley, 1986) noted lesion sites within certain sequence contexts at which bypass (translesion synthesis) occurred. More recently we have developed methods for the quantitative introduction of a single *cis*-thymine glycol lesion at a specific site in DNA oligomers and have used this system to extend our analysis to eucaryotic polymerases (Clark & Beardsley, 1987). We have also used molecular mechanical calculations and computer graphics methods to evaluate the structural perturbations introduced into DNA by thymine glycol (Clark et al., 1987b). In this paper we describe the effects of several parameters, including template length, DNA sequence context, and 3′–5′ exonuclease activity, that modulate replicative bypass of *cis*-thymine glycol lesions in vitro.

Materials and Methods

Reagents. DNA oligonucleotides were synthesized and purified by gel electrophoresis as described (Clark & Beardsley, 1987). DNA polymerase I (Klenow fragment), T4 DNA polymerase, and avian myeloblastosis virus reverse transcriptase were purchased from International Biotechnologies, Inc. Human DNA polymerase α_2, purified from HeLa cells, was provided by Dr. Earl Baril (Worcester Foundation for Experimental Biology, Shrewsbury, MA). A mutant Klenow fragment (designated D355A, E357A) was provided by Dr. C. M. Joyce (Department of Molecular Biophysics and Biochemistry, Yale University). The polymerase activity of this enzyme is normal, but its 3′–5′ exonuclease activity is $\sim 1 \times 10^{-5}$ that of the wild type Klenow fragment (Derbyshire et al., 1988). Nucleotide precursors were obtained from Sigma.

Preparation of DNA Substrates. All DNA substrates were prepared as described (Clark & Beardsley, 1987) by annealing a ^{32}P-labeled 14-mer primer to the appropriate template strand.

† This work was supported by Grant RO1CA43200 from the National Cancer Institute. J.M.C. was supported by a Swebilius Cancer Research Award from the Yale Comprehensive Cancer Center and by NIH Training Grant T32 HL 07262.

* Address correspondence to this author.
‡ Present address: Laboratory of Molecular Genetics, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709.

0006-2960/89/0428-0775$01.50/0

The sequences of the primer and template strands, along with their corresponding designations, are

5′ CCTTTTCGTCGGCC 3′ (primer)

3′ GGAAAAGCAGCCGGGTCG 5′ (18C)

3′ GGAAAAGCAGCCGGGTAGAGAA 5′ (22A)

3′ GGAAAAGCAGCCGGGTCGAGAA 5′ (22C)

3′ GGAAAAGCAGCCGGGTGGAGAA 5′ (22G)

For convenience, the template sequences are denoted by their length in nucleotides (18 or 22) and by the particular nucleotide present at the position immediately 5′ to the unique T residue. Substrates in which *cis*-thymine glycol replaced thymine were prepared by chemical oxidation of the undamaged templates with osmium tetroxide as described. The specificity of the reaction chemistry was tested by oxidizing thymidine under the same conditions used for treating oligonucleotides. HPLC analysis of the reaction products showed that >90% of the oxidized material was identical with an authentic thymidine glycol marker prepared by $KMnO_4$ oxidation of thymidine (provided by Dr. Lawrence Sowers, Department of Molecular Biology, University of Southern California, Los Angeles).

DNA Synthesis Assays. Primer extension assays, gel electrophoresis, and autoradiography were carried out as described (Clark & Beardsley, 1987). The enzyme reactions typically contained 5–10 ng of DNA and 0.04–0.5 unit (as defined by the supplier) of polymease activity (see figure legends). The polymerase α_2 reactions, modified from the earlier procedure (Clark & Beardsley, 1987), contained all four dNTPs at a concentration of 400 μM and were carried out for 45 min at 37 °C.

Results

Effect of Template Length on Bypass. In previous studies from our laboratory, *cis*-thymine glycol was shown to be a nearly complete block to DNA synthesis catalyzed by several DNA polymerases, including DNA polymerase I (Klenow fragment), when the lesion was introduced into short (18-mer) oligonucleotide templates (Clark & Beardsley, 1987). In contrast, the Klenow fragment was able to synthesize past thymine glycol to a considerable extent when the lesion was introduced into a slightly longer (22-mer) template. Figure 1 shows the results of primer extension assays in which the labeled primer was annealed to lesion-containing or control templates having lengths of 18 and 22 nucleotides. The lesion frequency at the position corresponding to the single thymine residue in the template strands was determined by piperidine cleavage to be nearly 100% (data not shown). The method employs a ^{32}P 5′-end-labeled primer; thus, the band intensity at any given position is directly proportional to the number of molecules of the corresponding length. The relative frequency of bypass, defined as the proportion of primers that are extended beyond the lesion site (i.e., longer than 16 nucleotides), can be readily estimated by inspection of the autoradiogram. Synthesis on control templates resulted in extension of the majority of the primer population to full-length or nearly full-length products (Figure 1, lanes 1 and 3). Synthesis on the thymine glycol containing 18-mer template (Figure 1, lane 2) resulted in little or no extension beyond the lesion site (17- and 18-mers). In contrast, a significant fraction of the primer population was extended beyond the lesion site (notably to 21- and 22-mers) when synthesis was carried out on the lesion-containing longer template (Figure 1, lane 4). Since thymine glycol occurs at the same position relative to the primer terminus in both templates, the Klenow fragment was able to extend a substantial fraction of the primer population past the site of the lesion only when synthesis was carried out on the longer template. The ability of the Klenow fragment to synthesize past thymine glycol was only partial since a substantial fraction of the primer population was extended only to the site of the lesion even on the 22-mer template (Figure 1, lane 4).

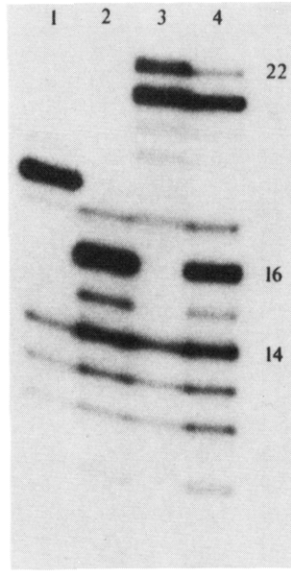

FIGURE 1: Effect of template length upon the frequency of translesion synthesis by the Klenow fragment. The figure shows an autoradiogram of the primer extension products sysnthesized by the Klenow fragment on 18-mer (18C) and 22-mer (22C) templates. These templates contain a C residue immediately 5′ to the unique T residue which was converted to thymine glycol by chemical oxidation (see Materials and Methods for complete sequence). Synthesis was carried out on control (lane 1) or oxidized (lane 2) 18-mers and on control (lane 3) or oxidized (lane 4) 22-mers. Each reaction contained all four dNTPs (100 μM) plus 5 units of enzyme and was carried out for 30 min at room temperature. The lengths of the reaction products are given alongside the autoradiogram; the unextended primer is 14 nucleotides in length.

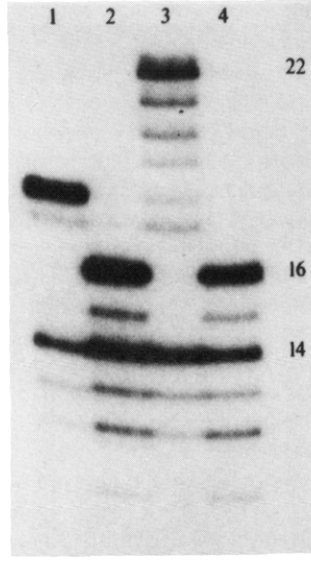

FIGURE 2: Autoradiogram of primer extension products synthesized by T4 DNA polymerase on the 18C and 22C templates. The DNA substrates used in each lane are identical with those in Figure 1. Each reaction contained 0.3 units of T4 DNA polymerase and was carried out at room temperature for 30 min.

A similar analysis was also carried out with T4 DNA polymerase (Figure 2). In contrast to Klenow fragment, this

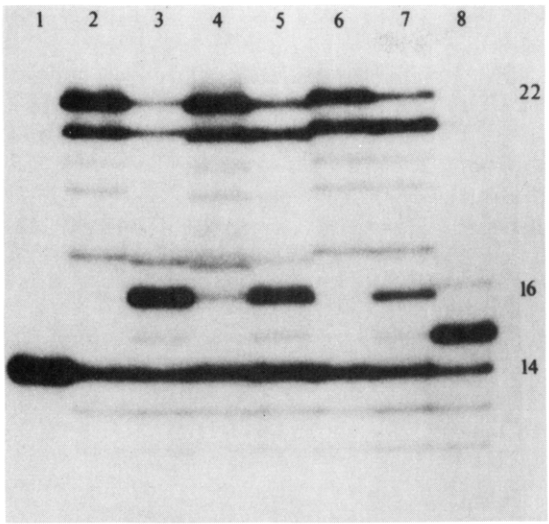

FIGURE 3: Effect of DNA sequence on the frequency of bypass by the Klenow fragment on the 22-mer templates. Primer extension assays were done with 22-mer templates having A, G, or C immediately 5′ to thymine or thymine glycol. Lane 1 shows the unextended 14-mer. The reactions were carried out on control (lane 2) or oxidized (lane 3) 22A templates, control (lane 4) or oxidized (lane 5) 22G templates, and control (lane 6) or oxidized (lane 7) 22C templates. Lane 8 shows the products of a reaction in which synthesis was carried out on the oxidized 22C template in the absence of dATP.

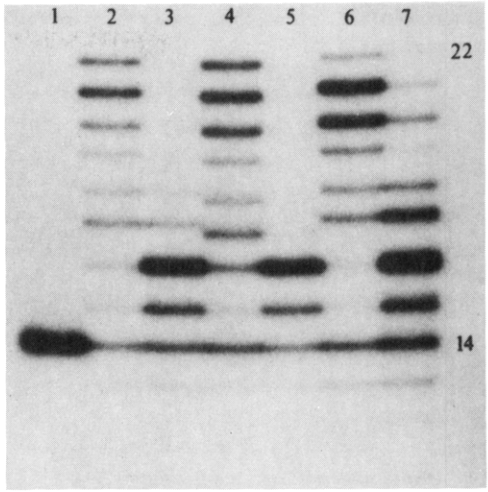

FIGURE 4: Effect of DNA sequence on the frequency of bypass by polymerase α_2 on the 22-mer templates. The DNA substrates used in each lane are identical with those in Figure 3. Each reaction contained 0.04 unit of polymerase α_2 and was carried out at 37 °C for 45 min.

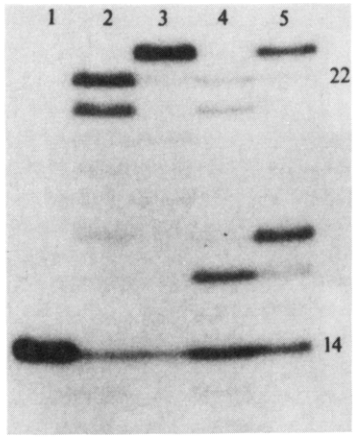

FIGURE 5: Comparison of the primer extension products synthesized by the wild-type Klenow fragment and a mutant form of the enzyme that is deficient in 3′–5′ exonuclease activity. The template used was the 22-mer having an A residue on the 5′ side of the lesion (22A). Lane 1 shows the 14-mer marker. The products synthesized on undamaged templates by the wild-type and mutant enzymes are shown in lanes 2 and 3, respectively. Lanes 4 (wild type) and 5 (mutant) show the corresponding patterns of synthesis on oxidized 22A templates. Each reaction contained 0.5 unit of enzyme (defined with poly[d(A-T)] as a substrate).

enzyme was unable to progress past thymine glycol lesions in either of the templates to any appreciable extent. The lack of significant lesion bypass with T4 DNA polymerase (Figure 2, lanes 2 and 4) also demonstrates that the lesion frequency is nearly 100% since undamaged templates in the lesion-containing population would allow synthesis to proceed to the end of the template as is seen with the controls (Figure 2, lanes 1 and 3).

Effect of DNA Sequence on Bypass. The effect of DNA sequence context on the frequency of bypass was investigated by varying the identity of the template base immediately 5′ to thymine glycol. Oligonucleotides containing adenine (22A), guanine (22G), or cytosine (22C) at this position were annealed to the primer and used as substrates for the Klenow fragment (Figure 3). The frequency of bypass was greatest when cytosine was present immediately 5′ to thymine glycol and least when adenine occurred at this position (compare lanes 3, 5, and 7, Figure 3). The increase in bypass frequency that occurs on the 22C template also shows a strong bias for the insertion of dAMP opposite the lesion since omitting dATP from the reaction results primarily in termination of synthesis one nucleotide before the lesion site (Figure 3, lane 8).

The results of similar experiments carried out with polymerase α_2 are shown in Figure 4. Although the products synthesized by this polymerase on undamanged templates were heterogeneous in length, the majority of the primer population was extended beyond the template thymine residue that represents the potential lesion site (Figure 4, lanes 2, 4, and 6). Synthesis on the lesion-containing 22A and 22G templates terminated at, or one nucleotide before, thymine glycol (Figure 4, lanes 3 and 5, respectively). However, a significant proportion of the primer population was extended beyond the lesion site when synthesis was carried out on the lesion-containing 22C template (Figure 4, lane 7). Bypass of thymine glycol lesions by polymerase α_2 therefore shows a dependence upon local DNA sequence that is qualitatively similar to that seen with the Klenow fragment. In contrast to the results obtained with the Klenow fragment or polymerase α_2, little or no bypass was observed with T4 DNA polymerase in any sequence context examined (data not shown).

Role of the 3′– 5′ Exonuclease Activity. Our earlier studies had suggested that increased nucleotide turnover opposite thymine glycol contributed to the arrest of synthesis provoked by this lesion (Clark & Beardsley, 1987). To test this possibility more directly, we examined the ability of a mutant Klenow fragment that is deficient in editing activity to synthesize past thymine glycol lesions. The mutant enzyme has normal levels of polymerase activity and a crystallographic structure that differs from that of the wild-type protein only in the 3′–5′ exonuclease site (Derbyshire et al., 1988). A comparison of the ability of the wild-type and exonuclease-deficient enzymes to synthesize past thymine glycol should therefore directly assess the role of editing in the arrest of synthesis provoked by this lesion. As shown in Figure 5, the exonuclease-deficient enzyme was able to extend a considerably larger fraction of the primer population past the lesion site than did the wild-type Klenow fragment on the 22A template. A similar increase in lesion bypass (on 18C and 22C templates)

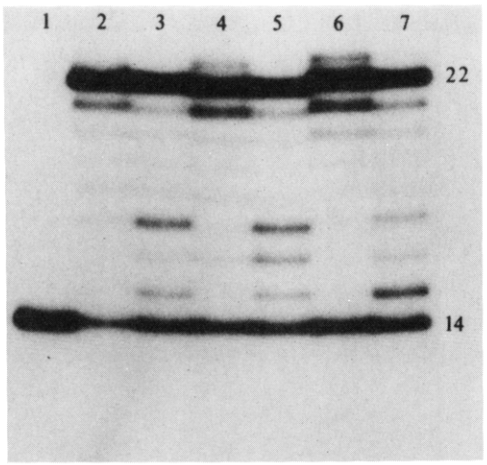

FIGURE 6: Autoradiogram of primer extension products synthesized by AMV reverse transcriptase on control and oxidized 22-mer templates having A, G, or C immediately 5′ to thymine or thymine glycol. Lane 1 is the 14-mer marker. Reactions were carried out on control (lane 2) or oxidized (lane 3) 22A templates, control (lane 4) or oxidized (lane 5) 22G templates, and control (lane 6) or oxidized (lane 7) 22C templates. Each reaction contained 0.5 unit of AMV reverse transcriptase and was carried out at 37 °C for 30 min.

was seen with the wild-type Klenow fragment when dAMP was used to suppress editing (data not shown). The products synthesized by the mutant polymerase on the lesion-containing 22-mer included fully extended primers as well as some partially extended primers in which synthesis terminated one nucleotide beyond the lesion site (Figure 5, lane 5). Surprisingly, the mutant polymerase also synthesized some products that were one nucleotide longer than expected given the length of available (undamaged) template. For example, synthesis on the undamaged 22A template resulted in the appearance of a band corresponding to a fragment length of 23 nucleotides (Figure 5, lane 3). A similar result was observed when synthesis was carried out on the damaged template (Figure 5, lane 5). These extra bands are due to a novel activity of the Klenow fragment that adds one or more nucleotides to the 3′-hydroxyl terminus of a blunt-ended DNA fragment. The product of this reaction is only formed in significant yield under conditions where the 3′–5′ exonuclease function of the polymerase is suppressed (Clark et al., 1987a).

The contribution of 3′–5′ exonuclease activity to synthesis arrest by thymine glycol lesions was also assessed with reverse transcriptase, a polymerase that lacks this function. The results of primer extension assays carried out with this enzyme on the 22-mer templates are shown in Figure 6. Synthesis by reverse transcriptase on the 22A template was virtually unimpeded by the presence of thymine glycol since the majority of the primer population was fully extended on both control (Figure 6, lane 2) and lesion-containing templates (Figure 6, lane 3). Similarly, synthesis on the 22G (lanes 4 and 5) and 22C (lanes 6 and 7) templates was relatively unaffected by the presence of thymine glycol. Thus the effect of DNA sequence context on the frequency of bypass was minimal for this enzyme.

Discussion

We have identified three parameters that affect the ability of DNA polymerases to synthesize past *cis*-thymine glycol lesions in vitro. These are the length of single-stranded template available to the polymerase, the identity of the template base immediately 5′ to thymine glycol, and the presence or absence of 3′–5′ exonuclease activity. Our conclusion that thymine glycol is responsible for the effects on DNA synthesis that we observe is based upon HPLC analysis of the products produced by osmium tetroxide oxidation of free thymidine. Moreover, a similar specificity of osmium tetroxide for producing this lesion in DNA has been reported (Beer et al., 1966). Thus we conclude that the replicative bypass that we observe represents synthesis past *cis*-thymine glycol lesions.

The requirement that a minimum length of template be available beyond the lesion site for significant bypass to occur with the Klenow fragment presumably accounts for the fact that sequence context dependent bypass was observed in the M13 system (Hayes & LeClerc, 1986; Clark & Beardsley, 1986) but not to any significant extent with the 18-mer oligonucleotide templates (Clark & Beardsley, 1987). Under our assay conditions, the rate at which the Klenow fragment added nucleotides up to the site of the lesion was similar for both short and long templates (data not shown). Therefore, the template length effect cannot be attributed simply to differences in the initial rates of synthesis on the two templates. The simplest explanation for the template length effect is that the availability of more nucleotides beyond the lesion site increases the affinity of the Klenow fragment for its substrate. The differential binding affinity could manifest itself as either a reduced rate of dissociation from the longer template when a block to synthesis is encountered or a higher rate of reassociation with the (partially extended) substrate. In either case the probability of bypass would be higher on the longer template. Consistent with this hypothesis, Joyce et al. (1986) have reported an increased binding of the Klenow fragment to (undamaged) DNA substrates that have 5′ single-stranded extensions compared to molecules that have flush ends.

A more interesting possibility is that the 3′–5′ exonuclease activity of the wild-type Klenow fragment contributes to the template length effect. It is possible that synthesis past a lesion in the 18-mer template generates an unstable duplex that is highly susceptible to fraying and subsequent exonucleotlytic degradation. According to this hypothesis, the increased bypass frequency seen with the longer template is due to the "locking in" of the lesion by the formation of additional base pairs beyond the lesion site. The increase in bypass frequency that occurs when editing is suppressed is consistent with this idea. These two possibilities are not mutually exclusive, however, and both processes may contribute to the overall template length effect.

The identity of the template base immediately 5′ to thymine glycol also has an appreciable effect on the frequency of bypass seen on the 22-mer templates with the Klenow fragment. In particular, the presence of cytosine at this position results in a greater frequency of bypass than is seen with either adenine or guanine at this position (Figure 3). This result is consistent with data obtained from the M13 system (Hayes & LeClerc, 1986). Polymerase α_2 shows a qualitatively similar dependence upon local sequence context (Figure 4) whereas reverse transcriptase synthesizes past the lesion at high frequency regardless of the DNA sequence (Figure 6). Our computer modeling studies suggest that the major structural perturbation introduced into DNA by *cis*-thymidine glycol is a rotation of the methyl group into an axial configuration with respect to the pyrimidine ring. For the particular cis diastereomer of thymidine glycol analyzed in the model (two are possible in DNA), the axial methyl group disrupts the stacking interactions between the adenine–thymine glycol base pair and the immediately adjacent base pair that would be formed during DNA synthesis past the lesion (Clark et al., 1987a,b). It is possible that cytosine, being sterically smaller than either of the purine bases, is less affected by steric hindrance with the

methyl group of thymine glycol and thus serves as a better template for bypass. It is also likely that other factors contribute significantly to the observed sequence context effects. For example, the additional hydrogen-bond energy provided by a G:C base pair 5′ to thymine glycol may contribute to the overall efficiency of bypass. Insertion of a purine (guanine) next to the adenine residue paired with thymine glycol should also provide additional stacking energy that may contribute to duplex stability and promote bypass.

The increase in bypass frequency that occurs when the 3′–5′ exonuclease activity of the Klenow fragment is suppressed either by mutation or by high concentrations of dAMP clearly demonstrates that this function of the polymerase contributes to the inhibitory effects of thymine glycol lesions on DNA synthesis. Polymerase α_2, which is capable of only a limited amount of bypass on the 22C template, also has an associated 3′–5′ exonuclease activity (Skarnes et al., 1986). Thus, it is likely that increased nucleotide turnover at, or beyond, the site of the lesion plays an important role in the arrest of synthesis caused by thymine glycol. This may be particularly true for T4 DNA polymerase, which has a very active 3′–5′ exonuclease activity (Huang & Lehman, 1972) and shows little or no lesion bypass on either short or long templates (Figure 2). Conversely, AMV reverse transcriptase, an enzyme that lacks an editing function, shows a high frequency of bypass on both the 18-mer (Clark & Beardsley, 1987) and 22-mer templates (Figure 5). The physicochemical basis for enhanced turnover at the lesion site is uncertain since our experimental (Clark & Beardsley, 1987) and computer modeling studies (Clark et al., 1987b) indicate that thymine glycol forms hydrogen bonds with adenine that have a reasonable geometry and are energetically favorable. Thymine glycol is therefore unlikely to be a highly mutagenic lesion. The requirement for the insertion of dAMP opposite the lesion during bypass on the 22C template by the Klenow fragment is consistent with this hypothesis and suggests that most of the translesion synthesis that we observe is nonmutagenic. However, we cannot exclude the possibility that thymine glycol may be mutagenic at frequencies below the detection limit of our assay (ca. 5%).

Acknowledgments

We thank Dr. Earl Baril for providing polymerase α_2 and Dr. Lawrence Sowers for providing the thymidine glycol HPLC standard. We are also grateful to Dr. Cathy Joyce for providing the mutant Klenow fragment and for a critical reading of the manuscript.

Registry No. 5′ CCTTTTCGTCGGCC, 117369-28-5; 3′ GGAAAAGCAGCCGGGTCG, 117369-29-6; 3′ GGAAAAGCAGCCGGGTAGAGAA, 117369-33-2; 3′ GGAAAAGCAGCCGGGTCGAGAA, 117369-31-0; 3′ GGAAAAGCAGCAGGGTGGAGAA, 117369-34-3; 3′ GGAAAAGCAGCCGGGT(glycol)CG, 117369-30-9; 3′ GGAAAAGCAGCCGGGT(glycol)AGAGAA, 117369-35-4; 3′ GGAAAAGCAGCCGGGT(glycol)CGAGAA, 117369-32-1; 3′ GGAAAAGCAGCCGGGT(glycol)GGAGAA, 117369-36-5; 5′ CCTTTTCGTCGGCC·3′ GGAAAAGCAGCCGGGT(glycol)CG, 117497-32-2; 5′ CCTTTTCGTCGGCC·3′ GGAAAAGCAGCCGGGT(glycol)AGAGAA, 117497-31-1; 5′ CCTTTTCGTCGGCC·3′ GGAAAAGCAGCCGGGT(glycol)CGAGAA, 117526-04-2; 5′ CCTTTTCGTCGGCC·3′ GGAAAAGCAGCCGGGT(glycol)GGAGAA, 117497-33-3; *cis*-thymine glycol, 1124-84-1; 3′–5′ exonuclease, 79393-91-2; DNA polymerase, 9012-90-2.

References

Beer, M., Stern, S., Carmalt, D., & Mohlhenrich, K. H. (1966) *Biochemistry 5*, 2283–2288.

Clark, J. M., & Beardsley, G. P. (1986) *Nucleic Acids Res. 14*, 737–749.

Clark, J. M., & Beardsley, G. P. (1987) *Biochemistry 26*, 5398–5403.

Clark, J. M., Joyce, C. M., & Beardsley, G. P. (1987a) *J. Mol. Biol. 198*, 123–127.

Clark, J. M., Pattabiraman, N., Jarvis, W., & Beardsley, G. P. (1987b) *Biochemistry 26*, 5404–5409.

Derbyshire, V., Freemont, P. S., Sanderson, M. R., Beese, L., Friedman, J. M., Joyce, C. M., & Steitz, T. A. (1988) *Science (Washington, D.C.) 240*, 199–201.

Hall, J. D., & Mount, D. W. (1981) *Prog. Nucleic Acid Res. Mol. Biol. 25*, 53–126.

Hanawalt, P. C., Cooper, P. K., Ganesan, A. K., & Smith, C. A. (1979) *Annu. Rev. Biochem. 48*, 783–836.

Hayes, R. C., & LeClerc, J. E. (1986) *Nucleic Acids Res. 14*, 1045–1061.

Huang, W. M., & Lehman, I. R. (1972) *J. Biol. Chem. 247*, 3139–3146.

Ide, H., Kow, Y. W., & Wallace, S. S. (1985) *Nucleic Acids Res. 13*, 8035–8052.

Joyce, C. M., Ollis, D. L., Rush, J., Steitz, T. A., Konigsberg, W. H., & Grindley, N. D. F. (1986) *UCLA Symp. Mol. Cell. Biol., New Ser. 32*, 197–205.

Lindahl, T. (1982) *Annu. Rev. Biochem. 518*, 61–87.

Moore, P. D., & Strauss, B. S. (1979) *Nature (London) 278*, 664–666.

Moore, P. D., Bose, K. K., Rabkin, S. D., & Strauss, B. S. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 110–114.

Moore, P. D., Rabkin, S. D., Osborn, A. L., King, C. M., & Strauss, B. S. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 7166–7170.

Piette, J., & Hearst, J. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 5540–5544.

Pinto, A. L., & Lippard, S. J. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 4616–4619.

Rouet, P., & Essigmann, J. M. (1985) *Cancer Res. 45*, 6113–6118.

Sagher, D., & Strauss, B. (1983) *Biochemistry 22*, 4518–4526.

Skarnes, W., Bonin, P., & Baril, E. (1986) *J. Biol. Chem. 261*, 6629–6636.

Strauss, B. S. (1985) *Adv. Cancer Res. 45*, 45–105.

Teoule, R., & Cadet, J. (1978) in *Effects of Ionizing Radiation on DNA* (Hutterman, J., Kohnlein, W., & Teoule, R., Eds.) pp 171–203, Springer-Verlag, Berlin.