Responses to the Major Acrolein-derived Deoxyguanosine Adduct in Escherichia coli*

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Acrolein, a reactive α,β-unsaturated aldehyde found ubiquitously in the environment and formed endogenously in mammalian cells, reacts with DNA to form an exocyclic DNA adduct, 3,4-dihydroxy-3-[(β-d-2′-deoxyribofuranosyl)-5,6,7,8-tetrahydroxypyrido[3,2-a]purine-9-one (γ-OH-Pdg). The cellular processing and mutagenic potential of γ-OH-Pdg have been examined, using a site-specific approach in which a single adduct is embedded in double-strand plasmid DNA. Analysis of progeny plasmid reveals that this adduct is excised by nucleotide excision repair. The apparent level of inhibition of DNA synthesis is ~70% in Escherichia coli xrecA, uvrA. The block to DNA synthesis can be overcome partially by recA-dependent recombination repair. Targeted G → T transversions were observed at a frequency of 7 × 10⁻³ translesion synthesis. Inactivation of polB, dinB, and umuDC genes coding for “SOS” DNA polymerases did not significantly the efficiency or fidelity of translesion synthesis. In vitro primer extension experiments revealed that the Klenow fragment of polymerase I catalyzes error-prone synthesis, preferentially incorporating dAMP and dGMP opposite γ-OH-Pdg. We conclude from this study that DNA polymerase III catalyzes translesion synthesis across γ-OH-Pdg in an error-free manner. Nucleotide excision repair, recombination repair, and highly accurate translesion synthesis combine to protect E. coli from the potential genotoxicity of this DNA adduct.

Exocyclic base adducts are formed when various endogenous and exogenous bifunctional agents react with DNA. Such lesions include etheno, ethano, propeno, propano, and chalcone dG, dA, and/or dC adducts (1, 2). Etheno and ethano adducts are formed when DNA reacts with chloroacetaldehyde, 1-substituted oxiranes, and/or enal epoxides; propeno and propano adducts, in reaction with malondialdehyde and enals, respectively; and cyclic adducts, in reactions with p-benzquinoxine, a stable metabolite of benzene (1). Etheno, propeno, and propano base adducts have been detected in DNA isolated from human and animal tissues (3–5). The reaction of peroxidation products of polysaturated fatty acids with DNA is suspected to be the major source of these endogenous adducts (3–5).

The ring structure of exocyclic DNA adducts prevents the formation of normal Watson-Crick hydrogen bonds. These adducts miscode in in vitro primer extension studies conducted with purified DNA polymerases (1, 6–9) and induce point mutations in Escherichia coli and mammalian cells (1, 10–17). Exocyclic etheno adducts are thought to be responsible for the mutations observed in the tumor suppressor p53 gene of humans and animals exposed to vinyl chloride (18, 19).

Acrolein, the simplest member of the α,β-unsaturated aldehyde family, is found widely in the environment and is formed in cells via lipid peroxidation (3). Acrolein was shown to initiate urinary bladder carcinogenesis in rats (20). Acrolein reacts with dG residues in DNA to form two sets of stereoisomeric propano adducts (see Fig. 1): αα and ββ isomers of 3,4-dihydroxy-3-[(β-d-2′-deoxyribofuranosyl)-5,6,7,8-tetrahydroxypyrido[3,2-a]purine-9-one (γ-OH-Pdg) (I) and αα and ββ isomers of 3,4-dihydroxy-3-[(β-d-2′-deoxyribofuranosyl)-5,6,7,8-tetrahydroxypyrido[3,2-a]purine-9-one (α-OH-Pdg) (II). The γ-OH-Pdg isomers are in greater abundance (3, 21). Chung et al. (3, 21, 22) detected background acrolein- and crotonaldehyde (methacrolein)-derived adducts in experimental animals and human tissues at levels ranging between 0.01 and 7.53 μmol/mol of guanines. These authors also reported that oxidative stress, enhanced lipid peroxidation, and decreased levels of glutathione increase markedly the tissue level of propano adducts (3).

1,1,N²-Propanodeoxyguanosine adducts appear to be ubiquitous in cellular DNA and may contribute to so-called spontaneous mutagenesis, thereby playing a role in aging and cancer. In this paper, we establish a genotoxic mechanism for γ-OH-Pdg in E. coli and describe the processing of this adduct in bacterial cells.

We have developed a novel experimental approach that allows us to explore cellular responses to DNA adducts at a mechanistic level (23). The plasmid vector used in this study employs strand-specific markers tagged with mismatches. An oligonucleotide containing a single DNA adduct is incorporated into heteroduplex (HD) DNA containing short stretches of mismatches at several locations. The modified DNA is introduced into mismatch repair-deficient hosts, and adduct-related events are measured. Progeny plasmid are analyzed for their marker sequences. Linkage analysis of marker sequences allows us to group progeny according to in vivo processing events,

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including excision repair, translesion DNA synthesis, and recombination repair.

**EXPERIMENTAL PROCEDURES**

**Enzymes**—Restriction enzymes, T4 polynucleotide kinase, and T4 DNA ligase were purchased from New England BioLabs. (γ-32P]ATP (3000 Ci/mmol) and polymerase chain reaction-grade dNTPs were obtained from Amersham Pharmacia Biotech and Roche Molecular Biochemicals, respectively. The 3′–5′-exonuclease-deficient Klenow fragment (KF exon) was purified from *E. coli* C374 containing pCJ141, an expression vector (gift from C. Joyce, Yale University), as described previously (24).

**Oligodeoxyribonucleotides**—The synthesis of oligodeoxyribonucleotides containing γ-OH-PdG or 1,N2-(1,3-propanediol-2)-deoxyguanosine (PdG) (IV) in Fig. 1) has been described previously (25, 26). Oligonucleotides containing the precursor of γ-OH-PdG (N2-[3,4-dihydroxybutyl] dG) or PdG were purified twice by high pressure liquid chromatography using a Waters Bondapak C18 column (3.9 × 300 mm) and acetonitrile in 100 mM triethylammonium acetate buffer, pH 6.8, at a flow rate of 1 ml/min with the dimethoxytrifluoromethyl-DNMT on and off. The acetonitrile gradient was 16–33% with DMT-on DNA and 0–20% with DMT-off DNA and applied over 40 min. To generate γ-OH-PdG, oligonucleotides containing N2-[3,4-dihydroxybutyl] dG were incubated in 100 mM sodium periodate for 2 h at room temperature (25). γ-OH-PdG-containing oligonucleotides were separated from the parental oligonucleotides by high pressure liquid chromatography as described above for DMT-off DNA. All oligonucleotides were subjected to electrophoresis in a denaturing 20% polyacrylamide gel. Bands were detected by UV shadowing. Oligonucleotides were excised from the gel then purified over a Sep-Pak column (Waters). Purified oligonucleotides were subjected to electrospray mass spectrometry analysis. The results were as follows: 13-mer with γ-OH-PdG m/z: observed, 2901.8 ± 0.47; calculated, 2901.6; 28-mer with γ-OH-PdG m/z: observed, 8510.15 ± 0.23; calculated, 8509.6; and 28-mer with PdG m/z: observed, 8494.37 ± 0.58; calculated, 8493.6.

**E. coli Strains and Vector**—*E. coli* strains used are shown in Table I; MO strains were constructed by P1 transduction (27) and are mismatch repair-deficient. Plasmids pS and pA have been described (23). These plasmids differ in DNA sequence at three regions but are otherwise identical; hence, HD DNA prepared from these plasmids contains three mismatched regions (see Fig. 3). A mismatched region involving a BamHI site is located 150 nucleotides upstream from the adduct site, the others, containing NheI and Spel/AarI sites, are located ~220 and 3100 nucleotides, respectively, downstream from the adduct (see Fig. 3). As described later, these mismatches serve as strand-specific markers.

**Construction of HD DNA Containing a Single γ-OH-PdG Adduct**—The scheme for this construction is shown in Fig. 2. Detailed procedures have been described previously (19). In brief, double-stranded (ds) pA was digested with EcoRV (Step I), and the linearized plasmid DNA was ligated to a blunt-ended duplex 13-mer, 5′-dAGGTACGTAGGAG/ 3′-dTCCATGCGCTCCTC), containing a SnblI site (5′-TACGTA) (Step II). Two constructs, each containing a single insert with opposite orientation, were isolated; one of these, pAl06, was used in this study.

Single-stranded (ss) pAl06 and its complementary strand (derived from ds pS) were annealed to form HD DNA containing a 13-nucleotide gap. An unmodified or modified 13-mer (3′-d(AGGTACGTAGGAG)/ 5′-d(TCCATGCGCTCCTC)), where X is dG or γ-OH-PdG) phosphorylated at the 5′-termini using T4 polynucleotide kinase and ATP, was annealed into the gap and ligated by T4 DNA ligase. The 13-mers are not fully complementary to the gap sequence, because they form mismatches at and adjacent (5′ and 3′) to the adduct site (Fig. 3). These mismatches, located opposite the SnblI site, also serve as a strand-specific marker. The ligation mixture was treated with Spel and EcoRV to remove residual ds pS. Closed circular ds DNA was purified by ultra centrifugation in a CsCl/ethidium bromide solution. DNA was concentrated by Centricon 30 (Amicon, Beverly, MA), and the concentration was determined spectrophotometrically.

In this HD construct, six and three base mismatches are formed at the Spel/AarI and SnblI sites, respectively. At the BamHI and NheI sites, one strand has six extra bases. These four mismatched regions serve as strand-specific markers: A/a (BamHI site), B/b (SnblI site), C/c

![Fig. 1. Exocyclic propano-dG adducts formed by the reaction of α,β-unsaturated aldehydes with DNA. I, γ-OH-PdG; II, α-OH-PdG; III, M2G; a, malondialdehyde-derived dG adduct; IV, PdG, a model for I, II, and III](image)

### Table I

| Strain          | Relevant genotype Source and/or reference |
|-----------------|------------------------------------------|
| AB1157          | EGSC                                      |
| AR1886          | EGSC                                      |
| ES1481          | EGSC, (33)                                |
| MO199           | AB1157 x P1(ES1481)                       |
| MO211           | RK1517 x P1(MO937)                        |
| MO220           | NR9232 x P1(RK1517)                       |
| MO221           | MO220 x P1(YG7210)                       |
| MO234           | AB1886 x P1(YG7210)                      |
| MO2031          | MO230 x P1(YG7210)                       |
| MO323           | MO231 x P1(YG7210)                       |
| MO324           | MO232 x P1(ES1481)                       |
| MO3034          | AB1886 x P1(ES1481)                      |
| MO3093          | MO933 x P1(MO937)                         |
| MO3096          | Volkert, M. a                           |
| MO3097          | Volkert, M. b                           |
| MO3098          | Schaeper, R. a                           |
| MV1932          | Koldoner, R. b                           |
| MV3912          | Goodman, M. F.                           |
| MV3912          | Nakami, T. c                           |

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*f.* National Institute of Health, Tokyo.

**Construction of HD DNA Containing a Single γ-OH-PdG Adduct**—
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(Table II)

| Progeny* | MO937, ΔrecA, wucA | MO938, ΔrecA, wucA | MO934, wucA | MO933, "wild" |
|----------------|------------------|------------------|--------------|--------------|
|                  | yOH-PdG | yOH-PdG | yOH-PdG | yOH-PdG |
|                  | dG      | dG      | dG     | dG     |
| SOS induction    | %       | %       | %      | %      |
| I                 | 32 (61)b  | 78 (140) | 40 (69) | 54 (100) | 55 (104) | 56 (108) | 41 (77) | 43 (80) |
| II                | 64 (122) | 17 (31) | 59 (102) | 12 (22) | 26 (49) | 30 (58) | 13 (24) | 11 (21) |
| III               | 0.5 (1) | 1 (2) | 0.6 (1) | 32 (58) | 2.6 (5) | 1.6 (3) | 35 (66) | 34 (64) |
| IV                | 0      | 0      | 0      | 0.5 (1) | 4.8 (9) | 2.1 (4) | 1.6 (3) | 2.1 (3) |
| V                 | 3.1 (6) | 3.1 (6) | 3.1 (6) | 3.1 (6) | 2.1 (4) | 1.6 (3) | 2.1 (3) | 1.6 (3) |
| VI                | 1.6 (3) | 1.7 (3) | 0.5 (1) | 1.6 (3) | 3.6 (7) | 3.7 (7) | 1.6 (3) | 2.1 (4) |
| Others            | 1.6 (3) | 1.7 (3) | 0      | 1 (2) | 4.2 (8) | 3.1 (6) | 4.2 (8) | 6.9 (13) |

* Linkages of marker sequences for progeny I–VI correspond to those in Fig. 4.

b Numbers represent percent. Actual numbers of plasmid detected are shown in parentheses.

c,d E. coli was pretreated with mitomycin C at 1 (c) or 10 (d) µg/ml.

DH5α (Life Technologies, Inc.). This second transformation segregates progeny plasmid derived from each strand of the HD DNA. Transformants were inoculated individually in 96-well plates and cultured for several hours. Bacterial cultures were stamped onto filter paper placed on a 1× YT-ampicillin plate and cultured overnight. The filter was treated with 0.5 M NaOH for 11 min, neutralized in 0.5 M Tris-HCl, pH 7.4, for 7 min, washed with 1× SSC (150 mM NaCl, 15 mM Na3 citrate, pH 7.2) then with ethanol, and baked at 80 °C for 2 h. Differential oligonucleotide hybridization (16, 23, 28) using the 32P-labeled probes shown in Fig. 3 was employed to detect strand-specific marker sequences and the base located at the position of the adduct. L and R probes were used to confirm the presence of the 13-mer insert.

In several experiments, mitomycin C was used to induce SOS functions in E. coli. Overnight cultures of MO strains were diluted 20-fold with prewarmed 2× YT and cultured for 2 h. Mitomycin C (1–10 µg/ml) was added to the cultures and incubated at 37 °C for 30 min with shaking. Cells were prepared for electrophoresis by repeated washings with H2O.

Interpretation of Results—When a HD construct bearing a single yOH-PdG residue is introduced into a host cell, various events may occur (Fig. 4). If the adduct is removed by excision repair before being replicated, the immediate 5’- and 3’-flanking mismatches are also removed. Gap-filling DNA synthesis converts 5’-CXA (b) to 5’-ACG (B); therefore, progeny derived from the repaired strand contain the linkage of a-b-c-d (progeny III) (Fig. 4, step 1). When the construct is replicated in the absence of DNA repair, progeny produced from the unmodified strand contain the A-B-C-D linkage (step 2), whereas those from the modified strand have a-b-c-d (step 4) following translesion synthesis (TLS). When DNA synthesis is blocked by the adduct, the block may be overcome by UmuD’C/RecA-assisted TLS or recombination repair (daughter strand gap repair) (29). In recombination repair, the ss gap is filled by strand transfer from the unmodified parental strand (step 5).

The 3’-end of the blocked nascent strand is used to replicate the transferred region (step 6). These processes create a Holliday junction. When the Holliday junction is migrated by the RuvA/RuvB complex, a Holliday junction-specific helicase, and resolved by RuvC, a Holliday junction-specific endonuclease (step 7), progeny with the linkage of A-B-C-D (progeny II), a-B-c-d (progeny IV), a-B-C-D (progeny V), and A-B-c-d (progeny VI) are created.

In Vivo Primer Extension Studies—Oligonucleotides used for primer extension studies are as follows: 28-mer templates, 5’-CTGCTCTCCT-ATACTACACGCTTGTAAC, which is dG, eOH-PdG, or PdG; and an 18-mer primer for incorporation studies, 5’-GTCTTAGCTGTTAGGTAT, 19-mer primers for extension experiments, 5’-GTCTTAGCTGTTAGGTATN, where N is dA, dG, dC, or dT; and a 16-mer primer for read-through experiments, 5’-GTCTTAGCTGTTAGGT. All oligonucleotides were purified by electrophoresis in a denaturing 20% polyacrylamide gel. The 5’-32P-end-labeled primers were hybridized to template DNA at a molar ratio of 1:1.2 in a buffer containing 50 mM Tris-HCl, pH 7.5, 5 mM EDTA, and 500 mM NaCl.

Annealing reactions were conducted by heating at 70 °C for 5 min followed by slow cooling.

Reaction mixtures (50 µl) contained 50 mM Tris-HCl, pH 7.4, 5 mM MgCl2, 65 mM primer/template, 200 or 300 µM dNTP, and 0–850 nM KF exo. The concentrations of dNTP were 200 µM for incorporation and extension experiments and 300 µM for read-through experiments. KF exo was diluted in a solution containing 50 mM Tris-HCl, pH 7.5, 0.5 mM KF.

* Numbers represent percent. Actual numbers of plasmid detected are shown in parentheses.

c,d E. coli was pretreated with mitomycin C at 1 (c) or 10 (d) µg/ml.

At each step, the nascent strand is filled by strand transfer from the unmodified parental strand (step 6). These processes create a Holliday junction. When the Holliday junction is migrated by the RuvA/RuvB complex, a Holliday junction-specific helicase, and resolved by RuvC, a Holliday junction-specific endonuclease (step 7), progeny with the linkage of A-B-C-D (progeny II), a-B-c-d (progeny IV), a-B-C-D (progeny V), and A-B-c-d (progeny VI) are created.
RESULTS

γ-OH-PdG Is a Substrate for Nucleotide Excision Repair—Pathway 1 depicted in Fig. 4 predicts that progeny III derived from plasmids subjected to excision repair will contain the a-B-c-d linkage. The unmodified control construct, which contained three base mismatches and no adduct, yielded <1% of progeny III in the uvrA (MO937) and wu+ (MO939) strains (Table II), indicating that the mismatches were not subjected to nucleotide excision repair. When the adducted construct was introduced into the uvrA strains (MO937 and MO939), progeny III accounted for <3% of plasmids analyzed. When the same construct was introduced into wu+ strains (MO939 and MO933), fractions of progeny III ranged from 32 to 35%. Thus, γ-OH-PdG is a substrate for UvrABC-catalyzed nucleotide excision repair in the presence of the 5‘- and 3‘-flanking mismatches.

γ-OH-PdG Adduct Inhibits DNA Synthesis in E. coli—The HD constructs were introduced into MO937 (ΔrecA, uvrA, alkA1, tag1). With the unmodified control construct, more than 96% of progeny were derived from replication of both strands (progeny I and II, Table II). The ratio of progeny I to II, however, was not 1:1 but twice that of progeny I. This result is consistent with that of a previous study (23). Using the modified construct, the number of progeny I derived from the unmodified strand (78%) markedly exceeded that of progeny II derived from TLS (17%). This result indicates that γ-OH-PdG is a strong but not complete block to DNA synthesis. The apparent efficiency of TLS is estimated to be 27% (17/64). The induction of SOS functions did not increase significantly the fraction of progeny II in MO934. The fractions of progeny II obtained in the wu+ strains (MO939 and MO933) were lower than those in the uvrA strains (MO937 and MO934) due to efficient repair of the adduct by nucleotide excision repair.

TLS across γ-OH-PdG Is Essentially Error-free—The adducted HD construct was introduced into MO933 (wild), MO934 (uvrA), MO230 (mutD5), and MO221 (mutD5, ΔumuDC) in the presence or absence of induced SOS functions. Initial numbers of transformants in the transformation mixtures were determined by plating a portion of the transformation mixture. This analysis revealed >1 × 10^6 and >1 × 10^5 transformants per transformation in the absence and presence of induced SOS functions, respectively. The lower transformation efficiency of mitomycin C-treated cells is thought to be due to DNA damage. Progeny plasmid DNA was purified following overnight culture of the transformation mixture in the presence of ampicillin and then digested with SnaBI, the site for which is located in marker B (Fig. 3). This digestion removes progeny derived from the unmodified strand and plasmids subjected to excision repair or recombination repair (see Fig. 4), facilitating analysis of TLS events. Targeted events were analyzed by differential oligonucleotide hybridization and DNA sequencing. This analysis revealed that almost all targeted events were γ-OH-PdG → dG, indicating accurate TLS. Only one γ-OH-PdG → dT transversion was observed among 282 transformants of SOS-uninduced MO933, yielding a miscoding frequency of 0.35%. The numbers of transformants analyzed were 144 for SOS-induced MO933; 190 each for SOS-induced and -uninduced MO934 and MO220, and 144 each for SOS-induced -uninduced MO221. Targeted mutations were not observed in these strains. When data for the four strains are combined, the frequencies of targeted mutations are 0.12% and <0.15% in the absence and presence of induced SOS functions, respectively. Thus, TLS across γ-OH-PdG is highly accurate in E. coli.

DNA Polymerase III and/or Pol I Catalyze Accurate TLS—To address the question of which DNA polymerase(s) is (are) responsible for the TLS, we constructed MO233, a strain that lacks all “SOS DNA polymerases” such as pol II (polB), pol IV (dinB), and pol V (umuDC), in addition to the mutS and uvrA genes. MO234 is a control strain that expresses all SOS DNA polymerases. The degree of TLS (number of progeny II) is not significantly different in MO233 and MO234 in the presence or absence of induced SOS functions (Table III). The analysis of targeted events revealed a single γ-OH-PdG → dT transversion in SOS-induced MO233, suggesting that pol III or pol I is responsible for this mutation. These results suggest that nons-SOS DNA polymerase(s) conduct(s) TLS across γ-OH-PdG with high fidelity.

DNA Polymerase I-catalyzed TLS Is Diminished and Error-prone—Incorporation studies using pol I (KF exo−), in which a
primer terminus was located one base 3' from the adduct site and a single dNTP was present, showed that this polymerase inserts dAMP and dGMP and, to a much lesser extent, dCMP and dTTP opposite γ-OH-PdG. Frequencies of incorporation were 93, 88, 7, and 5% for dATP, dGTP, dCTP, and dTTP, respectively, in a reaction using 85 nM KF exo (Fig. 5A). Primer extension studies, in which a primer terminus was located opposite γ-OH-PdG and all four dNTPs were present, showed that dAMP, dCMP, and dGMP termini were extended similarly and the dTTP terminus was extended poorly: Yields of extended products were 63, 57, 44, and 18% with the dA, dC, dG, and dT termini, respectively, in the reaction containing 85 nM enzyme (Fig. 5B). These results suggest that pol I promotes error-prone DNA synthesis across γ-OH-PdG. When the read-through experiments were conducted using a 16-mer primer in the presence of all four dNTPs, doublet bands, caused by differing migration rates due to the difference in the nucleotide inserted, were observed opposite the adduct (X) and the next base (C) (Fig. 5C). This result indicates that different nucleotides are inserted opposite this adduct and then extended. Effects of the nucleotide difference is obscured as the length increases. This result is consistent with those of incorporation and extension studies described above. Because dCMP is not the preferred nucleotide inserted, TLS catalyzed by this polymerase is likely to be error-prone.

Extension of primers beyond γ-OH-PdG or PdG was observed at 85 and 170 nM KF exo (Fig. 5C). The fractions of primers extended beyond these modified bases are 36% at 85 nM and 69% at 170 nM for γ-OH-PdG and 11% at 85 nM and 23% at 170 nM for PdG. These results indicate that PdG is more inhibitory than γ-OH-PdG.

**DISCUSSION**

In this report, we studied the genotoxicity of γ-OH-PdG in *E. coli* and the response of this organism to the presence of the adduct. Our results indicate that γ-OH-PdG is a good substrate for nucleotide excision repair; in this respect, it is similar to the structurally related PdG and malondialdehyde-derived propeno-dG adduct, pyrimido[1,2-a]purin-10(3H)-one (M1G) (III in...
Fig. 1) (30). γ-OH-PdG inhibits DNA synthesis; the apparent efficiency of TLS is 27% in the absence of induced SOS functions. The inhibition of DNA synthesis created by γ-OH-PdG is partly overcome by daughter strand gap repair (recombination repair) as determined by the increase in the number of recombinants of progenies IV, V, and VI (Tables II and III). Because the parental unmodified strand is used to fill in a gap generated as a result of the synthesis block (refer to Fig. 4), this repair is mechanistically accurate and therefore contributes to an error-free recovery from DNA synthesis block.

The frequency of progeny II derived from the TLS pathway accounts for >30% among all progeny in the strain (MO233) lacking all the SOS DNA polymerases (pol II, pol IV, and pol V), and the SOS induction did not significantly increase the number of progeny II in MO233 or MO234, a control strain containing the SOS DNA polymerases (Table III). These results suggest that one or more constitutive DNA polymerases, pol III and/or pol I, play the major role in this TLS, which is highly accurate. Only two γ-OH-PdG → T transversions were observed among the total of 2687 transformants of various E. coli strains obtained in the presence or absence of SOS induction. The overall targeted mutation frequency is 0.07%. Marnett and his colleagues also have observed the lack of mutagenicity of this adduct (accompanying article (39)). Because pol I is necessary for the replication of ColE1 origin-based plasmids (31), inactivation of this gene is not possible. Our *in vitro* primer extension studies show that this polymerase appears to catalyze error-prone TLS across γ-OH-PdG (Fig. 5), pol I is believed to catalyze the filling of small gaps and the formation of DNA primers in E. coli. Therefore, we speculate that pol III, but not pol I, is responsible for this highly accurate TLS. The accurate TLS was also observed in the strain (MO220) carrying a mutation in the mutD (dnaQ) gene that codes for the pol III-associated 3′→5′-exonuclease (ε subunit) (31). This exonuclease removes nucleotides from the 3′ terminus when incorrect nucleotides are inserted during pol III-catalyzed DNA synthesis. Therefore, if this TLS is catalyzed by pol III, the result of the accurate TLS in this strain suggests that correct dCMP is inserted almost exclusively opposite γ-OH-PdG and extended: The accurate TLS can be ascribed to the selection of the correct deoxyribonucleoside triphosphate, dCTP. Another possibility is that only γ-OH-PdG:dC pair, but not other incorrect pairs, can be extended by pol III.

Results of this study are very different from those reported for the model acrolein adduct PdG, which strongly blocks DNA synthesis and efficiently induces targeted PdG → T and PdG → A mutations in *E. coli* and simian kidney cells (12, 15). PdG presents a stronger block than γ-OH-PdG when tested by primer extension experiments *in vitro*. When a 25-mer containing γ-OH-PdG was incubated at room temperature with its complementary strand, two species migrating more slowly than the starting material appeared, accounting eventually for 5% of the total oligonucleotide. This observation suggests that interstrand cross-linking has occurred, an event that would take place only if γ-OH-PdG exists in a ring-open form similar to that reported for M2G paired with dC in duplex DNA (32). An NMR structural study (see accompanying article (40)) shows unequivocally that ring opening of γ-OH-PdG occurs when the adduct is located opposite dC in duplex DNA, in a conformation allowing formation of Watson-Crick bonds. Because γ-OH-PdG is located in a mismatched region in our HD DNA, the structure of the adduct cannot be stated with certainty; however, Watson Crick hydrogen bonding generated by ring opening of γ-OH-PdG would ac-