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DNA Polymerase II Supports the Replicative Bypass of N^2-Alkyl-2′-deoxyguanosine Lesions in *Escherichia coli* Cells

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Abstract

Alkylation represents a main form of DNA damage. The N^2 position of guanine is frequently alkylated in DNA. The SOS-induced polymerases have been shown to be capable of bypassing various DNA damage products in *Escherichia coli*. Herein, we explored the influences of four N^2-alkyl-dG lesions (alkyl = ethyl, *n*-butyl, isobutyl, or *sec*-butyl) on DNA replication in AB1157 E. *coli* cells and the corresponding strains with polymerases (Pol) II, IV, and V being individually or simultaneously knocked out. We found that N^2-Et-dG is slightly less blocking to DNA replication than the N^2-Bu-dG lesions, which display very similar replication bypass efficiencies. Additionally, Pol II and, to a lesser degree, Pol IV and Pol V are required for the efficient bypass of the N^2-alkyl-dG adducts, and none of these lesions was mutagenic. Together, our results support that the efficient replication across small N^2-alkyl-dG DNA adducts in E. *coli* depends mainly on Pol II.

Graphical Abstract

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Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.chemrestox.0c00478.
Detailed experimental conditions, MS and MS/MS of synthetic ODNs, and MS data and PAGE gel image for monitoring the restriction fragments of replication products of damage and lesion-free genomes (PDF)

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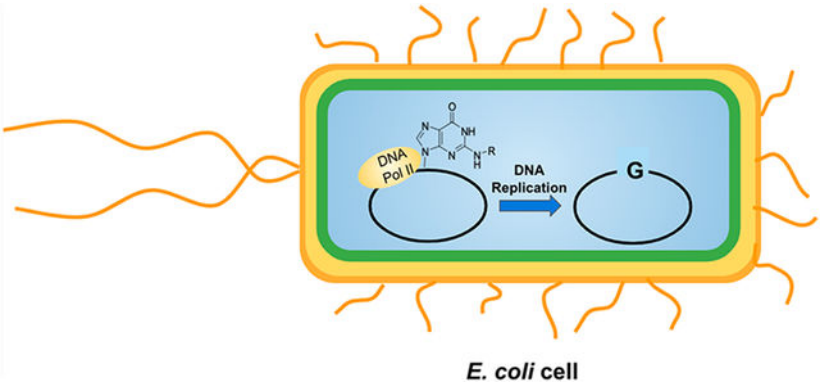

Cells are continuously exposed to alkylating agents that can damage DNA, and the N^2 position of guanine is a common site of alkylation.[1,2] For instance, the N^2 of guanine is susceptible to reaction with formaldehyde and acetaldehyde, which can be produced endogenously or present in external sources, including diesel exhaust, cigarette smoke, etc.[3,4] In addition, methylglyoxal, an air pollutant and a byproduct of endogenous glycolysis, can induce the stable N^2-(1-carboxyethyl)-2′-deoxyguanosine (N^2-CE-dG) in DNA.[5]

DNA adducts, if left unrepaired, can block DNA replication and induce nucleobase substitutions in that process; if mutations occur in oncogenes or tumor suppressor genes, these adducts may contribute to carcinogenesis.[5–7] To ameliorate the genotoxic effects of DNA lesions, cells are equipped with various DNA repair machineries to remove these lesions. Cells are also evolved with DNA damage tolerance pathways to cope with unrepaired lesions, where translesion synthesis (TLS) is one of these pathways for overcoming replication blockage conferred by DNA lesions.[1,8,9] In *Escherichia coli*, three TLS polymerases (Pol II, Pol IV, and Pol V) can be induced under SOS conditions; Pol II and Pol IV participate in mainly error-free TLS of specific DNA lesions, whereas Pol V can bypass a wide range of DNA lesions in a more error-prone manner.[10]

Several studies have been conducted to examine the effects of N^2-alkyl-dG adducts on DNA replication in cells. Yuan et al.[11] showed that the *R* diastereomer of N^2-CE-dG blocks DNA replication more strongly than the *S* diastereomer in *E. coli*, and replicative bypass of these lesions is largely error-free, where Pol IV is required for the accurate and efficient bypass of these lesions. In addition, faithful replication of N^2-CE-dG and a number of simple N^2-alkyl-dG lesions (alkyl = Me, Et, *n*Pr, and *n*Bu) in mammalian cells requires polymerases κ and ι, where Pol κ is the mammalian ortholog of *E. coli* Pol IV.[11,12] On the other hand, Shrivastav et al.[13] found that the replication across N^2-Me-dG and N^2-Et-dG is accurate and efficient in *E. coli* cells; depletion of Pol IV, however, does not perturb the efficiency or fidelity of replication across these lesions. It has not yet been investigated systematically how simple N^2-alkyl-dG lesions influence DNA replication in *E. coli* cells or how the three SOS-induced DNA polymerases modulate the replication across these lesions. To answer these questions, we examined the efficiencies and fidelities of replication across four N^2-alkyl-dG lesions with different sizes and various structures of alkyl groups (N^2-Et-dG, N^2-*n*Bu-dG, N^2-*i*Bu-dG, and N^2-*s*Bu-dG, Figure 1) in *E. coli* cells and the functions of the SOS-induced DNA polymerases in supporting their replication bypass.

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We utilized a modified competitive replication and adduct bypass (CRAB) assay[11] to explore how replication efficiency and fidelity of a single-stranded M13 plasmid are influenced by the presence of site-specifically inserted N^2-alkyl-dG lesions (Figure 2 and Figures S1–S5). In brief, lesion-containing single-stranded M13 plasmids were mixed individually with a lesion-free competitor plasmid at a specific molar ratio and allowed to replicate in SOS-induced AB1157 *E. coli* cells that are proficient in TLS or with one or more SOS-induced DNA polymerases being genetically depleted. In this respect, the competitor genome, which harbors three more nucleotides but is lesion-free, acts as an internal standard for measuring the replication efficiency across the damage site. After progeny genome isolation, PCR amplification, and restriction digestion, the released oligodeoxyribonucleotides (ODNs) were analyzed by LC-MS/MS and native PAGE to identify and quantify the replication products.

The results from native PAGE analyses of the ensuing radiolabeled fragments revealed that no mutagenic products were induced by any of the N^2-alkyl-dG lesions, and a similar finding was made from LC-MS/MS analysis of the corresponding non-radiolabeled restriction fragments (Figure 2B,C and Figures S3–S5). We also determined the bypass efficiencies of the N^2-alkyl-dG lesions by measuring the ratio of intensity of the 10mer band arising from the lesion-containing genome over that of the 13mer band emanating from the lesion-free competitor genome and further normalizing the ratio to that observed for the control dG-containing genome. The results showed that all four N^2-alkyl-dG lesions could impede DNA replication in SOS-induced *E. coli* cells, with the bypass efficiencies for N^2-Et-dG, N^2-nBu-dG, N^2-*i*Bu-dG, and N^2-*s*Bu-dG being 36.5, 31.5, 27.1, and 28.5%, respectively. Hence, the three N^2-Bu-dG lesions display blockage effects on DNA replication slightly stronger than that with N^2-Et-dG; the replication efficiencies across the three N^2-Bu-dG lesions are, nonetheless, not appreciably affected by the structures of the butyl groups (*n*Bu, *i*Bu, and *s*Bu) (Figure 2D).

It is worth noting that the bypass efficiency for N^2-Et-dG observed in the present study is lower than what was observed previously by Shrivastav et al.,[13] which could be attributed to different flanking sequences of the lesion employed in the two studies. In this vein, sequence contexts surrounding DNA damage sites are known to modulate the efficiencies and fidelities of DNA replication across these sites.[14,15] The bypass efficiencies for N^2-Et-dG and N^2-*n*Bu-dG obtained from this study were also lower than what Wu et al.[12] reported recently for the same lesions in HEK293T human embryonic kidney cells, which could be due to differences in replication machineries in E. *coli* and human cells.

We next examined the roles of Pol II, Pol IV, and Pol V in bypassing the N^2-alkyl-dG adducts by performing the replication experiments using the SOS-induced isogenic *E. coli* strains where these polymerases were individually or concurrently ablated. Except that depletion of Pol IV alone did not give rise to significant drops in bypass efficiencies for N^2-Et-dG or N^2-*n*Bu-dG, individual depletion of each of the three SOS-induced DNA polymerases led to substantial attenuations in bypass efficiencies for all four N^2-alkyl-dG lesions, with the most pronounced decreases being observed for the Pol II-deficient background (Figure 2D). Additional drops in bypass efficiencies were observed for the three

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N^2-Bu-dG lesions in Pol IV and Pol V double knockout background compared to those with depletion of either polymerase alone (Figure 2D).

For comparison, we also examined how these lesions modulate the replicative bypass of these lesions in wild-type and Pol II-depleted AB1157 cells without SOS induction. Our results showed that SOS induction led to augmented bypass efficiencies for all four lesions in AB1157 cells (Figure 2D,E). Depletion of Pol II, however, does not alter appreciably the bypass efficiencies for any of the four N^2-alkyl-dG lesions in AB1157 cells without SOS induction (Figure 2E). It was shown previously that SOS induction could give rise to a 7-fold elevation in expression level of Pol II.[16] Thus, the lack of effect of Pol II deletion on the bypass efficiencies of these lesions in uninduced *E. coli* cells could be due to the relatively low level of expression of Pol II in wild-type AB1157 cells without SOS induction.

Exposure to endogenous and exogenous genotoxic agents can lead to the formation of various DNA lesions, many of which block replicative DNA polymerases and require TLS polymerases for their replicative bypass.[17] A large body of literature revealed the roles of B- and Y-family polymerases in modulating the cytotoxic and mutagenic properties of various DNA lesions.[18,19] In this vein, Pol IV and Pol V were found to participate in error-free TLS and induce a −1 frameshift mutation at the site of an N^2-dG adduct of benzo[*a*]pyrene; Pol II, however, is involved in bypassing the bulky N^2-dG adduct of acetylaminofluorene and elicits a −2 frameshift mutation at the lesion site.[20,21] In addition, previous in vitro biochemical studies showed that purified Pol IV preferentially inserts a dCMP opposite N^2-Et-dG, N^2-*i*Bu-dG, and N^2-CE-dG in template DNA,[11,22] though the kinetic parameters for nucleotide insertions opposite simple N^2-alkyl-dG lesions were not measured. It will be important to determine, in the future, the efficiencies and fidelities of Pol II- and Pol IV-catalyzed nucleotide incorporation opposite the N^2-alkyl-dG lesions.

The major finding from the present study is about the contributions of the three SOS-induced DNA polymerases, namely, Pol II, Pol IV, and Pol V, in bypassing four minor-groove N^2-alkyl-dG adducts (alkyl = Et, *n*Bu, *i*Bu, and *s*Bu) in *E. coli* cells. Similar to what were observed for N^2-furfuryl-dG and N^2-tetrahydrofuran-2-yl-methyl-dG lesions,[13] we found that the smaller N^2-Et-dG and N^2-Bu-dGs were not mutagenic in AB1157 cells or any of the isogenic polymerase-deficient strains tested. Our results support that all three TLS polymerases in *E. coli* are involved in the error-free TLS of these four lesions. A small alkyl group adducted to the N^2 of guanine does not strongly impair the base-pairing property of the nucleobase, whereas those adducts with large alkyl groups can induce DNA frameshifts or single-base substitutions during DNA replication.[20] Meanwhile the loss of Pol II results in the most marked diminutions in bypass efficiencies in SOS-induced AB1157 cells, underscoring the major role of this polymerase in overcoming the replication blockage imposed by these N^2-alkyl-dG lesions.

It is worth noting that Pol IV was found to be the major DNA polymerase responsible for bypassing N^2-CE-dG lesions in vivo,[11] whereas Pol II was the main polymerase involved in bypassing the N^2-dG lesions with small alkyl groups. The exact reason for these differences remains unclear and awaits further investigation. In this vein, the X-ray crystal structure of a ternary complex of *E. coli* Pol II, duplex DNA, and an incoming dCTP showed that the

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active site of the polymerase facing the minor-groove N^2 position of template dG is quite spacious,[23] which should be able to accommodate alkyl groups adducted to the N^2 of dG (Figure S6). This may explain Pol II's role in supporting the accurate and efficient bypass of N^2-alkyl-dG lesions. In this context, it is of note that minor-groove O^2-alkyl-dT lesions are highly mutagenic in *E. coli* cells and their efficient bypass mainly requires Pol V.[24]

These previously published results, together with the observations made from the present study, indicate that all three TLS polymerases can participate in bypassing minor-groove lesions; Pol V tends to transverse, in an error-prone manner, those lesions with the hydrogen bonding properties of the nucleobases being disrupted (e.g. the O^2-alkyl-dT lesions), whereas Pol II and Pol IV tend to bypass accurately minor-groove N^2-alkyl-dG lesions. Moreover, DNA Pol II, which is B-family DNA polymerase, was also found to participate in the error-free bypass of a major-groove N^6-benz[*a*]anthracene adenine adduct.[20] Along this line, several B-family polymerases were shown to possess a conserved motif that scans the DNA minor groove for lesions and misincorporations.[25] Therefore, Pol II is capable of bypassing accurately both major- and minor-groove alkylated DNA lesions. Together, the results from previous studies and the current work reveal that nuances of TLS can be modulated by the subtle differences in chemical structures of DNA lesions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

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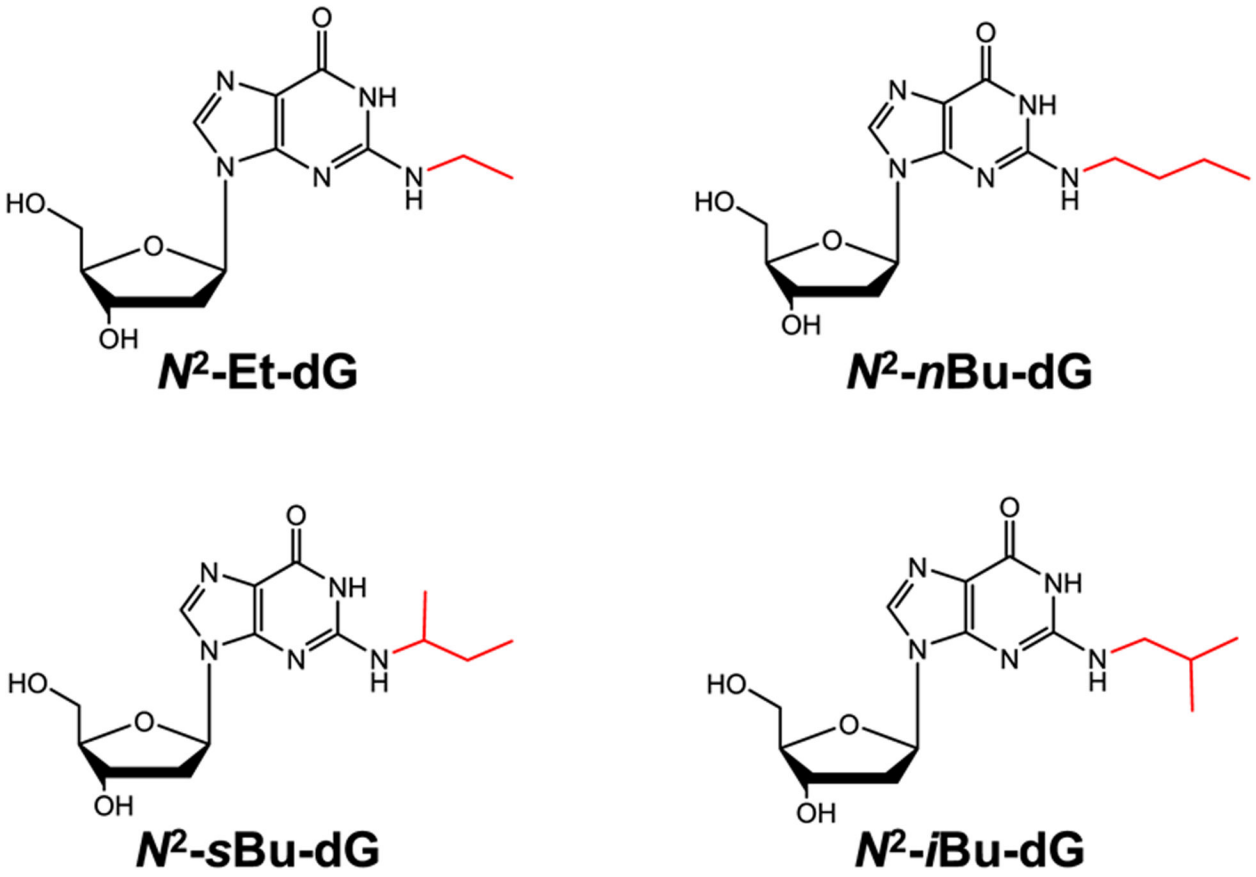

Figure 1.
N^2-Alkyl-dG lesions investigated in this study.

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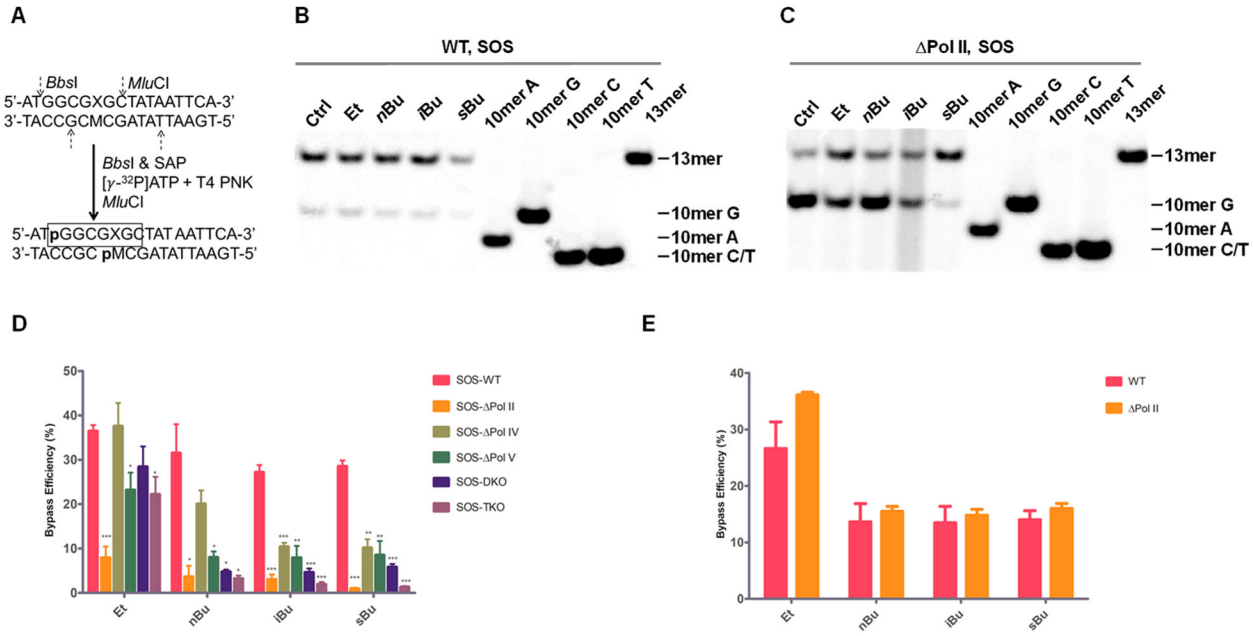

Figure 2.

Restriction digestion and postlabeling method for determining the cytotoxic and mutagenic properties of N^2-alkyl-dG lesions in wild-type AB1157 *E. coli* and the isogenic strains deficient in one or more SOS-induced DNA polymerases. (A) Restriction digestion and selective radiolabeling of the original lesion-containing strand or its complementary strand. (B,C) Representative gel images showing the *Bbs*I/*Mluc*I-produced restriction fragments of interest from the PCR products of progeny genomes of the indicated lesion- or control dG-containing plasmids isolated from SOS-induced wild-type (WT) and Pol II-deficient AB1157 *E. coli* cells. (D) Bypass efficiencies of N^2-alkyl-dG lesions in SOS-induced wild-type AB1157 cells and isogenic polymerase-deficient cells. (E) Bypass efficiencies of N^2-alkyl-dG lesions in wild-type and Pol II-deficient AB1157 E. coli cells without SOS induction. The bypass efficiency was calculated by using the following equation: bypass efficiency (%) = (10mer lesion signal/13mer competitor signal)/(10mer control signal/13mer competitor signal) × 100%. The data represent the mean ± SEM of results acquired from three independent replication experiments. *, $0.01 \le P < 0.05$; **, $0.001 \le P < 0.01$; ***, $P < 0.001$. The P values in (D) were calculated using a two-tailed, unpaired t test, and the values refer to the comparisons between SOS-induced WT and the isogenic TLS polymerase-deficient cells (listed above the columns for the polymerase-deficient cells).