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Backbone Flexibility Influences Nucleotide Incorporation by Human Translesion DNA Polymerase η opposite Intrastrand Cross-Linked DNA

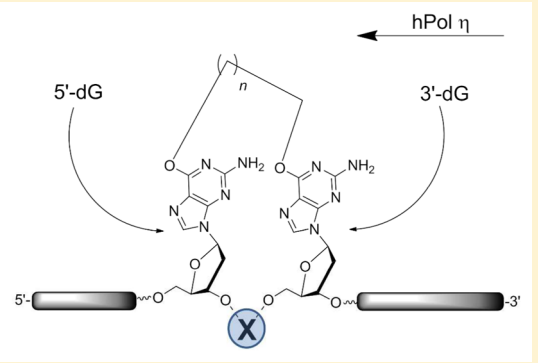
Derek K. O'Flaherty,[†] F. Peter Guengerich,[‡] Martin Egli,[*,‡] and Christopher J. Wilds[*,†]

[†]Department of Chemistry and Biochemistry, Concordia University, 7141 Sherbrooke Street West, Montréal, Québec, Canada H4B 1R6

[‡]Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0146, United States

Supporting Information

ABSTRACT: Intrastrand cross-links (IaCL) connecting two purine nucleobases in DNA pose a challenge to high-fidelity replication in the cell. Various repair pathways or polymerase bypass can cope with these lesions. The influence of the phosphodiester linkage between two neighboring 2′-deoxyguanosine (dG) residues attached through the O^6 atoms by an alkylene linker on bypass with human DNA polymerase η (hPol η) was explored *in vitro*. Steady-state kinetics and mass spectrometric analysis of products from nucleotide incorporation revealed that although hPol η is capable of bypassing the 3′-dG in a mostly error-free fashion, significant misinsertion was observed for the 5′-dG of the IaCL containing a butylene or heptylene linker. The lack of the phosphodiester linkage triggered an important increase in frameshift adduct formation across the 5′-dG by hPol η, in comparison to the 5′-dG of IaCL DNA containing the phosphodiester group.



Chemotherapeutic agents such as the platinum-containing drugs used in the treatment of cancer exert their therapeutic effect mainly via the formation of cytotoxic DNA damage. The lesions that these agents produce have been identified primarily as intrastrand cross-links (IaCL) between the N7 atoms of purines with the distribution of these IaCL determined to be 65% 1,2(GpG), 25% 1,2(ApG), and 5−10% 1,3(GpTpG).[1−9] In addition, formation of minor products, including interstrand cross-links (ICL), monoadducts, and DNA−protein cross-links, occur.[9] The presence of these adducts on the DNA scaffold impedes vital cellular processes such as DNA replication and transcription, ultimately leading to cell death. Drugs used in cancer regimens, other than platinum-containing agents, such as mechlorethamine,[10,11] mitomycin C,[12,13] and busulfan,[14] have also been shown to introduce IaCL into DNA, in particular between adjacent purine nucleobases. Using drugs that act directly on DNA to treat cancer has intrinsic and acquired drug resistance as a major limitation, which is mediated by the cellular response processes of DNA repair and translesion DNA synthesis (TLS).

The four TLS DNA polymerases identified in humans are Pol η, Pol κ, Pol ι, and Rev1. The most widely studied of these is Pol η given its crucial involvement in bypassing UV-induced intrastrand cross-linked DNA lesions. Disruption of the proper function of the *POLH* gene leads to xeroderma pigmentosum variant (XPV), a disease characterized by hypersensitivity to UV irradiation and an increased incidence of skin cancer.[15] As suspected, *POLH* knockout mice demonstrated heightened incidences of skin cancer compared to the control group upon being exposed to UV irradiation.[16] XPV cell extracts displayed replication inhibition of plasmid DNA containing a single (6−4) pyrimidone photoproduct lesion.[17] Moreover, human cells deficient in Pol η revealed a greater number of cell death events upon being treated with platinum-based chemotherapeutic agents.[18−21] Exposure of DNA to γ-irradiation leads to the formation of a mixture of the IaCL lesions G[8,5]C and G[8,5]T, among others, formed via a radical mechanism.[22] Their bypass by yeast and/or human Pol η demonstrated reduced fidelity and processivity, in particular across the dG portion of the lesion.[23−25] Accounts of Pol η bypass are numerous, and the search for other biologically relevant DNA damage, or mimics thereof, is ongoing.

DNA alkylating agents such as *N*-nitroso-*N*-methylurea readily modify the N7 atom of dG and, to a lesser extent, the O^6 position. Lesions at the O^6 atom of dG have also been detected after exposure to the methylating and chloroethylating chemotherapeutic drugs temozolimide and carmustine. Endogenous formation of O^6-MedG by *S*-adenosylmethionine is estimated at 10−30 damage events per cell per day.[26] The

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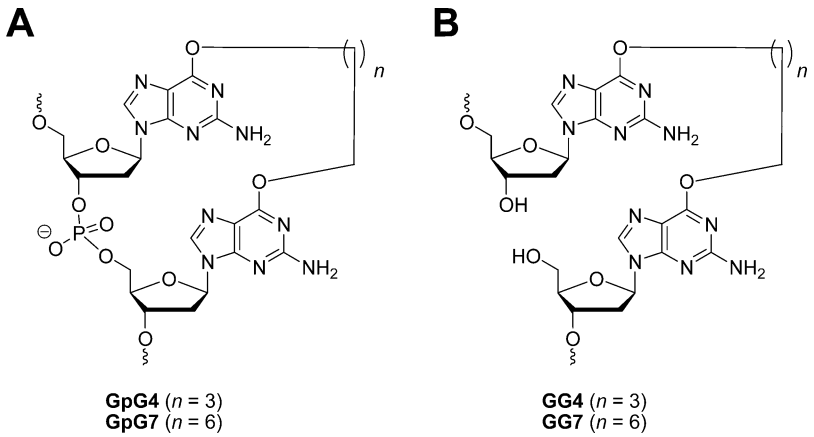

Figure 1. Structures of the O^6-dG-alkylene-O^6-dG IaCL containing (A) and lacking (B) the phosphodiester linkage between the O^6-linked nucleotides.

introduction of covalent appendages at the N7 atom of a purine nucleotide in DNA reduces chemical stability, leading to modifications such as the introduction of abasic sites and/or formation of formamidopyrimidine lesions. Alkyl modifications introduced at the O^6 position of dG are chemically stable and, if left uncorrected, lead to stalls or significant G:C to A:T transitions by DNA polymerases, including those of the Y-family.[27−32] The importance of this site of alkylation has been demonstrated by *in vivo* studies that revealed the direct link between O^6-MedG and carcinogenesis.[33] The disruption of high-fidelity DNA polymerase activity in the presence of O^6-MedG and other O^6-alkyldG lesions has been attributed to disruption of Watson−Crick base pairing.

Much less is known about the bypass of IaCL containing more flexible lesions such as an alkylene linker, in comparison to more rigid systems, including platinum-based IaCL DNA. In this study, we investigated whether Pol η was capable of efficiently bypassing an O^6-dG-alkylene-O^6-dG IaCL containing butylene or heptylene tethers (Figure 1A). Our phosphoramidite synthetic strategy used to generate such IaCL DNA probes allowed us to engineer identical sets of adducted DNA lacking the phosphodiester linkage at the cross-linked site (Figure 1B), which were also evaluated.[34] The absence of the phosphodiester linkage presumably confers increased flexibility to the IaCL DNA. This study set out (i) to investigate the ability of hPol η to bypass a malleable IaCL lesion that can disrupt the fidelity of Watson−Crick base pairing (at the O^6 position of dG as opposed to the N7 position). The second objective was (ii) to investigate the effect of IaCL linker length (butylene vs heptylene) on the processivity of hPol η. The chain lengths of the IaCL linkers were inspired from DNA adducts formed from exposure to the alkyl sulfones busulfan and hepsulfam, known to generate butylene and heptylene DNA adducts, respectively.[14,35] It should be noted, however, that a specific IaCL connected at O^6 atoms of 5′-d(GG) sequences has not been identified to date. Our model IaCL DNAs serve as chemically stable probes that can be prepared on scales and at purities amenable for biochemical studies. The final objective of the study was (iii) to investigate the effect of phosphodiester linkage deletion on IaCL DNA bypass by hPol η.

MATERIALS AND METHODS

Chemical Synthesis and Characterization of Modified DNA Oligonucleotides. See the Supporting Information for details regarding the synthesis of **GG4**, **GG7**, **GpG4**, and **GpG7**.

Steady-State Kinetics. All primer extension assays were conducted using a template strand with the sequence 5′-AC **XX** CT CAC ACT (where **XX** denotes the cross-linked dGG residues or dGG for the unmodified control) and a fluorescently labeled primer 5′-(FAM)TAG TGU GAG (where FAM is 6-carboxyfluorescein and *U* is 2′-deoxyuridine). Steady-state kinetic experiments were conducted as previously described.[36−39] Briefly, assays were generally performed at 37 °C in 40 mM Tris-HCl buffer (pH 7.5) containing 100 mM KCl, 5% glycerol (v/v), 10 mM dithiothreitol (DTT), 5 mM $MgCl_2$, and 100 μg mL^{-1} bovine serum albumin (BSA). The 5′-fluorescently labeled (FAM) primer-template (9/13-mer) duplex (5 μM) was extended using 1.9−500 nM hPol η in the presence of various concentrations of a single dNTP (0−1 mM, at 7−10 different dNTP concentrations) at 37 °C for 5−20 min. Reactions were quenched using a solution containing 20 mM EDTA (pH 8.0), 95% formamide (v/v), bromophenol blue, and xylene cyanol dyes. Substrates and products were resolved on 18% (w/v) polyacrylamide electrophoresis gels containing 7.5 M urea. Gels were monitored by a Typhoon Scanner (GE Healthcare) and analyzed by fluorescence intensity using ImageJ (National Institutes of Health, Bethesda, MD). The values of k_{cat} and K_m were estimated by nonlinear regression analysis (hyperbolic fit) with Prism (GraphPad, La Jolla, CA).

LC−MS/MS Analysis of Fully Extended Products. All primer extension assays were conducted using identical sequences as described in the preceding section. DNA primers were extended in the presence of all four dNTPs followed by analysis via mass spectrometry. Primer sequences were specifically designed to contain a 2′-deoxyuridine (*U*) insert to produce shorter oligonucleotide fragments that are formed by treatment with uracil DNA glycosylase followed by hot piperidine. These were subsequently analyzed by an LC−MS/MS method (ion-trap mass spectrometer), as previously described.[36,37,40−42] DNA primer extension was accomplished by combining hPol η (95 pmol, 0.95 μM for unmodified duplexes; 340 pmol, 3.4 μM for IaCL-containing duplexes) with template-primer duplex (2 nmol, 20 μM) and a mixture of

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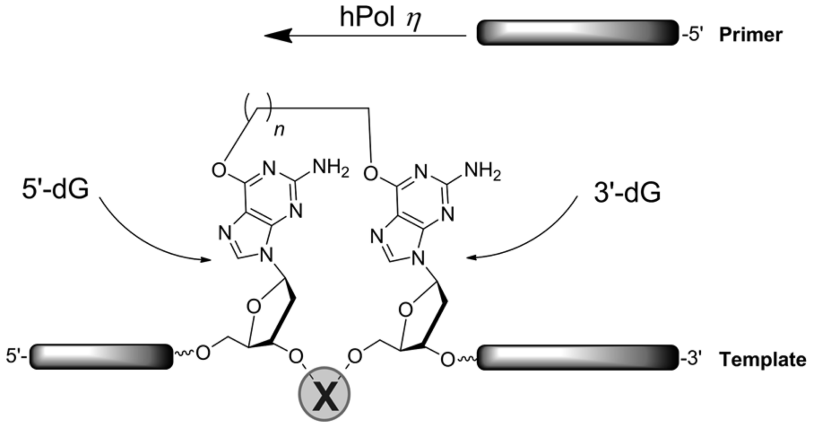

Figure 2. Pictorial representation of the primer extension assay using hPol η, where **X** represents the presence, or absence, of a phosphodiester linkage. Frameshift adduct formation (−1), observed primarily at the 5′-dG residue for **GG4** and **GG7**, is characterized by hPol η skipping the template nucleotide.

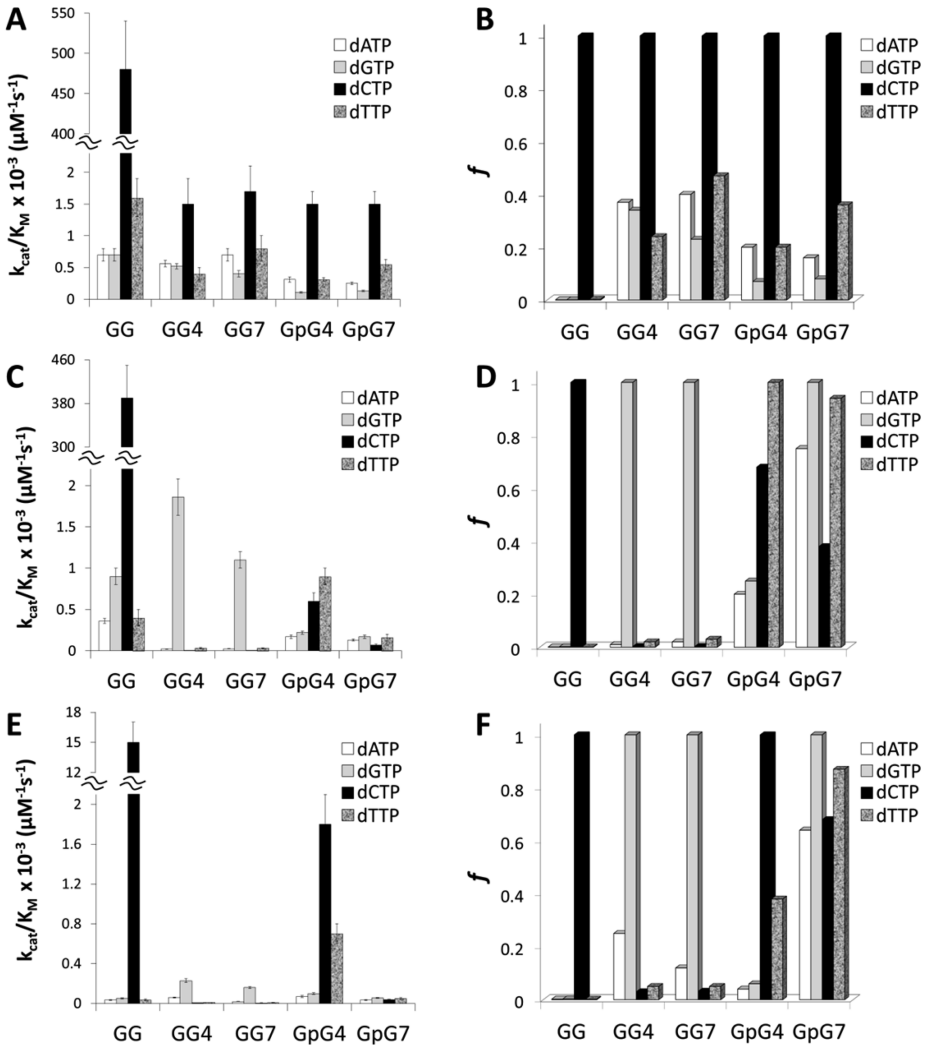

Figure 3. Summary of steady-state kinetics of incorporation of dNTP opposite IaCL-containing template **GG4**, **GG7**, **GpG4**, and **GpG7** (5′-AC **XX** CT CAC ACT) and an unmodified template (**GG**) by hPol η. DNA primer sequences were 3′-GA GUG TGA T(FAM)-5′ (A and B), 3′-**C** GA GUG TGA T(FAM)-5′ (C and D), or 3′-**T** GA GUG TGA T(FAM)-5′ (E and F). Tabulated values are reported in the Supporting Information.

dATP, dCTP, dGTP, and dTTP (1 mM each) at 37 °C for 0.5−1.5 h in 50 mM Tris-HCl buffer (pH 7.5), 50 mM NaCl, 5 mM DTT, 5 mM $MgCl_2$, and 50 μg/mL bovine serum albumin (BSA). The reactions were terminated by spin column separations (Micro Bio-Spin 6 Columns from Bio-Rad) to extract the dNTPs and Mg^{2+}. The extent of the extension was monitored by electrophoresis and fluorography prior to LC−MS/MS analysis (Figure S6). The extension products were

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treated with 25 units of uracil DNA glycosylase followed by 0.25 M piperidine and subjected to LC−MS/MS analysis using an Acquity UPLC system (Waters) interfaced to a Thermo-Finnigan LTQ mass spectrometer (Thermo Scientific, San Jose, CA) equipped with a negative ion electrospray source.[36,37,40−42] Chromatographic separation was conducted with an Acquity UPLC BEH octadecylsilane (C18) column (2.1 mm × 100 mm, 1.7 μm). The LC solvent system was as follows: mobile phase A, 10 mM $CH_3CO_2NH_4$ in 98% H_2O; mobile phase B, 10 mM $CH_3CO_2NH_4$ in 90% CH_3CN (v/v). The following gradient (v/v) was used with a flow rate of 300 μL min^{-1} at a temperature of 50 °C: linear gradient from 0 to 3% B (v/v) in 3 min, followed by a linear increase to 20% B (v/v) from 3 to 5 min, and then 20 to 100% B (v/v) from 5 to 6 min, which was held for 2 min. The column was re-equilibrated for 3 min with 0% B (v/v) for subsequent analysis. Mass spectrometry conditions were as follows: source voltage, 4 kV; source current, 100 μA; capillary voltage, −49 V; capillary temperature, 350 °C; tube lens voltage, −90 V. Product ion spectra were recorded over the range of m/z 300−2000, and the most abundant species (−2 charge) was used for collision-induced dissociation (CID) analysis. The calculation for the oligonucleotide sequence CID fragmentation was conducted using Mongo Oligo Mass Calculator version 2.06 from The RNA Institute (College of Arts and Science, University at Albany, State University of New York, Albany, NY). The relative yields of various products were calculated on the basis of the peak areas of extracted ion chromatograms from LC−MS analyses. The sum of the peak areas was used for multicharged species.

RESULTS AND DISCUSSION

Bypass studies with hPol η have revealed its ability to process a wide variety of DNA modified at the nucleobase, including O^6-alkyl-dG adducts,[28] 1,N^2-etheno-2′-deoxyguanosine,[43] and 8-oxo-7,8-dihydro-2′-deoxyguanosine.[36] Bulkier forms of DNA damage, including intrastrand lesions induced by cisplatin,[44,45] UV radiation,[15,46] and the dG[8,5-Me]dT IaCL[23] have also been the subject of bypass studies with hPol η. Bis-alkylating drugs such as busulfan can introduce IaCL that may have increased flexibility at the modified site relative to the bulkier lesions described above. Given the versatility of hPol η to bypass a variety of lesions by TLS, we decided to explore the influence of flexibility at the modified site with O^6-dG-alkylene-O^6-dG IaCL containing either a butylene or heptylene linkage between the bases. To further probe the influence of flexibility, template strands lacking and containing a phosphodiester linkage between the linked nucleobases have been prepared (see Figures S2−S5 for ESI-MS of the template strands). DNA duplexes containing an O^6-dG-alkylene-O^6-dG IaCL and lacking a phosphodiester linkage between the tethered nucleotides have been shown to be substrates of the human DNA repair protein O^6-alkylguanine DNA alkyltransferase (hAGT), which repairs the linkage restoring dG.[34] In this process, the first reaction with AGT forms two products, a DNA−protein cross-linked (DPC) species and a repaired oligonucleotide. The DPC is a very weak substrate for a second repair event that releases the second unmodified (repaired) DNA fragment. Butylene-linked IaCLs were observed to be resistant to the action of hAGT using extended reaction times (8 h) and 5 molar equiv of the protein. However, the heptylene-linked IaCL DNA analogues were almost completely consumed under these conditions.[34] The template strands employed in this study were designed on the basis of cisplatin-adducted DNA sequences studied by Zhao and co-workers.[45]

Steady-state kinetic evaluations of nucleotide incorporations opposite IaCL lacking a phosphodiester linkage between the attached nucleobases (**GG4** and **GG7**, where 4 and 7 are butylene- and heptylene-linked), containing a phosphodiester linkage (**GpG4** and **GpG7**), and unmodified **GG** were conducted with the catalytic core construct of hPol η (amino acids 1−432). The primer extension assay is illustrated in Figure 2. In all cases, the IaCL modification blocked DNA synthesis by hPol η relative to the unmodified control. The level of incorporation of the correct dCMP nucleotide by hPol η opposite the first 3′-alkylated dG of **GG4**, **GG7**, **GpG4**, and **GpG7** was reduced by approximately 320-, 280-, 320-, and 320-fold, respectively, relative to that of **GG** (see Figure 3 and Table S1). These results differ significantly from those for the cisplatin-adducted GG templates where a 1.2-fold decrease was observed,[45] which suggests that modification of the Watson−Crick hydrogen-bonding face poses a challenge to the incorporation efficiency of hPol η. Moreover, they correlate with studies of hPol η bypass with an O^6-MedG-containing template, which demonstrated a significant decrease (10-fold) in efficiency for incorporation of the correct dCMP nucleotide.[28] The reduced efficiency of incorporation may be attributed to hindrance due to the presence of the alkylene tether. Bulkier lesions at the O^6 atom such as the benzyl and 4-oxo-4-(3-pyridyl)butyl lesion have been shown to reduce incorporation efficiency by factors of ∼65 and 250, respectively, for hPol η.[28] The alkylene linkage to a subsequent nucleotide, found in IaCL DNA, could also contribute to the observed reduced relative kinetic efficiencies. Significant misinsertions by *Saccharomyces cerevisiae* Pol η have also been observed across the dG nucleotide of the dG[8,5-Me]dT IaCL, whereas hPol η mostly incorporated the correct dNMPs across both nucleotides of this IaCL.[23,25,47] Steady-state data for our IaCL DNA showed a preference for dCMP insertion across the first 3′-dG-alkylated residue. However, an overall decrease in selectivity (f coefficients are shown in Figure 3 and listed in Table S1) was observed, particularly for the **GG4** and **GG7** templates. Relative insertions (f coefficients) of dAMP, dGMP, and dTMP increased to 0.37, 0.34, and 0.24 for **GG4** and 0.40, 0.23, and 0.47 for **GG7** compared to 0.001, 0.001, and 0.003 for **GG**, respectively. Lower f coefficients were generally observed for **GpG4** and **GpG7** than for **GG4** and **GG7**, respectively, suggesting the added flexibility inherent to **GG4** and **GG7** posed an issue for hPol η fidelity. It was interesting to observe an increased efficiency and selectivity for the butylene linkers (**GG4** and **GpG4**) in comparison to those of the heptylene linkers (**GG7** and **GpG7**), substantiating the idea that increased steric bulk hinders processivity by hPol η. It was noted that in all cases, except **GG4**, dTMP was incorporated most efficiently from the misinsertion pool across the 3′-dG position.

Postinsertion extension across the second alkylene-linked site (5′-dG) using primers that contain dC or dT directly across from the 3′-dG of the IaCL-containing templates, as well as the unmodified **GG**, was studied. The results are summarized in Figure 3 and listed in Tables S2 and S3. Extension across the second O^6-alkylene-linked dG was generally slower than the first regardless of the identity of the additional nucleotide (dC or dT) in the primer. An interesting feature for the **GG4** and **GG7** templates was the apparent proficient misinsertion of dGTP across the O^6-alkylated 5′-dG residue. It was suspected that frameshift adduct formation had occurred, which was

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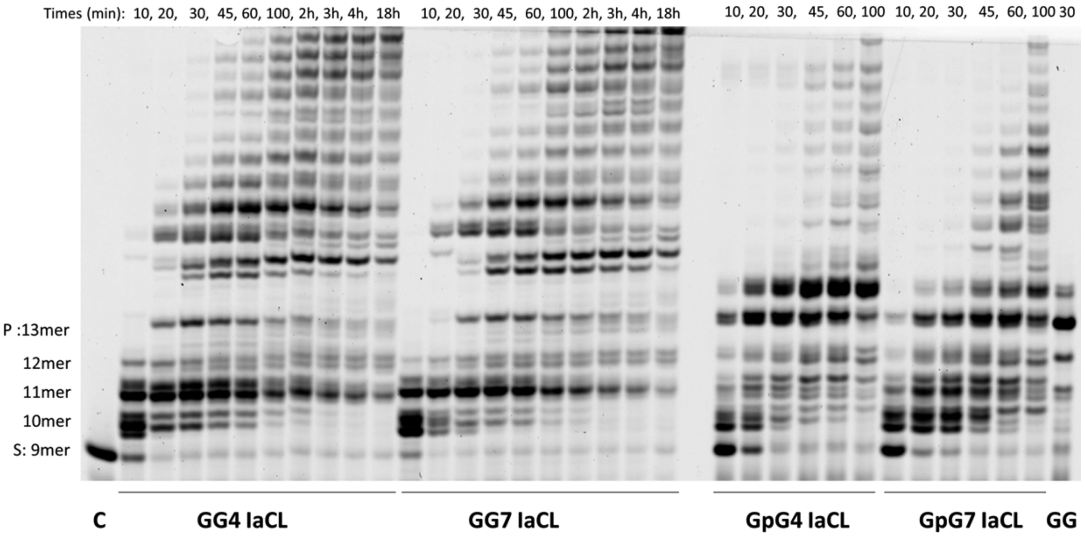

Figure 4. Time course assay of primer full extension (25 μM annealed DNA) by hPol η (3.4 μM for damaged and 1 μM for undamaged DNA) in the presence of all four dNTPs (1 mM mixture). DNA duplexes were composed of IaCL-containing template **GG4**, **GG7**, **GpG4**, or **GpG7** (5′-AC XX CT CAC ACT) or unmodified template (**GG**), annealed with the DNA primer [3′-GA GUG TGA T(FAM)-5′]. The DNA template used is indicated at the bottom, with the product length identified on the left-hand side; reaction times are indicated at the top (in minutes unless stated otherwise). Note that only the 30 min reaction time is shown for the undamaged control (**GG**) (data for other time points not shown). Extended products were resolved by 17% denaturing PAGE (19:1) and visualized via fluorography.

confirmed by full extension analysis by LC−MS/MS, given that dC is the subsequent nucleotide in the template strand. dNTPs other than dGTP were not efficiently incorporated in the case of **GG4** and **GG7**. The postextension profiles for **GpG4** and **GpG7** displayed a clear decrease in selectivity compared to that of insertion across the first (3′) O^6-alkylated-dG, with an almost complete loss of dNTP preference for **GpG7**. To be noted is the preference of dTTP over dCTP for **GpG4** and **GpG7**. The 3′-end O^6-alkylated-dG·dT mismatch significantly lowered the efficiency of incorporation across the subsequent O^6-alkylated-dG for all IaCL DNAs except for insertion of dCMP in the case of **GpG4**. The steady-state results showed an overall reduction in incorporation efficiency and fidelity, particularly across the 5′-dG of the IaCL. This occurrence is not observed for hPol η bypass of platinum-based dGG adducts or the dG[8,5-Me]dT adduct.[48] The reduction of incorporation efficiency and fidelity across the 5′-dG may be the result of the alkylene linker adopting an orientation that disrupts efficient DNA primer extension.

Analysis of single insertions by a DNA polymerase is useful for kinetic analysis in terms of identifying the extent of blockage by a given modification in a quantitative manner. However, this may not reflect insertion profiles in the presence of all four dNTPs as well as extension past the damaged sites. The fidelity of hPol η and processivity across and past the damage site were assessed via analysis of extended products by LC−MS/MS, using a reported methodology.[28,36,40−42] This method provides insight regarding hPol η's preference for incorporation of dNTP across the IaCL site (Tables S4 and S5). The PAGE analysis of fully extended products (Figure 4) revealed blockage of hPol η after insertion across the **GG4** and **GG7** IaCL modification (e.g., extension was stalled after incorporation of the second dNTP). Similar results were observed for replication across and past DNA templates containing a single abasic site insert and may represent slippage of hPol η during replication.[37] Our study suggests that hPol η bypass requires an intact phosphodiester DNA backbone for processivity, particularly for IaCL. In contrast, hPol η replicated past the IaCL of **GpG4** and **GpG7** to reach an accumulation of the fully extended (13 nucleotides) and overextended (14 nucleotides) product (Figure 4). hPol η exhibited similar bypass extension profiles for other O^6-alkyl-dG lesions, which suggested that the IaCL containing a phosphodiester linkage may be processed as two adjacent monoadducted dG inserts.

Insertion profiles opposite the 3′-dG and 5′-dG of **GG4**, **GG7**, **GpG4**, and **GpG7** and the unmodified control are shown in Figure 5. Insertion of dCMP across the 3′-end of the O^6-dG-alkylene-O^6-dG was preferred by hPol η in all cases, which was in agreement with the steady-state kinetic data. Although the DNA replication machinery has been shown to misinsert dTMP opposite O^6-MedG,[28−31] only a slight increase (3−12%) in the efficiency of insertion of dTMP by hPol η across the 3′-

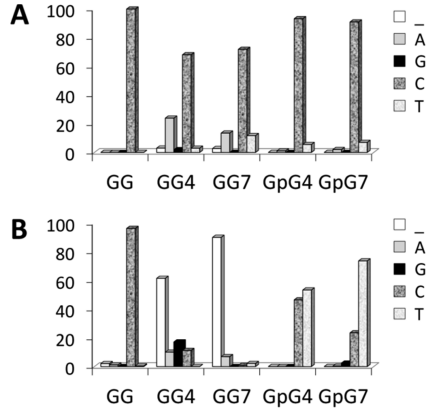

Figure 5. Insertion profiles by hPol η opposite (A) the 3′-dG and (B) the 5′-dG of the O^6-dG-alkylene-O^6-dG IaCL and unmodified control. DNA sequence contexts were as follows: IaCL-containing template (**GG4**, **GG7**, **GpG4**, **GpG7**, or unmodified template **GG**) had sequence 5′-AC XX CT CAC ACT, and the DNA primer sequence was 3′-GA GUG TGA T(FAM)-5′. Tabulated LC−MS extension products and percentages can be found in the Supporting Information.

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dG relative to the unmodified control was observed. Interestingly, the MS-based analysis revealed that dAMP incorporation occurs at levels of 24 and 14% for **GG4** and **GG7**, respectively, and in only negligible amounts for **GpG4**, **GpG7**, and the unmodified control. Similar erroneous dAMP insertions by hPol η have been observed opposite abasic sites (also known as the "purine rule") in addition to frameshift adduct formation.[37,49−51] Minor products (<3%) corresponded to frameshift adduct formation opposite 3′-dG lesions.

Nucleotide insertion profiles for the 5′-end of the dG lesion by hPol η revealed a proclivity for frameshift adduct formation in the case of **GG4** and **GG7**, whereas a pyrimidine insertion was preferred for **GpG4** and **GpG7**. These results were in agreement with the steady-state kinetic evaluation of dGMP insertion across the 5′-end of the dG lesion of **GG4** and **GG7**. We suspect that the added flexibility incurred from the lack of a phosphodiester linkage is responsible for large discrepancies observed between the IaCL DNA. hPol η incorporated few to no purines opposite the 5′-end of the dG lesion in **GpG4** and **GpG7**, but levels of 10 and 17% for dAMP and dGMP, respectively, were measured for **GG4**. Only 7% dAMP was detected for extension across the O^6-alkylated 5′-dG in **GG7**, with no dGMP insertion detected. Misinsertion of dTMP was preferred by hPol η in the case of **GpG4** and **GpG7** (53 and 74%, respectively). Correct insertion of dCMP accounted for 47 and 24% opposite the O^6-alkylated 5′-dG in **GpG4** and **GpG7**, respectively, and only a minor dCMP insertion was observed for **GG4** and **GG7** (11 and 1%, respectively).

CONCLUSION

In conclusion, both the steady-state primer extension assays and LC−MS analysis of the fully extended product demonstrated that extension opposite the 5′-end of the O^6-dG-alkylene-O^6-dG was more problematic than that opposite the 3′-end. An increase in the level of frameshift adduct formation by hPol η was observed at the 5′-dG of IaCL DNA lacking the phosphodiester linkage, compared to those IaCLs containing this functional group. To be noted was the decrease in selectivity by hPol η for longer heptylene IaCL versus the butylene analogue, regardless of the presence of the phosphodiester group at the cross-linked site. Structural insights into the ternary complexes (DNA, hPol η, and incoming nucleoside triphosphate) by X-ray crystallography are being initiated and will shed light on the error-free bypass and error-prone behavior of hPol η toward these IaCLs.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biochem.5b01078.

Example of IaCL DNA mentioned herein, characterization by ESI-MS of the modified oligomers, and LC−MS analysis of most abundant full-length extension products opposite modifications in the DNA template by hPol η in the presence of all four dNTPs (PDF)

AUTHOR INFORMATION

Corresponding Authors

*E-mail: martin.egli@vanderbilt.edu.
*E-mail: chris.wilds@concordia.ca.

Author Contributions

All authors conceived and designed the experiments. D.K.O. performed all the experiments and analyzed the data. All authors interpreted the results and wrote the manuscript.

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Notes

The authors declare no competing financial interest.

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DEDICATION

Dedicated to the memory of Dr. Richard Armstrong.

ABBREVIATIONS

dG, 2′-deoxyguanosine; 3′-dG, dG residue at the 3′-end of the IaCL; 5′-dG, dG residue at the 5′-end of the IaCL; TLS, translesion synthesis; Pol, DNA polymerase; XPV, xeroderma pigmentosum variant; UV, ultraviolet; O^6-MedG, O^6-methyl-2′-deoxyguanosine; ESI-MS, electrospray ionization mass spectrometry; PAGE, polyacrylamide gel electrophoresis; hPol η, human DNA polymerase η; CID, collision-induced dissociation; dNTP, 2′-deoxynucleoside triphosphate.

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