LC-MS/MS Identification and Yeast Polymerase η Bypass of a Novel γ-Irradiation-Induced Intrastrand Cross-Link Lesion G[8−5]C†

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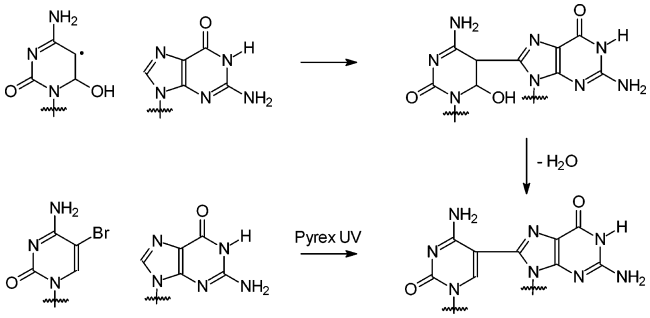
ABSTRACT: Reactive oxygen species can give rise to intrastrand cross-link lesions, where two neighboring nucleobases are covalently bonded. Here, we employed LC-MS/MS and demonstrated for the first time that γ irradiation of a synthetic duplex oligodeoxyribonucleotide can give rise to an intrastrand cross-link lesion G[8−5]C, where the C8 carbon atom of guanine and the C5 carbon atom of its 3′-neighboring cytosine are covalently bonded. We also carried out in vitro replication studies of a substrate containing a site-specifically incorporated G[8−5]C, and our results showed that yeast *Saccharomyces cerevisiae* DNA polymerase η (pol η) was able to replicate past the cross-link lesion. Steady-state kinetic analyses for nucleotide incorporation by pol η showed that the 3′-cytosine moiety of the cross-link did not significantly affect either the efficiency or the fidelity of nucleotide incorporation. The 5′ guanine portion of the cross-link lesion, however, markedly reduced both the efficiency and the fidelity of nucleotide incorporation; the insertion of dGMP or dAMP was slightly favored over the insertion of the correct nucleotide, dCMP, which was in turn favored over the insertion of dTMP. The above results support that the oxidative cross-link lesion, if not repaired, can be mutagenic.

Reactive oxygen species are produced by both endogenous and exogenous pathways, and they can damage cellular components including DNA (*1*−*3*). Box, Cadet, their co-workers (*4*−*10*), and we (*11*) have identified several intrastrand cross-link lesions of CG, TG, and mCG (mC is 5-methylcytosine) formed by γ irradiation under anaerobic conditions. These cross-link lesions have been shown to be induced from a single pyrimidine radical (*5*, *6*, *8*, *10*, *11*). In this respect, the hydroxyl radical can attack the C5 and C6 carbon atoms of cytosine, thymine, and 5-methylcytosine, as well as the methyl carbon atom of the latter two, which gives rise to pyrimidine-base-centered radicals (*12*).

Among these cross-link lesions, the one at the CG site was isolated by Box and co-workers (*7*) from the X-ray irradiation mixture of a tetranucleotide d(CGTA), and it has a covalent bond between the C5 carbon atom of cytosine and the C8 carbon atom of its 3′ adjacent guanine (*7*). This lesion was proposed to arise from the dehydration of the coupling product between guanine and the 6-hydroxy-5,6-dihydrocytosin-5-yl radical (Scheme 1) (*7*). The formation of the cross-link lesion between cytosine and guanine, however, has never been demonstrated in duplex DNA. To evaluate the significance of this type of cross-link lesion in ionizing radiation-induced mutagenesis and its implication in cancer radiotherapy, it is important to determine whether the cross-link lesion can form in duplex DNA and, if so, to examine its mutagenic properties.

Scheme 1



Not all lesions can be repaired efficiently prior to DNA replication. To prevent acute cell death through blocked DNA replication, cells have evolved a translesion synthesis mechanism, which allows DNA synthesis to proceed past the lesions (*13*). Recently, several eukaryotic DNA polymerases participating in translesion synthesis have been discovered (*13*, *14*). Among these polymerases, eukaryotic DNA polymerase η (pol η), which is the gene product of Rad30 in budding yeast *Saccharomyces cerevisiae* (*15*) and the variant form of *Xeroderma pigmentosum* (XP-V)[1] in humans (*16*), is extensively studied. It has been demonstrated that yeast and human pol η can efficiently bypass a *cis*,*syn*-cyclobutane thymine dimer (T[*c*,*s*]T) in an error-free manner (*15*, *17*).

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[1] Abbreviations: PAGE, polyacrylamide gel electrophoresis; MS/MS, tandem mass spectrometry; XP-V, variant form of *Xeroderma pigmentosum*; T[*c*,*s*]T, *cis*-,*syn*-cyclobutane thymine dimer; ODN, oligodeoxyribonucleotide.

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The intrastrand cross-link lesions induced by γ irradiation and dimeric DNA photoproducts induced by sunlight irradiation (*18*) are similar in that they both have neighboring nucleobases being covalently bonded with each other. Although the mutagenic properties of the latter have been extensively investigated, those of the former remain unexplored.

In this paper, we will demonstrate by LC-tandem mass spectrometry (MS/MS) that the cross-link lesion, in which the C8 carbon atom of guanine and the C5 carbon atom of cytosine are covalently bonded, can form in duplex DNA upon γ irradiation under anaerobic condition. In addition, we will discuss the steady-state kinetic analyses for nucleotide incorporation across the two damaged bases by yeast *S. cerevisiae* DNA pol η.

EXPERIMENTAL PROCEDURES

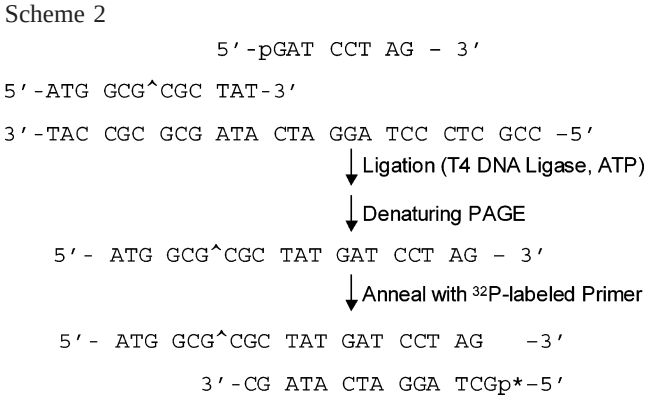
All oligodeoxyribonucleotides (ODNs) used in this paper were purchased from Integrated DNA Technologies (Coraville, IA). [γ-^{32}P]ATP was obtained from Amersham Biosciences Co. (Piscataway, NJ). All other chemicals unless otherwise notified were obtained from Sigma−Aldrich (St. Louis, MO).

γ Irradiation, Enzymatic Digestion, and LC-MS/MS. A self-complementary ODN d(CCGGCCGGCCGGCCGG) (25 nmol) was dissolved in a 25-μL aqueous solution containing 50 mM NaCl and 50 mM phosphate (pH 6.8). The ODN was annealed by heating to 90 °C and cooling slowly to room temperature. The ODN solution was then diluted to 2.5 mL with doubly distilled water, dispersed in a 50-mL round-bottom flask, and degassed by three cycles of freeze−pump−thaw. The flask was then filled with argon and exposed to a Mark I ^{137}Cs Irradiator (JL Shepherd and Associates, San Fernando, CA) for a total dose of 40 Gray (Gy) at a dose rate of 0.93 Gy/min.

Two units of nuclease P1 was then added to the irradiation sample, and the digestion was carried out at room temperature overnight. The resulting solution was dried by using a Savant Speed-vac (Savant Instruments Inc., Holbrook, NY), and the dried residue was redissolved in a 100-μL solution containing 100 mM Tris (pH 8.0) and 50 mM NaCl. The 5′-phosphate groups in the nucleotides resulting from nuclease P1 digestion were removed by overnight incubation with 2 units of alkaline phosphatase at room temperature. The digestion mixture was extracted with an equal volume of chloroform to remove the enzymes, and the aqueous layer was dried by using the Speed-vac and redissolved in water prior to LC-MS/MS analysis.

A 0.32 × 250 mm C18 column with a particle size of 5 μm and a pore size of 300 Å (Micro-Tech Scientific, Vista, CA) was employed for the separation, and a 100-min gradient of 0−35% acetonitrile in 10 mM ammonium acetate was used. A homemade precolumn splitter was employed, and the flow rate was 4−6 μL/min after splitting. The effluent from HPLC was coupled directly to an LCQ Deca XP ion-trap mass spectrometer (ThermoFinnigan, San Jose, CA). The spray voltage was 4.0 kV, and the capillary temperature was maintained at 200 °C. The mass spectrometer was set up to monitor the fragmentation of the $[M + H]^+$ ion (*m*/*z* 555) of the cross-link lesions d(C[5−8]G) and d(G[8−5]C) (The "[5−8]" and "[8−5]" denote that the C5 carbon atom of cytosine and the C8 carbon atom of guanine are covalently bonded in these two dinucleoside monophosphates). The digestion mixture of 2-nmol γ-irradiated ODN (amount determined in the single-stranded form) was injected in each run. Standard d(C[5−8]G) and d(G[8−5]C) (1 pmol) were also injected into the column in separate LC-MS/MS runs with an identical experimental setup as the γ-irradiation mixture.

Scheme 2



Preparation of the Substrate for in Vitro Replication Studies. Recently, we discovered that Pyrex-filtered UV light irradiation of duplex DNA containing an adjacent guanine 5-bromocytosine can lead to the facile formation of G[8−5]C (Scheme 1) (*19*). To prepare an ODN substrate containing G[8−5]C for in vitro replication studies, we first isolated a 12mer lesion-bearing ODN by HPLC from the Pyrex-filtered UV irradiation of duplex ODNs containing a 5-bromocytosine as described in a recent paper (*19*). Briefly, an Apollo reverse-phase C18 column (4.6 × 250 mm, 5 μm in particle size, and 300 Å in pore size; Alltech Associates Inc., Deerfield, IL) was used. The flow rate was 0.8 mL/min, and a gradient of 35-min 6−12% acetonitrile in a 50 mM triethylammonium acetate (TEAA) buffer (pH 6.8) was used for the separation of the irradiation mixtures of the duplex ODNs.

The lesion-bearing substrate was then ligated with a 5′-phosphorylated 8mer ODN d(GATCCTAG) in the presence of a template ODN, d(CCGCTCCCTAGGATCATAGCG-CGCCAT), by using previously described procedures (*20*) (Scheme 2). The desired lesion-containing 20mer ODN was purified by 20% denaturing polyacrylamide gel electrophoresis (PAGE) and desalted by using an NAP-25 Column (Amersham Bioscience Co.) The two-step purification, i.e., HPLC purification of the 12mer and the denaturing PAGE purification of the 20mer, ensures the purity of the lesion-bearing substrate. The purity of the product was further confirmed by PAGE analysis (data not shown).

For primer extension under standing-start conditions, the 20mer lesion-containing template or normal template (10 nM) with GC in lieu of the G[8−5]C cross-link was annealed with a 5′-[^{32}P]-labeled 14mer primer (20 nM, Scheme 2), to which duplex mixture was then added individual dNTPs or a mixture of all four dNTPs as well as a DNA polymerase. The reaction was carried out at room temperature in a buffer containing 10 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, and 7.5 mM dithiothreitol for 60 min or for another time period as indicated. The reaction was then terminated by adding a 2-volume excess of formamide gel-loading buffer (80% formamide, 10 mM ethylenediaminetetraacetic acid at pH 8.0, 1 mg/mL xylene cyanol, and 1 mg/mL bromophenol

blue). Primer extension under running-start conditions was carried out in a similar way except that a shorter primer d(GCTAGGATCA) was used. The products were resolved on 20%, 1:19 cross-linked denaturing polyacrylamide gels containing 8 M urea. Gel-band intensities of the substrates and products were quantified by using a Typhoon 9410 Variable Mode Imager (Amersham Biosciences Co.) and ImageQuant 5.2 software (Amersham Biosciences Co.).

Steady-State Kinetics Measurement. The C-terminal catalytic core of yeast pol η, which was an N-terminal His6-tagged fusion protein (*21*), was used. This protein and exonuclease-free (exo$^-$) T7 DNA polymerase were kindly provided by Prof. John-Stephen A. Taylor at Washington University in St. Louis, MO. HIV reverse transcriptase was a kind gift from Prof. John Termini at the City of Hope, Duarte, CA.

We followed the previously described procedure (*22*, *23*) for the steady-state kinetic analyses. In this measurement, the primer-template complex (10 nM) was incubated with yeast pol η (2 nM) in the presence of individual dNTP at various concentrations. The reaction was carried out at room temperature for 10 or 30 min with the same reaction buffer as described above. The dNTP concentration and reaction time were optimized for different insertion reactions to allow for less than 20% primer extension (*23*). The V_{obs} or the observed rate of dNTP incorporation was plotted as a function of the dNTP concentration, and the apparent K_m and V_{max} steady-state kinetic parameters for the incorporation of both correct and incorrect nucleotides were determined by fitting the data with the Michaelis−Menten equation:

$$V_{obs} = \frac{V_{max} \times [\text{dNTP}]}{K_m + [\text{dNTP}]}$$

The efficiency of nucleotide incorporation was calculated from the ratio of V_{max}/K_m. The fidelity of nucleotide incorporation was then determined by the frequency of misincorporation (f_{inc}) with the following equation:

$$f_{inc} = \frac{(V_{max}/K_m)_{incorrect}}{(V_{max}/K_m)_{correct}}$$

RESULTS

Formation of the G[8−5]C Cross-Link Lesion in Duplex DNA upon γ Radiation. Recently, we discovered that the Pyrex-filtered UV light irradiation of dinucleoside monophosphate d(^{Br}CG) yielded the d(C[5−8]G) as the major product (*19*). We also demonstrated that the similar irradiation of a duplex dodecamer d(ATGGCG^{Br}CGCTAT)/d(ATAGCGCGCCAT) can lead to the facile formation of a cross-link lesion between the guanine at the sixth position and cytosine at the seventh position (*19*). Nuclease P1 and alkaline phosphatase digestion of d(ATGGCG[8−5]CGCTAT) gives rise to d(G[8−5]C). The availability of authentic cross-link lesion-containing d(C[5−8]G) and d(G[8−5]C) facilitates us to demonstrate the formation of this type of cross-link lesion in duplex DNA.

We irradiated a 16mer self-complementary duplex ODN d(CCGGCCGGCCGGCCGG) with a γ ray under anaerobic conditions and degraded the irradiation product with nuclease P1 and alkaline phosphatase. As demonstrated with the

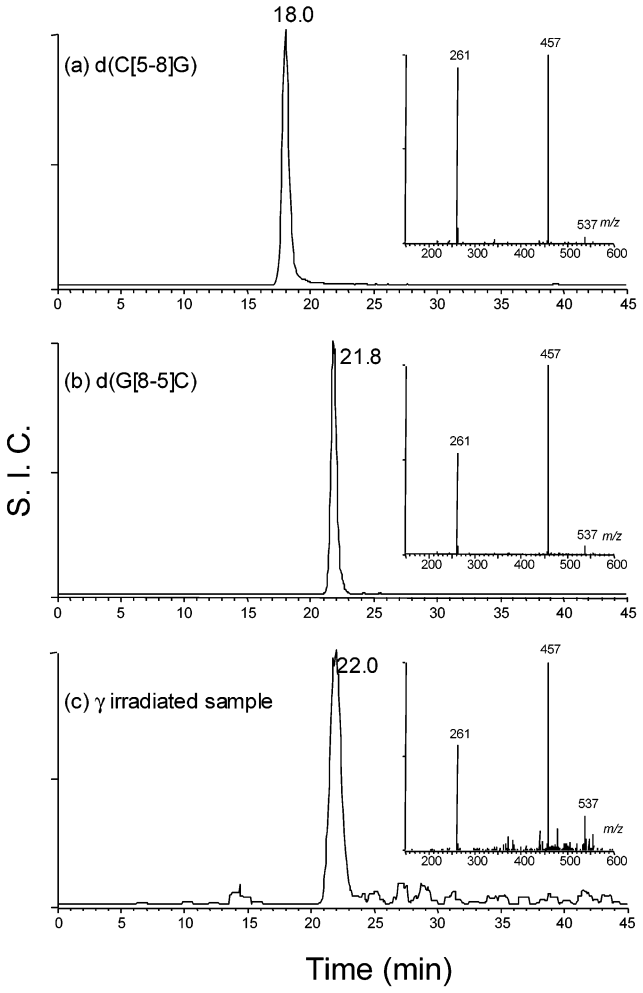


FIGURE 1: LC-MS/MS analysis of 1-pmol authentic d(C[5−8]G) (a), d(G[8−5]C) (b), and the digestion product of 2-nmol γ-irradiation (total dose = 40 Gy) mixture of duplex ODN d(CCGG)$_4$ under anaerobic conditions (c). The mass spectrometer was set to monitor the fragmentation of the $[M + H]^+$ ion (*m*/*z* 555) of the cross-link lesion (product-ion spectra are shown in the insets).

d(ATGGCG[8−5]CGCTAT), the cross-link lesion formed is liberated as a dinucleoside monophosphate under this enzymatic digestion condition (data not shown). The selected-ion chromatogram for the 555 → 261 transition (the ion of *m*/*z* 261 is the protonated ion of the cross-linked nucleobase moiety) showed a peak with a retention time of 22.0 min for the enzymatic digestion product of the γ-irradiation mixture (Figure 1c). This retention time is very close to the peak with the injection of the authentic cross-link lesion d(G[8−5]C) (Figure 1b). Moreover, the product-ion spectra are almost identical for the ion of *m*/*z* 555 for the standard d(G[8−5]C) and the γ-irradiation sample. The above results demonstrate that γ irradiation under anaerobic conditions can give rise to the cross-link lesion G[8−5]C in double-stranded ODNs. From the peak area in the selected-ion chromatograms (*m*/*z* 555 → 261) for the analyses of the authentic cross-link lesion and the γ-irradiation sample, we estimated that the yield is approximately 3×10^{-7} lesion at one GC site. The *G* value for the formation of the lesion was calculated to be 0.05, which is comparable to that for the formation of 8,5′-cyclo-2′-deoxyguanosine from the γ irradiation of 2′-deoxyguanosine (dG) in a N_2O-saturated solution (*G* value = 0.06) (*12*). The *G* value is approximately 4−5-fold lower

than that for the formation of 8-oxo-2′-deoxyguanosine (*G* value = 0.24) from the similar γ irradiation of dG (*12*). These results demonstrate that the formation of this type of intrastrand cross-link lesion during γ irradiation is a biologically significant process.

Interestingly, we were unable to detect the corresponding lesion at the CG site with the γ irradiation at this dose (40 Gy), indicating that the formation of the lesion at the CG site is much less facile than that at the GC site. This is consistent with the distances between the C5 carbon atom of cytosine and the C8 carbon atom of guanine at these two sites, which are 3.87 and 5.13 Å at the GC and CG sites, respectively (the distances were determined based on structures built in the software package Insight II using standard B-DNA geometry). These results are also in accordance with the more facile formation of the same cross-link lesion from the $G^{Br}C$ site than the ^{Br}CG site in duplex ODNs (*19*). In addition, previous work by Bellon and co-workers (*10*) demonstrated that, in dinucleoside monophosphates and duplex DNA, the intrastrand cross-link lesion with a covalent bond formed between the methyl carbon atom of thymine and the C8 carbon atom of adenine or guanine is produced more abundantly at the adjacent purine/thymine site than at the adjacent thymine/purine sites. We also demonstrated that in dinucleoside monophosphates the methyl radical of 5-methylcytosine leads to a more facile formation of an intrastrand cross-link lesion in the d(GmC) sequence than that in the d(mCG) sequence, where the methyl carbon atom of mC and the C8 carbon atom of guanine are covalently bonded (*11*). All of these observations have been correlated with a shorter distance for the formation of a covalent bond at an adjacent purine/pyrimidine than at a neighboring pyrimidine/purine site in duplex B-form DNA (*10*, *11*).

In Vitro Replication Studies with Yeast DNA Pol η. We next examined the mutagenic properties of G[8−5]C by carrying out primer extension assays with several DNA polymerases. We constructed a 20mer lesion-containing substrate by ligating d(ATGGCG[8−5]CGCTAT) with a 8mer ODN as described in the Experimental Procedures. Primer extension assays with the yeast DNA pol η, exo$^-$ T7 DNA polymerase, HIV reverse transcriptase, and exo$^-$ Klenow fragment of *Escherichia coli* DNA polymerase I show that yeast pol η is the only polymerase that can partially bypass G[8−5]C when all four dNTPs are present (Figure 2). The other three polymerases, however, stop synthesis mostly after the incorporation of the first nucleotide opposite the 3′-modified cytosine moiety for the lesion-bearing substrate (data shown in the Supporting Information).

Next, we determined the steady-state kinetic parameters for nucleotide incorporation opposite the damaged nucleobases and the undamaged bases in the control substrate by yeast pol η (Figure 3; Michaelis−Menten plots for one set of measurements are shown in the Supporting Information). When the data for the lesion-containing and the undamaged substrates are compared (Table 1), the efficiency for the incorporation of the correct nucleotide, dGMP, opposite the 3′-cytosine moiety is not drastically reduced by the presence of the lesion. Moreover, among the four nucleotides, the insertion of dGMP is the most efficient for both the lesion-containing and the undamaged substrates (Table 1).

The efficiency for the incorporation of the correct nucleotide, dCMP, opposite the 5′-guanine moiety, however, is markedly reduced ($\sim 10^3$ times difference) by the presence of the cross-link lesion (a 15mer primer with a dG at the 3′ terminus was used for this experiment; Table 1). The efficiencies for the insertion of dAMP and dGMP opposite the guanine portion of the cross-link lesion, however, are higher than those for the incorporation of these two nucleotides opposite the undamaged guanine. As a result, the fidelity of nucleotide incorporation is significantly reduced; the incorporation of dGMP or dAMP is slightly favored over the insertion of the correct nucleotide, dCMP, which is in turn more favored over the insertion of dTMP. This result indicates that the hydrogen-bonding properties of the guanine portion of the lesion are not conferred during nucleotide incorporation by yeast pol η.

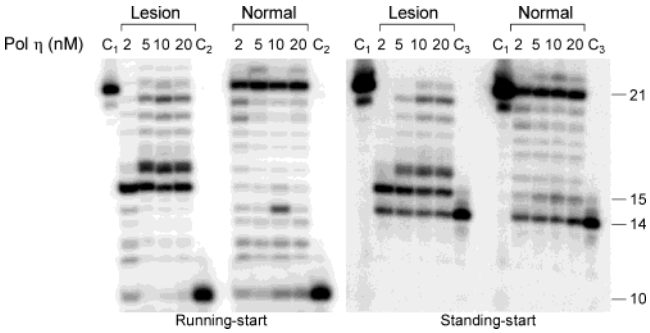


FIGURE 2: Primer extension assays for nucleotide incorporation opposite a G[8−5]C-bearing substrate and its control undamaged substrate with yeast pol η. Left, running-start experiments, in which 5′-[^{32}P]-labeled d(GCTAGGATCA) was used as the primer; right, standing-start experiments, in which 5′-[^{32}P]-labeled d(GCTAGGATCATAGC) was used as the primer. Pol η with the indicated concentrations was incubated with 10 nM substrate and 100 μM dNTPs at room temperature for 60 min. Controls are C_1, the full-length 20mer product; C_2, the running-start primer; and C_3, the standing-start primer.

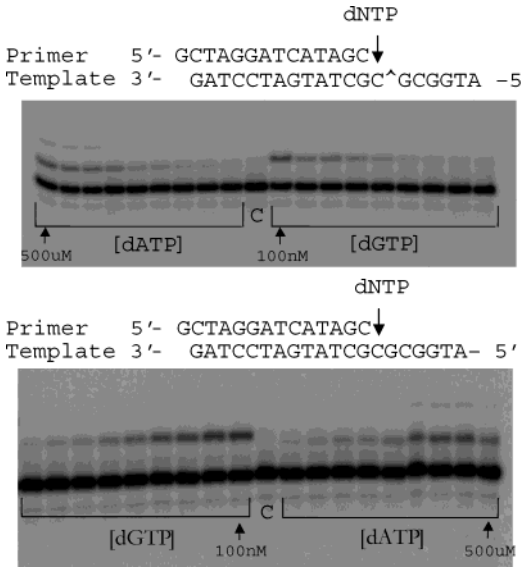


FIGURE 3: Steady-state kinetic measurements for dAMP and dGMP incorporation opposite the cytosine portion of G[8−5]C (top) or the cytosine at an undamaged GC site (bottom). Yeast pol η (2 nM) was incubated with 10 nM DNA substrate at room temperature for 10 min. The highest dNTP concentration was shown in the figure, and the concentration ratio of dNTP between the adjacent lanes was 0.6−1.0. The "C" indicates the control, where no dNTP was added.

Table 1: Fidelity of Nucleotide Incorporation by Yeast Pol η on an Undamaged Substrate and a G[8−5]C-Containing Substrate (Scheme 2) as Determined by Steady-State Kinetic Measurements[a]

dNTP	V_{max} (nM/min)	K_m (μM)	V_{max}/K_m	f_{inc}
Undamaged Substrate, 14mer Primer (5′-GCTAGGATCATAGC-3′)				
dATP	0.14 ± 0.01	96 ± 11	1.5×10^{-3}	3.0×10^{-4}
dGTP	0.50 ± 0.09	0.10 ± 0.03	5.0	1
dCTP	0.40 ± 0.02	40 ± 8	1.0×10^{-2}	2.0×10^{-3}
dTTP	0.47 ± 0.03	450 ± 50	1.0×10^{-3}	2.0×10^{-4}
Damaged Substrate, 14mer Primer				
dATP	0.22 ± 0.02	250 ± 40	8.8×10^{-4}	3.0×10^{-4}
dGTP	1.1 ± 0.2	0.38 ± 0.08	2.9	1
dCTP	0.024 ± 0.002	180 ± 50	1.3×10^{-4}	4.5×10^{-5}
dTTP	0.41 ± 0.05	790 ± 130	5.2×10^{-4}	1.8×10^{-4}
Damaged Substrate, 15mer-G Primer (5′-GCTAGGATCATAGCG-3′)				
dATP	0.30 ± 0.02	190 ± 20	1.6×10^{-3}	1.5
dGTP	0.42 ± 0.02	190 ± 20	2.2×10^{-3}	2.0
dCTP	0.26 ± 0.02	240 ± 30	1.1×10^{-3}	1
dTTP	0.088 ± 0.008	430 ± 80	2.1×10^{-4}	1.9×10^{-1}
Undamaged Substrate, 15mer-G Primer				
dATP	0.17 ± 0.01	210 ± 20	7.9×10^{-4}	8.7×10^{-4}
dGTP	0.10 ± 0.01	520 ± 70	1.8×10^{-4}	2.0×10^{-4}
dCTP	0.74 ± 0.09	0.82 ± 0.04	0.90	1
dTTP	0.43 ± 0.04	400 ± 70	1.1×10^{-3}	1.2×10^{-3}

[a] The K_m and V_{max} were average values based on three independent measurements.

DISCUSSION

Previous work by Box, Cadet, and us (*4*−*11*, *24*) demonstrated that several intrastrand cross-link lesions can be induced by γ irradiation or one-electron photooxidation. This is the first demonstration that G[8−5]C, which has a covalent bond formed between the C8 carbon atom of guanine and the C5 carbon atom of its 3′ adjacent cytosine, can form in duplex DNA by γ irradiation. Interestingly, the corresponding lesion at the CG site was not detectable under 40-Gy γ irradiation, and we attributed the difference in formation of the cross-link lesion at the CG and GC sites to the shorter distance between the C5 carbon atom of cytosine and the C8 carbon atom of guanine at the latter site.

Studies in the past have demonstrated that, depending on the lesion structure, translesion synthesis by eukaryotic DNA pol η can be either error-prone or error-free. Similar to the results for T[*c*,*s*]T (*15*, *25*), studies with the intrastrand cross-linked cisplatin-GG adduct show that yeast pol η can bypass the lesion and incorporates the correct nucleotides opposite both damaged bases (*26*−*28*). In contrast, human pol η efficiently bypasses a template 8-oxoguanine and incorporates a dAMP or dCMP opposite the lesion with similar efficiencies (*29*). In addition, the same polymerase partially bypasses a template (+)-*trans*-*anti*-benzo[*a*]pyrere-N^2-dG and predominantly incorporates a dAMP opposite the lesion (*29*). Our results with the G[8−5]C cross-link lesion show that the 3′-cytosine moiety does not compromise either the efficiency or the fidelity of nucleotide insertion by yeast pol η. The presence of the 5′-guanine portion of the lesion, however, drastically decreases the efficiency for the incorporation of the correct nucleotide, dCMP. The efficiencies for the insertion of the two purine nucleotides, dGMP and dAMP, were slightly higher than that for the insertion of the correct nucleotide, dCMP. The much lower efficiency of nucleotide incorporation opposite the damaged 5′-guanine moiety than the damaged 3′-cytosine moiety is consistent with that Klenow fragment of *E. coli* DNA polymerase I, exo$^-$ T7 DNA polymerase, and HIV reverse transcriptase stop synthesis after the insertion of the first nucleotide across the 3′-cytosine moiety of the lesion (data shown in the Supporting Information).

It is interesting that the 5′-guanine and 3′-cytosine portions of the cross-link lesions exhibit distinct behaviors upon nucleotide incorporation by yeast pol η; we attributed this to the unique structure of the cross-link lesion. The high-resolution X-ray or NMR structure of duplex DNA containing the lesion will offer us insight into the impact of the lesion on the double-helix structure DNA. Before such a structure is available, we optimized the gas-phase structure of the dinucleoside monophosphate d(G[8−5]C) by using the semiempirical PM3 method with Spartan 5.1.3 (Wave functions Inc., Irvine, CA). It turns out that the covalent bond formed between the C8 carbon atom of guanine and the C5 carbon atom of cytosine significantly distorts the structure of the dinucleoside monophosphate. Although the glycosidic linkage of the 3′ nucleoside remains in the anti conformation, that of the 5′ nucleoside assumes a syn conformation exclusively (structure shown in the Supporting Information). Even though this result is only based on the dinucleoside monophosphate, we reason that a similar structural distortion is expected in duplex DNA. This may explain why the hydrogen-bonding property of the 5′-guanine portion is not conferred during nucleotide insertion by the yeast pol η. Under such circumstances, purine nucleotides can conceivably be incorporated more efficiently than pyrimidine nucleotides because the former nucleotides have a stronger stacking interaction with the 3′ nucleotide of the primer than the latter nucleotides (*30*). In addition, recent studies by Kool and Matray (*31*) showed that steric complementarity is also important in the fidelity of nucleotide insertion by DNA polymerases. Therefore, the more favorable incorporation of purine nucleotides might also be attributed to a better steric match of purine than pyrimidine bases with the 5′-guanine portion of the lesion. Moreover, the significant structural distortion of the dinucleoside monophosphate implicates that the lesion may distort the double-helix structure of DNA. The lesion, therefore, might be a good substrate for the nucleotide-excision repair pathway (*32*).

Given the steady-state kinetic parameters discussed above and that pol η is the only eukaryotic DNA polymerase that can efficiently bypass the structurally related T[*c*,*s*]T and cisplatin-GG adduct, it may suggest that the oxidative cross-link lesion plays an important role in γ-radiation-induced mutagenesis.

When the translesion synthesis of T[*c*,*s*]T-bearing substrate by yeast pol η is compared to our results, it showed that the synthesis across the 3′-cytosine portion of the d(G[8−5]C) is very similar to the bypass of the T[*c*,*s*]T-bearing substrate (*15*, *17*); i.e., the efficiencies for nucleotide incorporation opposite the damaged and undamaged bases are comparable. In contrast to the observation with the bypass of the T[*c*,*s*]T (*15*, *17*), our results showed that the efficiency for nucleotide insertion opposite the 5′-guanine moiety of G[8−5]C is significantly lower than that for the corresponding nucleotide incorporation across an undamaged base. This result may implicate that other polymerase(s) might also be involved in the bypass of this lesion in vivo. In this respect, it has

been demonstrated previously that eukaryotic DNA polymerases ξ and ι can act together in bypassing several lesions (*33*). Likewise, yeast polymerases η and ξ together are able to bypass an abasic site, whereas either of the two polymerases cannot bypass the lesion on its own (*29*). Therefore, it is conceivable that a combination of pol η with another polymerase might be important in the efficient bypass of both the cytosine and guanine moieties of the cross-link lesion in vivo.

ACKNOWLEDGMENT

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SUPPORTING INFORMATION AVAILABLE

Primer extension assays in Figures S1 and S2, Michaelis−Menten plots in Figures S3−S6, and the structure of d(G[8−5]C) in Figure S7. This material is available free of charge via the Internet at http://pubs.acs.org.

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