Repair of DNA Containing Fapy·dG and Its β-C-Nucleoside Analogue by Formamidopyrimidine DNA Glycosylase and MutY†

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ABSTRACT: Fapy·dG is produced in DNA as a result of oxidative stress. Under some conditions Fapy·dG is formed in greater yields than 8-oxodG from a common chemical precursor. Recently, Fapy·dG and its C-nucleoside analogue were incorporated in chemically synthesized oligonucleotides at defined sites. Like 8-oxodG, Fapy·dG instructs DNA polymerase to misincorporate dA opposite it in vitro. The interactions of DNA containing Fapy·dG or the nonhydrolyzable analogue with Fpg and MutY are described. Fpg excises Fapy·dG (K_M = 2.0 nM, k_{cat} = 0.14 min^{-1}) opposite dC ~17-fold more efficiently than when mispaired with dA, which is misinserted by DNA polymerase in vitro. Fpg also prefers to bind duplexes containing Fapy·dG·dC or β-C-Fapy·dG·dC compared to those in which the lesion is opposite dA. MutY incises dA when it is opposite Fapy·dG and strongly binds duplexes containing the lesion or β-C-Fapy·dG. Incision from Fapy·dG·dA is faster than from dG·dA mispairs but slower than from DNA containing 8-oxodG opposite dA. These data demonstrate that Fapy·dG closely resembles the interactions of 8-oxodG with two members of the GO repair pathway in vitro. The similar effects of Fapy·dG and 8-oxodG on DNA polymerase and repair enzymes in vitro raise the question as to whether Fapy·dG elicits similar effects in vivo.

DNA is constantly subjected to oxidative stress from both exogenous (e.g., UV light, γ-radiolysis) and endogenous (i.e., aerobic metabolism, lipid peroxidation) sources. Oxidative stress creates a variety of base lesions, which are often cytotoxic or mutagenic. The accumulation of lesions in DNA is believed to play a role in aging and various diseases, including cancer (*1*−*6*). To protect the genome from oxidative stress, an elaborate set of enzymes have evolved to repair damaged DNA. One class of these, the base excision repair (BER)[1] proteins, remove the aberrant base through a glycosylase activity (*7*, *8*). This is followed by a lyase activity possessed either by the BER enzyme or by a distinct AP endonuclease, generating a single strand break. DNA repair is completed by polymerase filling in the gap and ligase sealing the nick. This process becomes more complicated if the BER enzyme detects a lesion after DNA polymerase has bypassed the damaged site. If a polymerase is unfaithful when bypassing a lesion, the repair enzyme must distinguish between the correct and a misincorporated nucleotide opposite the lesion if it is to protect the genome from mutagenesis. Removal of the damaged base from a promutagenic base pair results in a permanent mutation. For this reason, mismatch-repair proteins exist to remove the misincorporated nucleotide before the lesion is repaired. We wish to describe the in vitro characterization of BER repair of an important DNA lesion, Fapy·dG and its β-C-nucleoside analogue.

Of the base lesions formed as a result of oxidative stress, 8-oxoguanine is generally considered to be the most deleterious in terms of its high rate of formation and mutagenicity (*9*, *10*). However, the 8-oxopurines (8-oxodA and 8-oxodG) are formed in competition with the formamidopyrimidines (Fapy·dA and Fapy·dG) from a common intermediate (Scheme 1) (*11*, *12*). The endogenous cellular levels and yields of these lesions following exposure to oxidative stress in vitro or in vivo are controversial and are dependent upon a number of factors (*13*−*17*). The relative yields of the 8-oxopurine and formamidopyrimidine lesions formed when chromatin is exposed to γ-radiolysis vary as a function of oxygen and are consistent with mechanisms proposed to describe their formation (*12*). Measurements of these lesions in vivo are more variable. Fapy·dG is present in greater amounts than 8-oxodG in human leukemia cells exposed to ionizing radiation (*18*). However, the relative endogenous levels of Fapy·dG and 8-oxodG depended upon the type of cells examined (*17*, *19*). The availability of chemically synthesized oligonucleotides containing the 8-oxopurines at defined sites has greatly facilitated extensive studies of these important lesions (*20*). In contrast, little was known about the mutagenicity of the formamidopyrimidines until recently,

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[1] Abbreviations: Fapy·dG, 2′-deoxyguanosine formamidopyrimidine; Fapy·dA, 2′-deoxyadenosine formamidopyrimidine; 8-oxodG, 8-oxodeoxyguanosine; 8-oxodA, 8-oxodeoxyadenosine; Fpg, formamidopyrimidine DNA glycosylase; BER, base excision repair; AP, abasic site.

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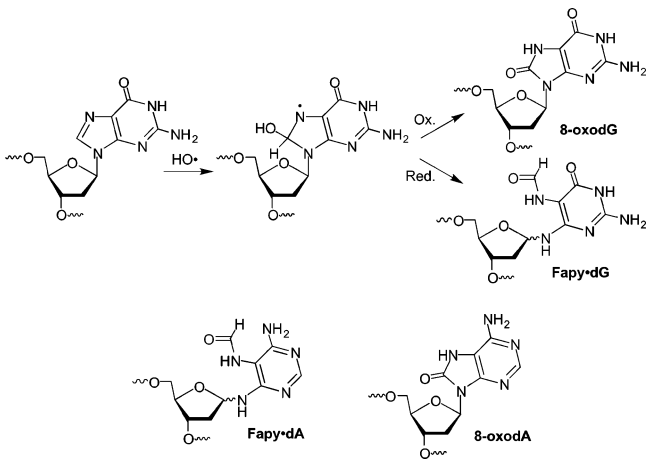
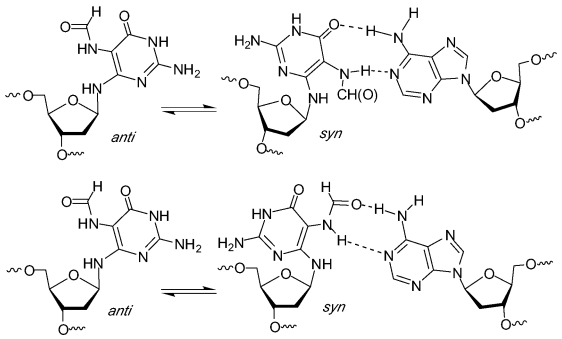
Scheme 1: Fapy·dG and 8-OxodG Arise from a Common Intermediate



Scheme 2: Proposed Hydrogen-Bonding Arrangement between Fapy·dG and dA



because a method for preparing oligonucleotides containing Fapy·dA and Fapy·dG site specifically was unavailable (*21*−*23*). Conclusions regarding the effects of Fapy·dG were inferred from studies involving *N*7-MeFapy·dG, an analogue that can be incorporated at a defined site in an oligonucleotide. On the basis of experiments showing that *N*7-MeFapy·dG is a significant block to replication, the formamidopyrimidines were believed by some researchers to be nonmutagenic and not given much attention (*24*−*26*). In contrast, Fapy·dG has been of greater interest to those researchers who have reported the relatively efficient excision of this lesion by many of the same BER enzymes that recognize 8-oxodG (*27*−*31*). This opinion is validated by a recent study on an oligonucleotide containing Fapy·dG site specifically, which has shown that polymerase (Klenow exo$^-$) efficiently bypasses the lesion and misinserts dA (*32*). Klenow exo$^-$ incorporates dA opposite Fapy·dG and extends past the mismatch 80 million times more efficiently than when the template strand is composed of native nucleotides. Furthermore, the level of dA misincorporation opposite Fapy·dG by Klenow exo$^-$ is comparable to that observed opposite 8-oxodG (*33*). Structural information describing the pairing between Fapy·dG and dA is not available, but we hypothesize that the syn conformation of the lesion provides a thymidine-like hydrogen-bonding pattern to dA (Scheme 2) (*32*).

These in vitro experiments suggest that Fapy·dG is a premutagenic lesion. Hence, its proper repair is critical in order to maintain the integrity of the genome. Given the similarity in structure of Fapy·dG and 8-oxodG, the lesions are excised by a number of the same BER enzymes. 8-OxodG repair is well characterized and is achieved through the synergistic effects of three BER enzymes known as the GO repair system (*7*, *34*). Two of these enzymes act on the lesion in DNA and are the subject of this study. Formamidopyrimidine DNA glycosylase (Fpg, MutM) is a bifunctional glycosylase/AP endonuclease, which excises 8-oxodG when the lesion is base paired to dC. Repair of an 8-oxodG·dA base pair is inefficient by Fpg (*35*, *36*). This prevents the formation of G → T transversions by allowing MutY to repair the mismatch. MutY is an adenine glycosylase, which incises dA from 8-oxodG·dA base pairs (*37*, *38*). In vivo assays have confirmed the importance of these enzymes in protecting cells from 8-oxodG since the mutation frequency at the site of the lesion greatly increases in cells deficient in Fpg and/or MutY (*39*, *40*). The third enzyme that makes up the GO system is MutT, a nucleoside triphosphate pyrophosphohydrolase, which is believed to cleanse the nucleotide pool by hydrolyzing 8-oxodGTP to 8-oxodGMP (*41*). In addition to these three enzymes, Nei (EndoVIII) has recently been proposed as a fourth component of the GO repair system (*42*). *Escherichia coli* cells deficient in Fpg, MutY, and Nei exhibit a higher level of spontaneous G → T transversions than Fpg, MutY double mutants (*42*). In vitro assays revealed that Nei excises 8-oxodG from duplexes containing an 8-oxodG·dA base pair (*9*). Although this seems to undermine the protection afforded by MutY, Nei is believed to repair 8-oxodG·dA mismatches resulting from misincorporation of 8-oxodGTP (*9*, *10*).

EXPERIMENTAL PROCEDURES

Materials and General Methods. Oligonucleotides were prepared on an Applied Biosystems Inc. 394 DNA synthesizer. Commercially available DNA synthesis reagents were obtained from Glen Research Inc. Oligonucleotides containing Fapy·dG or β-C-Fapy·dG were prepared as previously described (*21*−*23*). All others were synthesized using standard protocols. DNA manipulations were carried out using standard procedures. T4 polynucleotide kinase was obtained from New England Biolabs. Radionuclides were obtained from Amersham Pharmacia. Fpg was obtained from Trevigen.

Excision of Fapy·dG by Fpg. A solution of Fpg (final concentration = 1.755 nM, 1×10^{-4} unit) and **1a** in which the strand containing Fapy·dG was labeled with ^{32}P at its 5′-terminus (final concentration = 0.5−10 nM) was incubated in 1× Rec buffer (10 mM HEPES−KOH, pH 7.4, 100 mM KCl, 10 mM EDTA, 0.1 mg/mL BSA) at 25 °C. After 3 min, the reaction was quenched with formamide loading buffer (20 μL), denatured (90 °C, 1 min), and immediately cooled to 0 °C. The cleavage products were separated by 20% denaturing PAGE. Each substrate concentration was run in triplicate. The measured velocities were plotted versus the substrate concentration, and K_M and k_{cat} values were determined using a nonlinear curve fitting procedure (Origin 6.1). Each value is the average of three independent experiments. Experiments with duplex **1b** were performed as described above except that the reactions were quenched after 5 min.

Active Site Titration of MutY (38). A solution of 20 nM **2** in 1× MutY reaction buffer (20 mM Tris-HCl, pH 7.5, 10

mM EDTA, 0.1 mg/mL BSA) was equilibrated at 37 °C for 10 min. MutY was diluted in 20 mM Tris-HCl, pH 7.5, 10 mM EDTA, and 20% glycerol before being added to the DNA solution (final concentration = 1.3, 2.6, or 3.9 nM, based on the Bradford assay). The solution was incubated at 37 °C. Aliquots (10 μL) were removed over the course of 1 h and added to 2 μL of 1 M NaOH. The aliquots were heated at 90 °C for 5 min to cleave the abasic site and denatured (10 μL of formamide, 90 °C for 3 min, and immediate cooling to 0 °C). Cleavage products were separated by 20% denaturing PAGE. The data were plotted as the concentration of product versus time. The data in the plateau region were fit to a straight line, and the *y*-intercept obtained corresponds to the amount of active enzyme. The active site concentration was calculated as a percentage of the total protein concentration, based on the Bradford assay.

Single Turnover Kinetics for Glycosylase Activity of MutY. Experiments were performed as described for the active site titration. The corrected concentration of MutY used was 30 nM, but the DNA concentration remained 20 nM. Aliquots were taken over the course of 1 h for **1b** and 1.5 h for **1d**. The data were plotted as the concentration of product versus time and fit to eq 1, where A_0 is the amplitude of the exponential curve.

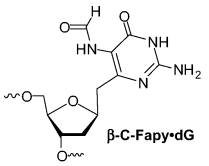
$$[P]_t = A_0[1 - \exp(-kt)] \quad (1)$$

Multiple Turnovers of MutY. These experiments were performed as described above. MutY was used at an active site concentration of 2.7 nM, and the concentration of DNA was 50 nM. Aliquots were taken periodically over 2 h.

Gel Shift Assays of DNA Binding to Fpg and MutY. Fpg was diluted in 20 mM Tris-HCl, pH 7.8, 100 mM NaCl, 1 mM EDTA, 50% (v/v) glycerol, and 1 mM DTT. Fpg was added at varying concentrations to a solution (20 μL) of 50 pM DNA and 10% (v/v) glycerol in 1× Rec buffer (10 mM HEPES−KOH, pH 7.4, 100 mM KCl, 10 mM EDTA, and 0.1 mg/mL BSA). The solution was incubated at room temperature for 10−20 min. Nondenaturing glycerol loading buffer (2 μL, 30% glycerol, 0.5× TBE, 1% xylene cylanol, and 1% bromophenol blue) was added before the samples were loaded on a 6% nondenaturing gel (1.5 mm thick) run at 500 V for 2 h at 4 °C. Fpg concentrations were varied from 0 to 100 nM for **1a**, 0 to 2 μM for **1b**, 0 to 300 nM for **1c**, and 0 to 2 μM for **1d**. Binding at each concentration was measured in triplicate. The percent bound was plotted versus the enzyme concentration on a semilog plot and fit sigmoidally (Origin 6.1). The K_D is defined as the enzyme concentration at which 50% is bound and is the average of three independent experiments. Binding of DNA by MutY was determined in a similar manner, except that MutY was diluted in 20 mM Tris-HCl (pH 7.5), 10 mM EDTA, and 20% glycerol before being added to a solution DNA and 10% (v/v) glycerol in 20 mM Tris-HCl, pH 7.5, 10 mM EDTA, and 0.1 mg/mL BSA. The concentrations of MutY used were 0−100 nM for **1b** and 0−1 μM for **1d**.

RESULTS

Interaction of Fapy·dG with Fpg. Klenow exo$^-$ incorporates dA opposite Fapy·dG more readily than it does opposite dG in an otherwise identical template (*32*). These data suggest that DNA-containing Fapy·dG·dC and Fapy·dG·dA base pairs are biologically relevant. The efficiency of repair of these duplexes by Fpg was measured through the formation of direct strand breaks due to the enzyme's β,δ-lyase activity. Michaelis−Menten kinetic constants for the excision of Fapy·dG by Fpg were obtained through a nonlinear least-squares analysis of a plot of the initial velocity versus substrate (DNA) concentration (Figure 1). In contrast to the repair of Fapy·dA (*43*), Fpg was selective when excising Fapy·dG opposite either dC or dA. Excision of the lesion from a Fapy·dG·dC (**1a**) base pair was preferred to cleavage of Fapy·dG opposite dA (**1b**) by a factor of 17 (Table 1). This is almost identical to the selectivity observed in the reactivity of Fpg with 8-oxodG base pairs (*36*).



5'-d(AGG CGT TCA ACG TGC AGT XAC AGC ACG TCC CAT GGT)
3'-d(TCC GCA AGT TGC ACG TCA YTG TCG TGC AGG GTA CCA)

1a: X = Fapy·dG, Y = dC
1b: X = Fapy·dG, Y = dA
1c: X = β-C-Fapy·dG, Y = dC
1d: X = β-C-Fapy·dG, Y = dA

5'-d(AGG CGT TCA ACG GCT CTG XGT CGT ACG TCC CAT GGT)
3'-d(TCC GCA AGT TGC CGA GAC ACA GCA TGC AGG GTA CCA)

2: X = 8-oxodG

This selectivity for a translesional dC is further reflected in the binding of duplexes containing Fapy·dG by Fpg. Gel shift experiments revealed that Fpg binds duplexes containing Fapy·dG·dC base pairs 20 times tighter than when the lesion is opposite dA (Figure 2 and Table 2). Again, this is mirrored in the recognition of 8-oxodG by Fpg, where binding strength is dependent upon the translesional base (*36*).

Recognition of β-C-Fapy·dG by Fpg. Although β-C-Fapy·dG is not a substrate for Fpg due to its nonhydrolyzable glycosidic bond, the enzyme tightly binds to duplexes containing the analogue (Figure 3, Table 2). Fpg binds duplexes containing the C-nucleoside analogue only slightly more weakly than the true formamidopyrimidine when the lesion is opposite dC. This suggests that, like β-C-Fapy·dA, β-C-Fapy·dG is a strong inhibitor of Fpg (*43*). Furthermore, as is the case with Fapy·dG, Fpg is selective in its binding to duplexes containing β-C-Fapy·dG opposite the native nucleotides. While duplexes containing a β-C-Fapy·dG·dC (**1c**) base pair were bound tightly, only weak binding was observed between Fpg and duplexes containing a β-C-Fapy·dG·dA (**1d**) base pair. Complete binding of the β-C-Fapy·dG·dA duplex (**1d**) was not observed at Fpg concentrations up to 2 μM.

Cleavage of dA Opposite Fapy·dG and β-C-Fapy·dG by MutY. The ability of the GO system to protect against Fapy·dG-induced mutations was examined further in vitro by determining the ability of MutY to repair a Fapy·dG·dA base pair. Since MutY does not possess lyase activity, duplexes were treated with NaOH following reaction with the enzyme to yield single strand breaks. When dA is incised opposite 8-oxodG, MutY exhibits strong product inhibition and remains bound to the deglycosylated product (*38*). The same behavior was observed during the repair of Fapy·dG·dA

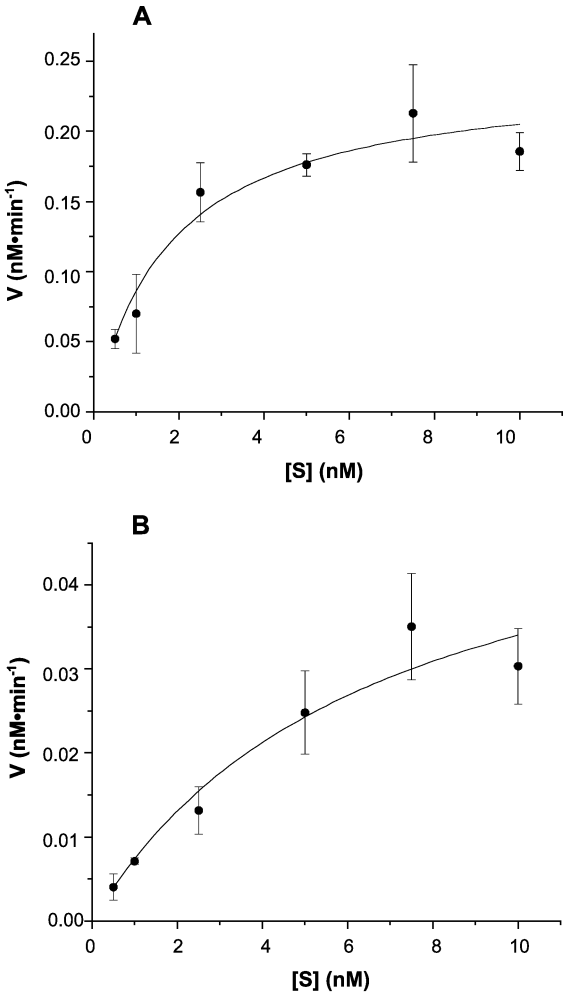


FIGURE 1: Fapy·dG excision by Fpg when the lesion is opposite (A) dC or (B) dA.

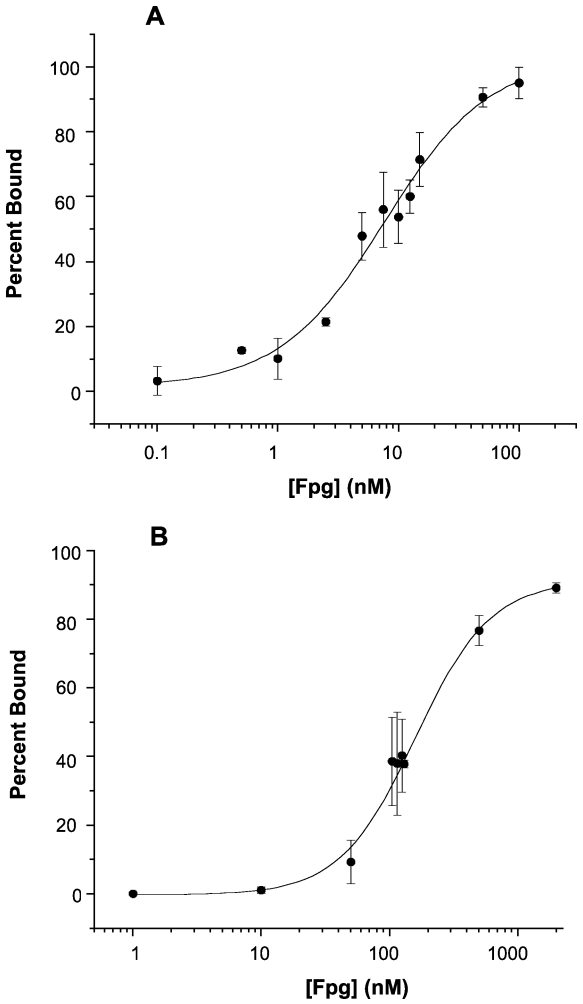


FIGURE 2: Gel shift analysis of Fpg binding to duplex DNA containing (A) Fapy·dG·dC or (B) Fapy·dG·dA base pairs.

Table 1: Kinetic Parameters for the Excision of Fapy·dG or 8-OxodG from DNA by Fpg[a]

base pair	k_{cat} (min^{-1})	K_M (nM)	$k_{cat}/K_M \times 10^{-3}$ (min^{-1}·nM^{-1})
Fapy·dG·dC	0.14 ± 0.004	2.0 ± 0.4	70.0 ± 14.9
Fapy·dG·dA	0.02 ± 0.01	4.7 ± 1.9	4.3 ± 2.9
8-oxodG·dC[b]	0.13 ± 0.01	14 ± 6	9.3 ± 3.9
8-oxodG·dA[b]	0.10 ± 0.02	190 ± 74	0.5 ± 0.3

[a] Reactions carried out at 298 K. [b] Data taken from ref *36*.

Table 2: Dissociation Constants for Fpg Binding to Duplex DNA Containing a Single Lesion[a]

base pair	K_D (nM)
Fapy·dG·dC	6.3 ± 1.5
Fapy·dG·dA	128.5 ± 27.3
β-C-Fapy·dG·dC	11.5 ± 1.9
β-C-Fapy·dG·dA	N/D
8-oxodG·dC[b]	8.9 ± 2.1
8-oxodG·dA[b]	340 ± 29

[a] Binding carried out at 298 K. [b] Data taken from ref *36*.

DNA (data not shown). Therefore, the rate of cleavage was measured using single turnover conditions. The amount of product formed was plotted as a function of time (Figure 4). Duplex DNA containing a Fapy·dG·dA (**1b**) base pair is a good substrate for MutY, showing a k_{obs} of 4.2 min^{-1} (Table 3). This is faster than the repair of a duplex containing a dG·dA mispair by approximately 2.5-fold but slower than the rate of deglycosylation of adenine opposite 8-oxodG (*38*). When β-C-Fapy·dG is present, MutY recognizes the mispair and successfully removes adenine. However, dA is cleaved at a rate that is approximately 50-fold slower than when the true lesion is present in the duplex.

Binding of MutY to Fapy·dG·dA and β-C-Fapy·dG·dA Base Pairs. MutY exhibits tight binding to duplexes containing Fapy·dG and β-C-Fapy·dG (Figure 5). A duplex containing a Fapy·dG·dA base pair was bound significantly more tightly than an analogous duplex containing β-C-Fapy·dG. Single turnover kinetics (Table 3) indicate that in the former the complexes observed undoubtedly contain the lesion opposite the AP site, whereas in experiments using the C-nucleoside a mixture of starting material and product should be present. The binding of MutY to duplexes containing a Fapy·dG·dA base pair was comparable to when 8-oxodG was present opposite dA (*44*). Both Fapy·dG and β-C-Fapy·dG were bound more tightly by MutY than a duplex containing native nucleotides. Moreover, the binding between MutY and Fapy·dG (**1b**) was ~175-fold stronger than DNA containing Fapy·dA, indicating that the enzyme is selective with respect to what lesions it recognizes (*43*).

DISCUSSION

Through studies involving randomly generated DNA lesions, it is well established that Fapy·dG is a substrate for

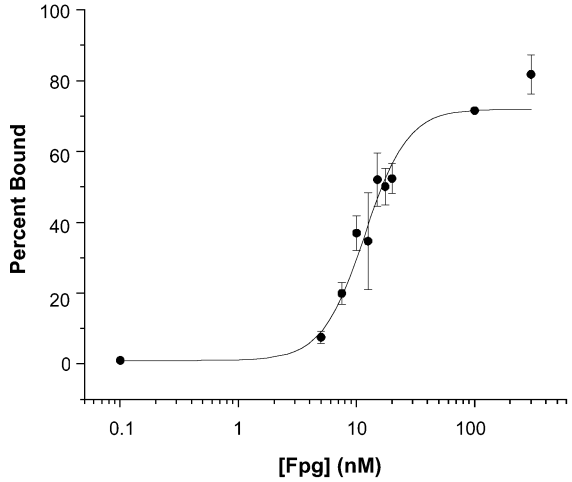


FIGURE 3: Gel shift analysis of Fpg binding to duplex DNA containing a β-C-Fapy·dG·dC base pair.

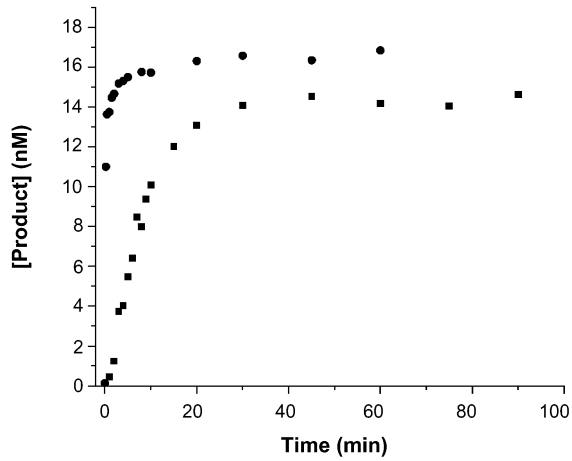


FIGURE 4: Single turnover kinetics of dA incision by MutY from DNA containing Fapy·dG·dA (●) or β-C-Fapy·dG·dA (■) base pairs.

Table 3: Binding Strength and Reactivity of MutY for Duplex DNA Containing a Translesional Adenine[a]

base pair	k_{obs} (min^{-1})	K_D (nM)
Fapy·dG·dA	4.20 ± 0.46	0.25 ± 0.01
β-C-Fapy·dG·dA	0.09 ± 0.01	5.41 ± 1.22
8-oxodG·dA[b]	16 ± 2	0.2 ± 0.1
dG·dA[b]	1.6 ± 0.2	35 ± 7

[a] Binding carried out at 315 K. [b] Data taken from refs *38*, *44*, and *45*.

Fpg (*27*, *46*). However, due to the limitations in studying randomly generated lesions, the absolute efficiency of this repair was not known. Furthermore, the effect of the translesional base on Fpg excision of Fapy·dG could not be examined until oligonucleotides containing formamidopyrimidines site specifically were prepared (*21*−*23*). The studies reported here reveal that Fpg exhibits high selectivity when removing Fapy·dG from duplex DNA opposite either dC or dA. Excision of Fapy·dG opposite the other two nucleotides was not examined since these base pairs are not expected to be biologically relevant based on in vitro polymerase fidelity assays (*32*). Although the lesions are present in different sequences, these data reveal that Fpg exhibits comparable discrimination between duplexes containing Fapy·dG opposite dA and dC as it does 8-oxodG (Table 1) (*36*). The

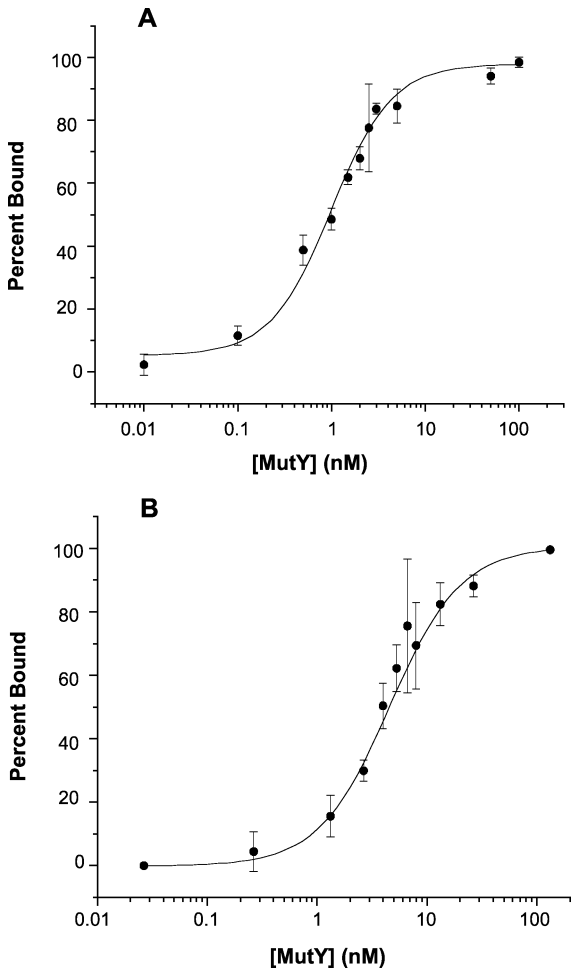


FIGURE 5: Gel shift analysis of MutY binding to duplex DNA containing (A) Fapy·dG·dA or (B) β-C-Fapy·dG·dA base pairs.

lesion is removed 17 times more efficiently when it is base paired to the correct nucleotide (dC) than when dA is present opposite the lesion. This is in contrast to the repair of Fapy·dA where the translesional base has no effect on the efficiency of repair by Fpg (*43*). The selectivity observed with 8-oxodG and Fapy·dG likely arose as a means of protecting the genome from mutagenesis. If a Fapy·dG·dA base pair is repaired by Fpg, the only source of genetic information would be the misincorporated dA, which would yield a G → T transversion.

Fpg exhibits comparable selectivity with respect to the identity of the nucleotide opposite Fapy·dG when binding to DNA. Twentyfold tighter binding to Fapy·dG is observed when the lesion is placed in a duplex opposite dC (**1a**) than when it is base paired to dA (**1b**). This suggests that the selectivity observed in Fpg's reactivity with Fapy·dG is due in part to preferential recognition of the lesion when it is opposite dC. This selectivity is mimicked by the recognition of the C-nucleoside analogue of Fapy·dG by Fpg. Significantly tighter binding is observed when dC is the translesional base. In fact, a K_D could not be determined for the β-C-Fapy·dG·dA base pair since incomplete binding was observed up to 2 μM Fpg. The fact that the β-C-nucleoside is recognized by Fpg suggests that this configuration of Fapy·dG is recognized in DNA. The binding of Fpg also suggests that β-C-Fapy·dG may be a useful BER inhibitor.

Fpg's reluctance to excise Fapy·dG when it is opposite dA suggests that the GO system evolved to protect against

the potential mutagenic effects of this lesion, which is necessary based upon the polymerase's infidelity in vitro (*32*). This proposal is affirmed by the interaction of MutY with Fapy·dG·dA DNA. MutY incises dA opposite the formamidopyrimidine faster than in a dG·dA mispair and shows the same tight binding to the abasic site product. These data resemble the behavior exhibited by MutY when it encounters an 8-oxodG·dA base pair, in which tight binding is believed to protect the abasic site from premature cleavage (*38*). Recently, it was shown that MutY bound to DNA containing a dG·AP base pair is displaced by some endonucleases but that the respective complex containing 8-oxodG is not (*47*). It is not known at this time whether MutY protects the Fapy·dG·AP in a similar fashion, but the above results lead us to predict that Fapy·dG will copy 8-oxodG's behavior in this respect as well.

CONCLUSIONS

These data show that two of the GO pathway BER enzymes protect against possible mutations that may result from unfaithful replication of templates containing Fapy·dG. In contrast to Fpg's recognition of Fapy·dA, the BER enzyme discriminates between nonmutagenic and promutagenic Fapy·dG base pairs (*43*). Another difference between the Fapy lesions is the ability of MutY to incise dA when it is opposite Fapy·dG. The interactions of Fapy·dG containing DNA with Fpg and MutY resemble those of 8-oxodG far more than they do Fapy·dA (*36*, *37*). The effect of Fapy·dG on polymerase fidelity and the in vitro results presented above suggest that Fapy·dG is deleterious to the cell (*32*). It is tempting to extrapolate the similarities in the in vitro repair of Fapy·dG and 8-oxodG by Fpg and MutY to what happens in *E. coli*. The role of the GO repair system in vivo is seen in the case of 8-oxodG where the mutagenicity of the lesion increases up to 8-fold when the lesion is placed in either Fpg- or MutY-deficient cells. When both BER enzymes are removed, the mutagenicity of 8-oxodG increases another 1.4-fold over the sum of the individual mutation frequencies, indicating the synergistic relationship between the enzymes (*39*). In vivo analysis of the effects of Fapy·dG in a repair-dependent fashion have not yet been completed, but it would not be surprising if the in vitro effects carry over into *E. coli*.

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