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Excision of formamidopyrimidine lesions by endonucleases III and VIII is not a major DNA repair pathway in *Escherichia coli*

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ABSTRACT

Proper maintenance of the genome is of great importance. Consequently, damaged nucleotides are repaired through redundant pathways. We considered whether the genome is protected from formamidopyrimidine nucleosides (Fapy•dA, Fapy•dG) via a pathway distinct from the *Escherichia coli* guanine oxidation system. The formamidopyrimidines are produced in significant quantities in DNA as a result of oxidative stress and are efficiently excised by formamidopyrimidine DNA glycosylase. Previous reports suggest that the formamidopyrimidine nucleosides are substrates for endonucleases III and VIII, enzymes that are typically associated with pyrimidine lesion repair in *E.coli*. We investigated the possibility that Endo III and/or Endo VIII play a role in formamidopyrimidine nucleoside repair by examining Fapy•dA and Fapy•dG excision opposite all four native 2′-deoxyribonucleotides. Endo VIII excises both lesions more efficiently than does Endo III, but the enzymes exhibit similar selectivity with respect to their action on duplexes containing the formamidopyrimidines opposite native deoxyribonucleotides. Fapy•dA is removed more rapidly than Fapy•dG, and duplexes containing purine nucleotides opposite the lesions are superior substrates compared with those containing formamidopyrimidine–pyrimidine base pairs. This dependence upon opposing nucleotide indicates that Endo III and Endo VIII do not serve as back up enzymes to formamidopyrimidine DNA glycosylase in the repair of formamidopyrimidines. When considered in conjunction with cellular studies [J. O. Blaisdell, Z. Hatahet and S. S. Wallace (1999) *J. Bacteriol.*, 181, 6396–6402], these results also suggest that Endo III and Endo VIII do not protect *E.coli* against possible mutations attributable to formamidopyrimidine lesions.

INTRODUCTION

Proper repair of damaged DNA is critical to maintaining the integrity of the genome. Cells have developed multiple approaches for repairing DNA damage and there is increasing evidence for overlap between these repair systems (1). For instance, nucleotide incision repair complements base excision repair (BER) for some lesions (2,3). Effective DNA repair requires the enzyme to discriminate among substrates based upon opposing nucleotide. Excision of a lesion within a promutagenic base pair leaves only the incorrect nucleotide to direct native nucleotide incorporation and will result in a mutation. We examined the feasibility that BER enzymes found in *Escherichia coli* back up and/or augment the repair of the formamidopyrimidine family of lesions. The formamidopyrimidine nucleosides (Fapy•dA and Fapy•dG) (Figure 1) are formed in DNA as a result of oxidative stress. Compared with other DNA lesions, they often are produced in high yields, especially under O_2-deficient conditions (4–7). The lesions are a substrate for a BER enzyme (formamidopyrimidine DNA glycosylase, Fpg) typically associated with modified purine repair in *E.coli* (8,9). Recent reports describe the excision of modified purine nucleotides by Endo III and Endo VIII from randomly damaged DNA, as well as from oligonucleotides containing MeFapy•dG or OxodG (Figure 1) (10–14). These studies led us to investigate Fapy•dA and Fapy•dG excision by Endo III and Endo VIII as a function of opposing nucleotide in order to determine whether these enzymes protect *E.coli* against the formamidopyrimidines.

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The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors

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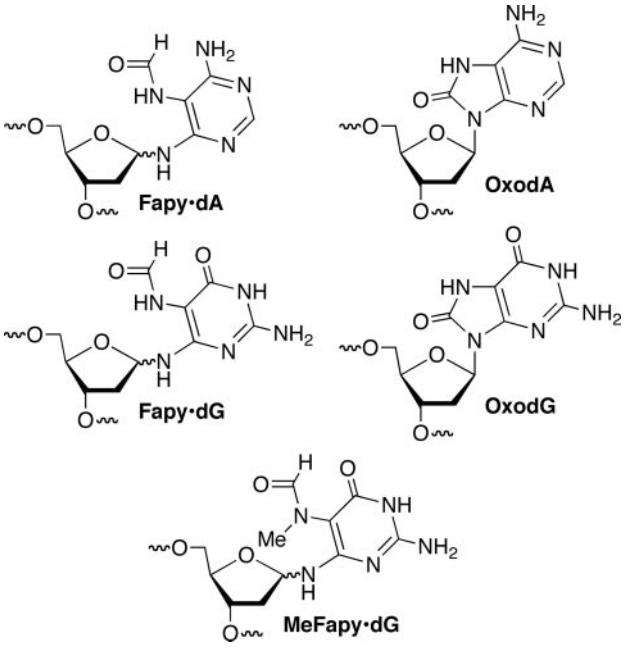

Figure 1. Structures of purine lesions.

The formamidopyrimidine nucleosides are structurally unique. Facile isomerization of the monomeric lesions between α- and β-nucleotides is a distinctive formamidopyrimidine nucleoside property (15,16). However, a recently reported biochemical study suggests that the β-configurations of Fapy•dA and Fapy•dG predominate in duplex DNA (17). Although they are derived from purines, as their name implies they are amino pyrimidines (homopyrimidines). The suggested relationship to pyrimidine lesions is consistent with reports that MeFapy•dG (Figure 1) oligonucleotides and DNA substrates containing randomly produced formamidopyrimidines are repaired by Endo III and Endo VIII, BER enzymes that typically excise pyrimidine lesions in *E.coli* (12–14). Both enzymes preferentially excise MeFapy•dG when it is opposite purine nucleotides. However, enzymes typically associated with the repair of damaged purines also recognize the formamidopyrimidine nucleosides (8,12–15,18–26). These experiments revealed that the guanine oxidation (GO) family enzymes Fpg and MutY efficiently repair Fapy•dG:dC and Fapy•dG:dA base pairs, respectively, which protects against G→T transversions (9). This protection against the promutagenic FapydG:dA base pair is consistent with *in vitro* replication experiments, which showed that dA is misincorporated opposite the lesion (27).

Despite their readily apparent structural differences, the formamidopyrimidines and 8-oxo-7,8-dihydropurines (OxodA, OxodG, Figure 1) are believed to arise from a common intermediate (28). The connection between these families of DNA lesions is reflected in their biochemistry, thermodynamic preferences for base pairing, and penchant for those derived from 2′-deoxyguanosine (Fapy•dG and OxodG) to instruct polymerases to misincorporate dA opposite themselves (18,25,27,29–31). Although there are many similarities in the recognition of formamidopyrimidine and 8-oxopurine lesions, their repair by all BER enzymes has not been studied equally. For example, MeFapy•dG excision by Endo VIII and Endo III is well characterized (14). In contrast, OxodG excision by Endo III has not been reported, and there are conflicting reports concerning the action of Endo VIII on this lesion. One group found that Endo VIII excised OxodG slightly more efficiently when the lesion was opposite a purine compared with 2′-deoxycytidine (11). This opposing base preference for OxodG excision by Endo VIII gave rise to the hypothesis that it might be the fourth member of the GO family of BER enzymes that guard against GO (11). Its proposed role is to repair OxodG:dA mispairs that arise from misincorporation of OxodGTP. Thus, one would expect large increases in T→G transversions owing to OxodG incorporation in *nth nei* deficient *E.coli*. However, increases in these mutations are not observed in *nth nei* mutants, suggesting that Endo III and Endo VIII do not play such a role in protecting *E.coli* against this lesion (10). In accordance with this, Blaisdell *et al*. (10) reported preferential excision of OxodG when it was opposite dC and suggested that Endo VIII is a back up repair system for Fpg. Additional information is available regarding the excision of modified purines by Endo III and Endo VIII from studies on DNA substrates containing randomly distributed lesions (12,13). In these experiments, Fapy•dA excision from randomly damaged DNA is detected, whereas Fapy•dG, OxodA and OxodG are not, despite their formation under the oxidation conditions. Studies on randomly damaged DNA provide a picture of the competition by various lesions for BER enzymes. However, these experiments do not allow one to discern if the enzyme is able to discriminate with respect to opposing nucleotide. An important consequence of this is that such experiments do not provide insight into the enzyme's ability to prevent mutations. Recently, chemical syntheses of oligonucleotides containing Fapy•dA or Fapy•dG were reported (22,23,32). We have taken advantage of these substrates to examine formamidopyrimidine excision by Endo III and Endo VIII when the lesions are opposite each of the four native nucleotides. Consequently, we are able to evaluate the possible roles of these enzymes in protecting *E.coli*'s genome against the formamidopyrimidine lesions.

MATERIALS AND METHODS

General methods

Oligonucleotides were prepared on an Applied Biosystems Inc. 394 DNA synthesizer. Commercially available DNA synthesis reagents were obtained from Glen Research Inc (Sterling, VA). Oligodeoxyribonucleotides containing Fapy•dG or Fapy•dA were prepared as described previously (22,23,32). Oligodeoxyribonucleotides containing Tg or 5,6-dihydro-2′-deoxyuridine (dHU) were prepared using commercially available reagents and protocols provided by the manufacturer (Glen Research). All others were synthesized using standard protocols. DNA manipulations were carried out using standard procedures (33). T4 polynucleotide kinase was obtained from New England Biolabs (Beverly, MA). Radionuclides were obtained from Amersham Pharmacia (Piscataway, NJ). Plasmids containing the *nth* or *nei* genes were obtained from Professors Kow and Hazra, respectively. Hexa-his tagged Endo III was isolated using the Novagen bugbuster kit and purified using a His•Bind column (Novagen, Madison, WI). Endo VIII was isolated as described previously (13). 5′-^{32}P-labeled duplexes were prepared by hybridizing

unlabeled complementary strand to that containing the lesion (200–300 nm) for 5 min at 55°C for Fapy•dA or 90°C for Fapy•dG, followed by slow cooling to room temperature (8,9).

Active site titration of Endo III and Endo VIII (34)

$NaCNBH_3$ (50 mM) was added to **3** (30–100 nM) in 1× Endo III buffer (10 mM HEPES–KOH, pH 7.4, 100 mM KCl, and 10 mM EDTA). Endo III (81.5 μM as established by Bradford assay) in 1× Endo III buffer was added to the DNA solution (total volume: 10 μl) and incubated at 37°C for 30 min. The reactions were quenched with 10 μl of 2× SDS–PAGE loading buffer (100 mM Tris, pH 6.8, 4% SDS and 20% glycerol) and denatured (90°C for 1 min and cooled to 0°C) before being separated by 12.5% SDS–PAGE. The active site concentration was calculated as a percentage of the total protein concentration (12.75%) and represents the average of three experiments.

The active site concentration of Endo VIII (9.5% of the total protein concentration) was determined in a similar manner using 11.5 μM Endo VIII (as established by the Bradford assay) and **3** (20–100 nM) in 1× Endo VIII buffer (20 mM Tris, pH 7.5, 50 mM NaCl, 1 mM EDTA, 0.1 mM DTT and 0.1 mg/ml BSA).

Kinetics for excision of Tg (4) and Fapy•dA (1a) opposite dA by Endo III

Reaction mixtures containing varying concentrations of DNA (0.5–150 nM for **4** and 100–1300 nM for **1a**) were prepared by mixing the corresponding 2× DNA solution (5 μl) with a 2× enzyme solution (5 μl) containing Endo III (0.5 nM for **4** and 10 nM for **1a**) in 20 mM HEPES–KOH (pH 7.4), 200 mM KCl and 20 mM EDTA. Reactions were run for 5 min at 37°C and then quenched with 95% formamide loading buffer (20 μl). Samples were then denatured for 1 min at 90°C, cooled on ice and separated by 20% denaturing PAGE. To account for the adventitious deglycosylation of Fapy•dA, a control was carried out where the substrate (20 nM) was treated with 1 M NaOH for 20 min at 37°C. Samples were neutralized with 1 M HCl, diluted with formamide loading buffer (20 μl) and denatured for 1 min at 90°C. The fraction of cleaved duplex (≤7%) in the control was subtracted from that in the reactions. Kinetic parameters reported represent the results of three separate experiments each carried out in triplicate.

Excision of Fapy•dG/Fapy•dA by Endo III and Endo VIII as a function of opposing nucleotide

An enzyme–substrate solution containing Endo III (10 nM) in 1× Endo III buffer, or Endo VIII (0.1 nM for **1a–d**; 1 nM for **2a–d**) in 1× Endo VIII buffer and radiolabeled DNA (50 nM) was incubated at 37°C (5 μl total volume). After 20 min, the reaction was quenched with 5 μl of 95% formamide loading buffer. Samples were denatured for 1 min at 90°C and separated by 20% denaturing PAGE. Reactions were carried out in triplicate. Adventitious deglycosylation of Fapy•dA (≤7%)/dG (≤3%) was accounted for as described above.

Single turnover kinetics of Fapy•dG opposite dA and dC (2a, 2b) by Endo VIII (35)

An enzyme–substrate solution containing Endo VIII (20 nM) in 1× Endo VIII buffer, and radiolabeled DNA (5 nM) was incubated at 37°C. Aliquots (10 μl) were taken after 0.5, 1, 3, 5, 15 and 30 min and quenched with 20 μl formamide loading buffer. Background cleavage was accounted for as described previously. Samples were denatured for 1 min at 90°C and separated by 20% denaturing PAGE. The data were plotted as product concentration versus time and fit to Equation 1 to find k_{obs}. A_0 is the amplitude of the exponential curve.

$$A_t = A_0\left(1 - e^{-kt}\right) \quad \mathbf{1}$$

Kinetics for Fapy•dA (1a, 1c) and Fapy•dG (2a, 2d) excision by Endo VIII

Reaction mixtures containing varying concentrations of **1a** (0.5–10 nM), **1c** (0.5–10 nM), **2a** (25–400 nM) or **2d** (0.5–2 μM) were prepared by mixing a solution (5 μl) containing the appropriate duplex with a 2× enzyme solution (5 μl) containing Endo VIII (0.1 nM for **1a**, **1c** or 20 nM for **2a**, **2d**) in 2× Endo VIII buffer. After incubation at 37°C for the appropriate time (**1a**, 0.5 min; **1c**, 1 min; **2a**, 1 min; **2d**, 15 min), the reaction was quenched with formamide loading buffer (10 μl). Samples were denatured for 1 min at 90°C, cooled on ice and then separated by 20% denaturing PAGE. Background cleavage was accounted for as described above. Kinetic parameters reported represent the results of three separate experiments carried out in triplicate.

Time course for excision of Fapy•dA (1a,c) by Endo VIII

An enzyme–substrate solution containing Endo VIII (1 nM) in 1× Endo VIII buffer, and radiolabeled DNA (50 nM) was incubated at 37°C. Aliquots (5 μl) were taken over the course of 20 min and quenched with formamide loading buffer (5 μl). Background cleavage was accounted for as described above. Samples were denatured for 1 min at 90°C and separated by 20% denaturing PAGE. Reactions were carried out in triplicate.

Table 1. Oligonucleotide duplexes used in this study

5'-d(CGT TCA ACG TGC ACT **Fapy•dA**TC AGC ACG TCC CAT)
3'-d(GCA AGT TGC ACG TGA X AG TCG TGC AGG GTA)

	X
1a	A
1b	G
1c	T
1d	C

5'-d(AGG CGT TCA ACG TGC AGT **Fapy•dG**TC AGC ACG TCC CAT GGT)
3'-d(TCC GCA AGT TGC ACG TCA X AG TCG TGC AGG GTA CCA)

	X
2a	A
2b	G
2c	T
2d	C

5'-d(GAC GAA TTC GCG ATC **dHU**TC GAC TCG AGC TCA G)
3'-d(CTG CTT AAG CGC TAG A AG CTG AGC TCG AGT C)

3

5'-d(GAG CTA GCT CGA CCT **Tg**TA GGA CCT GCA GCT)
3'-d(CTC GAT CGA GCT GGA AAT CCT GGA CGT CGA)

4

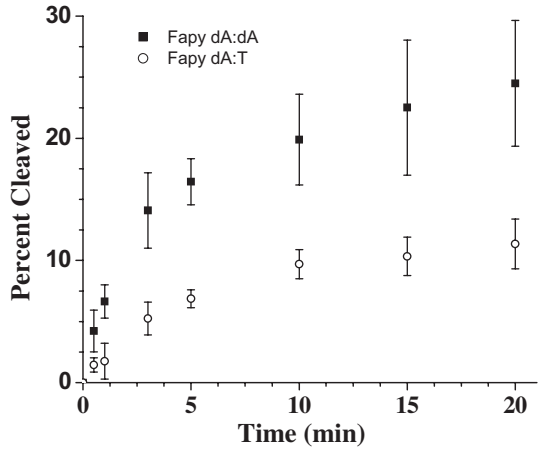

Figure 2. Excision of Fapy•dA (50 nM) by Endo VIII (1 nM) as function of time when opposed by dA or dC.

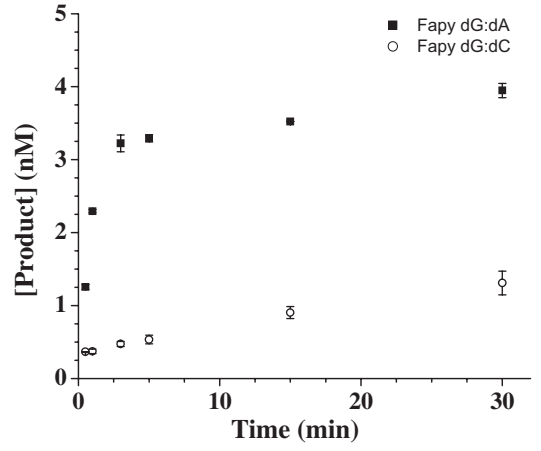

Figure 3. Endo VIII (20 nM) excision of Fapy•dG opposite dA (**2a**, 5 nM) or dC (**2d**, 5 nM) under single turnover conditions.

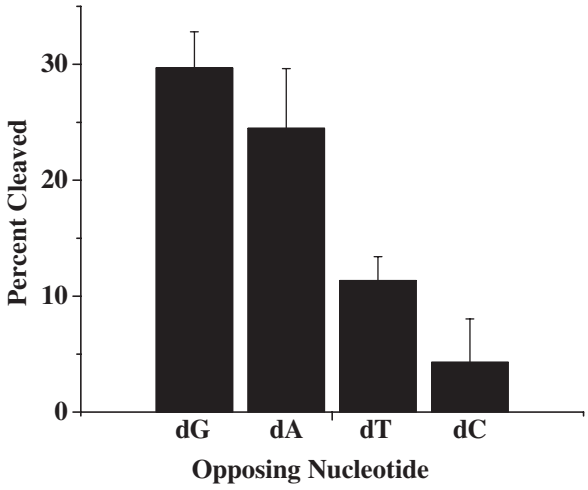

Figure 4. Excision of Fapy•dA (50 nM) by Endo VIII (0.1 nM) as a function of translesional nucleotide (**1a–d**).

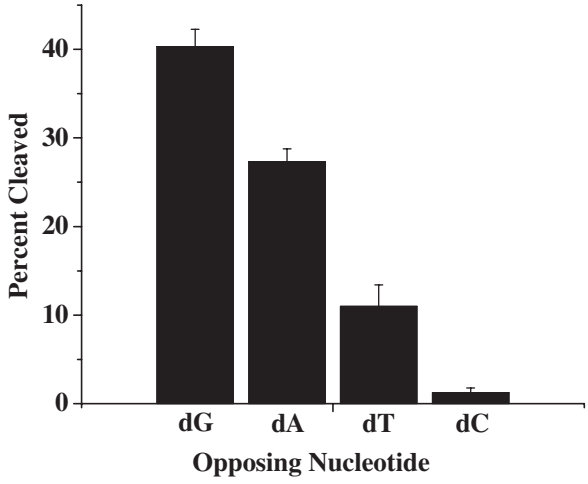

Figure 5. Excision of Fapy•dG (50 nM) by Endo VIII (1 nM) as a function of translesional nucleotide (**2a–d**).

RESULTS

Fapy•dA and Fapy•dG excision by Endo VIII

Steady-state kinetic analysis for Fapy•dA excision was carried out when the lesion was opposite the 2 nt [dA (**1a**) and dT (**1c**)] most frequently incorporated opposite it by Klenow exo$^-$, and which form the most stable duplexes (Table 2) (25). Multiple turnovers were observed in a relatively short time for both substrates (Figure 2). The kinetic constants measured for Endo VIII excision of Fapy•dA indicate that the lesion is excised about twice as efficiently when it is opposite dA than dT. This is attributable to a greater k_{cat} for reaction with **1a**. The measured k_{cat} for the excision of Fapy•dA opposite the correct nucleotide (**1a**) is much larger than that observed for dHU (**3**) and accounts for most of the ~50-fold greater specificity constant. The excision of dHU proceeds with a specificity constant (k_{cat}/K_m), comparable with that reported previously, verifying its accuracy (11).

Quantitative analysis of Fapy•dG excision opposite dA reveals that repair of this lesion is ~500 times less efficient than repair of Fapy•dA by Endo VIII (Table 2). When comparing formamidopyrimidine substrates containing dA opposite the respective lesions, the predominant difference in kinetic parameters is owing to a significantly higher K_m (>70-fold) for the duplex containing Fapy•dG. Steady-state experiments on Fapy•dG:dC (**2d**) excision verified that this substrate is considerably poorer than that containing a Fapy•dG:dA base pair. We were unable to saturate the enzyme even when using as much as 2 μM Fapy•dG:dC containing DNA (**2d**). Hence, the K_m expressed is a lower limit (Table 2). These experiments were discontinued due to the biological irrelevance of such an unfavorable process. The excision of Fapy•dG opposite dC (**2d**) was so low that the enzyme did not turnover during the course of the reaction (data not shown), although multiple turnovers were observed for **2a** in <5 min. In order to eliminate selective product inhibition following excision of Fapy•dG opposite dC, single turnover experiments (excess Endo VIII) were carried out (Figure 3) (35). These data were consistent with those obtained under multiple turnover conditions. There is a burst of Fapy•dG excision when

opposite dA. More than 65% of the lesion is removed within 5 min (k_{obs} = 0.5 min^{-1}), and 80% after 30 min. In contrast, a burst is not observed when Fapy•dG is opposite dC, and <30% of the lesion is excised in 30 min. An upper limit for k_{obs} was estimated by fitting the product concentration versus time to a first order process. This provided an upper limit for k_{obs} = 0.02 min^{-1}, confirming that Fapy•dG:dA is a superior substrate for Endo VIII.

Although kinetic parameters for Fapy•dA and Fapy•dG excision opposite each of the four native nucleotides under Michaelis–Menten conditions were not determined, the amount of strand scission by Endo VIII was measured as a function of opposing nucleotide at constant DNA and enzyme concentration (Figures 4 and 5). The substrates were present in excess in all experiments. A proportionally smaller amount of enzyme, indicative of its more efficient excision, was employed for cleavage of Fapy•dA containing DNA (Figure 4). Excision of Fapy•dA and Fapy•dG (Figure 5) showed similar trends with respect to opposing nucleotide. Repair was more efficient when a purine was opposite the respective lesion. Furthermore, the rank order for repair as a function of opposing nucleotide was dG > dA > dT > dC when the lesion was either Fapy•dA (Figure 4) or Fapy•dG (Figure 5).

Fapy•dA and Fapy•dG excision by Endo III

Many of the trends noted above for formamidopyrimidine excision by Endo VIII were observed with Endo III. Fapy•dA excision opposite thymidine (**1c**) was examined under Michaelis–Menten conditions, but saturation could not be achieved giving a K_m > 1 μM (data not shown). This is much higher than the K_m for Fapy•dA repair by Fpg and quantitative analysis was not pursued further (8). Excision of Fapy•dA opposite dA (**1a**) was much more efficient (Figure 6). The kinetic parameters for repair of the Fapy•dA:dA duplex by Endo III (k_{cat}/K_m = 6.9 ± 1.0 × 10^{-2} nM^{-1} min^{-1}) describe a process that is comparable in efficiency with excision of thymidine glycol opposite dA (**4**, k_{cat}/K_m = 6.0 ± 1.9 × 10^{-2} nM^{-1} min^{-1}). The latter value is comparable with that reported in a different sequence context (36). However, excision of Fapy•dA by Endo III is >200 times less efficient than by Endo VIII (Table 1). The K_m for Endo III excision of Fapy•dA opposite dA (144.8 ± 34.2 nM) is ~60-fold higher than repair by Endo VIII. A significantly lower k_{cat} (7.9 ± 2.1 min^{-1}) also contributes to the differences in specificity constants.

One of the trends shared between Endo III and Endo VIII is the more efficient excision of Fapy•dA than Fapy•dG. The data presented above reveal that Fapy•dA repair by Endo III is much less efficient than excision by Endo VIII. Initial experiments indicated that Fapy•dG repair by Endo III is significantly less efficient than that of Fapy•dA and could not compete with the excision kinetics exhibited by Fpg or Endo VIII (above) (9). Consequently, the dependency of Fapy•dG excision by Endo III on opposing nucleotide was investigated at constant DNA and enzyme concentration for a fixed time (Figure 7). Duplexes containing purines opposite Fapy•dG (**2a**, **b**) were cleaved more extensively than those containing Fapy•dG:Pyr base pairs (**2c**, **d**), which are very poor substrates. Preferential excision of Fapy•dA by Endo III was also observed when the lesion was paired with a purine (Figure 8). One minor difference is that the rank order of

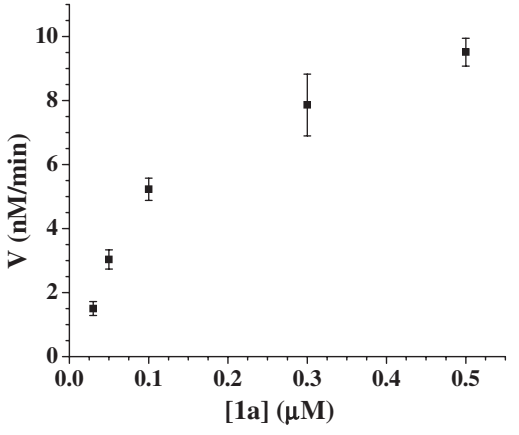

Figure 6. Representative velocity versus substrate concentration plot for excision of Fapy•dA opposite dA (**1a**) by Endo III (5 nM).

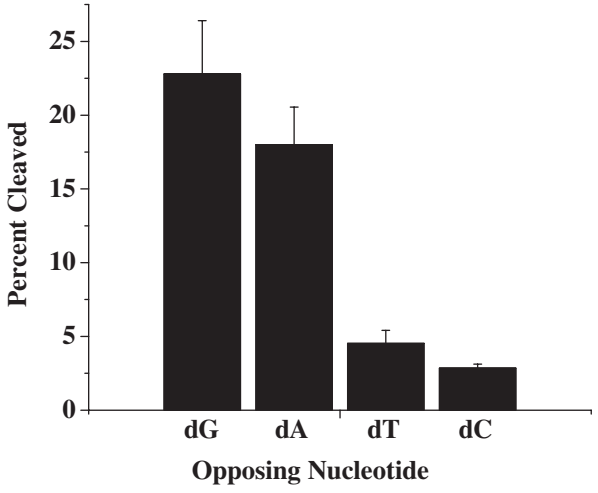

Figure 7. Excision of Fapy•dG (50 nM) by Endo III (10 nM) as a function of translesional nucleotide (**2a–d**).

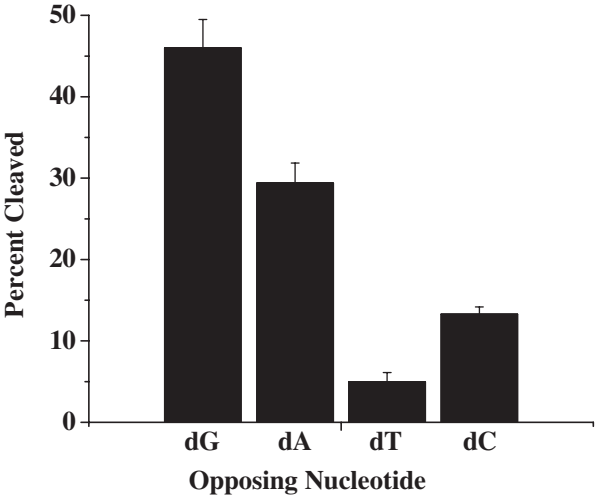

Figure 8. Excision of Fapy•dA (50 nM) by Endo III (10 nM) as a function of translesional nucleotide (**1a–d**).

Fapy•dA excision by Endo III was dG > dA > dC > dT, and not dG > dA > dT > dC as was observed for Fapy•dG repair by the same enzyme and removal of either lesion by Endo VIII.

DISCUSSION

The discovery of alternative repair pathways for DNA lesions, such as nucleotide incision repair, suggests that organisms have developed redundancy in systems designed to protect their genomes (2,3). Although Endo III and Endo VIII are typically associated with modified pyrimidine repair in *E.coli*, examination of DNA containing random distributions of DNA lesions revealed that these enzymes also excise Fapy•dA (12,13). Here, the excision of Fapy•dA and Fapy•dG by Endo III and Endo VIII was examined as a function of opposing native nucleotide by taking advantage of chemically synthesized oligonucleotides containing the lesions at defined sites. We were motivated to determine whether Endo III and/or Endo VIII play a role in repairing formamidopyrimidines in *E.coli*.

Overall, the reactivity trends observed using synthetic oligonucleotide duplexes containing a single formamidopyrimidine nucleoside were consistent with experiments using substrates containing a random distribution of lesions (12,13). Steady-state analyses and single time point experiments indicate that Endo III and Endo VIII excise Fapy•dA more efficiently than Fapy•dG. When the lesions were opposite dA, Fapy•dA was excised by Endo VIII >500 times more efficiently than Fapy•dG (Table 2). The more facile hydrolysis of Fapy•dA lesions was evident in single time point experiments using Endo VIII, in which 10-fold less enzyme was needed to excise Fapy•dA than Fapy•dG (Figures 3 and 4). The reason for the more efficient cleavage of Fapy•dA than Fapy•dG is uncertain and could be attributable to multiple issues, including the higher lability of its *N*-glycosidic bond (15). In addition to correctly predicting the relative reactivity of Fapy•dA and Fapy•dG in homogeneous substrates, Dizdaroglu's experiments revealed Endo VIII's greater effectiveness at excising the lesions than that of Endo III (12,13). However, the studies described here using purified DNA substrates containing a single lesion revealed a much larger difference between the two enzymes. This was evident in steady-state analyses of reactions with DNA containing a Fapy•dA:dA base pair (**1a**) where Endo VIII was >800 times more efficient than Endo III and from experiments where significantly less Endo VIII could cleave comparable amounts of substrates (Figures 4, 5, 7 and 8). Furthermore, the absolute values for k_{cat}/K_m of Fapy•dA opposite dA (where a direct comparison could be made) by Endo III and Endo VIII were significantly greater than when the lesion was present in a milieu of DNA damage products (12,13). This could be attributable to the presence of lesions that compete with one another for the BER enzymes in such experiments.

Table 2. Steady-state analysis of Endo VIII excision

Base pair (duplex)	K_m (nM)	k_{cat} (min^{-1})	k_{cat}/K_m (min^{-1} nM^{-1})
Fapy•dA:dT (**1c**)	2.1 ± 1.3	13.6 ± 4.6	8.3 ± 4.1
Fapy•dA:dA (**1a**)	2.5 ± 0.6	35.0 ± 9.4	14.5 ± 4.3
Fapy•dG:dA (**2a**)	178.1 ± 14.3	5.0 ± 0.5	$2.8 \pm 0.1 \times 10^{-2}$
Fapy•dG:dC (**2d**)	>1000	>0.6	—
DHU:dA (**3**)	3.2 ± 0.6	0.5 ± 0.1	$1.7 \pm 0.2 \times 10^{-1}$

In order for a BER enzyme to protect the genome, it must discriminate against excising DNA lesions when they are part of promutagenic base pairs (e.g. Fapy•dG:dA) that result from unfaithful replication. For instance, Fpg excises OxodG and Fapy•dG ~20 times more efficiently when they are opposite dC than dA (9,30). However, it is interesting to note that OxodG is excised from duplexes in which it is opposite dG or dT even more rapidly. Neither of these nucleotides are reported to be incorporated efficiently opposite OxodG by *E.coli* polymerases *in vitro* or *in vivo* (29,37–39). Hence, the biological relevance of BER enzyme specificity should be interpreted in the context of replication experiments. Consequently, we examined each enzyme's excision of the formamidopyrimidine nucleosides opposite their correct nucleotide (e.g. Fapy•dA:dT), as well as from duplexes in which the lesions are opposite dA. *In vitro* experiments indicate that dA is the nucleotide most likely to be misincorporated opposite the formamidopyrimidines (25,27). Both lesions were excised less efficiently when paired with their respective native nucleotide's match than when opposite dA. This indicates that it is unlikely that either Endo III or Endo VIII serves as a back up for formamidopyrimidine nucleoside repair by Fpg in *E.coli*. In order to fulfill such a role, the enzymes would have to preferentially excise the lesions when they are opposite the 'correct' nucleotide (e.g. dC opposite Fapy•dG).

One possible explanation for the preference for Endo VIII and Endo III excision of Fapy•dA and Fapy•dG when the lesions are mispaired with dA is that the enzymes protect against formamidopyrimidine incorporation in the nascent strand. This has been proposed for Endo VIII repair of OxodG, resulting in its consideration as the fourth enzyme for protecting the *E.coli* genome against GO (11). This proposal has not been unanimously embraced (10). For instance, other researchers found that OxodG was excised opposite dC more rapidly than when opposed by dA (10). In addition, in contrast to OxodGTP, it is not known whether the nucleotide triphosphates of the formamidopyrimidines are incorporated opposite dA by polymerases (40). Before one can propose that the BER enzymes guard against formamidopyrimidine incorporation in the nascent strand, one must be sure that the lesions are indeed substrates for this process. Moreover, protecting *E.coli* against formamidopyrimidine lesion incorporation in the nascent strand is an unlikely role for Endo III or Endo VIII, because of the lack of an increase in the corresponding mutations (e.g. T→A from Fapy•dA, T→G from Fapy•dG) in *nth nei* deficient cells (10).

Alternatively, one can rationalize formamidopyrimidine excision by Endo III and Endo VIII based upon the enzymes' proclivity to repair damaged pyrimidines, and the structural similarity between the families of lesions. The dependence of repair by these enzymes upon opposing nucleotide is quite similar for the formamidopyrimidines and damaged pyrimidines (36). The comprehensive comparison of formamidopyrimidine repair dependency on opposing nucleotide consistently showed that Endo III and Endo VIII distinguish between purines and pyrimidines (Figures 4, 5, 7 and 8). However, the enzymes did not significantly distinguish one purine from another, or between individual pyrimidines. Furthermore, formamidopyrimidine repair efficiency by Endo III

and Endo VIII rivals that of pyrimidine lesions. Fapy•dA repair is as efficient as thymidine glycol excision by Endo III (36). Fapy•dA is repaired by Endo VIII more efficiently than is dHU, and Fapy•dG is excised ~6-fold less efficiently than the pyrimidine lesion (11). The structural relationship between the two families of DNA lesions stems from cleavage of the purine's imidazole ring en route to formamidopyrimidine formation. This produces a pyrimidine ring linked to the anomeric position of the 2′-deoxyribose via a nitrogen atom. In the parlance of organic chemistry, the formamidopyrimidine lesions are 'homopyrimidines'. Hence, we propose that Endo III and Endo VIII excise formamidopyrimidine lesions because they are recognized as modified pyrimidines. Because pyrimidines are typically paired with purines, this also explains why Endo III and Endo VIII preferentially recognize Fapy•dA:Pur and Fapy•dG:Pur base pairs.

CONCLUSIONS

Endo III and Endo VIII excise Fapy•dG and Fapy•dA efficiently compared with dihydropyrimidine lesions. The formamidopyrimidines are excised more efficiently opposite purines than pyrimidines. This could be an indication that these enzymes are involved in the removal of these lesions from the nascent strands of DNA, but related studies in *E.coli* do not support this concept (10). The selectivity exhibited by Endo III and Endo VIII with respect to opposing nucleotide also does not support a role for either enzyme as a back up to Fpg repair of the formamidopyrimidine nucleosides in *E.coli*. Instead, the data indicate that these enzymes recognize formamidopyrimidines owing to their structural similarity to damaged pyrimidines. These experiments highlight the importance of examining the dependence of DNA lesion repair on opposing nucleotide identity.

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