Aflatoxin B₁ formamidopyrimidine adducts are preferentially repaired by the nucleotide excision repair pathway *in vivo*

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Aflatoxin B₁ (AFB₁), the most potent member of the aflatoxin family of hepatocarcinogens, upon metabolic activation reacts with DNA and forms a population of covalent adducts. The most prevalent adduct, 8,9-dihydro-8-(N⁷-guanyl)-9-hydroxyaflatoxin (AFB₁-N⁷-dG), as well as the AFB₁ formamidopyrimidine adduct (AFB₁-FAPY), resulting from imidazole ring opening of the major adduct, are thought to be responsible for mutations caused by AFB₁. The AFB₁-N⁷-dG lesions are rapidly removed in *Escherichia coli* and mammals, whereas the AFB₁-FAPY lesions persist in mammalian cells, which along with the higher stability of this lesion suggests that AFB₁-FAPY might significantly contribute to the observed toxicity and carcinogenicity of AFB₁ in higher organisms. Other workers have shown *in vitro* evidence that AFB₁-FAPY lesions are substrates for both nucleotide excision repair (NER) and base excision repair (BER). The present study, done *in vivo*, utilized a modified host cell reaction assay and showed that AFB₁-FAPY lesions are preferentially repaired in *E.coli* by NER. Comparisons of repair in wild-type, NER-deficient (uvrA), BER-deficient (mutM) and NER/BER double mutant *E.coli* strains transformed with plasmids enriched for AFB₁-N⁷-dG or AFB₁-FAPY lesions indicate that both lesions are efficiently repaired by NER-proficient cells (both wild-type and BER-deficient strains). We have found that the level of activity of the reporter gene is significantly affected by the presence of either lesion in NER-deficient strains due to the lack of repair. This effect is similar in NER-deficient and NER/BER-deficient strains indicating that BER (specifically in the strains we investigated) does not contribute significantly to the repair of these lesions *in vivo*. Consistent with this finding, *in vitro* analysis of AFB₁-FAPY adduct excision by purified MutM and its functional analog human 8-oxoguanine DNA glycosylase using site-specifically modified oligonucleotides indicates that this lesion is a poor substrate for both proteins compared with canonical substrates for these enzymes, such as 7,8-dihydro-8-oxoguanine and methylformamidopyrimidine.

**Introduction**

Aflatoxin B₁ (AFB₁) (Figure 1) is produced by several fungal species that contaminate food supplies (reviewed in ref. 1). Exposure to AFB₁ is especially high in underdeveloped countries with a warm, moist climate (2). Developed countries have set strict standards for the aflatoxin levels in food, but AFB₁ contamination is nevertheless responsible for substantial economic losses to corn crops in the southern and mid-western USA (3). AFB₁ is cytotoxic and mutagenic in bacteria, and toxic and carcinogenic in animals (1,4). Epidemiological studies link AFB₁ exposure to hepatocellular carcinoma incidence, especially in the situations in which AFB₁ exposure is accompanied with hepatitis B virus infection (5–9). Interestingly, in more than half of these latter cases, a characteristic G to T mutation at the third position of codon 249 of the p53 gene is present (10). In addition, some epidemiological data suggest a link between inhalation of AFB₁ contaminated grain dusts and increased lung cancer risks in certain occupational settings (11).

AFB₁ is activated by cytochrome P450 enzymes (12) forming the AFB₁-8,9-epoxide (Figure 1), which is capable of covalent modification of DNA, preferentially at the N⁷ position of guanine (dG). The resulting cationic adduct is labile and can depurinate or, alternatively, undergo a base-catalyzed reaction during which the imidazole ring of dG opens and a stable 8,9-dihydro-8(2,6-diamino-4-oxo-3,4-dihydropyrimidin-5-yl-formamido)-9-hydroxyaflatoxin B₁ (AFB₁-FAPY) adduct is formed (4,13). It is possible that all three lesions contribute to the observed biological effects of AFB₁ exposure. Using site-specific, lesion-directed mutagenesis experiments, it has been shown that both cationic AFB₁-N⁷-dG, AFB₁-FAPY and AFB₁-FAPY adducts cause the same types of mutations (mostly G to T transversions) at the site of the lesion and some mutations 5′ to the lesion (14,15). In these studies, the AFB₁-FAPY adduct was found to be six times more mutagenic and caused a much stronger blocking effect on DNA replication than the AFB₁-N⁷-dG adduct (14).

Nuclear magnetic resonance (NMR) studies have been performed on the structures of the aflatoxin adducts in double-helical DNA (16–21). Both AFB₁-N⁷-dG and AFB₁-FAPY adducts form similar structures in which the AFB₁ moiety is intercalated on the 5′ side of the modified base, which remains in H-bonding alignment with the complementary cytosine. The differences between the structures of DNA containing each of the two studied aflatoxin adducts are minor, despite the fact that a significantly different extent of DNA duplex

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**Abbreviations:** AFB₁, aflatoxin B₁; AFB₁-FAPY, 8,9-dihydro-8(2,6-diamino-4-oxo-3,4-dihydropyrimidin-5-yl-formamido)-9-hydroxyaflatoxin B₁; AFB₁-N⁷-dG, 8,9-dihydro-8-(N⁷-guanyl)-9-hydroxyaflatoxin B₁; BER, base excision repair; dG, 2'-deoxyguanosine; FAPY, formamidopyrimidine; Fpg, formamidopyrimidine DNA glycosylase; HCR, host cell reactivation; hOGG1, human 8-oxoguanine DNA glycosylase; HPLC, high pressure liquid chromatography; Me-FAPY, 2,6-diamino-4-oxo-5-(N'-methyl)formamidopyrimidine; NER, nucleotide excision repair; NMR, nuclear magnetic resonance; 8-oxoG, 7,8-dihydro-8-oxoguanine; PAGE, polyacrylamide gel electrophoresis; TEAAC, tetraethyl ammonium acetate.
stabilization by those adducts is observed (the difference is up to 12°C in terms of melting temperatures) (22). One goal of the present work was to elucidate if the differences between AFB₁-N⁷-dG and AFB₁-FAPY adduct structures are significant enough for repair enzymes to recognize and repair these lesions differentially, especially as it has been shown that in mammalian cells (but not in bacteria) AFB₁-FAPY adducts persist for many days to weeks, while the AFB₁-N⁷-dG adducts are efficiently removed (23-25). Nucleotide excision repair (NER) plays an important role in removing many bulky adducts in organisms from bacteria to humans (26), and its importance upon AFB₁ exposure has been established (27-31). Also, it has been demonstrated that E.coli uvrABC enzymes bind and incise both AFB₁-N⁷-dG and AFB₁-FAPY with equal efficiency in vitro (32). In contrast, it has been shown that the formamidopyrimidine DNA glycosylase (Fpg) protein (MutM), a base excision repair (BER) glycosylase that removes a variety of oxidation-derived lesions including purine imidazole ring-opened species (33), is also capable of removing AFB₁-FAPY, but not AFB₁-N⁷-dG, in vitro (34). It has not been established if NER or BER is the preferred biologically relevant pathway for removal of AFB₁-FAPY lesions in vivo.

In order to determine the preferred pathway of AFB₁ adduct removal in vivo, we used a modified host cell reactivation (HCR) assay (35,36) in which E.coli cells with different repair backgrounds were transformed with plasmids bearing a reporter gene. Prior to transformation of cells, the plasmids were enriched for either AFB₁-N⁷-dG or AFB₁-FAPY lesions. The results of these experiments suggest that AFB₁-FAPY lesions are repaired in vivo by NER, whereas the role of the Fpg protein in this case is negligible. In addition, our in vitro comparison of the cleavage efficiencies of the damaged DNA by the Fpg protein and its human ortholog 8-oxoguanine DNA glycosylase (hOGG1) suggests that the AFB₁-FAPY lesion is a surprisingly poor substrate for these enzymes, compared with the canonical substrates 7,8-dihydro-8-oxoguanine (8-oxoG) and methyl-formamidopyrimidine (Me-FAPY) (37).

Materials and methods

Materials

Fpg and hOGG1 proteins were gifts from Gregory L.Verdine (Harvard University). Fpg protein was also purchased from Trevigen (Gaithersburg, MD). The Klenow fragment (KF) of E.coli DNA polymerase I was from Amersham Pharmacia Biotech (Piscataway, NJ). The protein had been over-produced and purified from a strain carrying a double mutation D355A, E357A, which results in ~10³-fold reduction of endogenous 3'-5'exonuclease activity (38).

Plasmids pSP72 (2462 bp) bearing the ampicillin resistance gene were purchased from Promega (Madison, WI). To amplify the plasmids, competent DH5α cells were transformed with the pSP72 plasmids and re-grown from a single colony in ampicillin-containing media as described in (39); the plasmids were purified using a Qiagen (Valencia, CA) plasmid kit according to the manufacturer’s protocol.

Oligonucleotides were obtained from Qiagen. dNTPs were purchased from Promega. [γ-³²P]ATP was from ICN Biomedicals (Irvine, CA). AFB₁ was purchased from Sigma. AFB₁ labeled with tritium was from Moravek Biochemicals (Brea, CA). All other general reagents and chemicals were obtained from Fisher (Pittsburgh, PA), Aldrich (Milwaukee, WI) and VWR (West Chester, PA).

Synthesis and purification of the AFB₁-modified DNA

An oligonucleotide (a 13mer, 5'-CCTCTTCGAACTC-3') containing a single dG was purified by 20% denaturing polyacrylamide gel electrophoresis (PAGE) in the presence of 8 M urea according to the procedures described in (40). The oligonucleotides were detected under UV light; the relevant area was cut out, eluted overnight and desalted by spin-dialysis using centricon-3 microconcentrators (Amicon, Bedford, MA) according to the manufacturer’s protocol. The oligonucleotide was allowed to react with AFB₁-8,9-epoxide (obtained as described in ref. 41) to produce either AFB₁-N⁷-dG or AFB₁-FAPY adducts as described in detail (14,32,42). Briefly, the DNA (~90 nmol) was resuspended in 100 µl of 10 mM phosphate buffer (Na₂HPO₄) containing 100 mM NaCl, 50 mM EDTA (pH 7) and 50 µl of acetone solution containing 5.6 µmol of AFB₁-epoxide was added. The mixture was shaken at 4°C for 15 min, after which another 50 µl of the same AFB₁-epoxide solution was added and incubated for another 15 min. The unreacted AFB₁ products were
mock-treated plasmids was the same as determined from agarose gel analysis (data not shown).

**Host cell reaction assay**

The E. coli strains used were JM105 (wild-type), BH 410 [JM 105 fpg::Kan<sup>R</sup>], BH 430 [JM 105 uvrA::Tet<sup>R</sup>], BH 420 [JM 105 fpg::Kan<sup>R</sup>, uvrA::Tet<sup>R</sup>]. The cells were prepared for transformation as described (15). For each transformation, 100 µl of the cell suspension was added to 0.1 µg of the plasmid DNA (pSP72 unmodified, or containing from 0 to 50 adducts per plasmid of either AFB<sub>1</sub>-N<sup>2</sup>-dG or AFB<sub>1</sub>-FAPY). The mixture was transferred to a cold Bio-Rad (Hercules, CA) gene pulser cuvette (0.2 cm) and electroporations were performed with a BTX Electro Cell Manipulator 600 set at 2.5 kV and 129 Ω. Immediately after electroporation, 900 µl of M9 media was added and after 3 h of recovery at 37°C, the cells were plated on LB plates containing ampicillin.

The percentage of survival was determined as a ratio of the normalized number of colonies that formed from the cells transformed with modified plasmid to that of cells transformed with the mock-treated plasmid. The survival of the cells transformed with the mock-treated plasmid (0 adducts/plasmid) was set as 100%. To account for plating efficiency, the number of colonies on the ampicillin-containing plates was normalized by dividing by the number of colonies that grew on plates without ampicillin. The percentage of survival was plotted as a function of the number of adducts per plasmid.

**Interactions of MutM and hOGG1 proteins with DNA containing 7,8-dihydro-8-oxoguanine, methyl-formamidopyrimidine or AFB<sub>1</sub>-FAPY lesions in vitro**

A 20mer oligonucleotide (5'-CCACCTCCXCATCTACC-3') containing 8-oxoG was synthesized using the phosphoramidite method, purified using reverse-phase HPLC as described in (33) and desalted with Centricron-3 microconcentrators (Amicon) according to the manufacturer’s protocol. The Me-FAPY lesions were generated by primer extension with KF using procedures similar to those described in (37). A 3<sup>22</sup>P-labeled primer oligonucleotide (5'-CCACCTCGCTGTTGAATGG-3') (100 pmol) and an equimolar 25mer complementary template (5'-GGTGTAATGCCAGT-GAAAGG-3') with a 5' overhang was annealed by heating and slow cooling in 50 mM Tris-Cl, pH 7.5, 10 mM MgCl<sub>2</sub>, 1 mM dithiothreitol and 0.05 ng/ml bovine serum albumin. The annealed primer-template duplex was added to 7methyl-2'-deoxynosine 5'-triphosphate (200 µM), dATP (20 µM), dTTP (20 µM), dCTP (20 µM) and 20 U of Klenow Fragment (final volume 30 µl). After 45 min at 37°C, the full-length replication product (20mer) was purified by 20% PAGE in the presence of 8 M urea.

**Results**

**Repair of AFB<sub>1</sub> lesions in vivo**

The HCR assay has been used previously to study repair of different lesions in cells with different repair status (35,36). Here we used pSP72 plasmids carrying a β-lactamase gene, which allowed transformed cells to grow in the presence of ampicillin. Prior to introduction into cells, plasmids were covalently modified with AFB<sub>1</sub>-epoxide so that either AFB<sub>1</sub>-N<sup>2</sup>-dG or AFB<sub>1</sub>-FAPY adducts were introduced. The survival of the cells transformed with modified plasmids on ampicillin-containing media is possible only when complete or at least...
was significantly less than that of the wild-type and the mutant cells. For example, essentially non-viable, whereas deficient cells (both single and fpg)

There is no evidence that the Fpg protein participates in repair of bulky adducts with an intact purine imidazole ring, such as found in AFB1-N7-dG adducts; thus, it is not surprising that the survival of cell strains of different repair backgrounds on selective media allows one to establish which pathway or pathways are essential for repair AFB1-N7-dG or AFB1-FAPY adducts in vivo.

Figure 3 shows the survival of the E.coli cells transformed with the plasmids containing AFB1-N7-dG adducts on ampicillin-containing plates. The survival of the NER-deficient cells (both single uvrA and double uvrA/fpg mutants) was significantly less than that of the wild-type and the fpg mutant cells. For example, uvrA and uvrA/fpg cells transformed with a plasmid containing ~25 adducts/plasmid were essentially non-viable, whereas >50% of the wild-type cells and fpg cells transformed with the same plasmid were able to survive. This result was predictable, as it has been shown that bulky adducts including the AFB1 lesions are efficiently removed by NER enzymes in vitro and in vivo (27–32). There is no evidence that the Fpg protein participates in repair of bulky adducts with an intact purine imidazole ring, such as found in AFB1-N7-dG adducts; thus, it is not surprising that the survival of the wild-type and the fpg cells are essentially the same. The decrease of survival observed for the double mutant cells (uvrA/fpg) compared with that of the wild-type is caused by the deficiency of NER and not by the absence of the Fpg protein, as a similar survival is observed for cells carrying the single uvrA mutant allele (Figure 3A).

It has been shown that the Fpg protein is capable of incising the AFB1-FAPY adducts in vitro (34), so we performed the HCR assay to determine if this protein contributes to the repair of these lesions in vivo. If true, one would expect to see the decreased survival in fpg mutant cells transformed with the plasmids enriched for this type of lesion compared with survival of wild-type cells, as well as a decrease of survival of the double uvrA/fpg mutant compared with that of the single uvrA mutant cells. The results presented in Figure 3B suggest, however, that this is not the case. The results show that both the wild-type and the fpg mutant are similarly resistant to the presence of the AFB1-FAPY adduct-containing plasmids used for the transformation. For example, >12% survival was observed when the wild-type and fpg cells were transformed with plasmids containing at average 18 adducts, compared with the uvrA mutant and the double uvrA/fpg mutant cells, which could not tolerate >10 adducts/plasmid. The similarity of the survival curves obtained for the latter two cell lines also suggests that the contribution of the Fpg protein to the repair of AFB1-FAPY adducts in vivo is negligible.

Our results suggest that AFB1-N7-dG and AFB1-FAPY adducts, both of which are bulky lesions, are good substrates for E.coli NER proteins, which are able to remove the lesions even at the basal level of NER expression without SOS induction. It is also noteworthy that, overall, all cell lines tolerated the AFB1-N7-dG lesion better than the AFB1-FAPY lesions (compare Figure 3A and B). This finding can be correlated with higher DNA replication blocking caused by AFB1-FAPY adduct observed in a different system (14).

Interactions of MutM and hOGG1 proteins with DNA containing 8-oxoG, Me-FAPY or AFB1-FAPY lesions in vitro Fpg is a DNA glycosylase with a broad substrate-specificity, and is capable of incising a variety of DNA lesions, most of which are derived from oxidative damage (33). Fpg is also reported to remove AFB1-FAPY adducts in vitro (34). To compare the substrate specificity of Fpg protein for different types of DNA damage, we used an in vitro assay in which the double-stranded DNA substrates with a 32P-labeled strand containing the damage are allowed to interact with purified Fpg or its human analog, hOGG1 (Figure 4A). Under the test conditions, efficient cleavage was observed in the cases of 8-oxoG and Me-FAPY lesions, verifying the activity of the enzymes on known substrates. Consistent with previous data (37) 6-elimination products observed in the case of the Fpg protein migrated faster than β-elimination products generated by hOGG1 protein, which lacks homology with the Fpg protein and has a different AP lyase mechanism. No cleavage, however, was observed in the case of the AFB1-FAPY lesion under the same experimental conditions (Figure 4A).

The assay shown in Figure 4A measures the DNA nicking efficiency due to the associated lyase activity that follows the glycosidic cleavage event. As a caveat to the experiment described above (Figure 4A), it is possible that Fpg or hOGG1 displayed some glycosylase activity against the AFB1-FAPY lesion, but the protein may not have displayed detectable lyase activity, leading to a strand break. This would parallel the observation made by Hazra and coworkers that the lyase activity of hOGG1 against the 8-oxoG is weak (44). We addressed this possibility by utilizing NaBH4, which reduces the Schiff base intermediates, thus producing a stable covalent cross-link between Fpg or hOGG1 and abasic sites. Covalent enzyme–DNA complexes were detected only when the substrates contained 8-oxoG and Me-FAPY lesions, but not with the AFB1-FAPY (Figure 4B) nor with the unmodified...
either undergo a base-catalyzed conversion into imidazole ring-opened AFB1-FAPY adducts or depurinate forming abasic sites. The deoxyglycosidic bond of AFB1-FAPY adduct is much more stable than that of the cationic AFB1-N7-dG adduct; this enhanced stabilization of the duplex by AFB1-FAPY could be a factor in its ability to evade repair (16,18,19,22). However, it has been shown that the bacterial uvrABC endonuclease, which bears a significant extent of homology with the mammalian NER system, binds and cleaves both lesions with equal efficiency in vivo (32).

One possible explanation for the more efficient repair of AFB1-FAPY lesions in bacteria versus mammals is the observation that the bacterial Fpg glycosylase (MutM) is able to incise AFB1-FAPY lesions in vitro (34). It was reasonable to extrapolate that Fpg would contribute to the repair of AFB1-FAPY lesions in bacteria, but not in mammals, where proteins serving similar functions lack the homology with Fpg protein (reviewed in refs 45,46). Our results obtained by the ICR assay, however, suggest that it is not the case. When isogenic E.coli cells with different repair backgrounds were transformed with plasmids bearing an ampicillin resistance gene and containing either AFB1-N7-dG or AFB1-FAPY adducts, the observed survival trends on selective ampicillin media were the same for both adducts. Wild-type cells and fpg mutants were relatively resistant to the presence of both lesions, compared with the uvrA and the uvrA/fpg mutants (Figure 3). This suggests that in E.coli both adducts are repaired preferentially by NER and the contribution of the Fpg protein, if any, is negligible.

Fpg protein has a broad substrate-specificity, including 8-oxoG and imidazole ring-opened purines (33). A recently published analysis of the crystal structure of the E.coli Fpg protein bound to oxidatively damaged DNA (47) revealed that there is a compact negatively charged hydrophobic pocket formed in the active site of the protein into which the damaged DNA base is everted prior to the cleavage event (see figure 3 in ref. 47). Positioning of a bulky AFB1 moiety into close proximity of this pocket would possibly cause significant distortion of the structure and presumably preclude recognition and/or flipping and/or cleavage of the AFB1-FAPY base, despite the existence of the same structural features as the FAPY and Me-FAPY due to the open imidazole ring of the modified dG. In the case of eukaryotic hOGG1 protein (48), which lacks sequence homology with the E.coli Fpg protein, the compact damage-specificity pocket is also present and it is unlikely that the bulky AFB1-FAPY adduct can be efficiently ejected from the helix and excised by the hOGG1 protein. Other than the prokaryotic proteins, the hOGG1 protein was shown to recognize the 8-oxoG damage through interactions on the N7 atom. In addition, as the AFB1-FAPY was shown to stabilize the DNA double-helix presumably due to stacking interactions (19,22), significantly more energy would be required to flip the damaged base out of the helix. Overall, the structural data on the DNA glycosylases, whether prokaryotic or eukaryotic, suggest that the bulky adducts should be poor substrates for those proteins, consistent with our results obtained in vivo. Based on the fact that AFB1-FAPY adducts persist in mammals, one can speculate that these lesions are not removed efficiently by newly discovered enzymes from the NEIL family, even though it has been shown that at least one protein from this family (NEIL1) efficiently excises ring-open purines (reviewed in ref. 49).
Also consistent with that view, our in vitro analysis of the interaction of purified Fpg and hOGG1 proteins with double-stranded DNA containing 8-oxoG, Me-FAPY or AFB1-FAPY suggests that the latter lesion is a poor substrate for both enzymes. No cleavage was observed when the DNA contained AFB1-FAPY lesion, whereas the 8-oxoG and Me-FAPY promoted efficient cleavage by both glycosylases (Figure 4). Furthermore, reduction of the Schiff base reaction intermediates revealed covalent enzyme-DNA complexes only for the 8-oxoG and Me-FAPY reactions, suggesting that the AFB1-FAPY base was not excised by either enzyme.

In contrast to our work, Chetsanga and Frenette (34) reported in their early study that radioactively labeled AFB1-FAPY is cleaved by purified Fpg protein in vitro. Another group (32) also observed nicking of the DNA containing AFB1-FAPY adducts by purified Fpg protein using an in vitro analysis similar to that used in our study. However, others (50) reported only very inefficient cleavage of AFB1-FAPY adducts by the Fpg protein. No comparison of the cleavage efficiency of the AFB1-FAPY adducts with other substrates have been made in any of these studies. Previously, it has been shown that the cleavage efficiency of another bulky lesion, the ring-opened C8-guanine-aminofluorene adduct, by the Fpg protein in vitro is >30-fold less than that of the 8-oxoG lesion (33), a difference that might also account for our results. In experiments by Chetsanga (34) and Oleykowski (32) where the cleavage of the AFB1-FAPY adducts by the Fpg protein was observed, significantly longer DNA substrates (calf thymus DNA and 115 bp double-stranded DNA, respectively) were used, so our 20mer substrates may not have been optimal for Fpg and hOGG1 cleavage. However, the fact that under exactly the same conditions, efficient cleavage was observed for 8-oxoG and Me-FAPY, still suggests that AFB1-FAPY is comparatively a poor substrate for those DNA glycosylases.

It is possible that the different rotamers of AFB1-FAPY adducts (discussed in ref. 14) are recognized differentially by the Fpg protein. In early studies of Fpg protein substrate specificity (33), which were later confirmed by crystallographic analysis of the Fpg protein structure from Thermus thermophilus (51), it was shown that the C8 and C6 keto groups of the damaged purine bases are important for recognition by the enzyme. Other imidazole ring-opened species, FAPY and Me-FAPY, which are substrates for the Fpg protein also form similar rotameric structures (52,53). It has been proposed but not proven (33) that the rotamer that resembles the conformation of 8-oxoG (anti) is preferentially cleaved by the enzyme. The structure of the AFB1-FAPY in double-stranded DNA that has been determined so far by NMR (19) presumably corresponds to the major rotamer, while the structure of the minor rotamer is unknown. A comparison of this structure with the crystal structure of 8-oxoG (54) suggests that the positions of the C8 carboxyl group in those two cases are different. In the case of AFB1-FAPY, a hydrogen bond was predicted between the C8 carboxyl group and NH2 atom of the 3'-adjacent adenine, so it is not clear what would be the position of this functional group in other sequence contexts. Theoretically, it is possible that the minor rotamer positions the C8 carboxyl group in a more favorable position for the Fpg protein and, consequently, may be a better substrate for it. According to Giri and Stone (22), an NMR structure of the minor rotamer is currently under investigation, which may clarify this issue. However, even if the minor rotamer is a better substrate for the Fpg protein, it seems unlikely that it would be meaningful in vivo as this rotamer is unstable and easily converts into the major form at room temperature (14,22).

In summary, we observed that the AFB1-FAPY lesions are preferentially repaired by NER and not BER in vivo. Thus, the difference in repair of the AFB1-FAPY lesions in bacteria versus mammals must be explained by reasons other than differential contribution of the Fpg versus hOGG1 proteins. As it has been proposed earlier (32), possible explanations for this are differential repair of those adducts by mammalian and bacterial NER, or differences in repair and accumulation in different parts of mammalian genome. As with many other DNA synthesis-blocking lesions (55), lesions induced by AFB1 were shown to be recombiningenic (56,57), and it remains to be determined if differential recombination could account for the observed differences between mammals and bacteria.

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References


