Substrate Specificity of *Deinococcus radiodurans* Fpg Protein†

Sema Sentürker,‡,§,|| Cécile Bauche,||,⊥ Jacques Laval,⊥ and Miral Dizdaroglu*,‡

Chemical Science and Technology Laboratory, National Institute of Standards and Technology, Gaithersburg, Maryland 20899-8311, and UMR 8532 CNRS, Institut Gustave Roussy, 94805 Villejuif Cedex, France

Received March 23, 1999; Revised Manuscript Received June 1, 1999

ABSTRACT: A DNA repair enzyme has recently been isolated from the ionizing radiation-resistant bacterium *Deinococcus radiodurans* [Bauche, C., and Laval, J. (1999) *J. Bacteriol. 181*, 262−269]. This enzyme is a homologue of the Fpg protein of *Escherichia coli*. We investigated the substrate specificity of this enzyme for products of oxidative DNA base damage using gas chromatography/isotope-dilution mass spectrometry and DNA substrates, which were either γ-irradiated or treated with H_2O_2/Fe(III)−EDTA/ascorbic acid. Excision of purine lesions 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua), 4,6-diamino-5-formamidopyrimidine (FapyAde), and 8-hydroxyguanine (8-OH-Gua) was observed among 17 lesions detected in damaged DNA substrates. The extent of excision was determined as a function of enzyme concentration, time, and substrate concentration. FapyGua and FapyAde were excised with similar specificities from three DNA substrates, whereas 8-OH-Gua was the least preferred lesion. The results show that *D. radiodurans* Fpg protein and its homologue *E. coli* Fpg protein excise the same modified DNA bases, but the excision rates of these enzymes are significantly different. Formamidopyrimidines are preferred substrates of *D. radiodurans* Fpg protein over 8-OH-Gua, whereas *E. coli* Fpg protein excises these three lesions with similar efficiencies from various DNA substrates. Substrate specificities of these enzymes were also compared with that of *Saccharomyces cerevisiae* Ogg1 protein, which excises FapyGua and 8-OH-Gua, but not FapyAde.

The repair of oxidatively damaged DNA bases is thought to be primarily mediated by base excision repair (*1*). The first step in this repair pathway is the excision of a damaged base by a DNA *N*-glycosylase (*1*). In *Escherichia coli*, the principal DNA glycosylases involved in the repair of oxidized bases are Nth and Nei proteins (endonucleases III and VIII, repectively) and Fpg protein. Nth and Nei proteins process a variety of pyrimidine-derived lesions, whereas Fpg protein acts primarily at purine modifications (*1*−*6*). Functional homologues of these DNA glycosylases have been identified in eukaryotes (reviewed in ref *7*).

Deinococcus radiodurans is a bacterium, which is extremely resistant to the lethal and mutagenic effects of ionizing radiation (*8*, *9*), as well as other physical and chemical DNA-damaging agents, including reactive oxygen species (*10*−*12*). It is believed that this bacterium possesses highly efficient DNA repair mechanisms, explaining this unusual resistance (*10*, *13*), although these mechanisms have not yet been formally established. Therefore, a study of the properties of the proteins responsible for DNA repair in *D. radiodurans* may explain this unusual resistance, and also provide important information about the pathogenesis of cancer and aging. We have previously isolated two different proteins from *D. radiodurans*, which excise oxidized purines by a DNA glycosylase mechanism and are endowed with an AP lyase activity (*14*). Moreover, the results strongly suggested that one of those proteins could be the homologue of *E. coli* Fpg protein, while the other, which is endowed with a thymine glycol DNA glycosylase activity, could be the homologue of the *E. coli* Nei protein (*14*). The *D. radiodurans* Fpg gene, which encodes a protein with 273 amino acids and a molecular mass of 34.8 kDa, was cloned (*14*). This protein, designated *D. radiodurans* Fpg protein, possesses both a DNA glycosylase activity and an AP lyase activity. In contrast to *E. coli* Fpg protein (*15*), *D. radiodurans* Fpg protein is able to recognize and repair the 8-oxoG/A mismatch at a detectable rate.

The objective of this study was to identify the modified DNA bases that are excised from oxidatively damaged DNA by *D. radiodurans* Fpg protein. Furthermore, we investigated the kinetics of excision of these lesions, and thus provide a quantitative comparison of the specificity of this enzyme with that of *E. coli* Fpg protein under similar conditions. We utilized the technique of gas chromatography/isotope-dilution mass spectrometry (GC/IDMS)[1] to detect the modified bases and to measure their excision rates. This technique permits

† This work (in the laboratory of J.L.) was supported by Communautés Européennes, Association pour la Recherche sur le Cancer, by Contrat Radioprotection from Electricité de France, and by a fellowship to C.B. from Ligue contre le Cancer du Val de Marne.

* To whom correspondence should be addressed: National Institute of Standards and Technology, 100 Bureau Dr., Stop 8311, Gaithersburg, MD 20899-8311. Telephone: (301) 975-2581. Fax: (301) 975-8505. E-mail: miral@nist.gov.

‡ National Institute of Standards and Technology.

§ Present address: Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD 20892.

|| These two authors contributed equally to the work.

⊥ Institut Gustave Roussy.

[1] Abbreviations: GC/IDMS, gas chromatography/isotope-dilution mass spectrometry; asc, ascorbic acid; FapyGua, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; FapyAde, 4,6-diamino-5-formamidopyrimidine; 8-OH-Gua, 7,8-dihyro-8-oxoguanine or 8-hydroxyguanine; yOgg1, *Saccharomyces cerevisiae* Ogg1 protein.

10.1021/bi990680m CCC: $18.00
Published on Web 06/23/1999

an extensive and simultaneous identification and quantification of numerous base lesions in a given DNA sample (*16*), and is well suited for the determination of the substrate specificity of DNA repair enzymes (*4*, *5*, *17*−*22*).

EXPERIMENTAL PROCEDURES

Materials.[2] Modified DNA bases, their stable isotope-labeled analogues, and other materials for GC/IDMS were obtained as described previously (*16*). Calf thymus DNA was purchased from Sigma. Calf thymus DNA was dissolved in 10 mM phosphate bufFer (pH 7.4, 0.3 mg/mL). Aliquots of this solution were bubbled with air or N_2O and then γ-irradiated (80 Gy, dose rate of 43 Gy/min), or were treated with H_2O_2/Fe(III)−EDTA/ascorbic acid (asc) as described previously (*19*). DNA solutions were dialyzed against 10 mM phosphate buffer for 18 h at 4 °C. The cloning of the *D. radiodurans* Fpg gene will be reported elsewhere. The isolation of the recombinant *D. radiodurans* Fpg protein was carried out as described previously (*14*). *E. coli* Fpg protein was isolated as described previously (*4*).

Enzymatic Assays and GC/IDMS. Aliquots of DNA samples (100 μg) were dried in a SpeedVac under vacuum and were then dissolved in 100 μL of phosphate buffer (50 mM final concentration, pH 7.4) containing 100 mM KCl, 2 mM EDTA, and 2 mM dithiothreitol. Depending on the experiment, various amounts of *D. radiodurans* Fpg protein were added to the mixture and three replicates of each mixture were incubated at 37 °C for various periods of time. As controls, DNA samples were incubated with the heat-inactivated enzyme or without the enzyme. The kinetic parameters were determined as described previously (*18*). The amount of *D. radiodurans* Fpg protein was 1 μg per 100 μg of DNA in 100 μL of the incubation mixture. This amount corresponded to an enzyme concentration of 260 nM. In an additional experiment, aliquots of three DNA substrates were incubated with *E. coli* Fpg protein (1 μg/100 μg of DNA) at 37 °C for 30 min. Following incubation, DNA samples were precipitated with 260 μL of cold ethanol (kept at −20 °C) and centrifuged at 15000*g* for 30 min at 4 °C. Subsequently, DNA pellets and supernatant fractions were separated. Aliquots of stable isotope-labeled analogues of modified DNA bases were added as internal standards to pellets with known DNA amounts and to supernatant fractions. Both fractions were analyzed by GC/IDMS as described previously (*17*−*22*).

RESULTS

Three different DNA substrates, which were prepared by γ-irradiation under air or N_2O, or by treatment with H_2O_2/Fe(III)−EDTA/asc, were used to investigate the ability of *D. radiodurans* Fpg protein to recognize and release modified bases from oxidatively damaged DNA. Seventeen modified bases in these DNA substrates were identified and quantified using GC/IDMS (*17*). Of these lesions, *D. radiodurans* Fpg protein excised 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua), 4,6-diamino-5-formamidopyrimidine (FapyAde), and 8-hydroxyguanine (8-OH-Gua). Control experiments showed that essentially no excision of FapyGua, FapyAde, and 8-OH-Gua was observed when DNA samples were incubated in the presence of the heat-inactivated enzyme or without the enzyme. The remaining 14 lesions were not significantly excised from DNA substrates by the active enzyme.

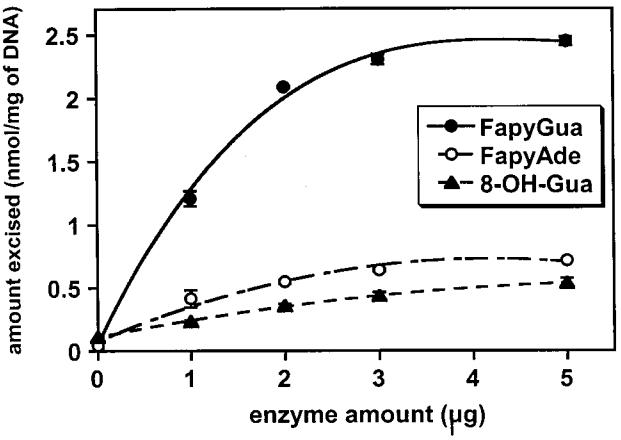

FIGURE 1: Excision of FapyGua, FapyAde, and 8-OH-Gua by *D. radiodurans* Fpg protein as a function of enzyme amount. DNA γ-irradiated under N_2O (100 μg) was used as a substrate. The incubation time was 30 min. The amounts given on the *y*-axis represent those found in the supernatant fractions. One nanomole of a lesion corresponds to ≈32 lesions/10^5 DNA bases.

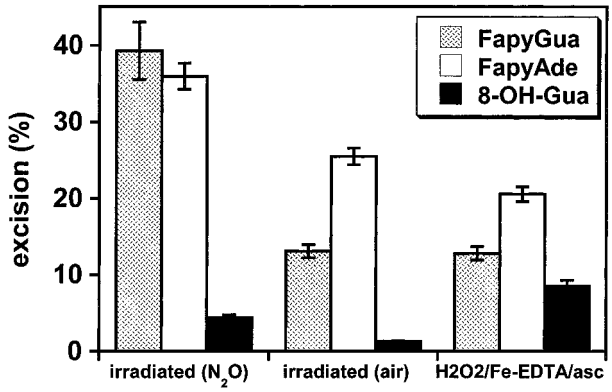

FIGURE 2: Percent excision of FapyGua, FapyAde, and 8-OH-Gua by *D. radiodurans* Fpg protein from three DNA substrates. The incubation time was 30 min. The enzyme amount was 1 μg/100 μg of DNA.

Figure 1 illustrates excision of FapyGua, FapyAde, and 8-OH-Gua from γ-irradiated DNA as a function of enzyme amount. The levels of the lesions increased with the increasing enzyme amount, approaching a plateau above 3 μg of the enzyme. For all subsequent experiments, 1 μg of *D. radiodurans* Fpg protein/100 μg of DNA was used. The extent of excision increased as a function of incubation time and approached a plateau at 30 min of incubation (data not shown).

Percent excisions (excised amount of the lesion × 100/amount of the lesion in DNA) of the lesions from three DNA substrates are shown in Figure 2. 8-OH-Gua was the least preferred lesion. At this enzyme concentration, the percent of excision of 8-OH-Gua from γ-irradiated DNA substrates was approximately 4% (N_2O) and 1.5% (air). In the case of

[2] Certain commercial equipment or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

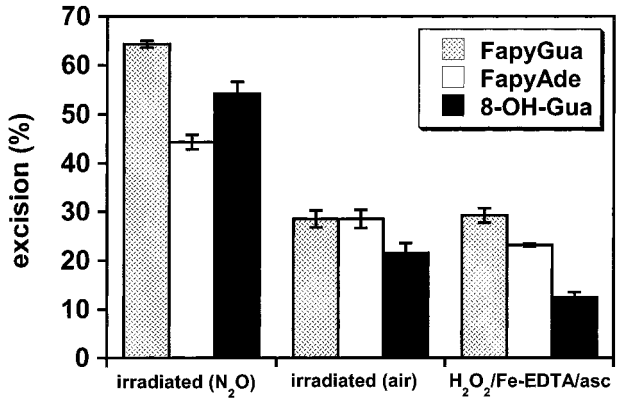

FIGURE 3: Percent excision of FapyGua, FapyAde, and 8-OH-Gua by *E. coli* Fpg protein from three DNA substrates. The incubation time was 30 min. The enzyme amount was 1 μg/100 μg of DNA.

Table 1: Maximum Velocities for Excision of Purine Lesions by *D. radiodurans* Fpg Protein from DNA Treated with Various Free Radical-Generating Systems

	V_{max} (nM min^{-1})[a]		
	irradiation (N_2O)	irradiation (air)	H_2O_2/ Fe(III)−EDTA/asc
FapyGua	$135 \pm 23^{b,c}$	$31.0 \pm 2.1^{c,d}$	$56.6 \pm 1.9^{d,e}$
FapyAde	$99.5 \pm 17^{b,c}$	20.8 ± 1.5	29.2 ± 2.9^{e}
8-OH-Gua	−	−	14.2 ± 1.0

[a] Values represent the mean ± standard deviation ($n = 6$). [b] Statistically different from the value in column 2 ($P < 0.05$). [c] Statistically different from the value in column 3 ($P < 0.05$). [d] Statistically different from the value in line 2 ($P < 0.05$). [e] Statistically different from the value in line 3 ($P < 0.05$).

DNA treated with H_2O_2/Fe(III)−EDTA/asc, a greater percent of excision of 8-OH-Gua was observed. However, this lesion was again the least preferred substrate. As a control, a similar experiment was carried out using *E. coli* Fpg protein instead of *D. radiodurans* Fpg protein. Figure 3 illustrates the percent excision of FapyGua, FapyAde, and 8-OH-Gua by *E. coli* Fpg protein from three DNA substrates. In contrast to that with *D. radiodurans* Fpg protein, 8-OH-Gua was significantly excised and its percent excision was comparable to those of FapyGua and FapyAde.

Kinetic parameters were obtained from measurements at six different concentrations of each lesion (*18*). Concentration ranges were as follows: 0.6−4.1 μM for FapyGua, 0.2−1.5 μM for FapyAde, and 0.5−3.1 μM for 8-OH-Gua in DNA γ-irradiated under N_2O, 0.4−1.7 μM for FapyGua, 0.2−0.65 μM for FapyAde, and 0.6−3.4 μM for 8-OH-Gua in DNA γ-irradiated under air, and 1.5−5.5 μM for FapyGua, 0.2−1.0 μM for FapyAde, and 0.6−1.8 μM for 8-OH-Gua in DNA treated with H_2O_2/Fe(III)−EDTA/asc. Initial velocities were estimated on the basis of the time dependency of excision. The *N*-glycosylase activity of *D. radiodurans* Fpg protein on the excised lesions followed Michaelis−Menten kinetics. The kinetic constants and standard deviations ($n = 6$) were determined using linear Lineweaver−Burk plots (*23*) and a linear least-squares analysis of the data. The kinetic parameters are given in Tables 1−3. Small amounts of 8-OH-Gua excised from γ-irradiated DNA substrates prevented an accurate determination of the kinetic constants of this compound. Table 3 also shows a comparison of the specificity constants for excision by *D. radiodurans* Fpg protein with those by *E. coli* Fpg and *Saccharomyces cerevisiae* Ogg1 (yOgg1) proteins.

Table 2: Michaelis Contants for Excision of Purine Lesions by *D. radiodurans* Fpg Protein from DNA Treated with Various Free Radical-Generating Systems

	K_M (nM)[a]		
	irradiation (N_2O)	irradiation (air)	H_2O_2/ Fe(III)−EDTA/asc
FapyGua	$6030 \pm 1060^{b,c}$	$3169 \pm 1016^{c,d}$	1975 ± 103^{d}
FapyAde	$5904 \pm 1004^{b,c}$	751 ± 138^{c}	1050 ± 113^{e}
8-OH-Gua	−	−	1710 ± 142

[a] Values represent the mean ± standard deviation ($n = 6$). [b] Statistically different from the value in column 2 ($P < 0.05$). [c] Statistically different from the value in column 3 ($P < 0.05$). [d] Statistically different from the value in line 2 ($P < 0.05$). [e] Statistically different from the value in line 3 ($P < 0.05$).

Maximum velocities (V_{max}) of FapyGua and FapyAde excisions from DNA γ-irradiated under N_2O were similar. The maximum velocity of FapyGua excision was greater than that of FapyAde excision from the other two DNA substrates, and than that of 8-OH-Gua excision from DNA treated with H_2O_2/Fe(III)−EDTA/asc. A similar order was observed with the K_M values, with the exception of DNA treated with H_2O_2/Fe(III)−EDTA/asc. In this case, the K_M values for FapyGua and 8-OH-Gua excisions were similar, and the specificity constants (k_{cat}/K_M) of FapyGua and FapyAde excision were approximately 3.5-fold greater than that of 8-OH-Gua. For all three DNA substrates, the k_{cat}/K_M values of FapyGua excision were similar. The specificity of the enzyme for FapyAde excision was lowest in the case of DNA γ-irradiated under N_2O.

DISCUSSION

The results show the ability of *D. radiodurans* Fpg protein to excise three purine-derived lesions from damaged DNA. Low extents of excision of 8-OH-Gua from γ-irradiated DNA substrates prevented an accurate determination of the kinetic parameters for these substrates. FapyGua and FapyAde were the preferred substrates of *D. radiodurans* Fpg protein over 8-OH-Gua. In each case of DNA substrates, FapyGua and FapyAde were excised with similar specificities, indicating a similar preference of the enzyme for these compounds. FapyGua was excised from all three DNA substrates with a similar preference. The excision of FapyAde from DNA γ-irradiated under N_2O was less preferred than those from the other two DNA substrates, indicating a dependence of excision on the nature of the DNA substrate. Such a dependence was also observed in the case of other DNA glycosylases (*17*, *19*, *20*, *22*).

It should be pointed out that the K_M values for excision of FapyGua, FapyAde, and 8-OH-Gua by *D. radiodurans* Fpg protein are greater than those reported for excision of 2,6-diamino-4-hydroxy-5-*N*-methylformamidopyrimidine (Me-FapyGua) and 8-OH-Gua from oligonucleotides by the same enzyme (*14*). This difference may relate to the specific assay and different DNA substrates used in each case. When DNA substrates such as those in this work are used, the enzyme acts on multiple lesions simultaneously. In this case, each modified base may act as a competitor and an inhibitor with respect to others for excision. As a result, the kinetics of excision from DNA substrates with multiple lesions may be

Table 3: Comparison of the Specificity Contants for Excision of Purine Lesions by *E. coli* and *D. radiodurans* Fpg Proteins and yOgg1 Protein from DNA Treated with Various Free Radical-Generating Systems

	$k_{cat}/K_M \times 10^5$ (min^{-1} nM^{-1})[a]								
	irradiation (N_2O)			irradiation (air)			H_2O_2/Fe(III)−EDTA/asc		
	E. coli	yOgg1	*D. radio.*	*E. coli*	yOgg1	*D. radio.*	*E. coli*	yOgg1	*D. radio.*
FapyGua	$16.5 \pm 2.6^{b-i}$	$1.5 \pm 0.1^{c-j}$	$8.6 \pm 0.9^{e,h}$	$5.0 \pm 1.0^{e,h}$	$2.7 \pm 0.6^{f,g,i}$	7.8 ± 0.3^{h}	9.0 ± 0.9^{h}	3.7 ± 0.2^{i}	11.0 ± 0.2^{j}
FapyAde	$11.2 \pm 1.2^{c,d,g}$	−	6.5 ± 0.6^{f}	$5.2 \pm 0.2^{f,i}$	−	10.7 ± 0.4^{g}	6.5 ± 0.6	−	10.7 ± 0.6^{j}
8-OH-Gua	$11.4 \pm 1.2^{b,d,e,i}$	$6.5 \pm 0.4^{e,i}$	−	5.5 ± 0.5^{h}	3.5 ± 0.2^{g}	−	7.4 ± 1.2^{i}	−	3.2 ± 0.1

[a] Values represent the mean ± standard deviation ($n = 6$). $k_{cat} = V_{max}$/[enzyme]. [*E. coli* Fpg] = 280 nM. [*D. radiodurans* Fpg] = 260 nM. [yOgg1] = 212 nM. [b] Statistically different from the value in column 2 ($P < 0.05$). [c] Statistically different from the value in column 3 ($P < 0.05$). [d] Statistically different from the value in column 4 ($P < 0.05$). [e] Statistically different from the value in column 5 ($P < 0.05$). [f] Statistically different from the value in column 6 ($P < 0.05$). [g] Statistically different from the value in column 7 ($P < 0.05$). [h] Statistically different from the value in column 8 ($P < 0.05$). [i] Statistically different from the value in column 9 ($P < 0.05$). [j] Statistically different from the value in line 3.

different from those obtained with oligonucleotides containing a single lesion only. High K_M values observed in this work are in agreement with previous work on specificities of other DNA glycosylases (*17*−*22*).

FapyGua, FapyAde, and 8-OH-Gua are also substrates of *E. coli* Fpg protein (*3*, *4*, *17*). As *D. radiodurans* Fpg protein, this enzyme does not recognize any other lesions in damaged DNA (*4*, *17*), although the excision of some pyrimidine-derived lesions from small oligonucleotides containing one single lesion has been reported (*1*). Since we had previously determined the kinetic parameters for excision of FapyGua, FapyAde, and 8-OH-Gua by *E. coli* Fpg protein using DNA substrates similar to those in this work (*17*), a comparison of excision kinetics of both enzymes under similar experimental conditions was possible. Table 3 shows the comparison of the specificity constants. Specificity constants for excision of FapyGua and 8-OH-Gua by yOgg1 protein from similar DNA substrates (*19*) are also included in Table 3. This enzyme does not excise FapyAde (*19*). The comparison shows that there are no statistically significant differences between the k_{cat}/K_M values for excision of FapyGua, FapyAde, and 8-OH-Gua by *E. coli* Fpg protein from each of the DNA substrates. This indicates the same preference of the enzyme for all three lesions in each case. The specificity constants of FapyGua and FapyAde excisions are similar for both enzymes in each case of the DNA substrates. In contrast, there is an approximately 3.5-fold difference between the k_{cat}/K_M values of 8-OH-Gua and those of FapyGua and FapyAde in the case of *D. radiodurans* Fpg protein and DNA substrate treated with H_2O_2/Fe(III)−EDTA/asc. No specificity constants for excision of 8-OH-Gua from γ-irradiated DNA substrates could be determined because of low extents of excision. In contrast, *E. coli* Fpg protein excises FapyGua, FapyAde, and 8-OH-Gua from all three DNA substrates with a similar specificity (Table 3 and ref *17*). This comparison shows a significant difference between the activities of these enzymes in terms of 8-OH-Gua excision. Percent excisions shown in Figures 2 and 3 lend credence to this fact.

There were also significant differences between the two enzymes pertaining to the excison of a lesion from the same DNA substrate. The specificity constants for excision of FapyGua and FapyAde by *E. coli* Fpg protein from DNA γ-irradiated under N_2O were significantly greater (2-fold) than those by *D. radiodurans* Fpg protein, indicating the preferred excision of these lesions by the former enzyme. However, this was reversed in the case of DNA γ-irradiated under air. Similar specificity constants for both enzymes were observed in the case of DNA treated with H_2O_2/Fe(III)−EDTA/asc, indicating a similar preference of both enzymes for excision of FapyGua and FapyAde, whereas *E. coli* Fpg protein had a significantly greater preference for excision of 8-OH-Gua. The excision of FapyGua was less preferred by yOgg1 protein than by *E. coli* and *D. radiodurans* Fpg proteins. In the case of γ-irradiated DNA substrates, 8-OH-Gua was excised more preferentially by *E. coli* Fpg protein than by yOgg1 protein. There were significant differences between *D. radiodurans* Fpg and yOgg1 proteins in terms of 8-OH-Gua excision, as this compound was excised by yOgg1 protein from γ-irradiated DNA substrates, but not from DNA treated with H_2O_2/Fe(III)−EDTA/asc. These comparisons clearly indicate significant differences between the substrate specificities of these homologous enzymes from three different organisms.

In conclusion, the results show that *D. radiodurans* Fpg protein excises three purine-derived lesions from oxidatively damaged DNA substrates. Other oxidized bases detected in DNA substrates were not excised. Excision rates of FapyGua and FapyAde are significantly greater than that of 8-OH-Gua, indicating the preference of *D. radiodurans* Fpg protein for formamidopyrimidines over 8-OH-Gua. Its homologue *E. coli* Fpg protein also excises the same lesions from damaged DNA. However, there are significant differences between the excision kinetics of these enzymes. A comparison of excision rates of *E. coli* and *D. radiodurans* Fpg proteins and yOgg1 protein shows that these homologous enzymes possess different substrate specificities.

REFERENCES

1. Wallace, S. S. (1998) *Radiat. Res. 150*, S60−S79.
2. Breimer, L. H. (1984) *Nucleic Acids Res. 12*, 6359−6367.
3. Tchou, J., Kasai, H., Shibutani, S., Chung, M. H., Laval, J., Grollman, A. P., and Nishimura, S. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 4690−4694.
4. Boiteux, S., Gajewski, E., Laval, J., and Dizdaroglu, M. (1992) *Biochemistry 31*, 106−110.
5. Dizdaroglu, M., Laval, J., and Boiteux, S. (1993) *Biochemistry 32*, 12105−12111.
6. Jiang, D., Hatahet, Z., Melamede, R. J., Kow, Y. W., and Wallace, S. S. (1997) *J. Biol. Chem. 272*, 32230−32239.
7. Laval, J., Jurado, J., Saparbaev, M., and Sidorkina, O. (1998) *Mutat. Res. 402*, 93−102.
8. Anderson, A. W., Norden, H. C., Cain, R. F., Parrish, G., and Duggan, D. (1956) *Food Technol. 10*, 575−578.
9. Battista, J. R. (1997) *Annu. Rev. Microbiol. 51*, 203−224.
10. Moseley, B. E. B. (1983) *Photochem. Photobiol. Rev. 7*, 223−274.
11. Murray, R. G. E., and Brooks, B. W. (1986) in *Bergey's Manual of Systematic Bacteriology* (Sneath, P., Mair, N.,

Sharpe, M., and Holt, J., Eds.) Vol. 2, pp 1035−1043, Williams and Wilkins, Baltimore.
12. Smith, M. D., Masters, C. I., and Moseley, B. E. B. (1992) in *Molecular Biology and Biothechnology of Extremophiles* (Herbert, R. A., and Sharp, R. J., Eds.) pp 258−280, Chapman & Hill, New York.
13. Minton, K. W. (1994) *Mol. Microbiol. 13*, 9−15.
14. Bauche, C., and Laval, J. (1999) *J. Bacteriol. 181*, 262−269.
15. Castaing, B., Geiger, A., Seliger, H., Nehls, P., Laval, J., Zelwer, C., and Boiteux, S. (1993) *Nucleic Acids Res. 21*, 2899−2905.
16. Dizdaroglu, M. (1994) *Methods Enzymol. 234*, 3−16.
17. Karakaya, A., Jaruga, P., Bohr, V. A., Grollman, A. P., and Dizdaroglu, M. (1997) *Nucleic Acids Res. 25*, 474−479.
18. Deutsch, W. A., Yacoub, A., Jagura, P., Zastawny, T. H., Madden, C., and Dizdaroglu, M. (1997) *J. Biol. Chem. 272*, 32857−32860.
19. Karahalil, B., Girard, P. M., Boiteux, S., and Dizdaroglu, M. (1998) *Nucleic Acids Res. 26*, 1228−1232.
20. Karahalil, B., Roldan-Arjona, T., and Dizdaroglu, M. (1998) *Biochemistry 37*, 590−595.
21. Sentürker, S., Auffret van der Kemp, P., You, H.-J., Doetsch, P. W., Dizdaroglu, M., and Boiteux, S. (1998) *Nucleic Acids Res. 26*, 5270−5276.
22. Dizdaroglu, M., Karahalil, B., Sentürker, S., Buckley, T. J., and Roldán-Arjona, T. (1999) *Biochemistry 38*, 243−246.
23. Gutfreund, H. (1972) *Enzymes: Physical Principals*, pp 116−175, Wiley-Interscience, London.

BI990680M