Substrate Specificity of the *Escherichia coli* Fpg Protein (Formamidopyrimidine–DNA Glycosylase): Excision of Purine Lesions in DNA Produced by Ionizing Radiation or Photosensitization†

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ABSTRACT: We have investigated the excision of a variety of modified bases from DNA by the *Escherichia coli* Fpg protein (formamidopyrimidine–DNA glycosylase) [Boiteux, S., O'Connor, T. R., Lederer, F., Gouyette, A., & Laval, J. (1990) *J. Biol. Chem. 265*, 3916–3922]. DNA used as a substrate was modified either by exposure to ionizing radiation or by photosensitization using visible light in the presence of methylene blue (MB). The technique of gas chromatography/mass spectrometry, which can unambiguously identify and quantitate pyrimidine- and purine-derived lesions in DNA, was used for analysis of hydrolyzed and derivatized DNA samples. Thirteen products resulting from pyrimidines and purines were detected in γ-irradiated DNA, whereas only the formation of 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) and 8-hydroxyguanine (8-OH-Gua) was observed in visible light/MB-treated DNA. Analysis of γ-irradiated DNA after incubation with the Fpg protein followed by precipitation revealed that the Fpg protein significantly excised 4,6-diamino-5-formamidopyrimidine (FapyAde), FapyGua, and 8-OH-Gua. The excision of a small but detectable amount of 8-hydroxyadenine was also observed. The detection of these products in the supernatant fractions of the same samples confirmed their excision by the enzyme. Nine pyrimidine-derived lesions were not excised. The Fpg protein also excised FapyGua and 8-OH-Gua from visible light/MB-treated DNA. The presence of these products in the supernatant fractions confirmed their excision. The results suggest that one of the biological roles of the Fpg protein, which is present in bacteria as well as in mammalian cells, is the repair of DNA damage caused by free radicals or by other oxygen-derived species such as singlet oxygen. The Fpg protein appears to be specific for recognition of imidazole ring opened purines and 8-hydroxypurines in DNA and may complement pyrimidine-specific enzymes in repair of DNA damage in vivo.

Oxygen-derived species such as superoxide radical (O_2^-),[1] H_2O_2, and singlet oxygen ($^1\Delta_gO_2$) are formed in cells during aerobic metabolism and during oxidative stress [for a review see Halliwell and Gutteridge (1989)]. Generation of these species by endogenous and exogenous sources may result in damage to biomolecules including DNA. Thus oxygen-derived species may be mutagenic and carcinogenic (Ames, 1983; Cerutti, 1985; Weitzman et al., 1985; Decuyper-Debergh et al., 1987; Breimer, 1990). However, neither O_2^- nor H_2O_2 under physiological conditions produces any DNA damage (Lesko et al., 1980; Rowley & Halliwell, 1983; Sagripanti & Kraemer, 1989; Aruoma et al., 1989; Blakely et al., 1990). Thus the toxicity of these species is thought to result from their metal ion catalyzed conversion into the highly reactive hydroxyl radical (·OH) [for a review see Halliwell and Gutteridge (1989)]. Ionizing radiation also exerts its biological effects mainly by free radicals (·OH, H atom, hydrated electron) produced from cellular water, in particular by ·OH [for a review see von Sonntag (1987)]. Hydroxyl radicals generate base and sugar modifications in DNA and DNA–protein cross-links in nucleoprotein [for reviews see Têoule and Cadet (1978), von Sonntag (1987), and Oleinick et al. (1987)]. On the other hand, $^1\Delta_gO_2$ is not a free radical, but it can be formed in radical reactions or during oxidative stress simultaneously with radical production (Sies, 1986; Halliwell & Gutteridge, 1989). It is also generated by photosensitization when certain molecules including some found in vivo are illuminated with light of a specific wavelength. Photosensitization reactions, probably involving $^1\Delta_gO_2$, are important in many biological situations including several diseases (Halliwell & Gutteridge, 1989). Studies indicated that $^1\Delta_gO_2$ is the predominant species, which damages DNA in photosensitization (Epe et al., 1988). Recently, photosensitization by visible light in the presence of methylene blue (MB) has been reported to cause the formation of 8-hydroxyguanine (8-OH-Gua) in DNA (Floyd et al., 1989; Schneider et al., 1990). So far, formation of other products has not been reported.

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[1] Abbreviations: O_2^-, superoxide radical; $^1\Delta_gO_2$, singlet oxygen; ·OH, hydroxyl radical; Fapy, formamidopyrimidine; MB, methylene blue; 5,6-diHThy, 5,6-dihydrothymine; 5-OH-5-Me-Hyd, 5-hydroxy-5-methylhydantoin; 5-OH-Hyd, 5-hydroxyhydantoin; 5-OH-6-HThy, 5-hydroxy-6-hydrothymine; 5-OH-6-HCyt, 5-hydroxy-6-hydrocytosine; 5-OHMe-Ura, 5-(hydroxymethyl)uracil; Cyt, cytosine; Thy, thymine; 5,6-diOH-Cyt, 5,6-dihydroxycytosine; FapyAde, 4,6-diamino-5-formamidopyrimidine; 8-OH-Ade, 8-hydroxyadenine; FapyGua, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; 8-OH-Gua, 8-hydroxyguanine; 8-OH-dG, 8-hydroxy-2′-deoxyguanosine; BSTFA, bis(trimethylsilyl)trifluoroacetamide; GC/MS-SIM, gas chromatography/mass spectrometry with selected-ion monitoring; Gy, gray ($J \cdot kg^{-1}$); nd, not detected.

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DNA damage caused by oxygen-derived species is thought to contribute to the mutagenic, carcinogenic, and lethal effects of these species [for a review see Breimer (1990)]. Specific repair enzymes in cells are known to eliminate some types of DNA damage [for reviews see Wallace (1988) and Sancar and Sancar (1988)]. Among the repair enzymes, a DNA glycosylase from *Escherichia coli* [the formamidopyrimidine (Fapy)–DNA glycosylase] has been shown to recognize purines with an opened imidazole ring (Chetsanga & Lindahl, 1979; Breimer, 1984; Boiteux et al., 1984). This enzyme also exists in mammalian cells (Margison & Pegg, 1981) and has been partially purified (Breimer, 1983; Laval et al., 1990a). The excision by the Fapy–DNA glycosylase of 4,6-diamino-5-formamidopyrimidine (FapyAde), an ·OH-induced product of adenine, from γ-irradiated polydeoxyribonucleotides of adenine has been demonstrated (Breimer, 1984). Moreover, various damaged pyrimidines and purines in DNA are repaired in vitro and in vivo by the UvrABC complex (Lin & Sancar, 1989; Kow et al., 1990; Czeczot et al., 1991).

The *E. coli fpg*$^+$ gene coding for the Fapy–DNA glycosylase has been cloned recently, and the Fpg protein has been purified to homogeneity (Boiteux et al., 1987, 1990). The Fapy–DNA glycosylase also excises the imidazole ring opened form of guanine modified at the N-7 or C-8 position by various DNA-damaging agents (Boiteux et al., 1989; Laval et al., 1990b). In addition to its DNA glycosylase activity, this protein has an associated β-lyase activity that nicks DNA at apurinic/apyrimidic (AP) sites (O'Connor & Laval, 1989; Bailly et al., 1989). Since the Fapy–DNA glycosylase from *E. coli* possesses a broad substrate specificity and two enzyme activities, it has been named the Fpg protein (Boiteux et al., 1990). The Fpg protein also recognizes $^1\Delta_g O_2$-induced DNA base modifications, which have not yet been identified (Müller et al., 1990). The excision of 8-OH-Gua by the Fpg protein from small duplex oligonucleotides containing a single 8-OH-Gua residue has been reported (Tchou et al., 1991). However, the excision by the Fpg protein of this lesion or any other free radical induced or $^1\Delta_g O_2$-induced products of pyrimidines and purines in DNA has not been demonstrated.

In the present work, we have investigated the ability of the Fpg protein to excise modified pyrimidine and purine bases from DNA exposed to visible light in the presence of MB or to ionizing radiation. The products excised (or nonexcised) from DNA by the Fpg protein have been unambiguously characterized by gas chromatography/mass spectrometry (GC/MS).

Experimental Procedures

Materials.[2] Calf thymus DNA, 6-azathymine, and 8-azaadenine were purchased from Sigma Chemical Co. Dialysis membranes with a molecular weight cutoff of 3500 were obtained from Fisher Scientific Co. Acetonitrile and bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane were from Pierce Chemical Co. Formic acid (88%) was purchased from Mallinckrodt.

Irradiations. Calf thymus DNA was dissolved in 10 mM phosphate buffer (pH 7.4) (0.25 mg of DNA/mL) and then dialyzed extensively against 10 mM phosphate buffer. Subsequently, DNA solutions were saturated with N_2O for 30 min and then irradiated in a ^{60}Co γ-source (dose rate 98.4 Gy/min). The total radiation dose applied was 42 or 150 Gy. After irradiation, samples were dialyzed against phosphate buffer (pH 7.4). Aliquots of unirradiated and irradiated DNA samples were dried in a SpeedVac under vacuum at room temperature.

Treatment with Visible Light and Methylene Blue. A solution of calf thymus DNA (0.1 mg/mL) in 10 mM phosphate buffer (pH 7.4) was extensively dialyzed against the same buffer. Subsequently, 10 mL of the DNA solution in a 10-cm glass dish was supplemented with methylene blue (MB) (10 μg/mL final concentration) and then illuminated for 60 min at 4 °C with two 100-W lamps (Phillips), which were placed at a distance of 20 cm from the DNA solution. The visible light/MB-treated DNA was precipitated twice with ethanol, redissolved in water, and dialyzed against 10 mM phosphate buffer (pH 7.4).

Purification of the Fpg Protein. The Fpg protein was isolated from overproducing *E. coli* strain harboring the pFPG230 plasmid and purified to apparent homogeneity as previously described (Boiteux et al., 1990). The concentration of the fraction used was 1 mg/mL, and the specific activity was 150000 units/mg, which was measured using DNA containing the imidazole ring opened form of N^7-methylguanine as a substrate (Boiteux et al., 1984).

Enzymatic Assays. The standard reaction mixture (100 μL final volume) contained 50 mM phosphate buffer (pH 7.4), 100 mM KCl, 50 μg of γ-irradiated DNA or visible light/MB-treated DNA, and 6 μg of the homogeneous Fpg protein. When indicated (boiled enzyme), the Fpg protein was inactivated by heat treatment at 90 °C for 10 min. Reaction mixtures were incubated at 37 °C for 20 min. Subsequently, 250 μL of cold ethanol (−20 °C) was added to each sample, and the resulting mixture was kept at −20 °C for 30 min. The sample was centrifuged for 15 min at 4 °C in an Eppendorf microfuge. The precipitated DNA (pellet) and the supernatant fraction were separated and dried in a SpeedVac under vacuum at room temperature.

Hydrolysis and Derivatization. Pellets were dissolved in water and the absorbance at 260 nm of each sample was measured in order to calculate the amount of DNA (absorbance of 1 = 50 μg of DNA/mL). To supernatant fractions and to ≈30 μg of DNA pellets was added 0.5 nmol each of 6-azathymine and 8-azaadenine as internal standards. Samples were then dried in a SpeedVac under vacuum at room temperature. Dried pellets were hydrolyzed with 0.5 mL of formic acid (70%) in evacuated and sealed tubes for 30 min at 140 °C. This was followed by lyophilization. Dried supernatant fractions were not hydrolyzed. Subsequently, the samples were trimethylsilylated in poly(tetrafluoroethylene)-capped hypovials (Pierce Chemical Co.) with 60 μL of a mixture of BSTFA and acetonitrile (2/1 v/v) by heating for 30 min at 130 °C.

Gas Chromatography/Mass Spectrometry (GC/MS). An aliquot (4 μL) of each derivatized sample was injected without further treatment onto the injection port of the gas chromatograph by means of an automatic injector. Separations were carried out on a fused silica capillary column (12.5 m × 0.2 mm i.d.) coated with cross-linked 5% phenylmethylsilicone gum (film thickness 0.33 μm) (Hewlett-Packard). The amount of DNA in pellets injected onto the column for each analysis was ≈0.2 μg. The temperature of the column was programmed from 150 to 260 °C at 8 °C/min after 2 min at 150 °C. Equipment and other details of analysis were as described elsewhere (Fuciarelli et al., 1989; Dizdaroglu, 1990, 1991).

Results

The objective of this work was to investigate the ability of

[2] Certain commercial equipment or materials are identified in this paper in order 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.

Table I: Yields of Products in Pellets of DNA and in Supernatant Fractions

product (nmol/mg of DNA)[a]	treatment[b] 1	2	3	4	5
5,6-diHThy	nd	0.116 ± 0.015	0.107 ± 0.032	0.096 ± 0.016	nd
5-OH-5-Me-Hyd	0.056 ± 0.010	0.686 ± 0.165	0.495 ± 0.092	0.692 ± 0.150	nd
5-OH-Hyd	0.014 ± 0.020	0.207 ± 0.040	0.163 ± 0.019	0.161 ± 0.019	nd
5-OH-6-HThy	nd	0.204 ± 0.013	0.177 ● 0.011	0.192 ± 0.008	nd
5-OH-6-HCyt	nd	0.159 ± 0.020	0.176 ± 0.018	0.155 ± 0.016	nd
5-OHMe-Ura	nd	0.135 ± 0.050	0.135 ± 0.015	0.115 ± 0.026	nd
Cyt glycol	0.061 ± 0.011	0.645 ● 0.021	0.665 ± 0.059	0.650 ± 0.080	nd
Thy glycol	0.045 ± 0.013	0.675 ± 0.017	0.540 ± 0.070	0.575 ± 0.082	nd
5,6-diOH-Cyt	nd	0.078 ± 0.010	0.064 ± 0.009	0.060 ± 0.015	nd
FapyAde	0.085 ± 0.013	1.22 ± 0.024	1.13 ± 0.168	0.678 ± 0.102	0.630 ± 0.240
8-OH-Ade	0.204 ± 0.023	1.12 ± 0.056	1.11 ± 0.079	0.977 ± 0.059	0.166 ± 0.010
FapyGua	0.136 ± 0.003	1.76 ± 0.333	1.63 ± 0.244	0.761 ± 0.056	1.39 ± 0.510
8-OH-Gua	0.347 ± 0.059	2.67 ± 0.084	2.86 ± 0.155	1.17 ± 0.217	2.33 ± 0.470

[a] Values represent the mean ± standard deviation from three independent measurements. [b] Treatment: 1, unirradiated DNA (pellet); 2, γ-irradiated DNA (pellet); 3, pellet from γ-irradiated DNA after incubation with the boiled Fpg protein; 4, pellet from γ-irradiated DNA after incubation with the native Fpg protein; 5, supernatant fraction from γ-irradiated DNA after incubation with the native Fpg protein.

the *E. coli* Fpg protein (Boiteux et al., 1990) to excise various modified DNA bases. DNA used as a substrate for this purpose was modified either by ionizing-radiation-generated free radicals or by a nonradical pathway, namely, by photosensitization using visible light in the presence of MB. Ionizing-radiation-generated free radicals are known to produce a variety of products of pyrimidines and purines in DNA, whereas photosensitization by visible light/MB has been reported to produce only 8-OH-Gua in DNA by a mechanism involving $^1\Delta_gO_2$ (see above). In the present work, the technique of GC/MS with selected-ion monitoring (SIM) was used to analyze the DNA samples. This technique provides unequivocal identification and quantitation of a large number of modified bases in DNA [for reviews see Dizdaroglu (1990, 1991)]. This capability of GC/MS-SIM permitted us to examine whether the Fpg protein excises various modified pyrimidine and purine bases from DNA.

Specificity of the Fpg Protein on γ-Irradiated DNA. Irradiation of DNA was carried out in N_2O-saturated aqueous solution. When N_2O is present in solution, hydrated electrons react with N_2O at a diffusion-controlled rate to give additional hydroxyl radicals. The system then consists of ≈90% ·OH and ≈10% H atom in terms of radical species [for a review see von Sonntag (1987)]. Irradiated DNA was incubated with either the native enzyme or the boiled enzyme and subsequently ethanol-precipitated. The precipitates were analyzed by GC/MS-SIM after acidic hydrolysis and trimethylsilylation. The supernatant fractions of the samples were analyzed after lyophilization and trimethylsilylation without acidic hydrolysis. Irradiated DNA and unirradiated DNA without incubation with the enzyme but after precipitation in the incubation buffer were also analyzed as controls. Thirteen modified DNA bases were identified and quantitatively measured. The amounts of the modified bases are given in Table I. DNA used in these studies contained some of the modified bases at detectable levels (Table I, column 1) as was reported previously (Dizdaroglu & Bergtold, 1986). Irradiation of DNA at a dose of 42 Gy significantly increased the amounts of those products above the background levels present in unirradiated DNA and caused formation of other products (Table I, column 2). The amount of 8-OH-Gua, which was the major product in γ-irradiated DNA, corresponds to ≈1 modified base per 10^3 DNA nucleotides. The boiled Fpg protein excised none of the pyrimidine- and purine-derived lesions from γ-irradiated DNA (compare columns 2 and 3 in Table I). None of these modified bases was detectable in the supernatant fraction. In contrast, ≈42% of FapyAde, ≈55% of FapyGua, and ≈58% of 8-OH-Gua were excised by the native enzyme under the conditions used (Table I, column 4). The excision of these compounds was confirmed by their presence in the supernatant fraction (Table I, column 5). The amounts found in the supernatant fraction roughly corresponded to the amounts excised (compare columns 3, 4, and 5). A small amount (≈10–15%) of 8-OH-Ade also appeared to be removed by the native enzyme. This was confirmed by a small but detectable amount of this compound found in the supernatant fraction (Table I, column 5). We did not observe any excision of the other modified bases in significant amounts (compare columns 2, 3, and 4). This was also confirmed by the absence of those modified bases in the supernatant fraction. Excision of modified bases from DNA irradiated at a higher dose than 42 Gy was also investigated. In DNA irradiated at 150 Gy, the amounts of 8-OH-Gua (≈3.2 molecules/10^3 DNA bases) and of other modified bases were ≈2–3-fold higher than those in DNA irradiated at 42 Gy. Similar to the results discussed above, FapyAde and FapyGua were excised by the Fpg protein to an extent of ≈37% and ≈40%, respectively, from DNA irradiated at 150 Gy. In contrast, the excision of 8-OH-Gua and 8-OH-Ade by the Fpg protein was not observed from DNA irradiated at 150 Gy.

Specificity of the Fpg Protein on Visible Light/MB-Treated DNA. Analysis of visible light/MB-treated DNA by GC/MS-SIM revealed the formation of FapyGua in addition to the previously observed 8-OH-Gua (Floyd et al., 1989; Schneider et al., 1990). The amount of 8-OH-Gua was about 20 times greater than that of FapyGua (Figure 1). The formation of FapyGua in DNA by photosensitization has not been reported previously. Other products listed in Table I were not formed by this type of treatment of DNA under the conditions used. Similar to γ-irradiated DNA, the native enzyme excised FapyGua and 8-OH-Gua from visible light/MB-treated DNA (Figure 1). The presence of FapyGua and 8-OH-Gua in the supernatant fraction of the sample confirmed their excision by the native enzyme. The amounts of DNA bases measured in the supernatant fraction corresponded to the amounts excised (Figure 1). The boiled enzyme did not excise these modified bases (Figure 1). Figure 2 illustrates that the Fpg protein excised 8-OH-Gua and FapyGua from visible light/MB-treated DNA at similar rates.

Discussion

The results obtained in the present work demonstrate that photosensitization by visible light/MB gives rise to the formation of FapyGua and 8-OH-Gua in DNA. The formation

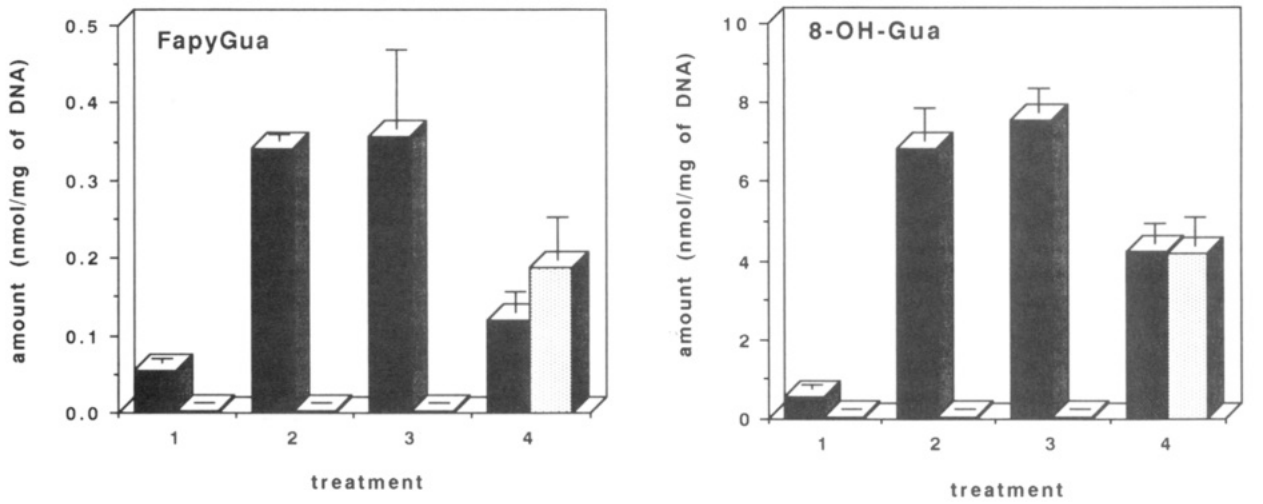

FIGURE 1: Yields of FapyGua and 8-OH-Gua in pellets and in supernatant fractions of visible light/MB-treated DNA. Treatment: 1, control (not exposed) DNA; 2, visible light/MB-treated DNA; 3, visible light/MB-treated DNA after incubation with the boiled Fpg protein; 4, visible light/MB-treated DNA after incubation with the native Fpg protein. Dark bars and dotted bars represent pellets and supernatant fractions, respectively. Graphs represent the mean ± standard deviation from three independent measurements. For assay conditions see Experimental Procedures.

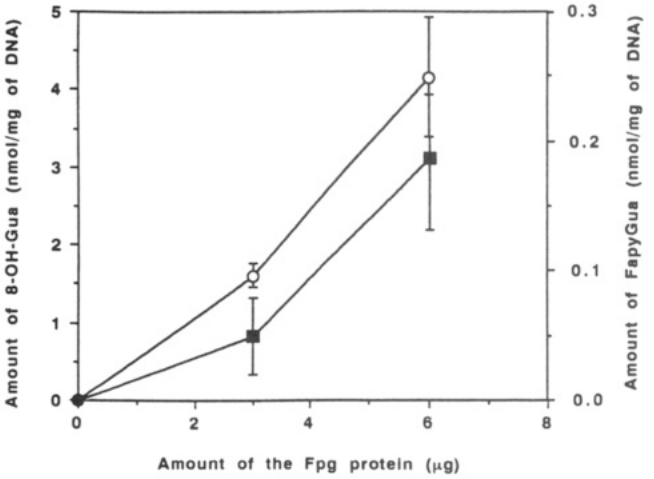

FIGURE 2: Excision of 8-OH-Gua and FapyGua from visible light/MB-treated DNA by different amounts of the Fpg protein. Data indicate the amounts of the products found in the supernatant fractions after the ethanol precipitation of visible light/MB-treated DNA. The graph represents the mean ± standard deviation from three independent measurements. (○) 8-OH-Gua; (■) FapyGua.

of 8-OH-Gua in DNA by this system has been reported previously (Floyd et al., 1989; Schneider et al., 1990) but not that of FapyGua. Furthermore, 8-OH-dG has been reported to result from dG upon exposure to $^1\Delta_gO_2$-producing systems other than visible light/MB (Ravanat et al., 1991; Devasagayam et al., 1991). In the present work, we report that adenine and pyrimidines were not significantly affected under the conditions used. The formation of FapyGua and 8-OH-Gua might be explained by an electron transfer from the guanine ring to $^1\Delta_gO_2$, resulting in generation of a guanine radical cation and O_2^-. Further reactions of the guanine radical cation may result in the formation of a C-8 OH adduct radical of guanine (Symons, 1987). The C-8 OH adduct radicals of purines are well-known to produce 8-hydroxypurines upon one-electron oxidation and formamidopyrimidines upon ring opening followed by one-electron reduction [for a review see Steenken (1989)]. On the other hand, the formation of 8-OH-dG from dG by $^1\Delta_gO_2$ has also been suggested to occur by a mechanism involving the addition of $^1\Delta_gO_2$ across the imidazole ring of guanine (Ravanat et al., 1991).

The results show that FapyGua in both γ-irradiated and visible light/MB-treated DNA is recognized and excised by the Fpg protein. This is the first demonstration of the excision of FapyGua by the Fpg protein. In addition, the present work demonstrates the ability of the Fpg protein to recognize and excise FapyAde not only from homopolymers of 2′-deoxyadenosine (dA) (Breimer, 1984) but also from DNA. While the present work was in progress, Tchou et al. (1991) reported the excision of 8-OH-Gua by the Fpg protein from a small synthetic oligonucleotide containing a single 8-OH-Gua residue. Our results demonstrate the ability of the Fpg protein to excise 8-OH-Gua from DNA as well. In addition to the excision of significant amounts of formamidopyrimidines and 8-OH-Gua, the native Fpg protein excised a small but detectable amount of 8-OH-Ade from γ-irradiated DNA. By inference to 8-OH-Gua, the excision of 8-OH-Ade by the Fpg protein would be expected. However, the small amount excised appears to be insignificant in order to draw any conclusion on the substrate specificity of the Fpg protein concerning this lesion. The excision of 8-hydroxypurines by the Fpg protein was inhibited from DNA irradiated at a higher dose, although formamidopyrimidines were excised. These results imply that the structural requirement for the recognition of formamidopyrimidines by the Fpg protein is somewhat different from that for the recognition of 8-hydroxypurines. The rationale for this difference remains unclear.

The results support the view that the biological role of the Fpg protein is the repair of DNA damage caused by free radicals or other oxygen-derived species. This enzyme appears to be specific for purine-derived bases and may complement pyrimidine-specific repair enzymes such as endonuclease III (Breimer & Lindahl, 1984; Asahara et al., 1989). The excision of 8-OH-Gua implies that imidazole ring opening is not an absolute requirement for the recognition of modified purines by the Fpg protein. As was suggested previously (Dizdaroglu, 1985), the analytical approach used in this work might be useful for the unequivocal characterization of various pyrimidine- and purine-derived lesions recognized (or not recognized) by DNA repair enzymes and thus for the elucidation of mechanisms underlying the repair of DNA damage.

Registry No. MB, 61-73-4; 5,6-diHThy, 696-04-8; 5-OH-5-Me-Hyd, 10045-58-6; 5-OH-Hyd, 29410-13-7; 5-OH-6-HThy, 1123-21-3; 5-OH-6-HCyt, 123472-58-2; 5-OHMe-Ura, 4433-40-3; Cyt glycol,

13484-98-5; Thy glycol, 2943-56-8; 5,6-diOH-Cyt, 3914-34-9; FapyAde, 5122-36-1; 8-OH-Ade, 21149-26-8; FapyGua, 51093-31-3; 8-OH-Gua, 5614-64-2; 8-OH-dG, 88847-89-6; Fapy-DNA glycosylase, 78783-53-6.

REFERENCES

Ames, B. N. (1983) *Science 221*, 1256–1264.

Aruoma, O. I., Halliwell, B., & Dizdaroglu, M. (1989) *J. Biol. Chem. 264*, 13024–13028.

Asahara, H., Wistort, P. M., Bank, J. F., Bakerian, R. H., & Cunningham, R. P. (1989) *Biochemistry 28*, 4444–4449.

Bailly, V., Verly, W. G., O'Connor, T., & Laval, J. (1989) *Biochem. J. 262*, 581–589.

Blakely, W. F., Fuciarelli, A. F., Wegher, B. J., & Dizdaroglu, M. (1990) *Radiat. Res. 121*, 338–343.

Boiteux, S., Belleney, J., Roques, B. P., & Laval, J. (1984) *Nucleic Acids Res. 12*, 5429–5439.

Boiteux, S., O'Connor, T. R., & Laval, J. (1987) *EMBO J. 6*, 3177–3183.

Boiteux, S., Bichara, M., Fuchs, R. P. P., & Laval, J. (1989) *Carcinogenesis 10*, 1905–1909.

Boiteux, S., O'Connor, T. R., Lederer, F., Gouyette, A., & Laval, J. (1990) *J. Biol. Chem. 265*, 3916–3922.

Breimer, L. H. (1983) *Biochemistry 22*, 4192–4197.

Breimer, L. H. (1984) *Nucleic Acids Res. 12*, 6359–6367.

Breimer, L. H. (1990) *Mol. Carcinogenesis 3*, 188–197.

Breimer, L. H., & Lindahl, T. (1984) *J. Biol. Chem. 259*, 5543–5548.

Cerutti, P. A. (1985) *Science 227*, 375–381.

Chetsanga, C. J., & Lindahl, T. (1979) *Nucleic Acids Res. 6*, 3673–3684.

Czeczot, H., Tudek, B., Lambert, B., Laval, J., & Boiteux, S. (1991) *J. Bacteriol. 173*, 3419–3424.

Decuyper-Debergh, D., Piette, J., & van de Vorst, A. (1987) *EMBO J. 8*, 3155–3161.

Devasagayam, T. P. A., Steenken, S., Obendorf, M. S. W., Schulz, W. A., & Sies, H. (1991) *Biochemistry 30*, 6283–6289.

Dizdaroglu, M. (1985) *Anal. Biochem. 144*, 593–603.

Dizdaroglu, M. (1990) *Methods Enzymol. 193*, 842–857.

Dizdaroglu, M. (1991) *Free Radical Biol. Med. 10*, 225–242.

Dizdaroglu, M., & Bergtold, D. S. (1986) *Anal. Biochem. 156*, 182–188.

Epe, B., Mützel, P., & Adam, A. (1988) *Chem.-Biol. Interact. 67*, 149–165.

Floyd, R. A., West, M. S., Eneff, K. L., & Schneider, J. E. (1989) *Arch. Biochem. Biophys. 273*, 106–111.

Fuciarelli, A. F., Wegher, B. J., Gajewski, E., Dizdaroglu, M., & Blakely, W. F. (1989) *Radiat. Res. 119*, 219–231.

Halliwell, B., & Gutteridge, J. M. C. (1989) *Free Radicals in Biology and Medicine*, 2nd ed., Clarendon, Oxford.

Kow, Y. W., Wallace, S. S., & van Houten, B. (1990) *Mutat. Res. 235*, 147–156.

Laval, J., Boiteux, S., & O'Connor, T. R. (1990a) *Mutat. Res. 233*, 73–79.

Laval, J., O'Connor, T. R., d'Hérin-Lagravère, C., Auffret van der Kemp, P., & Boiteux, S. (1990b) in *Repair Mechanisms and Their Biological Implications in Mammalian Cells* (Laval, J., & Lambert, M., Eds.) pp 25–36, Plenum Press, New York.

Lesko, S. A., Lorentzen, R. J., & Ts'o, P. O. P. (1980) *Biochemistry 19*, 3023–3028.

Lin, J.-J., & Sancar, A. (1989) *Biochemistry 28*, 7979–7984.

Margison, G. P., & Pegg, A. E. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 861–865.

Müller, E., Boiteux, S., Cunningham, R. P., & Epe, B. (1990) *Nucleic Acids Res. 18*, 5969–5973.

O'Connor, T. R., & Laval, J. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 5222–5226.

Oleinick, N. L., Chiu, S., Ramakrishnan, N., & Xue, L. (1987) *Br. J. Cancer 55* (Suppl. VIII), 135–140.

Ravanat, J.-L., Berger, M., Buchko, G. W., Benard, J.-F., van Lier, J., & Cadet, J. (1991) *J. Chim. Phys. 88*, 1069–1076.

Rowley, D. A., & Halliwell, B. (1983) *Biochim. Biophys. Acta 761*, 86–93.

Sagripanti, J. L., & Kraemer, K. H. (1989) *J. Biol. Chem. 264*, 1729–1734.

Sancar, A., & Sancar, G. B. (1988) *Annu. Rev. Biochem. 57*, 29–67.

Schneider, J. E., Price, S., Maidt, L., Gutteridge, J. M. C., & Floyd, R. A. (1990) *Nucleic Acids Res. 18*, 631–635.

Sies, H. (1986) *Angew. Chem., Int. Ed. Engl. 25*, 1058–1072.

Steenken, S. (1989) *Chem. Rev. 89*, 503–520.

Symons, M. C. (1987) *J. Chem. Soc., Faraday Trans. 1 83*, 1–11.

Tchou, J., Kasai, H., Shibutani, S., Chung, M.-H., Laval, J., Grollman, A. P., & Nishimura, S. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 4690–4694.

Têoule, R., & Cadet, J. (1978) in *Effects of Ionizing Radiation on DNA* (Hüttermann, J., Köhnlein, W., Têoule, R., & Bertinchamps, A. J., Eds.) pp 171–203, Springer-Verlag, Berlin.

von Sonntag, C. (1987) *The Chemical Basis of Radiation Biology*, Taylor & Francis, London.

Wallace, S. S. (1988) *Environ. Mol. Mutagen. 12*, 431–477.

Weitzman, S. A., Weitberg, A. B., Clark, E. P., & Stossel, T. P. (1985) *Science 227*, 1231–1233.