Arabidopsis thaliana Ogg1 Protein Excises 8-Hydroxyguanine and 2,6-Diamino-4-hydroxy-5-formamidopyrimidine from Oxidatively Damaged DNA Containing Multiple Lesions

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ABSTRACT: A functional homologue of eukaryotic Ogg1 proteins in the model plant *Arabidopsis thaliana* has recently been cloned, isolated, and characterized [Garcia-Ortiz, M. V., Ariza, R. R., and Roldan-Arjona, T. (2001) *Plant Mol. Biol. 47*, 795−804]. This enzyme (*At*Ogg1) exhibits a high degree of sequence similarity in several highly conserved regions with *Saccharomyces cerevisiae*, *Drosophila melanogaster*, and human Ogg1 proteins. We investigated the substrate specificity and kinetics of *At*Ogg1 for excision of modified bases from oxidatively damaged DNA that contained multiple pyrimidine- and purine-derived lesions. Two different DNA substrates prepared by exposure to ionizing radiation in aqueous solution under N_2O or air were used for this purpose. Gas chromatography/isotope-dilution mass spectrometry was applied to identify and quantify modified bases in DNA samples. Of the 17 modified bases identified in DNA samples, only 8-hydroxyguanine and 2,6-diamino-4-hydroxy-5-formamidopyrimidine were significantly excised from both DNA substrates. This is in agreement with the substrate specificities of other eukaryotic Ogg1 proteins that had previously been studied under identical conditions. Excision depended on incubation time, enzyme concentration, and substrate concentration and followed Michaelis−Menten kinetics. A significant dependence of excision on the nature of DNA substrate was observed in accord with previous studies on other DNA glycosylases. A comparison of excision kinetics pointed to significant differences between *At*Ogg1 and other Ogg1 proteins. We also investigated the effect of base-pairing on the excision using double-stranded oligodeoxynucleotides that contained 8-OH-Gua paired with each of the four DNA bases. The activity of *At*Ogg1 was most effective on the 8-OH-Gua:C pair with some or very low activity on other pairs in agreement with the activity of other Ogg1 proteins. The results unequivocally show that *At*Ogg1 possesses common substrates with other eukaryotic Ogg1 proteins albeit significant differences between their excision kinetics.

Oxygen-derived species such as free radicals and other oxidizing agents generate a multiplicity of lesions in DNA comprising modified bases and sugars, DNA-protein cross-links, strand breaks, and base-free sites (reviewed in refs *1* and *2*). A variety of repair pathways exist in cells to combat DNA damage and to maintain genomic integrity (reviewed in ref *3*). The repair of modified bases in DNA of both eukaryotes and prokaryotes primarily occurs via the base-excision repair (BER)[1] pathway (reviewed in ref *4*), which is conserved throughout all species. DNA glycosylases are involved in the first step of BER and remove modified bases from DNA by catalyzing the hydrolysis of the glycosidic bond between the modified base and the sugar moiety. In *Escherichia coli*, formamidopyrimidine DNA glycosylase (Fpg) is a DNA glycosylase/lyase that excises 8-hydroxyguanine (8-OH-Gua), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua), and 4,6-diamino-5-formamidopyrimidine (FapyAde) from DNA and processes the resulting abasic site by cleaving both 3'- and 5'-phosphodiester bonds by successive β- and δ-eliminations (*5*−*7*). Fpg shows a clear preference for Cyt as the base opposite 8-OH-Gua, with 8-OH-Gua:Ade being a particularly poor substrate (*8*). This specificity avoids repair of 8-OH-Gua:Ade mispairs to T:A, which would cause a GC → TA transversion. The repair of 8-OH-Gua:Ade mispairs is initiated instead by MutY, a DNA

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[1] Abbreviations: BER, base-excision repair; *At*Ogg1, *Arabidopsis thaliana* Ogg1 protein; Fpg, formamidopyrimidine DNA glycosylase; *Dr*Fpg, *Deinococcus radiodurans* Fpg; *y*Ogg1, *Saccharomyces cerevisiae* Ogg1; *d*Ogg1, *Drosophila* Ogg1; *d*S3, *Drosophila* ribosomal protein S3; GC/IDMS, gas chromatography/isotope-dilution mass spectrometry; 8-OH-Gua, 7,8-dihydro-8-oxoguanine, 8-hydroxyguanine (also known as 8-oxoguanine); FapyGua, 2,6-diamino-4-hydroxy-5-formamido-pyrimidine; FapyAde, 4,6-diamino-5-formamidopyrimidine.

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glycosylase that catalyzes excision of misincorporated Ade (*9*). Animals and yeast cells possess 8-OH-Gua DNA glycosylases that do not share significant sequence identity with bacterial Fpg proteins. The first of these Fpg analogues was identified in *Saccharomyces cerevisiae* and designated *y*Ogg1 (*10*, *11*). This enzyme possesses a similar substrate specificity to Fpg and removes 8-OH-Gua and FapyGua, but not FapyAde in contrast to Fpg (*12*), and displays a preference for 8-OH-Gua opposite Cyt but not Ade (*10*, *11*, *13*). Genes encoding proteins, which share significant sequence identity with *y*Ogg1, have been subsequently cloned and characterized in humans and other mammals (*14*−*20*). Genome analyses revealed proteins similar to Ogg1 in Archaea but not in any bacterial species (*21*). *Arabidopsis thaliana* has become a very attractive model system for study of conserved DNA repair pathways because of the practical advantages of small size and rapid life cycle in conjunction with the recent development of powerful tools to study its genome. In addition, there are important differences between the life strategies of plants and most eukaryotes. For example, plants do not have a reserve germ line, and their gametes differentiate late in development from somatic cells. This and other differences may shed light on crucial aspects of genome-maintenance functions in eukaryotes.

We recently isolated and characterized an Ogg1 orthologue in the model plant *A. thaliana* (*At*Ogg1) (*22*). Our finding was of particular interest since an 8-OH-Gua DNA glycosylase (*At*MMH) encoded by a gene named *AtMMH*, which is an orthologue of *E. coli*'s gene *Fpg*, had previously been isolated and characterized in this model plant (*23*). The discovery of *At*Ogg1 established plants as the only organisms, where the presence of both Fpg and Ogg1 homologues exist. On the other hand, *Arabidopsis* is not the only example of an organism with two different enzymes for the repair of 8-OH-Gua. In *Drosophila melanogaster*, both the ribosomal protein S3 and an Ogg1 protein (*d*Ogg1), which is a true orthologue of other Ogg1 proteins, possess DNA glycosylase/β-lyase activity capable of releasing 8-OH-Gua and FapyGua from damaged DNA with multiple lesions (*24*, *25*). In the case of *Arabidopsis*, the relative roles of *At*Ogg1 and *At*MMH might be connected to their likely different phylogenetic origin since the latter may be the result of a gene transfer from an ancestral chloroplast genome to the nucleus (*23*). Although there are putative nuclear targeting signals in the predicted amino acid sequences of *At*Ogg1 and *At*MMH, the subcellular localization of both enzymes remains to be determined. The possibility exists that *At*Ogg1 removes modified bases for oxidatively damaged DNA in the nucleus, while *At*MMH continues performing an essential DNA repair activity in the chloroplast genome.

In the present work, we report the substrate specificity and excision kinetics of *At*Ogg1 using oxidatively damaged DNA containing a multiplicity of lesions. Excision by *At*Ogg1 of modified DNA bases was studied using the technique of gas chromatography/isotope-dilution mass spectrometry (GC/IDMS). Furthermore, an oligodeoxynucleotide containing a single 8-OH-Gua residue at a defined position, which was paired with each of the four DNA bases, was used to investigate the paired-base effect on the repair activity of this protein.

MATERIALS AND METHODS

Materials.[2] Modified DNA bases, their stable isotope-labeled analogues, and other materials for GC/IDMS were obtained as described (*26*, *27*). Calf thymus DNA was purchased from Sigma. The preparation of N_2O-saturated aqueous solutions of DNA and their exposure to ionizing radiation in a ^{60}Co γ-source were performed as described (*28*). Aliquots of nonirradiated and irradiated DNA samples (100 μg) were dried in a SpeedVac under vacuum.

Expression and Purification of AtOgg1. *At*Ogg1 was overexpressed in *E. coli* strain BL21(DE3) and purified as previously described (*22*).

DNA Substrates. A PAGE-purified 33-mer oligodeoxynucleotide containing a single 8-OH-Gua at position 20 (underlined) (5'-ATTCGTGACGATCTGCAGTGCCTTCTGCAGGCA-3') was purchased from Synthegen. The oligodeoxynucleotide (50 ng) was ^{32}P-labeled at the 5′ terminus with T4 polynucleotide kinase (Roche) and [γ-^{32}P]ATP (Amersham). To prepare double-stranded substrates, this oligodeoxynucleotide (5 pmol) was annealed in 10 mM Tris-HCl, pH 8.0, and 20 mM NaCl with a complementary one (10 pmol) containing either Cyt, Ade, Thy, or Gua opposite to the 8-OH-Gua residue. Annealing was performed by heating at 95 °C for 5 min, followed by slow cooling to room temperature.

Assay for the Repair of 8-OH-Gua. Double-stranded oligodeoxynucleotides (24 fmol) were incubated at 37 °C for 1 h in a reaction mixture containing 40 mM HEPES-KOH at pH 8.0, 100 mM KCl, 0.5 mM EDTA, 0.5 mM DTT, 0.2 mg/mL BSA, and different amounts of the enzyme in a total volume of 10 μL. Reactions were stopped by adding an equal volume of formamide dye mix (80% formamide, 1 mg/mL bromophenol blue, and 10 mM EDTA); the products were separated by 15% denaturing PAGE containing 8 M urea and visualized by autoradiography.

Preparation of DNA Samples, Enzymic Assays, and GC/IDMS. Enzymic assays were performed as described (*29*). For the measurement of excision kinetics, DNA solutions were γ-irradiated at 2.5, 5, 10, 20, 40, and 60 Gy. Aliquots (100 μg) of irradiated DNA samples were dried in a SpeedVac under vacuum. Two sets of these samples with three replicates were prepared. One set of the samples was incubated with 1 μg of *At*Ogg1 at 37 °C for 30 min. The amount of the enzyme corresponded to a concentration of 226 nM. After incubation, 250 μL of cold ethanol (−20 °C) was added to the samples to stop the reaction and precipitate DNA. The samples were kept at −20 °C for 2 h. Aliquots of stable isotope-labeled analogues of modified DNA bases as internal standards and an additional 180 μL of cold ethanol (−20 °C) were added. Following storage at −20 °C for 2 h, the samples were centrifuged at 15 000*g* for 30 min at 4 °C. DNA pellets and supernatant fractions were separated. Ethanol was removed from supernatant fractions under vacuum in a SpeedVac. Aqueous supernatant fractions were lyophilized to dryness for 18 h.

[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.

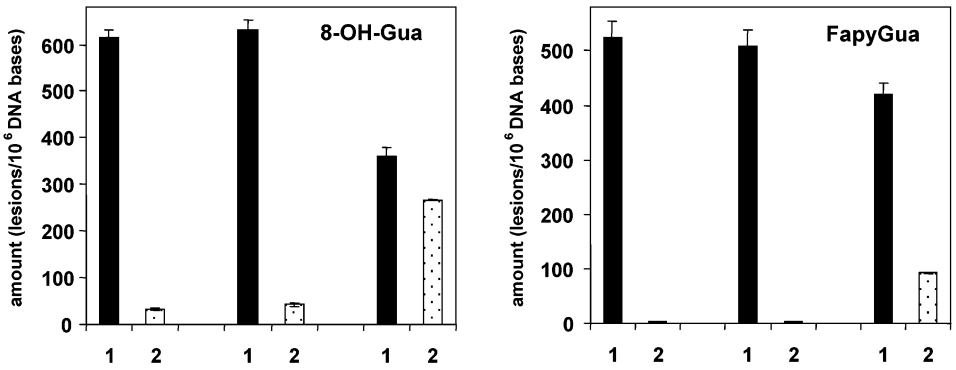

FIGURE 1: Excision of 8-OH-Gua and FapyGua by *At*Ogg1 from DNA irradiated under air. Dark columns (1), pellets; light columns (2), supernatant fractions. Left: DNA incubated with no enzyme. Middle: DNA incubated with heat-inactivated enzyme. Right: DNA incubated with active enzyme. The enzyme amount was 2 μg/100 μg of DNA. The incubation time was 30 min at 37 °C. Each column represents the mean ± standard deviation of the values obtained by GC/IDMS analysis of three independently prepared samples.

The other set of irradiated samples was used to determine the levels of modified DNA bases in each sample. Following addition of aliquots of stable isotope-labeled analogues of modified bases as internal standards, the samples were dried in a SpeedVac under vacuum and hydrolyzed with 0.5 mL of 60% formic acid in evacuated and sealed tubes for 30 min at 140 °C. The hydrolysates were frozen in liquid nitrogen and lyophilized for 18 h.

GC/MS measurements were performed using a gas chromatograph (Model 6890 Series)−mass selective detector (Model 5973N) system (Agilent Technologies, Rockville, MD) according to the published procedures for trimethylsilylation of dried supernatant fractions and DNA hydrolysates and for the use of GC column and other experimental conditions (*30*).

RESULTS

Substrates of AtOgg1. The glycosylase activity of *At*Ogg1 for modified heterocyclic bases in oxidatively damaged DNA was tested using DNA samples that had been γ-irradiated in buffered aqueous solution under N_2O (anoxic) or air (oxic). Using GC/IDMS, 17 and 13 modified bases were identified and quantified in DNA γ-irradiated under anoxic conditions and oxic conditions, respectively (*28*). Heterocyclic bases in DNA are modified by reactions of radiation-generated hydroxyl radical, with the exception of 5,6-dihydropyrimidines, which result from reactions of a radiation-generated H atom under anoxic conditions only. Furthermore, hydroxyl radical-induced 5-hydroxy-6-hydropyrimidines are not formed under oxic conditions because of diffusion-controlled reactions of molecular oxygen with hydroxyl radical-adduct radicals of pyrimidines (reviewed in refs *1* and *2*).

DNA samples were incubated with either active enzyme or heat-inactivated enzyme or no enzyme. Supernatant fractions and pellets of DNA samples were analyzed by GC/IDMS. Of the modified bases in DNA, *At*Ogg1 efficiently excised 8-OH-Gua and FapyGua only. No significant excision of other modified bases was observed. The identification and quantification of 8-OH-Gua and FapyGua was achieved using GC/MS with the selected-ion monitoring (SIM) mode by monitoring the characteristic ions of their trimethylsilyl derivatives and those of the trimethylsilyl derivatives of their stable isotope-labeled analogues (*31*, *32*). The excision of 8-OH-Gua and FapyGua was established by their appearance in supernatant fractions and by reduction of their levels in pellet fractions of DNA samples incubated with active enzyme when compared with DNA samples incubated with inactivated enzyme or no enzyme. Figure 1 illustrates the levels of these compounds in supernatant and pellet fractions. The amount of each compound removed from the pellet fraction by the active enzyme was similar to its amount found in the supernatant fraction; unequivocally proving that 8-OH-Gua and FapyGua are substrates of *At*Ogg1. Heat-inactivated enzyme displayed no activity as the results were identical to those obtained with samples incubated without the enzyme. Excision of 8-OH-Gua and FapyGua was also determined as a function of the enzyme amount and incubation time. Figure 2 illustrates the dependence of excision on the enzyme amount. Excision steadily increased and reached a plateau at about 4 μg of *At*Ogg1/100μg of DNA. Time dependence of excision was measured using 1 μg of the enzyme/100μg of DNA and incubation times of 10, 20, 30, and 45 min. Excision increased depending on incubation time and reached a plateau after 45 min (Figure 3).

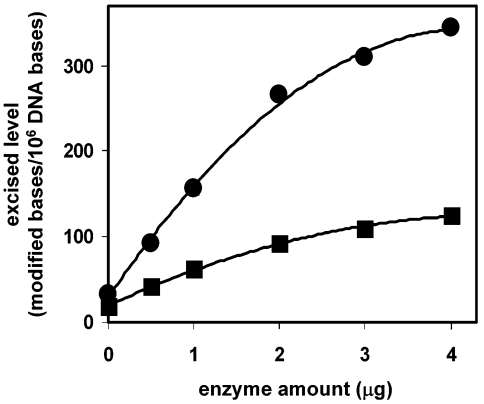

FIGURE 2: Dependence of excision on the enzyme amount. The incubation time was 30 min at 37 °C. The levels given on the *y* axis represent those found in the supernatant fractions. The calculation of the level of each lesion per 10^6 DNA bases was based on the known total level of each lesion in DNA samples prior to enzymic hydrolysis. Each data point represents the mean ± standard deviation of the values obtained by GC/IDMS analysis of three independently prepared DNA samples (100 μg) γ-irradiated under air at 60 Gy. ●: 8-OH-Gua; ■: FapyGua. The standard deviations are not seen in this graph because they were less than 5% of the corresponding data points.

Excision Kinetics. To measure the excision kinetics of *At*Ogg1, DNA samples that had been irradiated at six different radiation doses were used. This enabled the

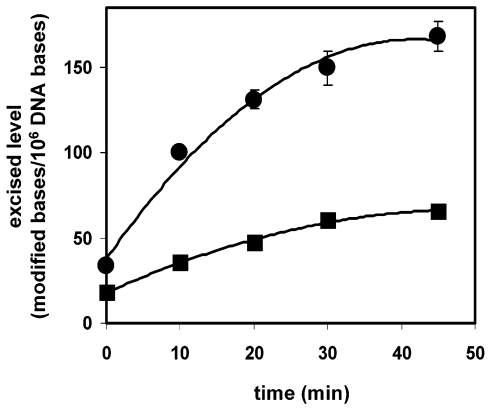

FIGURE 3: Dependence of excision on the incubation time. The enzyme amount was 1 μg/100 μg of DNA. Other details are as in Figure 2. ●: 8-OH-Gua; ■: FapyGua. In some cases, the standard deviations are not seen in this graph because they were less than 5% of the corresponding data points.

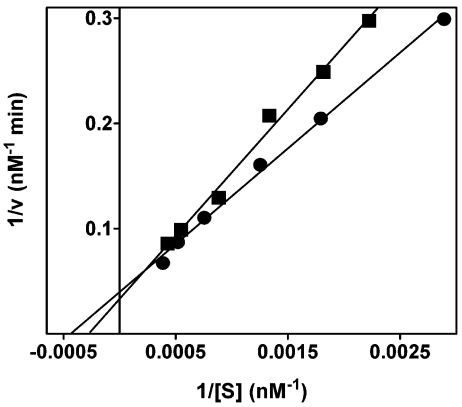

FIGURE 4: Lineweaver−Burk plots for excision of 8-OH-Gua (●) and FapyGua (■) by *At*Ogg1 from DNA γ-irradiated under air. The incubation time was 30 min at 37 °C. The enzyme amount was 1 μg/100 μg of DNA. Amounts of products found in supernatant fractions were used for initial velocity. *S*, concentration of 8-OH-Gua or FapyGua; *v*, initial velocity of excision of 8-OH-Gua or FapyGua.

measurement of the dependence of excision on substrate concentration. Concentration ranges of 8-OH-Gua and FapyGua were 0.35−2.6 and 0.45−2.3 μM, respectively, in DNA samples irradiated under air and 0.7−3.1 μM and 1.1−5.3 μM, respectively, in DNA samples irradiated under N_2O. The amounts measured in supernatant fractions were used for the determination of the kinetic parameters. Initial velocities were obtained using the plots of excision dependence on incubation time. Excision followed Michaelis−Menten kinetics (*33*). A computer program with the linear least-squares analysis of the data was used to calculate the kinetic constants and standard deviations ($n = 6$). Figure 4 illustrates Lineweaver−Burk plots of excision of 8-OH-Gua and FapyGua from DNA samples irradiated under air. Only low excision of 8-OH-Gua from DNA samples irradiated under N_2O was observed. Thus, no kinetic parameters for this substrate could be determined. Kinetic constants are given in Table 1. *At*Ogg1 excised 8-OH-Gua and FapyGua with similar maximum velocity (V_{max}) from DNA irradiated under air. However, the specificity constant (k_{cat}/K_M) for excision of 8-OH-Gua was slightly but significantly greater than that of excision of FapyGua. This indicates the somewhat greater preference of this enzyme for excision of 8-OH-Gua. On the other hand, 8-OH-Gua was not sufficiently excised from DNA irradiated under N_2O to obtain kinetic parameters. In contrast, FapyGua was efficiently removed. Furthermore, the k_{cat}/K_M value of excision of FapyGua from this DNA substrate was significantly greater (about 2-fold) than that from DNA irradiated under air. This means that *At*Ogg1 displayed a greater preference for excision of FapyGua from DNA irradiated under N_2O than from DNA irradiated under air.

Tables 2 and 3 show a comparison of the specificity constants of *At*Ogg1 with those of other prokaryotic and eukaryotic DNA glycosylases that specifically excise 8-OH-Gua and FapyGua from DNA. Kinetic parameters of *d*Ogg1, *d*S3, hOgg1-Ser^{326}, hOgg1-Cyt^{326}, hOgg1-Gln^{46}, and hOgg1-His^{154} using DNA irradiated under air were not reported. It should be pointed out that prokaryotic enzymes *E. coli* Fpg and *Deinococcus radiodurans* Fpg remove FapyAde from DNA in addition to 8-OH-Gua and FapyGua (*6*, *34*, *35*). As the comparison in Table 2 shows, *y*Ogg1 and *d*Ogg1 exhibit preference for 8-OH-Gua over FapyGua, whereas hOgg1-Ser^{326} and hOgg1-Cyt^{326} preferentially excise FapyGua. On the other hand, hOgg1-Gln^{46}, hOgg1-His^{154}, and *d*S3 excise both compounds with similar preference. *At*Ogg1 almost exclusively prefers FapyGua in the case of DNA irradiated under N_2O (Table 2). In contrast, *At*Ogg1 as *y*Ogg1 possesses a similar preference for excision of both 8-OH-Gua and FapyGua from the other DNA substrate (Table 3).

Cleavage of 33-mer Double-Stranded Oligodeoxynucleotides Containing 8-OH-Gua Paired with Each of the DNA Bases. We tested purified *At*Ogg1 for its capacity to cleave a 33-mer double-stranded oligodeoxynucleotide, which contained 8-OH-Gua paired with C, T, G, or A at a defined position. Incubation of these oligodeoxynucleotides with *At*Ogg1 resulted in the generation of a cleavage product that migrated slightly more slowly than the δ-elimination product generated by Fpg under the same reaction conditions (Figure 5). This is consistent with AP-site cleavage via β-elimination, as previously reported (*22*). Consistent with previous studies (*5*, *8*), 8-OH-Gua paired with A was poorly recognized by Fpg, whereas 8-OH-Gua paired with other bases (G, C, and T) was a good substrate. *At*OGG1 recognizes 8-OH-Gua paired with C as the most efficiently cleaved substrate. In contrast to the data obtained with Fpg, however, a very low activity was observed when 8-OH-Gua was paired with T, and only residual activity when paired with purines (A or G). These results are in accord with those reported for other eukaryotic Ogg1 proteins (*14*, *16*, *18*, *25*, *36*−*38*).

DISCUSSION

The results show the ability of *At*Ogg1 to efficiently excise 8-OH-Gua and FapyGua from DNA containing multiple lesions that are generated from pyrimidines and purines by ionizing radiation-generated hydroxyl radicals and H atoms. No significant excision of any other lesion was observed under the experimental conditions used. Thus, this work unequivocally established that *At*Ogg1 possesses common substrates with its functional homologues *S. cerevisiae*, *Drosophila*, and human Ogg1 proteins that had previously been investigated under identical experimental conditions (*12*, *25*, *38*, *39*). Amino acid sequences of eukaryotic Ogg1 proteins including *At*Ogg1 show a high degree of sequence

Table 1: Kinetic Constants for Excision of 8-OH-Gua and FapyGua by *At*Ogg1 from DNA γ-Irradiated under Air or N_2O

	V_{max} (nM min^{-1})[a]		K_M (nM)[a]		$k_{cat}/K_M \times 10^5$ (min^{-1} nM^{-1})[a]	
substrate	air	N_2O	air	N_2O	air	N_2O
8-OH-Gua	25.1 ± 2.6	*c*	2287 ± 242[b]	*c*	4.9 ± 0.3[b]	*c*
FapyGua	30.0 ± 6.7	32.1 ± 2.1	3600 ± 824[d]	2147 ± 183	3.7 ± 0.4[e]	6.6 ± 0.2

[a] Values represent the mean ± standard deviation ($n = 6$). ($k_{cat} = V_{max}$/[enzyme]). The enzyme amount was 1 μg/100 μg of DNA, corresponding to an enzyme concentration of 226 nM. The concentration ranges of 8-OH-Gua and FapyGua were 0.35−2.6 μM and 0.45−2.3 μM, respectively, in DNA samples irradiated under air and 0.7−3.1 μM and 1.1−5.3 μM, respectively, in DNA samples irradiated under N_2O. [b] Statistically different from the value in line 2 ($P < 0.05$). [c] Not determined because of low excision. [d] Statistically different from the value in column 2 ($P < 0.05$). [e] Statistically different from the value in column 2 ($P < 0.05$).

Table 2: Comparison of the Specificity Constants [$k_{cat}/K_M \times 10^5$ (min^{-1} nM^{-1})][a] for Excision of 8-OH-Gua and FapyGua from DNA by *At*Ogg1, *E. Coli* Fpg (*34*), *D. radiodurans* Fpg (*Dr*Fpg) (*35*), *S. cerevisiae* Ogg1 (*y*Ogg1) (*12*), *Drosophila* Ogg1 (*d*Ogg1) (*25*), *Drosophila* Protein S3 (*d*S3) (*24*), and Human Ogg1 (hOgg1-Ser^{326}, hOgg1-Cyt^{326}, hOgg1-Gln^{46}, and hOgg1-His^{154}) (*38*, *39*)

	DNA irradiated under N_2O									
substrate	*At*Ogg1	*E. coli* Fpg	*Dr*Fpg	*y*Ogg1	*d*Ogg1	*d*S3	hOgg1-Ser^{326}	hOgg1-Cyt^{326}	hOgg1-Gln^{46}	hOgg1-His^{154}
8-OH-Gua	*b*	13.0 ± 1.3[c,d]	*b*	6.5 ± 0.4[c,e]	21.0 ± 0.4[c,f]	2.6 ± 0.6[g]	4.5 ± 0.1[c,h]	2.8 ± 0.1[c]	5.9 ± 0.1	4.3 ± 0.04
FapyGua	6.6 ± 0.2[i]	25.7 ± 2.9[j]	8.6 ± 0.9[k]	1.5 ± 0.1[e]	11.2 ± 0.2[f]	2.5 ± 0.4[l]	9.0 ± 0.2[m]	4.4 ± 0.1	4.7 ± 0.1	3.9 ± 0.1

[a] Values represent the mean ± standard deviation ($n = 6$). [b] Not determined because of low excision. [c] Statistically different from the value in line 2 ($P < 0.05$). [d] Statistically different from the values in columns 4−10 ($P < 0.05$). [e] Statistically different from the values in columns 5−8 and 10 ($P < 0.05$). [f] Statistically different from the values in columns 6−10 ($P < 0.05$). [g] Statistically different from the value in columns 7, 9, and 10 ($P < 0.05$). [h] Statistically different from the value in column 8 ($P < 0.05$). [i] Statistically different from the values in columns 2 and 4−10 ($P < 0.05$). [j] Statistically different from the values in columns 3−10 ($P < 0.05$). [k] Statistically different from the values in columns 4, 6, and 8−10 ($P < 0.05$). [l] Statistically different from the values in columns 7−10 ($P < 0.05$). [m] Statistically different from the values in columns 8−10 ($P < 0.05$).

Table 3: Comparison of the Specificity Constants [$k_{cat}/K_M \times 10^5$ (min^{-1} nM^{-1})][a] for Excision of 8-OH-Gua and FapyGua from DNA by *At*Ogg1, *E. Coli* Fpg (*34*), *Dr*Fpg (*35*), and *y*Ogg1 (*12*)

	DNA irradiated under air			
substrate	AtOgg1	*E. coli* Fpg	*Dr*Fpg	*y*Ogg1
8-OH-Gua	4.9 ± 0.3[b,c]	9.0 ± 0.3[b,d]	*e*	3.5 ± 0.2
FapyGua	3.7 ± 0.4[f]	5.3 ± 1.1[g]	7.8 ± 0.3[d]	2.7 ± 0.6

[a] Values represent the mean ± standard deviation ($n = 6$). [b] Statistically different from the value in line 2 ($P < 0.05$). [c] Statistically different from the values in columns 2 and 4 ($P < 0.05$). [d] Statistically different from the value in column 4 ($P < 0.05$). [e] Not determined because of low excision. [f] Statistically different from the values in columns 2−4 ($P < 0.05$). [g] Statistically different from the values in columns 3 and 4 ($P < 0.0$).

homology with several highly conserved regions (*22*, *25*, *40*−*42*). Despite common substrates and extensive structural conservation among these functional homologues, the comparison of excision kinetics of *At*Ogg1 and other eukaryotic Ogg1 proteins (as shown in Tables 1 and 2) reveals that there exist significant differences with respect to kinetic parameters. There are subtle variations among eukaryotic Ogg1 proteins in their highly conserved regions and sequences (*11*, *22*, *25*, *40*−*44*). These variations may account for the differences between excision kinetics of Ogg1 proteins.

We used two different DNA substrates that had been damaged either in the presence of oxygen or its absence. It is well-known that experimental conditions profoundly affect the types and yields of free radical-induced products in DNA (reviewed in ref *45*). Oxygen inhibits the formation of H atom-induced products and those formed by addition of hydroxyl radical to pyrimidines followed by reduction. However, it increases the yields of products that result from oxidation of hydroxyl radical-adduct radicals of both pyrimidines and purines. Although formed by reduction of hydroxyl radical-adduct radicals of purines, formamidopyrimidines FapyGua and FapyAde are generated both in the presence and absence of oxygen with their yields being greater under the former condition than under the latter one. The yields of 8-OH-Gua and FapyGua in two different types of DNA samples are given in the Results section. The yields of other pyrimidine- and purine-derived lesions in DNA irradiated under N_2O or air can be found in previous publications, of which experimental conditions were similar to those in this work (see e.g., refs *35* and *46*). *At*Ogg1 efficiently removed 8-OH-Gua and FapyGua from DNA irradiated under air. Their concentration ranges were similar in DNA samples used for the measurement of kinetic constants. There was no significant difference between the maximum velocities (V_{max}) for excision of these compounds. Because of the greater K_M value of FapyGua, however, the specificity constant of 8-OH-Gua was slightly but significantly greater than that of FapyGua. This indicates that the enzyme might have a preference for excision of 8-OH-Gua over that of FapyGua from DNA irradiated under air. In contrast, excision of 8-OH-Gua from DNA irradiated under N_2O was low so that no kinetic parameters could be determined. Thus, FapyGua was clearly the preferred substrate in this case. Furthermore, a noteworthy difference was observed between the two DNA substrates in terms of the preference for FapyGua. The specificity constant for excision of FapyGua from DNA irradiated under N_2O was significantly greater (2-fold) than that from DNA irradiated under air. These results indicate a profound dependence of excision by *At*Ogg1 on the nature of damaged DNA substrate. Other DNA glycosylases, which had recently been investigated under similar conditions as in this work, exhibited the same characteristic (see e.g., ref *28*). However, the reason for this phenomenon is not known. It may well be that different damaging conditions generate a different, sequence-dependent distribution of lesions in DNA. The recognition of the lesions by the enzyme may depend on this distribution. Furthermore, different product yields in DNA that depend

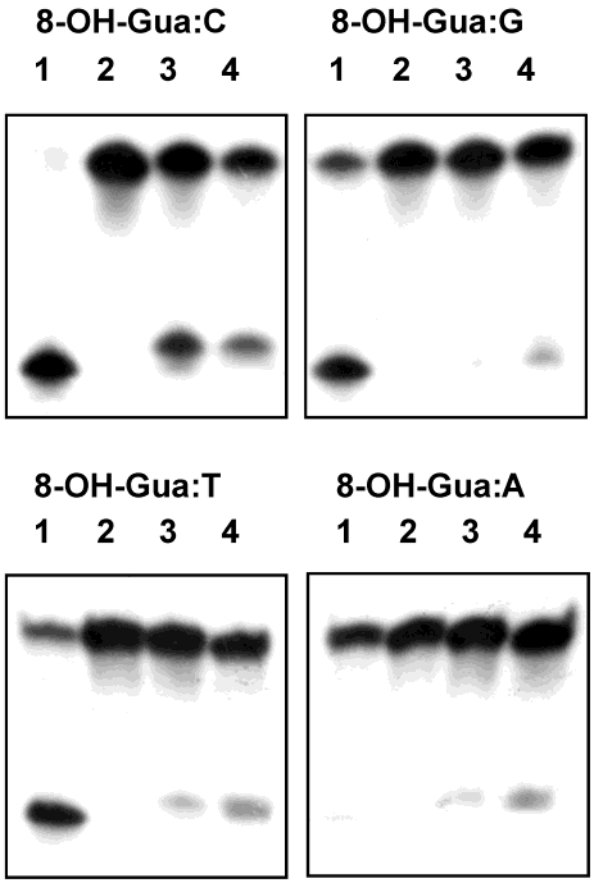

FIGURE 5: Effect of the paired-base on the excision of 8-OH-Gua by *At*Ogg1 and *E. coli* Fpg. Double-stranded oligodeoxynucleotides containing 8-OH-Gua:C, 8-OH-Gua:G, 8-OH-Gua:T, or 8-OH-Gua:A were incubated with 10 ng of *E. coli* Fpg (lane 1), no enzyme (lane 2), 0.1 μg of *At*Ogg1 (lane 3), and 1 μg of *At*Ogg1 (lane 4). After incubation at 37 °C for 1 h, the reaction products were separated by electrophoresis in a denaturing 15% polyacrylamide gel and visualized by autoradiography.

on the experimental conditions (see above) may affect the competition among products for the active site of the enzyme. As was indicated above, two DNA substrates used in this work contained different amounts of 8-OH-Gua and FapyGua.

Furthermore, clustered damaged sites and tandem lesions are generated by ionizing radiation in DNA in solution and cells (*47*−*54*). These sites that were shown to have base lesions and/or single-strand breaks in close proximity, but no double-strand breaks, or two covalently bound base lesions may interfere with the action of DNA glycosylases and other DNA repair systems (*54*−*61*). The distribution of clustered damaged sites or tandem lesions along DNA is not known and may depend on different irradiation conditions as used in this paper. In the same context, tandem lesions consisting of two DNA bases may not be formed in the presence of oxygen because of rapid reaction of oxygen with precursor hydroxyl radical-adduct radicals of DNA bases. In addition, tandem lesions 8,5′-cyclo-2′-deoxynucleosides are formed under anoxic conditions only (reviewed in ref *62*). All these factors may affect the excision of 8-OH-Gua and FapyGua by *At*Ogg1 or by any other DNA glycosylase from different DNA substrates.

The results obtained with double-stranded oligodeoxynucleotides containing 8-OH-Gua paired with each of the four DNA bases showed that the activity of *At*Ogg1 was most effective on the 8-OH-Gua:C pair. Some appreciable base excision was also detected for other substrates, particularly that with the 8-OH-Gua:T and 8-OH-Gua:A pairs. Oligodeoxynucleotides containing 8-OH-Gua:G were excised to a lower extent. These results are in agreement with those from the previous studies on *S. cerevisiae*, *Drosophila*, and human Ogg1 proteins (*14*, *16*, *18*, *25*, *36*−*38*). This clearly shows that *At*Ogg1 not only possesses common substrates with other eukaryotic Ogg1 proteins but also exhibits a similar dependence of excision on the pairing of 8-OH-Gua with intact DNA bases. Removal of 8-OH-Gua from mispairs might potentially lead to harmful biological consequences since it might contribute to fix mutations in DNA. Particularly relevant is the fact that the 8-OH-Gua:A pair was a very poor substrate of *At*Ogg1. Since 8-OH-Gua can pair with A, mispairs 8-OH-Gua:A are formed with high frequency during replication, the removal of 8-OH-Gua across A would cause fixation of mutation with high frequency. In analogy, the excision of FapyGua, which is the other substrate of Ogg1 proteins, might also depend on its pairing with DNA bases, with excision from the natural FapyGua:C pair being the highest. Due to the lack of oligodeoxynucleotides containing FapyGua, however, it was not possible to study the excision of this compound from oligodeoxynucleotides using any of the Ogg1 proteins. Recent successful synthesis of oligodeoxynucleotides, which contains FapyGua embedded at a specific position (*63*, *64*), might enable such studies in the future.

In conclusion, this work identifies 8-OH-Gua and FapyGua as the only substrates of *At*Ogg1 for excision from DNA with multiple pyrimidine- and purine-derived lesions. This means that *At*Ogg1 possesses common substrates with its functional yeast, *Drosophila*, and human homologues that had previously been studied under identical conditions. Moreover, the dependence of excision from oligodeoxynucleotides containing 8-OH-Gua on the pairing of this lesion with four intact DNA bases is in agreement with the corresponding characteristics of other Ogg1 proteins. On the other hand, kinetics of excision from DNA significantly differ among Ogg1 proteins, indicating a possible role of subtle differences in the sequence of active sites of these enzymes in their catalytic activity.

REFERENCES

1. Dizdaroglu, M. (1992) *Mutat. Res. 275*, 331−342.
2. Breen, A. P., and Murphy, J. A. (1995) *Free Radical Biol. Med. 18*, 1033−1077.
3. Lindahl, T., and Wood, R. D. (1999) *Science 286*, 1897−1905.
4. McCullough, A. K., Dodson, M. L., and Lloyd, R. S. (1999) *Annu. Rev. Biochem. 68*, 255−285.
5. 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.
6. Boiteux, S., Gajewski, E., Laval, J., and Dizdaroglu, M. (1992) *Biochemistry 31*, 106−110.
7. Bhagwat, M., and Gerlt, J. A. (1996) *Biochemistry 35*, 659−665.
8. Castaing, B., Geiger, A., Seliger, H., Nehls, P., Laval, J., Zelwer, C., and Boiteux, S. (1993) *Nucleic Acids Res. 21*, 2899−2905.
9. Michaels, M. L., Cruz, C., Grollman, A. P., and Miller, J. H. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 7022−7025.
10. Auffret van der Kemp, P., Thomas, D., Barbey, R., De Oliveira, R., and Boiteux, S. (1996) *Proc. Natl. Acad. Sci. U.S.A. 93*, 5197−5202.

11. Nash, H. M., Bruner, S. D., Schärer, O. D., Kawate, T., Addona, T. A., Sponner, E., Lane, W. S., and Verdine, G. L. (1996) *Curr. Biol. 6*, 968−980.
12. Karahalil, B., Girard, P. M., Boiteux, S., and Dizdaroglu, M. (1998) *Nucleic Acids Res. 26*, 1228−1233.
13. Girard, P. M., D'Ham, C., Cadet, J., and Boiteux, S. (1998) *Carcinogenesis 19*, 1299−1305.
14. Roldán-Arjona, T., Wei, Y. F., Carter, K. C., Klungland, A., Anselmino, C., Wang, R. P., Augustus, M., and Lindahl, T. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 8016−8020.
15. Radicella, J. P., Dherin, C., Desmaze, C., Fox, M. S., and Boiteux, S. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 8010−8015.
16. Bjorås, M., Luna, L., Johnson, B., Hoff, E., Haug, T., Rognes, T., and Seeberg, E. (1997) *EMBO J. 16*, 6314−6322.
17. Arai, K., Morishita, K., Shinmura, K., Kohno, T., Kim, S. R., Nohmi, T., Taniwaki, M., Ohwada, S., and Yokota, J. (1997) *Oncogene 14*, 2857−2861.
18. Aburatani, H., Hippo, Y., Ishida, T., Takashima, R., Matsuba, C., Kodama, T., Takao, M., Yasui, A., Yamamoto, K., Asano, M., Fukasawa, K., Yoshinari, T., Inoue, H., Ohtsuka, E., and Nishimura, S. (1997) *Cancer Res. 57*, 2151−2156.
19. Lu, R., Nash, H. M., and Verdine, G. L. (1997) *Curr. Biol. 7*, 397−407.
20. Rosenquist, T. A., Zharkov, D. O., and Grollman, A. P. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 7429−7434.
21. Eisen, J. A., and Hanawalt, P. C. (1999) *Mutat. Res. 435*, 171−213.
22. Garcia-Ortiz, M. V., Ariza, R. R., and Roldan-Arjona, T. (2001) *Plant Mol. Biol. 47*, 795−804.
23. Ohtsubo, T., Matsuda, O., Iba, K., Terashima, I., Sekiguchi, M., and Nakabeppu, Y. (1998) *Mol. Gen. Genet. 259*, 577−590.
24. Deutsch, W. A., Yacoub, A., Jaruga, P., Zastawny, T. H., and Dizdaroglu, M. (1997) *J. Biol. Chem. 272*, 32857−32860.
25. Dherin, C., Dizdaroglu, M., Doerflinger, H., Boiteux, S., and Radicella, J. P. (2000) *Nucleic Acids Res. 28*, 4583−4592.
26. Dizdaroglu, M. (1994) *Methods Enzymol. 234*, 3−16.
27. Nelson, V. C. (1996) *J. Label. Comput. Radiopharm. 38*, 713−723.
28. Dizdaroglu, M., Bauche, C., Rodriguez, H., and Laval, J. (2000) *Biochemistry 39*, 5586−5592.
29. Jaruga, P., Jabil, R., McCullough, A. K., Rodriguez, H., Dizdaroglu, M., and Lloyd, R. S. (2002) *Photochem. Photobiol. 75*, 85−91.
30. Jaruga, P., Birincioglu, M., Rodriguez, H., and Dizdaroglu, M. (2002) *Biochemistry 41*, 3703−3711.
31. Dizdaroglu, M. (1985) *Anal. Biochem. 144*, 593−603.
32. Dizdaroglu, M. (1993) *FEBS Lett. 315*, 1−6.
33. Copeland, R. A. (1996) *Enzymes*: *A Practical Introduction to Structure, Mechanism, and Data Analysis*, pp 93−119, VCH Publishers, Inc., New York.
34. Karakaya, A., Jaruga, P., Bohr, V. A., Grollman, A. P., and Dizdaroglu, M. (1997) *Nucleic Acids Res. 25*, 474−479.
35. Sentürker, S., Bauche, C., Laval, J., and Dizdaroglu, M. (1999) *Biochemistry 38*, 9435−9439.
36. Girard, P. M., Guibourt, N., and Boiteux, S. (1997) *Nucleic Acids Res. 25*, 3204−3211.
37. Asagoshi, K., Yamada, T., Terato, H., Ohyama, Y., Monden, Y., Arai, T., Nishimura, S., Aburatani, H., Lindahl, T., and Ide, H. (2000) *J. Biol. Chem. 275*, 4956−4964.
38. Audebert, M., Radicella, J. P., and Dizdaroglu, M. (2000) *Nucleic Acids Res. 28*, 2672−2678.
39. Dherin, C., Radicella, J. P., Dizdaroglu, M., and Boiteux, S. (1999) *Nucleic Acids Res. 27*, 4001−4007.
40. Boiteux, S., and Radicella, J. P. (1999) in *Advances in DNA Damage and Repair*: *Oxygen Radical Effects, Cellular Protection, and Bilogical Consequences* (Dizdaroglu, M., and Karakaya, A. E., Eds.) pp 35−45, Kluwer Academic/Plenum Publishers, New York.
41. Boiteux, S., and Radicella, J. P. (2000) *Arch. Biochem. Biophys. 377*, 1−8.
42. Boiteux, S., Gellon, L., and Guibourt, N. (2002) *Free Radical Biol. Med. 32*, 1244−1253.
43. Nash, H. M., Lu, R., Lane, W. S., and Verdine, G. L. (1997) *Chem. Biol. 4*, 693−702.
44. Bruner, S. D., Norman, D. P., and Verdine, G. L. (2000) *Nature 403*, 859−866.
45. Dizdaroglu, M. (2000) in *Free Radicals in Chemistry, Biology and Medicine* (Yoshikawa, T., Toyokuni, S., Yamamoto, Y., and Naito, Y., Eds.) pp 58−76, OICA International, London.
46. Karahalil, B., Roldan-Arjona, T., and Dizdaroglu, M. (1998) *Biochemistry 37*, 590−595.
47. Ward, J. F. (1995) *Radiat. Res. 142*, 362−368.
48. Budzinski, E. E., Dawidzik, J. D., Wallace, J. C., Freund, H. G., and Box, H. C. (1995) *Radiat. Res. 142*, 107−109.
49. Cunniffe, S., and O'Neill, P. (1999) *Radiat. Res. 152*, 421−427.
50. Sutherland, B. M., Bennett, P. V., Sidorkina, O., and Laval, J. (2000) *Proc. Natl. Acad. Sci. U.S.A. 97*, 103−108.
51. Box, H. C., Patrzyc, H. B., Dawidzik, J. B., Wallace, J. C., Freund, H. G., Iijima, H., and Budzinski, E. E. (2000) *Radiat. Res. 153*, 442−446.
52. Jenner, T. J., Fulford, J., and O'Neill, P. (2001) *Radiat. Res. 156*, 590−593.
53. Box, H. C., Dawidzik, J. B., and Budzinski, E. E. (2001) *Free Radical Biol. Med. 31*, 856−868.
54. Blaisdell, J. O., and Wallace, S. S. (2001) *Proc. Natl. Acad. Sci. U.S.A. 98*, 7426−7430.
55. Chaudhry, M. A., and Weinfeld, M. (1995) *J. Mol. Biol. 249*, 914−922.
56. Harrison, L., Hatahet, Z., Purmal, A. A., and Wallace, S. S. (1998) *Nucleic Acids Res. 26*, 932−941.
57. Harrison, L., Hatahet, Z., and Wallace, S. S. (1999) *J. Mol. Biol. 290*, 667−684.
58. David-Cordonnier, M. H., Laval, J., and O'Neill, P. (2000) *J. Biol. Chem. 275*, 11865−11873.
59. David-Cordonnier, M. H., Laval, J., and O'Neill, P. (2001) *Biochemistry 40*, 5738−5746.
60. David-Cordonnier, M. H., Boiteux, S., and O'Neill, P. (2001) *Biochemistry 40*, 11811−11818.
61. David-Cordonnier, M. H., Boiteux, S., and O'Neill, P. (2001) *Nucleic Acids Res. 29*, 1107−1113.
62. Dizdaroglu, M., Jaruga, P., Birincioglu, M., and Rodriguez, H. (2002) *Free Radical Biol. Med. 32*, 1102−1115.
63. Haraguchi, K., and Greenberg, M. M. (2001) *J. Am. Chem. Soc. 123*, 8636−8637.
64. Haraguchi, K., Delaney, M. O., Wiederholt, C. J., Sambandam, A., Hantosi, Z., and Greenberg, M. M. (2002) *J. Am. Chem. Soc. 124*, 3263−3269.

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