Nucleic Acids Research, 1999, Vol. 27, No. 20 **4001–4007**

Excision of oxidatively damaged DNA bases by the human α-hOgg1 protein and the polymorphic α-hOgg1(Ser326Cys) protein which is frequently found in human populations

Claudine Dherin, J. Pablo Radicella, Miral Dizdaroglu[1] and Serge Boiteux*

CEA, DSV, DRR, UMR217 CNRS-CEA, Radiobiologie Moléculaire et Cellulaire, 60 rue du Général Leclerc, BP6, 92265-Fontenay aux Roses, France and [1]Chemical Science and Technology Laboratory, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA

Received July 14, 1999; Revised and Accepted August 30, 1999

ABSTRACT

We have investigated the substrate specificity of the major nuclear form of the human Ogg1 protein, referred as α-hOgg1, for excision of damaged bases from DNA exposed to γ-irradiation. Excision products were identified and quantified using gas chromatography/ isotope dilution mass spectrometry (GC/IDMS). The GST–α-hOgg1 protein used in this study is a fusion of α-hOgg1 to the C-terminus of the GST protein. The results show that GST–α-hOgg1 protein excises 8-hydroxyguanine (8-OH-Gua) and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) from DNA exposed to γ-irradiation in a solution saturated with N_2O or air. Fourteen other lesions, including oxidised purines and pyrimidines, were not excised from these substrates. Catalytic constants were measured for the excision of 8-OH-Gua and FapyGua from DNA γ-irradiated under N_2O. The k_{cat}/K_m values for excision of 8-OH-Gua and FapyGua were 4.47×10^{-5} and 8.97×10^{-5} (min^{-1} nM^{-1}), respectively. The substrate specificity and the catalytic parameters of the wild-type GST–α-hOgg1 protein were compared to that of a polymorphic form of α-hOgg1 harbouring a Ser→Cys mutation at codon 326. In the Japanese population, 47.6% of individuals possess both alleles coding for the wild-type α-hOgg1-Ser^{326} and mutant α-hOgg1-Cys^{326} proteins. The GST–α-hOgg1-Cys^{326} protein was purified and its substrate specificity was determined by GC/IDMS analysis. The results show that the GST–α-hOgg1-Cys^{326} protein efficiently excises 8-OH-Gua and FapyGua from γ-irradiated DNA. The k_{cat}/K_m values for excision of 8-OH-Gua and FapyGua were 2.82×10^{-5} and 4.43×10^{-5} (min^{-1} nM^{-1}), respectively. Furthermore, we compared the capacity of these two forms of α-hOgg1 to act on substrates containing 2,6-diamino-4-hydroxy-5-*N*-methylformamidopyrimidine (Me-FapyGua). The k_{cat}/K_m values for excision of Me-FapyGua were 278×10^{-5} and 319×10^{-5} (min^{-1} nM^{-1}), respectively. Cleavage of 34mer oligodeoxyribonucleotides containing 8-OH-Gua, 8-hydroxyadenine or an apurinic/apyrimidinic site paired with a cytosine was also investigated. The results show that both GST–α-hOgg1-Ser^{326} and GST–α-hOgg1-Cys^{326} catalyse the various cleavage reactions at very similar rates. Furthermore, both proteins efficiently complement the mutator phenotype of the *fpg mutY* mutant of *Escherichia coli*.

INTRODUCTION

DNA damage generated by reactive oxygen species (ROS) has been implicated in mutagenesis and carcinogenesis and may play a role in the pathogenesis of aging (1–4). Most of these lesions are substrates for DNA repair systems in prokaryotes and eukaryotes (5). Oxidised bases in DNA are primarily repaired by the base excision repair (BER) pathway (5–8). The first step in this ubiquitous repair pathway is the recognition and removal of the altered base by a DNA glycosylase catalysing cleavage of the glycosylic bond between the modified base and the sugar moiety, leaving an abasic apurinic/apyrimidinic (AP) site in DNA. Subsequently, the resulting AP site is incised and repair is completed by the successive actions of a phosphodiesterase, a DNA polymerase and a DNA ligase (5–8). An oxidatively damaged form of guanine, 8-hydroxyguanine (8-OH-Gua), is a highly mutagenic DNA lesion yielding GC→TA tranversions (9,10). *Escherichia coli* possesses two DNA glycosylases that prevent mutagenesis by 8-OH-Gua: the Fpg protein which excises 8-OH-Gua in damaged DNA and the MutY protein which excises the adenine residues incorporated by DNA polymerases opposite 8-OH-Gua (11–13). Inactivation of both the *fpg* (*mutM*) and *mutY* (*micA*) genes of *E.coli* results in a strong GC→TA mutator phenotype (14–16). In *Saccharomyces cerevisiae*, the *OGG1* gene encodes a DNA glycosylase activity that catalyses the removal of 8-OH-Gua and and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) from damaged DNA (17–19). Furthermore, Ogg1-deficient strains of *S.cerevisiae* exhibit a mutator phenotype and specifically accumulate GC→TA transversions (20,21). These results

*To whom correspondence should be addressed. Tel: +33 1 46 54 88 58; Fax: +33 1 46 54 88 59; Email: boiteux@dsvidf.cea.fr

strongly suggest that BER of 8-OH-Gua, by the Fpg or Ogg1 proteins, protects genomes from the mutagenic action of ROS in prokaryotes or in the simple eukaryote *S.cerevisiae* (22).

Two human cDNAs encoding proteins of 345 and 424 amino acids showing strong sequence similarities with the yeast Ogg1 protein have been cloned (23–30). These proteins, which we named α-hOgg1 and β-hOgg1, have 316 identical amino acids at the N-terminus but exhibit completely different sequence at the C-terminus (reviewed in 22). Both the α-hOgg1 and β-hOgg1 proteins catalyse the cleavage of DNA duplexes containing 8-OH-Gua paired with a cytosine and complement the mutator phenotype of a *fpg mutY* strain of *E.coli* (23–30). These two forms of hOgg1 are the result of an alternative splicing after transcription of the *hOGG1* gene localised on chromosome 3p25 (reviewed in 22). Recent experiments have shown that α-hOgg1 and β-hOgg1 are targeted to the nucleus and the mitochondrion, respectively (31,32). The nuclear α-hOgg1 protein is a DNA glycosylase/AP lyase which releases 8-OH-Gua and 2,6-diamino-4-hydroxy-5-*N*-methylformamidopyrimidine (Me-FapyGua) and catalyses strand cleavage at the 3′-side of an AP site via a β-elimination reaction (23–26,28–30). Furthermore, α-hOgg1 incises 34mer DNA fragments containing 8-hydroxyadenine (8-OH-Ade) mispaired with a cytosine, as does the yeast Ogg1 protein (33). The catalytic mechanism of α-hOgg1 involves the formation of a transient covalent imino enzyme–DNA intermediate involving Lys249, which can be trapped in the presence of sodium borohydride (34). The biological function of Fpg and yeast Ogg1 proteins is to prevent mutations induced by endogeneous ROS (reviewed in 22). By analogy, the hOgg1 protein may also have an anti-mutator function in human cells and its inactivation may be involved in the complex process of carcinogenesis. Therefore, human tumours have been analysed for expression and mutation of the *hOGG1* gene. Indeed, somatic and polymorphic mutations of *hOGG1* have been found in human lung and kidney tumours (35,36). A genetic polymorphism at codon 326 (Ser326Cys) was frequently found in the Japanese population, in both healthy individuals and lung cancer patients (35). The same polymorphism was also found at a similar frequency in European patients with head and neck or kidney tumours (37; unpublished results). A reduced activity of the mutant α-hOgg1-Cys^{326} protein compared to that of the wild-type (Ser^{326}) was also reported (35).

The objective of the present study was to analyse the substrate specificity of the human α-hOgg1 protein for a variety of oxidatively damaged purines and pyrimidines in DNA exposed to γ-irradiation. We utilized the technique of gas chromatography/isotope dilution mass spectrometry with selected ion monitoring (GC/IDMS-SIM) to determine the excision of lesions and their kinetic parameters (38–42). The results show that α-hOgg1 protein excises 8-OH-Gua and FapyGua from DNA exposed to γ-irradiation. Furthermore, we compared the repair capacity of wild-type α-hOgg1 with that of the polymorphic version α-hOgg1-Cys^{326}. Our results show that the two enzymes are functional and display the same substrate specificity, releasing 8-OH-Gua, FapyGua and Me-FapyGua in damaged DNA. Furthermore, they both incise 34mer DNA duplexes containing 8-OH-Gua, 8-OH-Ade and AP sites paired with a cytosine.

MATERIALS AND METHODS

Materials

Modified DNA bases, their stable isotope-labelled analogues and other materials for GC/IDMS were obtained as described previously (40–42). Calf thymus DNA and poly(dG-dC)·poly-(dG-dC) were purchased from Sigma and Boehringer. Restriction endonucleases, DNA polymerases and T4 DNA ligase were from New England Biolabs and Boehringer. Uracil-DNA glycosylase from *E.coli* was from our laboratory stock.

Preparation of DNA substrates

The preparation of DNA samples exposed to a ^{60}Co γ-ray source at a dose of 80 Gy under N_2O or air was described elsewhere (42). DNA solutions were dialysed against 10 mM phosphate buffer for 18 h at 4°C. Phosphate buffer was changed three times during the course of dialysis. The [^{3}H]Me-FapyGua-poly-(dG-dC)·poly(dG-dC) substrate was prepared as previously described (43). The 34mer oligodeoxyribonucleotides used in this study have the following sequence: 5′-GGCTTCATCG-TTGTCXCAGACCTGGTGGATACCG-3′ with X = 8-OH-Gua, 8-OH-Ade or uracil, respectively. Oligodeoxyribonucleotides containing 8-OH-Gua or 8-OH-Ade were a kind gift of Drs A. Guy and J. Cadet (CEA-Grenoble, France) (44). Oligodeoxyribonucleotides containing uracil and the complementary sequence with a cytosine placed opposite X in the duplex were purchased from OligoExpress (Grenoble, France). To generate the AP sites, the 34mer DNA containing uracil was incubated in the presence of uracil-DNA glycosylase (45).

Expression and purification of GST–α-hOgg1-Ser^{326} and GST–α-hOgg1-Cys^{326} proteins

The open reading frame coding for α-hOgg1was excised from plasmid pPR59 (26) as a *Hin*dIII (rendered blunt by filling in with Klenow)–*Eco*RI DNA fragment. This 1 kb fragment was cloned into pGEX-4T 1 (Pharmacia Biotech) using the *Xho*I (filled in by Klenow) and *Eco*RI sites of the polylinker, yielding plasmid pPR71. This plasmid allows the expression of a fusion protein, GST–α-hOgg1, where the α-hOgg1 protein is fused to the C-terminus of GST protein. To express the GST–α-hOgg1-Cys^{326} protein, pPR71 was mutagenized using the QuickChange site-directed mutagenesis kit (Stratagene) according to the manufacturer's protocol to generate plasmid pPR180. The oligodeoxyribonucleotides used were: 5′-CGACCTGCGCCAAT-GCCGCCATGCTCAGG-3′ and its complementary sequence. The sequence change, as well as the integrity of the rest of the sequence were confirmed.

Escherichia coli BH410 (*fpg*$^-$) harbouring plasmid pPR71 or pPR180 was grown at 37°C in LB broth (5 l) containing 500 μg/ml ampicillin until the absorbance at 600 nm reached 0.3 and induced for 3 h at 37°C in the presence of IPTG (0.5 mM). Cells were collected, 8 or 9 g (w/w), and stored at –80°C. Lysis was performed as previously described (41). After centrifugation of the cell lysate, the supernatant fraction (fraction 1) was dialysed against phosphate-buffered saline (PBS) and applied to a glutathione–Sepharose 4B (Pharmacia Biotech) column equilibrated with PBS. The column was washed with PBS and eluted with a buffer containing 50 mM Tris–HCl pH 8.0 and 10 mM reduced glutathione. Fractions containing the enzyme activity were pooled (fraction 2) and dialysed against a buffer containing 20 mM Tris–HCl pH 7.6,

2 mM Na_2EDTA, 50 mM NaCl and 5% glycerol (v/v) and applied to a MonoS PC1.6/5 column (SMART System; Pharmacia Biotech). The column was eluted with a linear salt gradient (50–800 mM NaCl). Fractions containing enzyme activity were pooled (fraction 3) and the concentration was adjusted to 1 mg/ml. Glycerol was added to 50% and the protein solution was stored at –20°C. Both proteins were purified using the excision of [^{3}H]Me-FapyGua from [^{3}H]Me-FapyGua-poly-(dG-dC)·poly(dG-dC) as an activity assay (43). One unit releases 1 pmol of Me-FapyGua in 15 min at 37°C. The protein concentration was determined using the method of Bradford (46).

Enzymatic assays for GC/IDMS analysis

Irradiated and control DNA samples (100 µg) were dried under vacuum. DNA samples were dissolved in phosphate buffer (50 mM final concentration, pH 7.4) containing 100 mM KCl, 2 mM Na_2EDTA and 2 mM dithiothreitol. Depending on the experiment, various amounts of GST–α-hOgg1-Ser^{326} or GST–α-hOgg1-Cys^{326} protein were added to the mixture. The total volume of the mixture was 110 µl. Three replicates of each mixture were incubated at 37°C for periods of time depending on the experiment. As controls, DNA samples were incubated with heat-inactivated enzyme (140°C for 15 min) or without enzyme. The kinetic constants were determined as described (40–42). The amount of GST–α-hOgg1-Ser^{326} or GST–α-hOgg1-Cys^{326} protein was 1 µg/100 µg DNA in 110 µl of the incubation mixture, corresponding to a concentration of 138 nM. Three replicates of DNA samples were incubated with or without each enzyme at 37°C. Following incubation, DNA samples were precipitated with 270 µl of cold ethanol, kept at –20°C for 2 h, and centrifuged at 15 000 *g* for 30 min at 4°C. Subsequently, DNA pellets and supernatant fractions were separated. Aliquots of stable isotope-labelled analogues of modified DNA bases were added as internal standards to pellets with known DNA amounts and to supernatant fractions. Pellets were dried under vacuum in a SpeedVac and then hydrolysed with 0.5 ml of 60% formic acid in evacuated and sealed tubes at 140°C for 30 min. The hydrolysates were lyophilised in vials for 18 h. Supernatant fractions were freed from ethanol under vacuum in a SpeedVac and subsequently lyophilised for 18 h without prior hydrolysis. Both lyophilised supernatant fractions and hydrolysates of DNA pellets were derivatised and analysed by GC/IDMS (39–42).

Assays for cleavage of 8-OH-Gua:C or 8-OH-Ade:C 34mer DNA duplexes

The DNA strand containing 8-OH-Gua or 8-OH-Ade was ^{32}P-labelled at the 5′-end and annealed with the complementary sequence yielding the 8-OH-Gua:C or 8-OH-Ade:C duplexes as described (45). In a standard reaction (10 µl final volume), 50 fmol of ^{32}P-labelled 8-OH-Gua:C or 8-OH-Ade:C duplex were incubated in reaction buffer (25 mM Tris–HCl pH 7.6, 2 mM Na_2EDTA, 50 mM NaCl) with the α-hOgg1 proteins. The reactions were performed at 37°C for 15 min. Reactions were stopped by adding 6 µl of formamide dye and subjected to 7 M urea–20% PAGE (45). Gels were scanned and quantified using a Bio-Rad PhosphorImager.

Assay for cleavage of AP site:C 34mer DNA duplex

The DNA strand containing uracil was ^{32}P-labelled at the 5′-end, annealed to the complementary sequence and incubated with purified uracil-DNA glycosylase to generate the AP:C substrate (45). Reactions were performed at 37°C for 15 min as described for 8-OH-Gua:C. Separation of products and quantification were as described for 8-OH-Gua-containing duplex.

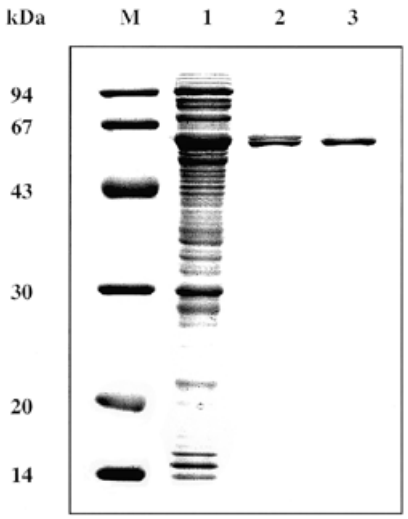

Figure 1. SDS–PAGE analysis of the GST–α-hOgg1-Ser^{326} protein purification fractions. Lane M, molecular weight markers (Amersham-Pharmacia Biotech); lanes 1–3, purification steps of the GST–α-hOgg1-Ser^{326} protein. Purification step and amounts of protein are as follows: lane 1, cell free extract (50 µg); lane 2, eluate from glutathione–Sepharose 4B (2 µg); lane 3, eluate from MonoS PC1.6/5 (SMART; Pharmacia Biotech) (2 µg). The gel was 15% acrylamide, 0.4% bis-acrylamide and stained with Coomassie brilliant blue.

Mutagenesis experiments

Complementation of the spontaneous mutator phenotype of an *E.coli fpg mutY* double mutant (PR195) by expression of the different GST fusion proteins was analysed by determining the frequency of rifampicin-resistant cells in 20 independent cultures (26).

RESULTS

Expression and purification of the GST–α-hOgg1-Ser^{326} and GST–α-hOgg1-Cys^{326} proteins

The major nuclear form of the hOgg1 protein in human cells is composed of 345 amino acids and is referred as α-hOgg1 (22). To overproduce α-hOgg1, its cDNA coding sequence was PCR amplified and cloned into vector pGEX-4T 1, yielding plasmid pPR71 expressing α-hOgg1 fused to the C-terminus of GST protein. The wild-type version of the α-hOgg1 protein possesses a serine residue at position 326 (26); it will be referred as α-hOgg1-Ser^{326}. The GST–α-hOgg1-Ser^{326} protein was purified from *E.coli* BH410 (*fpg*$^-$) using the release of Me-FapyGua from [^{3}H]Me-FapyGua-poly(dG-dC)·poly(dG-dC) as an activity assay (43). The purity of the protein was assessed by the observation of a single protein band on SDS–PAGE with a molecular mass of ~65 kDa, which agreed well with the expected mass (65.6 kDa) of the fusion protein (Fig. 1). Digestion of the GST–α-hOgg1-Ser^{326} protein by thrombin resulted in

extensive degradation of the protein (data not shown). Therefore, the GST–α-hOgg1-Ser^{326} fusion protein was used in all experiments reported in this study. The same procedure was used to purify GST–α-hOgg1-Cys^{326} protein (data not shown). Kinetic constants for GST–α-hOgg1-Ser^{326} and GST–α-hOgg1-Cys^{326} proteins for the excision of Me-FapyGua were determined. Table 1 shows that the specificity constants K_{cat}/K_m are very similar, 278×10^{-5} and 319×10^{-5} (nM^{-1} min^{-1}), respectively.

Table 1. Kinetic constants for excision of Me-FapyGua by GST–α-hOgg1-Ser326 or GST–α-hOgg1-Cys326 protein

Protein	$k_{cat} \times 10^3$ (min^{-1})	K_m (nM)	$k_{cat}/K_m \times 10^5$ (min^{-1} nM^{-1})
GST–α-hOgg1-Ser^{326}	89	32	278
GST–α-hOgg1-Cys^{326}	67	21	319

A Lineweaver–Burk plot was used for the determination of kinetic constants for GST–α-hOgg1-Ser^{326} or GST–α-hOgg1-Cys^{326} protein for excision of Me-FapyGua. The substrate concentration [S] was given as the concentration of Me-FapyGua in [^{3}H]Me-FapyGua-poly(dG-dC)·poly(dG-dC).

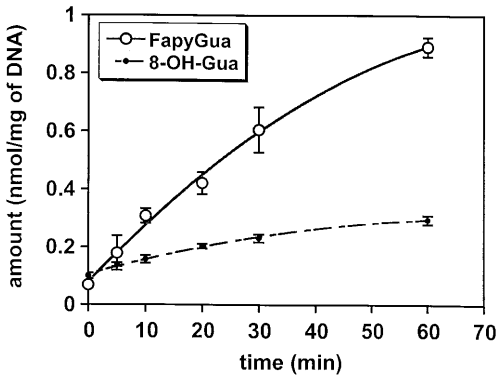

Figure 2. Excision of 8-OH-Gua and FapyGua from DNA γ-irradiated under N_2O as a function of the incubation time by wild-type GST–α-hOgg1-Ser^{326} protein. Purified GST–α-hOgg1-Ser^{326} protein (1 μg) was incubated with 100 μg of DNA exposed to γ-irradiation under NO_2. The amounts of products given on the *y*-axis represent those found in the supernatant fractions. Each data point represents the mean (± SD) from the analysis of three independently prepared samples. A value of 1 nmol lesion/mg DNA corresponds to 32 lesions/10^5 DNA bases.

Excision of modified bases by the wild-type GST–α-hOgg1-Ser^{326} protein from γ-irradiated DNA: GC/IDMS analysis

A DNA glycosylase activity of α-hOgg1 protein has been previously demonstrated. It releases 8-OH-Gua from DNA fragments containing a single lesion and Me-FapyGua from alkylated DNA (23–30). However, no other modified bases have been tested as substrates of this enzyme, especially using oxidatively damaged DNA containing a multiplicity of pyrimidine- and purine-derived lesions. In this work, we used DNA substrates damaged by γ-irradiation under N_2O or air. Sixteen and 12 modified bases were identified and quantified by GC/IDMS in DNA samples damaged under anoxic (irradiation under N_2O) and oxic (irradiation under air) conditions, respectively (39,40). These were FapyGua, 8-OH-Gua, 4,6-diamino-5-formamidopyrimidine (FapyAde), 8-OH-Ade, 2-hydroxyadenine, 5-hydroxy-5-methylhydantoin, 5-hydroxyhydantoin, 5-OH-Ura, 5-OH-Cyt, 5-(hydroxymethyl)uracil, thymine glycol, 5,6-dihydroxyuracil, 5,6-dihydrothymine, 5,6-dihydrouracil, 5-hydroxy-6-hydrothymine and 5-hydroxy-6-hydrouracil. The presence of oxygen in an aqueous solution of DNA during γ-irradiation modified the types and yields of these compounds and thus the latter four compounds are not produced in DNA irradiated under air, due to inhibition of their formation by oxygen (38). On the other hand, products such as thymine glycol are formed at higher yield in air-irradiated DNA (38). Of the 16 modified bases identified in these damaged DNA substrates, the GST–α-hOgg1-Ser^{326} protein efficiently excised 8-OH-Gua and FapyGua. Figure 2 illustrates excision of 8-OH-Gua and FapyGua from DNA γ-irradiated under N_2O as a function of the incubation time. Similar results were obtained using DNA γ-irradiated under air (data not shown). Other purine- or pyrimidine-derived lesions were not significantly excised from these DNA substrates. The excision of 8-OH-Gua and FapyGua was assessed by their appearance in supernatant fractions of DNA substrates incubated with active enzyme. The amounts of these modified bases in DNA pellets incubated with active protein were significantly reduced when compared to those in DNA pellets incubated with inactivated enzyme or without enzyme (data not shown). The amounts found in the supernatant fractions of DNA substrates incubated with active enzyme were similar to those removed from the pellets of the same DNA substrate, demonstrating excision of the lesions.

Excision was determined as a function of the concentration of 8-OH-Gua and FapyGua in the DNA substrate. Kinetic constant values were obtained from measurements at six different concentrations of each lesion in DNA γ-irradiated under N_2O. Lineweaver–Burk plots representing the reciprocal of initial velocity versus the reciprocal of substrate concentration were utilized to determine the kinetic constants (40–42). Initial velocities were estimated using the plots of excision as a function of time for each excised lesion. Figure 3 illustrates Lineweaver–Burk plot analysis for the excision of FapyGua by GST–α-hOgg1-Ser^{326} and GST–α-hOgg1-Cys^{326} proteins. The k_{cat} and K_m values for excision of 8-OH-Gua and FapyGua are given in Table 2. These results suggest that FapyGua may be a better substrate than 8-OH-Gua in N_2O γ-irradiated DNA for the wild-type GST–α-hOgg1-Ser^{326} protein.

Excision of modified bases by the mutant GST–α-hOgg1-Cys^{326} protein from γ-irradiated DNA: GC/IDMS analysis

We have purified GST–α-hOgg1-Cys^{326} as described for the wild-type protein. The substrate specificity of the polymorphic version of α-hOgg1 was determined using γ-irradiated DNA under N_2O as substrate and GC/IDMS. The results show that GST–α-hOgg1-Cys^{326} efficiently excises 8-OH-Gua and FapyGua from γ-irradiated DNA as a function of incubation time and enzyme concentration. Kinetic constants for GST–α-hOgg1-Cys^{326} were determined as described for the wild-type protein. The k_{cat}/K_m values for excision of 8-OH-Gua and FapyGua are given in Table 2. These results also indicate a greater specificity for excision of FapyGua compared to 8-OH-Gua for

Table 2. Kinetic constants for excision of 8-OH-Gua and FapyGua lesions from DNA exposed to γ-irradiation under N_2O by wild-type GST–α-hOgg1-Ser^{326} and mutant GST–α-hOgg1-Cys^{326} proteins

Protein	$k_{cat} \times 10^3$ (min^{-1})		K_m (nM)		$k_{cat}/K_m \times 10^5$ (min^{-1} nM^{-1})	
	8-OH-Gua	FapyGua	8-OH-Gua	FapyGua	8-OH-Gua	FapyGua
GST–α-hOgg1-Ser^{326}	83.4 ± 1.6[a,b]	211.4 ± 4.0[a]	1863 ± 144	2356 ± 184	4.47 ± 0.09[a,c]	8.97 ± 0.17[a]
GST–α-hOgg1-Cys^{326}	65.5 ± 2.2[b]	111.3 ± 1.3	2319 ± 305	2513 ± 112	2.82 ± 0.09[c]	4.43 ± 0.05

Values represent the means ± SD (n = 6) ($k_{cat} = V_{max}$/[enzyme]). Both enzymes were used at a concentration of 138 nM. Numbers of lesions in DNA γ-irradiated under N_2O (nmol/mg DNA) were similar to those reported previously (42). The concentration ranges of the two lesions used for determination of the kinetic constants were as follows: 0.86–2.75 μM for 8-OH-Gua; 0.91–5.13 μM for FapyGua.

[a]Statistically different from the value in line 2 ($P < 0.05$).

[b]Statistically different from the values in column 2 ($P < 0.05$).

[c]Statistically different from the values in column 6 ($P < 0.05$).

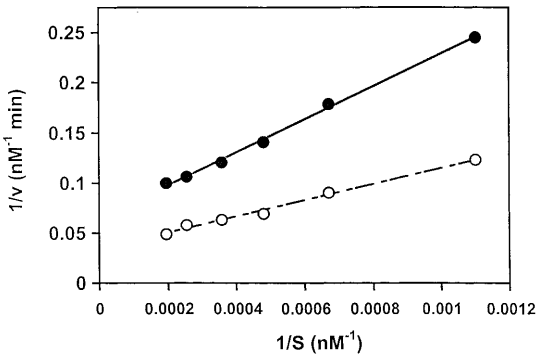

Figure 3. Lineweaver–Burk plots for excision of FapyGua by wild-type GST–α-hOgg1-Ser^{326} and GST–α-hOgg1-Cys^{326} protein from DNA γ-irradiated under N_2O. The enzyme amount was 1 μg/100 μg DNA. S, concentration of FapyGua (0.91–5.13 μM); v, initial velocity. The amounts of product found in the supernatant fraction were used for initial velocity. (Open circle), GST–α-hOgg1-Ser^{326}; (filled circle), GST–α-hOgg1-Cys^{326}.

the mutant protein, as was shown for the wild-type protein. The k_{cat}/K_m values for excision of FapyGua and 8-OH-Gua are 2.0- and 1.6-fold lower for the mutant protein compared to the wild-type (Table 2).

Cleavage of 34mer DNA duplexes containing 8-OH-Gua, 8-OH-Ade or an AP site paired with a cytosine by GST–α-hOgg1-Ser^{326} and GST–α-hOgg1-Cys^{326} proteins

The catalytic properties of GST–α-hOgg1-Ser^{326} and GST–α-hOgg1-Cys^{326} proteins were also compared using DNA lesions embedded in 34mer DNA duplexes as substrates. The lesion, either 8-OH-Gua, 8-OH-Ade or an AP site, was localised at the same position of the 34mer oligodeoxyribonucleotide. After 5′-end-labelling, the DNA strand containing the lesion was hybridised with a complementary sequence containing a cytosine paired with the lesion in the DNA duplex. Figure 4 shows that both GST–α-hOgg1-Ser^{326} and GST–α-hOgg1-Cys^{326} proteins efficiently cleave the three 34mer DNA duplexes containing 8-OH-Gua:C (Fig. 4A), 8-OH-Ade:C (Fig. 4B) or an AP site:C (Fig. 4C). Comparison of the efficacy of the two isoforms of α-hOgg1 for cleavage of the three substrates does not reveal significant differences. The GST–α-hOgg1-Ser^{326} protein does not cleave 34mer oligodeoxyribonucleotide duplexes containing 8-OH-Ade paired with a thymine, a guanine or an adenine (data not shown). This last result explains why 8-OH-Ade is not released from γ-irradiated DNA substrates. In addition, a 34mer DNA duplex containing a modified pyrimidine (5,6-dihydrothymine) paired with a cytosine or an adenine was not incised (data not shown), which is in agreement with our GC/IDMS analysis.

Expression of GST–α-hOgg1-Ser^{326} and GST–α-hOgg1-Cys^{326} proteins in *E.coli fpg mutY* complements the mutator phenotype

Plasmids expressing GST–α-hOgg1-Ser^{326} or GST–α-hOgg1-Cys^{326} protein were transformed into an *E.coli* strain PR195 in which the *mutY* and *fpg* genes are disrupted. This strain displays a strong spontaneous mutator phenotype due to its incapacity to eliminate errors induced by the presence of 8-OH-Gua in its DNA. The rates of mutation to rifampicin resistance were determined. Table 3 shows that expression of the human proteins in this strain strongly reduces the mutation frequencies for Rif^R, partially complementing the mutator phenotype. Furthermore, GST–α-hOgg1-Ser^{326} and GST–α-hOgg1-Cys^{326} proteins display the same capacity to complement the mutator phenotype (Table 3).

Table 3. Frequencies of spontaneous mutation to rifampicin resistance in *E.coli fpg mutY* expressing GST–α-hOgg1-Ser^{326} or GST–α-hOgg1-Cys^{326} protein

Protein expresssed	Rif^r/10^8
GST	257 ± 35
GST–α-hOgg1-Ser^{326}	137 ± 23
GST–α-hOgg1-Cys^{326}	100 ± 17

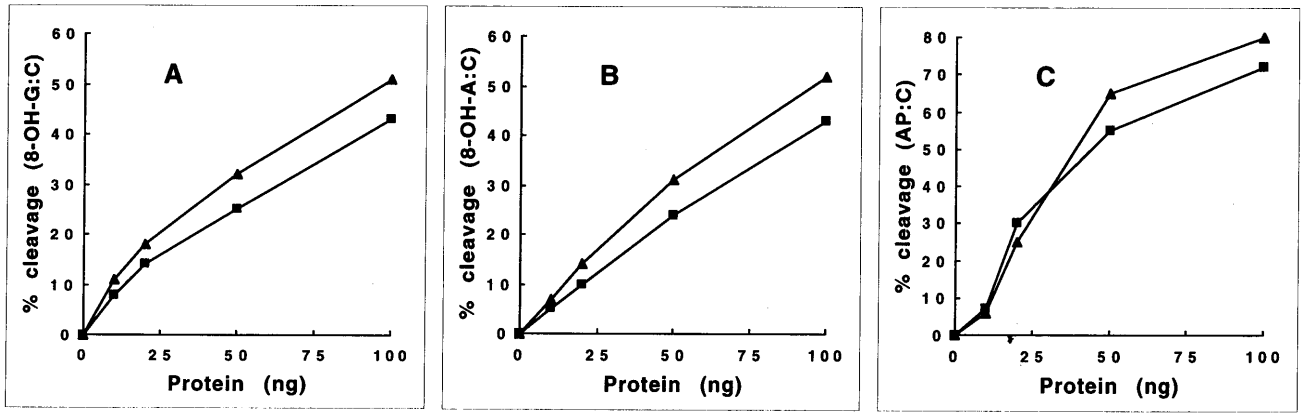

Figure 4. Cleavage of 34mer oligodeoxyribonucleotide duplexes containing 8-OH-Gua, 8-OH-Ade or an AP site paired with a cytosine by wild-type GST–α-hOgg1-Ser^{326} or mutant GST–α-hOgg1-Cys^{326} protein. The 8-OH-Gua-, 8-OH-Ade- or AP site-containing strands were ^{32}P-labelled at the 5′-end and annealed with the complementary sequence carrying a cytosine placed opposite the lesion. These DNA duplexes (50 fmol) were incubated at 37°C for 15 min with of GST–α-hOgg1-Ser^{326} protein (triangle) or GST–α-hOgg1-Cys^{326} protein (square). The reaction was terminated as described in Materials and Methods. The products of the reaction were separated by denaturing 20% PAGE with 7 M urea. Reaction products were quantified using a Bio-Rad PhosphorImager. (**A**) 8-OH-Gua:C duplex; (**B**) 8-OH-Ade:C duplex; (**C**) AP site:C duplex.

DISCUSSION

The substrate specificity of the major nuclear form of the human Ogg1 protein, α-hOgg1, was investigated using γ-irradiated DNA substrates and GC/IDMS. The results show that the wild-type GST–α-hOgg1-Ser^{326} protein efficiently excises 8-OH-Gua and FapyGua from DNA exposed to γ-irradiation under N_2O or air. In contrast, 14 other lesions, including FapyAde and 8-OH-Ade and a variety of oxidised pyrimidines, are not released from irradiated DNA by the GST–α-hOgg1-Ser^{326} protein. The specificity constant for excision of FapyGua from DNA γ-irradiated under N_2O was greater than that for excision of 8-OH-Gua, indicating the preference of α-hOgg1 protein for excision of the former lesion from this DNA substrate. The GST–α-hOgg1-Ser^{326} protein also releases Me-FapyGua and incises 34mer oligodeoxyribonucleotide duplexes containing 8-OH-Ade or an AP site paired with a cytosine. To the best of our knowledge, these results indicate that α-hOgg1 and yeast Ogg1 proteins have a similar substrate specificity.

The biological function of the yeast Ogg1 protein is to protect the genome from the mutagenic action of ROS. In *S.cerevisiae*, Ogg1-deficient strains accumulate GC→TA transversions, probably due to the presence of unrepaired 8-OH-Gua residues in DNA (20). By analogy, we suggest that the biological function of α-hOgg1 is to protect the genetic material in the nucleus from the mutagenic action of endogenous ROS. Since mutation events are associated with cancer, it has been proposed that a mutator phenotype might be involved at some point in the multistage process of carcinogenesis (2). This model has been actually confirmed by the finding that hereditary non-polyposis colorectal cancer (HNPCC) is associated with defects in the gene coding for a homologue of the bacterial mismatch repair protein MutS (47–50). The expected antimutator activity of the α-hOgg1 protein suggests that this protein may play a role in the prevention of some cancer pathologies. A frequent polymorphism at codon 326 corresponding to an amino acid substitution (Ser326Cys) was found in human populations (35,37). The allelic frequencies for the wild-type (Ser^{326}) and the mutant (Cys^{326}) are 59.5 and 40.5% in the healthy Japanese population. These allelic frequencies (Ser^{326}/Cys^{326}) are not significantly different in Japanese patients with lung cancer (35). However, the authors stated that repair activity of the α-hOgg1-Ser^{326} protein was greater than that of the α-hOgg1-Cys^{326} protein (35). In this study, we purified both wild-type and the Ser326Cys form of GST–α-hOgg1 and analysed their substrate specificities and determined their catalytic constants. The results show that both GST–α-hOgg1-Ser^{326} and GST–α-hOgg1-Cys^{326} proteins excise 8-OH-Gua and FapyGua from γ-irradiated DNA. The k_{cat}/K_m values for excision of FapyGua and 8-OH-Gua are 2.0- and 1.6-fold lower for the mutant protein compared to the wild-type. On the other hand, both proteins excise Me-FapyGua and incise 34mer DNA duplexes containing 8-OH-Gua, 8-OH-Ade or an AP site paired with a cytosine at nearly identical rates. Since the proteins used in this study are tagged and expressed in bacteria, these minor differences in catalytic constants may not reflect a reduced repair capacity of the Cys^{326} allele in the human cell context. In addition, both forms of GST–α-hOgg1 complement to similar extent the mutator phenotype of a *fpg mutY* strain of *E.coli*. From these results, we conclude that both the α-hOgg1-Ser^{326} and α-hOgg1-Cys^{326} proteins are functional and probably do not exhibit significant differences in repair activities. Therefore, the polymorphism at codon 326 is probably neutral, which is in agreement with the fact that tumours with loss of heterozygosity at 3p25 do not preferentially retain the allele coding for α-hOgg1-Cys^{326} protein (35,37).

ACKNOWLEDGEMENTS

This work was supported by the Centre National de la Recherche Scientifique (CNRS), the Commissariat à l'Energie

Atomique (CEA) and the Association pour la Recherche sur le Cancer (ARC). 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.

REFERENCES

1. Breimer,L.H. (1990) *Mol. Carcinog.*, **3**, 188–197.
2. Feig,D.I., Reid,T.M. and Loeb,L.A. (1994) *Cancer Res.*, **54** (suppl.), 1890–1894.
3. Wiseman,H. and Halliwell,B. (1996) *Biochem. J.*, **313**, 17–29.
4. Beckman,K.B. and Ames,B.N. (1997) *J. Biol. Chem.*, **272**, 19633–19636.
5. Friedberg,E.C., Walker,G.C. and Siede,W. (1995) *DNA Repair and Mutagenesis*. ASM Press, Washington, DC.
6. Boiteux,S. and Laval,J. (1997) In Hickson,I.D. (ed.), *Base Excision Repair of DNA Damage*. Landes Bioscience-Springer, Austin, TX, pp. 31–44.
7. Lindahl,T., Karran,P. and Wood,R.D. (1997) *Curr. Opin. Genet. Dev.*, **7**, 158–169.
8. Krokan,H.E., Standal,R. and Slupphaug,G. (1997) *Biochem. J.*, **325**, 1–16.
9. Grollman,A.P. and Moriya,M. (1993) *Trends Genet.*, **9**, 246–249.
10. Moriya,M. and Grollman,A.P. (1993) *Mol. Gen. Genet.*, **239**, 72–76.
11. Tchou,J., Kasai,H., Shibutani,S., Chung,M.-H., Laval,J., Grollman,A.P. and Nishimura,S. (1991) *Proc. Natl Acad. Sci. USA*, **88**, 4690–4694.
12. Michaels,L.M., Tchou,J., Grollman,A.P. and Miller,J.H. (1992) *Biochemistry*, **31**, 10964–10968.
13. Boiteux,S., Gajewski,E., Laval,J. and Dizdaroglu,M. (1992) *Biochemistry*, **31**, 106–110.
14. Radicella,J.P., Clark,E.A. and Fox,M.S. (1988) *Proc. Natl Acad. Sci. USA*, **85**, 9674–9678.
15. Michaels,M.L., Cruz,C., Grollman,A.P. and Miller,J.H. (1992) *Proc. Natl Acad. Sci. USA*, **89**, 7022–7025.
16. Duwat,P., De Oliveira,R., Ehrlich,D.S. and Boiteux,S. (1995) *Microbiology*, **141**, 411–417.
17. Auffret van der Kemp,P., Thomas,D., Barbey,R., de Oliveira,R. and Boiteux,S. (1996) *Proc. Natl Acad. Sci. USA*, **93**, 5197–5202.
18. Nash,H.M., Bruner,S.D., Schärer,O.D., Kawate,T., Addona,T.A., Spooner,E., Lane,W.S. and Verdine,G.L. (1996) *Curr. Biol.*, **6**, 968–980.
19. Karahalil,B., Girard,P.M., Boiteux,S. and Dizdaroglu,M. (1998) *Nucleic Acids Res.*, **26**, 1228–1232.
20. Thomas,D., Scott,A., Barbey,R., Padula,M. and Boiteux,S. (1997) *Mol. Gen. Genet.*, **254**, 171–178.
21. Bruner,S.D., Nash,H.W., Lane,W.S. and Verdine,G.L. (1998) *Curr. Biol.*, **8**, 393–403.
22. Boiteux,S. and Radicella,J.P. (1999) *Biochimie*, **81**, 59–67.
23. 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.
24. Lu,R., Nash,H.M. and Verdine,G.L. (1997) *Curr. Biol.*, **7**, 397–407.
25. 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.
26. Radicella,J.P., Dhérin,C., Desmaze,C., Fox,M.S. and Boiteux S. (1997) *Proc. Natl Acad. Sci. USA*, **94**, 8010–8015.
27. Roldan-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. USA*, **94**, 8016–8020.
28. Rosenquist,T.A., Zharkov,D.O. and Grollman,A.P. (1997) *Proc. Natl Acad. Sci. USA*, **94**, 7429–7434.
29. Bjoras,M., Luna,L., Johnsen,B., Hoff,E., Haug,T., Rognes,T. and Seeberg,E. (1997) *EMBO J.*, **16**, 6314–6322.
30. Shinmura,K., Kasai,H., Sasaki,A., Sugimura,H. and Yokota,J. (1997) *Mutat. Res.*, **385**, 75–82.
31. Takao,M., Aburatani,H., Kobayashi,K. and Yasui,A. (1998) *Nucleic Acids Res.*, **26**, 2917–2922.
32. Nishioka,K., Ohtsubo,T., Oda,H., Fujiwara,T., Kang,D., Sugimachi,K. and Nakabeppu,Y. (1999) *Mol. Biol. Cell*, **10**, 1637–1652.
33. Girard,P.M., D'Ham,C., Cadet,J. and Boiteux,S. (1998) *Carcinogenesis*, **19**, 1299–1305.
34. Nash,H.M., Rongzhen,L., Lane,W.S. and Verdine,G.L. (1997) *Chem. Biol.*, **4**, 693–702.
35. Kohno,T., Shinmura,K., Tosaka,M., Tani,M., Kim,S.R., Sugimura,H., Nohmi,T., Kasai,H. and Yokota,J. (1998) *Oncogene*, **16**, 3219–3225.
36. Chevillard,S., Radicella,J.P., Levalois,C., Lebeau,J., Poupon,M.F., Oudard,S., Dutrillaux,B. and Boiteux,S. (1998) *Oncogene*, **16**, 3083–3086.
37. Blons,H., Radicella,J.P., Laccoureye,O., Brasnu,D., Beaune,P., Boiteux,S. and Laurent-Puig,P. (1999) *Mol. Carcinog.*, in press.
38. Dizdaroglu,M. (1992) *Mutat. Res.*, **275**, 331–342.
39. Dizdaroglu,M., Laval,J. and Boiteux,S. (1993) *Biochemistry*, **32**, 12105–12111.
40. Karakaya,A., Jaruga,P., Bohr,V.A., Grollman,A.P. and Dizdaroglu,M. (1997) *Nucleic Acids Res.*, **25**, 474–479.
41. Deutsch,W.A., Yacoub,A., Jaruga,P., Zastawny,T.H. and Dizdaroglu,M. (1997) *J. Biol. Chem.*, **272**, 32857–32860.
42. Senturker,S., Auffret van der Kemp,P., Ho Jin You, Doetsch,P.W. and Boiteux,S. (1998) *Nucleic Acids Res.*, **26**, 5270–5276.
43. Boiteux,S., Belleney,J., Roques,B.P. and Laval,J. (1984) *Nucleic Acids Res.*, **12**, 5429–5439.
44. Guy,A., Duplaa,A.M., Harel,P. and Teoule,R. (1988) *Helv. Chim. Acta*, **71**, 1566–1572.
45. Girard,P.M., Guibourt,N. and Boiteux,S. (1997) *Nucleic Acids Res.*, **25**, 3404–3411.
46. Bradford,M.M. (1976) *Anal. Biochem.*, **72**, 248–254.
47. Fishel,R., Lescoe,R.K., Rao,M.R.S., Copeland,N.G., Jenkins,N.A., Garber,J., Kane,M. and Kolodner,R. (1993) *Cell*, **75**, 1027–1038.
48. Leach,F.S., Nicolaides,N.C., Papadopoulos,N., Liu,B., Jen,J., Parsons,R., Peltomaki,P., Sistonen,P., Aaltonen,L.A., Nystrom-Lahti,M. *et al*. (1993) *Cell*, **75**, 1215–1225.
49. Parsons,R., Li,G.M., Longley,M.J., Papadopoulos,N., Jen,J., de la Chapelle,A., Kinzler,K.W., Vogelstein,B. and Modrich,P. (1993) *Cell*, **75**, 1227–1236.
50. Jiricny,J. (1994) *Trends Genet.*, **10**, 164–168.