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The presence of two distinct 8-oxoguanine repair enzymes in human cells: their potential complementary roles in preventing mutation

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ABSTRACT

8-Oxoguanine (8-oxoG), induced by reactive oxygen species (ROS) and ionizing radiation, is arguably the most important mutagenic lesion in DNA. This oxidized base, because of its mispairing with A, induces GC→TA transversion mutations often observed spontaneously in tumor cells. The human cDNA encoding the repair enzyme 8-oxoG-DNA glycosylase (OGG-1) has recently been cloned, however, its activity was never detected in cells. Here we show that the apparent lack of this activity could be due to the presence of an 8-oxoG-specific DNA binding protein. Moreover, we demonstrate the presence of two antigenically distinct OGG activities with an identical reaction mechanism in human cell (HeLa) extracts. The 38 kDa OGG-1, identical to the cloned enzyme, cleaves 8-oxoG when paired with cytosine, thymine and guanine but not adenine in DNA. In contrast, the newly discovered 36 kDa OGG-2 prefers 8-oxoG paired with G and A. We propose that OGG-1 and OGG-2 have distinct antimutagenic functions *in vivo*. OGG-1 prevents mutation by removing 8-oxoG formed in DNA *in situ* and paired with C, while OGG-2 removes 8-oxoG that is incorporated opposite A in DNA from ROS-induced 8-oxodGTP. We predict that OGG-2 specifically removes such 8-oxoG residues only from the nascent strand, possibly by utilizing the same mechanism as the DNA mismatch repair pathway.

INTRODUCTION

Reactive oxygen species (ROS) are ubiquitous oxidizing agents that are generated in all organisms, both endogenously as by-products of respiration and during the inflammatory response and also exogenously after exposure to a variety of agents, including ionizing radiation (IR) (1–3). ROS have been implicated in the etiology of a wide variety of diseases, including arthritis and cancer, and also in aging (4–6). ROS are genotoxic and induce a variety of DNA lesions, including oxidized bases, abasic (AP) sites and DNA strand breaks (7). Because of its strong mutagenic potential, 8-oxoguanine (8-oxoG or G*) is commonly used as the marker of ROS damage (8). The spontaneous mutations observed in selected genes from human tumors and IR-induced mutations in hamster cells include a significant fraction of transversions of GC→TA type (9–11). Such mutations can be explained by the pairing of G* to A during DNA replication, as has been experimentally observed (12–15).

Michaels and Miller have coined the term GO system consisting of three enzymes, MutM, MutY and MutT, that prevent spontaneous mutagenesis in *Escherichia coli* due to G* (16). Fpg (MutM), an 8-oxoG-DNA glycosylase/AP lyase of *E.coli*, removes G* preferably from the G*·C pair in duplex DNA, which results from *in situ* oxidation of G (17–19). Unrepaired G* can form the mutagenic G*·A pair during subsequent DNA replication. MutY (or its human homolog hMYH), a G* (or G)·A-specific adenine-DNA glycosylase, removes A from the G*·A pair and thus provides a second opportunity to prevent mutation (16,20). However, the situation with G*-induced mutagenesis is more complex than originally envisioned, because G* is not only generated *in situ* in DNA, but may also be incorporated into DNA from ROS-induced 8-oxodGTP. MutT (or its human homolog hMTH), a ubiquitous 8-oxodGTPase, may block incorporation of 8-oxodGMP (21) and thus prevent AT→CG transversion mutations, which are in fact observed in tumor cells, although at a low frequency (10). That the spontaneous mutation frequency (mutator phenotype) of the *E.coli mutT* mutant is much higher than that of the *mutM* or *mutY* mutant (21,22) indicates a significant potential for mutation due to G* incorporation opposite A during DNA replication. In fact, removal of A by MutY in such a situation will fix rather than prevent mutation. This paradox suggests the need for differential removal of G* when incorporated into DNA, versus being generated *in situ*.

The 8-oxoG-DNA glycosylase (Ogg1) of yeast, unrelated to Fpg in sequence, was recently cloned (23,24). Based on sequence homology with yeast Ogg1, the cDNA of human OGG (hOGG-1), including several splice variants, has since been cloned independently by several groups and the recombinant proteins have been expressed in *E.coli* (25–30). The mammalian and yeast OGGs are 8-oxoG-DNA glycosylase/AP lyases like

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E.coli Fpg, which remove the base lesion from DNA and concomitantly cleave the DNA strand at the resulting AP site via β-elimination (β,δ for Fpg) (23–29; Fig. 1B). Surprisingly, the cloning of mammalian OGG cDNA was not preceded by identification of the enzyme in mammalian cell-free extracts. Preliminary evidence for the presence of a mitochondrial OGG in rat liver has recently been published, but its relationship to the cloned enzyme is not clear (31). In an earlier study, an 8-oxoG-specific DNA endonuclease and a monofunctional 8-oxoG-DNA glycosylase were identified in HeLa cells (32), however, neither activity appears to be identical to that of the cloned hOGG-1.

The recombinant yeast Ogg1 and hOGG-1 excise G* preferentially when it is paired with C in DNA and are inactive with a G*·A pair (23–29). However, a second 8-oxoG-DNA glycosylase Ogg2, identified in yeast, cleaves 8-oxoG preferentially in G*·purine base pairs (23,33) and was later identified to be an endonuclease III (Ntg1; 34).

In this report, we show for the first time that human cell extracts possess two distinct, antigenically unrelated OGG activities. These appear to account for most of the cellular 8-oxoG repair activity. We propose further that they act differentially in removing 8-oxoG generated directly in DNA versus that incorporated from the nucleotide pool.

MATERIALS AND METHODS

Enzymes

Escherichia coli endonuclease IV (Nfo) and *E.coli* MutM (Fpg) enzymes were provided by Drs Y. W. Kow and B. Van Houten.

Preparation of extracts from HeLa cells

HeLa and the human lymphoblastoid TK6 cells were grown in suspension to late log phase (~5 × 10^5 cells/ml) in S-MEM medium and RPMI 1640 (Gibco BRL), respectively, containing 10% bovine serum (Hyclone). Whole cell extracts (WCE), nuclear extracts (NE) and mitochondrial extracts (ME) were prepared according to the protocols of Manley *et al.* (35), Shapiro *et al.* (36) and Croteau *et al.* (31), respectively, and stored in aliquots at –80°C.

Preparation of substrate DNA

The sequences of oligodeoxynucleotides used as substrates are shown in Figure 1. The oligonucleotide containing a single 8-oxoG at position 16 (Seq. 1), purified by HPLC, was purchased from Midland. Other oligonucleotides (Seq. 2) complementary to Seq. 1 but for A, G, C and T opposite G* were synthesized locally. The oligonucleotides were 5′-^{32}P-end-labeled using [γ-^{32}P]ATP (Amersham) and T4 polynucleotide kinase (Pharmacia). The 3′-terminus of Seq. 1 oligo was labeled by incorporation of a single residue of [^{32}P]dCMP with Klenow DNA polymerase after annealing with the 32mer complementary strand.

Assay of lesion-specific DNA strand incision

The incision assay was carried out at 37°C for 45 min in a reaction mixture (50 µl) containing 10 fmol labeled duplex oligonucleotide, 30–35 µg extract (WCE, NE or ME) or 50–150 ng of purified proteins, 25 mM HEPES, pH 7.9, 50 mM KCl, 2.5 mM EDTA, 2 mM DTT and 2.5% glycerol. Unlabeled substrate (~1.5 pmol) was added only in the assays with NE and WCE, but not with purified enzymes. After termination of the reaction with phenol/chloroform and ethanol precipitation, the oligonucleotides were separated by electrophoresis in 15% denaturing polyacrylamide gels containing 7 M urea, 90 mM Tris–borate, 2 mM EDTA (pH 8.0). The structures of OGG products are schematically shown in Figure 1B. The gels were dried and the radiolabeled intact and cleaved oligo fragments were quantitated by PhosphorImager analysis (Molecular Dynamics).

A

5'-GACGAATTCGCGATCG*TCGACTCGAGCTCAG-3' (Seq 1)
5'-CTGAGCTCGAGTCGANGATCGCGAATTCGTC-3' (Seq 2)
5'-GACGAATTCGCGATC-3' (Seq 3)
5'-TCGACTCGAGCTCAGC-3' (Seq 4)

B

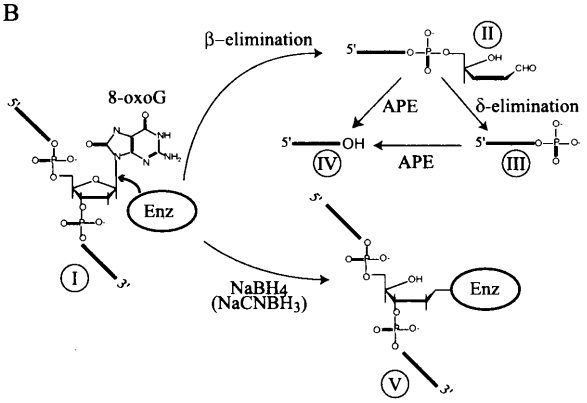

Figure 1. (**A**) Sequences of oligonucleotides used in this study. Seq. 1, 31mer bearing 8-oxoG (G*)- or G-containing base pair at position 16; Seq 2, complementary strand of Seq 1, where N represents A, T, G or C; Seq. 3 and Seq. 4, 5′-upstream and 3′-downstream segments of Seq. 1, respectively, up to the damaged site, used as markers wherever necessary. (**B**) A schematic diagram of various products generated by OGGs. **I**, an oligonucleotide containing 8-oxoG. Thick bars denote the DNA strand. **II**, 3′-phospho-α,β-unsaturated aldehyde resulting from β-elimination reaction by OGG (Enz). **III**, 3′-phosphate via δ-elimination (observed with Fpg but not hOGG-1). **IV**, 3′-OH terminus generated from either **II** or **III** by phosphoesterase (APE) activity. **V**, DNA–enzyme covalent complex trapped by reduction with $NaBH_4$ or $NaCNBH_3$.

Purification of 8-oxoG-DNA glycosylases from HeLa cells

Whole cell extract (10 ml containing 150–180 mg protein from 4 × 10^9 cells) was centrifuged (80 000 *g*, 30 min) and then diluted in a buffer containing 25 mM HEPES, pH 7.4, 2 mM DTT, 100 mM KCl to reduce the initial glycerol concentration from 17 to 5%, then loaded onto 5 ml HiTrap Q (5 ml) and SP (Pharmacia) columns connected in tandem. After initial washing with buffer A (25 mM HEPES, pH 7.4, 2 mM DTT, 5% glycerol) containing 100 mM KCl, the HiTrap SP column was disconnected and the proteins were eluted from the column in a stepwise fashion with 15 ml each of 0.2, 0.3, 0.4 and 0.5 M KCl in buffer A. Fractions of 0.4 M KCl with OGG activity were pooled, diluted and then loaded onto a 1 ml HiTrap SP column. After initial washing, a 50 ml linear gradient of KCl (0.15–0.45 M) in buffer A was used to elute OGGs in 0.35–0.38 M KCl. Using G*·C and G*·A duplex oligos

as substrates, two OGG peaks were identified which were pooled separately. These peaks (OGG-1 and OGG-2) were further purified by chromatography on a 1 ml HiTrap heparin column (Pharmacia), using a 0.1–0.45 M KCl gradient. OGG-2 fractions were stored at –80°C. OGG-1 fractions were further size fractionated on Superdex 75 in buffer A containing 0.4 M KCl and then stored at –80°C.

Assay of 8-oxoG release from 8-oxoG·C-containing oligonucleotide

Aliquots of OGG-1 (10 µl with 0.2 µg protein) were incubated with 10 pmol substrate oligonucleotide (G*·C pair; Seq 1 annealed with Seq. 2) in a 100 µl reaction mixture containing 50 mM Tris–HCl, pH 7.9, 50 mM KCl, 2.5 mM EDTA at 37°C for 1 h. After removal of DNA by ethanol precipitation, an aliquot (10 µl) was analyzed in a HPLC/ECD system for G* base (37) and quantitated by comparing with a standard having a retention time of 20 min.

Analysis of DNA glycosylase/AP lyase trapped complex

DNA trapping reactions were performed by incubating at 37°C for 30 min 3–4 fmol ^{32}P-labeled 8-oxoG-containing oligo with 20 ng OGG-1 or 50 ng OGG-2 or 5 ng Fpg in a reaction mixture (10 µl) containing 25 mM HEPES, pH 7.9, 2 mM DTT, 50 mM KCl, 2.5 mM EDTA, 50 mM $NaCNBH_3$. The incubated samples were mixed with gel loading buffer, heated at 100°C for 5 min, then analyzed by SDS–PAGE (12% polyacrylamide). The gels were dried on DE-81 paper for PhosphorImager analysis of radioactivity.

Preparation of anti-hOGG-1 antibody and western blot analysis

A synthetic peptide, DKSQASRPTPDELEAVRKC, corresponding to the deduced amino acid residues 81–98 of the cloned hOGG-1 (25–30), was chemically coupled to keyhole limpet hemocyanin (KLH) and used for raising polyclonal antibody in rabbits (Alpha Diagnostic International, San Antonio), which was used for western blot analysis to identify OGG using the enhanced chemiluminescence system (Amersham).

RESULTS

Detection of 8-oxoG-specific DNA glycosylase/AP lyase activity in HeLa cell extracts

Strand-specific cleavage of the 8-oxoG-containing oligonucleotide was observed after incubating duplex oligonucleotides (G*·C) with WCE and NE (Fig. 2A, lanes 3 and 5), versus no incision of the control G·C oligo (Fig. 2A, lanes 2 and 4). Incision occurred in the absence of Mg^{2+} or any other exogenous cofactor. Our early efforts did not detect 8-oxoG-specific nicking activity of crude extracts with oligonucleotide at 0.2 nM. Paradoxically, 8-oxoG-strand incision was detected only when the concentration of the oligonucleotide was increased to 40 nM by addition of unlabeled substrate, which in fact reduced the specific activity of the labeled substrate by 200-fold. Incision was absolutely dependent on the specific presence of 8-oxoG-containing oligonucleotide at higher concentration, because the activity was not observed with the same concentration of the control oligo (data not shown). As shown later, an inhibitor or a G*-binding protein may be responsible for the requirement of high substrate with crude but not partially purified enzyme.

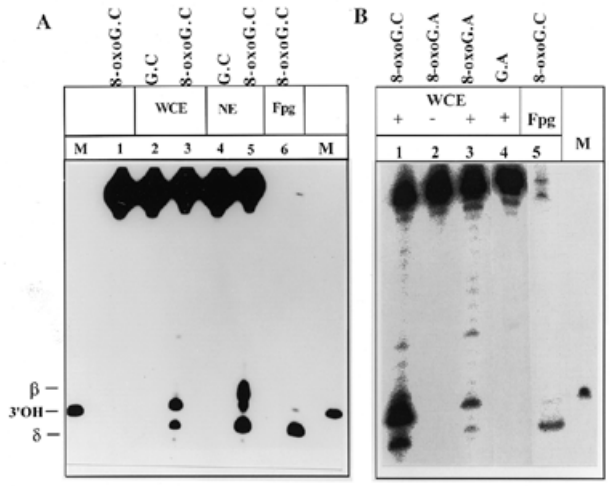

Figure 2. 8-OxoG-specific strand incision by HeLa cell extracts: 5′-^{32}P-labeled 31mer containing Seq. 1 with a G* or G, annealed to Seq. 2 (C or A for N) and used in nicking assays with WCE or NE. Other details are provided in Materials and Methods. (**A**) Lane 1, G* oligo only (Seq. 1); lanes 2 and 4, control oligo G·C; lanes 3 and 5, products of G*·C after incubation with WCE or NE; lane 6, Fpg-digested product as δ-elimination marker. M denotes the 5′-^{32}P-labeled 15mer marker (Seq. 3). (**B**) Lanes 1 and 3, reaction products of G*·C and G*·A oligos, respectively, after incubation with WCE; lane 2, oligo only; lane 4, G·A oligo; lane 5, Fpg product. M, 15mer marker.

Incubation of unlabeled G*-containing duplex oligo annealed with a 5′-labeled complementary strand (C opposite to G*) showed no radioactive cleavage products with HeLa extracts (data not shown). Thus the strand scission activity of HeLa extract was specific for the 8-oxoG-containing strand in the duplex oligonucleotide.

Analysis of the cleavage products

The products of OGG-catalyzed strand incision 5′ to the lesion were 15 nt long but were present as a mixture of fragments with 3′-phosphate and 3′-OH termini when WCE (Fig. 2A, lane 3) was used, but as 3′-OH, 3′-phosphate and 3′-phospho α,β-unsaturated deoxyribose aldehyde ends after reaction with NE (Fig. 2A, lane 5). It appears likely that the 3′-phospho sugar moiety and 3′-phosphate were removed by an EDTA-resistant Nfo-type AP-endonuclease (APE) or a non-specific phosphoesterase to yield 3′-OH terminus-containing fragments (Fig. 1B). This enzyme, however, could not be the major APE, which has an absolute requirement for Mg^{2+} and is inactive in the presence of EDTA (7). In any event, this activity was removed during OGG purification.

Detection of G*·A-specific DNA glycosylase activity in HeLa whole cell extract

We were able to detect for the first time a G*·A mismatch-specific G* strand nicking activity only in HeLa WCE (Fig. 2B, lane 3). Surprisingly, this enzyme was barely detectable in NE (data not shown). Furthermore, this activity was much weaker than the G*·C repair activity. However, the products of the two different substrates appeared to be identical and high substrate concentration

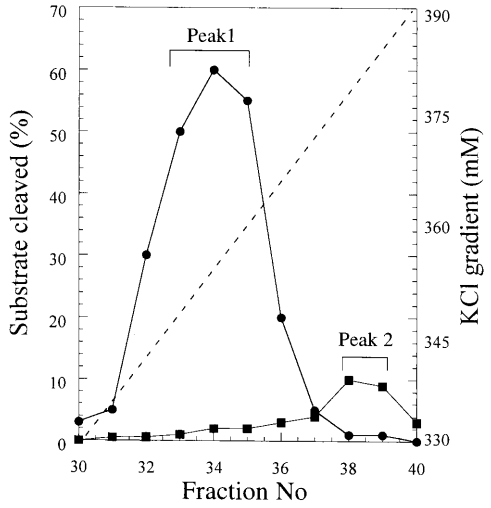

Figure 3. Separation of OGG-1 and OGG-2. The initial Hi-Trap SP fraction was further fractionated on the same column (1 ml) using a 0.15–0.45 M KCl gradient (---). Fractions (1 ml) were collected and assayed for OGG-1 (●) with G*·C-containing duplex oligo and for OGG-2 (■) with a G*·A-containing oligo.

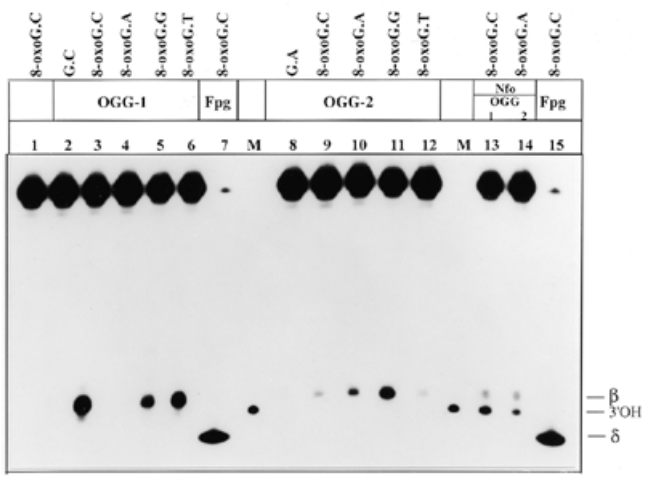

Figure 4. Substrate specificity and AP lyase activity of purified OGG-1 and OGG-2. OGG-1 and OGG-2 were incubated with 5′-end-labeled G*- or G-containing duplex oligos with C, A, G or T in the complementary strands opposite G*. Lane 1, no enzyme; lanes 2–6, incubation of OGG-1 with different substrates; lanes 7 and 15, control G*·C oligo with Fpg; lanes 8–12, incubation of OGG-2 with different substrates; lanes 13 and 14, incubation of Nfo with OGG-1 (G*·C) and OGG-2 (G*·A) reaction products; M, 15mer marker (Seq. 3).

(30–40 nM) was necessary for both activities in WCE (Fig. 2B, lane 3). As expected, no activity was observed with the G·A control oligo (Fig. 2B, lane 4).

In order to check whether the same enzyme nicks the G*-containing strand in both G*·C and G*·A oligo duplexes, extensive purification of OGGs was carried out from HeLa WCE. Two distinct activity peaks (1 and 2) were identified after elution from a HiTrap SP column (Fig. 3), which were pooled separately and further purified by chromatography on heparin–Sepharose. The G*·A-specific activity in peak 2, named OGG-2, was purified ~750-fold, while the G*·C-specific activity, named OGG-1 (peak 1) was purified further to ~1500-fold by FPLC on Superdex 75.

Nature of products generated by purified OGGs

The *E.coli* Fpg, *S.cerevisiae* Ogg1 and the recently cloned hOGG-1 act as DNA glycosylase/AP lyases at 8-oxoG residues base paired with C, but not with A (25–29). The recombinant hOGG-1 catalyzes a β-elimination reaction at the AP site produced by excision of the 8-oxoG and thus generates 3′-phospho α,β-unsaturated sugar at the incision site (depicted in Fig. 1B). The different termini were identified by distinct electrophoretic mobilities of the oligo fragments (27,29). We examined the ability of the endogenous OGG-1 (50 ng) and OGG-2 (150 ng) to cleave duplex oligos containing 8-oxoG paired with four different bases (Fig. 4). OGG-1 strongly preferred G*·C substrate, followed by G*·T and G*·G, but had no detectable G*·A-specific strand cleavage; OGG-2 cleaved mainly G*·G- and G*·A-containing oligos and less efficiently G*·T- and G*·C-containing substrates. In all cases, the incision product results from a β-elimination reaction and further treatment with Nfo (5 ng) generated products identical to the marker 3′-OH-containing 15mer oligo (Seq. 3) (Fig. 4, lanes 13 and 14).

We have also determined the size of the downstream cleavage product of both OGG-1 (Fig. 5, lanes 1–3) and OGG-2 (lanes 4–6) using the oligonucleotide labeled with ^{32}P at the 3′-terminus.

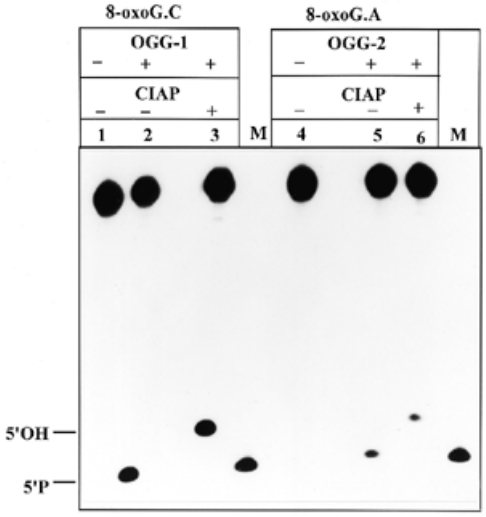

Figure 5. Characterization of the 3′-termini at the OGG cleavage sites. A 32mer oligo containing G* at position 16 and labeled with ^{32}P at the 3′-end was incubated with OGG-1 or OGG-2 and the labeled fragment analyzed as described in Materials and Methods. The presence of a phosphate residue at its 5′-end was confirmed by CIAP treatment. M, 5′-^{32}P-labeled 16mer marker (Seq. 4).

Both OGG-1 (lane 2) and OGG-2 (lane 5) generated a 16mer fragment containing 5′-phosphate as indicated by its identical electrophoretic mobility with that of the marker (M). The change in mobility of the fragment after removal of the terminal phosphate residue with 0.1 U calf intestinal phosphatase (CIAP; Gibco BRL) further confirmed its structure (Fig. 5, lanes 3 and 6).

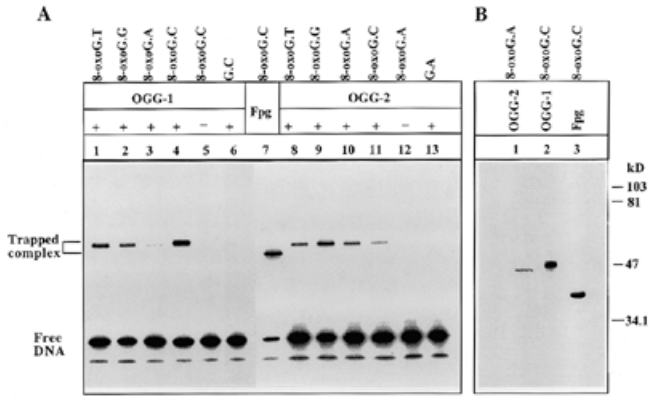

Figure 6. Trapping analysis of OGG-1 and OGG-2. (**A**) Lanes 1–4 and 6, trapping assay of OGG-1 with G*·T, G*G, G*·A, G*·C and normal G·C; lane 5, no protein; lanes 8–11 and 13, trapping assay of OGG-2 with G*·T, G*·G, G*·A, G*·C and G·A oligo, respectively; lane 7, trapped complex of Fpg with G*·C oligo; lane 12, no protein. Trapped complex and free DNA are indicated. Other details are provided in Materials and Methods. (**B**) Trapping assay of OGG-2 with G*·A (lane 1), OGG-1 with G*·C (lane 2) and Fpg with G*·C (lane 3). The extended gel electrophoresis was carried out to detect the size difference of OGG-1 and OGG-2. Protein size markers are indicated.

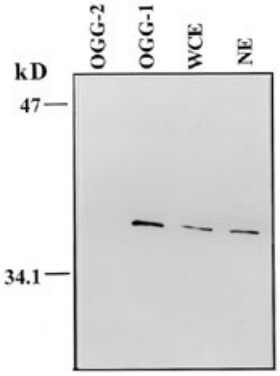

Figure 7. Western blot analysis of purified OGGs, WCE and NE using anti-hOGG-1 peptide antibody.

Release of 8-oxoG from DNA by OGG-1

In order to confirm that HeLa OGG-1 is indeed a DNA glycosylase that releases 8-oxoG, we tested for the free base in the reaction mixture. After incubation with G*·C-containing oligonucleotide, purified OGG-1 released a product that had the same retention time of 20 min as the authentic G*. About 6.6 pmol of 8-oxoG was released in a reaction while in a duplicate reaction 7.2 pmol DNA strand incision product was generated. The close equivalence of the two different assay products indicates that for OGG-1, the glycosylase and lyase act in concert so that no free AP site is generated. Although the release of 8-oxoG from DNA by OGG-2 was not investigated, the identical nature of products of the two enzymes suggests the same reaction mechanism.

OGG-1 and OGG-2 form Schiff base intermediates

A DNA glycosylase/AP lyase forms a transient Schiff base (imino) intermediate that can be trapped by $NaCNBH_3$ or $NaBH_4$ to generate a covalent enzyme–DNA complex as depicted in Figure 1B (23,38). Trapping assays with HeLa OGG-1 and OGG-2 show that OGG-1 has a distinct preference for C and T opposite G* (Fig. 6A). On the other hand, OGG-2 formed complexes with oligos containing G* paired with all four bases in the order of preference, G > A > C and T. After extended electrophoresis, the trapped complex of OGG-1 and G*·C duplex with an apparent molecular mass of ~48 kDa had a slightly lower mobility than that of OGG-2 and G*·A duplex, whose apparent molecular mass was calculated to be 46 kDa (Fig. 6B, lanes 2 and 1). A parallel trapping assay with *E.coli* Fpg (Fig. 6B, lane 3) showed that the 31mer oligonucleotide contributed ~10 kDa to the mass of the trapped complex. After correcting for the contribution of the oligo (23), we estimated that HeLa OGG-1 and OGG-2 have molecular masses of 38 and 36 kDa, respectively. Thus HeLa cell extract exhibits two distinct activities specific for the base opposite G*.

Identification of hOGG-1 by western blotting

Western blot analysis showed the presence of an ~38 kDa protein band in WCE (20 µg), NE (20 µg) and HeLa OGG-1 fraction (60 ng), but not in the OGG-2 fraction (250 ng) (Fig. 7). This protein is most likely identical to the cloned OGG-1a variant (29). The absence of any cross-reacting band indicates that OGG-2 is distinct from any of the four variant isoforms of hOGG-1 predicted from the mRNA sequence (29).

The presence of a G*-binding protein in HeLa cells

We were intrigued by our initial observation about the need to use a high concentration (40 nM) of G*-containing substrate in order to detect activity of both OGGs in crude cell extracts. The fact that robust activity of partially purified enzymes was observed with 0.2 nM substrate (Fig. 8, lanes 1 and 4) suggested the presence of an OGG inhibitor or a G*-binding protein in HeLa extracts. This was confirmed in a mixing experiment which shows that activity of both OGG-1 and OGG-2 with 0.2 nM substrate was eliminated by addition of HeLa extract (Fig. 8, lanes 2 and 5). That this inhibition was removed by heating the extract (95°C, 5 min; lanes 3 and 6) suggested a proteinaceous nature of the inhibitor. The inhibition was also eliminated by addition of 40 nM unlabeled substrate as predicted from the early studies with WCE (data not shown).

DISCUSSION

Although several 8-oxoG repair enzymes had been identified earlier in human cells, none of those is identical to the recently cloned hOGG-1. We deemed it necessary to re-examine the 8-oxoG repair activity in human cells in order to determine if hOGG-1 (isoform a) is indeed the major 8-oxoG repair enzyme in these cells. Detection of OGG activity in HeLa cells and its subsequent purification showed the presence of only two measurable activity peaks, of which the major peak corresponds to a 38 kDa protein and the minor enzyme to a 36 kDa protein. Western blot analysis indicates that the 38 kDa species is identical to the product of the predominant splice variant 1a mRNA of the cloned hOGG-1 gene. Furthermore, the 36 kDa OGG-2, antigenically unrelated to hOGG-1, has a distinct substrate specificity compared with OGG-1.

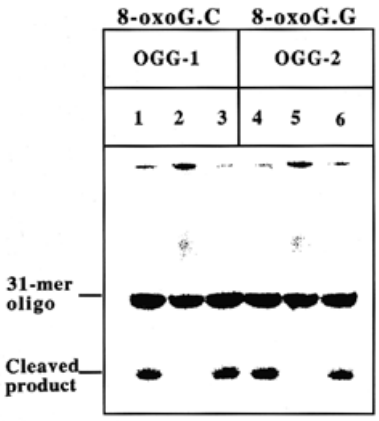

Figure 8. Inhibition of purified OGGs by WCE. 5′-^{32}P-labeled G*·C- (lanes 1–3) or G*·G-containing (lanes 4–6) duplex oligos (10–12 fmol) were incubated at 37°C for 45 min with 50 ng OGG-1 (G*·C oligo) or 150 ng OGG-2 (for G*·G oligo) in the absence (lanes 1 and 4) or presence of 35 µg WCE before (lanes 2 and 5) and after heating (lanes 3 and 6). The oligonucleotides were separated by electrophoresis in 20% polyacrylamide containing 7 M urea.

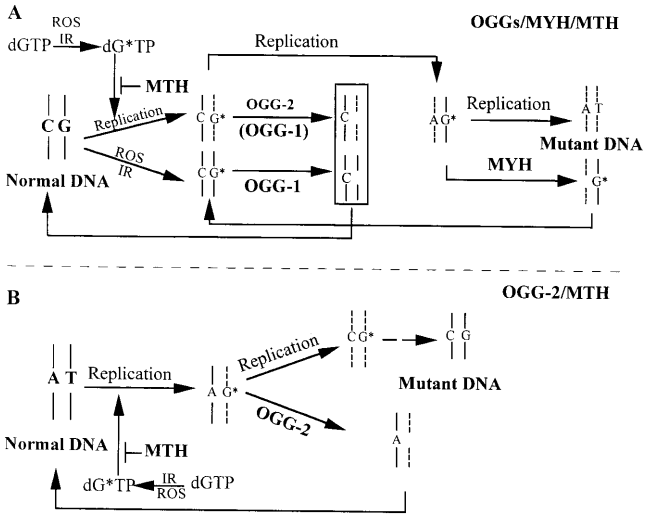

Figure 9. A model for bipartite antimutagenic processing of 8-oxoG. (**A**) G* formed *in situ* in DNA by ROS/IR is present as G*·C in template DNA (—) and subject to removal by OGG-1. Unrepaired G* may pair with C or A during replication. A is removed from the newly synthesized strand (---) in G*·A pair by MYH and completion of repair may generate G*·C. (**B**) Incorporation of G* from 8-oxodGTP (which may be prevented due to the latter's hydrolysis by MTH) opposite A will generate G*·A pair from which G* is removed by OGG-2.

Our serendipitous discovery of a OGG activity in crude HeLa extracts that could not be detected with low substrate concentrations led to the discovery of a protein which is a G*-specific binding protein or an inhibitor specific for both OGGs. This may explain the earlier failures to detect OGG in human cell extracts.

On the basis of identification of the reaction products, both OGGs appear to act as DNA glycosylase/AP lyases. Specifically, an excellent correlation between the amount of released free G* and the G*-containing DNA strand cleavage, that resulted in 3′-phospho α,β-unsaturated aldehyde and 5′-phosphate termini, rigorously proved the identity of OGG-1 as a DNA glycosylase/AP lyase. Purified OGGs catalyzed only β-elimination (Fig. 4). The 3′-phosphate termini generated after reaction with crude WCE and NE (Fig. 2, lanes 3 and 5) apparently resulted from a subsequent δ-elimination of the OGG products by a non-specific AP lyase. Further evidence for AP lyase activity of both OGGs was provided by the formation of covalent enzyme–substrate complexes. The 38 kDa molecular mass of HeLa OGG-1, determined by two independent methods, was identical to the predicted size of the major variant 1a of recombinant hOGG-1 (29). Finally, the immunological evidence directly confirms the identity of endogenous OGG-1 with the recombinant enzyme.

The presence of a mitochondrial OGG from rat liver has recently been reported (31). Because the OGG-2 activity was detected in WCE but barely in NE, it appeared possible that OGG-2 is a mitochondrial enzyme. In order to exclude this possibility, we tested for OGG activity in extracts of mitochondria purified from human TK6 cells. Although low OGG activity was indeed observed with 35–40 µg mitochondrial extract, this could not be due to OGG-2 because: (i) it was of the OGG-1 type and (ii) its specific activity per cell was much less than that calculated for OGG-2 (data not shown). It is expected that OGGs should be nuclear proteins. Although the OGG-2 activity was observed in WCE with very little in NE, it is possible that OGG-2, like some other nuclear enzymes (e.g. DNA polymerase α), leaked out during nuclei preparation (39). Furthermore, simple DNA glycosylases, including *N*-methylpurine-DNA glycosylase and an endonuclease, which were shown earlier to possess 8-oxoG repair activity, are unlikely to contribute significantly to the total cellular OGG activity (32,40). Reardon *et al*. recently showed a low level of *in vitro* 8-oxoG repair via the nucleotide excision repair pathway (41). The human OGG is distinct from the S3 ribosomal protein, which, unlike its *Drosophila* homolog (42), does not have G*-DNA glycosylase activity (M.R.Kelley, personal communication).

The most important finding documented in this paper is the presence of OGG-2, a new 8-oxoG-DNA glycosylase. We confirmed that HeLa OGG-1, like the recombinant hOGG-1, preferentially cleaved G* when paired with C and T and had a lower activity for G*·G pairs. More significantly, OGG-1 did not remove G* when paired with A in a duplex oligo. In contrast, the activity of OGG-2 in cleaving G* was highest when G* was paired with G or A and in the order G > A >> C and T. In general, the activity of OGG-2 was much lower than that of OGG-1 in HeLa cells.

We have discussed in the Introduction a rationale for the presence of a second G* repair enzyme which removes the lesion when incorporated in nascent DNA and present as a G*·A pair. OGG-2 fulfills the criteria for such an enzyme. We now propose a model (Fig. 9) which shows two pathways for repair of G* representing a two-stage protection system against mutagenesis due to this critical lesion. OGG-1 may be a housekeeping enzyme which removes G* from DNA of non-dividing cells. When A is incorporated opposite G* in a dividing cell, hMYH removes it to prevent mutation fixation. However, G* can be incorporated in nascent DNA, opposite A in the template, from the dG*TP pool when hMTH fails to destroy dG*TP. This will lead to AT→CG mutations, which have indeed been found in some human tumor lines (10). OGG-2 would remove G* from such a G*·A pair. Although a similar mechanism was postulated earlier for yeast Ogg2 (43), Bruner *et al*. proposed that G* repair in yeast is

distinct from that in *E.coli* and mammals (34). On the contrary, our results and the model derived therefrom provide a unified mechanism for antimutagenic processing of G* in all eukaryotes.

A recent study on site-specific mutations induced by 8-oxo-guanine, when paired with A or C in plasmids after their replication in primate cells, showed that the G*·C pair was barely mutagenic while the G*A pair gave rise to about two thirds T·A and one third G·C sequences in the progeny molecules (44). The authors thus concluded that G* repair in the G*·C pair was highly efficient. In contrast, the G*·A pair was either replicated to generate a T·A mutation or it produced G·C mutation due to removal of A by MYH. In the absence of knowledge about OGG-2, the authors did not consider possible repair of G* from the G*·A pair. However, OGG-2 will also contribute to the observed generation of T·A from the G*·A pair. While our model proposes that OGG-2 is active on G* only when present in the nascent strand, it is possible that such strand discrimination may be relaxed for extrachromosomal DNA.

Why the G*·G pair is the best substrate for both yeast and human OGG-2 is not clear. Although an earlier study showed that G* can pair with all four normal bases (15), Shibutani *et al.* showed later that G* preferentially pairs with C or A during *in vitro* DNA synthesis (13). On the other hand, the significant increase in both GC→CG and TA transversions in *E.coli* and human cells after ROS treatment (11,45) suggests that such mutations could result from G*·G and G*·A pairing, respectively, during DNA replication. In the light of recent observations, the base pairing properties of G* should be re-examined.

The primary requirement for OGG-2 and MYH should be that these act specifically on the nascent strand. This specificity for nascent DNA repair is strongly reminiscent of the mismatch repair process in *E.coli* and mammals (46) and suggests a common mechanism for strand discrimination in mismatch repair and G* excision repair. It should be possible to test this prediction once the human OGG-2 gene is cloned. With the availability of active OGGs, it will also be important to elucidate the structural basis for the subtle but profound discrimination of these enzymes in recognizing the same lesion.

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