Substrate specificity and excision kinetics of natural polymorphic variants and phosphomimetic mutants of human 8-oxoguanine-DNA glycosylase

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Abbreviations:

8-oxoGua, 8-oxo-7,8-dihydroguanine; BER, base excision repair; DTT, dithiothreitol; EDTA, ethylenediamine tetraacetate; FapyAde, 2,4-diamino-5-formamidopyrimidine; FapyGua, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; GC/MS, gas chromatography/mass spectrometry.

Abstract

Human 8-oxoguanine-DNA-glycosylase (OGG1) efficiently removes mutagenic 8-oxoguanine (8-oxoGua) and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) when paired with cytosine in damaged DNA. Excision of 8-oxoGua mispaired with adenine may lead to G→T transversions. Posttranslational modifications such as phosphorylation could affect cellular distribution and enzymatic activity of OGG1. Mutations and polymorphisms of OGG1 may affect enzymatic activity and have been associated with increased risk of several cancers. In this study, using double-stranded oligodeoxynucleotides containing 8-oxoGua:Cyt or 8-oxoGua:Ade pairs, as well as γ-irradiated calf thymus DNA, we have investigated the kinetics and substrate specificity of several known OGG1 polymorphic variants and phosphomimetic Ser→Glu mutants. Among the polymorphic variants, A288V and S326C displayed opposite-base specificity similar to that of wild type OGG1, and OGG1-D322N was 2.5-fold more specific for the correct opposite base than the wild type enzyme. S231E, S232E, S231/232E, and S280E phosphomimetic mutants had slightly lower activity and specificity similar to the wild type enzyme in both assays. OGG1-S326C efficiently excised 8-oxoGua from oligodeoxynucleotides and FapyGua from γ-irradiated DNA but excised 8-oxoG rather inefficiently from the latter. Otherwise k_{cat} values for 8-oxoGua excision obtained from both types of experiments were broadly similar for all OGG1 variants studied. It is known that human AP endonuclease APEX1 can stimulate OGG1 activity by increasing its turnover rate. However, when wild type OGG1 was replaced with one of the phosphomimetic mutants, very little stimulation of 8-oxoGua removal was observed in the presence of APEX1.

8-Oxo-7,8-dihydroguanine (8-oxoGua) and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) are pre-mutagenic DNA lesions that appear in DNA damaged by reactive oxygen species such as hydroxyl radical of endogenous and environmental origin (1). During replication, 8-oxoGua directs misincorporation of dAMP (2) and thereby induces G→T transversions, which in mammals can activate oncogenes or inactivate tumor suppressor genes (3,4). Likewise, FapyGua pairs with Ade and leads to G→T transversions in mammalian cells (5,6). A causal role of oxidative damage to DNA in human cancer development has not been demonstrated directly; nevertheless, oxidatively induced DNA lesions, including 8-oxoGua, may be responsible for mutations that play a role in carcinogenesis (7).

8-oxoGua and FapyGua are removed from DNA by base excision repair (BER) (8). As a part of this process, all organisms possess an enzymatic system that averts mutagenic load caused by these two lesions. In humans, a system has been described that consists of three enzymes: 8-oxoGua-DNA glycosylase (OGG1). mismatched adenine-DNA glycosylase (MUTYH), and 8-oxo-7,8-dihydro-2'-deoxyguanosine triphosphatase NUDT1 (MTH1) (recently reviewed in (9)). OGG1 excises 8-oxoGua paired with Cyt, the context in which this oxidized base is naturally formed, but not from 8-oxoGua:Ade pairs that appear following misincorporation of dAMP opposite 8-oxoGua or by insertion of 8-oxodGTP opposite Ade. MUTYH removes Ade from 8-oxoGua:Ade pairs followed by additional repair processes that convert this mispair into 8-oxoGua:Cyt, which is repaired by OGG1. In parallel, NUDT1 hydrolyzes 8-oxodGTP, preventing its misincorporation during DNA replication. In addition to 8-oxoGua, human and other OGG1 proteins efficiently remove FapyGua from DNA with similar excision kinetics to that of removal of 8-oxoG (10-13). In agreement with this fact, FapyGua paired with Cyt is also efficiently removed by human OGG1 from synthetic

oligodeoxynucleotides (14). Simultaneous inactivation of OGG1 and MUTYH in transgenic mice predisposes these animals to lymphomas, lung and ovarian tumors, associated with many G→T transversions in codon 12 of the *K-ras* protooncogene (15).

Ultimately, the fidelity of the 8-oxoGua repair system depends on discrimination between 8-oxoGua:Cyt and 8-oxoGua:Ade pairs by OGG1. This enzyme possesses two catalytic activities, a strong DNA glycosylase activity specific for 8-oxoGua and FapyGua, and a relatively weak apurine/apyrimidine (AP) lyase activity which, after base excision, cleaves the DNA backbone by elimination of the 3'-phosphate of the damaged deoxynucleotide (β-elimination) (11,16,17). Due to the weak AP lyase activity and high affinity for the AP product, the turnover of OGG1 is low but the enzyme is stimulated by the major human apurine/apyrimidine endonuclease APEX1 (18-21). OGG1 is highly selective for 8-oxoGua:Cyt substrates and discriminates against 8-oxoGua:Thy, 8-oxoGua:Gua, and especially 8-oxoGua:Ade, both at the level of its glycosylase and the AP lyase activity (22,23). The C/A specificity of OGG1 is influenced by several factors including ionic strength, presence of Mg^{2+} ions (24), and interactions with APEX1 (25).

Many single-nucleotide polymorphisms of *OGG1* gene have been found in human populations and deposited in the NCBI dbSNP database (26) or reported individually (27-30). Of these, 14 change the sequence of its major protein isoform OGG1-1a (A3P, P27T, A53T, A85S, R131Q, R154H, R229Q, E230Q, A288V, G308E, S320T, D322N, S326C). Two more variants, R46Q and S232T, have so far been reported only from human tumors (27,31). Few mutant proteins encoded by these variants have been characterized with respect to their function, kinetics and substrate specificity. So far, most attention has been given to the OGG1-S326C variant, which is associated with an increased risk of lung, and possibly gastrointestinal, cancer,

especially in patients exposed to environmental factors such as smoking or animal protein consumption ((32,33) and references therein). Still, functional characterization of this protein has been inconclusive. In *E. coli* mutator strain complementation tests, OGG1-S326C has been reported as either being less efficient than wild-type OGG1 (34) or providing normal complementation (11). Cell extracts from lymphocytes from OGG1-S326 and OGG1-S326 homozygous individuals show similar abilities to excise 8-oxoGua (35). When γ-irradiated DNA was used, OGG1-S326C exhibit less efficient excision of 8-oxoGua and FapyGua than the wild-type enzyme (11) and show less proficiency in excising 8-oxoGua from oligodeoxynucleotides (36). Of other OGG1 polymorphic variants, limited kinetic information is available for R46Q, A53T, R154H, and A288V (30,37).

Many BER proteins undergo posttranslational modification, including acetylation and phosphorylation (38). OGG1 interacts physically with protein kinases CDK4, c-ABL and PKC, with CDK4 and PKC being able to modify OGG1 *in vitro* (39,40). Phosphorylation of OGG1 by CDK4 was reported to activate the enzyme (40), while phosphorylation by PKC had no effect on OGG1 activity (39), suggesting that several sites in OGG1 may be phosphorylated. In no case has the site of OGG1 phosphorylation been identified. Additionally, the OGG1-S326C variant, which shows aberrant intracellular sorting, can be rescued by mutating residue 326 to glutamate, a substitution approximating the bulk and charge of phosphoserine (41).

In this report, we analyze the activity, substrate specificity and kinetics of two naturally occurring polymorphic variants of OGG1, A288V and D322N, and compare them with wild-type and S326C variants of the enzyme. We also use a neural network trained on a large set of experimentally proven protein phosphorylation sites to predict additional sites of high phosphorylation probability in OGG1, then introduce phosphomimetic Ser→Glu substitutions at

these positions, determining changes in the activity, substrate specificity, and interactions with AP endonuclease of the resulting enzyme variants.

Materials and methods

Enzymes and oligodeoxynucleotides. The 8-oxoGua-containing oligodeoxyribonucleotide 5'-d(CTCTCCCTTCXCTCCTTTCCTCT)-3' (X = 8-oxoGua) and its complementary strand 5'-d(AGAGGAAAGGAGNGAAGGGAGAG)-3' (N = Ade or Cyt) were synthesized by Operon Biotechnologies (Huntsville, AL). The 8-oxoGua-containing strand was ^{32}P-labeled using γ[^{32}P]ATP and phage T4 polynucleotide kinase (New England Biolabs, Beverly, MA) according to the manufacturer's protocol, then annealed to a complementary strand to produce duplexes containing an 8-oxoGua:Cyt or 8-oxoGua:Ade pair. His_6-tagged human AP endonuclease APEX1 was purified as described (21).

Construction and purification of OGG1 mutants. OGG1 mutants were produced using a QuikChange Multi site-directed mutagenesis kit (Stratagene, Cedar Creek, TX) with pET-15b-hOGG1-1a plasmid (42) as a template. The presence of target mutation and the absence of other mutations were confirmed by sequence analysis. Plasmids carrying the mutant *ogg1* coding sequence were used to transform *E. coli* BL21(DE3)RIL. Wild-type and mutant His_6-tagged OGG1 were purified as described (42), except that pre-charged Ni-NTA chelating resin (Qiagen, Venlo, the Netherlands) was used for affinity chromatography. The concentration of the active wild-type enzyme was determined from burst phase kinetic experiments as described (21).

Kinetics of OGG1 mutants on oligodeoxynucleotide substrates. The standard reaction mixture (20 μl) included 20 mM HEPES-NaOH (pH 7.5), 50 mM KCl, 1 mM DTT, 1 mM EDTA, and radioactively labeled 8-oxoGua:Cyt substrate (2–400 nM) or 8-oxoGua:Ade substrate (5–1500 nM). The cleavage reaction was initiated by adding wild-type or mutant OGG1 (10–20 nM for 8-oxoGua:Cyt; 20–50 nM for 8-oxoGua:Ade), allowed to proceed for 20 min (8-oxoGua:Cyt) or 30 min (8-oxoGua:Ade) and terminated by addition of putrescine-

HCl, (pH 8.0) to a final concentration of 125 mM and heating at 95°C for 5 min to fully cleave the AP site product of the OGG1 glycosylase reaction. An equal volume of formamide dye was added, the mixture was heated for 3 min at 95°C, and the reaction products were separated by electrophoresis in a denaturing polyacrylamide gel. The bands were quantified using a Storm 840 system and ImageQuant v5.2 software (GE Healthcare Life Sciences, Uppsala, Sweden). The kinetic constants, K_M and k_{cat}, were determined by non-linear least-square fitting using SigmaPlot v8.0 software (SPSS Inc., Chicago, IL).

Kinetics of OGG1 mutants on γ-irradiated DNA substrates. Calf thymus DNA (Sigma-Aldrich, St. Louis, MO) was dissolved in phosphate buffer (pH 7.4) at a concentration of 0.3 mg/ml. Aliquots of this solution were bubbled with N_2O and γ-irradiated at doses 2.5, 5, 10, 20, 40 and 60 Gy. Irradiated samples were dialyzed against water for 18 h at 4°C. Aliquots of dialyzed samples (50 μg) were dried under vacuum. For enzymatic hydrolysis, DNA samples (50 μg) were dissolved in 50 μl of an incubation buffer consisting of 50 mM phosphate buffer (pH 7.4), 100 mM KCl, 1 mM EDTA and 0.1 mM dithiotreitol. Aliquots of 8-oxoGua-$^{13}C,^{15}N_3$, FapyGua-$^{13}C,^{15}N_2$ and 4,6-diamino-5-formamidopyrimidine-$^{13}C,^{15}N_2$ (FapyAde-$^{13}C,^{15}N_2$) were added as internal standards. Samples were incubated with 1 μg of an enzyme for 30 min at 37°C in a water bath, and then processed and analyzed by gas chromatography/mass spectrometry (GC/MS) as described (43,44). Time dependence of excision was measured by incubation of DNA samples, which were irradiated at 20 Gy, with 1 μg of the enzyme for 0, 10, 20 and 30 min. The measurement of excision kinetics was performed by using DNA samples γ-irradiated at 2.5, 5, 10, 20, 40 and 60 Gy (43). The kinetic constants and standard deviations were calculated by non-linear least square fitting.

Stimulation of OGG1 by APEX1. The standard reaction mixture (20 μl) included 20 mM HEPES-NaOH (pH 7.5), 50 mM KCl, 1 mM DTT, 5 mM $MgCl_2$, 50 nM substrate and 10 nM wild-type or mutant OGG1. The reaction was allowed to proceed for 20 min., aliquots were withdrawn at the required time and quenched by heating with putrescine-HCl as described above. Reaction products were analyzed by electrophoresis in denaturing polyacrylamide gel followed by phosphorimaging.

Results

Selection of amino acid residues for mutagenesis

Association of *OGG1* polymorphisms with succeptibility to human cancer and other diseases is an area of active research (32,45). Among known polymorphic variants, S326C, associated with the increased risk of lung cancer, has been extensively studied, since the frequency of this allele in the general population is ~0.25. Several functional defects have been found in this form of the OGG1 protein, including abnormal cell cycle-dependent localization (41), protein dimerization, changes in opposite-base specificity and inability to be stimulated by APEX1 (36). Therefore, we have used OGG1-S326C as a "reference" mutant, with which to compare other enzyme variants. Of other polymorphic OGG1 forms, we have chosen OGG1-A288V and OGG1-D322N for structural reasons. In the OGG1-DNA complex (46), Ala-288 forms direct contacts to DNA, while a highly conserved Asp-322 is involved in positioning the imidazole ring of an absolutely conserved His-270 residue, which in turn binds to the 5'-phosphate of the damaged nucleotide monophosphate (Fig. 1). The A288V polymorphism in the germline has been found in Alzheimer's disease patients and the activity of the OGG1-A288V has been reported to be lower than of the wild-type enzyme (30). The activity of OGG1-D322N has not previously been addressed.

Phosphorylation of OGG1 can affect its biological functions at several levels, including the intrinsic activity and intracellular localization (40,41). The sites of phosphorylation in this enzyme are presently unknown. Thus, to select residues for phosphomimetic Ser/Thr modifications, we used the NetPhos 2.0 server (www.cbs.dtu.dk/services/NetPhos/), a neural network that predicts the probability of phosphorylation at a given site using a constantly

updated learning set based on the sequences of experimentally proven phosphorylation sites (47). In Table 1, we summarize the results of an analysis of overall phosphorylation probability within the OGG1 sequence. It should be noted that the NetPhos score is not the exact probability, but rather a function of the probability of a site being phosphorylated. A NetPhos score > 0.5 is generally considered a threshold for prediction of a Ser/Thr residue as a possible phosphorylation site, and the higher the score, the higher the probability of the site being phosphorylated (48). Therefore, we have chosen S231, S232, S280, and S326, the residues with overall scores > 0.99, for biochemical characterization of the phosphomimetic Ser to Glu substitution. Additionally, a double mutant S231E/S232E, mimicking double phosphorylation at two adjacent sites, was studied. All of these residues are located at the surface of the OGG1 protein globule far away from the protein–DNA interface; Ser-326 is missing from the OGG1–DNA crystal structure (46) but is inferred to be distant from DNA (Fig. 1).

Activity and substrate specificity of OGG1 mutants on oligodeoxynucleotide substrates

OGG1 is part of an enzymatic system responsible for prevention of mutations generated by 8-oxoGua and FapyGua (9). Since 8-oxoGua directs pre-mutagenic misincorporation of dAMP during replication, a distinguishing feature of OGG1 is its preference for removal of 8-oxoGua from 8-oxoGua:Cyt pairs compared with that from 8-oxoGua:Ade pairs (22,23,49). To study the effect of amino acid substitutions on the activity and opposite-base specificity of OGG1, we determined the kinetic constants k_{cat} and K_M for reaction of cleavage of 8-oxoGua:Cyt and 8-oxoGua:Ade substrates by wild-type and mutant OGG1 enzymes. Fig. 2 shows a typical dependence of the reaction velocity on the substrate concentration in double reciprocal coordinates for the wild-type enzyme. The specificity constant, $k_{sp} = k_{cat}/K_M$, was calculated for

each enzyme and substrate, and the ratio of the k_{sp} for 8-oxoGua:Cyt to the k_{sp} for 8-oxoGua:Ade was used as a measure of the biologically relevant opposite-base specificity (C/A specificity) (24). In the wild-type enzyme, the C/A specificity of 4.9 was due mostly to the lower value of K_M for the 8-oxoGua:Cyt substrate (Table 2, Table 3), similar to what was reported in the literature (23,49). The K_M values for cleavage of 8-oxoGua:Cyt by OGG1-A288V and OGG1-D322N were higher than that for wild-type OGG1. Due to a concomitant increase in k_{cat} for OGG1-A288V, no significant difference in k_{sp} and C/A specificity was observed for this form of the enzyme (Table 2 and Table 3). Interestingly, the activity of OGG1-D322N towards the 8-oxoGua:Cyt substrate was the lowest of all polymorphic variants studied, but this mutant showed an even lower activity on the 8-oxoGua:Ade substrate. As a result, the overall C/A specificity of OGG1-D322N was 12, 2.4-fold higher than the C/A specificity of wild-type OGG1 (Table 2 and Table 3). In the OGG1-S326C variant, the K_M value for the cleavage of 8-oxoGua:Cyt substrate was nearly the same as for the wild-type OGG1, and decreased for the 8-oxoGua:Ade substrate in the mutant, but, as the k_{sp} value decreased for both 8-oxoGua:Cyt and 8-oxoGua:Ade, the C/A specificities of wild-type OGG1 and OGG1-S326C were similar (Table 2 and Table 3). Thus, of all studied natural variants of the enzyme, OGG1-D322N demonstrated the highest C/A specificity. The values of kinetic constants found for cleavage of 8-oxoGua:Cyt by OGG1-A288V and OGG1-S326C were in an overall agreement with published data (30,36).

In the reaction of 8-oxoGua:Cyt cleavage by phosphomimetic mutants of OGG1, we observed an increase in both K_M and k_{cat} for OGG1-S231E, OGG1-S232E and OGG1-S231S/S232E, and a decrease in k_{cat} for OGG1-S280E and OGG1-S326E, as compared with wild-type OGG1 (Table 2). Overall, the decrease in k_{sp} for all phosphomimetic mutants of OGG1 but OGG1-S231E reveals that these enzymes are ~2-fold less active than wild-type OGG1. For

OGG1-S231E, the increase in K_M was compensated by an increase in k_{cat}, leading to only a marginal decrease in the activity of the mutant enzyme. For the 8-oxoGua:Ade substrate, the K_M value in the phosphomimetic mutants either decreased in comparison with that for wild-type OGG1 (OGG1-S231E and OGG1-S280E), or did not change (OGG1-S232E, OGG1-S231/232E, OGG1-S326E). The k_{cat} value decreased in all cases; as a result, all phosphomimetic mutants excised 8-oxoGua from 8-oxoGua:Ade pairs less efficiently than did the wild-type enzyme (Table 3). The C/A specificity for all phosphomimetic mutants of OGG1 resembled closely that of the wild-type enzyme (Table 3).

Activity and substrate specificity of OGG1 mutants on γ-irradiated DNA

In addition to measuring kinetic constants of DNA glycosylases on oligodeoxynucleotide substrates containing 8-oxoGua, the substrate specificity of these enzymes may be analyzed using high molecular weight DNA damaged by γ-irradiation or other treatment, with the following analysis of excised bases by GC/MS with isotope dilution (50). This assay reveals the spectrum of damaged bases released by a given enzyme, including those not easily introduced into oligodeoxynucleotides, such as formamidopyrimidines. Applied to wild-type human OGG1 and its mutant forms R46Q, R154H, and S326C, OGG1 excises only 8-oxoGua and FapyGua out of more than 20 oxidized bases detected in this system (11,37). Both OGG1 and OGG1-S326C excise 8-oxoGua and FapyGua, with k_{cat} and k_{sp} for OGG1-S326C being about 2-fold lower than for wild-type OGG1 (11).

To determine the full spectrum of substrate bases excised from their naturally occurring base pairs by OGG1 and its variants, we used γ-irradiated calf thymus DNA and employed *E. coli* Fpg protein, a functional counterpart but not a structural homolog of OGG1, with well established specificity for 8-oxoGua, FapyGua, and FapyAde (51,52), as an additional control.

All studied OGG1 variants were able to excise FapyGua and 8-oxoGua from DNA, with OGG1-S326C being the least active for excision of 8-oxoGua (Table 4). Figures 3A and 3B illustrate excision of 8-oxoGua, FapyGua and FapyAde by OGG1 and Fpg, respectively. In agreement with previous results, OGG1 excised 8-oxoGua and FapyGua, but not FapyAde (11), whereas all three products were removed by Fpg from DNA (51,52). Other modified bases monitored by GC/MS were not excised, indicating that mutant OGG1 forms do not acquire broader substrate specificity compared with the wild-type enzyme.

The values of kinetic constants for excision of FapyGua and 8-oxoGua by various forms of OGG1 are summarized in Table 4. Excision of 8-oxoGua by OGG1-A288V was characterized by a somewhat lower k_{cat} than that for the wild-type enzyme but, due to a concomitant decrease in K_M, the value of k_{sp} for OGG1 and OGG1-A288V were very similar. The values of k_{cat} and K_M for FapyGua excision were higher than for 8-oxoGua excision by both OGG1 and OGG1-A288V, making these two forms of the enzyme equally well suited for excision of both lesions. The polymorphic variant OGG1-D322N showed notably lower k_{cat} and k_{sp} for excision of both lesions, with a more pronounced effect on 8-oxoGua excision. In this case, the k_{sp}(WT)/k_{sp}(mutant) ratio was 4.3 for 8-oxoGua excision and 1.6 for FapyGua excision, consistent with a decrease in OGG1-D322N activity observed with oligodeoxynucleotide substrates. Interestingly, OGG1-S326C was the least active variant in excising 8-oxoGua while retaining appreciable activity towards FapyGua. For the latter substrate, the value of k_{cat} decreased 3.8-fold in comparison with that for the wild-type enzyme, but due to a concomitant decrease in K_M for OGG1-S326C, the k_{sp} value for FapyGua excision by this mutant was only 2-fold lower than the k_{sp} for FapyGua excision by OGG1. In contrast, k_{sp} for 8-oxoG excision by OGG1-S326C was 6.2-fold lower than that of wild-type OGG1.

All phosphomimetic mutants of OGG1 demonstrated reduced abilities to excise FapyGua and especially 8-oxoGua when compared to the wild-type enzyme. Both k_{cat} and K_M for 8-oxoGua excision by OGG1-S231E, OGG1-S232E, OGG1-S231E/S232E, OGG1-S280E, and OGG1-S326E were elevated in comparison with the kinetic constants for wild-type OGG1; as a result, k_{sp} was 2.2–3.6-fold lower for all phosphomimetic mutants than for wild-type OGG1. The reduction in k_{sp} for FapyGua excision also was evident although not as pronounced (1.1–1.7-fold) as in the case of 8-oxoGua (Table 4). For OGG1-S326E, k_{sp} characterizing the excision of both 8-oxoGua and FapyGua was lowered in comparison with the wild-type OGG1 due to an increase in K_M with a much less effect on k_{cat}. Overall, k_{cat} values of 8-oxoGua excision from irradiated DNA are in a good agreement with data for the cleavage of 8-oxoGua:Cyt oligodeoxynucleotide substrates (compare Tables 2 and 4). Much higher values obtained for apparent K_M in the irradiated DNA assay are due to a much lower concentration of damaged bases in this substrate, which causes K_M to increase due to longer lesion search time and a correspondingly lower association rate constant in the Michaelis–Menten equation as discussed previously (53).

Stimulation of OGG1 phosphomimetic mutants by AP endonuclease

Regulation of protein–protein interactions by post-translational modification, including phosphorylation, is widely encountered in nature. We and others have shown that human AP endonuclease APEX1 stimulates the activity of wild-type OGG1, most likely through DNA-mediated protein–protein interactions (18-21). Therefore, we asked whether putative phosphorylation of OGG1 at sites of high phosphorylation probability could influence the ability of APEX1 to stimulate OGG1. To address this question, we investigated the activity of phosphomimetic mutants of OGG1 in the presence and in the absence of APEX1. All forms

showed a significantly lower ability to be stimulated by APEX1 than the wild-type enzyme (Fig. 4). APEX1 elicited only a moderate stimulation of OGG1-S326E, OGG1-S231E and OGG1-S232E, whereas the activity of OGG1-S280E and OGG1-S321E/S232E in the presence and in the absence of the AP endonuclease was nearly indistinguishable. Also, OGG1-S280E, OGG1-S326E, and possibly OGG1-S231E, lacked a pronounced burst phase characteristic of wild-type OGG1 (compare panel A with panels B–D in Fig. 4). This result may indicate that reaction rates are limited by chemical steps of the reaction rather than by the product release step, as had been suggested for cleavage of suboptimal substrates, including 8-oxoGua:Ade, by wild-type OGG1 (25).

Discussion

Relatively few polymorphisms affecting the protein sequence of OGG1 have been characterized with respect to their function. Population data are available for only five polymorphisms that deviate from the reference sequence (26). By far, the most widely encountered variant is *OGG1* 326C allele (refSNP ID rs1052133), the frequency of which varies from ~0.1 in African Americans to > 0.5 in some Japanese populations (26). The other alleles are much less common: the reported frequency of the *OGG1* 85S allele (refSNP ID rs17050550) is ~0.04 (Centre d'Etude du Polymorphisme Human population sample, Caucasian origin), and of the 229Q allele (refSNP ID rs1805373), 0.008 (NIEHS HSP_GENO_PANEL population sample, ethnic origin not specified) to 0.1 (NIEHS YRI_GENO_PANEL population sample, Sub-Saharan African). The *OGG1* 288V and 322N alleles also are rare; in the NIH PDR90 population sample, the global frequency of *OGG1* 288V allele (refSNP ID rs1805373) is 0.011, and the global frequency of *OGG1* 322N allele is 0.006 (26). Given the functional defects reported for OGG1-S326C and OGG1-R229Q proteins (34,36,41,54-56), it was interesting to analyze various aspects of activity of other variants of OGG1. We have selected OGG1-A288V and OGG1-D322N as the variants in which, as deduced from the structural data (46), the DNA-binding interface of the protein could be affected.

The OGG1-A288V variant has been observed in patients with Alzheimer's disease (30). A very limited kinetic analysis of this variant has been reported, revealing that K_M of OGG1-A288V is moderately higher than that of the wild-type enzyme (30). In our experiments, A288V was ~30% less efficient (in terms of k_{sp}) than wild-type OGG1 in the oligodeoxynucleotide cleavage assay (8-oxoGua:Cyt substrate) but virtually indistinguishable from wild-type enzyme in the irradiated DNA assay. Little difference was observed in cleavage of 8-oxoGua:Ade

substrate between wild-type OGG1 and OGG1-A288V, making the latter the least specific form of all OGG1 variants studied. In the OGG1–DNA complex (46), the Ala-288 backbone amide forms a hydrogen bond with an internucleoside phosphate $p^{(5)}$ residing in the non-damaged strand and remote from the active site. Additionally, the side chain methyl group of Ala-288 makes van der Waals contacts with non-bridging oxygens of the same phosphate. While the hydrogen bond may be lost in the lesion search complex (57) and in some late complexes (58), the van der Waals contacts are present in all reported OGG1-DNA complexes (46,57-63). The bulkier isopropyl side chain of Val may induce local distortion in the region of $p^{(5)}$, partly destabilizing the OGG1–DNA complex. However, it is not clear whether the moderate decrease in the activity and C/A-specificity of OGG1-A288V, as measured on oligodeoxynucleotide substrates, may impair the activity of this variant *in vivo* and contribute to the pathogenesis of Alzheimer's disease.

Of all variants studied, OGG1-D322N possessed the highest C/A specificity. In the crystal structure of the complex of DNA with catalytically inactive OGG1 (46), and in several other structures of OGG1, either free or bound to DNA (57-64), the side chain carboxyl group of Asp-322 forms a hydrogen bond with the Nδ1 atom of His-270. The Nε2 atom of the His-270 imidazole ring in turn hydrogen bonds to a non-bridging oxygen of the phosphodiester bond immediately 5' to the damaged deoxynucleoside (Fig. 1B). Substitutions of Ala or Leu for His-270 drastically decrease OGGl activity (65). The structures of OGG1/DNA complexes approximating other intermediates of the catalytic cycle suggest considerable dynamics of His-270, which stacks with undamaged G in the lesion search complex (57), disengages from this interaction in the early and advanced lesion detection complexes (62,66), and stacks with Phe-319 in the late abasic product complex (59) and in the free enzyme (64). In all these cases,

however, the bond between Asp-322 and either Nδ1 or Nε2 of His-270 is maintained. Donating two hydrogen bonds to acidic moieties requires the imidazole ring of His-270 to be in the doubly protonated, positively charged state, which may be important in interactions of His-270 with the negatively charged DNA backbone or transient stacking of His-270 with DNA bases during lesion search and recognition. Substitution of Asn for Asp-322 would likely maintain the hydrogen bonding with His-270 but eliminate the positive charge. This change appears to destabilize modestly the Michaelis complex with the 8-oxoGua:Cyt substrate while not affecting the catalytic constant (Table 2), suggesting that correct adjustment of catalytic residues in the OGG1-D322N Michaelis complex is preserved. In contrast, with the incorrect 8-oxoGua:Ade substrate, the K_M value is nearly the same in both wild-type and OGG1-D322N while k_{cat} is reduced, possibly reflecting disorganization of the active site when the incorrect substrate binds to the mutant enzyme. On the other hand, in the irradiated DNA assay, k_{cat} rather than K_M was affected for OGG1-D322N, most probably because the reaction pathway leading to the Michaelis complex is different for short oligodeoxynucleotides carrying a single lesion and long DNA with interspersed lesions, In the latter case, K_M is dominated by sliding to and from the lesion rather than by direct binding of the lesion (53). As substrate recognition by OGG1 proceeds through at least three kinetically stable intermediate complexes (42,49), it is also possible that the D322N mutation may have an impact on selected steps of this process and/or on the sliding of the enzyme along DNA.

The *OGG1* 326C allele has been associated with an increased cancer risk in a number of epidemiological studies (reviewed in (32,33)). The activity of OGG1-S326C variant has been studied; however, the precise nature of the functional defects in this enzyme has not been established. The comparison of the ability of wild-type and S326C enzymes to counteract

spontaneous or induced mutagenesis in *E. coli*, *Salmonella*, and cultured human cells showed either the functional equivalence of these two variants (11,67) or a functional deficiency in OGG1-S326C (34,55). Extracts of lymphocytes from individuals homozygous for either form of OGG1 have the same ability to excise 8-oxoGua from DNA (35). No significant differences in the kinetic parameters of wild-type OGG1 and OGG1-S326C as glutathione-*S*-transferase fusion proteins has been found using the oligodeoxynucleotide cleavage assay while both k_{cat} and k_{sp} were reported ~2-fold lower than those for wild-type OGG1 in the γ-irradiated DNA cleavage assay (11). Unlike wild-type OGG1, OGG1-S326C is prone to dimerization, potentially producing a non-functional enzyme that is inefficiently stimulated by AP endonuclease (36). On the other hand, the functional impairment in OGG1-S326C may be due not to lower enzyme activity but to incorrect cell localization during the cell cycle (41).

In this study, we found that OGG1-S326C have a ~30% lower activity (in terms of k_{sp}) than the wild-type OGG1 acting on 8-oxoGua:Cyt and 8-oxoGua:Ade oligodeoxynucleotide substrates. A different picture was observed in the irradiated DNA assay. While the removal of FapyGua lesions by OGG1-S326C was only ~2-fold lower than by wild-type OGG1, the S326C variant was much less efficient (~6-fold lower) than the wild-type in its ability to remove 8-oxoGua from high-molecular-weight DNA. Thus, our findings are in general agreement with an earlier study of the activity and substrate specificity of OGG1-S326C (11), confirming the usefulness of this mutant form as a reference point for kinetics of other OGG1 mutants. Differences in the relative efficiency of excision of certain damaged bases from oligodeoxynucleotide substrates and from high-molecular-weight DNA by the same enzyme is rather common for DNA glycosylases. In particular, such differences have been observed before for Fpg, a bacterial enzyme overlapping with OGG1 in its substrate specificity except for

excision of FapyAde, which is not removed by OGG1 from DNA or oligodeoxynucleotides (14,52,53,68). It is possible that the S326C substitution more significantly affects the ability of OGG1 to participate in the repair of 8-oxoGua and thus represents a risk factor in carcinogenesis.

Phosphorylation represents an established mechanism for regulating the function of certain proteins, including enzymatic activity, protein–protein interactions, cell sorting, etc. (69). As obtaining pure proteins phoshorylated at a defined site is hard to achieve, replacement of Ser and Thr residues with acidic residues, Asp or Glu, is often used as a convenient tool to study potential effects of phosphorylation in a diverse set of proteins. Such phosphomimetic mutations reproduce accurately both the structural and the functional consequences of phosphorylation (70-72). OGG1 contains several Ser and Thr residues located in sequences with a high probability of phosphorylation (Table 1), and has been shown to be phosphorylated, although the modified residues have not been specifically identified (39,40). In fact, one of the putative phosphorylation residues is Ser-326, and the inability of OGG1-S326C to be phosphorylated at this site has been proposed as a possible cause of the functional deficiency of this OGG1 form (41). The phosphomimetic strategy was employed to explore the consequences of Ser-326 phosphorylation for cell sorting of OGG1 (41). However, data on the activity or substrate specificity of this phosphomimetic mutant other than confirmation that the OGG1-like activity is present in nuclear extracts of transfected HeLa cells are unavailable. In this paper, we have constructed and analyzed a series of phosphomimetic mutants at sites with the highest probability of phosphorylation (Table 1). All mutants were ~2-fold lower in activity than the wild-type protein in the oligodeoxynucleotide assay and 1.1–3.6-fold lower in the irradiated DNA assay, indicating that phosphorylation of OGG1 is not likely to be involved in regulating its activity. This result contrasts to the moderate activation of OGG1 by another posttranslational modification,

acetylation at Lys-338/Lys-341 in the C-terminal tail of the protein (73). In other human DNA glycosylases, phosphorylation have been shown to increase the activity of MUTYH (74,75) and uracil-DNA glycosylase (UNG) (76,77).

Protein–protein interactions are important in the coordination of sequential BER steps; also as a potential target for regulation by phosphorylation. The ability of OGG1 to be stimulated by APEX1 is abrogated by the S326C substitution (36). We have shown that the same is true for phosphomimetic mutants of OGG1 (Fig. 4). Since Ser-231, Ser-232, Ser-280, and Ser-326 residues are located a significant distance apart on the surface of OGG1 globule, it is unlikely that all these mutations disrupt the OGG1–APEX1 interaction interface. However, the phosphomimetic mutations could alter the structure of some transient intermediate protein–DNA complexes that occurs during displacement of OGG1 by APEX1. The nature of such complexes is currently under investigation in our laboratory using stopped-flow enzyme kinetics. If regulation of functional interactions with APEX1 is indeed affected by phosphorylation of OGG1, this reaction may be involved in switching between APEX1-assisted and NEIL1-assisted subpathways of OGG1-initiated BER (78).

Other processes involving DNA glycosylases may be affected by protein phosphorylation. For instance, phosphorylation regulates proteasomal degradation of UNG (77,79). In the case of OGG1, phosphorylation may be required for association with chromatin (39) and localization in the nucleolus (41). It remains to be seen whether phosphomimetic mutants of OGG1 differ from wild-type protein in these aspects or in other properties such as intracellular trafficking, interactions with other BER components, etc.

The C/A specificity of OGG1 is important in preventing 8-oxoGua-induced mutagenesis. We have shown that the C/A specificity of OGG1 and Fpg is highest under nearly-physiological

conditions, due to a sharp decrease in the enzyme's activity on 8-oxoGua:Ade substrates with increasing ionic strength and and Mg^{2+} concentration (24), and that APEX1 stimulates OGG1 to a higher degree on 8-oxoGua:Cyt than on 8-oxoGua:Ade substrates (25). Compared with these factors, the natural variations and phosphomimetic mutations in OGG1 had a lower impact on the C/A specificity, which varied between 70–240% of the specificity of the wild-type enzyme. Therefore, it is unlikely that the erroneous repair of 8-oxoGua:Ade mispairs by the studied forms of OGG1 would contribute significantly to the mutagenic load, or that phosphorylation of OGG1 could be used by the cell to regulate the enzyme's opposite-base specificity.

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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. von Sonntag, C. (2006) *Free-Radical-Induced DNA Damage and Its Repair: A Chemical Perspective*, Springer, Berlin - Heidelberg
2. Shibutani, S., Takeshita, M., and Grollman, A. P. (1991) *Nature* **349**, 431-434
3. Kamiya, H., Miura, K., Ishikawa, H., Inoue, H., Nishimura, S., and Ohtsuka, E. (1992) *Cancer Res.* **52**, 3483-3485
4. Moriya, M. (1993) *Proc. Natl Acad. Sci. U.S.A.* **90**, 1122-1126
5. Wiederholt, C. J., and Greenberg, M. M. (2002) *J. Am. Chem. Soc.* **124**, 7278-7279
6. Kalam, M. A., Haraguchi, K., Chandani, S., Loechler, E. L., Moriya, M., Greenberg, M. M., and Basu, A. K. (2006) *Nucleic Acids Res.* **34**, 2305-2315
7. Olinski, R., Gackowski, D., Rozalski, R., Foksinski, M., and Bialkowski, K. (2003) *Mutat. Res.* **531**, 177-190
8. Friedberg, E. C., Walker, G. C., Siede, W., Wood, R. D., Schultz, R. A., and Ellenberger, T. (2006) *DNA Repair and Mutagenesis*, ASM Press, Washington, D.C.
9. Tsuzuki, T., Nakatsu, Y., and Nakabeppu, Y. (2007) *Cancer Sci.* **98**, 465-470
10. Karahalil, B., Girard, P.-M., Boiteux, S., and Dizdaroglu, M. (1998) *Nucleic Acids Res.* **26**, 1228-1232
11. Dherin, C., Radicella, J. P., Dizdaroglu, M., and Boiteux, S. (1999) *Nucleic Acids Res.* **27**, 4001-4007
12. Dherin, C., Dizdaroglu, M., Doerflinger, H., Boiteux, S., and Radicella, J. P. (2000) *Nucleic Acids Res.* **28**, 4583-4592
13. Morales-Ruiz, T., Birincioglu, M., Jaruga, P., Rodriguez, H., Roldan-Arjona, T., and Dizdaroglu, M. (2003) *Biochemistry* **42**, 3089-3095

14. Krishnamurthy, N., Haraguchi, K., Greenberg, M. M., and David, S. S. (2008) *Biochemistry* **47**, 1043-1050

15. Xie, Y., Yang, H., Cunanan, C., Okamoto, K., Shibata, D., Pan, J., Barnes, D. E., Lindahl, T., McIlhatton, M., Fishel, R., and Miller, J. H. (2004) *Cancer Res.* **64**, 3096-3102

16. Rosenquist, T. A., Zharkov, D. O., and Grollman, A. P. (1997) *Proc. Natl Acad. Sci. U.S.A.* **94**, 7429-7434

17. Radicella, J. P., Dherin, C., Desmaze, C., Fox, M. S., and Boiteux, S. (1997) *Proc. Natl Acad. Sci. U.S.A.* **94**, 8010-8015

18. Hill, J. W., Hazra, T. K., Izumi, T., and Mitra, S. (2001) *Nucleic Acids Res.* **29**, 430-438

19. Vidal, A. E., Hickson, I. D., Boiteux, S., and Radicella, J. P. (2001) *Nucleic Acids Res.* **29**, 1285-1292

20. Saitoh, T., Shinmura, K., Yamaguchi, S., Tani, M., Seki, S., Murakami, H., Nojima, Y., and Yokota, J. (2001) *Mutat. Res.* **486**, 31-40

21. Sidorenko, V. S., Nevinsky, G. A., and Zharkov, D. O. (2007) *DNA Repair* **6**, 317-328

22. Bjørås, M., Luna, L., Johnsen, B., Hoff, E., Haug, T., Rognes, T., and Seeberg, E. (1997) *EMBO J.* **16**, 6314-6322

23. Zharkov, D. O., Rosenquist, T. A., Gerchman, S. E., and Grollman, A. P. (2000) *J. Biol. Chem.* **275**, 28607-28617

24. Sidorenko, V. S., Mechetin, G. V., Nevinsky, G. A., and Zharkov, D. O. (2008) *FEBS J.* **275**, 3747-3760

25. Sidorenko, V. S., Nevinsky, G. A., and Zharkov, D. O. (2008) *Biochem. Biophys. Res. Commun.* **368**, 175-179

26. NCBI dbSNP: http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp

27. Chevillard, S., Radicella, J. P., Levalois, C., Lebeau, J., Poupon, M.-F., Oudard, S., Dutrillaux, B., and Boiteux, S. (1998) *Oncogene* **16**, 3083-3086

28. Blons, H., Radicella, J. P., Laccourreye, O., Brasnu, D., Beaune, P., Boiteux, S., and Laurent-Puig, P. (1999) *Mol. Carcinog.* **26**, 254-260

29. Pieretti, M., Khattar, N. H., and Smith, S. A. (2001) *Mutat. Res.* **432**, 53-59

30. Mao, G., Pan, X., Zhu, B.-B., Zhang, Y., Yuan, F., Huang, J., Lovell, M. A., Lee, M. P., Markesbery, W. R., Li, G.-M., and Gu, L. (2007) *Nucleic Acids Res.* **35**, 2759-2766

31. Audebert, M., Chevillard, S., Levalois, C., Gyapay, G., Vieillefond, A., Klijanienko, J., Vielh, P., El Naggar, A. K., Oudard, S., Boiteux, S., and Radicella, J. P. (2000) *Cancer Res.* **60**, 4740-4744

32. Weiss, J. M., Goode, E. L., Ladiges, W. C., and Ulrich, C. M. (2005) *Mol. Carcinog.* **42**, 127-141

33. Hung, R. J., Hall, J., Brennan, P., and Boffetta, P. (2005) *Am. J. Epidemiol.* **162**, 925-942

34. 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

35. Janssen, K., Schlink, K., Götte, W., Hippler, B., Kaina, B., and Oesch, F. (2001) *Mutat. Res.* **486**, 207-216

36. Hill, J. W., and Evans, M. K. (2006) *Nucleic Acids Res.* **34**, 1620-1632

37. Audebert, M., Radicella, J. P., and Dizdaroglu, M. (2000) *Nucleic Acids Res.* **28**, 2672-2678

38. Fan, J., and Wilson, D. M., III. (2005) *Free Radic. Biol. Med.* **38**, 1121-1138

39. Dantzer, F., Luna, L., Bjorås, M., and Seeberg, E. (2002) *Nucleic Acids Res.* **30**, 2349-2357

40. Hu, J., Imam, S. Z., Hashiguchi, K., de Souza-Pinto, N. C., and Bohr, V. A. (2005) *Nucleic Acids Res.* **33**, 3271-3282

41. Luna, L., Rolseth, V., Hildrestrand, G. A., Otterlei, M., Dantzer, F., Bjørås, M., and Seeberg, E. (2005) *Nucleic Acids Res.* **33**, 1813-1824

42. Kuznetsov, N. A., Koval, V. V., Zharkov, D. O., Nevinsky, G. A., Douglas, K. T., and Fedorova, O. S. (2005) *Nucleic Acids Res.* **33**, 3919-3931

43. Roy, L. M., Jaruga, P., Wood, T. G., McCullough, A. K., Dizdaroglu, M., and Lloyd, R. S. (2007) *J. Biol. Chem.* **282**, 15790-15798

44. Jaruga, P., Kirkali, G., and Dizdaroglu, M. (2008) *Free Radic. Biol. Med.* **45**, 1601-1609

45. Goode, E. L., Ulrich, C. M., and Potter, J. D. (2002) *Cancer Epidemiol. Biomarkers Prev.* **11**, 1513-1530

46. Bruner, S. D., Norman, D. P. G., and Verdine, G. L. (2000) *Nature* **403**, 859-866

47. Blom, N., Gammeltoft, S., and Brunak, S. (1999) *J. Mol. Biol.* **294**, 1351-1362

48. Blom, N., Sicheritz-Pontén, T., Gupta, R., Gammeltoft, S., and Brunak, S. (2004) *Proteomics* **4**, 1633-1649

49. Kuznetsov, N. A., Koval, V. V., Nevinsky, G. A., Douglas, K. T., Zharkov, D. O., and Fedorova, O. S. (2007) *J. Biol. Chem.* **282**, 1029-1038

50. Dizdaroglu, M., Jaruga, P., Birincioglu, M., and Rodriguez, H. (2002) *Free Radic. Biol. Med.* **32**, 1102-1115

51. Boiteux, S., Gajewski, E., Laval, J., and Dizdaroglu, M. (1992) *Biochemistry* **31**, 106-110

52. Karakaya, A., Jaruga, P., Bohr, V. A., Grollman, A. P., and Dizdaroglu, M. (1997) *Nucleic Acids Res.* **25**, 474-479

53. Zaika, E. I., Perlow, R. A., Matz, E., Broyde, S., Gilboa, R., Grollman, A. P., and Zharkov, D. O. (2004) *J. Biol. Chem.* **279**, 4849-4861

54. Hyun, J.-W., Choi, J.-Y., Zeng, H.-H., Lee, Y.-S., Kim, H.-S., Yoon, S.-H., and Chung, M.-H. (2000) *Oncogene* **19**, 4476-4479

55. Yamane, A., Kohno, T., Ito, K., Sunaga, N., Aoki, K., Yoshimura, K., Murakami, H., Nojima, Y., and Yokota, J. (2004) *Carcinogenesis* **25**, 1689-1694

56. Hill, J. W., and Evans, M. K. (2007) *Cancer Detect. Prev.* **31**, 237-243

57. Banerjee, A., Yang, W., Karplus, M., and Verdine, G. L. (2005) *Nature* **434**, 612-618

58. Fromme, J. C., Bruner, S. D., Yang, W., Karplus, M., and Verdine, G. L. (2003) *Nat. Struct. Biol.* **10**, 204-211

59. Norman, D. P. G., Bruner, S. D., and Verdine, G. L. (2001) *J. Am. Chem. Soc.* **123**, 359-360

60. Norman, D. P. G., Chung, S. J., and Verdine, G. L. (2003) *Biochemistry* **42**, 1564-1572

61. Chung, S. J., and Verdine, G. L. (2004) *Chem. Biol.* **11**, 1643-1649

62. Banerjee, A., and Verdine, G. L. (2006) *Proc. Natl Acad. Sci. U.S.A.* **103**, 15020-15025

63. Radom, C. T., Banerjee, A., and Verdine, G. L. (2007) *J. Biol. Chem.* **282**, 9182-9194

64. Bjørås, M., Seeberg, E., Luna, L., Pearl, L. H., and Barrett, T. E. (2002) *J. Mol. Biol.* **317**, 171-177

65. Auffret van der Kemp, P., Charbonnier, J.-B., Audebert, M., and Boiteux, S. (2004) *Nucleic Acids Res.* **32**, 570-578

66. Lee, S., Radom, C. T., and Verdine, G. L. (2008) *J. Am. Chem. Soc.* **130**, 7784-7785

67. Kim, S.-R., Matsui, K., Yamada, M., Kohno, T., Kasai, H., Yokota, J., and Nohmi, T. (2004) *Mutat. Res.* **554**, 365-374

68. Zharkov, D. O., Shoham, G., and Grollman, A. P. (2003) *DNA Repair* **2**, 839-862

69. Pawson, T., and Scott, J. D. (2005) *Trends Biochem. Sci.* **30**, 286-290

70. Wittekind, M., Reizer, J., Deutscher, J., Saier, M. H., and Klevit, R. E. (1989) *Biochemistry* **28**, 9908-9912

71. Morrison, P., Takishima, K., and Rosner, M. R. (1993) *J. Biol. Chem.* **268**, 15536-15543

72. Maciejewski, P. M., Peterson, F. C., Anderson, P. J., and Brooks, C. L. (1995) *J. Biol. Chem.* **270**, 27661-27665

73. Bhakat, K. K., Mokkapati, S. K., Boldogh, I., Hazra, T. K., and Mitra, S. (2006) *Mol. Cell. Biol.* **26**, 1654-1665

74. Gu, Y., and Lu, A.-L. (2001) *Nucleic Acids Res.* **29**, 2666-2674

75. Parker, A. R., O'Meally, R. N., Sahin, F., Su, G. H., Racke, F. K., Nelson, W. G., DeWeese, T. L., and Eshleman, J. R. (2003) *J. Biol. Chem.* **278**, 47937-47945

76. Lu, X., Bocangel, D., Nannenga, B., Yamaguchi, H., Appella, E., and Donehower, L. A. (2004) *Mol. Cell* **15**, 621-634

77. Hagen, L., Kavli, B., Sousa, M. M. L., Torseth, K., Liabakk, N. B., Sundheim, O., Pena-Diaz, J., Otterlei, M., Hørning, O., Jensen, O. N., Krokan, H. E., and Slupphaug, G. (2008) *EMBO J.* **27**, 51-61

78. Mokkapati, S. K., Wiederhold, L., Hazra, T. K., and Mitra, S. (2004) *Biochemistry* **43**, 11596-11604

79. Fischer, J. A., Muller-Weeks, S., and Caradonna, S. (2004) *DNA Repair* **3**, 505-513

80. DeLano, W. L. (2002) The PyMOL molecular graphics system.

Table 1. NetPhos scores for Ser/Thr phosphorylation of OGG1

Ser/Thr position	Peptide context	Score	Predicted phosphorylation
15	MGHRTLAST	0.228	–
18	RTLASTPAL	0.004	–
19	TLASTPALW	0.060	–
25	ALWASIPCP	0.012	–
31	PCPRSELRL	0.860	+
41	LVLPSGQSF	0.065	–
44	PSGQSFRWR	0.232	–
51	WREQSPAHW	0.792	+
56	PAHWSGVLA	0.010	–
65	DQVWTLTQT	0.211	–
67	VWTLTQTEE	0.881	+
69	TLTQTEEQL	0.185	–
76	QLHCTVYRG	0.046	–
83	RGDKSQASR	0.368	–
86	KSQASRPTP	0.917	+
89	ASRPTPDEL	0.986	+
105	QLDVTLAQL	0.011	–
115	HHWGSVDSH	0.059	–
118	GSVDSHFQE	0.032	–
143	ECLFSFICS	0.155	–
147	SFICSSNNN	0.006	–
148	FICSSNNNI	0.005	–
156	IARITGMVE	0.806	+
177	LDDVTYHGF	0.025	–
183	HGFPSLQAL	0.006	–
209	ARYVSASAR	0.943	+
211	YVSASARAI	0.950	+
231	**QLRESSYEE**	**0.996**	**+**
232	**LRESSYEEA**	**0.997**	**+**
248	PGVGTKVAD	0.834	+
280	**QRDYSWHPT**	**0.994**	**+**
284	SWHPTTSQA	0.374	–
285	WHPTTSQAK	0.311	–
286	HPTTSQAKG	0.032	–
292	AKGPSPQTN	0.415	–
295	PSPQTNKEL	0.980	+
305	NFFRSLWGP	0.014	–
320	AVLFSADLR	0.003	–
326	**DLRQSRHAQ**	**0.990**	**+**
340	RRKGSKGPE	0.986	+

The sequences in boldface mark the position of Ser residues selected for mutagenesis.

Table 2. K_M, k_{cat}, and k_{sp} values for the cleavage of 8-oxoGua:Cyt oligodeoxynucleotide substrates by wild-type and mutant OGG1 proteins

OGG1	K_M, nM	k_{cat}, min^{-1}, $\times 10^2$	k_{sp}, $nM^{-1} \times min^{-1}$, $\times 10^3$	k_{sp}(WT)/k_{sp}(mutant)
WT	3.4 ± 0.6	3.0 ± 0.1	8.9	1
A288V	8.6 ± 1.2	5.5 ± 0.3	6.4	1.4
D322N	6.1 ± 1.2	2.8 ± 0.1	4.6	1.9
S326C	3.4 ± 0.8	2.2 ± 0.1	6.5	1.4
S231E	5.7 ± 1.2	4.2 ± 0.2	7.4	1.2
S232E	9.2 ± 1.5	3.9 ± 0.2	4.2	2.1
S231E/S232E	10 ± 1	4.1 ± 0.2	4.0	2.2
S280E	7.4 ± 1.6	2.9 ± 0.2	4.0	2.2
S326E	7.5 ± 1.4	3.2 ± 0.1	4.3	2.1

Means of 3–5 independent experiments are shown. Uncertainties are standard deviations. WT, wild-type.

Table 3. K_M, k_{cat}, and k_{sp} values for the cleavage of 8-oxoGua:Ade oligodeoxynucleotide substrates by wild-type and mutant OGG1 proteins

OGG1	K_M, nM	k_{cat}, min^{-1}, $\times 10^2$	k_{sp}, $nM^{-1} \times min^{-1}$, $\times 10^3$	k_{sp}(WT)/k_{sp}(mutant)	C/A specificity*
WT	23 ± 5	4.1 ± 0.3	1.8	1	4.9
A288V	18 ± 4	3.2 ± 0.2	1.8	0.97	3.6
D322N	22 ± 6	0.9 ± 0.1	0.4	4.5	12
S326C	13 ± 3	1.6 ± 0.1	1.2	1.4	5.4
S231E	14 ± 4	2.0 ± 0.1	1.4	1.3	5.3
S232E	23 ± 4	2.3 ± 0.1	1.0	1.8	4.2
S231E/S232E	25 ± 5	2.4 ± 0.1	1.0	1.8	4.0
S280E	18 ± 3	1.6 ± 0.1	0.9	2.1	4.4
S326E	24 ± 2	1.6 ± 0.0	0.7	2.6	6.1

Means of 3–5 independent experiments are shown. Uncertainties are standard deviations. WT, wild-type.

*See the definition of C/A specificity in the main text

Table 4. K_M, k_{cat}, and k_{sp} values for excision of FapyGua and 8-oxoGua from γ-irradiated calf thymus DNA by wild-type and mutant OGG1 proteins

OGG1	FapyGua			8-oxoGua		
	K_M, μM	k_{cat}, min^{-1}, $\times 10^2$	k_{sp}, $nM^{-1}\times min^{-1}$, $\times 10^5$	K_M, μM	k_{cat}, min^{-1}, $\times 10^2$	k_{sp}, $nM^{-1}\times min^{-1}$, $\times 10^5$
WT	3.6 ± 0.2	15 ± 1	4.1 ± 0.2	1.4 ± 0.1	6.5 ± 0.4	4.7 ± 0.3
A288V	4.1 ± 0.4	16 ± 1	4.0 ± 0.3	1.1 ± 0.2	5.1 ± 0.4	4.6 ± 0.4
D322N	3.0 ± 0.4	7.9 ± 0.8	2.6 ± 0.3	1.1 ± 0.1	1.3 ± 0.1	1.1 ± 0.1
S326C	2.0 ± 0.2	4.0 ± 0.4	2.0 ± 0.2	3.2 ± 1.0	2.4 ± 0.6	0.7 ± 0.2
S231E	3.5 ± 0.4	13 ± 1	3.7 ± 0.3	6.0 ± 0.3	12 ± 0.5	2.0 ± 0.1
S232E	3.6 ± 0.2	11 ± 1	3.1 ± 0.1	7.6 ± 2.9	9.8 ± 0.4	1.3 ± 0.5
S231E/S232E	4.8 ± 0.7	13 ± 2	2.6 ± 0.3	4.5 ± 1.0	9.1 ± 1.8	2.1 ± 0.4
S280E	2.8 ± 0.1	9.3 ± 0.3	3.3 ± 0.1	8.2 ± 1.2	12 ± 2	1.4 ± 0.2
S326E	4.6 ± 0.3	11 ± 1	2.4 ± 0.2	3.5 ± 0.3	5.5 ± 0.3	1.6 ± 0.1

Mean of 3 independent experiments are shown. Uncertainties are standard deviations. WT, wild-type.

Figure legends

Fig. 1. A, localization of the mutated residues in the three-dimensional structure of OGG1 (Protein Data Bank reference number 1EBM, Ref. (46)). DNA is shown as a stick model, the protein, as a cartoon. The residues investigated in this study are shown in dotted spheres. Ser-326 is absent from the structure but is presumably located near its C-terminus. The figure was prepared using PyMOL (80). B, Asp-322–His-270–8-oxodGMP bridge in the active site of OGG1.

Fig. 2. Lineweaver–Burk plot for the cleavage of 8-oxoGua:Cyt (●) and 8-oxoGua:Ade (○) substrates by wild-type OGG1 Means of 3–4 independent experiments are shown. Uncertainties are standard deviations.

Fig. 3. Excision of 8-oxoGua and FapyGua by wild-type OGG1 and Fpg from γ-irradiated calf thymus DNA. A, time course of excision of 8-oxoGua (●), FapyGua (○), and FapyAde (■) by OGG1. B, time course of excision of 8-oxoGua (●), FapyGua (○), and FapyAde (■) by Fpg. Means of 3 independent experiments are shown. Uncertainties are standard deviations.

Fig. 4. Time course of 8-oxoGua:Cyt substrate cleavage by wild-type OGG1 and its phosphomimetic mutants alone (●) or in the presence of APEX1 (○). A, wild-type OGG1; B, OGG1-S280E; C, OGG1-S326E; D, OGG1-S231E; E, OGG1-S232E; F, OGG1-S231E/S232E. The scale of the Y axis (product accumulation) is the same in all plots. Means of 2 independent experiments are shown. [P], concentration of the AP product.

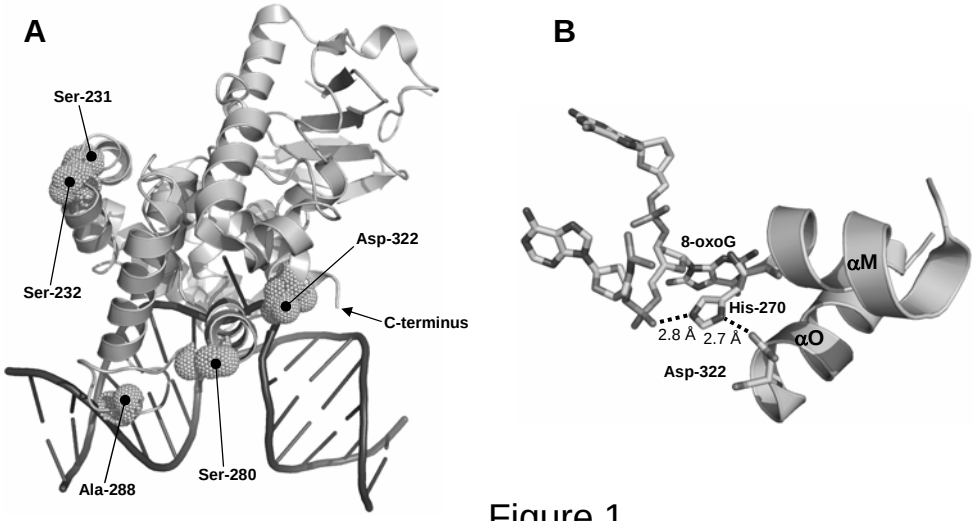
A
Ser-231
Ser-232
Asp-322
C-terminus
Ala-288
Ser-280
B
8-oxoG
αM
His-270
2.8 Å
2.7 Å
αO
Asp-322

Figure 1

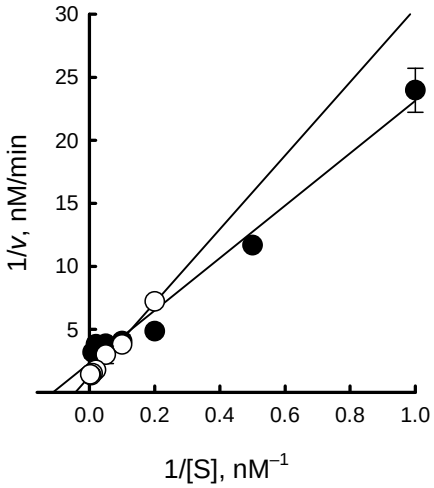

Figure 2

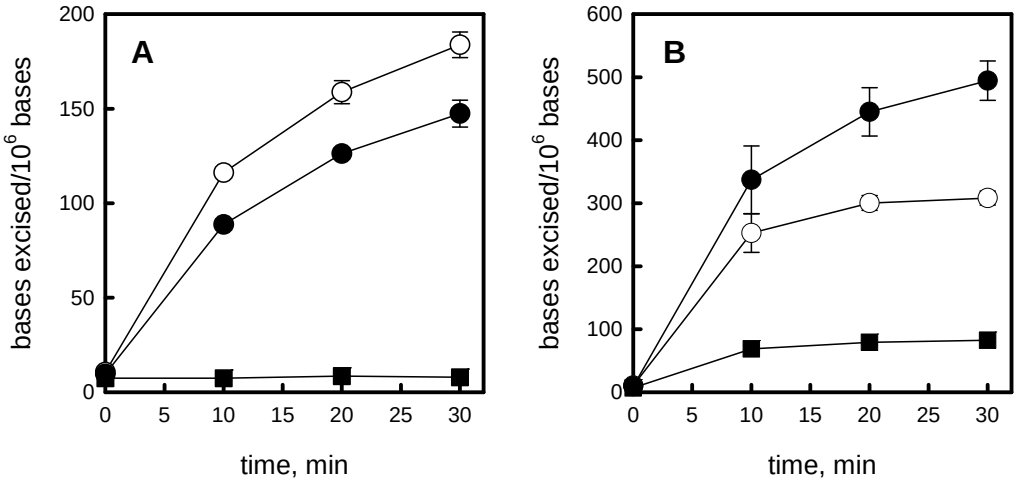
A
B
bases excised/10^6 bases
time, min
0
50
100
150
200
300
400
500
600
5
10
15
20
25
30

Figure 3

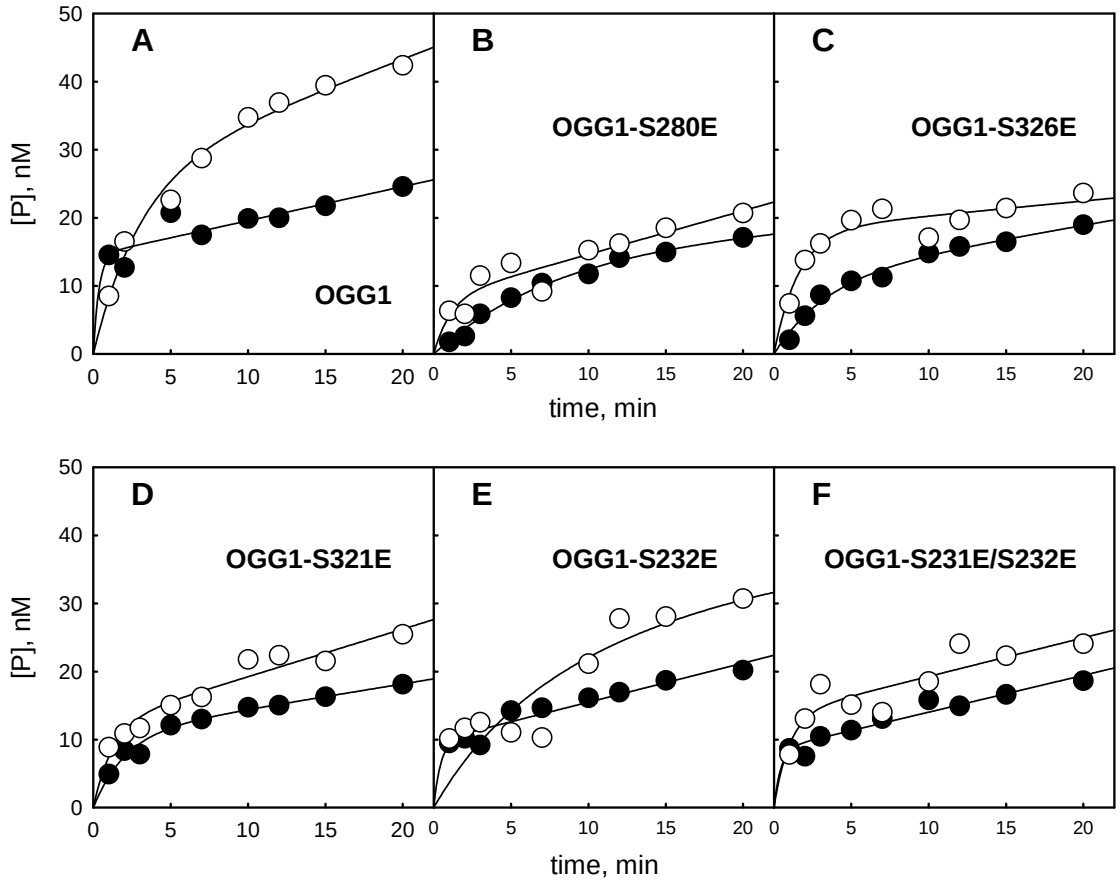
A
B
C
OGG1
OGG1-S280E
OGG1-S326E
[P], nM
time, min
D
E
F
OGG1-S321E
OGG1-S232E
OGG1-S231E/S232E
[P], nM
time, min

Figure 4