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ARTICLE *in* BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS · APRIL 2008

Impact Factor: 2.3 · DOI: 10.1016/j.bbrc.2008.01.076 · Source: PubMed

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Specificity of stimulation of human 8-oxoguanine–DNA glycosylase by AP endonuclease

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Received 13 January 2008
Available online 24 January 2008

Abstract

Human 8-oxoguanine–DNA glycosylase OGG1 is an enzyme that removes abundant oxidative lesion 8-oxoguanine (8-oxoG) from DNA. Excision of 8-oxoG by OGG1 is inhibited by the abasic DNA reaction product and is stimulated by AP endonuclease APEX1. Besides 8-oxoG, OGG1 shows activity towards several other base lesions. Here we report that APEX1 efficiently stimulates OGG1 on good substrates (8-oxoadenine, 8-oxoinosine, or 6-methoxy-8-oxoguanine opposite to cytosine) but the stimulation is low or absent with poor OGG1 substrates (8-oxoadenine or 8-oxoinosine opposite to thymine; 8-oxoG or 8-aminoguanine opposite to adenine; 8-oxonebularine, 8-metoxyguanine, inosine or guanine opposite to cytosine). APEX1 significantly improves the ability of OGG1 to excise 8-aminoguanine from its naturally occurring pair with cytosine, making it possible that OGG1 repairs this lesion. Overall, APEX1 serves to improve specificity of OGG1 for its biologically relevant substrates.

Keywords: DNA repair; 8-oxoguanine–DNA glycosylase; AP endonuclease; Protein–protein interactions

Base excision repair is an important process that involves several enzymes acting sequentially to remove damaged bases from DNA and replace them with regular ones. At the first step of this process, DNA glycosylases hydrolyze the *N*-glycosidic bond of the damaged deoxynucleoside, leaving an apurine/apyrimidine (AP) site. The AP site is then cleaved by AP endonucleases, and the repair is completed by the action of a DNA polymerase, DNA ligase and several accessory proteins [1]. Eleven DNA glycosylases specific for different kinds of base lesions are known from human cells [2]. One of them, 8-oxoguanine–DNA glycosylase (OGG1), initiates the repair of 8-oxoguanine (8-oxoG), an abundant pre-mutagenic DNA lesion [3–5].

Acting alone, OGG1 is inhibited by its product, the AP site in DNA. However, this inhibition can be relieved by the major human AP endonuclease APEX1 [6–8], resulting in an apparent stimulation of OGG1. The mechanism of stimulation is likely direct displacement of the glycosylase by APEX1 from the OGG1–product complex [9].

While 8-oxoGua is considered the main substrate for OGG1, the enzyme can remove some other modified purine lesions from DNA with variable efficiencies; some of them are better substrates than 8-oxoGua, while others are less favored [10]. In addition, OGG1 has a strong preference for excision of 8-oxoG paired with C and is less efficient when this lesion is found opposite A in DNA, presumably to avoid mutations due to misincorporation of dAMP opposite 8-oxoG during replication [3]. Stimulation of OGG1 by APEX1 on substrates other than 8-oxoG:C has never been addressed. Since the presence of APEX1 in the complete BER system may influence the action of OGG1 on various damaged DNA substrates, here we have analyzed OGG1 activity towards several naturally occurring and synthetic base lesions in the absence and in the presence of APEX1.

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doi:10.1016/j.bbrc.2008.01.076

Materials and methods

Enzymes and oligonucleotides. OGG1 and APEX1 were purified as described [9,11]; T4 polynucleotide kinase was from New England Biolabs. Oligonucleotides, synthesized using established protocols [12–15], were purchased from Operon Biotechnologies or kindly provided by Dr. Francis Johnson (SUNY at Stony Brook). The modified 23-mer d(CTCTCCCTTCXCTCCTTTCCTCT), where X was 8-oxoG, 8-oxoadenine (8-oxoA) 8-methoxyguanine (8-MeOG), 8-oxonebularine (8-oxoN), 6-*O*-methyl-8-oxoguanine (6-OMe-8-oxoG), 8-aminoguanine (8-aminoG), 8-oxohypoxanthine (8-oxoI), hypoxanthine (I), or guanine (G; see Fig. 1 for the structures of the lesions), was 5′-labeled using $\gamma[^{32}P]$-ATP and polynucleotide kinase and annealed to the complementary strand, which contained C, T, or A opposite the lesion.

Stimulation assay. The reaction mixture contained 20 mM Hepes–NaOH (pH 7.5), 1 mM DTT, 50 mM KCl, 5 mM $MgCl_2$, 50 nM double-stranded oligonucleotide substrate, 20 nM OGG1, and 100 nM APEX1. The reaction was initiated by simultaneous addition of OGG1 and APEX1, and allowed to proceed for 1–20 min. Aliquots were withdrawn at required times, mixed with putrescine–HCl (pH 8.0, 100 mM final concentration), heated for 5 min at 95 °C, and stored on ice until analysis. Immediately before loading onto the gel, the samples were heated again at 95 °C for 3 min. The substrates incubated with no enzyme or with APEX1 alone for the same amount of time were used as controls. After separation by 20% denaturing PAGE, the reaction products were quantified by phosphorimaging using a Storm 840 system and ImageQuant v5.2 software.

Results and discussion

OGG1 is known to display a burst phase kinetics on 8-oxoG:C substrates, with a fast increase in the product concentration followed by slow product accumulation indicative of a limited turnover due to tight binding of the enzyme to the AP reaction product [6,9]. Analysis of the time course of cleavage of an 8-oxoG:C substrate by OGG1 revealed a clear burst phase (Fig. 2A), the amplitude of which, as expected, was proportional to the concentration of the enzyme in the reaction mixture (not shown). In line with the literature data [6,9], addition of excess APEX1 relieved the product inhibition, causing a 1.8-fold increase in the apparent OGG1 turnover rate (as calculated from the slope of the linear parts of the curves in Fig. 2A; Table 1).

The time course of cleavage by OGG1 was very different for the 8-oxoG:A substrate (Fig. 2B). No burst phase was observed in this case, suggestive of either lower chemical step rate than for 8-oxoG:C, or higher product release rate. The apparent steady-state turnover rate, determined from the initial phase of the time course, was actually higher than for the 8-oxoG:C substrate (Table 1); however, the turnover rates for these two substrates cannot be directly compared as they are limited by different processes, the product release in the case of 8-oxoG:C and the chemical steps in the case of 8-oxoG:A. APEX1 caused a more modest enhancement of cleavage of 8-oxoG:A than of 8-oxoG:C (Fig. 2A and B, Table 1). Overall, 8-oxoG:C was a better substrate than 8-oxoG:A both with and without APEX1. The better activation of OGG1 by APEX1 on 8-

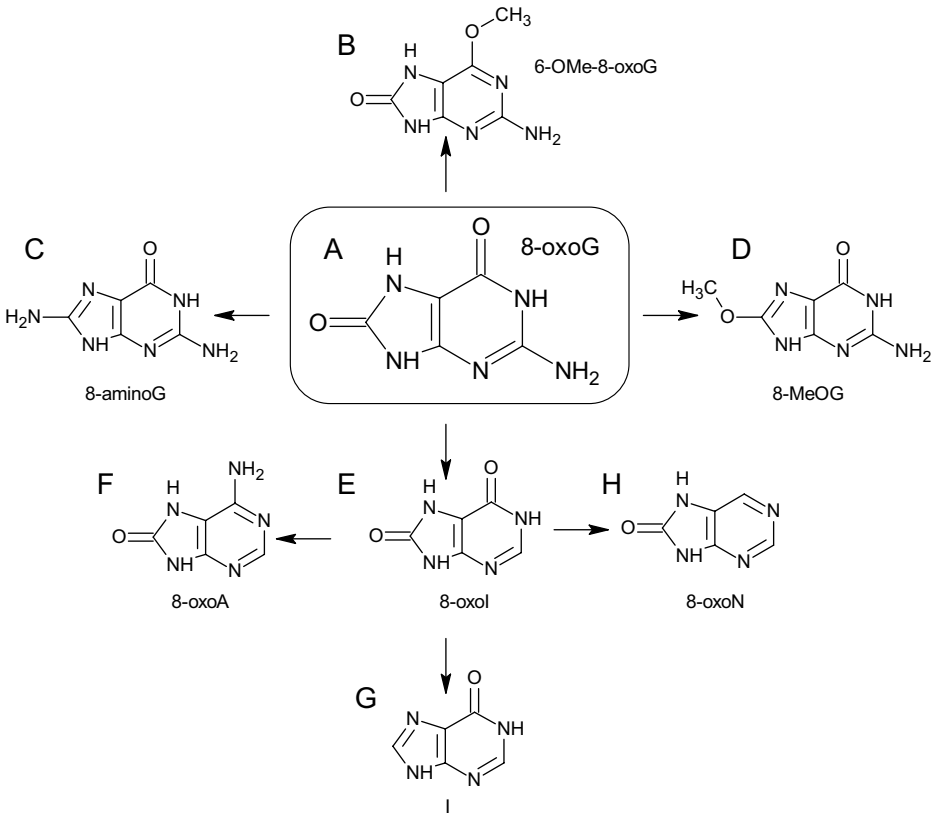

Fig. 1. Base lesions used in this work. (A) 8-oxoguanine, (B) 6-*O*-methyl-8-oxoguanine, (C) 8-aminoguanine, (D) 8-methoxyguanine, (E) 8-oxohypoxanthine, (F) 8-oxoadenine, (G) hypoxanthine, (H) 8-oxonebularine. Arrows schematically denote changes in the substituents at the purine ring between different nucleobases.

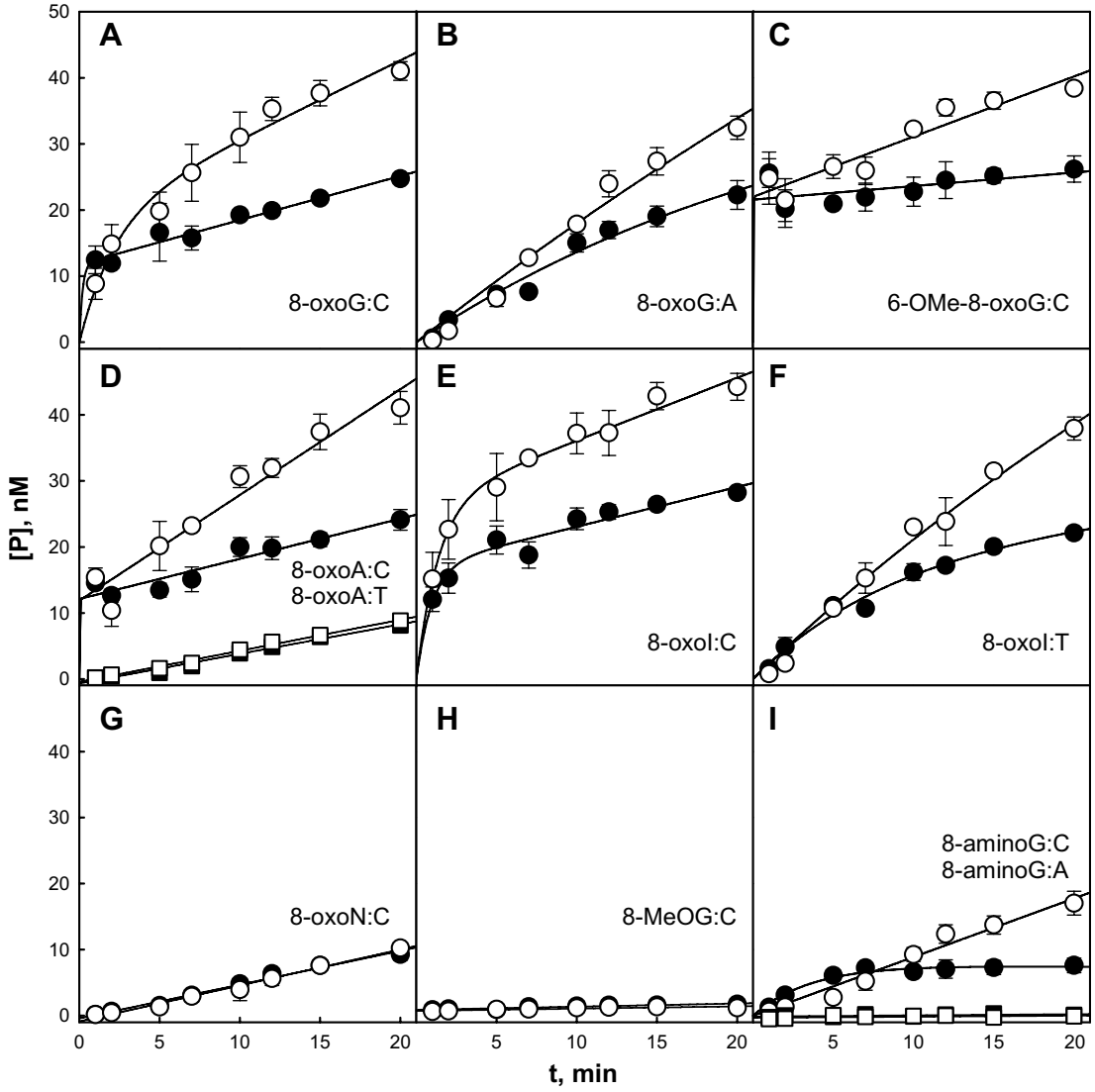

Fig. 2. Stimulation of OGG1 by APEX1 on various substrates. Time courses of substrate cleavage by OGG1 in the absence (black symbols) or in the presence (white symbols) of APEX1 are shown for substrates 8-oxoG:C (A), 8-oxoG:A (B), 6-OMe-8-oxoG:C (C), 8-oxoA:C (D, circles), 8-oxoA:T (D, squares), 8-oxoI:C (E), 8-oxoI:T (F), 8-oxoN:C (G), 8-MeOG:C (H), 8-aminoG:C (I, circles) and 8-aminoG:A (I, squares). The scale of all graphs is the same. Mean ± SE of three independent experiments is shown; error bars may be covered by the symbols. In all cases, the lines represent least-square fitting to the burst phase kinetics.

Table 1
Apparent turnover rate of OGG1 on different substrates under the standard conditions

Substrate	–APEX1, nM/min	+APEX1, nM/min	Fold increase
8-oxoG:C[a]	0.67 ± 0.11	1.2 ± 0.3	1.8
8-oxoG:A[b]	1.4 ± 0.1	1.8 ± 0.1	1.3
6-OMe-8-oxoG:C[a]	0.21 ± 0.12	0.91 ± 0.13	4.3
8-oxoA:C[a]	0.61 ± 0.08	1.6 ± 0.1	2.6
8-oxoA:T[b]	0.50 ± 0.02	0.52 ± 0.03	1.0
8-oxoI:C[a]	0.60 ± 0.13	0.95 ± 0.25	1.6
8-oxoI:T[b]	1.5 ± 0.2	2.0 ± 0.1	1.3
8-oxoN:C[b]	0.51 ± 0.03	0.54 ± 0.04	1.1
8-MeOG:C[b]	0.034 ± 0.008	0.047 ± 0.012	1.4
8-aminoG:C[a]	0.077 ± 0.064	0.93 ± 0.07	12
8-aminoG:A[b]	0.018 ± 0.015	0.016 ± 0.011	0.89

[a] Calculated from the linear parts of the burst-phase time courses.
[b] Calculated from the initial parts of the time courses with no burst phase.

oxoG:C than on 8-oxoG:A substrates may be an additional factor contributing to specificity of OGG1 for the former mispair, which is biologically significant because 8-oxoG:A pairs are pre-mutagenic if repaired by OGG1 [3].

Several modified purine bases (6-OMe-8-oxoG, 8-oxoI, 8-oxoA) are good substrates for OGG1, but only if paired with C [10]. Although of these only 8-oxoA has been shown to appear in cellular DNA as a natural lesion [16], they provide a useful tool to analyze structural elements of the damaged base required for the activity of OGG1. We have inquired whether cleavage of 6-OMe-8-oxoG-, 8-oxoI-, and 8-oxoA-containing substrates followed burst phase kinetics, and whether it could be enhanced by APEX1. When all these bases were paired with C, a burst phase and slow product accumulation was observed (Fig. 2C–E, Table 1). With 6-OMe-8-oxoG:C and 8-oxoA:C substrates,

as with 8-oxoG:C, the burst phase was so quick that only the following steady-state rise could be seen, while with 8-oxoI:C, the onset of the linear phase was slower. Interestingly, while the burst amplitude for 8-oxoA:C and 8-oxoI:C was close to that of 8-oxoG:C, it increased nearly 2-fold (with the same total enzyme concentration) for 6-OMe-8-oxoG:C, and the slope of the linear part decreased, indicating that the product release is even more limiting for this substrate. APEX1 stimulated the cleavage of all these substrates by OGG1. The highest stimulation, in terms of the increase in the turnover rate, was observed for 6-OMe-8-oxoG:C and 8-oxoA:C, the substrates with the most pronounced burst phase, while the initial burst amplitude in these cases did not change (Fig. 2C and D). In contrast, APEX1 had less influence on the turnover rate for 8-oxoI:C but the amplitude of the burst seemed to increase (Fig. 2E).

Mirroring the situation with 8-oxoG paired with either G (good substrate) or A (poor substrate), the bases efficiently excised by OGG1 from mispairs with C can be poorly excised from pairs with other bases. The structure of OGG1 in complex with DNA [17] suggests that its preference for C opposite the lesion is due to a number of specific contacts formed with this base in the undamaged strand, estranged after eversion of the damaged base into the enzyme's active site. To assess the effect of APEX1 on such suboptimal substrates, we have investigated cleavage of 8-oxoA:T (the natural context of 8-oxoA, which appears in DNA by oxidation of A) and 8-oxoI:T by OGG1 in the presence or in the absence of the endonuclease (Fig. 2D and F). The 8-oxoA:T substrate was cleaved by OGG1 alone less efficiently than 8-oxoA:C, with the most pronounced difference in the time courses being the absence of the burst with 8-oxoA:T. Strikingly, APEX1 had no effect whatsoever on the cleavage of 8-oxoA:T (Fig. 2D, squares). The situation with the 8-oxoI:T substrate was very similar to that for 8-oxoG:A (compare Fig. 2A and B and Fig. 2E and F): the burst phase for OGG1 cleaving 8-oxoI:T was absent, and the enzyme was modestly (1.3-fold) stimulated by APEX1.

Next, we have addressed the question whether the presence of APEX1 can improve excision of bases normally excised poorly or not excised by OGG1 even from pairs with C, such as undamaged G, natural lesions I or 8-aminoG, and synthetic purine analogs 8-oxoN and 8-MeOG [10]. Of these G and I were not excised at all neither with nor without APEX1 (not shown). The cleavage of 8-MeOG was barely over the detection limit (Fig. 2H), and, although it was apparently slightly stimulated by APEX1 (Table 1), this stimulation is likely insignificant due to the large error of fitting to a very shallow slope. The cleavage of 8-oxoN:C substrate (Fig. 2G) showed the efficiency similar to that for 8-oxoA:T, demonstrated no burst phase, and was not stimulated by APEX1.

Of poor OGG1 substrates, 8-aminoG presented the most interesting case. This lesion is induced in DNA by nitrogen-containing environmental pollutants [18] and in mammalian cells produces mostly G→T transversions, suggestive of dAMP misincorporation opposite 8-aminoG during replication [19]. The mechanism of repair of 8-aminoG is not known; this damaged base is excised by OGG1 much less efficiently than 8-oxoG [10]. The time course of 8-aminoG:C cleavage by OGG1 revealed a slow increase in the product concentration at the beginning but then the product accumulation ceased (Fig. 2I, black circles). When APEX1 was added to the reaction, the product accumulation time course became linear, and, although the overall efficiency of cleavage was still worse than for regular OGG1 substrates, it significantly improved compared with OGG1 acting alone (Fig. 2I, white circles). In contrast, OGG1 possessed an extremely low activity towards 8-aminoG:A substrates either in the presence or in the absence of APEX1 (Fig. 2I, squares).

The comparison of OGG1 activity on all tested substrates reveals that they fall in two groups, those with and without a pronounced burst phase. The former group includes the mispairs usually regarded as good substrates for OGG1, and the latter, as poor substrates [4,10,20]. Our observations suggest that the substrate preference of OGG1, acting alone, depends on the chemical step of the reaction rather than on the overall steady-state turnover rate, which may be dominated by different reaction stages for different substrates. Moreover, the stimulation of good OGG1 substrates by APEX1 is more pronounced than stimulation of poor substrates, and in most cases is caused by an increase in the turnover rate, suggesting a relief of product inhibition. Two models of OGG1 stimulation by APEX1 have been suggested, the product sequestering model, in which APEX1 binds or cleaves the AP product released by OGG1 and stimulates the glycosylase simply by preventing it from re-binding the product, and the direct displacement model, with APEX1 actively disengaging OGG1 from the complex with the product (see [9] and the discussion therein). Our observation that processing of some substrates by OGG1 is not stimulated (8-oxoA:T, 8-oxoN:C) or poorly stimulated (8-oxoG:A, 8-oxoI:T) by APEX1 further supports the direct displacement model, because the rival mechanism, AP product sequestering, would not depend on the nature of the initial substrate as soon as the base have been excised, and therefore should stimulate OGG1 on all substrates.

The stimulation of OGG1 by APEX1 on 8-aminoG:C substrate suggests that OGG1 could be involved in repair of this lesion in the context of the complete BER system. However, 8-aminoG:A mispair, arising by misincorporation of dAMP during replication, was not substrate for OGG1. In this respect, 8-aminoG is possibly subject to the same type of repair by a two-glycosylase system (OGG1 and MUTYH in human cells, Fpg and MutY in bacteria [3]) as 8-oxoG, with inefficient excision of a damaged purine from a mispair with A. If 8-aminoG is excised from the 8-aminoG:A mispair, a G→T transversion would result; therefore, such a substrate may be subject to removal of A by the MUTYH glycosylase.

In conclusion, our data suggest that stimulation of OGG1 by APEX1 proceeds by direct displacement and could be an important factor improving the specificity of this DNA glycosylase for its biologically relevant substrates.

Acknowledgments

We thank Dr. Francis Johnson (SUNY at Stony Brook) for the gift of modified oligonucleotides. This research was supported by Russian Academy of Sciences (Programs 10.5 and 12.2), an Integrative Grant from the Siberian Branch of the Russian Academy of Sciences (Program 6), Russian Foundation for Basic Research (07-04-00395, 08-04-00596), and Russian Ministry of Science and Education (02.512.11.2194). The authors are grateful to Dr. Arthur Grollman (SUNY at Stony Brook) in whose laboratory this work was partly done.

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