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Mechanism of interaction between human 8-oxoguanine-DNA glycosylase and AP endonuclease

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ARTICLE INFO

Article history:
Received 4 August 2006
Received in revised form
20 October 2006
Accepted 24 October 2006
Published on line 27 November 2006

Keywords:
DNA repair
Oxidative damage
8-Oxoguanine-DNA glycosylase
AP endonuclease
Protein–protein interactions

ABSTRACT

Human 8-oxoguanine-DNA glycosylase (OGG1) is the main human base excision protein that removes a mutagenic lesion 8-oxoguanine (8-oxoG) from DNA. Since OGG1 has DNA glycosylase and weak abasic site (AP) lyase activities and is characterized by slow product release, turnover of the enzyme acting alone is low. Recently it was shown that human AP endonuclease (APE1) enhances the activity of OGG1. This enhancement was proposed to be passive, resulting from APE1 binding to or cleavage of AP sites after OGG1 dissociation. Here we present evidence that APE1 could actively displace OGG1 from its product, directly increasing the turnover of OGG1. We have observed that APE1 forms an electrophoretically detectable complex with OGG1 cross-linked to DNA by sodium borohydride. Using oligonucleotide substrates with a single 8-oxoG residue located in their 5′-terminal, central or 3′-terminal part, we have demonstrated that OGG1 activity does not increase only for the first of these three substrates, indicating that APE1 interacts with the DNA stretch 5′ to the bound OGG1 molecule. In kinetic experiments, APE1 enhanced the product release constant but not the rate constant of base excision by OGG1. Moreover, OGG1 bound to a tetrahydrofuran analog of an abasic site stimulated the activity of APE1 on this substrate. Using a concatemeric DNA substrate, we have shown that APE1 likely displaces OGG1 in a processive mode, with OGG1 remaining on DNA but sliding away in search for a new lesion. Altogether, our data support a model in which APE1 specifically recognizes an OGG1/DNA complex, distorts a stretch of DNA 5′ to the OGG1 molecule, and actively displaces the glycosylase from the lesion.

1. Introduction

8-Oxo-7,8-dihydroguanine (8-oxoG) is one of the most abundant base lesions generated when DNA is attacked by reactive oxygen species, which are produced by cellular oxidative metabolism or by certain environmental challenges such as ionizing radiation [1–4]. 8-OxoG can easily adopt *syn* conformation and pair with A [5,6], and therefore is miscoding [7] and mutagenic [8,9], promoting G → T transversions due to misincorporation of adenine during replication. This lesion has been

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Abbreviations: AP, apurinic/apyrimidinic; BER, base excision repair; BSA, bovine serum albumin; DTT, dithiothreitol; EMSA, electrophoretic mobility shift assay; ODN, oligodeoxyribonucleotide; PAGE, polyacrylamide gel electrophoresis; ss, single-stranded; ds, double-stranded

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doi:10.1016/j.dnarep.2006.10.022

associated with human cancers [10–12]; in culture, transfection of human cells with c-Ha-*ras* sequence containing a single specifically placed 8-oxoG residue significantly increases the number of transformed foci [13].

In eukaryotes, 8-oxoG and the related redox lesions, formamidopyrimidines, which are also mutagenic [14,15], are repaired by base excision repair (BER) system [16]. The central element of this system is 8-oxoguanine-DNA glycosylase, OGG1 [17–19], an enzyme that recognizes 8-oxoG paired with C and excises the 8-oxoG base, forming an abasic (AP) site. The following steps are common for all kinds of damage removed by BER: DNA is cleaved 5′ to the nascent AP site by AP endonuclease (APE1 in mammals), the resulting hanging deoxyribose-5′-phosphate is excised by the deoxyribose phosphate lyase function of DNA polymerase β, which then fills the gap, and DNA ligase seals the nick afterwards [16]. Protein–protein interactions detected between several major and accessory BER proteins [20] suggest that these steps are not completely independent but may involve some kind of substrate channeling [21].

OGG1 possesses two enzymatic activities: DNA glycosylase and AP lyase, the latter catalyzing β-elimination at the nascent AP site with formation of a nick in DNA flanked by a 3′-terminal 4-hydroxy-2-pentenal moiety and a 5′-terminal phosphate [19,22,23]. However, the AP lyase activity of OGG1 is much weaker than its glycosylase activity [23]. After removal of the base, OGG1 remains associated with an intact AP site for minutes [23], perhaps due to persistence of a covalent Schiff base intermediate formed during catalysis between C1′ of the damaged nucleotide and the ε-amino group of the enzyme's active site (Lys-249 in human OGG1) [24]. It has been shown that human APE1 stimulates the activity of OGG1 [25–27] as well as several other human or murine DNA glycosylases [28–32]. The mechanism of such stimulation, however, remains controversial (discussed in [20]). For OGG1/APE1, in particular, it was argued that APE1 simply cleaves or otherwise sequesters AP sites or products of β-elimination released by OGG1 and prevents OGG1 re-association with them (*product sequestering* mechanism [25]). This conclusion was corroborated by the observations that (i) OGG1 is also stimulated by *E. coli* endonuclease IV (Nfo), which is not related to APE1 neither in sequence nor in the three-dimensional structure [33], and (ii) no stable ternary complex between OGG1, APE1 and DNA has been detected. Alternatively, APE1 could directly displace OGG1 from the nascent AP site in a manner similar to the "hand-off" model first proposed by Tainer and colleagues for human uracil-DNA glycosylase and APE1 [21].

To clarify the mechanism of stimulation of OGG1 by APE1 here we analyze this process using a series of oligonucleotide substrates with lesions placed in different positions. A clear position effect on the stimulation is observed. Rate constants for individual steps of OGG1-catalyzed reaction in the absence and in the presence of APE1 are determined. We have also used sodium borohydride-stabilized covalent complex between OGG1 and DNA to analyze its binding to APE1 into a ternary complex. In addition, we show that OGG1 stimulates the activity of APE1 on a tetrahydrofuran analog of AP site (F). Altogether, our results suggest that rather than being a passive "product scavenger", APE1 may act in an *active displacement* mode, dislodging OGG1 from the nascent AP site and thus enhancing the activity of OGG1 by increasing its turnover.

2. Materials and methods

2.1. Proteins

His_6-tagged OGG1 was purified as described [34]. *E. coli* Nfo protein was kindly provided by Dr. A.A. Ischenko; bacteriophage T4 endonuclease V (DenV) was a gift from R. Stephen Lloyd. *E. coli* uracil-DNA glycosylase (Ung) and bacteriophage T4 polynucleotide kinase were purchased from New England Biolabs (Beverly, MA), and lysozyme, from Reachim (Moscow, Russia). Wild-type recombinant APE1 was expressed in a pXC53 plasmid (courtesy of Dr. P.R. Strauss) and purified in a procedure adopted from [35]. To purify recombinant His_6-tagged APE1, *E. coli* BL21(DE3)RIL cells transfected with a pET-28b(+) plasmid carrying a APE1 insert (a kind gift from Dr. B. Demple) was grown to $A_{600} = 1.0$ at 37 °C with vigorous shaking in 2× YT supplemented with 50 μg/ml kanamycin and 25 μg/ml chloramphenicol. Fifty milliliters of this culture were used to inoculate 1 l of the same medium, and the cells were allowed to reach $A_{600} = 1.0$, then induced by adding isopropyl-β-D-1-thiogalactopyranoside to 1 mM and grown for 6 h. The cells were harvested by centrifugation (2.3 g wet weight) and resuspended in 30 ml of lysis buffer (100 mM Tris–HCl pH 7.5, 1 mM EDTA) supplemented with 1 mM phenylmethanesulfonyl fluoride. The cells were lysed by 30 min treatment with 0.5 mg/ml lysozyme at room temperature followed by 30 min after addition of NaCl to 1 M. The lysis was then completed by sonicating the suspension on ice using an UZDN-2T Ultrasound Dispergator (SELMI, Ukraine), with 10 pulses of 30 s at the highest power setting, with 90 s interval between pulses. The lysate was clarified by centrifugation (12,000 × *g*, 4 °C, 30 min) and the proteins were precipitated by $(NH_4)_2SO_4$ at 80% saturation. The precipitate was collected by centrifugation, dissolved in Buffer A (25 mM potassium phosphate pH 7.4) supplemented with 1 M NaCl and diluted with 20 volumes of Buffer A. The solution was applied to a 5 ml HiTrap SP HP column (Pharmacia) equilibrated with Buffer A plus 50 mM NaCl. The column was washed with the same buffer and then with Buffer A plus 800 mM NaCl. The eluate was loaded to a 5 ml HiTrap Chelating column (Pharmacia) charged with Ni^{2+}, and the column was washed with Buffer A containing 500 mM NaCl and 50 mM imidazole. His_6-tagged polypeptides were eluted in Buffer A with 500 mM NaCl and 500 mM imidazole. The eluate was diluted 20-fold with Buffer A and applied to a 5 ml HiTrap heparin column (Pharmacia) equilibrated in Buffer A with 50 mM NaCl. The column was developed with a gradient of 50–800 mM NaCl in Buffer A; the fractions absorbing at 280 nm were collected, supplemented with DTT to 1 mM, and analyzed by 12% discontinuous gel electrophoresis (Laemmli system). Fractions of at least 90% purity were pooled and dialyzed for 20 h against two changes of storage buffer (50 mM Tris–HCl pH 7.5, 400 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 50% (v/v) glycerol). The protein was stored at −20 °C. Concentration of APE1 was determined spectrophotometrically using an extinction coefficient calcu-