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Slow base excision by human alkyladenine DNA glycosylase limits the rate of formation of AP sites and AP endonuclease 1 does not stimulate base excision

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8-oxoG, 7,8-dihydro-8-oxo-2′deoxyguanosine; AAG, alkyladenine DNA glycosylase; AP, apurinic/apyrimidinic site; APE1, apurinic/apyrimidinic endonuclease 1; BER, base excision repair; εA, 1,N^6-ethenoadenine; ENDOIV, endonuclease IV; Hx, hypoxanthine; MYH, MutY homolog; TDG, thymine DNA glycosylase; MED1, methyl-CpG-binding endonuclease 1 a.k.a.; MBD4, methyl-CpG-binding domain 4; OGG1, 8-oxoguanine DNA glycosylase 1; UDG, uracil DNA glycosylase

ABSTRACT

The base excision repair pathway removes damaged DNA bases and resynthesizes DNA to replace the damage. Human alkyladenine DNA glycosylase (AAG) is one of several damage-specific DNA glycosylases that recognizes and excises damaged DNA bases. AAG removes primarily damaged adenine residues. Human AP endonuclease 1 (APE1) recognizes AP sites produced by DNA glycosylases and incises the phophodiester bond 5′ to the damaged site. The repair process is completed by a DNA polymerase and DNA ligase. If not tightly coordinated, base excision repair could generate intermediates that are more deleterious to the cell than the initial DNA damage. The kinetics of AAG-catalyzed excision of two damaged bases, hypoxanthine and 1,N^6-ethenoadenine, were measured in the presence and absence of APE1 to investigate the mechanism by which the base excision activity of AAG is coordinated with the AP incision activity of APE1. 1,N^6-ethenoadenine is excised significantly slower than hypoxanthine and the rate of excision is not affected by APE1. The excision of hypoxanthine is inhibited to a small degree by accumulated product, and APE1 stimulates multiple turnovers by alleviating product inhibition. These results show that APE1 does not significantly affect the kinetics of base excision by AAG. It is likely that slow excision by AAG limits the rate of AP site formation *in vivo* such that AP sites are not created faster than can be processed by APE1.

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1. Introduction

The bases of DNA can undergo chemical modification from both endogenous and exogenous sources (reviewed in [1,2]). Biological organisms have evolved many repair mechanisms to ensure the stability of DNA and the accurate transmission of genetic information. The base excision repair pathway (BER) serves to repair the genome via the removal and replacement of damaged bases (reviewed in [3,4]). DNA glycosylases initiate BER by recognizing and excising damaged bases through cleavage of the C1′–N glycosidic bond to leave an abasic (AP) site. Some DNA glycosylases, termed bifunctional, also posses AP-lyase activity and incise the deoxyribophosphate backbone on the 3′ side to leave a single-stranded break. DNA glycosylases are damage-specific in that each recognizes a particular damaged base, for example uracil, or class of damaged bases, for example alkylated purines. The products made by these DNA glycosylases are substrates for the next enzyme in the pathway, an AP endonuclease. AP endonucleases incise the sugar-phosphate backbone 5′ to AP sites to create a 3′ hydroxyl on one side of the nick and 5′ deoxyribose phosphate residue on the other side. AP endonucleases also remove sugar residues left by the action of bifunctional DNA glycosylases. Minimally, a DNA polymerase and DNA ligase are needed to complete the repair process.

Human alkyladenine DNA glycosylase (AAG [5,6], a.k.a. *N*-methylpurine DNA glycosylase (MPG, [7]) and alkyl-*N*-purine DNA glycosylase (ANPG, [8])) removes damaged purines from DNA by flipping the base 180° out of the double helix and catalyzing the hydrolysis of the C1′–N glycosidic bond to leave an abasic site. Human AAG excises a notably diverse series of damaged bases most of which are modified adenines. Studies conducted with mouse AAG knockout cells indicate that AAG is the major glycosylase responsible for the removal of 3-methyladenine (3MeA), hypoxanthine (Hx), and ethenoadenine (εA) [9]. *In vitro*, all three of theses bases are excised efficiently [5,7,8,10–14] as well as 7-methylguanine [5,7,8,10] and to a much lesser extent undamaged guanine and adenine [15]. The substrate specificity of AAG reflects an interplay between binding affinity for damaged bases, the lability of the C1′–N glycosidic bond, and the ease with which damaged bases are flipped from the DNA helix [8,15–21].

Human apurinic/apyrimidinic endonuclease1 (APE1) (also called redox effector factor 1 (Ref1)) is the major AP endonuclease in humans and is homologous to the *Escherichia coli* AP endonuclease, ExoIII [22–24]. The activities of APE1 are essential for repairing baseless sugar residues generated by DNA glycosylases, and APE1 knockout mice are inviable [25]. APE1 cleaves the sugar-phosphate backbone in DNA 5′ to AP sites leaving a 3′OH as a substrate for a polymerase [22,23,26,27]. In addition, APE1 possesses 3′ phosphodiesterase and 3′ phosphatase activities to repair 3′ ends created by bifunctional DNA glycosylases or ionizing radiation [26,28,29].

Intermediates formed during base excision repair can be more deleterious to the cell than the initial damage if repair is not completed. In many cases, damaged DNA bases are less toxic, but lead to mutagenesis during DNA replication. For example, uracil arising from deaminated C promotes G→A transitions, hypoxanthine T→C transitions, and 8-oxoguanine G→T transversions. On the other hand, unrepaired AP sites are cytotoxic lesions that block DNA replication. In addition, single-stranded breaks can form at AP sites that lead to double-stranded breaks during replication. The harmful effects of these BER intermediates has led to the proposal that the pathway must be coordinated to ensure that once started, the repair process is completed [30–32]. There are a dozen or so DNA glycosylases that initiate repair of different damaged bases to produce the same AP products, which are then repaired by a common set of enzymes beginning with the activity of APE1. A key question is how do these different DNA glycosylases interact with the same enzyme, APE1, to coordinate the initial stages of BER. This question has been addressed for several DNA glycosylases [33–40].

In this paper, we examine the coordination of the initial stages of the BER through kinetic analysis of the effects of APE1 on base excision by AAG. Excision of two different damaged bases, εA and Hx, was measured to determine if APE1 affected both in the same manner. In addition, differences in the activity of AAG on εA- and Hx-DNA are experimentally advantageous in defining the effects of APE1 on different kinetics steps of the excision reaction. Our results demonstrate that a slow step leading to base excision limits the rate at which AAG produces AP sites, and addition of APE1 does not affect this rate. An earlier report suggests that AAG-catalyzed excision of Hx is stimulated significantly in the presence of APE1 [41], however, our further experiments indicate that this likely reflects instability of AAG *in vitro* rather than a mechanism for coordinating AAG and APE1 activities. These results suggest that coordination of AAG and APE1 activities relies on slow excision by AAG such that AP sites are not created at a rate greater than can be processed by APE1.

2. Materials and methods

2.1. DNA substrates

Oligonucleotides (25 mers) were synthesized using standard phosphoramidite chemistry as in [17]. The sequence was: 5′GCGTCAAAATGTDGGTATTTCCATG3′ in which the nucleotide (*D*) at position 13 was hypoxanthine (Hx), etheonadenine (εA), reduced AP site (AP), or adenine (A). Oligonucleotides were 5′-end labeled with ^{32}P through incubation with T4 polynucleotide kinase (Invitrogen, Carlsbad, CA) and ATPγ ^{32}P (Amersham Biosciences, Piscataway, NJ) for 1 h at 37 °C. The kinase was heat inactivated at 95 °C for 10 min. Labeled oligonucleotides were annealed to complementary oligonucleotides by adding a two-fold excess of complement, heating to 80 °C, and slowly cooling to room temperature.

2.2. Cloning and expression of human AP endonuclease 1

The coding sequence for APE1 was PCR-amplified from a normal human liver cDNA library (Invitrogen, Carlsbad, CA) using primers (below) specific for the 5′ and 3′ ends of the coding sequence. The PCR primers contained additional sequence