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Human endonuclease V as a repair enzyme for DNA deamination

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ABSTRACT

The human endonuclease V gene is located in chromosome 17q25.3 and encodes a 282 amino acid protein that shares about 30% sequence identity with bacterial endonuclease V. This study reports biochemical properties of human endonuclease V with respect to repair of deaminated base lesions. Using soluble proteins fused to thioredoxin at the N-terminus, we determined repair activities of human endonuclease V on deoxyinosine (I)-, deoxyxanthosine (X)-, deoxyoxanosine (O)- and deoxyuridine (U)-containing DNA. Human endonuclease V is most active with deoxyinosine-containing DNA but with minor activity on deoxyxanthosine-containing DNA. Endonuclease activities on deoxyuridine and deoxyoxanosine were not detected. The endonuclease activity on deoxyinosine-containing DNA follows the order of single-stranded I > G/I > T/I > A/I > C/I. The preference of the catalytic activity correlates with the binding affinity of these deoxyinosine-containing DNAs. Mg^{2+} and to a much less extent, Mn^{2+}, Ni^{2+}, Co^{2+} can support the endonuclease activity. Introduction of human endonuclease V into *Escherichia coli* cells deficient in *nfi*, *mug* and *ung* genes caused three-fold reduction in mutation frequency. This is the first report of deaminated base repair activity for human endonuclease V. The relationship between the endonuclease activity and deaminated deoxyadenosine (deoxyinosine) repair is discussed.

1. Introduction

Endonuclease V (endo V encoded by *nfi* gene) was initially discovered in *Escherichia coli* (*E. coli*) as a nuclease that acted on a variety of DNA damage [1,2]. In the course of identifying hypoxanthine DNA repair activity, *E. coli* endo V was rediscovered as a deoxyinosine 3′ endonuclease [3,4]. Endo V in general hydrolyzes the second phosphodiester bond 3′ to the aberrant site [3,5–11]. Inosine (known as hypoxanthine as a base), xanthosine and oxanosine, and uridine are deamination products derived from adenosine, guanosine, and cytidine [12–15]. In addition to endonuclease activity on DNA containing deoxyinosine [5,7,9,16], endo V was also found to be active on deoxyxanthosine [5,17–19], deoxyuridine [2,4,5,7,20], and deoxyoxanosine [5,6,17]. Genetic analysis indicates that bacterial endo V is involved in repair of deoxyinosine, deoxyxanthosine, and N^6-hydroxylaminopurine [21–23]. Homologs from endo V family proteins are diverse in substrate specificity. While bacterial endo V enzymes exhibit broad endonuclease activity toward different deaminated bases, endo V proteins from the archaeal species *Archaeoglobus fulgidus* and mouse seem to be active only on deoxyinosine-containing DNA [8,9]. Endo V from *Salmonella typhimurium* appears to possess high affinity to deaminated bases as it is the only endo V enzyme tested that shows detectable binding to deoxyoxanosine-containing DNA [5,6]. Interestingly, bacterial endo V enzymes also demonstrate endonuclease activity on mismatches [5,7,10,24], small insertions/deletions (indels) [11], flap and pseudo-Y structures [11]. The mismatch and indel cleavage activity has been exploited for development of mutation identification or scanning methods [25–28].

The structure-function relationship has been extensively studied using endo V from the thermophilic bacterium *Thermotoga maritima* (Tma) as a model system. Sequence alignment has identified seven conserved motifs in endo V family proteins (Fig. 1A). D43 in motif II, E89 in motif III, and D110 in motif IV are identified as catalytic residues involved in coordination of a metal ion [17,29]. Through a large-scale site-directed mutagenesis analysis, Y80, G83, L85 in motif III, G113, H116, R118 and G121 in motif IV, G136 and A138 in motif V, and S182 in motif VI were identified as residues that affect protein-DNA interactions [17]. The importance of Y80 in base recognition has been demonstrated by switching of base preference in mismatch cleavage by Y80A mutant [24]. The recently solved crystal structures of Tma endo V reveal that Y80 is situated in the PYIP wedge that occupies the space vacated by the flipped out hypoxanthine base [30].

Endo V family proteins are ubiquitous in bacteria, archaea, and eukaryotes (Fig. 1A). The human genome contains an endo V homolog located in chromosome 17q25.3. The biochemical

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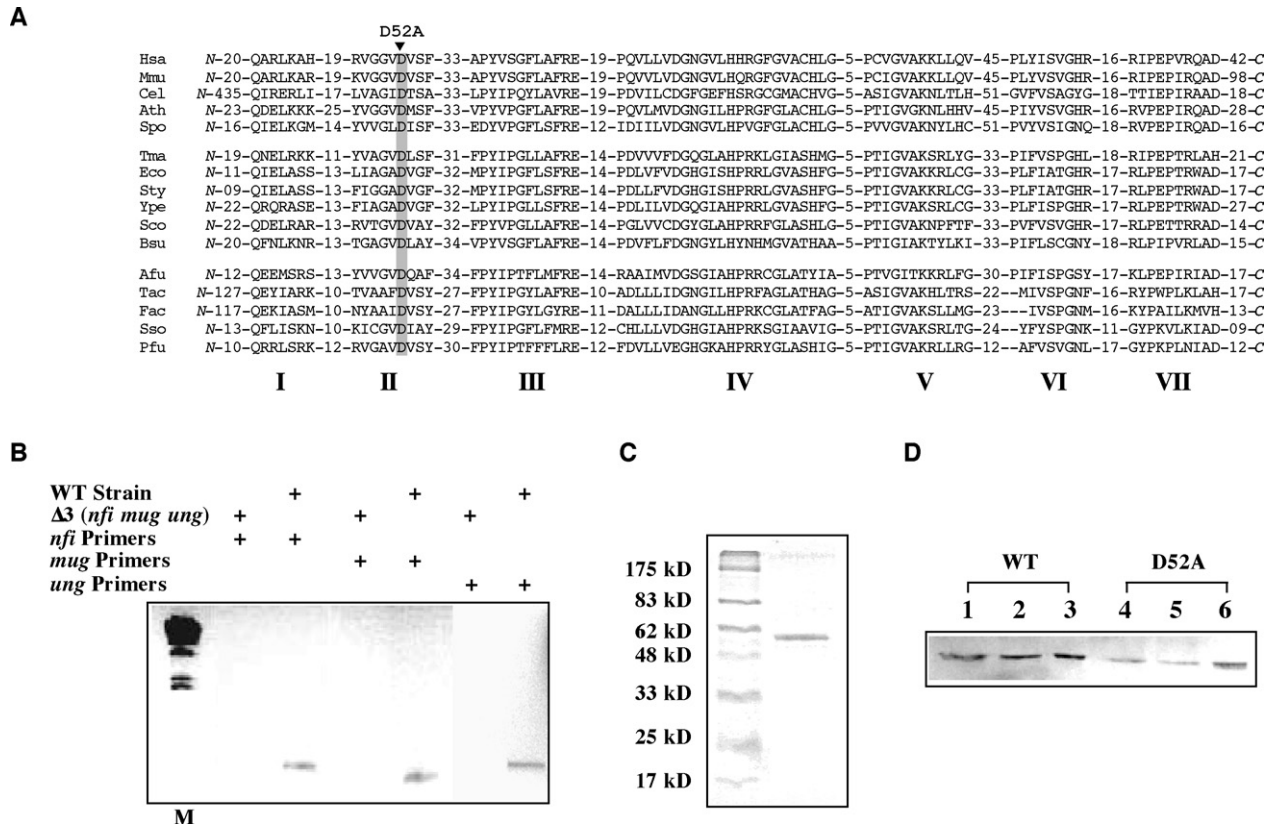

Fig. 1. Expression of human endonuclease V. A. Sequence alignment of endonuclease V. The amino acid residue selected for site-directed mutagenesis is highlighted and the resulting mutant is indicated above the arrows. Genbank accession numbers are shown after the species names. Hsa: *Homo sapiens*, BAC04765; Mmu: *Mus musculus*, XP_203558; Cel: *Caenorhabditis elegans*, 1731299; Ath: *Arabidopsis thaliana*, T10669; Spo: *Schizosaccharomyces pombe*, 1723511; Tma: *Thermotoga maritima*, NP_229661; Eco: *Escherichia coli*, NP_418426; Sty: *Salmonella typhimurium*, NP_463037; Ype: *Yersinia pestis*, NP_667835; Sco: *Streptomyces coelicolor*, CAB40676; Bsu: *Bacillus subtilis*, BSUB0019; Afu: *Archaeoglobus fulgidus*, NP_068968; Tac: *Thermoplasma acidophilum*, CAC11602; Fac: *Ferroplasma acidarmanus*, ZP_00001774; Sso: *Sulfolobus solfataricus*, NP_343804; Pfu: *Pyrococcus furiosus*, NP_578716. B. Confirmation of the genotype of BL21(DE3)Δ3 by PCR. WT strain: *E. coli* K-12 strain. C. SDS-PAGE analysis of the wt human endo V. Purified protein (~1 μg) was electrophoresed on a 15% polyacrylamide gel containing 0.1% SDS. Protein bands were visualized by Coomassie staining. The molecular weight of the fused human endo V protein is 50.2 kD. D. Western blot analysis of the wt human endo V and mutant D52A proteins. Lanes 1 and 4: 10 μl whole cell extract. Lanes 2 and 5: Supernatant collected after sonication (equivalent to 30 μl cell culture). Lanes 3 and 6: purified protein (~0.5 μg).

and enzymatic properties of human endo V are not known. In this study, we report that human endo V is a deoxyinosine and deoxyxanthosine endonuclease. The single-stranded deoxyinosine endonuclease activity is 14-fold stronger than the single-stranded deoxyxanthosine endonuclease activity. Human endo V is active with Mg^{2+}, and to a much less extent with Mn^{2+}, Ni^{2+} and Co^{2+} as a metal cofactor. The mutation frequency in *E. coli* is suppressed by the presence of the wt human endo V, but not the active site D52A mutant. The biochemical and genetic analysis indicates that human endo V possesses deoxyinosine endonuclease activity that plays an important role for repair of deaminated purine damage *in vivo*.

2. Materials and methods

2.1. Reagents, media and strains

All routine chemical reagents were purchased from Sigma Chemicals (St. Louis, MO), Fisher Scientific (Suwanee, GA), or VWR (Suwanee, GA). Restriction enzymes, *Taq* DNA polymerase, Phusion hifidelity polymerase and T4 DNA ligase were purchased from New England Biolabs (Beverly, MA). BSA and dNTPs were purchased from Promega (Madison, WI). Anti-His (N-term) antibody and anti-rabbit IgG HRP-linked antibody were purchased from Cell Signaling Technology (Danvers, MA). The horseradish peroxidase substrate Opti-4CN and PVDF membrane for Western blot analysis were purchased from Bio-Rad (Hercules, CA). HiTrap chelating and Q columns were purchased from GE Healthcare (Piscataway, NJ). Oligodeoxyribonucleotides were ordered from Integrated DNA Technologies Inc. (Coralville, IA). LB medium was prepared according to standard recipes. Human endo V sonication buffer consisted of 50 mM Tris–HCl (pH 7.4), 1 mM EDTA (pH 8.0), 2.5 mM DTT, 0.15 mM PMSF, 10% glycerol and 50 mM NaCl. GeneScan stop buffer consisted of 80% formamide (Amresco, Solon, OH), 50 mM EDTA (pH 8.0), and 1% blue dextran (Sigma Chemicals). TB buffer (1×) consisted of 89 mM Tris base and 89 mM boric acid. TE buffer consisted of 10 mM Tris–HCl (pH 8.0), and 1 mM EDTA. *E. coli* host strain BL21(DE3)Δ3 [F^-, *ompT*, $hsdS_B$, (r_B^- m_B^-), *gal*, *dcm*, *sly*, (DE3), *nfi*, *ung*, *mug*] and JM109 [e14$^-$(McrA$^-$) *endA1*, *recA1*, *gyrA96*, *thi-1*, *hsdR17* (r_k^-, m_k^+), *supE44*, *relA1* Δ(*lac-proAB*), [F^-, *traD36*, *proAB*, *lacI*^{q}ZΔ*M15*]] are from our laboratory collection. Plasmid pET28a-hnfi was constructed by PCR amplification of human cDNA (Fig. S1). *E. coli* wild type K-12 strain was obtained from *E. coli* Genetic Stock Center at Yale University (New Haven, CT).

2.2. Construction of E. coli BL21(DE3)Δ3 and confirmation of the genotype

The strain *E. coli* BL21 (DE3) *mug*$^-$ *ung*$^-$ *nfi*$^-$ was constructed *via* transduction using the bacteriophage P1 *vir*. The individual mutant genes were obtained from the Keio Collection in the form of gene knockouts carrying a kanamycin gene cartridge [31]. A mutant gene was transduced into *E. coli* BL21(DE3) using a selection for kanamycin resistance. The resistance cartridge was then flipped out using flp sequences surrounding the kanamycin cartridge and flp recombinase supplied from the plasmid pCP20 [32]. Three rounds of transduction followed by kanamycin gene cartridge removal yielded the final strain.

The *E. coli* BL21(DE3)Δ3 strain was confirmed by PCR with wild type *E. coli* K-12 stain as positive control using the following primers: Ec.NFI.F, 5′- TAA AGT ACC CCA TGG GTG ATT ATG GAT CTC GCG TC-3′ (the *Nco*I site is underlined); Ec.NFI.R, 5′-TAA AGG GTG GAT CCT AGG GCT GAT TTG CTG T-3′ (the *Bam*HI site is underlined); Ec.MUG.F, 5′-TGG GGT ACC CCA TGG GTT GAG GAT ATT TTG GCT CCA GGG-3′ (the *Nco*I site is underlined); Ec.MUG.R, 5′-CCC GGA TCC TTA TCG CCC ACG CAC TAC CAG CGC CTG GTC-3′ (the *Bam*HI site is underlined); Ec.UNG.F, 5′-GGG AAT TCC ATA TGG CTA ACG AAT TAA CCT GGC ATG AC-3′ (the *Nde*I site is underlined); Ec.UNG.R 5′-CCC AAG CTT CTC ACT CTC TGC CGG TAA TAC TGG-3′ (the *Hind*III site is underlined). The PCR mixtures (50 μl) contained 40 ng of genomic DNA as template, 200 nM each primer pair, 50 μM each dNTP, 1× *Taq* DNA polymerase buffer, and 1 unit of *Taq* DNA polymerase. The PCR procedure was composed of a predenaturation step at 95 °C for 2 min, 30 cycles with each cycle consisting of denaturation at 94 °C for 15 s,