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EXPERIMENTAL ARTICLES

Functional Changes in a Novel Uracil-DNA Glycosylase Determined by Mutational Analyses[1]

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Abstract—Uracil-DNA glycosylase (UDG) is a ubiquitous enzyme found in bacteria and eukaryotes, which removes uracil residues from DNA strands. *Methanococcus jannaschii* UDG (*Mj*UDG), a novel monofunctional glycosylase, contains a helix–hairpin–helix (HhH) motif and a Gly/Pro rich loop (GPD region), which is important for catalytic activity; it shares these features with other glycosylases, such as endonuclease III. First, to examine the role of two conserved amino acid residues (Asp150 and Tyr152) in the HhH-GPD region of *Mj*UDG, mutant *Mj*UDG proteins were constructed, in which Asp150 was replaced with either Glu or Trp (D150E and D150W), and Tyr152 was replaced with either Glu or Asn (Y152E and Y152N). Mutant D150W completely lacked DNA glycosylase activity, whereas D150E displayed reduced activity of about 70% of the wild type value. However, the mutants Y152E and Y152N retained unchanged levels of UDG activity. We also replaced Glu132 in the HhH motif with a lysine residue equivalent to Lys120 in endonuclease III. This mutation converted the enzyme into a bifunctional glycosylase/AP lyase capable of both removing uracil at a glycosylic bond and cleaving the phosphodiester backbone at an AP site. Mutant E132K catalyzes a β-elimination reaction at the AP site via uracil excision and forms a Schiff base intermediate in the form of a protein-DNA complex.

Key words: DNA glycosylase, UDG, DNA repair, mutagenesis.

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DNA glycosylases catalyze the major repair process: the base excision repair (BER) pathway, which exists in almost all living organisms and is initiated by removal of the damaged base. There are two families of DNA glycosylases, monofunctional (only glycosylase activity) and bifunctional (glycosylase/AP lyase activity) [1]. Uracil-DNA glycosylases (UDGs) recognize uracils in DNA and catalyze their removal by cleaving the N-glycoside bond between the base and the DNA backbone to generate an apyrimidinic DNA [1]. UDGs are monofunctional DNA glycosylases. Several families of UDGs have been recently described based on the differences in their amino acid sequences [2, 3].

We have characterized a UDG (*Mj*UDG) of a novel family from *Methanococcus jannaschii* [3]. Although *Mj*UDG shows a low degree of similarity in the primary amino acid sequence with such DNA glycosylases as endonuclease III (EndoIII) or the 8-oxoguanine DNA glycosylase (Ogg) family, interestingly, *Mj*UDG contains a similar helix–hairpin–helix (HhH) motif and a Gly/Pro rich loop (GPD region). This HhH superfamily of BER glycosylases includes a diverse assortment of proteins capable of specifically recognizing and excising the damaged bases and mismatched base pairs [1, 4]. However, unlike EndoIII and Oggs, *Mj*UDG is a monofunctional DNA glycosylase. Several studies have led to elucidation of the catalytic mechanism for bifunctional DNA glycosylases/AP lyases such as EndoIII and Ogg [5, 6]. These enzymes act through a nucleophilic attack on the sugar of the damaged base using an imino group as the attacking nucleophile. A lysine is a crucial residue for the catalytic activities of an AP lyase [7]. Amino acid sequence alignment of Endo III with *Mj*UDG revealed that the lysine residue involved in AP lyase activity of EndoIII was replaced by a Glu (E132) residue in *Mj*UDG [3]. The HhH-GPD motif that is likely to be implicated in the catalytic activity has also been identified in several other DNA glycosylases [1]. *Mj*UDG shares an Asp (D150) in the HhH-GPD region with EndoIII and the MIG family [3]. However, a unique conserved amino acid residue (Y152) differs from Thr and Asn of EndoIII and the MIG family, respectively. In this study we have attempted to investigate the role of D150 and Y152 for glycosylase activity using site-directed *Mj*UDG mutant proteins. We have also examined the AP lyase activity and catalytic mechanism of an E132K mutant of *Mj*UDG.

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MATERIALS AND METHODS

Materials. *Escherichia coli* BL21 (DE3) and plasmid vector pET28a were purchased from Novagen (United States). Restriction enzymes were purchased from New England Biolabs (United States). *Taq* DNA polymerase and T4 polynucleotide kinase were obtained from Takara (Japan) and *E. coli* Fpg was obtained from Trevigen (United States). The Fast Protein Liquid Chromatographu (FPLC) system, columns, and the [γ-^{32}P]ATP were obtained from Amersham Biosciences (Sweden).

Construction of *Mj*UDG mutants. To construct *Mj*UDG mutants (D150E, D150W, Y152E, Y152N, E132K, and E132S), we performed the first PCR using pET28a-*Mj*UDG as a template with the oligomer sets listed below. The forward primer (5'-CGTTCACATATGAAAGAGAACAAA-3') of *Mj*UDG ORF and one of the reverse primers for mutant proteins, D150E–1 (5'-GGTATAGGCCTCAACAACAAAGC-3'), D150W–1 (5'-GGTATAGGCCCAAACAACAAAGC-3'), Y152E–1 (5'-CTTTTGGTCTCGGCATCAACAAC-3'), Y152N–1 (5'-CTTTTGGTATTGGCATCAACAAC-3'), E132K–1 (5'-ATCAGCTGTTTTCTTTCCCACTCC-3'), and E132S–1 (5'-ATCAGCTGTAGACTTTCCCACTCC-3') were used to obtain a PCR product (P1). The reverse primer (5'-CATGTCAAGCTTTTACTTTGAGAGCAGAA-3') of *Mj*UDG ORF and one of the forward primers for mutant proteins, D150E–2 (5'-GCTTTGTTGTTGAGGCCTATACC-3'), D150W–2 (5'-GCTTTGTTGTTTGGGCCTATACC-3'), Y152E–2 (5'-GTTGTTGATGCCGAGACCAAAAG-3'), Y152N–2 (5'-GTTGTTGATGCCAATACCAAAAG-3'), E132K–2 (5'-CGAGTGGGAAAGAAAACAGCTGATAGTATT-3'), and E132S–2 (5'-CGAGTGGGAAAGTCTACAGCTGATAGTATT-3') were also used to obtain a PCR product (P2). The PCR products were eluted using agarose gel, and a second PCR was performed using the products (P1 and P2). The second PCR products were digested with *Nde*I and *Hin*dIII and ligated with pET28a vector. DNA sequences were confirmed using an ABI 373 automatic DNA sequencer (United States).

Protein expression and purification of *Mj*UDG mutants. The recombinant plasmids were introduced into *E. coli* BL21 (DE3). *E. coli* BL21 harboring pET-*Mj*UDG mutants (D150W, D150E, Y152E, Y152N, E132K or E132S) was grown in Luria–Bertani (LB) broth with 50 μg/ml of kanamycin at 37°C. When OD_{600} of the culture reached 0.5, the recombinant protein was induced with 0.2 mM isopropyl-β-D-thiogalactopyranoside (IPTG). After incubation for an additional 4 h at 37°C, cells were harvested, resuspended in buffer A (50 mM NaH_2PO_4, 300 mM NaCl, pH 8.0), and lysed by sonication. After centrifugation at 15000 rpm for 30 min at 4°C, the supernatant was loaded onto a Ni-NTA agarose affinity column (Qiagen, Germany), and the His-tagged protein was eluted with linear gradient (0–500 mM imidazole) in buffer A. The fusion protein was then cleaved with thrombin (10 U/mg of fusion protein) for 16 h at 4°C and the cleavage mixture was dialyzed in buffer A. The resulting mixture was applied to a Superdex–75 gel filtration FPLC column. The fractions containing homogeneous mutant protein were collected using a fraction collector and concentrated by ultrafiltration using an YM10 membrane (Millipore, United States).

DNA substrates. The oligonucleotides used in this work were 32-mers (5'-GGATCCTCTAGAGTCXACCTGCAGGCATGCAA-3'), where X denotes uracil (U) or 8-oxoguanine (8-oxoG). These oligonucleotides containing a single modified base at position 16 were purchased from Bio-Synthesis, Inc. (United States). Oligonucleotides with a complementary base (A, T, G, or C) opposite the X were used as complementary strands. The oligonucleotides containing an X were 5'-end-labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase. Unincorporated [γ-^{32}P]ATP was removed using the QIAquick Nucleotide Removal Kit (Qiagen, Germany). Duplexes were prepared by annealing those using a 1.5-fold molar excess of unlabeled complementary strand in the buffer (20 mM Tris–HCl, pH 7.4, 100 mM NaCl, 1 mM DTT, 1 mM EDTA, and 3% glycerol). The annealing mixtures were heated to 75°C for approximately 5 min and slowly cooled to room temperature. The annealed DNA was then precipitated with ethanol, dried, and resuspended in double-distilled water.

Assay of DNA glycosylase activity. DNA cleavage by *Mj*UDG mutant proteins was assayed at 50°C using 5 pmol of protein and 1 pmol of radiolabeled 32-mer oligonucleotide duplexes in a reaction buffer containing 20 mM MES pH 6.0, 80 mM NaCl, 1 mM DTT, 1 mM EDTA, and 3% glycerol. The reaction mixture was subjected to hot alkaline treatment with 50 mM NaOH at 95°C for 15 min. The reaction mixture was then neutralized by adding 30 mM Tris, and then an equal volume of formamide loading buffer with 0.05% bromophenol blue and 0.05% xylene cyanol was added to the mixture. The sample solutions were then heated at 95°C for 5 min and cooled on ice immediately. The samples were subjected to electrophoresis on a denaturing 15% polyacrylamide gel containing 7 M urea in 1× TBE buffer (89 mM Tris, 89 mM boric acid and 2 mM EDTA). The gel was dried, placed on an imaging plate, and DNA cleavage products were quantified using a BAS2500 image analyzer (Fuji, Japan).

AP lyase activity assay of mutant E132K. *Mj*UDG mutant E132K was incubated in a reaction buffer (20 mM MES pH 6.0, 80 mM NaCl, 1 mM DTT, 1 mM EDTA, and 3% glycerol) with 1 pmol of radiolabeled 32-mer oligonucleotide duplex containing a U : T mismatch at 50°C for 30 min. An equal volume of the formamide loading buffer (0.05% bromophenol blue and 0.05% xylene cyanol) was added to samples with or without subsequent alkaline treatment (50 mM NaOH) at 95°C for 15 min. The samples were then heated at 95°C for 5 min and cooled on ice immediately. The

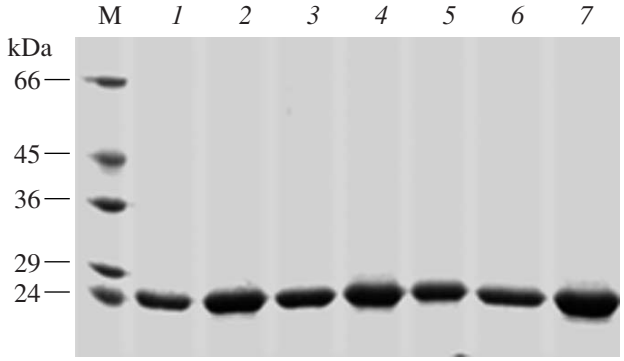

Fig. 1. Purification of *Mj*UDG and its mutants. The proteins were overexpressed in *E. coli*, analyzed on a 12.5% SDS-polyacrylamide gel, and detected by Coomassie blue staining. Lane M, protein molecular weight markers; lane *1*, wild-type *Mj*UDG; lane *2*, D150E; lane *3*, D150W; lane *4*, Y152E; lane *5*, Y152N; lane *6*, E132K; lane *7*, E132S.

reaction samples were analyzed using denaturing 15% polyacrylamide gel electrophoresis in a BAS2500 image analyzer.

DNA trapping assay with $NaBH_4$. *Mj*UDG mutant E132K protein was incubated with 1 pmol of radiolabeled 32-mer oligonucleotide duplex containing a U : T mismatch in the presence of 20 mM MES, pH 6.0, 1 mM EDTA, 1 mM DTT, and 50 mM $NaBH_4$ in a total volume of 10 μl. The reaction mixtures were incubated at 50°C for 30 min and mixed with 2.5 μl of the 5× SDS loading buffer (100 mM Tris–HCl, pH 6.8, 10% SDS, 20% glycerol, 5% 2-mercaptoethanol and 0.2% bromophenol blue). The samples were heated at 90°C for 5 min, followed by electrophoresis on a 12.5% SDS-polyacrylamide gel. The trapping efficiency was analyzed using a BAS2500 image analyzer.

RESULTS AND DISCUSSION

Expression and purification of *Mj*UDG mutant proteins. DNA sequences encoding the wild type and mutant variants (D150E, D150W, Y152E, Y152N, E132K, and E132S) of the *Mj*UDG were subcloned into the pET28a vector which places a hexa-histidine tag and a thrombin cleavage site at the upstream of the cloning site. These constructs were introduced into competent *E. coli* BL21 (DE3) cells and overexpressed as hexa-histidine-tagged recombinant proteins by the addition of isopropyl β-D-1-thiogalactopyranoside (IPTG) to the growth medium. The histidine-tagged proteins were eluted from the Ni-NTA affinity column with imidazole. After cleavage of the N-terminus of histidine-tagged proteins with thrombin, the protein samples were purified with Superdex–75 gel filtration FPLC. The final preparation of *Mj*UDG mutant proteins produced a single band of more than 95% purity (Fig. 1). The molecular mass of the mutant proteins determined on a denaturing gel was 24 kDa, corresponding to the theoretically calculated value. Finally, the purified protein sample was heated for 10 min at 70°C; endogenous *E. coli* proteins are completely denatured by this treatment, while *Mj*UDG is a thermostable protein and high temperature does not alter the enzyme activity [3].

Uracil-DNA glycosylase activity of wild type and mutant proteins. Although duplexes where the uracil is positioned opposite the common bases of a complementary strand were incised with a similar efficiency in all double-stranded substrates, *Mj*UDG preferentially excises on U : T mispair [3]. So, all activity tests of *Mj*UDG mutants were performed using a [γ-^{32}P]-labeled oligonucleotide duplex containing a U : T mispair. Although they share no common substrates, *Mj*UDG is closer to the endonuclease III (EndoIII) family than to the typical UDG families [3]. Interestingly, *Mj*UDG also has a helix–hairpin–helix (HhH) motif found in a diverse range of structurally related DNA repair proteins such as EndoIII, mismatch-specific DNA glycosylase (MIG), and 8-oxoguanine DNA glycosylase (Ogg) families (Fig. 2). Based on the amino acid sequence alignments in the HhH motif of four protein families, it is suggested that Asp150 of *Mj*UDG may be involved in the catalytic activity (Fig. 2). The Asp150 residue was then mutated to Glu and Trp, and mutant proteins were expressed in *E. coli* and purified to >95% apparent homogeneity (Fig. 1). To evaluate the uracil-DNA glycosylase activity, *Mj*UDG and its mutants were compared using a 32-mer DNA duplex containing a U : T mispair at position 16. After the DNA glycosylase reaction with *Mj*UDG or its mutants, samples were treated with NaOH for cleavage at the internal AP site following removal of uracil. Since the oligonucleotide containing uracil was labeled on its 5'-end with [γ-^{32}P]ATP, cleaved products could be observed in the gel. Wild-type *Mj*UDG catalyzed excision of the uracil mispaired with thymine (Fig. 3a). The reaction rate of mutant D150W was significantly reduced (Fig. 3a), indicating the importance of this residue in the catalytic reaction. The mutant D150E had a slightly decreased activity of about 70% of the wild-type value (Fig. 3b), suggesting that the effect of these residues was electrostatic due to the negative charge. Aspartic acid was shown to be the catalytic residue in the cases of most DNA glyosylases, including UDG, AlkA, Ogg, and EndoIII [5, 8–10]. It is interesting to note that D150 of *Mj*UDG is conserved in the HhH motif of EndoIII family (Fig. 2). Furthermore, this aspartic acid residue of human Ogg–1 (D268) is located at nearly superimposable positions in the co-crystal structure [11]. Crystal structures of other DNA glycosylases of the HhH family show that the aspartic acid of the active site deprotonates water, thereby activating it for nucleophilic attack [9, 12]. Aspartic acid (Asp152) in mouse *N*-methylpurine-DNA glycosylase (MPG) was also shown to be involved in catalysis by donating a proton to the substrate base [13].

In the case of several UDGs, conserved residues have been shown to be involved in the catalytic reaction

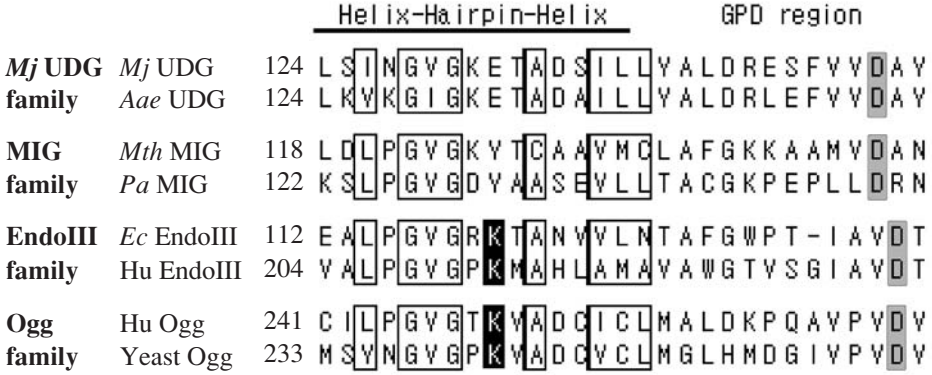

Fig. 2. Alignment of the helix–hairpin–helix (HhH) and Gly/Pro-rich (GPD) region of *Mj*UDG with those of other proteins. Hydrophobic residues important for achieving the HhH structure are shown in boxes. Aspartic acid residues important for achieving the catalytic reaction of DNA glycosylase are shaded. Black boxes indicate the lysine residue involved in AP lyase activity. *Aae* UDG, *A. aeolicus* UDG; *Mth*MIG, *M. thermautotrophicus* mismatch-specific DNA glycosylase; *Pa*MIG, *P. aerophilum* mismatch-specific DNA glycosylase; *Ec* EndoIII, *E. coli* endonuclease III; Hu EndoIII, human endonuclease III; Hu Ogg, human 8-oxoguanine DNA glycosylase; Yeast Ogg, *S. cerevisiae* 8-oxoguanine DNA glycosylase. Multiple alignments of amino acid sequences were performed using the Clustal program.

and lesion recognition. The biochemical characteristics and the protein structure of *E. coli* UDG (UDG family I) provided an insight regarding the role of the histidine residue of motif B in the catalytic mechanism of glycosyl bond cleavage [14, 15]. This histidine residue is conserved in motif B of UDG families I, III, IV, and V [16]. A mutant form of *Pyrobaculum aerophilum* UDG (Pa-UDG) (UDG family V) at the position corresponding to the histidine residue in motif B of other UDG families displayed reduced enzymatic activity [17]. Family I UDGs share a distinct tyrosine residue in motif A (-GQDPYH-), which is believed to be responsible for the enzyme activity [16]. A previous report on the human UDG involving mutation analysis suggested that tyrosine (Tyr147) is a significant residue involved in protein–uracil base interaction as a specificity determinant [18]. Furthermore, in *E. coli* UDG, Tyr66 at the position which corresponds to Tyr147 of the human UDG was involved specifically in uracil base selectivity and catalysis [19, 20]. However, *Mj*UDG is closer to the MIG family DNA glycosylases than the EndoIII family in terms of sequence similarity in the HhH motif and lack of AP lyase activity. In *Mth*MIG, a DNA glycosylase of MIG family, Asp144 at the position corresponding to *Mj*UDG Asp150 is positioned to interact with T : G mismatch-containing substrates, and its mutant form displays significantly reduced activity compared to the wild type protein [21]. Two mutant forms (Glu or His) of the position Asn146 of *Mth*MIG also showed severely decreased catalytic activity. This Asn residue at position 146 of *Mth*MIG was replaced by tyrosine (Tyr152) in *Mj*UDG (Fig. 2). Because Tyr152 is a unique amino acid residue in the HhH-GPD region of the *Mj*UDG family and a Tyr residue is crucial for efficient catalysis of UDG [18–20], we obtained two mutants with Tyr152 of *Mj*UDG replaced by Glu and Asn, respectively. To verify the role of Tyr152, enzyme activities of wild type *Mj*UDG and its mutants (Y152E and Y152N) were compared using DNA duplexes containing a U : T mismatch. However, these mutant proteins retained the same level of UDG activity and did not exhibit novel enzyme activity such as TDG activity (capacity for removal of T:G mismatches) (data not shown).

AP lyase activity of *Mj*UDG E132K mutant protein. There are two classes of DNA glycosylases with distinct substrate specificities: monofunctional glycosylases and glycosylases associated with apurine/apyrimidine (AP) lyase activity [1]. Most UDGs are known to be monofunctional without AP lyase activity [17]. UDGs catalyze the excision of a uracil mispaired with base and results in formation of an AP site. When this DNA product is treated with NaOH, cleavage of the phosphodiester bond at the internal AP site is created, resulting in increased DNA fragment production. Wild type *Mj*UDG also displayed a monofunctional activity. After the reaction of wild type *Mj*UDG protein with the uracil-containing oligonucleotide duplex, the DNA backbone at the AP site was cleaved only when treated with NaOH (Fig. 4a). This phosphodiester backbone cleavage was not observed when NaOH was not applied.

The EndoIII and Ogg families have an AP lyase activity and contain a conserved lysine residue (Fig. 2). In *Mj*UDG, the position corresponding to this lysine residue of EndoIII and Ogg is Glu132 (Fig. 2). It was therefore suggested that monofunctional *Mj*UDG could be converted to a bifunctional glycosylase/AP lyase by conversion of Glu132 to Lys. *Mj*UDG mutant protein E132K, in which Glu132 was substituted with Lys, was capable of processing the substrate to yield 16-mer strands as the cleavage product, in the absence of NaOH (Fig. 4a). Under the same conditions, *Mj*UDG mutant E132S was also tested for AP lyase activity and

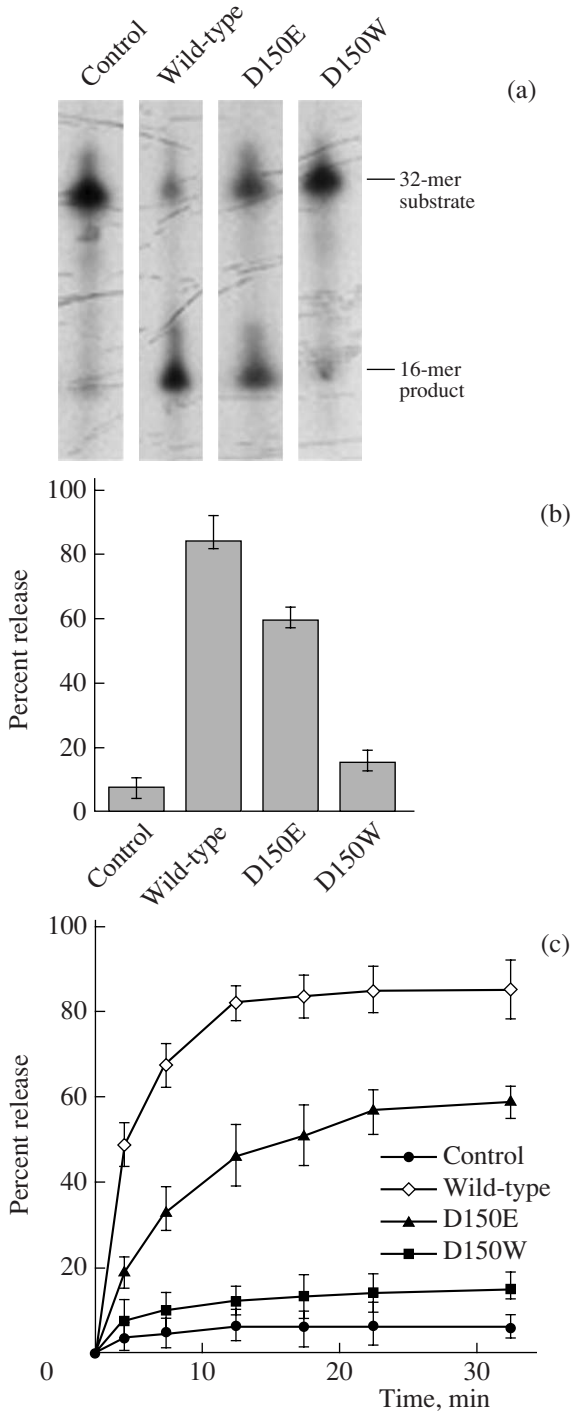

Fig. 3. Uracil-DNA glycosylase activity of wild-type *Mj*UDG and its mutants. (a) Purified proteins (5 pmol) were incubated with 1 pmol of a 5'-end-labeled 32-mer oligonucleotide duplex containing a U : T mismatch at 50°C for 30 min. The reaction mixture was treated with 50 mM NaOH and incubated at 95°C for 15 min. In the bottom graph, quantitative data were obtained from at least three independent experiments. (b) Reactions were carried out at 50°C for 30 min with an oligonucleotide duplex containing a U : T mismatch. At the indicated times, reaction mixtures were treated with 50 mM NaOH at 95°C for 15 min. Quantitative data were obtained from at least three independent experiments.

exhibited no detectable lyase activity (data not shown); this result confirms that the lysine in this position is responsible for emergence of AP lyase activity in *Mj*UDG E132K. As shown in Fig. 4b, the 16-mer DNA product of a NaOH-untreated sample ran slower than that of a NaOH-treated sample after mutant E132K reaction, suggesting that the E132K protein catalyzes a β-elimination reaction at the AP site and thus generates 3'-α, β-unsaturated aldehyde sugar termini at the incision site. The different reaction mechanisms of DNA glycosylase/AP lyase enzymes, β-elimination or δ-elimination, can be identified by the different electrophoretic mobilities of the oligonucleotide products. Eukaryotic Ogg enzymes [6] and archaeal Ogg [22] form 5'-phosphate and 3'-α, β-unsaturated aldehyde via β-elimination, whereas *E. coli* Fpg [23] produces 5' and 3' phosphate termini, resulting from a δ-elimination reaction. The gel mobility of the cleavage product of the E132K-catalyzed reaction in the absence of hot alkaline treatment was lower than that of *E. coli* Fpg (Fig. 4b). This result suggests that E132K mutant protein catalyzes a β-elimination reaction at the AP site by uracil excision and thus generates 3'-α,β-unsaturated aldehyde sugar termini at the incision site. To confirm this β-elimination reaction mechanism of E132K protein, we examined the DNA cleavage reaction under piperidine treatment. Piperidine induces cleavage of modified DNA by strand scission at the AP site through β-δ-elimination [24], producing 5' and 3' phosphate termini of the original product. So DNA strands migrate slightly faster than the β-elimination product. After the cleavage reaction of E132K protein with the substrate, the aliquots were treated with 10% piperidine at 90°C. In the case of piperidine treatment, reaction products of E132K protein were converted to δ-elimination products migrating slightly faster than β-elimination products according to time course (Fig. 4c).

Formation of a Schiff base intermediate by the *Mj*UDG mutant E132K. To determine whether *Mj*UDG E132K was able to form a Schiff base, the protein was incubated with a substrate containing uracil in the presence of sodium borohydride. Covalent protein-DNA Schiff base intermediates are formed with an active amine residue of a bifunctional glycosylase/lyase by reduction with sodium borohydride [25]. They can be detected by SDS-polyacrylamide gel electrophoresis as stable protein-DNA complexes. As expected, a DNA trapping assay showed that *Mj*UDG mutant E132K, rather than the wild type *Mj*UDG, formed a covalent protein-DNA adduct with a uracil-containing DNA substrate (Fig. 4d). It has been shown that the lysine residue of DNA glycosylases is indeed critical for catalytic activity and formation of a covalent complex with DNA in EndoIII [5] and Ogg [26]. Our results suggest that, in the case of *Mj*UDG, a monofunctional DNA glycosylase can be converted to bifunctional glycosylase/AP lyase by a single amino acid change. Our study implies that *Mj*UDG possibly evolved into a monofunctional DNA glycosylase through mutation of the lysine

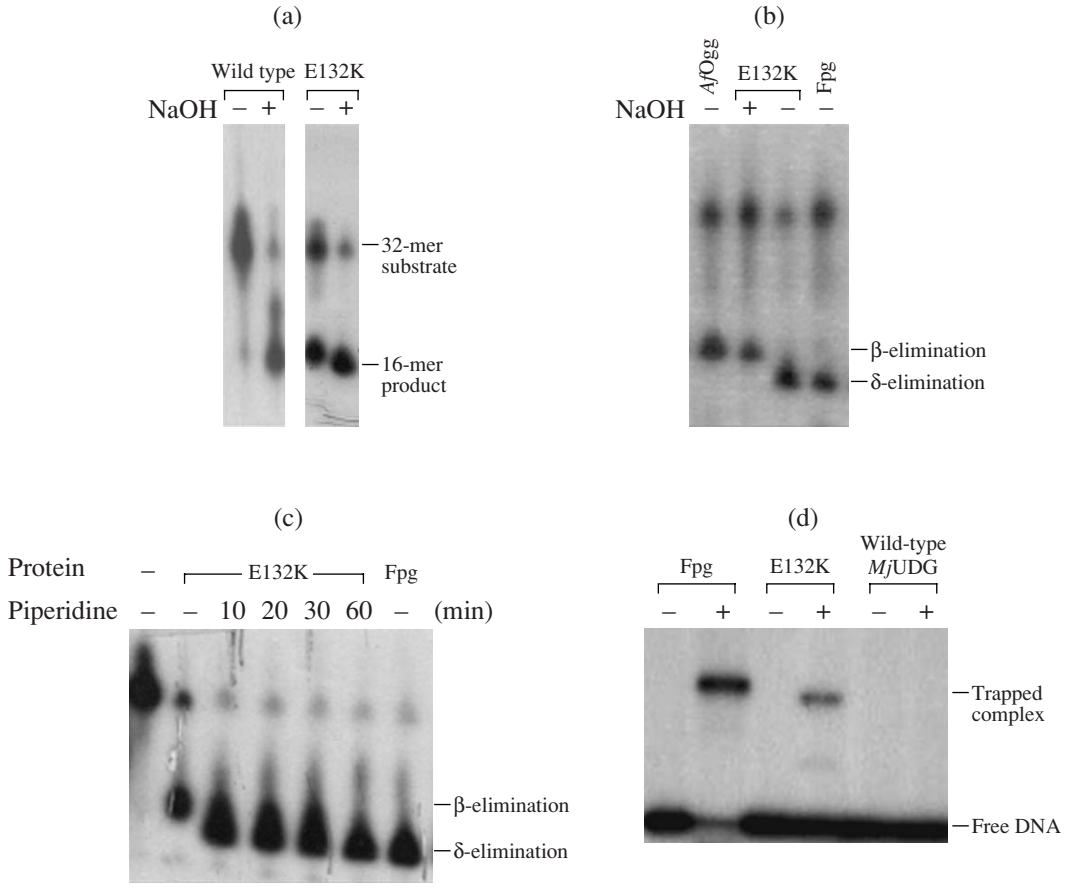

Fig. 4. AP lyase activity and Schiff base intermediate formation of *Mj*UDG mutant E132K. (a) Purified proteins (5 pmol) were incubated with 1 pmol of a 5'-end-labeled 32-mer oligonucleotide duplex containing a U : T mismatch at 50°C for 30 min. The reaction mixture was then incubated at 95°C for 10 min in the absence or presence of 50 mM NaOH. (b) Purified proteins (5 pmol) were incubated for 30 min at 50°C with 1 pmol of a 5'-end-labeled 32-mer oligonucleotide duplex containing a U : T mismatch. *Archaeoglobus fulgidus* 8-oxoguanine DNA glycosylase (*Af*ogg) and *E. coli* Fpg were used as control of β-elimination and β-elimination, respectively. (c) After enzymatic reaction with E132K mutant protein, the samples were treated with 10% piperidine at 90°C according to the indicated time course (0–60 min). *Af*Ogg and Fpg used as a positive control of reaction were reacted with 8-oxoguanine-containing substrate. (d) Mutant E132K was incubated with an oligonucleotide duplex containing a U : T mismatch in the presence of 50 mM $NaBH_4$ at 50°C for 30 min. *E. coli* Fpg protein used as a positive control of protein-DNA complex was reacted with 8-oxoguanine-containing substrate at 37°C.

residue which is crucial for the catalytic activity of AP lyase.

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REFERENCES

1. Krokan, H.E., Standal, R., and Slupphaug, G., DNA Glycosylases in the Base Excision Repair of DNA, *Biochem. J.*, 1997, vol. 325, pp. 1–16.

2. Laurence, H.P., Structure and Function in the Uracil-DNA Glycosylase Superfamily, *Mutat. Res.*, 2000, vol. 460, nos. 3-4, pp. 165–181.

3. Chung, J.H., Im, E.K., Park, H.Y., Kwon, J.H., Lee, S., Oh, J., Hwang, K.C., Lee, J.H., and Jang, Y., A Novel Uracil-DNA Glycosylase Family Related to the Helix-Hairpin-Helix DNA Glycosylase Superfamily, *Nucleic Acids Res.*, 2003, vol. 31, no. 8, pp. 2045–2055.

4. Denver, D.R., Swenson, S.L., and Lynch, M., An Evolutionary Analysis of the Helix-Hairpin-Helix Superfamily of DNA Repair Glycosylases, *Mol. Biol. Evol.*, 2003, vol. 20, no. 10, pp. 1603–1611.

5. Ikeda, S., Biswas, T., Roy, R., Izumi, T., Boldogh, I., Kurosky, A., Sarker, A.H., Seki, S., and Mitra, S., Purification and Characterization of Human NT, a Homolog of *Escherichia coli* Endonuclease III. Direct Identification of Lys-212 as the Active Nucleophile Residue, *J. Biol. Chem.*, 1998, vol. 273, no. 34, pp. 21585-21593.

6. Leipold, M.D., Workman, H., Muller, J.G., Burrows, C.J., and David, S.S., Recognition and Removal of Oxidized Guanines in Duplex DNA by the Base Excision Repair Enzymes hOGGI, yOGG1, and yOGG2, *Biochemistry*, 2003, vol. 42, no. 38, pp. 11373-11381.

7. Girard, P.M., Guibourt, N., and Boiteux, S., The Ogg1 Protein of *Saccharomyces cerevisiae*: a 7,8-Dihydro-8-Oxoguanine DNA Glycosylase/AP Lyase Whose Lysine 241 Is a Critical Residue for Catalytic Activity, *Nucleic Acids Res*., 1997, vol. 25, no. 16, pp. 3204–3211.

8. Savva, R., McAuley-Hecht, K., Brown, T., and Pearl, L., The Structural Basis of Specific Base-Excision Repair by Uracil-DNA Glycosylase, *Nature*, 1995, vol. 373, no. 6514, pp. 487–493.

9. Labahn, J., Scharer, O.D., Long, A., Ezaz-Nikpay, K., Verdine, G.L., and Ellenberger, T.E., Structural Basis for the Excision Repair of Alkylation-Damaged DNA, *Cell*, 1996, vol. 86, no. 2, pp. 321–329.

10. Bruner, S.D., Nash, H.M., Lane, W.S., and Verdine, G.L., Repair of Oxidatively Damaged Guanine in *Saccharomyces cerevisiae* by an Alternative Pathway, *Curr. Biol*., 1998, vol. 8, no. 7, pp. 393–403.

11. Bruner, S.D., Norman, D.P., and Verdine, G.L., Structural Basis for Recognition and Repair of the Endogenous Mutagen, 8-Oxoguanine in DNA, *Nature*, 2000, vol. 403, no. 6772, pp. 859–866.

12. Guan, Y., Manuel, R.C., Arvai, A.S., Parikh, S.S., Mol, C.D., Miller, J.H., Lloyd, S., and Tainer, J.A., MutY Catalytic Core, Mutant and Bound Adenine Structures Define Specificity for DNA Repair Enzyme Superfamily, *Nat. Struct. Biol*., 1998, vol. 5, no. 12, pp. 1058–1064.

13. Roy, R., Biswas, T., Lee, J.C., and Mitra, S., Mutation of a Unique Aspartate Residue Abolishes the Catalytic Activity but not Substrate Binding of the Mouse N-Methylpurine-DNA Glycosylase (MPG), *J. Biol. Chem*., 2000, vol. 275, no. 6, pp. 4278–4282.

14. Putnam, C.D., Shroyer, M.J., Lundquist, A.J., Mol, C.D., Arvai, A.S., Mosbaugh, D.W., and Tainer, J.A., Protein Mimicry of DNA from Crystal Structures of the Uracil-DNA Glycosylase Inhibitor Protein and Its Complex with *Escherichia coli* Uracil-DNA Glycosylase, *J. Mol. Biol*., 1999, vol. 287, no. 2, pp. 331–346.

15. Shroyer, M.J., Bennett, S.E., Putnam, C.D., Tainer, J.A., and Mosbaugh, E.W., Mutation of an Active Site Residue in *Escherichia coli* Uracil-DNA Glycosylase: Effect on DNA Binding, Uracil Inhibition and Catalysis, *Biochemistry*, 1999, vol. 38, no. 15, pp. 4834–4845.

16. Sartori, A.A., Fitz-Gibbon, S., Yang, H., Miller, J.H., and Jiricny, J., A Novel Uracil-DNA Glycosylase with Broad Substrate Specificity and an Unusual Active Site, *EMBO J*., 2002, vol. 21, no. 12, pp. 3182-3191.

17. Caradonna, S. and Muller-Weeks, S., The Nature of Enzymes Involved in Uracil-DNA Repair: Isoform Characteristics of Proteins Responsible for Nuclear and Mitochondrial Genomic Integrity, *Curr. Protein Pept. Sci*., 2001, vol. 2, no. 4, pp. 335–347.

18. Kavli, B., Slupphaug, G., Mol, C.D., Arvai, A.S., Peterson, S.B., Tainer, J.A., and Krokan, H.E., Excision of Cytosine and Thymine from DNA by Mutants of Human Uracil-DNA Glycosylase, *EMBO J*., 1996, vol. 15, no. 13, pp. 3442–3447.

19. Handa, P., Acharya, N., and Varshney, U., Effects of Mutations at Tyrosine 66 and Asparagine 123 in the Active Site Pocket of *Escherichia coli* Uracil DNA Glycosylase on Uracil Excision from Synthetic DNA Oligomers: Evidence for the Occurrence of Long-Range Interactions between the Enzyme and Substrate, *Nucleic Acids Res*., 2002, vol. 30, no. 14, pp. 3086–3095.

20. Acharya, N., Talawar, R.K., Saikrishnan, K., Vijayan, M., and Varshney, U., Substitutions at Tyrosine 66 of *Escherichia coli* Uracil DNA Glycosylase Lead to Characterization of an Efficient Enzyme That Is Recalcitrant to Product Inhibition, *Nucleic Acids Res.,* 2003, vol. 31, no. 24, pp. 7216–7276.

21. Mol, C.D., Arvai, A.S., Begley, T.J., Cunningham, R.P., and Tainer, J.A., Structure and Activity of a Thermostable Thymine-DNA Glycosylase: Evidence for Base Twisting To Remove Mismatched Normal DNA Bases, *J. Mol. Biol*., 2002, vol. 315, no. 3, pp. 373–384.

22. Chung, J.H., Suh, M.J., Park, Y.I., Tainer, J.A., and Han, Y.S., Repair Activities of 8-Oxoguanine DNA Glycosylase from *Archaeoglobus fulgidus*, a Hyperthermopilic Archaeon, *Mutat. Res*., 2001, vol. 486, no. 2, pp. 99–111.

23. Bhagwat, M. and Gerlt, J.A., 3'- and 5'-Strand Cleavage Reactions Catalyzed by the Fog Protein from *Escherichia coli* Occur via Successive Beta- and Delta-Elimination Mechanisms, Respectively, *Biochemistry*, 1996, vol. 35, no. 2, pp. 659–665.

24. Burrows, C.J. and Muller, J.G., Oxidative Nucleobase Modifications Leading to Strand Scission, *Chem. Rev*., 1998, vol. 98, no. 3, pp. 1109–1151.

25. Dodson, M.L., Michaels, M.L., and Lloyd, R.S., Unified Catalytic Mechanism for DNA Glycosylases, *J. Biol. Chem*., 1994, vol. 269, no. 52, pp. 32709–32712.

26. Nash, H.M., Lu, R., Lane, W.S., and Verdine, G.L., The Critical Active-Site Amine of the Human 8-Oxoguanine DNA Glycosylase, hOgg1: Direct Identification, Ablation and Chemical Reconstitution, *Chem. Biol*., 1997, vol. 4, no. 9, pp. 693–702.