Determination of Active Site Residues in *Escherichia coli* Endonuclease VIII*

Sarah Burgess‡, Pawel Jaruga§, M. L. Dodson¶, Miral Dizdaroglu¶, and R. Stephen Lloyd‖

From the ‡Department of Human Biological Chemistry and Genetics and the §Sealy Center for Molecular Science, University of Texas Medical Branch, Galveston, Texas 77555-1071 and the ‖Chemical Science and Technology Laboratory, National Institute of Standards and Technology, Gaithersburg, Maryland 20899-8311

Endonuclease VIII from *Escherichia coli* is a DNA glycosylase/lyase that removes oxidatively damaged bases. EndoVIII is a functional homologue of endonuclease III, but a sequence homologue of formamidopyrimidine-DNA glycosylase (Fpg). Using multiple sequence alignments, we have identified six target residues in endoVIII that may be involved in the enzyme’s glycosylase and/or lyase functions: the N-terminal proline, and five acidic residues that are completely conserved in the endoVIII-Fpg proteins. To investigate the contribution of these residues, site-directed mutagenesis was used to create seven mutants: P2T, E3D, E3Q, E6Q, D129N, D160N, and E174Q. Each mutant was assayed both for lyase activity on abasic (AP) sites and for glycosylase/lyase activity on 5-hydroxymethyluracil, thymine glycol, and γ-irradiated DNA with multiple lesions. The P2T mutant did not have lyase or glycosylase/lyase activity but could efficiently form Schiff base intermediates on AP sites. E6Q, D129N, and D160N behaved essentially as endoVIII in all assays. E3D, E3Q, and E174Q retained significant AP lyase activity but had severely diminished or abolished glycosylase/lyase activities on the DNA lesions tested. These studies provide detailed predictions concerning the active site of endoVIII.

Oxidative damage to cellular DNA results from its interaction with free radical species, which can originate from endogenous aerobic respiration or from exogenous free radical-generating agents. Oxidation of bases results in a variety of damages, many of which are potentially mutagenic; additionally, oxidative DNA damage has been implicated in aging and diseases, such as cancer (1–4). Base excision repair (BER) is the primary pathway by which oxidative DNA damage is repaired, and this process is initiated by removal of the damaged base by a DNA glycosylase (5). In *Escherichia coli*, there are three known BER glycosylases that recognize and remove oxidized bases. Formamidopyrimidine-DNA glycosylase (Fpg) primarily removes oxidized purines such as 7,8-dihydro-8-oxoguanine (OG) and formamidopyrimidines (6, 7). Endonuclease III (endoIII) is the glycosylase primarily responsible for removal of oxidized pyrimidines, such as thymine glycol, 5,6-dihydrouracil, and 5,6-dihydrothymine (8, 9). The third *E. coli* BER glycosylase that removes oxidized bases is endonuclease VIII (endoVIII) (10, 11). EndoVIII has highly overlapping substrate specificity with endoIII, as evidenced through biochemical analyses (12) and the analyses of mutator phenotypes of *E. coli* strains deficient in one or both of these enzymes (11, 13). Although cells lacking functional genes for either endoIII (nth gene) or endoVIII (neo gene) have a weak mutator phenotype (13, 14), cells lacking both glycosylases have a much higher spontaneous mutation frequency (11) and a greater H2O2 sensitivity (13).

There is evidence that endoVIII also has slight substrate overlap with Fpg. Studies using oligonucleotides with site-specific damage have shown that there are common substrates between the two enzymes, including GO and 4,6-diamino-5-formamidopyrimidine (FapyAde) (6, 15–17). However, recent work showed that endoVIII does not remove OG in any detectable amount from radiation-damaged DNA but that FapyAde was indeed a substrate for endoVIII (12).

Despite differences in substrate specificity, endoVIII and Fpg have significant similarities in their catalytic activities. Both glycosylases possess lyase activity, catalyzing β-δ elimination to generate a single-strand break at the site of the removed base with both 5′- and 3′-phosphates (18, 19). A unified catalytic mechanism has been presented for glycosylase/lyase enzymes (20), in which the enzyme uses an amine as an active site nucleophile to break the glycosyl bond at the C1′ of the sugar moiety. As a result, the enzyme and DNA form an imino Schiff base intermediate, and this protein-DNA complex can be covalently trapped in the presence of a reducing agent, such as NaBH₄. Both endoVIII and Fpg use the secondary amine of their N-terminal proline residues as the nucleophile (21, 22). In addition to an active site nucleophile, glycosylase/lyase enzymes require acidic side chains to perform catalysis on damaged bases. Mutagenesis of acidic residues in two pyrimidine dimer glycosylases and *E. coli* Fpg severely diminishes or eliminates enzymatic activity of these enzymes (23–25), even with the active site nucleophile intact.

EndoVIII does not share sequence homology with its functional homologue, endoIII, but its sequence is significantly homologous to members of the Fpg family. In particular, we have found that the conserved glutamates and aspartates in Fpg that are critical for catalysis (25) are also conserved in endoVIII proteins from *E. coli* and from other microorganisms. Fig. 1 (see below) shows an alignment between *E. coli* endoVIII and five Fpg proteins, illustrating the conservation of several
end of the reaction time, NaBH₄ was added to a final concentration of 100 mM. Reducing agents were added from freshly prepared stock solutions. All reactions were stopped by the addition of an equal volume of 95% formamide/1% SDS loading buffer and immediately loaded onto a denaturing 8% urea/15% polyacrylamide gel; substrate and product DNAs were separated by electrophoresis at 20 watts for 4.5 h. Images were generated and quantified with PhosphorImager (Molecular Dynamics) analysis.

**Thymine Glycol Plasmid Nicking Assays**—Thymine glycols were introduced into supercoiled pBR322 plasmid by the method of Kow and Wallace (27). Plasmid DNA was incubated with 0.05% OsO₄ for 10 min at 70 °C. The mixture was then cooled on ice and dialyzed overnight into 1× TE (10 mM Tris-HCl, pH 8, 1 mM EDTA) at 4 °C to remove any possible AP sites that may have been created as a consequence of the OsO₄ treatment, the DNA was dialyzed into 50 mM NaBH₄ overnight at 4 °C to reduce AP sites. The DNA was then dialyzed into 1× TE overnight to remove the reducing agent. For plasmid nicking assays, ~1.5 μg of DNA was incubated with 1, 5, or 10 mM enzyme for 15 min at 37 °C, followed by addition of 0.5× volume of agarose gel loading buffer (40% sucrose, 50 mM Tris-HCl, pH 7.5, 20 mM EDTA, 2% SDS, 0.02% bromphenol blue), and samples were electrophoresed in a 0.85% agarose gel in 1× TBE (90 mM Tris borate, 2 mM EDTA).

**Analysis of Glycosylase/Lyase Activity on γ-Irradiated DNA by Gas Chromatography/Isotope Dilution Mass Spectrometry**—Preparation of γ-irradiated DNA and enzymatic assays were performed as previously described (12). Aliquots of 100 μg of DNA samples were incubated with 0.5 μg of enzyme at 37 °C for 30 min, and the supernatant fractions from the assays were analyzed by GC/IDMS as previously described (12, 28).

**RESULTS**

To identify amino acids that may be important for enzyme catalysis, multiple sequence alignments were generated from sequences of *E. coli* endoVIII, putative endoVIII proteins from other microorganisms, and several Fpg proteins. A representative alignment between *E. coli* endoVIII and five Fpg proteins is shown in Fig. 1, and the five completely conserved aspartates and glutamates are highlighted, as well as the conserved N-terminal proline. The following mutant endoVIII proteins were constructed: Pro-2 → Thr (P2T), Gln-3 → Asp (E3Q), Glu-3 → Gln (E3Q), Glu-6 → Gln (E6Q), Asp-129 → Asn (D129N), Asp-160 → Asn (D160N), and Glu-174 → Gln (E174Q). While verifying the sequence of each of the mutated pET22b-nei plasmid constructs, an additional, yet identical, sequence change was detected in each of the constructs. The previously published nei sequence codes for a leucine at position 90 (11); however, all of our constructs had a T → C transition that changed amino acid 90 from Leu to Ser. Upon further sequencing, we discovered that in fact our template nei gene had this transition, and this appeared to be a naturally arising variant of endoVIII. A recent study (12) compared the relative catalytic activities of the L90S variant with the previously published endoVIII protein, and the results show that this variation does not alter enzyme activity or substrate specificity, although there were slight variations in kinetic parameters on some substrates. Thus all of the mutated proteins within this study are in the endoVIII-L90S variant background. The activities of the mutants listed above are compared with endoVIII-L90S, which in this paper will be designated endoVIII.

The endoVIII proteins were purified from a pfg-nei* E. coli strain to avoid contaminating activities. Each of the constructs overexpressed well and behaved normally during the standard purification used for all of the endoVIII proteins. However, despite good purification yields, some proteins had a very low percentage of active molecules as determined by AP site trapping, such as E3Q at ~30% and E174Q at ~20%. Each protein has been determined to be ~95% pure (data not shown) by SDS-PAGE analysis.

**Oligonucleotide Nicking and Trapping Activities**—Two oligonucleotide substrates were used in this study: AP sites to test for lyase activity and 5-OH-Ura to test for glycosylase/lyase activity on modified bases.

**EXPERIMENTAL PROCEDURES**

**Materials**—Uracil- and 5-hydroxyuracil-containing oligonucleotides and their complements were synthesized and purified by Midland Certified Reagent Co. The pBR322 plasmid was purified by University of Texas Medical Branch (UTMB), NEIHS/National Institutes of Health, Center Molecular Biology Core. The pET22b-nei plasmid construct was a gift from Dr. Tapas Hazra (UTMB, Galveston, TX) (17).

**Targeted Mutagenesis of EndoVIII Protein**—Point mutations were made in the pET22b-nei construct using the QuikChange site-directed mutagenesis kit 1 per kit in instructions. The residues to be altered were identified by multiple sequence alignments using ClustalW (BCM Search Launcher, available at searchlauncher.bcm.tmc.edu). Mutations were made at the N-terminal proline, at glutamates 3, 6, and 174, and at aspartates 129 and 160. The nei gene sequence was verified byideoxy sequencing at SeqWright (Houston, TX).

**Expression and Purification of Recombinant EndoVIII**—Expression and purification of all endoVIII proteins was performed as previously described (12). Briefly, the nei gene in the pET22b vector (Novagen, Madison, WI) was transformed into *E. coli* DE884 nei mutM* cells. Cells were grown in 1 liter of LB broth containing 5 μM ZnCl₂ and 100 μg/ml ampicillin. At an A₆₀₀ of 0.5, isopropyl-1-thio-β-D-galactopyranoside was added to a final concentration of 1 mM, and the culture was grown overnight at room temperature. Cells were harvested by centrifugation at 8000 rpm for 15 min. Pelleted cells were lysed by BugBuster protein extraction reagent (Novagen) following the manufacturer’s instructions. Cell debris was pelleted by centrifugation, and the supernatant was treated with 0.1% Polymyx F, followed by ammonium sulfate precipitation (60% final saturation). The ammonium sulfate precipitate was resuspended and dialyzed overnight into buffer A (20 mM Tris-HCl, pH 7.5, 1 mM dithiothreitol, 10% glycerol, 100 mM NaCl). The dialysate was loaded onto a 5-ml HitTrap-SP column (Amersham Biosciences, Inc., Piscataway, NJ), followed by extensive washing with buffer A. EndoVIII was eluted in a 0.1–0.5 mM NaCl gradient in buffer A. Fractions were checked for purity by SDS-PAGE. Pure fractions were pooled and dialyzed into storage buffer (same as buffer A but with 50% glycerol) and then stored at −20 °C. Protein concentrations were determined by Bio-Rad protein assay kit.

**Determination of Fraction of Active Molecules**—The fraction of active molecules for each enzyme preparation was determined following the procedure previously outlined (28). Briefly, 100 nM AP-containing DNA (see the 26-mer below) was incubated with 25, 50, 100, 150, or 200 nM enzyme in the presence of 50 mM NaCNBH₃, 85 mM sodium Hepe, pH 7.5, and 100 μg/ml bovine serum albumin at 37 °C for 30 min. Maximum complex formation was determined by quantitating trapped complex for each enzyme concentration.

**EndoVIII Oligonucleotide Nicking and Trapping Assays**—Enzyme AP site activity was tested on a 26-mer oligonucleotide with the following sequence: 5'-CATCCGCTGCGAGAATAGAACTGCGATGCTAC-3', where X = 5-hydroxyuracil (5-OH-Ura). The substrate DNA strand was radioactively labeled at the 5' end and then annealed to its complementary strand. Oligonucleotides and trapping buffer were carried out in 85 mM sodium Hepe, pH 7.5, 100 μg/ml bovine serum albumin, and 1 mM DNA, with the indicated amount of the various enzymes at 37 °C for 30 min. All enzyme concentrations were based on the determined concentration of active enzyme for that preparation. For trapping reactions, NaCNBH₃ was added to a final concentration of 50 mM at the start of the reactions, simultaneously with the addition of the enzyme; at the
activity. Each mutant was tested for its ability to nick or trap on both substrates. Each assay was performed in three separate experiments, and the mean values with standard deviations were calculated. To simplify data presentation, mutant enzyme responses in oligonucleotide assays were clustered into groups that gave similar responses, and each cluster is presented by a separate figure. In each data set, AP trapping data are represented by three lines: AP DNA in trapped complex (closed circles), AP DNA cleaved via β-elimination (open circles), and the sum of the DNA trapped and nicked (inverted, solid triangles). As mentioned above, NaCNBH₃ was used as the reducing agent of the Schiff base intermediate, which is a much slower reductant than NaBH₄. Therefore, significant amounts of Schiff base intermediates formed in that reaction. Determining both trapped and product DNA shows the propensity of each Schiff base to either β-eliminate or be reduced, depending on the enzyme involved.

Substitution of the N-terminal Proline of EndoVIII—The data in Fig. 2 compares the activities of endoVIII (top row) and P2T (bottom row) on AP sites and 5-OH-Ura lesions in double-stranded oligonucleotides. Changing the N-terminal proline to a threonine eliminates almost all of the lyase activity on AP stranded oligonucleotides. Changing the N-terminal proline to P2T (data in Fig. 2) compares the activities of endoVIII (enzyme involved. with an efficiency equal to endoVIII, as shown in the first graphs). The mutants in this grouping all cleaved AP sites with an efficiency equal to endoVIII, as shown in the first column. All four enzymes had an equal propensity to form Schiff bases on AP sites, as shown by the summation lines in the second column of graphs. EndoVIII and D129N both had approximately equal amounts of trapped and nicked DNA, while E6Q and D129N had a higher fraction of the DNA trapped than nicked. E6Q, D129N, and D160N all retain significant 5-OH-Ura glycosylase/lyase and trapping activity as compared with endoVIII, as illustrated in the third and fourth columns of Fig. 3.

Glutamate Substitutions That Significantly Affect Enzyme Activities—The data in Fig. 4 illustrate the importance of Glu-3 and Glu-174 for endoVIII activity. Again, the endoVIII data are duplicated here for comparison. E3D, E3Q, and E174Q all retain lyase activity on AP sites (first column), although E3Q has a reduced efficiency. An interesting result that was consistently observed was that E3D gave significant amounts of β-elimination products (30–40%) in addition to β-elimination products (60–70%) on AP sites. As shown in the second column, all three of these mutants have diminished capacity for forming the Schiff base intermediate on AP sites; E3Q is the most deleterious mutation among these enzymes. Both changes at position 3 to aspartate (E3D) and glutamine (E3Q) almost completely abolish the nicking and trapping activities of endoVIII on 5-OH-Ura (third and fourth columns). E174Q retains slight 5-OH-Ura nicking activity but does not appreciably trap on this substrate.

Ability to Nick Thymine Glycol-containing Plasmid DNA—Thymine glycols were introduced into supercoiled pBR322 DNA, and the ability of the proteins to nick form I (supercoiled) DNA was monitored by the relaxation of supercoiled DNA via single-strand breaks. The percentage of incised DNA was determined by summing the amounts of form II (relaxed circular) and form III (linear) DNAs, and calculating that value as a percentage of total DNA in the reaction. Fig. 5 shows the results of this assay, which closely parallel parallel 5-OH-Ura-containing oligonucleotide assays. Mutations at the aspartates (D129N and D160N) did not affect enzyme activity on thymine glycol containing DNA, which agrees with the 5-OH-Ura data presented in Fig. 3. Both E6Q and E174Q have significant, albeit diminished, glycosylase activity as compared with endoVIII. P2T, E3D, and E3Q have negligible thymine glycol nicking activity; the ∼20% level of incised DNA in these three reactions was not dependent on enzyme concentration.

Enzymatic Activity as Analyzed by GC/IDMS—Aqueous solutions of calf thymus DNA were γ-irradiated under N₂O to create a multitude of oxidatively damaged bases. Upon incubation with active enzyme, those lesions that are substrates for the enzyme are released from the DNA and can be identified
Fig. 2. Relative oligonucleotide activities of endoVIII and P2T. DNA concentration was 1 nM, and enzyme concentrations were 0.1, 0.5, 1, 5, and 10 nM. Results are mean values ± S.D., n = 3. A, AP site nicking and trapping activities are shown for endoVIII (top row) and P2T (bottom row). AP trapping results are represented by three lines: AP DNA in trapped complex (●), AP DNA cleaved via β-δ-elimination (○), and the sum of the DNA trapped and nicked (▼). B, 5-OH-Ura nicking and trapping for endoVIII (top row) and P2T (bottom row) are shown. In control experiments with up to 2000× enzyme over 5-OH-Ura substrate, the maximum DNA cleaved never exceeded 60% and averaged 53% product.

and quantified by GC/IDMS as previously described (12, 28). Fig. 6 shows the results obtained for excision of FapyAde by the each enzyme, which is representative of analyses of various lesions. E6Q, D129N, and D160N retain glycosylase activity, although E6Q and D129N have significantly diminished activity. P2T, E3D, E3Q, and E174Q do not release any FapyAde residues above the background levels of the controls that have either no enzyme or heat-inactivated endoVIII. Analysis of the release of other substrates of endoVIII (12) showed trends similar to that of FapyAde for each enzyme.

**DISCUSSION**

The purpose of this study was to investigate the role of absolutely conserved amino acids in endoVIII catalysis. To better understand the results obtained and the contribution of these side chains, we have developed a minimal kinetic scheme for endoVIII, which is shown in Scheme 1. The formation of the Schiff base intermediate (E*SB) from enzyme (E) and substrate (S) is represented by the rate constant $k_1$, which encompasses many steps, including binding, damage recognition, base flipping, and the nucleophilic attack to form the covalent intermediate. From the Schiff base intermediate there are three pathways: $k_{1a}$, reversal of the imine to enzyme and substrate that are no longer covalently joined; $k_2$, hydrolysis of the Schiff base leading to the β-δ elimination product (P), and dissociation from product; or $k_3$, reduction of the Schiff base to produce a stable covalently trapped complex (ES$_{cov}$). The propensity of an enzyme to follow any of these paths is governed by...
the rate constants of each path for that enzyme. Reaction rates can be dependent on the pKₐ values of the ionizing side chains involved in the chemistry. Creating mutations in the active site can alter the electrostatic environment of the active site pocket and modulate the pKₐ values of neighboring functional groups, thus altering enzyme rate constants. The fourth path shown \(k_4\) is the rate of reduction of the AP site prior to enzymatic processing, which renders the reduced abasic DNA non-cleavable (designated rAP). In our experiments, NaCNBH₃ was used as a reducing agent, because it has a smaller \(k_4\) value than...
Covalent intermediate to either the N-terminal proline (in correlating conserved residues from likely to be located within endoVIII. The side chains of the residues of endoVIII that were investigated here are shown in color with side chains. The N-terminal proline is in red, and the five conserved asparagines of T. thermophilus are in blue. A, a close-up view of the active site pocket. The side chains of the relevant amino acids are labeled, using the numbers for the Fpg protein. Pro-2, Glu-3, and Glu-6 have the same numbers in endoVIII. The numbers of the three other corresponding residues in endoVIII are noted.

Three of the conserved acidic residues, Glu-6, Asp-129, and Asp-160, appear to be dispensable for catalytic activity on AP sites (31). Fpg P2G and P2T proteins did not possess lyase or glycosylase activities, demonstrating no significant nicking on oligonucleotides with either an AP site or OG lesion. However, Fpg P2G showed very efficient trapping on AP sites (31).

The results presented here can also be interpreted relative to their likely location in the structure of endoVIII. Recently, the crystal structure of a member of the endoVIII-Fpg family, Fpg from Thermus thermophilus, was determined previously (30). Assuming structural similarity between these two enzymes, we can approximate where the residues investigated here are likely to be located within endoVIII. The side chains of the correlating conserved residues from T. thermophilus Fpg are illustrated and colored on the structure shown in Fig. 7. The N-terminal proline (in red) sits in the proposed active site, and this residue has already been identified in endoVIII as the residue that cross-links to DNA (21), and the role of this proline in E. coli Fpg activity has been well documented (22, 31). Our results confirm that indeed the N-terminal proline of endoVIII is necessary for lyase and glycosylase activity as shown in Figs. 2, 5, and 6. Substituting the secondary amine of proline with the primary α-amino of threonine greatly decreases cleavage at AP sites and at three damaged bases, 5-OH-Ura, thymine glycols, and FapyAde. Although the P2T mutant was unable to efficiently catalyze β-elimination, it was very efficient in forming Schiff base intermediates on AP sites. It appears that the P2T mutation creates a very small $k_2$ value, shunting the covalent intermediate to either the $k_3$ or $k_{-1}$ pathway, while retaining a $k_1$ value approximately equal to endoVIII. These data do not provide sufficient information to know if the $k_4$ or $k_{-1}$ values for P2T are altered from endoVIII. P2T must have a significant $k_{-1}$ value (greater than or equal to $k_1$) in the absence of reductant, because no covalent complex was observed in reactions without NaCNBH₃ or NaBH₄. These results are consistent with the findings of mutations at the N-terminal proline of E. coli Fpg (31). Fpg P2G and P2T proteins did not possess lyase or glycosylase activities, demonstrating no significant nicking on oligonucleotides with either an AP site or OG lesion. However, Fpg P2G showed very efficient trapping on AP sites (31).

The glutamate at position three is critical for enzyme glycosylase catalysis. The results presented here for the E3D and E3Q mutants are expected, given the proximity of Glu-3 to the active site (Fig. 7). This glutamate side chain inserts directly into the active site of the enzyme and is right next to the active site nucleophile. Mutating Glu-3 to either aspartate or glutamine retains AP lyase activity, suggesting that Glu-3 is not directly involved in simple lyase reactions. However, Schiff base formation is hindered in E3D and E3Q, suggesting a lower $k_1$ rate for these enzymes (Fig. 4). Interestingly, the shorter yet still negatively charged aspartate at position 3 (E3D) consistently gives a significant amount of β-elimination product (~30–40%) on AP sites, in addition to the typical β-δ-elimination.
tion product (~60–70%, data not shown). These data suggest that moving the carboxyl group by the distance of a methylene group may allow a water molecule to occupy the vacated space. The water molecule may then hydrolyze the imine more rapidly than if a glutamate occupied this position, effectively stopping the β-elimination at only β-elimination.

EndoVIII cannot tolerate alterations at Glu-3 and still retain glycosylase/lyase activity on 5-OH-thymine glycols, or FapyAde as shown in Figs. 4–6. These results are in agreement with Glu-3 mutational analysis of E. coli Fpg (25). In endoVIII, eliminating this negative charge (E3Q) or shortening the side chain by one methylene group (E3D) prevents the enzyme from removing damaged bases from their deoxyribose rings. Hence, Glu-3 must be directly involved in the glycosylase activity of endoVIII. The role of acidic side chains in glycosylase enzymes is not clear, and several proposals have been made (32–34). One possibility is that the negative charge serves to stabilize a positively charged oxocarbenium ion intermediate, or to stabilize the positively charged Schiff base glycosylase product. Another possibility is that the acidic side chains serves to directly protonate either the damaged base or the O4’ of the sugar to facilitate glycosidic bond cleavage.

An interesting result is the effect of the E174Q mutation on endoVIII, which appears to be significantly deleterious. E174Q enzyme preparations consistently have a low percentage of active molecules, and even after correcting for the concentration of active sites, we still observe low activity of this enzyme on oligonucleotides. Structurally, the corresponding glutamate (Glu-166) in T. thermophilus is in the D helix of the helix-two-turns-helix motif (Fig. 7), which is removed from the active site. The side chain of Glu-166 points away from the pocket and appears that it would not interact with the substrate lesion at all. Nonetheless, this residue is completely conserved in this family, and the results of this study and of Lavrukhin and Lloyd (25) show that glycosylase/lyase activity of both endoVIII and Fpg are dependent on an intact glutamate at this position. Because it is unlikely that Glu-174 in endoVIII participates directly in the catalytic chemistry, we suggest two possible roles: to maintain structural integrity within the enzyme through interactions with other residues or to alter the electrostatics and pKa values within the active site.

The results of this study confirm the involvement of the N-terminal proline as the active site nucleophile and identify two conserved glutamates that are critical for the glycosylase activity of endoVIII. Pro-2 is necessary for both the glycosylase reaction and the lyase reaction, and our results are consistent with the role of the nucleophile in glycosylase enzymes. The most catalytically important amino acids of the conserved aspartates and glutamates are Glu-3 and Glu-174, which mediate the removal of a damaged base in the glycosylase reaction but are not necessary for the enzyme to perform the lyase mechanism on AP sites. Additionally, this work strongly underscores the similarities in catalytic mechanism between members of the endoVIII-Fpg family, despite broad differences in substrate specificity.

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