Functional Analysis of the Amino-terminal 8-kDa Domain of DNA Polymerase β as Revealed by Site-directed Mutagenesis

DNA BINDING AND 5′-DEOXYRIBOSE PHOSPHATE LYASE ACTIVITIES

(Received for publication, January 27, 1998)

Rajendra Prasad, William A. Beard, Jan Y. Chyan, Mark W. Maciejewski, Gregory P. Mullen, and Samuel H. Wilson

From the Laboratory of Structural Biology, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709, Sealy Center for Molecular Science, University of Texas Medical Branch, Galveston, Texas 77555, and Department of Biochemistry, University of Connecticut Health Center, Farmington, Connecticut 06032

The amino-terminal 8-kDa domain of DNA polymerase β functions in binding single-stranded DNA (ssDNA), and as a 5′-deoxyribose phosphate (dRP) lyase. NMR and x-ray crystal structures of this domain have suggested several residues that may interact with ssDNA or play a role in the dRP lyase reaction. Nine of these residues were altered by site-directed mutagenesis. Each mutant was expressed in Escherichia coli, and the recombinant protein was purified to near homogeneity. CD spectra of these mutant proteins indicated that the alteration did not adversely affect the global protein structure. Single-stranded DNA binding was probed by photochemical cross-linking to oligo(dT)₁₆. Several mutants (F25W, K35A, K60A, and K68A) were impaired in ssDNA binding activity, whereas other mutants (H34G, E71Q, K72A, E75A, and K84A) retained near wild-type binding activity. The 5′-phosphate recognition activity of these mutants was examined by UV cross-linking to a 5-nucleotide gap DNA where the 5′ terminus in the gap was either phosphorylated or unphosphorylated. The results indicate that Lys⁸⁵ is involved in 5′-phosphate recognition of DNA polymerase β. Finally, the dRP lyase activity of these mutants was evaluated using a preincised apurinic/apyrimidinic DNA. Alanine mutants of Lys⁸⁵ and Lys⁶⁸ are significantly reduced in dRP lyase activity, consistent with the lower ssDNA binding activity. More importantly, alanine substitution for Lys⁷² resulted in a greater than 90% loss of dRP lyase activity, without affecting DNA binding. Alanine mutants of Lys⁶⁸ and Lys⁸⁴ had wild-type dRP lyase activity. The triple alanine mutant, K35A/K68A/K72A, was devoid of dRP lyase activity, suggesting that the effects of the alanine substitution at Lys⁷² and Lys⁶⁸ were additive. The results suggest that Lys⁷² is directly involved in formation of a covalent imino intermediate and are consistent with Lys⁷² as the predominant Schiff base nucleophile in the dRP lyase β-elimination catalytic reaction.

Genomic DNA is constantly exposed to various endogenous and external environmental agents leading to DNA base loss and/or damage. To remove such damage and retain genome stability, the base excision DNA repair pathway has been maintained in essentially all organisms. Base excision repair was initially described in Escherichia coli (1) and later in mammalian cells (2). This repair pathway is initiated by enzymatic removal of an inappropriate base or spontaneous hydrolysis of bases through cleavage of the N-glycosyl bond (3, 4). The resulting apurinic/apyrimidinic (AP) site is cleaved by a class II AP endonuclease (5), which incises the phosphodiester backbone 5′ to the AP site resulting in a 3′-hydroxyl and 5′ 2-deoxyribose 5-phosphate (dRP) containing termini. To complete repair, the dRP moiety is removed so that a single-nucleotide gap with a 3′-hydroxyl and 5′-phosphate is generated (6, 7). DNA polymerase β (β-pol) replaces the missing nucleotide (7–9), and DNA ligase I seals the nicked product (9, 10). These enzymatic activities should be coordinated for efficient base excision repair.

β-pol is a multifunctional enzyme consisting of an 8-kDa amino-terminal domain with dRP lyase activity (11, 12) and a 31-kDa carboxy-terminal domain with nucleotidyltransferase activity (13). The crystal and solution structures of the amino-terminal 8-kDa domain have been determined (14, 15). The 8-kDa domain (residues 1–87) is formed by four helix-hairpin-helix (HhH) motifs, and as a 5′-deoxyribose 5-phosphate (dRP) containing termini. To complete repair, the dRP moiety is removed so that a single-nucleotide gap with a 3′-hydroxyl and 5′-phosphate is generated (6, 7). DNA polymerase β (β-pol) replaces the missing nucleotide (7–9), and DNA ligase I seals the nicked product (9, 10). These enzymatic activities should be coordinated for efficient base excision repair.

The abbreviations used are: AP, apurinic/apyrimidinic; β-pol, DNA polymerase β; dRP, 2′-deoxyribose 5′-phosphate; HhH, Helix-hairpin-Helix; HPLC, high pressure liquid chromatography; UDG, uracil-DNA glycosylase; ssDNA, single-stranded DNA; PAGE, polyacrylamide gel electrophoresis.

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Present address: Dept. of Pathology, University of South Alabama, Mobile, AL 36617.

To whom correspondence should be addressed: Laboratory of Structural Biology, National Institute of Environmental Health Sciences, 111 T.W. Alexander Drive, Bldg. 101, Rm. B246, Research Triangle Park, NC 27709. Tel.: 919-541-3267; Fax: 919-541-2260; E-mail: wilson5@niehs.nih.gov.
four lysine residues (residues 35, 68, 72, and 84) in this region of the protein coordinate the DNA 5'-phosphate that may exist in a gapped DNA (17). However, in structures of β-pol bound to a one-nucleotide DNA gap only Lys35 and Lys68 coordinate the 5'-phosphate in the short gap (21). Based on information available from the crystal and NMR structures of the 8-kDa domain and from biochemical studies of the protein (14–16, 22), we have now conducted site-directed mutagenesis to alter 9 residues in the 8-kDa domain of β-pol that appear to contribute key interactions to ssDNA binding, 5'-phosphate recognition in a DNA gap, and dRP lyase activity. The results allow us to identify two critical residues, Lys35 and Lys72, for 5'-phosphate recognition and dRP lyase chemistry, respectively.

EXPERIMENTAL PROCEDURES

Materials—Synthetic oligodeoxyribonucleotides purified by HPLC were obtained from Operon Technologies, Inc. Unphosphorylated oligodeoxythymidylylate, (dT)16, was from Pharmacia. [α-32P]dATP and [γ-32P]ATP (3000 Ci/mmol) were from Amersham. Terminal deoxynucleotidyltransferase and T4 polynucleotide kinase were from Promega. Human AP endonuclease and uracil-DNA glycosylase (UDG), with 84 amino acids deleted from the amino terminus, were purified as described (23, 24).

Mutagenesis, Expression, and Purification of the Recombinant Wild-type 8-kDa and Mutant Proteins—Oligodeoxynucleotide site-directed mutagenesis was performed using a procedure described previously (25). Recombinant amino-terminal 8-kDa domain and the mutant proteins were overexpressed and purified as described (22).

Circular Dichroism Spectroscopy—For CD analysis, the wild-type 8-kDa domain of β-pol and the mutants were further purified by gel filtration on HPLC using a Bio-Sel SEC-125 (300 × 7.8 mm) size exclusion column (Bio-Rad). Buffer consisted of 5 mM Tris-HCl, pH 7.2, and 500 mM NaCl. The chromatogram for the mutants was compared with the chromatogram of the highly purified wild-type 8-kDa domain in selecting the pooled peak fraction for each mutant. The concentrations of the mutant proteins were determined by UV absorption at 280 nm (ε280 = 5440 M⁻¹ cm⁻¹). CD measurements were performed on a Jasco J715 spectropolarimeter in a 1-cm cell at 25 °C. The CD spectra were collected from 260 to 200 nm at a resolution of 5 nm every 5 scans. The per residue molar ellipticity (deg cm² dmol⁻¹) was calculated from the concentration for the 87-residue polypeptide.

5'-End Labeling—Unphosphorylated oligodeoxyribonucleotide (P₂) was labeled by T4 polynucleotide kinase using [γ-32P]ATP as described (26, 27).

Template-Primer Annealing—Lyophilized oligodeoxynucleotides were resuspended in 10 mM Tris-HCl, pH 7.4, and 1 mM EDTA, and the concentrations were determined from their UV absorbance at 260 nm. Template-primers were annealed as described previously (27). The sequences of the 5'-nucleotide gapped DNA used was as follows.

(P₂)17-mer

5'-GCTCGGTACCGGCGATCCGTCTAAAAAACGCCACGGTCC-3' 
3'-GCTCGGTACCGGCGATCCGTCTAAAAAACGCCACGGTCC-5' 
(T39-mer) 
SEQUENCE 1

UV Cross-linking to Gapped DNA—Purified wild-type 8-kDa domain or the mutant protein (1.4 μM) was mixed with the gapped DNA template-primer (0.7 μM) in a reaction mixture containing 20 mM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA, and 5 mM MgCl₂ and incubated at room temperature for 15 min. The samples were irradiated, and the photochemical cross-linked 8-kDa protein-[32P]dT₆₄ complexes were separated and analyzed as described (22). To quantify cross-linking, the dried gels were scanned on a PhosphorImager 450 (Molecular Dynamics), and the data were analyzed using ImageQuant software.

UV Cross-linking of Oligo(dT)₆₄—Typically, wild-type 8-kDa domain or a mutant protein (50 μM) was mixed with [32P]dT₆₄ (14 μM) in a 15-μl reaction mixture containing 20 mM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA, and 5 mM MgCl₂. The samples were irradiated, and the photochemical cross-linked 8-kDa protein-[32P]dT₆₄ complexes were separated and analyzed as described (22). To quantify cross-linking, the dried gels were scanned on a PhosphorImager 450 (Molecular Dynamics), and the data were analyzed using ImageQuant software.

RESULTS AND DISCUSSION

Structure-guided Site-directed Mutagenesis and Purification of Mutant Proteins—To probe the functional importance of residues in the amino-terminal 8-kDa domain, 9 residues were selected from x-ray (14, 17, 21) and NMR (15, 16) structural analyses and altered by site-directed mutagenesis as described previously (25). These residues were Phe25, His15, Lys35, Lys60, Glu71, Lys72, and Lys84. The residues and alterations selected for mutagenesis were based on the proposed role of each residue in DNA binding (15, 22) or putative role in dRP lyase chemistry, as discussed in detail previously (11, 12, 28). Briefly, the primary structure of a portion of the HhH motif (residues 55–79) in the 8-kDa domain is similar to that of the HhH motif in E. coli endonuclease III glycosylase/AP lyase (17–20). In addition, significant intermolecular nuclear Overhauser effects and chemical shift changes were observed in the 1H-15N heteronuclear single quantum correlation NMR spectra for surface-exposed residues upon addition of ssDNA (15), including residues Phe25, Lys84, Glu71, Lys72, and Lys84. Lys84 is adjacent to residues showing chemical shift changes. Lys68 is in the NMR unstructured linker region between the 8-kDa and 31-kDa domains but is coordinated with the 5'-phosphate in one of the crystal structures, along with Lys35, Lys60, and Lys72 (16, 17). In the NMR structure, the adjacent flexible ß-loop residue Lys35 contributes to the basal surface charge potential and is adjacent to Lys68 and Lys72 (15). Also in the ß-loop, His44 has been shown to cross-link with ssDNA (22) and to stack with a template strand base in the crystal structure of β-pol bound to DNA (22).

The positions of the 9 residues selected for mutagenesis in the crystal structure of β-pol bound to one-nucleotide gapped DNA are illustrated in Fig. 1. Single mutations were constructed at residues Phe25, His34, Glu71, and Glu72 and at the lysines at positions 35, 60, 68, 72, and 84. His34 was changed to glycine, and Glu72 was changed to glutamine to remove the base-stacking potential and carbohydrate moieties, respectively. Double mutants were constructed at Lys68 and Lys72 (K68A/K72A) and Lys84 and Glu71 (K68A/E71Q), and a triple alanine...
Mutagenesis of dRP Lyase Catalytic Site of β-pol

The wild-type 8-kDa domain. Similar results for the K35A/K68A/K72A triple mutant were also observed. On the basis of these results, we conclude that the substitutions chosen at these surface-exposed residues do not adversely affect the overall structure of the 8-kDa domain and that the effects of the mutations on ssDNA binding, 5'-phosphate recognition in gapped DNA, and dRP lyase activity are likely the result of the loss of specific side chain functionality.

Single-stranded DNA Binding Activity—The ssDNA binding activity of the 39-kDa enzyme has been demonstrated to reside in the 8-kDa domain (22). The ssDNA binding activities of the wild-type 8-kDa domain and mutant proteins were examined by an assay involving photochemical cross-linking to oligo(dT)$_{16}$ as described previously (22). To assay ssDNA binding activity, purified protein was mixed with $[^{32}P]$(dT)$_{16}$ and irradiated with UV light to covalently cross-link the bound ligand. The cross-linked products were separated by SDS-PAGE and scored by autoradiography and PhosphorImager scanning (Fig. 3 and Table I). Under the conditions of the assay, the level of cross-linking is proportional to the equilibrium association constant, $K_a$ (22). Quantitative analysis of the UV cross-linked products indicated that the single mutants K35A, K60A, K68A, and F25W, and the triple mutant K35A/K68A/K72A were reduced in ssDNA binding by approximately 60–75% compared with wild type (Fig. 3 and Table I). The other mutants had similar ssDNA binding as wild type. Alanine substitution for Lys 72 did not affect ssDNA binding activity. In the intact enzyme, Lys$^{72}$ has been shown to be a target for pyridoxal 5'-phosphate modification and was protected by dNTP binding (29). Lys$^{72}$ has also been implicated in forming a Schiff base intermediate with abasic site DNA (12). The mutant bearing a glycine substitution for His$^{34}$ was slightly reduced (≈25%) in ssDNA binding activity. This histi-

---

**Fig. 1.** Interactions between the amino-terminal 8-kDa domain of DNA polymerase β and the 5'-phosphate in a one-nucleotide DNA gap (PDB code 1BPY). This figure was generated using GRASP (30). The molecular surface of only the 8-kDa domain is illustrated. The 31-kDa domain, which possesses nucleotidyltransferase activity, is omitted for clarity. The DNA is orange, and the 5'-deoxyxynucleoside monophosphate in the one nucleotide gap is yellow. The 5'-phosphate of this terminal nucleotide binds near a lysine-rich pocket. Residues altered by site-directed mutagenesis are indicated. Lysine residues 35, 60, 68, 72, and 84 (blue) were altered individually or in combination. Additionally, residues in red have been implicated in ssDNA binding (Phe$^{25}$, His$^{34}$, Glu$^{71}$, and Glu$^{75}$) and were modified as outlined under “Experimental Procedures.” The Na$^+$ ion bound to the hairpin of the HhH motif is indicated (purple). The HhH structural motif has been identified by primary sequence alignment in several other DNA repair enzymes and suggested to play a role in DNA binding (17, 18).

---

**Fig. 2.** Circular dichroism spectra of the site-directed mutants of the β-pol 8-kDa domain. The CD spectra were collected as described under “Experimental Procedures” and are shown for the K35A, K68A, and K72A single mutants (top panel), the K68A/K72A double mutant, the K35A/K68A/K72A triple mutant, and the K64A single mutant (middle panel), and for the E71Q and E75A single mutants (bottom panel). The CD spectrum of the wild-type 8-kDa domain of β-pol is included in each panel.

---

Mutagenesis of dRP Lyase Catalytic Site of β-pol
FIG. 3. Single-stranded DNA binding activity of mutants of the β-pol 8-kDa domain. A photograph of an autoradiogram illustrating the results of UV cross-linking to [32P]dT16. Purified wild-type 8-kDa or mutant protein (50 μg) was mixed with [32P]dT16 (14 μg) and irradiated as described under “Experimental Procedures.” The cross-linked complexes were separated from free probe by 15% SDS-PAGE and analyzed by autoradiography. The positions of the UV cross-linked product (8-kDa domain-oligo(dT)16) and free probe are indicated. The gel was quantified as outlined under “Experimental Procedures,” and the results are summarized in Table I.

![Image](image)

**Summary of ssDNA binding, 5'-phosphate recognition, and dRP lyase activities of mutants of the 8-kDa domain of β-pol**

| Mutant          | dRP lyasea | ssDNA bindingb | 5'-Phosphate recognitionc |
|-----------------|------------|-----------------|---------------------------|
| K72A            | +          | +++++           | +                         |
| K68A/K72A       | + +        | +++;           +            |
| K35A/K68A/K72A  | --         | +; +           +            |
| K35A            | ++         | +               +            |
| K60A            | ++         | +               ++           |
| H34G            | ++         | +               ++           |
| E75A            | ++         | +               ++           |
| F25W            | ++         | +               ++           |
| K68A            | ++         | +               ++           |
| K84A            | ++         | +               ++           |
| E71Q            | ++         | +               ++           |
| K68A/E71Q       | ++         | +               ++           |

a Activity relative to wild-type: +++++, 75–100%; +++, 50–75%; ++, 25–50%; +, 1–25%; −, <1%; ND, not determined. Data were analyzed as described under “Experimental Procedures” and tabulated from the data in Figs. 3–5 for ssDNA binding, 5'-phosphate recognition, and dRP lyase activities, respectively.

b The relative activity is determined by the ability of the mutant 8-kDa domain to discriminate between a 5'-phosphorylated and unphosphorylated terminus in a gap as compared with wild type as described under “Experimental Procedures.”

d The finding that Lys 35 is a key residue in 5'-phosphate recognition activity, alanine substitutions of Lys35, Lys68, or Lys72 retained the recognition activity (Fig. 4A and Table I). The E75A mutant also displayed a diminished ability to discriminate between the phosphorylated and unphosphorylated gaps. The lower amount of cross-linking for the K60A and K68A mutants is consistent with the lower ssDNA binding activity of these mutants, but both mutants retained the 5'-phosphate recognition activity of wild type (Fig. 4A and Table I). Both NMR and crystallography data suggested that Lys35 and Lys68 are sites for 5'-phosphate contact (15, 17, 21). Our results on 5'-phosphate recognition with the K35A mutant support the conclusion that Lys35 coordinates the 5'-phosphate group, whereas Lys68 does not.
important for ssDNA binding, whereas Lys35 is critical for 5'-phosphate recognition. Lys35 and the exposed residues displaying significant NMR chemical shift changes upon binding (dT)₉, such as Glu71, had little or no influence on the DNA binding activities tested when mutated to alanine or glutamine (Figs. 3 and 4 and Table I).

dRP Lyase Activity—To examine the dRP lyase activity of wild-type and mutant enzymes, we utilized a 49-residue oligonucleotide duplex DNA, which contained a uracil residue at position 21. The uracil-containing strand was 3'-end labeled with [α-³²P]ddAMP and annealed to its complementary DNA strand. To prepare DNA substrate for the dRP lyase reaction, the ³²P-labeled duplex DNA was pretreated with UDG and AP endonuclease. Thus, the resulting DNA substrate contains a 5'-dRP group and a ³²P-labeled ddAMP residue at the 3'-end of the downstream DNA strand (Fig. 5B). Wild-type and mutant enzymes were incubated with this pretreated ³²P-labeled duplex DNA, and at the end of each reaction period the DNA product was stabilized by NaBH₄. The release of 5'-dRP from the ³²P-labeled substrate was determined by the appearance of a new radioactive electrophoretic band migrating approximately one-half nucleotide faster than the substrate (Fig. 5A). Results of dRP lyase activity of alanine substitutions are shown (Fig. 5A), but the results of all the mutants have been summarized (Fig. 5C and Table I). Our results indicate that Lys84 retained wild-type dRP lyase activity when mutated to alanine. We had previously considered that this residue was a candidate nucleophile involved in Schiff base formation during the lyase reaction, based on sequence alignment with endonuclease III (28). Mutagenesis of the corresponding lysine (residue 120) in the HhH motif of endonuclease III strongly reduced AP site lyase activity (20). In contrast, our results indicate that Lys84 is not a candidate for Schiff base formation in the dRP lyase reaction catalyzed by the 8-kDa domain of β-pol. While mutants E75A, K35A, H34G, and K60A retained approximately 40–75% dRP lyase activity, the K72A had less than 10% activity of wild type (Fig. 5C). There was no further decline in dRP lyase activity with the K68A/K72A double mutant over that of the K72A single mutant, suggesting that a Schiff base nucleophile role for Lys84 in the K72A mutant is unlikely.

Near wild-type dRP lyase activity was observed for F25W, K84A, E71Q, and the K68A/E71Q double mutant. The triple mutant, K35A/K68A/K72A, was essentially devoid of dRP lyase activity (<1%), and use of a 10-fold higher protein concentration also failed to reveal detectable activity (data not shown).

Our data suggest that Lys72 is the best candidate as the Schiff base-forming nucleophile. This interpretation must be considered in light of the results obtained by Piersen et al. (12), who demonstrated that a Schiff base intermediate could be formed with the K72A mutant of intact β-pol, albeit at a strongly reduced efficiency. Reduction in the dRP lyase activity of the alanine mutant of Lys60 may be attributed to a significant loss in ssDNA binding. A role of His84 in β-elimination chemistry has also been proposed, and the loss in dRP lyase activity of the H34G mutant is consistent with a role for this residue in lyase chemistry, as suggested previously (28).

The activities characterized for each 8-kDa domain mutant are summarized in Table I. Although Lys72 is the candidate for Schiff base formation during the dRP lyase reaction, the K72A mutant retained significant residual activity. Thus, Lys72 may be the preferred, but not obligatory, Schiff base nucleophile. The residual activity with K72A may be contributed from an alternate Schiff base nucleophile in the lysine-rich pocket formed in the wild type by Lys84, Lys68, Lys72, and Lys84 (21). In the absence of Lys72, partial deprotonation at a nearby amine, such as Lys84, could activate this amine and allow it to function as a backup Schiff base nucleophile. This explanation is strengthened by the observation of complete loss of dRP lyase activity in the triple mutant where lysines 35, 68, and 72 are changed to alanine. Taken together, our results clearly demonstrate that Lys72 and Lys84 are involved in the dRP lyase reaction catalyzed by the 8-kDa domain and that Lys84 is involved in 5'-phosphate recognition. Lys84 may stabilize the leaving group in the dRP lyase reaction through its interaction with the 5'-phosphate of the DNA terminus, and Lys84 could be activated in this role by His84.

In conclusion, it is interesting to compare the results of our site-directed mutagenesis with NMR and x-ray crystallography studies of the 8-kDa domain. Alanine substitution for lysine at residues 35, 60, and 72 resulted in loss of function for 5'-phosphate recognition, DNA binding, and dRP lyase, respectively. In each case, this loss of function was perfectly consistent with the structural data, e.g., Fig. 1. Similarly, Lys84 and Glu71 are distal to the dRP lyase active site and also to the DNA
binding interface, and the K84A and E71Q mutants are not altered in any of the activities tested. The I34G mutant shows loss of a portion of the dRP lyase activity, and this is consistent with predictions from NMR studies (27). In contrast, the E75A mutant has lower 5'-phosphate recognition, and this is not suggested by the structural data. The results with Lys68 are surprising. The K68A mutant, as with K35A and K60A, is lower in DNA binding activity. However, K68A is not lower in either 5'-phosphate recognition or dRP lyase activity, as expected.

REFERENCES
1. Franklin, W. A., and Lindahl, T. (1988) EMBO J. 7, 3617–3622
2. Price, A., and Lindahl, T. (1991) Biochemistry 30, 8631–8637
3. Lindahl, T., and Nyberg, B. (1974) Biochemistry 13, 3405–3410
4. Doetsch, P. W., Helland, D. E., and Haseltine, W. A. (1986) Biochemistry 25, 2212–2220
5. Doetsch, P. W., and Cunningham, R. P. (1990) Mutat. Res. 236, 173–201
6. Franklin, W. A., and Lindahl, T. (1988) EMBO J. 7, 8631–8637
7. Dianov, G., Price A., and Lindahl, T. (1992) Mol. Cell. Biol. 12, 1605–1612
8. Dianov, G., and Lindahl, T. (1994) Curr. Biol. 4, 1069–1076
9. Singhal, R. K., Prasad, R., Wilson, S. H. (1995) J. Biol. Chem. 270, 949–957
10. Kungland, A., and Lindahl, T. (1997) EMBO J. 16, 3341–3348
11. Matsumoto, Y., and Kim, K. (1995) Science 269, 699–702
12. Piersen, C. E., Prasad, R., Wilson, S. H., and Lloyd, R. S. (1996) J. Biol. Chem. 271, 17811–17815
13. Kumar, A., Abbotts, J., Karawya, E. M., and Wilson, S. H. (1990) Biochemistry 29, 7156–7159
14. Pelletier, H., Sawaya, M. R., Kumar, A., Wilson, S. H., and Kraut, J. (1994) Science 264, 1891–1903
15. Liu, D.-J., Prasad, R., Wilson, S. H., DeRose, E. F., and Mullen, G. P. (1996) Biochemistry 35, 6188–6200
16. Liu, D.-J., DeRose, E. F., Prasad, R., Wilson, S. H., and Mullen, G. P. (1994) Biochemistry 33, 9537–9545
17. Pelletier, H., Sawaya, M. R., Wolfe, W., Wilson, S. H., and Kraut, J. (1996) Biochemistry 35, 12742–12761
18. Seeberg, E., Edie, L., and Bjørnanglas, M. (1995) Trends Biochem. Sci. 20, 391–397
19. Mullen, G. P., and Wilson, S. H. (1997) Biochemistry 36, 4713–4717
20. Thayer, M. M., Ahern, H., Xing, D., Cunningham, R. P., and Tainer, J. A. (1995) EMBO J. 14, 4108–4120
21. Sawaya, M. R., Prasad, R., Wilson, S. H., and Kraut, J. (1997) Biochemistry 36, 11205–11215
22. Prasad, R., Kumar, A., Widen, S. G., Casas-Finet, J. R., and Wilson, S. H. (1993) J. Biol. Chem. 268, 22746–22755
23. Strauss, P. R., Beard, W. A., Patterson, T., and Wilson, S. H. (1997) J. Biol. Chem. 272, 1302–1307
24. Slupphaug, G., Eftedal, I., Kavli, B., Bharti, S., Helle, N. M., Haug, T., Levine, D. W., and Krokan, H. E. (1995) Biochemistry 34, 128–138
25. Kunkel, T. A., Roberts, J. D., and Zakour, R. A. (1987) Methods Enzymol. 154, 367–382
26. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., p. 11.31, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
27. Prasad, R., Beard, W. A., and Wilson, S. H. (1994) J. Biol. Chem. 269, 18966–18971
28. Mullen, G. P., Antuch, W., Maciejewski, M. W., Prasad, R., and Wilson, S. H. (1997) Tetrahedron 35, 12057–12066
29. Basu, A., Kedar, P., Wilson, S. H., and Modak, M. J. (1989) Biochemistry 28, 6305–6309
30. Nicholls, A., Sharp, K. A., and Henig, B. (1991) Proteins 11, 281–296