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# Deoxyribose Phosphate Excision by the N-Terminal Domain of the Polymerase β: The Mechanism Revisited†

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ABSTRACT: DNA polymerase β (Pol β) is one of the key enzymes in the base excision repair pathway. The amino-terminal 8 kDa domain of Pol β has an activity for excising a 5′-deoxyribose phosphate (dRP) group from preincised apurine/apyrimidine (AP) sites. Recent biochemical studies have identified the catalytic center of the 8 kDa domain and provided new insight into the mechanism of DNA repair by DNA polymerase β. By incorporating both structural and biochemical data, we present here a reaction mechanism for the 5′-dRP excision activity of the 8 kDa domain. This mechanism focuses on a catalytic groove near the helix−hairpin−helix (HhH) motif of the 8 kDa domain. Our model shows that the dRP group of the AP site can be stabilized in the catalytic groove through extensive interactions with the residues of the groove and be positioned close to the active center, Lys72, which catalyzes a β-elimination reaction by forming a Schiff base with the C1′ of the dRP group.

DNA damage caused by a myriad of environmental factors such as chemicals and UV or ionizing radiation is constantly being repaired by sophisticated DNA repair systems in almost all living organisms (*1*, *2*). These cellular defense systems include base excision repair (BER[1]), nuclear excision repair (NER), and DNA mismatch repair which corrects replication errors (*3*, *4*). Each DNA repair process requires the involvement of several proteins. In the base excision repair system, a set of proteins search the cellular DNA for the presence of damaged and/or modified bases, bind to these sites, and remove the bases by cleaving the N−C1′ glycosyl bond (*3*, *5*). The removal of the damaged and/or modified base creates an abasic or apyrimidine/apurine (AP) site on the DNA. These AP sites are recognized by another set of proteins which make phosphate backbone incisions at the 5′- and 3′-sides of the AP sites, removing the deoxyribose phosphate (dRP) group. The resultant gap is then filled with an appropriate nucleotide by DNA polymerase and sealed by a DNA ligase. Although most repair enzymes catalyze one specific step of the repair process, some of them have multiple functions. For example, bacterial endonuclease III (endo III) is a bifunctional enzyme with activities in DNA glycosylation and dRP excision (*6*). Mismatch-specific adenine glycosylase, MutY, on the other hand, is known as a monofunctional enzyme (*7*), i.e., removing the adenine base

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[1] Abbreviations: Pol β, DNA polymerase β; AP site, apurine/apyrimidine site; dRP, deoxyribose phosphate; HhH, helix−hairpin−helix; BER, base excision repair; NER, nuclear excision repair; endo III, endonuclease III.

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within G·A mismatches, although there are recent reports that suggest MutY also has dRP lyase activity (*8*, *9*).

DNA repair enzymes appear to be well-conserved machinery in cells throughout evolution. A number of eukaryotic homologues of bacterial repair enzymes have been discovered in recent years (*2*). Sequence homologues of *Escherichia coli* endo III have been found in both yeast and mammalian cells (*10*−*12*). These eukaryotic glycosylases appear to have the bifunctional DNA glycosylase/lyase activity. Not surprisingly, site-specific mutations on yeast (yOGG1) and mammanlian (mOGG1 and hOGG1) OG glycosylases at positions corresponding to the active site of the *E. coli* endo III have completely abolished the activity of these enzymes (*13*), suggesting that the functional mechanisms of these enzymes may be similar. It is conceivable that the basic principles realized in studying the bacterial DNA repair systems will be applicable to the studies of human diseases such as cancer, which may result from DNA repair deficiencies.

DNA polymerase $\beta$ (Pol $\beta$) is one of the key enzymes in the base excision repair system in eukaryotes (*14*). It specifically excises the 5′-terminal dRP group from a preincised AP site, which is generated after the removal of the modified and/or damaged base by DNA glycosylase and backbone incision by the AP-endonuclease at the 5′-side of the AP site, and then fills the gap with a single nucleotide complementary to the template (*15*, *16)*. Biochemical analyses have revealed that the protein mainly consists of two functional domains: an N-terminal 8 kDa domain which has a 5′-dRP excision activity and a 31 kDa polymerase domain (*15*). Structural studies have revealed that the polymerase domain of the Pol $\beta$ folds into a conformation similar to that of the Klenow fragment of *E. coli* DNA polymerase I with finger, palm, and thumb subdomains (*17*). The protein undergoes a large conformational change upon binding to its substrate. Before DNA binding, the Pol $\beta$ adopts an open conformation with its multiple domains enclosing a U-shaped cleft; upon binding to its DNA substrate, the protein shifts to a closed doughnut-like conformation where the N-terminal 8 kDa domain interacts with the C-terminal thumb subdomain (*18*). This conformational change brings the thumb subdomain close to the palm subdomain, where the DNA template binds, forming part of an active center for base incorporation. Being the smallest protein among five mammalian DNA polymerases and lacking an intrinsic exonuclease activity, Pol $\beta$ shows remarkable "accuracy" in filling appropriate nucleotides into short gaps in the base excision repair pathway (*19*). A detailed account of how Pol $\beta$ achieves such high fidelity was discussed in a number of recent articles (*18*, *20*, *21*). This paper focuses on the mechanism of 5′-dRP excision by the N-terminal 8 kDa domain.

The crystal structure of the Pol $\beta$ shows that the N-terminal 8 kDa domain is mainly α-helical with four α-helices termed A−D (Figure 1) (*17*). Helices A and B are aligned antiparallel to each other, while helix C is positioned at an angle of approximately 80° with respect to helices A and B. Helices C and D constitute a helix−hairpin−helix (HhH) motif, a novel DNA-binding motif found in a number of DNA repair enzymes (*5*). First identified in the structure of endo III (*22*, *23*), the HhH motif consists of two antiparallel α-helices aligned at an angle of approximately 25°. The hairpin loop connecting the two helices is a five-amino acid loop which forms a type II $\beta$-turn. This hairpin loop has unique sequence characteristics of XGV(I)GX, where X may be any residue, and its conformation is stabilized by a hydrogen bond between main chain atoms of residues at the first and fourth positions of the loop (*23*). Sequence analyses have revealed that at least 13 other families of DNA binding proteins contain the HhH motif (*24*). Since these proteins interact with a wide variety of both single- and double-stranded DNA sequences, the HhH motif is generally regarded as a non-sequence-specific DNA binding module.

The similarity between endo III and the 8 kDa domain of Pol $\beta$ has attracted much attention (*5*, *25*−*27*). The presence of HhH motifs near the active centers of both enzymes has led to the assumption that there is a general common mechanism for enzymatic reactions involving the HhH motif. On the basis of structural and biochemical data for endo III, Mullen and Wilson proposed a catalytic mechanism for 5′-dRP excision by the 8 kDa domain (*26*). In their mechanism, residue Lys68 of Pol $\beta$, which corresponds to the active center (Lys120) of endo III (*23*), was proposed to be the residue forming a Schiff base with C1′ of the dRP group. However, there are obvious differences between the structural environments of the HhH motifs of the two enzymes. The HhH motif from endo III constitutes part of a well-defined pocket in which the active site lies (*23*), whereas the HhH motif from the 8 kDa domain constitutes part of a catalytic groove formed between helices A, B, and D (see discussion later and Figure 1A,B). This catalytic groove is surrounded by lysine residues (Lys35, Lys68, Lys72, and Lys84) which form a distinctive positive patch on the surface of the domain (Figure 1A,B). The different structural appearances of the HhH motifs in these proteins strongly suggest that the two proteins may not share corresponding active centers and that the modes of protein−DNA interactions may be different. Indeed, these two proteins recognize completely different DNA substrates. Endo III, which has the dual activities of a DNA glycosylase and AP lyase, recognizes both damaged pyrimidine and intact AP sites, while the 8 kDa domain only processes preincised AP sites. In light of these observations, we carried out site-directed mutagenesis and in vitro DNA binding experiments. The results of these studies have unambiguously identified the active center of the 8 kDa domain (*28*) and offer new insight into the mechanism of the 5′-dRP excision by Pol $\beta$.

### *Specific Recognition of Substrate by Pol* $\beta$

Like many basic biological processes, the DNA repair process starts with specific DNA substrate recognition by repair enzymes. Although structural information on how the 8 kDa domain interacts with a preincised AP site is not yet available, crystal structures of Pol $\beta$ complexed with a nicked or a gapped DNA substrate do suggest some modes of interaction that may contribute to the specific DNA substrate recognition (*18*). The HhH motif of the 8 kDa domain is bound to the phosphate backbone of the DNA via direct hydrogen bondings between the polypeptide amide groups and the phosphate groups. There is no significant local conformational change in the hairpin loop before and after complex formation with the DNA, suggesting that DNA binding may not involve an "induced fit" mechanism. While these interactions may be common for all HhH motifs,

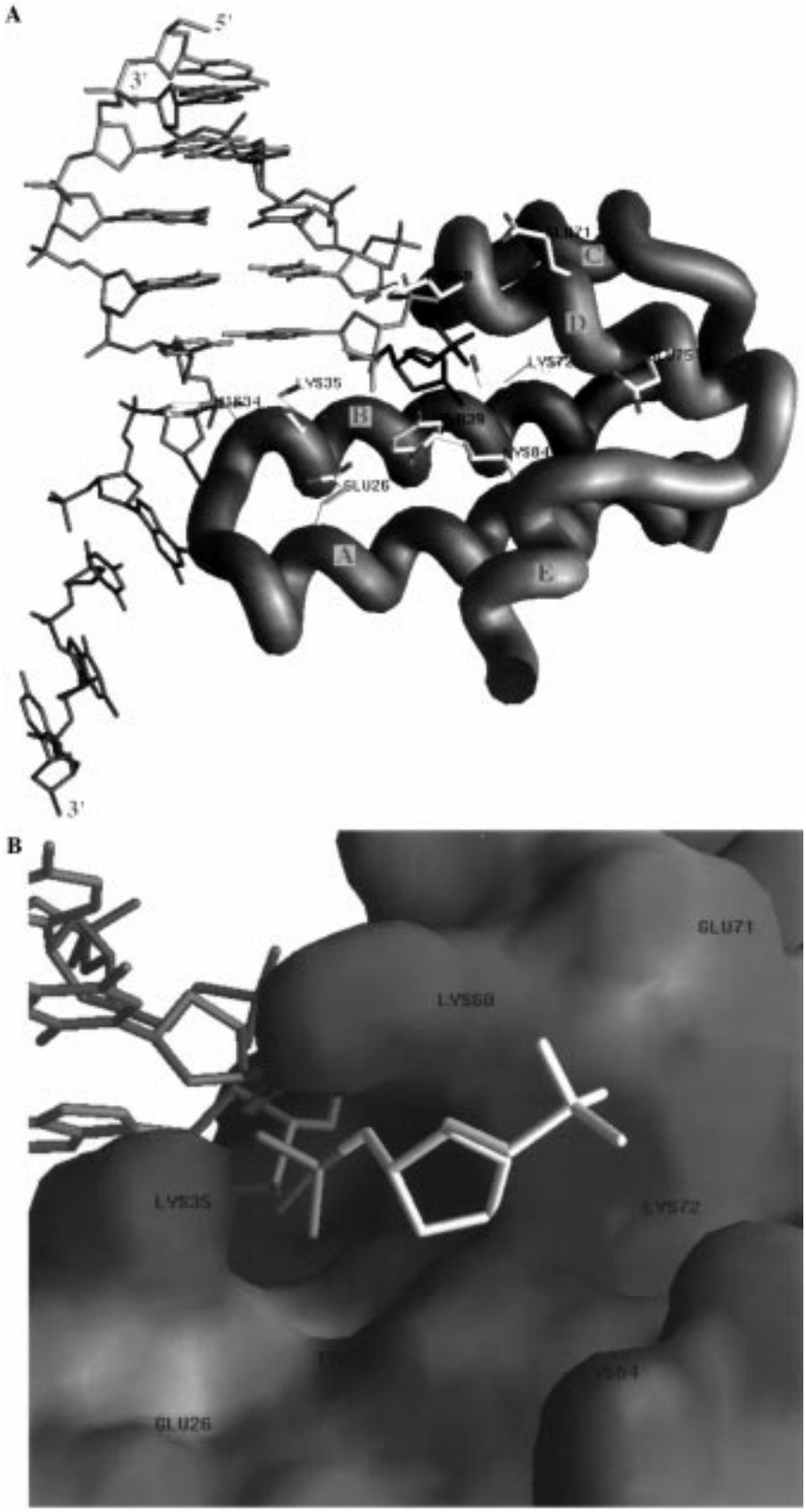


FIGURE 1: Computer-generated model of the 8 kDa domain bound to a preincised AP site. (A) A 5′-dRP group was modeled onto the crystal structure of the Pol $\beta$−gapped DNA complex. The DNA is represented in wire frames, and the 8 kDa domain is represented in ribbons. Residues relevant to the proposed catalytic mechanism are highlighted. The 5′-dRP group is dark gray. Four lysine residues, Lys35, Lys68, Lys72, and Lys84, constitute the distinctive positive patch at the surface of the 8 kDa domain. Residue Lys68, which interacts with the third phosphate from the 5′-terminal of the dRP group, guides the 5′-dRP group into a catalytic groove between helices A, B, and D. (B) A molecular surface representation of the active site showing the catalytic groove. Dark gray areas are positively charged, whereas light gray areas are negatively charged. Residues of interest are labeled to show their respective locations on the surface of the 8 kDa domain. The dRP group, in light gray, is positioned in the catalytic groove with the C1′ close to the active center, Lys72.

additional contacts are observed between residue Lys68 of the 8 kDa domain, one of the residues that makes up the positive patch near the HhH motif, and the 5′-phosphate group of the nicked or gapped substrate. Figure 1A shows a model of the 8 kDa domain bound to a preincised AP site, which is derived from the crystal structures of both Pol $\beta$−nicked DNA and Pol $\beta$−gapped DNA complexes, with a 5′-dRP group of the AP site positioned in the catalytic groove. In our model, the hairpin loop of the HhH motif is bound to the third and fourth phosphate groups from the dRP (Figure 1A). It is unlikely that the insertion of the dRP group into the catalytic groove will trigger any major conformational changes in the 8 kDa domain since the catalytic groove is large enough to accommodate the dRP and that the positive patch also provides an electrostatically favorable environment for the dRP binding. The side chains of residues Lys35, Glu26, Glu71, Lys72, and Lys84 could change conformation to establish hydrogen bonds with the terminal 5′-phosphate, and the hydroxyl groups of the deoxyribose ring. These contacts firmly anchor and properly orient the deoxyribose ring in close proximity to Lys72 located at the center of the catalytic groove. Tyr39 may also play a specific role in orienting the deoxyribose ring in the catalytic groove. On the basis of these structural data, we propose that the 8 kDa domain recognition of the preincised AP may be attributed to these additional contacts between amino acid residues in the catalytic groove and the dRP group. Indeed, in vitro DNA binding assays showed that the binding affinity of the 8 kDa domain for the nicked AP site is much higher than that for the AP nucleotide without nicking, implying that the 5′-phosphate was essential for recognition (*28*). Earlier studies of gapped DNA substrate binding by intact Pol $\beta$ also demonstrated the importance of the 5′-phosphate for specific protein−DNA interactions (*29*).

The specific DNA binding by intact Pol $\beta$ may also depend on the "bendability" of DNA. With its phosphate backbone incised at the 5′-side of the AP site, the DNA substrate of Pol $\beta$ is prone to deformation. As shown in the crystal structure, a 90° kink in DNA allows multiple domains of the Pol $\beta$ to interact with the substrate simultaneously, thus achieving efficient DNA processing; i.e., the 8 kDa domain removes the 5′-dRP group of the AP site, and the polymerase domain adds a new nucleotide to the 3′-end of the incision site. On the other hand, when Pol $\beta$ interacts with a regular B-DNA helix, where DNA bending is not readily achieved, no tight complex is formed. Therefore, the recognition process of Pol $\beta$ for a preincised AP site may involve two essential factors: enforced contacts between 8 kDa domain and the 5′ dRP group of the AP site and an induced fit mechanism involving sharply bent DNA and Pol $\beta$ (*18*).

### *Catalytic Mechanism of dRP Excision by the 8 kDa Domain*

Recent biochemical and mutagenesis studies have firmly established that Lys72 is the catalytic center for the dRP excision activity of the 8 kDa domain and that the dRP group is excised by $\beta$-elimination (*28*). Substitutions of Lys72 with Arg or Gln on the 8 kDa domain produced a 5′-dRP excision activity of less than 1% in comparison to that of the wild-type 8 kDa domain, whereas substrate binding affinity was not affected. Furthermore, these Lys72 mutants also formed significantly fewer Schiff base intermediates that could be trapped by reduction with sodium borohydride, thus providing evidence that Lys72 is the active center for Schiff base formation with C1′ of the deoxyribose. On the other hand, when residue Lys35, Lys68, or Lys84 was substituted with Arg or Gln, no significant reduction in 5′-dRP excision activity was observed.

On the basis of these data, we propose here a reaction mechanism for the 5′-dRP excision activity of the 8 kDa domain as depicted in Figure 2. The mechanism centers on a direct nucleophilic attack on C1′ by Lys72, which forms a Schiff base and facilitates subsequent cleavage of the dRP group by $\beta$-elimination. This direct nucleophilic attack of C1′ by Lys72 requires preopening of the deoxyribose ring. There are two possible ways of converting the deoxyribose into a ring-opened form. (1) After the removal of the damaged and/or modified base by a DNA glycosylase, the deoxyribose ring is rapidly interconverted between $\alpha$- and $\beta$-forms (*30*, *31*), and Lys72 may be able to attack C1′ in the intermediate ring-opened form. (2) One of the protonated lysine residues (Lys35 or Lys68) may donate a proton to the O4′ of the ribose ring and promote the deoxyribose ring opening. To be a good nucleophile, Lys72 has to be deprotonated before attacking C1′. This step may be facilitated by one of the nearby acidic residues, Glu71 or Glu75. After Schiff base formation, the p$K_a$ for the C2′ proton is lowered significantly, thus making it a target for proton abstraction by either one of the neighboring acidic residues, Glu71 and Glu26, or the deprotonated Lys35. The abstraction of the C2′ proton triggers a conjugation elimination event as depicted in Figure 2 and ultimately results in the cleavage of the 3′-C−O bond. The dRP group is subsequently released from the 8 kDa domain through a nucleophilic attack by a hydroxyl group which could be generated from a nearby water molecule by one of the neighboring acidic residues.

The proposed mechanism for the dRP excision activity of the 8 kDa domain is not only chemically consistent with the experimental data but also structurally plausible. The proposed interactions do not require large local conformational changes in both DNA and protein. The active center, Lys72, is partially shielded from the solvent as it is positioned at the bottom of the catalytic groove, as shown in the crystal structure (Figure 1A,B). This shielding effectively creates a chemical environment around Lys72 suitable for its catalytic activity. In vitro studies showed that a synthetic tripeptide of the sequence Lys-Trp(Tyr)-Lys can promote $\beta$-elimination at an aldehydic abasic site in DNA, suggesting that the excision reaction can occur spontaneously in an appropriate chemical environment (*32*). In our model (Figure 1), the dRP group is placed in the catalytic groove where residue Lys72 is in position to attack the C1′ of the dRP group. Residues Lys35, Lys68, Glu71, Glu75, and Glu26 are close to the deoxyribose ring, ready to donate protons to or accept protons from the reaction center. Residue His34 appears to play an important role in recognizing the AP site. In the crystal structures of Pol $\beta$−DNA complexes, His34 is inserted between the bases of the template strand at the gap site, promoting a 90° sharp kink in DNA and securing the DNA in "registry" with the Pol $\beta$, i.e., the binding of dRP group by the 8 kDa domain (*18*). Alternatively, His34 can be positioned in place of the missing base on the damaged strand as previously suggested (*26*) and establish

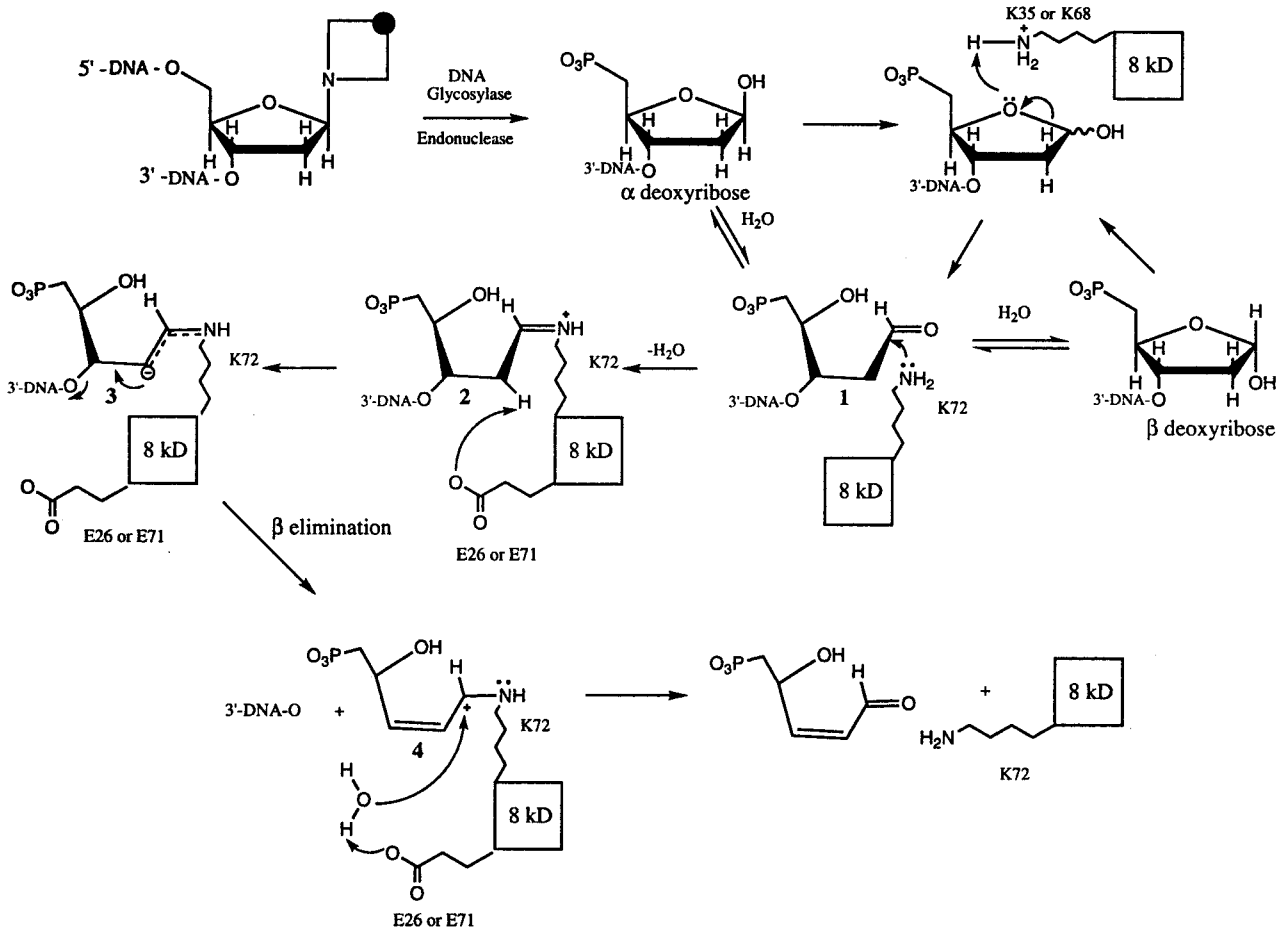


FIGURE 2: Schematics of our proposed reaction mechanism for the 5′-dRP excision by the 8 kDa domain. The damaged and/or modified base is represented by a square with a dot at the right upper corner. For the sake of clarity, only the relevant residues of the 8 kDa domain are included for each step of the reaction. The ring-opened form **1** can be generated by either water-mediated hydrolysis as the deoxyribose ring undergoes mutarotation or enzyme-assisted hydrolysis. Attack of an amine nucleophile (K72) on C1′ of **1** forms a covalently linked enzyme−substrate aminal intermediate, **2**. After facile rearrangement and generation of the Schiff base, the 2′-H of **2** is acidified. Abstraction of the 2′-H from **2** by one of the neighboring acidic residues (E26 or E71) yields an enamine, **3**, which undergoes a series of conjugate events, eliminating the dRP group by breaking the 3′-C−O bond. The α,β-unsaturated imine, **4**, is released from the 8 kDa domain as a result of a nucleophilic attack from a hydroxyl group.

stabilizing stacking energy with bases of the DNA helix. However, such a stacking interaction, resulting in sharp DNA bending by as much as 90°, has not yet been experimentally observed. DNA bending and induced fitting interactions between Pol $\beta$ and DNA are essential factors in precision DNA polymerization by Pol $\beta$ (*18*, *20*, *21*).

*Function of the HhH Motif*

The HhH motif has been generally known as a non-sequence-specific DNA binding motif (*24*). However, considering its presence in different families of proteins that are found in biological systems across the spectrum of evolution, it is likely that the HhH motif may play a specific role in regulating the function of these proteins. The HhH motif in the 8 kDa domain appears to play an important role in recognizing the preincised AP site. Residue Lys72, which has been identified as the residue which forms a Schiff base with C1′ of the dRP group (*28*), is located at the center of the catalytic groove formed between helices A, B, and D. In order to perform its lyase activity, the active center of 8 kDa must be brought as close to the DNA as possible. By binding the DNA backbone through its hairpin loop, the HhH motif brings the 8 kDa domain close to the DNA helix, thus enabling the dRP group of the AP site to interact with residues in the catalytic groove of the 8 kDa domain, particularly, the lysine residues of the positive patch discussed earlier. These additional contacts allow the HhH motif to have a "tighter" grip on this type of DNA structure than on a regular B-DNA. Therefore, the unique DNA binding features of the HhH motif of the 8 kDa domain assist the Pol $\beta$ in specifically recognizing its DNA substrate. On the other hand, not all HhH motifs can recognize similar types of DNA substrates. The sequence of the HhH motif and the residues of its surrounding structures should have a profound effect on its DNA binding activity. For example, the HhH motif of the endo III, which lacks many of the lysine residues found in the 8 kDa domain, binds DNA substrates with modified and/or damaged pyrimidine site without preincision (*23*).

Mullen et al. (*26*, *27*) previously proposed a reaction mechanism for the dRP excision activity of the 8 kDa domain. As mentioned earlier, their model was largely based on limited biochemical data on the active center of endo III and sequence homology between the HhH motifs of endo III and the 8 kDa domain. In that model, residue Lys68 was proposed to be the active center since it was found that

a mutation at the corresponding residue in endo III, Lys120, completely abolished its catalytic activity (the model was later modified to include Lys72 as a second candidate for Schiff base formation) (*27*). A "base checking" mechanism was also described for AP site recognition by the 8 kDa domain, in which hydrogen bonds were constantly being formed and broken between Lys68 and O2 of the pyrimidine base as the 8 kDa domain moved along the DNA helix. When the 8 kDa domain "detects" the AP site, where no hydrogen bond is formed, it stops moving and forms a hydrogen bond between Lys68 and O4′ of the deoxyribose ring which promotes ring opening for subsequent Schiff base formation (*26*, *27*).

One of the major differences between our model and the Mullen model is the location of the reaction center. According to the Mullen model (*26*, *27*), the reaction must occur in the proximity of His34, which is proposed to be positioned in place of the missing base in the DNA double helix. The side chain of the His34 is hydrogen-bonded to the aldehyde group of the ring-opened AP site; i.e., the reaction center is located within the boundary of the DNA double helix. If these geometrical constraints are necessary, it is difficult to place the catalytic center, Lys72, in the vicinity of C1′ of the dRP group, since Lys72 is located 6 Å from the DNA backbone or 8−9 Å from the C1′, unless the 8 kDa binds the DNA in a conformation that is considerably different from that of the crystal structures of Pol $\beta$−DNA complexes (*18*). On the other hand, our model requires a "side swing" of the dRP group into the catalytic groove next to the HhH motif of the 8 kDa domain and requires the group to be close to the active center, Lys72; i.e., the reaction occurs in the catalytic groove outside the boundary of the DNA double helix. The identity of the catalytic center, Lys68, of the Mullen model however was not supported by biochemical data for either the 8 kDa domain or intact Pol $\beta$ (*28*). One of the main weaknesses of the base checking mechanism is that it does not explain why the 8 kDa domain has a higher binding affinity for a DNA substrate with a preincised AP site than for a regular DNA substrate as shown by our in vitro binding assays (*28*).

*Concluding Remarks*

Recent studies of DNA polymerase $\beta$ have greatly improved our understanding of the basic mechanism of DNA repair by this enzyme. Pol $\beta$ employs a unique DNA recognition mechanism that is a combination of specific 5′-dRP group binding at the preincised AP site by the N-terminal 8 kDa domain and cooperative interaction between the DNA and the entire Pol $\beta$ protein, which result in major conformational changes in the protein and a sharp 90° kink in the DNA substrate. In contrast to previously proposed mechanisms, which either did not involve Lys72 in the catalytic reaction (*25*, *26*) or failed to identify the catalytic groove (*27*), both structural and biochemical data support a mechanism in which the catalytic reaction occurs at the center of a catalytic groove near the HhH motif of the 8 kDa domain (Figure 1A,B), and in which the 5′-dRP group swings away from the DNA helix and into the groove where Lys72 resides (*18*, *28*, *29*). On the other hand, more experimenal data are needed to construct a precise reaction mechanism for the base excision repair by Pol $\beta$, i.e., the identification of specific functional roles for every residue in the catalytic groove. Our model, which highlights the importance of the catalytic groove, presents a general framework from which one can design experiments for further elucidating each step of the reaction mechanism.

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## REFERENCES

1. Friedberg, E. C., Walker, G. C., and Siede, W. (1995) *DNA Repair and Mutagenesis*, pp 698, American Society for Microbiology, Washington, DC.
2. Wood, R. D. (1996) *Annu. Rev. Biochem. 65*, 135−168.
3. Sancar, A. (1996) *Annu. Rev. Biochem. 65*, 43−82.
4. Modrich, P., and Lahue, R. (1996) *Annu. Rev. Biochem. 65*, 101−134.
5. Seeberg, E., Eide, L., and Bjoras, M. (1995) *Trends Biochem. Sci. 20*, 391−397.
6. Kow, Y. W., and Wallace, S. S. (1987) *Biochemistry 26*, 8200−8206.
7. Bulychev, N. V., Varaprasad, C. V., Dorman, G., Miller, J. H., Eissenberg, M., Grollman, A. P., and Johnson, F. (1996) *Biochemistry 35*, 13147−13156.
8. Lu, A.-L, Tsai-Wu, J.-J., and Gillo, J. (1995) *J. Biol. Chem. 270*, 23582−23588.
9. Manuel, R. C., and Lloyd, R. S. (1997) *Biochemistry 36*, 11140−11152.
10. Hilbert, T. P., Boostein, R. J., Kung, H. C., Bolton, P. H., Xing, D., Cunningham, R. P., and Teebor, G. W. (1996) *Biochemistry 35*, 2505−2511.
11. Hilbert, T. P., Chaung, W., Boorstein, R. J., Cunningham, R. P., and Teebor, G. W. (1997) *J. Biol. Chem. 272*, 6733−6740.
12. Michaels, M. L., and Miller, J. H. (1992) *J. Bacteriol. 174*, 6321−6325.
13. Nash, H. M., Lu, R., Lane, W. S., and Verdine, G. L. (1997) *Chem. Biol. 4*, 693−702.
14. Sobol, R. W., Horton, J. K., Kuhn, R., Gu, H., Singhal, R. K., Prasad, R., Rajewsky, K., and Wilson, S. H. (1996) *Nature 379*, 183−186.
15. Beard, W. H., and Wilson, S. H. (1995) *Methods Enzymol. 262*, 98−107.
16. Matsumoto, Y., and Kim, K. (1995) *Science 269*, 699−702.
17. Pelletier, H., Sawaya, M. R., Kumar, A., Wilson, S. H., and Kraut, J. (1994) *Science 264*, 1891−1903.
18. Sawaya, M. R., Prasad, R., Wilson, S. H., Kraut, J., and Pelletier, H. (1997) *Biochemistry 36*, 11205−11215.
19. Beard, W. A., Osheroff, W. P., Prasad, R., Sawaya, M. R., Jaju, M., Wood, T. G., Kraut, J., Kunkel, T. A., and Wilson, S. H. (1996) *J. Biol. Chem. 271*, 12141−12144.
20. Kunkel, T. A., and Wilson, S. H. (1998*) Nat. Struct. Biol. 5*, 95−99.
21. Beard, W. H., and Wilson, S. H. (1998) *Chem. Biol. 5*, R7−R13.
22. Kuo, C.-F., McRee, D. E., Fisher, C. L., O'Handley, S. F., Cunningham, R. P., and Tainer, J. A. (1992) *Science 258*, 434−440.
23. Thayer, M. M., Ahern, H., Xing, D., Cunningham, R. P., and Tainer, J. A. (1995*) EMBO J. 14*, 4108−4120.
24. Doherty, A. J., Serpell, L. C., and Ponting, C. P. (1996*) Nucleic Acids Res. 24*, 2488−2497.
25. Pelletier, H., Sawaya, M. R., Wolfle, W., Wilson, S. H., and Kraut, J. (1996) *Biochemistry 35*, 12742−12761.
26. Mullen, G. P., and Wilson, S. H. (1997) *Biochemistry 36*, 4713−4717.
27. Mullen, G. P., Antuch, W., and Maciejewski, M. W. (1997) *Tetrahedron 53*, 12057−12066.

28. Matsumoto, Y., Kim, K., Katz, D. S., and Feng, J.-A. (1998) *Biochemistry 37*, 6456−6464.
29. Prasad, R., Beard, W. A., and Wilson, S. H. (1994) *J. Biol. Chem. 269*, 18096−18101.
30. Manoharan, M., Ransom, S. C., Mazumder, A., Gerlt, J. A., Wilde, J. A., and Bolton, P. H. (1988) *J. Am. Chem. Soc. 110*, 1620−1623.
31. Wilde, J. A., Bolton, P. H., Mazumder, A., Manoharan, M., and Gerlt, J. A. (1989) *J. Am. Chem. Soc. 111*, 1894.
32. Masumder, A., Gerlt, J. A., Absalon, M. J., Stubbe, J., Cunningham, R. P., Withka, J., and Bolton, P. H. (1991) *Biochemistry 30*, 1119−1126.

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