DNA polymerase $\beta$ (pol) cleaves the sugar-phosphate bond 3' to an intact apurinic/apyrimidinic (AP) site (i.e. AP lyase activity). The same bond is cleaved even if the AP site has been previously 5'-incised by AP endonuclease, resulting in a 5'-2-deoxyribose 5-phosphate (i.e. dRP lyase activity). We characterized these lyase reactions by steady-state kinetics with the amino-terminal 8-kDa domain of pol and with the entire 39-kDa polymerase. Steady-state kinetic analyses show that the Michaelis constants for both the dRP and AP lyase activities of pol are similar. However, $k_{\text{cat}}$ is approximately 200-fold lower for the AP lyase activity on an intact AP site than for an AP endonuclease-preincised site. The 8-kDa domain was also less efficient with an intact AP site than on a preincised site. The full-length enzyme and the 8-kDa domain efficiently remove the 5' dRP from a preincised AP site in the absence of Mg$^{2+}$, and the pH profiles of pol and 8-kDa domain dRP lyase catalytic efficiency exhibit a broad alkaline pH optimum. An inhibitory effect of pyridoxal 5'-phosphate on the dRP lyase activity is consistent with involvement of a primary amine (Lys$^{72}$) as the Schiff base nucleophile during lyase chemistry.

The cellular genome suffers extensive damage from exposure to ultraviolet light and ionizing radiation and also from alkylating agents and reactive oxygen species that accumulate in cells due to environmental stress and natural metabolic processes (1). These DNA lesions occur at a frequency far too high to be compatible with life unless they are repaired to allow faithful function and reproduction of the genome. To remove such damage and restore the integrity of the genome, specific DNA repair pathways have evolved. The major pathway, which protects cells from the deleterious effects of nucleotide base damage, is known as DNA base excision repair (BER).

BER is initiated by removal of a damaged or inappropriate base residue in DNA by cleavage of the N-glycosidic bond (1, 2). If not repaired, the resulting abasic or apurinic/apyrimidinic (AP) site may result in mutations, altered gene expression, chromo-

some breakage, apoptosis, and/or cell death.

Both procaryotes and eucaryotes possess class II AP endonucleases that cleave 5' to an AP site leaving a 3'-hydroxyl and a 5' 2-deoxyribose 5-phosphate (dRP) in the nicked DNA (Fig. 1). In Escherichia coli, two major AP endonuclease genes, xth and nfo, have been identified encoding exonuclease III and endonuclease IV, respectively (3, 4). Homologous genes to xth have been cloned from many sources, including Drosophila melanogaster and human cells (5–7). The major AP endonuclease in yeast, APN1, was shown to be an endonuclease IV homologue (8). These class II AP endonucleases also possess 3'-phosphodiesterase and 3'-phosphatase activities, and some members of the xth family carry 3' to 5' exonuclease activity (9). Subsequent to AP endonuclease cleavage in BER, the cleaved AP site must be processed further (Fig. 1). The enzymatic removal of the sugar-phosphate residue at the 5' terminus by a dRP lyase mechanism yields 4-hydroxy-2-pentenal-5-phosphate via β-elimination. Alternatively, hydrolytic cleavage releases dRP. The hydrolytic enzymes require Mg$^{2+}$ for activity (10–13).

Another class of AP endonuclease is termed AP lyase. These enzymes cleave the AP site 3' to the sugar by a β-elimination mechanism, leaving a nick with an unsaturated sugar-phosphate on the 3'-end and a 5'-phosphate (14–16). Traditionally, this AP lyase activity has been associated with DNA glycosylases such as thymine glycol-DNA glycosylase, formamidopyrimidine-DNA glycosylase, endonuclease V, and endonuclease VIII (4, 17–20). Subsequent β-elimination of the 3'-terminal sugar-phosphate produces 4-hydroxy-2-pentenal and 3'-phosphate (9) (Fig. 1B), which must be further processed to generate a 3'-hydroxyl required for DNA polymerase gap-filling synthesis. The single nucleotide gap is filled by DNA polymerase I in E. coli and by DNA polymerase β (pol) in mammalian cells (21–23). Since mammalian cells are known to contain multiple species of DNA ligase with broad substrate specificity, one of these enzymes completes the BER pathway by sealing the nicked DNA (24, 25).

DNA polymerase β is a 39-kDa monomeric enzyme. Limited proteolysis and physical studies have shown that pol consists of an independently folded amino-terminal domain of 8 kDa and a carboxyl-terminal domain of 31 kDa (26–28). The amino-terminal domain was originally characterized as a single-stranded DNA binding domain. Subsequently, it was found to possess binding specificity for the 5'-phosphate in gapped DNA (27–29) and a helix-hairpin-helix motif found in several other DNA repair enzymes (30, 31). Recently, Matsumoto and Kim (32) demonstrated that pol catalyzes removal of dRP from AP endonuclease-incised AP sites via β-elimination, as opposed to hydrolysis, and that this dRP lyase activity resides in the amino-terminal 8-kDa domain of pol. Further evidence that this reaction proceeds via β-elimination was obtained by Piersen et al. (33), who showed that a Schiff base intermediate...
is formed between the dRP-containing DNA substrate and the enzyme. The Schiff base nucleophile in the 8-kDa domain has been identified as Lys72 by site-directed mutagenesis (34). Surprisingly, the dRP lyase activity of β-pol was reported to be Mg2+-dependent (10, 32), whereas the β-elimination reactions described previously for other AP lyases do not require Mg2+ (11). In the present study, we investigated the catalytic efficiency and requirements of the β-pol dRP lyase activity using intact AP site-containing DNA substrates or substrates that have been preincised with AP endonuclease. The findings are discussed in the context of the NMR and crystal structures of the β-pol 8-kDa domain and the requirements of β-elimination chemistry in the dRP lyase reaction.

EXPERIMENTAL PROCEDURES

Materials—High pressure liquid chromatography-purified oligodeoxynucleotides were obtained from Operon Technologies, Inc. [α-32P]dATP (3000 Ci/mmol) was purchased from Amersham Pharmacia Biotech. Terminal deoxynucleotidyltransferase was from Promega. Recombinant human β-pol, the amino-terminal 8-kDa domain, and the carboxyl-terminal 31-kDa domain were overexpressed and purified as described previously (28, 35). Human AP endonuclease and uracil-DNA glycosylase with 84 amino acids deleted from the amino terminus were purified as described (36, 37). Antisera specific to intact β-pol and 8-kDa domain were raised in rabbits (22). Pyridoxal 5'-phosphate (PLP) was from Sigma. Alan E. Tomkinson (University of Texas Health Science Center, San Antonio, TX) provided human DNA ligase I that had been purified from baculovirus-infected insect cells (38).

γ,β-End Labeling—A 49-base pair (bp) oligodeoxynucleotide containing uracil at position 21 was labeled on its 3'-end by terminal deoxynucleotidyltransferase using [α-32P]dATP and annealed to its complementary strand by heating the solution at 90 °C for 3 min, followed by slow cooling to 25 °C. 32P-Labeled duplex oligodeoxynucleotide was separated from unincorporated [α-32P]dATP using a Nensorb-20 column according to the manufacturer's suggested protocol. The radiolabeled oligodeoxynucleotide was lyophilized, resuspended in H2O, and stored at −30 °C.

Preparation of DNA Substrates for AP Lyase and dRP Lyase Assays—32P-Labeled uracil-containing duplex oligodeoxynucleotide (62.5 nM) was pretreated for 20 min at 37 °C with uracil-DNA glycosylase (10 nM) in 100 μl of buffer containing 70 mM Hepes, pH 7.4, 0.5 mM EDTA, and 0.2 mM dithiothreitol. Due to the labile nature of the AP endonuclease-cleaved reaction is via β-elimination incising the AP site on the 3'-side of the sugar, AP endonuclease hydrolyzes the phosphodiester bond 5' to the AP site sugar (Fig. 2A). To study these reactions, we utilized a 49-bp oligodeoxynucleotide duplex DNA that contained a uracil residue at position 21. The uracil-containing treated DNA, the DNA substrate was prepared just prior to the dRP lyase assay.

Enzyme Assays—AP lyase activity was determined in a reaction mixture (10 μl) that contained 50 mM HEPES, pH 7.4, 2 mM dithiothreitol, with or without 5 mM MgCl2, and 20 nM 32P-labeled double-stranded oligodeoxynucleotide containing an AP site at position 21. The reaction was initiated by adding an appropriate dilution of β-pol or 8-kDa domain, as indicated in the figure legends, and incubated for 15 min at 37 °C. After the reaction was terminated, the product was stabilized by the addition of 2 mM NaBH4 to a final concentration of 340 mM and incubated for 30 min at 0 °C. The stabilized DNA product was recovered by ethanol precipitation in the presence of 0.1 mg/ml tRNA and resuspended in 10 μl of gel loading buffer (95% formamide, 20 mM EDTA, 0.02% bromphenol blue, and 0.02% xylene cyanol). After incubation at 75 °C for 2 min, the reaction products were separated by electrophoresis in a 20% polyacrylamide gel containing 8 M urea in 89 mM Tris·HCl, 89 mM boric acid, and 2 mM EDTA, pH 8.8, and visualized by autoradiography. To quantify the product, gels were scanned on a Molecular Dynamics PhosphorImager model 450, and the data were analyzed using ImageQuant software. dRP lyase activity was assayed essentially as described for the AP lyase reactions described above except that the uracil-DNA glycosylase-reacted DNA substrate was further treated with AP endonuclease in the presence of 5 mM MgCl2 to create a substrate containing a 5'-incised AP site (Fig. 1).

Effect of pH on dRP Lyase Activity of β-Pol and the 8-kDa Domain—The dRP lyase activity measurements were done in a multiple buffer system containing 25 mM acetic acid, 25 mM MES, and 50 mM Tris. The pH was adjusted with either HCl or NaOH as appropriate. The ionic strength of this buffer system is constant in the pH range used in this study. Each reaction pH at 37 °C was determined using a 400-μl mock-up reaction mixture.

RESULTS

Assay for Cleavage of AP Site-containing Double-stranded DNA by Human DNA Polymerase β and AP Endonuclease—Both β-pol and AP endonuclease cleave double-stranded DNA containing an AP site (4, 13–16, 32). Whereas the β-pol-catalyzed reaction is via β-elimination incising the AP site on the 3'-side of the sugar, AP endonuclease hydrolyzes the phosphodiester bond 5' to the AP site sugar (Fig. 2A). To study these reactions, we utilized a 49-bp oligodeoxynucleotide duplex DNA that contained a uracil residue at position 21. The uracil-containing
DNA strand was 3'-end-labeled with [32P]ddAMP and annealed to its complementary DNA strand. To generate an AP site, this 32P-labeled duplex DNA was treated with uracil-DNA glycosylase to quantitatively remove the uracil residue. This results in an AP site-containing strand where the 32P label is situated 3'-to the lesion (Fig. 2A). The AP site-containing DNA was incubated with either AP endonuclease or β-pol. Since AP endonuclease incises the AP site DNA strand 5'-to the BP site and β-pol incises 3', the AP endonuclease DNA product bears a nick with a 5'-dRP moiety and a 3'-hydroxyl. In contrast, the β-pol-incised DNA product bears a 3'-dRP moiety and a 5'-phosphate (Fig. 2A). To resolve the cleaved labeled DNA products bearing either a 5'-dRP moiety or a 5'-phosphate, the products were stabilized by NaBH4 reduction at the end of each reaction period, and the incised DNA products were separated by electrophoresis in a 20% denaturing polyacrylamide gel.

As expected, the AP endonuclease-cleaved labeled product with a 5'-dRP migrated slower than the β-pol-incised product bearing a 5'-phosphate (Fig. 2B). Since an AP lyase mechanism does not require Mg2+, the divalent cation requirement for the β-pol-dependent incision was examined. The results indicated that the AP incision activity of β-pol does not require Mg2+ (data not shown), whereas the AP endonuclease activity does. This is consistent with the idea that the β-pol AP lyase activity is catalyzed via β-elimination, as opposed to hydrolysis (10, 32).

The effect of Mg2+ on the β-pol dRP lyase activity will be considered in more detail below. The β-pol AP lyase activity was linear for at least 15 min and linearly dependent on enzyme concentration (Fig. 2C).

**dRP Lyase Releases 5'-Terminal Deoxyribose Phosphate from Preincised AP Site DNA**—Similar β-elimination lyase chemistry can be used for removal of the 5'-dRP from an AP endonuclease preincised AP site as with lyase cleavage of an intact AP site, as illustrated in Fig. 1. To study the dRP release reaction, the DNA substrate was prepared by pretreating 32P-labeled duplex DNA containing an AP site with AP endonuclease. The resulting DNA substrate with a dRP moiety at the 5'-end and a 32P-label at the 3'-end (Fig. 3A) was incubated with β-pol or its amino-terminal 8-kDa domain. As shown in Fig. 1, the dRP group can be cleaved from the preincised AP site either by hydrolysis or via a β-elimination reaction. β-Pol is proposed to catalyze the release of the 5'-dRP moiety from the cleaved AP site via a β-elimination mechanism, producing 4-hydroxy-2-pentenal-5-phosphate (Fig. 1D). Fig. 3B demonstrates that β-pol and the 8-kDa domain released dRP from the substrate DNA in a time-dependent manner. The release of the sugar-phosphate from the 5'-end of the 32P-labeled substrate is visualized by the appearance of a band migrating approximately one-half nucleotide faster than the substrate (Fig. 3). The time course of dRP removal was linear for at least 10 min (Fig. 3C).
Steady-state Kinetic Parameters of the dRP Lyase Reaction—To quantify the kinetic parameters for release of dRP from a preincised AP site-containing DNA, dRP release was examined as a function of AP site concentration (Fig. 4). The apparent $K_m$ and $k_{cat}$ for the dRP lyase activity of $\beta$-pol were 0.5 $\mu$M and 0.075 s$^{-1}$, respectively (Table I (top) and Fig. 4A). This results in a catalytic efficiency $(k_{cat}/K_m)$ of 0.15 $\mu$M$^{-1}$ s$^{-1}$. The dRP lyase activity of $\beta$-pol resides in its amino-terminal 8-kDa domain (32). In the current study, we did not detect any dRP lyase activity associated with the carboxyl-terminal 31-kDa domain of $\beta$-pol, even with much higher 31-kDa domain concentrations than that used with intact $\beta$-pol (see below). For the 8-kDa domain, dRP lyase activity was not saturated at the highest concentration of substrate examined (i.e. 2 $\mu$M). However, the catalytic efficiency, as measured by $k_{cat}/K_m$, was 7-fold lower than that of intact $\beta$-pol (Fig. 4B and Table I (top)). These results suggest that although the 8-kDa domain of $\beta$-pol can perform efficient cleavage of dRP from preincised AP site DNA, the 8-kDa domain requires the 31-kDa domain for full efficiency. Finally, attempts to restore 8-kDa dRP lyase activity to an efficiency similar to that of intact $\beta$-pol, by supplementing the 8-kDa domain with purified 31-kDa domain, were unsuccessful (data not shown).

DNA ligase I has been suggested to play a role in BER. We had shown earlier that $\beta$-pol and DNA ligase I are present in a BER-proficient complex isolated from bovine testis and that an interaction can be observed between $\beta$-pol and DNA ligase I when the purified enzymes are mixed in solution (39). Additionally, the interaction can be observed by analytical equilibrium ultracentrifugation of mixtures of either the two enzymes or the 8-kDa domain alone and DNA ligase I. When BER is reconstituted in vitro using purified human proteins (AP endonuclease, $\beta$-pol, and DNA ligase I), the overall BER reaction rate is stimulated modestly by the presence of DNA ligase I. Wang et al. (40) have reported that processing of the 5'-dRP moiety is the rate-limiting BER step in yeast. Therefore, DNA ligase I may influence $\beta$-pol dRP lyase activity. However, $K_m$ and $k_{cat}$ for the dRP lyase activity were not altered by the presence of DNA ligase I (Table I (top) and Fig. 4A). Since $k_{cat}$ and $K_m$ were not altered by the presence of DNA ligase I, it appears that the $\beta$-pol/DNA ligase I interaction has no detectable kinetic consequence (Fig. 4A and Table I).

Steady-state Kinetic Parameters of AP Lyase Using Intact AP Site DNA as Substrate—We also determined the kinetic parameters of $\beta$-pol and the 8-kDa domain utilizing an intact AP site-containing duplex DNA as substrate. DNA polymerase $\beta$ can incise intact AP sites but does so inefficiently as compared with a preincised AP site (Table I). The results show that while the $K_m$ for dRP and AP lyase activities of $\beta$-pol are similar, $k_{cat}$ is approximately 200-fold lower for the lyase activity on an intact AP site (Table I, Fig. 4C). As with $\beta$-pol, the apparent activity exhibited by the 8-kDa domain was over 2 orders of magnitude lower on an intact AP site, as compared with a preincised AP site, when assayed at similar substrate concentrations (Table I). As with the preincised AP site with the

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**Fig. 3. Release of 5′-dRP from preincised AP site-containing DNA.** A illustrates the DNA substrate that was generated by pretreatment of the $^{32}$P-labeled 49-bp oligonucleotide duplex with uracil DNA glycosylase and AP endonuclease and the expected products formed as a result of $\beta$-pol or 8-kDa domain-catalyzed incision of 5′-terminal deoxyribose-phosphate. B, the preincised DNA substrate (20 nM) was incubated either with $\beta$-pol (5 nM) or 8-kDa domain of $\beta$-pol (10 nM) as described under "Experimental Procedures." Aliquots were taken at the indicated time intervals, and the DNA product was stabilized and analyzed as in Fig. 2. The positions of the substrate and product are indicated. C, time courses of product formation for the 8-kDa domain [$\square$] and $\beta$-pol [■], illustrating linearity for at least 10 min.

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8-kDa domain, the elevated $K_{\text{m}}$ for substrate DNA precluded measurement of the Michaelis constant and $k_{\text{cat}}$.

Cation Dependence of the dRP Lyase Reaction—To determine whether the $\beta$-pol dRP lyase reaction required Mg$^{2+}$, uracil-containing $\beta$-pol dRP lyase activity. EDTA ap- 15267

TABLE I

Steady-state kinetic parameters for the dRP lyase and AP lyase activities of $\beta$-pol and the amino-terminal 8-kDa domain

| Enzyme(s)                          | Preincised AP site DNA substrate $^a$ | Preincised AP site DNA substrate $^b$ | Intact AP site DNA substrate $^c$ |
|-----------------------------------|---------------------------------------|---------------------------------------|-----------------------------------|
|                                   | $K_{\text{m}}$ (mM) | $k_{\text{cat}}$ (s$^{-1}$) | $k_{\text{cat}}/K_{\text{m}}$ | $K_{\text{m}}$ (mM) | $k_{\text{cat}}$ (s$^{-1}$) | $k_{\text{cat}}/K_{\text{m}}$ | $K_{\text{m}}$ (mM) | $k_{\text{cat}}$ (s$^{-1}$) | $k_{\text{cat}}/K_{\text{m}}$ |
| $\beta$-Pol                       | 0.5                                   | 75                                    | 0.15                              | 0.2                     | 0.4                           | 0.0013                       | NA                                  | NA                                  |
| $\beta$-Pol + DNA ligase I        | 0.5                                   | 77                                    | 0.15                              | 0.2                     | 0.4                           | 0.0010                       | NA                                  | NA                                  |
| 8-kDa domain                     | $>2.0^b$                           | $>0.2^c$                              | $0.0010$                           | NA                                  | NA                                  | $>2.0^b$                           | $>0.2^c$                              | $0.0010$                           |
| 31-kDa domain                    | NA                                  | 0                                     | NA                                  | NA                                  | NA                                  | NA                                  | NA                                  | NA                                  |

$^a$ DNA substrate was prepared by pretreating duplex AP site DNA with AP endonuclease as described under “Experimental Procedures.”

$^b$ Since activity was linearly related to substrate concentration (sub- strate concentration $<K_{\text{m}}$), the lower estimate is determined from the highest DNA concentration used in the assay.

$^c$ Since the highest concentration of substrate achievable was significantly below $K_{\text{cat}}$, $k_{\text{cat}}$ must be greater than $2 \times k_{\text{obs}}$ at the highest concentration assayed.

$^d$ NA, not applicable.

DNA substrate was prepared by pretreating the uracil-containing duplex DNA with UDG as described under “Experimental Procedures.”
pears to modulate an ionic strength requirement for activity of the intact protein. EDTA shows inhibitory effects on the dRP lyase activity of intact β-pol when added in an equimolar ratio to Mg\(^{2+}\), but this inhibitory effect can be overcome by NaCl. This effect requires the 31-kDa domain, since EDTA had no influence on the dRP lyase activity of the 8-kDa domain alone.

**Inhibition of dRP Lyase Activity by PLP and Antibodies to β-Pol**—Protein modification with PLP involves interaction of the formyl moiety of PLP with free amines on a protein forming a reversible imine or Schiff base. The Schiff base can be reduced to a stable, secondary amine with sodium borohydride. Thus, since Schiff base formation is involved in the dRP lyase reaction catalyzed by β-pol (33), treatment of the enzyme with PLP may be expected to inhibit the lyase reaction. To test this idea, the dRP lyase reaction was performed with samples of β-pol and 8-kDa domain that had been treated with PLP. PLP modification resulted in approximately 95 and 75% inhibition of the dRP lyase activity of β-pol and the 8-kDa domain, respectively (Fig. 6C). The results suggest that a PLP-reactive primary amine group is involved in dRP lyase catalytic activity. It had been shown earlier that Lys\(^{72}\) in intact β-pol is modified by PLP (41). Further, NMR studies of the 8-kDa domain have revealed that two lysines in the proposed lyase active site, Lys\(^{72}\) and Lys\(^{90}\), are preferentially modified by PLP (42). Thus, our present results on PLP inhibition of the dRP lyase activity are consistent with earlier results that β-pol can be PLP-modified. More recently, we have demonstrated that alanine substitution for Lys\(^{72}\) in the 8-kDa domain resulted in greater than 90% loss of the dRP lyase activity associated with this domain (34). In addition, the same mutation in full-length protein resulted in more than 80% loss of dRP lyase activity without altering DNA synthesis activity (data not shown).

Polyclonal antibodies prepared against intact β-pol and the 8-kDa domain are neutralizing against the DNA synthesis activity of β-pol (22). These antibodies can be used to distinguish β-pol DNA synthesis activity from that of other cellular DNA polymerases. The antibodies inhibit β-pol-dependent *in vitro* BER in cell or nuclear extracts (22, 43). We tested these polyclonal antibodies on the dRP lyase activity of β-pol and the 8-kDa domain. The results indicate that the dRP lyase activity is sensitive to the antibodies (Fig. 6B). These results confirm that the dRP lyase activity is intrinsic to β-pol and that the antibodies can not be used in crude cellular extracts to distinguish a β-pol DNA synthesis from a dRP lyase requirement in BER. Finally, dRP lyase activity associated with the 31-kDa domain was ruled out by the absence of dRP lyase activity in the presence of high concentrations of this domain (Fig. 6A).

**Effect of pH on the dRP Lyase Catalytic Efficiency of β-Pol and the 8-kDa Domain**—The relative pH profiles of the β-pol and 8-kDa domain dRP lyase catalytic efficiency were determined using a multiple buffer system, in order to maintain constant ionic strength across the pH range (Fig. 7). Apparent activity was also determined in the absence of enzyme to correct for pH effects on substrate stability.

In addition to BER, a number of alternative routes of enzymatic excision of the 5′-dRP moiety have been proposed (10). For example, the mammalian 5′ → 3′ exonuclease DNease IV was shown to release the 5′-terminal dRP residue as part of a small oligonucleotide product (4). Similarly, the 5′ → 3′ exonuclease activity of *E. coli* DNA polymerase I can remove 5′-
terminal dRP in the oligonucleotide form (44). Since these hydrolytic exonucleases remove 5′-dRP as part of a small oligonucleotide, it is possible that a repair patch size longer than one nucleotide results from repair in the presence of an unprocessed 5′-dRP. An alternate BER pathway exhibiting a longer DNA synthesis patch size (2–6 nucleotides) has been described (43). However, the predominant BER pathway in mammalian cells involves incorporation of a single nucleotide where the dRP moiety in the gap must be removed. Therefore, regulation and coordination of the enzymatic activities required to complete BER may depend on the removal or identity of the 5′-sugar-phosphate moiety. This could depend on the nature of the AP site (i.e. type of base damage or glycosylase that initiates BER). Our results show that β-pol efficiently removes dRP from the preincised AP site, consistent with the idea that the dRP lyase activity of β-pol removes the 5′-dRP during simple BER. These results are also consistent with the recent observations of Blaże et al. (45) and Fortini et al. (43) describing an alternate BER pathway that can utilize a DNA polymerase other than β-pol. Using nuclear extracts from wild-type and β-pol null mouse fibroblasts, they demonstrated that the alternate BER pathway repair patch size is 2–6 nucleotides.

Nonenzymatic attack at C1 through a lyase mechanism may be promoted by basic cellular macromolecules such as polyanines or histones and by other basic molecules including tripeptides such as Lys-Trp-Lys and Lys-Tyr-Lys (46). Such nonenzymatic cleavage generally occurs at a much lower efficiency than that observed with the dRP lyase of β-pol (47). The catalytic efficiency (kcat/Km) of the β-pol dRP lyase facilitates our understanding of the rate-determining steps in BER. For example, we found that kcat of the dRP lyase of intact β-pol is approximately 10-fold lower than kcat for DNA synthesis activity (34) and 100-fold lower than kcat of AP endonuclease (36). Assuming that these kcat values are similar to those operating in BER, these results indicate that the dRP lyase reaction would be the rate determining step in BER. Thus, there could be an accumulation of filled gaps (i.e. a single-nucleotide had been incorporated by β-pol) where the 5′-dRP is awaiting removal. Alternatively, if dRP removal is a strict requirement for DNA synthesis and/or ligation in BER, the β-pol dRP lyase activity could be critical in regulating not only the rate of simple BER but also alternate BER pathways.

A dRP lyase activity catalyzing the release of 5′-dRP from preincised AP site-containing DNA was reported by Lindahl and co-workers (10, 13) in E. coli and later in human cells. They showed that the reaction was catalyzed by a hydrolytic mechanism that required Mg2+. In contrast, Matsumoto and Kim (32) showed that a β-pol-mediated dRP lyase reaction was catalyzed via β-elimination. Surprisingly, this reaction also was Mg2+-dependent. Historically, the hydrolytic dRP lyases require Mg2+, whereas AP lyases, which catalyze via β-elimination, do not require Mg2+ (11). The results presented here demonstrate that the dRP lyase activity of β-pol removes 5′- dRP from the preincised AP site without a Mg2+ requirement. Interestingly, the dRP lyase activity of intact β-pol was inhibited by low concentrations of EDTA but not by high EDTA concentrations. The EDTA inhibition was reversed by NaCl. The dRP lyase activity of the β-pol 8-kDa domain was not affected significantly by the presence of EDTA. These results suggest that there is a Mg2+-dependent event, not related to dRP removal, that higher concentrations of Na+ can “complement" with full-length β-pol. Since the 31-kDa domain has been shown to change its conformation upon binding of monovalent or divalent ions (48–50), EDTA may indirectly inhibit the dRP lyase activity of intact β-pol by altering the 31-kDa domain. Our results indicate that the 31-kDa domain of β-pol promotes the dRP lyase activity, but the 31-kDa domain alone has no dRP lyase activity.

Since β-pol can incise intact AP sites, this activity may be important for the cell in removing AP sites in the absence of the major AP endonuclease. Recently, the Drosophila ribosomal protein S3 was reported to contain a dRP lyase activity (12) that can process 3′ or 5′ termini after the initial incision event. Interestingly, the release of 5′-dRP from the AP endonuclease-cleaved AP site is carried out by β-elimination, whereas the blocked 3′-hydroxyl generated from lysase cleavage of an intact AP site is processed through a Mg2+-dependent hydrolytic mechanism. Therefore, the Mg2+ dependence for AP site enzymology emerges as a distinguishing feature, and multiple alternate enzyme activities may be involved in BER in the absence of the three predominant enzymes (AP endonuclease, β-pol, and DNA ligase).

Structural characterization of the 8-kDa domain has identified a structural motif that binds a monovalent metal and interacts with the DNA backbone (51). This helix-hairpin-helix motif (residues 55–79) is also observed in endonuclease III, which has both glycosylase and AP lyase activities. The lyase activity of endonuclease III is through a β-elimination mechanism and results in a 3′-α, β-unsaturated aldehyde and a 5′-phosphate at the termini (Fig. 1A). Lys120 of endonuclease III has been identified as the primary amine that forms a Schiff base intermediate (52). Alignment of the endonuclease III and 8-kDa domain helix-hairpin-helix motifs suggest that Lys120 of β-pol may be the nucleophilic residue (51). However, alanine substitution for this lysine did not influence lyase activity (34).

Finally, the use of PLP inhibition and pH profile analysis of the β-pol and 8-kDa domain dRP lyase activities provides insight into residues involved in lyase reaction chemistry. Lys72 is preferentially modified by PLP in both β-pol and the 8-kDa domain (42, 53). Thus, this particular lysine residue can act as the Schiff base nucleophile attacking PLP at neutral pH. Site-directed mutagenesis of Lys72 to alanine in the recombinant 8-kDa domain was found to diminish dRP lyase activity nearly 2 orders of magnitude, indicating that this residue is the likely nucleophile that forms the Schiff base intermediate in the β-elimination reaction (34). This residue is observed to coordinate the 5′-phosphate in a DNA gap in crystal structures of β-pol (51, 54). The decrease in dRP lyase catalytic efficiency at low pH is consistent with protonation of the lysine nucleophile at low pH. The broad pH optimum extending into the physiological pH range suggests that the pK of the Lys72 ε-NH2 is diminished significantly. Lys72 is observed to be part of a lysine-rich pocket on the surface of the 8-kDa domain (51) that may reduce its pK. The low catalytic efficiency and sensitivity of the efficiency of the 8-kDa domain to pH indicates that the environment of the nucleophile is altered in the isolated 8-kDa domain. The crystal structure of β-pol bound to a one-nucleotide gap DNA substrate indicates that the 8-kDa and the carboxyl-subdomain of the 31-kDa domain interact (54).

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