Mammalian Abasic Site Base Excision Repair
IDENTIFICATION OF THE REACTION SEQUENCE AND RATE-DETERMINING STEPS*

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Base excision repair (BER) is one of the cellular defense mechanisms repairing damage to nucleoside 5'-monophosphate residues in genomic DNA. This repair pathway is initiated by spontaneous or enzymatic N-glycosidic bond cleavage creating an abasic or apurinic/apyrimidinic (AP) site in double-stranded DNA. Class II AP endonuclease, deoxyribonucleotide phosphate (dRP) lyase, DNA synthesis, and DNA ligase activities complete repair of the AP site. In mammalian cell nuclear extract, BER can be mediated by a macromolecular complex containing DNA polymerase β (β-pol) and DNA ligase I. These two enzymes are capable of contributing the latter three of the four BER enzymatic activities. In the present study, we found that AP site BER can be reconstituted in vitro using the following purified human proteins: AP endonuclease, β-pol, and DNA ligase I. Examination of the individual enzymatic steps in BER allowed us to identify an ordered reaction pathway: subsequent to 5'-“nicking” of the AP site-containing DNA strand by AP endonuclease, β-pol performs DNA synthesis prior to removal of the 5’-dRP moiety in the gap. Removal of the dRP flap is strictly required for DNA ligase I to seal the resulting nick. Additionally, the catalytic rate of the reconstituted BER system and the individual enzymatic activities was measured. The reconstituted BER system performs repair of AP site DNA at a rate that is slower than the respective rates of AP endonuclease, DNA synthesis, and ligation, suggesting that these steps are not rate-determining in the overall reconstituted BER system. Instead, the rate-limiting step in the reconstituted system was found to be removal of dRP (i.e., dRP lyase), catalyzed by the amino-terminal domain of β-pol. This work is the first to measure the rate of BER in an in vitro reaction. The potential significance of the dRP-containing intermediate in the regulation of BER is discussed.

Base excision repair (BER)**pathways are employed to repair damaged or modified bases in DNA. Because similar BER pathways are found in prokaryotic and eukaryotic cells, the extensive knowledge about prokaryotic BER has facilitated studies of this repair mechanism in mammalian cells. BER has been examined in vitro with crude extracts from Escherichia coli, Saccharomyces cerevisiae, Xenopus laevis oocyte, bovine testis, and various mammalian cells (1-5) and reconstituted using purified proteins from both prokaryotes and eukaryotes (1, 5-8).

Mammalian cells can repair abasic sites, an intermediate of BER, using at least two distinct pathways: one involving single nucleotide gap filling by DNA polymerase β (“simple” BER) and an “alternate” pathway that involves proliferating cell nuclear antigen (PCNA). In this latter pathway gap-filling DNA synthesis appears to be catalyzed by DNA polymerase δ or ε and results in a repair patch of 2–6 nucleotides (9). In addition, Kungl and Lindahl (10) have described a BER pathway that repairs reduced AP sites. This pathway also generates a repair patch 2–6 nucleotides in length, but in this case gap-filling DNA synthesis could be performed by DNA polymerase β (β-pol) or δ. Like the pathway described above (9), this BER pathway was stimulated by PCNA (10).

A working model for the simple BER pathway is outlined as follows (for review see Refs. 11 and 12). The glycosidic bond linking the damaged base and deoxyribose is cleaved either spontaneously or by a DNA glycosylase activity removing the inappropriate base to generate an abasic or AP site in double-stranded DNA. The phosphodiester backbone of the AP site is cleaved 5’ to the sugar moiety by AP endonuclease, leaving a 3’-hydroxyl group and a deoxyribose phosphate (dRP) group at the 5’ terminus. Excision of the deoxyribose phosphate group is catalyzed by 2-deoxyribose-5-phosphate lyase, an activity that is intrinsic to the amino-terminal 8-kDa domain of β-pol. The β-pol dRP lyase activity functions via β-elimination (13) and produces a single-nucleotide gap with a 3’-hydroxyl and 5’-phosphate at the gap margins. DNA polymerase β then fills the single-nucleotide gap, and a DNA ligase seals the resulting nick.

The identity of the DNA ligase that completes the simple BER pathway in mammalian cells is unresolved. There is genetic and biochemical evidence implicating the products of both the LIG1 and LIG3 genes in BER. Cell lines deficient in either DNA ligase I or DNA ligase III activity are hypersensitive to DNA alkylating agents (14, 15), and extracts from these cell lines are defective in BER (16, 17). Furthermore, protein-protein interactions between β-pol and XRCC1, the protein partner of DNA ligase III, and between β-pol and DNA ligase I have been characterized (7, 18, 19). Recently, we described the partial purification of a BER-proficient multiprotein complex from bovine testis nuclear extracts (19). DNA polymerase β and
DNA ligase I were identified as components of this complex, but no other ligases were present. Further studies have determined the stoichiometry and thermodynamic properties of this interaction and revealed that stable complex formation between DNA ligase I and β-pol is mediated through the noncatalytic amino-terminal domain of DNA ligase I and the 8-kDa amino-terminal domain of β-pol (20). Together, these results support the notion that a complex of β-pol and DNA ligase I catalyzes the latter steps of simple BER.

To define the influence of this and other putative protein-protein interactions on catalytic activities of enzymes that participate in simple BER, we reconstituted BER of a DNA substrate containing an AP site with three purified human enzymes: AP endonuclease, DNA polymerase β, and DNA ligase I. By characterizing isolated, individual reactions within the BER pathway, we determined the rate-limiting step. Because the overall repair of the AP site occurred at a rate similar to that of dRP removal (dRP lyase step), we suggest that β-pol dRP lyase activity could determine the choice between the simple and alternate BER pathways.

**EXPERIMENTAL PROCEDURES**

**Materials**—[α-32P]dCTP and γ-[32P]ddATP (specific activity, 6.6 × 10^6 dpm/μmol) were from Amersham Pharmacia Biotech. High performance liquid chromatography-purified synthetic 51-base oligodeoxyribonucleotides were obtained from Oligos Etc, Inc. (Wilsonville, OR): 5'-GCTTGGCATGCCTGACGAAATCACCTGAGATCCTCAGGGCCATGCGTCCAGCTG-3' and 3'-CGAACAGTCGAGTGCCCTGACGCTGACGATCTTGCTAGGGCCATGCGTCCAGCTG-5'.

The DNA substrate for the dRP lyase functional assay was a 49-base pair (bp) fragment constructed by annealing two oligodeoxyribonucleotides (Operon Technologies, Inc., Alameda, CA) to introduce a G-U base pair (bp) fragment constructed by annealing two oligodeoxyribonucleotides (Operon Technologies, Inc., Alameda, CA) to introduce a G-U base pair at position 22 in one strand (Fig. 1A). The DNA product was obtained from Oligos Etc, Inc. (Wilsonville, OR): 5'-AGATCTCCTAGGGGCCAAGACGGTCCGAGGTGATGGGCTTGCGTCCAGCTG-3' and 3'-CTGATTGCGTTAGCTACCATGCCTGCACGAA-5'. The uracil-containing strand was labeled at the 3'-end for the dRP lyase assay as described (21).

**Annealing**—Oligophosphorylated oligodeoxyribonucleotides were resuspended in 10 mM Tris-HCl, pH 7.4, and 1 mM EDTA, and the concentration was determined from their 260 nm absorbance at 260 nm. Complementary oligodeoxyribonucleotide or template primers were annealed by heating a solution of 10 μM template with an equivalent concentration of oligomers and primer to 90 °C for 3 min and incubating the solution for an additional 15 min at 50–60 °C, followed by slow cooling to room temperature.

**Recombinant Enzymes**—Human recombinant β-pol was overexpressed from plasmid pWL-11 and purified as described (22). Oligonucleotide site-directed mutagenesis was performed essentially as described previously (23). Human AP endonuclease was expressed in *E. coli* strain BL21/DE3pLysS from pXC53 carrying the *E. coli* lytic amino-terminal domain of DNA ligase I and the 8-kDa amino-terminal domain of DNA ligase I. The enzyme and substrate requirements for the DNA synthesis assays described above were obtained from Oligos Etc, Inc. (Wilsonville, OR): 5'-AGATCTCCTAGGGGCCAAGACGGTCCGAGGTGATGGGCTTGCGTCCAGCTG-3' and 3'-CTGATTGCGTTAGCTACCATGCCTGCACGAA-5'.

**Rate Limiting Step in Simple BER**

**Materials**—[α-32P]dCTP and γ-[32P]ddATP (specific activity, 6.6 × 10^6 dpm/μmol) were from Amersham Pharmacia Biotech. High performance liquid chromatography-purified synthetic 51-base oligodeoxyribonucleotides were obtained from Oligos Etc, Inc. (Wilsonville, OR): 5'-GCTTGGCATGCCTGACGAAATCACCTGAGATCCTCAGGGCCATGCGTCCAGCTG-3' and 3'-CGAACAGTCGAGTGCCCTGACGCTGACGATCTTGCTAGGGCCATGCGTCCAGCTG-5'. The DNA substrate for the dRP lyase functional assay was a 49-base pair (bp) fragment constructed by annealing two oligodeoxyribonucleotides (Operon Technologies, Inc., Alameda, CA) to introduce a G-U base pair (bp) fragment constructed by annealing two oligodeoxyribonucleotides (Operon Technologies, Inc., Alameda, CA) to introduce a G-U base pair at position 22 in one strand (Fig. 1A). The DNA product was obtained from Oligos Etc, Inc. (Wilsonville, OR): 5'-AGATCTCCTAGGGGCCAAGACGGTCCGAGGTGATGGGCTTGCGTCCAGCTG-3' and 3'-CTGATTGCGTTAGCTACCATGCCTGCACGAA-5'.

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accumulation of the unligated 22-residue intermediate was observed (lane 5). With all the enzymes present, the incorporation of \(^{32}\text{P}\)dCMP into BER products was examined as a function of time (Fig. 1C). The 22-mer intermediate accumulated modestly before being ligated to the downstream oligomer.

Conditions for Kinetic Studies of Base Excision Repair in Vitro—To identify potential regulatory steps in BER, we compared time courses of the reconstituted reaction and the individual steps in the reaction pathway at 37 °C. These kinetic studies were performed at the enzyme concentrations described in Fig. 1 (10 nm each UDG, AP endonuclease, and \(\beta\)-pol and 100 nm DNA ligase I) using saturating substrate concentrations (1 \(\mu\)M DNA, 10 \(\mu\)M dCTP, 4 nm ATP) to allow multiple catalytic turnovers. The decision to use a higher concentration of DNA ligase I than the other enzymes was based on preliminary results indicating that the unligated intermediate was accumulating during BER reactions performed using 10 nm DNA ligase I (data not shown). Because the persistence of this intermediate obscured study of enzymatic steps preceding the ligase step in this pathway (see below) and did not reflect results obtained with tissue nuclear extract (5), the higher DNA ligase I concentration was used throughout this work to facilitate study of the other steps. The time course of the reconstituted BER reaction revealed a slight lag phase preceding a linear steady-state rate (Fig. 2A). Extrapolation of the linear phase to the abscissa suggested a lag of approximately 5 s. These results are consistent with a model in which the concentration of an intermediate initially accumulates in the reaction mixture. After this lag, the overall base excision reaction proceeded at a velocity of 0.6 nm \(s^{-1}\) (Fig. 2A). The fact that there was a lag and that the rate of the overall reaction after the lag was relatively slow suggested that an intermediate accumulates and limits the overall reaction.

Identification of the Rate-determining Steps for AP Site BER in Vitro—To delineate which BER activities were rate-determining for product formation, we compared the rates of the various enzymatic activities (Table I). Note that the velocity measured for the overall BER reaction was 0.6 nm \(s^{-1}\). The first activity measured was the rate of DNA synthesis. With a single-nucleotide gapped DNA substrate, we found that \(\beta\)-pol formed the 22-residue product at a reaction velocity of 4.5 nm \(s^{-1}\) (Fig. 2B). Addition of DNA ligase I had a slight stimulatory effect on this DNA synthesis reaction rate (data not shown). Thus, the rate of the \(\beta\)-pol-mediated gap-filling reaction on a gapped DNA substrate was faster than the rate of the overall BER reaction and was not rate-limiting. The presence of a dRP group at the 5’ margin of the gap did not affect this rate (see Fig. 3A).

The velocity of the DNA ligase I step under conditions of the reconstituted system was then measured and found to be 4 nm \(s^{-1}\). Because the rate of ligation is faster than the rate of the reconstituted BER system (at high ligase concentration), one of the earlier intermediates must be accumulating during the BER reaction in vitro. Reducing the ligase concentration to 10 nm would result in a rate lower (0.4 nm \(s^{-1}\)) than the rate measured for the reconstituted BER reaction, confirming the observation noted above that DNA ligase I activity can limit the rate of BER when present at the lower concentration.

From Table I, the rate of in vitro BER can be compared with the velocity of each of the purified enzymes under the conditions of the in vitro BER system. The results discussed above establish that DNA synthesis was not rate-limiting. As noted in Table I, AP endonuclease was also found to possess high catalytic efficiency and was not rate-limiting. Consistent with this suggestion, we found that preincision of the AP site containing DNA did not alter the velocity of overall BER (data not shown). Instead, the rate of 51-mer product formation for in vitro BER was almost identical to that of the \(\beta\)-pol dRP lyase activity (Table I). This suggests that the dRP lyase step is rate-limiting during in vitro BER and that the dRP-containing intermediate accumulates in the BER pathway. These data are paralleled by \(k_{\text{cat}}/K_m\) determinations (Table I) that identify the dRP lyase step as the least efficient. Significantly, these data also indicate that despite several potentially beneficial protein-protein interactions in the reconstituted BER system, the overall BER rate is not faster than the slowest individual enzymatic activity.

The hypothesis that removal of the dRP moiety is the rate-determining step in the in vitro BER system was tested directly by measuring the velocity of polymerase-dependent nucleotide incorporation in the presence or absence of a dRP moiety. The presence or absence of the dRP group did not influence DNA synthesis by \(\beta\)-pol (velocity = 4 nm \(s^{-1}\)) (Fig. 3A). This was similar to the DNA synthesis velocity measured for \(\beta\)-pol in the absence of other BER proteins and was higher than the velocity of the dRP lyase itself (Table I). When a sample from the same
reaction mixture was analyzed by gel electrophoresis, the rate of 51-mer product formation was found to be lower for the reaction in which the dRP group had not been enzymatically removed prior to DNA synthesis. In fact, the velocity of 51-mer BER product formation was found to be lower for the reaction mixture in which the dRP group had not been enzymatically removed (closed circles, Fig. 3), indicating that the dRP moiety does not affect DNA synthesis by DNA polymerase (open circles) to create a single base gap bearing a dRP moiety. A portion of this substrate was then treated with UDG and AP endonuclease to create a single base gap bearing a dRP moiety at the 5'-phosphate in the gap. A portion of this substrate was then treated with dRP lyase activity of β-pol (in the absence of dCTP) to remove the deoxyribonucleotide phosphate group from the DNA. These enzymatic steps created the substrate intermediates present immediately before and after the dRP lyase activity of β-pol. A, DNA synthesis velocities on both substrates (dRP present (open circles) versus dRP group absent (closed circles)) were measured using the filter assay described under “Experimental Procedures.” The radioactivity present in the 22- and 51-nucleotide product bands was plotted on the ordinate. The observed velocities were similar (4.0 versus 3.9 nm s⁻¹, respectively), indicating that the dRP moiety does not affect DNA synthesis by DNA polymerase. B, the velocity of combined DNA synthesis, dRP lyase activity, and DNA ligase I activity was measured by loading the reactions used in A on a 12% polyacrylamide denaturing gel. The radioactivity present in the 51-nucleotide product bands was then measured by scintillation counting, and the product formed was plotted on the ordinate. For the substrate bearing the dRP group (open circles), the observed velocity was 0.8 nm s⁻¹, and for the substrate in which the dRP group was enzymatically removed (closed circles), the velocity was 2.7 nm s⁻¹.

**Fig. 2. Measurement of the velocities of AP site base excision repair, DNA synthesis, and DNA ligase I.** A, the velocity of abasic site BER was measured on a 51-bp substrate following pretreatment with UDG, AP endonuclease (10 nM), β-pol (10 nM), DNA ligase I (100 nM), [α-³²P]dCTP (10 μM), MgCl₂ (10 mM), and ATP (4 mM) were mixed with the substrate simultaneously, and the rate of formation of the 51-nucleotide product was visualized on a denaturing polyacrylamide gel. BER product formation in the 25-nucleotide substrate containing a uracil at position 22 was treated with UDG and the enzyme concentration of each enzyme was 10 nM, except DNA ligase I (100 nM). The initial velocity was 4.5 nM s⁻¹. B, the rate of DNA synthesis by β-pol was measured on the 51-nucleotide substrate containing a single nucleotide gap at an enzyme concentration of 10 nM. Substrate with incorporated [³²P]dCTP (10 μM) was quantitated using the filter binding assay described under “Experimental Procedures.” The initial velocity was 4.5 nM s⁻¹. C, the velocity of the ligase step was measured on the 51-nucleotide substrate containing a G-U mismatch (1 μM) that was preincubated in the presence of UDG, AP endonuclease, β-pol (10 nM each), and [α-³²P]dCTP (10 μM) to create an internally labeled nicked substrate. The rate of formation of the 51-mer ligation product was then quantified in the presence of DNA ligase I (10 nM) and ATP (4 mM). The observed velocity was 0.4 nM s⁻¹.

**Fig. 3. Measurement of the DNA synthesis and dRP lyase velocities of β-pol on a BER intermediate substrate.** The 51-nucleotide substrate containing an uracil at position 22 was treated with UDG and AP endonuclease to create a single base gap bearing a dRP moiety at the 5'-phosphate in the gap. A portion of this substrate was then treated with the dRP lyase activity of β-pol (in the absence of dCTP) to remove the deoxyribonucleotide phosphate group from the DNA. These enzymatic steps created the substrate intermediates present immediately before and after the dRP lyase activity of β-pol. A, DNA synthesis velocities on both substrates (dRP present (open circles) versus dRP group absent (closed circles)) were measured using the filter assay described under “Experimental Procedures.” The radioactivity present in the 22- and 51-nucleotide product bands was plotted on the ordinate. The observed velocities were similar (4.0 versus 3.9 nm s⁻¹, respectively), indicating that the dRP moiety does not affect DNA synthesis by DNA polymerase. B, the velocity of combined DNA synthesis, dRP lyase activity, and DNA ligase I activity was measured by loading the reactions used in A on a 12% polyacrylamide denaturing gel. The radioactivity present in the 51-nucleotide product bands was then measured by scintillation counting, and the product formed was plotted on the ordinate. For the substrate bearing the dRP group (open circles), the observed velocity was 0.8 nm s⁻¹, and for the substrate in which the dRP group was enzymatically removed (closed circles), the velocity was 2.7 nm s⁻¹.

**Table I**

| Human enzyme | k_cat | K_m | k_cat/K_m | Velocity under in vitro BER conditions* |
|--------------|-------|-----|----------|----------------------------------------|
| Uracil-DNA glycosylase | 42b | 0.1b | 420 | 420 |
| AP endonuclease | 10c | 0.1c | 100 | 100 |
| DNA polymerase (β-pol) | 0.45d | 0.3d | 1.5 | 4.5 |
| dRP lyase (β-pol) | 0.075d | 0.5d | 0.15 | 0.75 |
| DNA ligase I | 0.04 | 0.1c | 0.4 | 4.0 |
| BER system: AP endonuclease β-pol, DNA ligase I | | | | 0.6 |

*Repair of an AP site-containing oligodeoxynucleotide. The concentration of each enzyme was 10 nm, except DNA ligase I (100 nm).

Bavari et al. (21).
Bavari et al. (24).
Bavari et al. (43).
Bavari et al. (42).
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| BER system: AP endonuclease β-pol, DNA ligase I | | | | 0.6 |

*Repair of an AP site-containing oligodeoxynucleotide. The concentration of each enzyme was 10 nm, except DNA ligase I (100 nm).
result clearly indicates that the dRP lyase step is a rate-determining step in the in vitro base excision repair system. The rate of product formation observed for substrate lacking the dRP moiety was approximately 3.5-fold higher (velocity = 3 nm s⁻¹) and was similar to the rate of the DNA synthesis and DNA ligation step.

Sequence of Steps in the Reconstituted BER System—The sequence of the steps following incision by AP endonuclease was investigated using both [α-²²P]dCTP and ³²P-labeled DNA to simultaneously measure gap filling and dRP lyase activity, respectively, in the same reaction mixture. dRP lyase activity was measured by a difference for the 3' end labeled substrate and the product strands (Fig. 4, bands 2 and 3, respectively). The 29-residue product migrates slightly faster than the 29-residue substrate. The product of DNA synthesis is a 22-residue DNA strand that is well separated from the 3' end-labeled molecule. The resulting nicked DNA is then ligated. A wide variety of exogenous and endogenous chemicals damage DNA bases, initiating their removal by the numerous damage-specific DNA glycosylases present in cells (for review, see Ref. 29). Spontaneous cleavage of the N-glycosidic bond linking both undamaged and damaged nitrogenous bases to the deoxyribose sugar moiety generates additional abasic sites, estimated to total between 2,000 and 10,000 per day per human cell (30). These damaged sites must be repaired in a timely manner, because such noncoding lesions represent gaps in the templates used by polymerases, increasing the likelihood of mutation and aberrant RNA transcripts (31–33). The base excision repair systems in the cell are believed to constitute the primary mechanism for the repair of this form of DNA damage. This work demonstrates that base excision repair of an abasic site can be reconstituted in vitro using three human enzymes: AP endonuclease, DNA polymerase β, and DNA ligase I. This is the same repair system that was studied by Nicholl et al. (8) and is similar to the pathway described by Kubota et al. (7) containing AP endonuclease and β-pol but that utilizes a DNA ligase III-XRCC1 complex in place of DNA ligase I. The possibility that the DNA ligase III-XRCC1 complex can take the place of DNA ligase I in the simple BER pathway was not
directly examined in this work, so a role for DNA ligase III in BER is not excluded here. However, a BER-proficient complex containing β-pol and DNA ligase I, but not DNA ligase III, has been partially purified from bovine testis nuclear extract (19). Based on genetic and biochemical analysis of DNA ligase-deficient mammalian cell lines (14–17), it is possible that DNA ligase I and DNA ligase III-XRCC1 participate in distinct BER pathways whose in vivo substrate specificity remains to be elucidated. The simple BER pathway characterized in this study is distinct from the PCNA-dependent alternate BER pathways, components of which also play a role in semiconservative DNA replication and nucleotide excision repair (9, 34, 35).

Our interest in base excision repair stems from studies of mammalian β-pol, which had been proposed to be a DNA polymerase active in repairing short (1–6 nucleotide) gaps in DNA (5). The identification of interactions between β-pol and AP endonuclease (36) and between β-pol and DNA ligase I (19, 20), all of which are components of a BER-proficient complex, strongly supports the notion that the latter steps of simple BER are catalyzed by the sequential actions of these enzymes. Experiments by various groups (9, 27, 37) have shown that simple base excision repair is the predominant type of base excision repair used by human cells and mouse fibroblasts; therefore, it appears likely that the enzymes and reactions studied here constitute the predominant base excision repair pathway operating in human cells.

Importantly, this work measured the catalytic rate of the overall reconstituted BER reaction as well as individual steps, allowing identification of the dRP lyase step as the activity likely to be regulating this pathway. This is the first measurement of the rate of mammalian base excision repair in an in vitro reaction and, to our knowledge, the first rate determination of any mammalian DNA repair system. Within the reconstituted BER system, the dRP lyase activity was found to be the rate-determining step, because the velocity of this step (0.75 nM s⁻¹) was similar to the velocity measured for the overall BER reaction (Table I). The rate of DNA ligase I (4 nM s⁻¹) would be expected to be partially rate-limiting at lower DNA ligase I concentrations (e.g. 10 nM). DNA synthesis was rapid (velocity = 4.5 nM s⁻¹) and not rate-limiting. An additional finding was that the AP endonuclease product (dRP-containing intermediate) did not limit DNA synthesis. Thus, gap filling was not disrupted when the dRP moiety was still bound to the 5’-phosphate in the gap (see Figs. 3 and 4). Instead, the presence of a dRP group in the gap was found to inhibit DNA ligase I activity (see Fig. 4). After removal of the dRP, the rate of BER was similar to the rate of DNA synthesis and ligation (see Fig. 3). DNA ligase I was unable to seal the nick while the dRP flap was present, presumably because the flap interfered with ligation or DNA ligase binding. Alternatively, binding by β-pol at the gap may prohibit DNA ligase I from binding to the 5’-phosphate. Although it is known that the 8-kDa domain of β-pol possesses the dRP lyase active site and interacts with DNA ligase I (20, 28, 38), the precise molecular mechanism of this interaction has not been elucidated. Noting that the dRP lyase step is rate-determining in the BER system and that the dRP group must be removed prior to ligation, it is clear that dRP removal plays a significant functional role in the regulation of base excision repair. These observations also allow us to propose the following order for the AP site base excision repair enzymatic activities: AP endonuclease, DNA synthesis, dRP lyase activity, and then ligation (Fig. 5).

The identification of the dRP lyase step as a rate-determining step in simple base excision repair now permits a closer examination of the pathways capable of repairing AP sites in DNA. Data obtained from prokaryotic and eukaryotic systems (9, 10, 39–41) suggest that at least two classes of base excision repair pathway may operate in cells, including human cells: the simple, β-pol-mediated pathway described here, as well as alternate, PCNA-dependent pathways that can utilize DNA polymerase β, δ, and/or ε. It seems plausible that the choice of pathway would be linked to the status of the dRP group. Should the dRP be processed quickly by the dRP lyase activity of β-pol, the simple BER mechanism would likely complete the repair of the gap. If the dRP group were to persist in the DNA, however, it seems possible that the components of an alternate base excision repair pathway might bind to the dRP flap (with or without β-pol bound at the site) and complete the repair event. Thus, the status of the dRP group might function as a “switch” between the simple and alternate repair pathways. Although both pathways are known to occur in competition with each other in human cells (9), characterization of the signal that initiates each alternate BER pathway on gapped DNA has not been elucidated.

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