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Inhibition of Short Patch and Long Patch Base Excision Repair by an Oxidized Abasic Site&

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Abstract

5′-(2-Phosphoryl-1,4-dioxobutane) (DOB) is an oxidized abasic lesion that is produced by a variety of DNA damaging agents, including several antitumor antibiotics. DOB efficiently, and irreversibly inhibits DNA polymerase β, an essential base excision repair enzyme in mammalian cells. The generality of this mode of inhibition by DOB is supported by the inactivation of DNA polymerase λ, which may serve as a possible back-up for DNA polymerase β during abasic site repair. Protein digests suggest that Lys72 and Lys84, which are present in the lyase active site of DNA polymerase β are modified by DOB. Monoaldehyde analogues of DOB substantiate the importance of the 1,4-dicarbonyl component of DOB for efficient inactivation of Pol β and the contribution of freely diffusible electrophile liberated from the inhibitor by the enzyme. Inhibition of DNA polymerase β's lyase function is accompanied by inactivation of its DNA polymerase activity as well, which prevents long patch base excision repair of DOB. Overall, DOB is highly refractory to short patch and long patch base excision repair. Its recalcitrance to succumb to repair suggests that DOB is a significant source of the cytotoxicity of DNA damaging agents that produce it.

Keywords

DNA damage; base excision repair; inhibition; oxidized abasic site

Double strand breaks are the most deleterious form of DNA damage and molecules that efficiently produce them are highly cytotoxic (1–3). However, many DNA damaging agents that do not produce high yields of dsbs are effective at killing cells and molecules that produce dsbs also generate single strand breaks and/or DNA lesions (4). The reactivity, effects on polymerase activity, and interaction with repair enzymes of a specific lesion can contribute to the cytotoxicity of the agent that produces it. Abasic lesions are particularly interesting in these respects because they are highly mutagenic due to the absence of a heterocyclic (Watson-Crick) nucleobase to direct polymerases, are electrophilic, and are either formed concomitantly with a single strand break or are labile precursors to ssbs (5).

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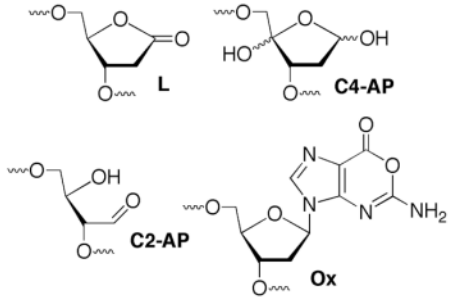
Supporting Information Available. Plots of Pol β excision of DOB and dRP under single turnover conditions, reaction of C2-AP with Pol β and FEN1, GluC and trypsin digests of Pol β following reaction with DOB. This material is available free of charge via the Internet at http://pubs.acs.org.

Apurinic/apyrimidinic sites are efficiently removed by base excision repair (Scheme 1). Excision of a 5′-deoxyribose phosphate (dRP) by Pol β via a lyase (β-elimination) mechanism is an integral step during base excision repair (6). Recently, we reported that 5′-(2-phosphoryl-1,4-dioxobutane) (DOB) efficiently, and irreversibly inhibits DNA polymerase β (7). The major pathway for DOB inactivation of Pol β involves covalent bond formation with the enzyme. Pol β modification by but-2-ene-1,4-dial released from DOB is a minor contributor to enzyme inactivation. A variety of anti-tumor agents that owe their cytotoxicity to their ability to oxidatively damage DNA produce the DOB lesion (8). Given that Pol β is overproduced in tumor cells and that this enzyme guards against DNA damaging cytotoxic agents, we postulated that irreversible inhibition of it by DOB provides a chemical basis for the cytotoxicity of the therapeutics that generate the lesion (9–11). We now demonstrate that DOB irreversibly inhibits Pol λ, resists long patch BER as well, and provide mechanistic evidence for the previously reported inhibition.

DOB is formed in competition with the 5′-aldehyde (T-al) following hydrogen atom abstraction from the C5′-position (Scheme 2) (12,13). Cyclonucleotides are also produced under anaerobic conditions from purines via C5′-radicals (14,15). However, these lesions have not been identified in reactions of antitumor agents that oxidatively damage DNA. Although the mechanism for its formation from the radical is uncertain, anoxic conditions and oxygen surrogates favor DOB formation. The C5′-hydrogens are readily accessible to diffusible species (16). Consequently, it is not surprising that ionizing radiation and Fe•EDTA, both of which generate hydroxyl radical also produce DOB (17). In addition to irreversibly inhibiting Pol β, DOB, which exists in equilibrium with its acyclic 1,4-dialdehyde form, yields DNA interstrand cross-links selectively with dA opposite the 3′-adjacent thymidine whose effects on DNA repair are unknown (18). The lesion is also chemically unstable, and undergoes spontaneous β-elimination to produce but-2-ene-1,4-dial (17–19). The latter forms promutagenic exocyclic adducts with dA, dC, and dG (20,21). All in all, DOB presents a variety of potential challenges directly and indirectly to a cell.

The discoveries regarding DOB reactivity are a subset of a growing number of examples of DNA modifications that spontaneously form potentially more deleterious lesions and/or inhibit BER. DOB, C4-AP, and AP have in common the ability to form DNA interstrand cross-links (22–24). A C4-AP interstrand cross-link induces bacterial nucleotide excision repair proteins (UvrABC) to mistakenly produce double strand breaks ~15% of the time (25). 2-Deoxyribonolactone (L) and oxanine (Ox) also inhibit BER proteins, although DOB is a much more potent irreversible inhibitor of Pol β than is L (26–29). In fact, DOB is a more potent irreversible inhibitor of BER than any DNA lesion of which we are aware.



Structures of L, C2-AP, C4-AP, and Ox

Materials and Methods

Materials and General Methods

Oligonucleotides were prepared on an Applied Biosystems Inc. 394 DNA synthesizer. Commercially available DNA synthesis reagents were obtained from Glen Research Inc. Oligonucleotides containing the photolabile DOB precursor and precursors of the monoaldehyde analogues used to prepare **4** and **5** were synthesized as previously described (7,18,19). Ternary complexes containing DOB (**1**), **4**, or **5** were comprised of a 30 nt template, 15 nt flanking oligonucleotide, and 15 nt containing one of the aforementioned modifications. The ternary complex containing dRP (**2**) was obtained by hybridizing a 37 nt template, 18 nt flanking oligonucleotide, and 19 nt oligonucleotide containing the dRP precursor. All others were synthesized and deprotected using standard protocols. T4 polynucleotide kinase, Glu C, and trypsin were obtained from New England Biolabs. DNA Pol β and FEN1 were obtained from Trevigen. DNA Pol λ was expressed and purified as previously described (30). Radionuclides were obtained from Perkin Elmer. ZipTips were from Millipore. Analysis of radiolabeled oligonucleotides was carried out using a Storm 840 Phosphorimager and ImageQuant 5.1 software. UPLC was carried out using an Agilent Infinity system. MALDI-TOF MS data were obtained on a Bruker AutoFlex spectrometer. Please note that either the 3′-terminus of the strand containing the modified nucleotide or the 5′-terminus of the flanking oligonucleotide is radiolabeled in all of the experiments described below.

The Preparation of ternary complex 3′-^{32}P-2

The hybridized ternary complex 3′-^{32}P-**3** (1 μM) was incubated with uracil-DNA glycosylase (UDG) (155 units) in UDG reaction buffer (100 μL, 20 mM Tris-HCl, pH 8.0, 1 mM DTT, 1 mM EDTA) at 37 °C for 10 min. The resulting solution of 3′-^{32}P-**2** was used immediately without further purification.

Pol β Active Site Titration Assay (31)

Freshly prepared ternary 3′-^{32}P-**2** (500 nM) was incubated with Pol β (5 nM, 10 nM and 15 nM) in HEPES buffer (50 mM, pH 7.4, containing 5 mM $MgCl_2$) at 25 °C for 20 min. Aliquots (5 μL) was removed at the indicated times (0.1, 0.25, 0.5, 1, 2, 5, 9 and 15 min) and quenched with $NaBH_4$ (2 μL, 500 mM) at 25 °C for 1 h. The samples were mixed with formamide loading buffer (15 μL, 90%, 10 mM EDTA). Aliquots (5 μL) of the mixture were loaded on to a 20% denaturing polyacrylamide gel and the product was analyzed using a phosphorimager. The amount of product versus time was fit using the equation: $y = A_0*(1 - \exp(-a*x)) + b*x$. The percentage of active Pol β (48.3 ± 3.8 %) was calculated from A_0/[Pol β].

Single-Turnover Kinetics of Pol β

Ternary complex 3′-^{32}P-**1** or 3′-^{32}P-**2** (40 nM) in HEPES buffer (460 μL, 50 mM, pH 7.4, containing 5 mM $MgCl_2$) was prepared. A solution of Pol β (200 nM) in HEPES buffer (460 μL, 50 mM, pH 7.4, containing 5 mM $MgCl_2$) was prepared and store on ice. Both solutions were loaded in 1 mL sterile disposable syringes and attached to KinTek rapid quench instrument. An experiment consisted of 7 time-points (0.02, 0.05, 0.1, 0.2, 0.5, 1 and 2 s for 3′-^{32}P-**2**, or 0.1, 0.5, 1, 5, 10, 30 and 60 s for 3′-^{32}P-**1**) carried out in triplicate. Prior to each measurement the reaction loop was rinsed with water, followed by methanol, and evacuated for 30 s by vacuum pump. A solution of DNA complex (15 μL) and enzyme (15 μL) were mixed and the reaction was performed at the indicated times before quenched by methanol. The reaction solution (300 μL) from the rapid quench was immediately mixed with $NaBH_4$ (20 μL, 1 M) and incubated at room temperature for 30 min. The sample was then mixed

with NaOAc (20 μL, 3 M, containing 200 μg/mL calf thymus DNA). The DNA was precipitated from ethanol, resuspended in formamide loading buffer (10 μL, 90%, 10 mM EDTA). The products were separated by 20% denaturing polyacrylamide gel and analyzed using a phosphorimager.

Pol β inhibition by DOB model compounds

Ternary complex 3′-^{32}P-**2** (dRP, 500 nM) was incubated with Pol β (7.5 nM) in the presence of **1**, **4** or **5** (60 nM) at 37 °C in HEPES buffer (200 μL, 50 mM, pH 7.4, containing 5 mM $MgCl_2$). Aliquots (10 μL) were removed at the indicated times (0, 5, 10, 15, 20 and 25 min) and quenched with $NaBH_4$ (2 μL, 500 mM) at 25 °C for 1 h. The samples were mixed with formamide loading buffer (20 μL, 90%, 10 mM EDTA). An aliquot of the mixture (5 μL) was loaded on to a 20% denaturing polyacrylamide gel and the product was analyzed using a phosphorimager.

Pol β extension reaction assay

Ternary complex 5′-^{32}P-**1** (200 nM) was incubated with Pol β (1 nM) in the absence or presence of FEN1 (25 nM) in HEPES buffer (60 μL, 50 mM, pH 7.4, containing 5 mM $MgCl_2$, 50 mM KCl, 0.2 mM EDTA) and dNTPs (50 μM, dATP, dGTP, dCTP and dTTP) at 37 °C for 1 h. Aliquots (5 μL) were removed at 0, 2, 5, 10, 15, 30, 45 and 60 min and quenched with 90% formamide loading buffer (10 μL). An aliquot of the mixture (5 μL) was loaded on to a 20% denaturing polyacrylamide gel and the product was analyzed using a phosphorimager.

Strand Excision by FEN1

Ternary complex 3′-^{32}P-**1** (200 nM) was incubated with Pol β (1 nM) in the absence or presence of FEN1 (25 nM) in HEPES buffer (60 μL, 50 mM, pH 7.4, containing 5 mM $MgCl_2$, 50 mM KCl, 0.2 mM EDTA) and dNTPs (50 μM, dATP, dGTP, dCTP and dTTP) at 37 °C for 1 h. Aliquots (5 μL) were removed at 0, 2, 5, 10, 15, 30, 45 and 60 min and quenched with 90% formamide loading buffer (10 μL). An aliquot of the mixture (5 μL) was loaded on to a 20% denaturing polyacrylamide gel and the product was analyzed using a phosphorimager.

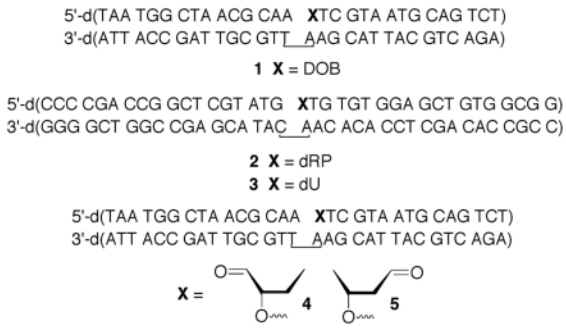
Pol λ Lyase Reaction with DOB and dRP

Freshly prepared ternary complex 3′-^{32}P-**1** or 3′-^{32}P-**2** (200 nM) was incubated with Pol λ (10 nM) in HEPES buffer (200 μL, 50 mM, pH 7.4, containing 5 mM $MgCl_2$) at 37 °C. Aliquots (5 μL) were removed at the indicated times (0, 2, 6, 10, 15, 20, 25, 30 and 40 min) and quenched with $NaBH_4$ (2 μL, 500 mM) at 25 °C for 1 h. The samples were mixed with formamide loading buffer (15 μL, 90%, 10 mM EDTA). An aliquot of the mixture (5 μL) was loaded on to a 20% denaturing polyacrylamide gel and the product was analyzed using a phosphorimager.

Stepwise Inhibition Pol λ Lyase Reaction by DOB

Freshly prepared ternary complex 3′-^{32}P-**1** (200 nM) was incubated with Pol λ (5 nM) in HEPES buffer (200 μL, 50 mM, pH 7.4, containing 5 mM $MgCl_2$) at 37 °C. Aliquots (5 μL) were removed at the indicated times (0, 5, 10 and 20 min) and quenched with $NaBH_4$ (2 μL, 500 mM) at 25 °C for 1 h. After 20 min another portion of Pol λ (10 μL, 100 nM, 1 pmol) was added to the solution. Aliquots (5 μL) were removed at the indicated times (25, 30, 35 and 40 min) and quenched with $NaBH_4$. After another 20 min Pol λ (10 μL, 100 nM, 1 pmol) was added to the solution. Aliquots (5 μL) were removed at the indicated times (45, 50, 55, 60, 65 and 70 min) and quenched with $NaBH_4$. The samples were mixed with formamide loading buffer (15 μL, 90%, 10 mM EDTA). An aliquot of each mixture (5 μL)

was loaded on to a 20% denaturing polyacrylamide gel and the product was analyzed using a phosphorimager.



Structures of ternary complexes 1-5

Results

DOB inhibition of Pol β

AP sites are not substrates for Pol β, but the Ape1 incision product dRP is. The presence of the 5′-phosphate is believed to be important for dRP recognition by Pol β (32,33). Hence, it was uncertain whether DOB would even be recognized by Pol β. The rate constant for DOB excision by Pol β in 3′-^{32}P-**1** was measured under single turnover conditions. The observed rate constant (k_{obs} = 0.04 s^{-1}) was 100-fold slower than that for dRP in 3′-^{32}P-**2** (k_{obs} = 4 s^{-1}). Despite reacting more slowly than dRP, DOB efficiently and irreversibly inhibited Pol β, as indicated by the K_I (12.8 ± 5.2 nM) and k_{inact} (4.2 ± 0.9 × 10^{-4} s^{-}1) for inhibition of the Pol β dRPase reaction with 3′-^{32}P-**2** by a ternary complex containing DOB (**1**) (7). The partition ratio for Pol β lyase inhibition by DOB is ~4, indicating that each Pol β molecule catalyzes ~4 lyase reactions on DOB prior to inactivation (34).

Pol β utilizes different binding sites to catalyze lyase and polymerase reactions. The latter is an integral component of short patch and long patch BER, and we determined whether irreversible inhibition of the former reaction also prevented primer extension by this enzyme. Consequently, 5′-^{32}P-**1** (200 nM) was subjected to reaction with Pol β (1 nM) in the presence of all 4 native dNTPs (Figure 1A). Less than 15% of the primer is extended one nucleotide in 1 h, and longer extension products are not detected. This amount of product corresponded to ~25 turnovers of Pol β. The presence of FEN1 had no effect on the amount or length of the extension product. Treatment of 3′-^{32}P-**1** (200 nM) with the above mixture, including FEN1 yielded 5% of 2 nt deletion product as the sole product (Figure 1B). In order to rule out experimental technique and/or poor quality enzyme(s) a comparable ternary complex containing C2-AP was subjected to the same reaction conditions (See Supporting Information). Long patch repair of C2-AP was previously reported (35). More than 50% of the corresponding 5′-^{32}P-labeled ternary complex (200 nM) was extended 1 and 2 nucleotides by Pol β. Furthermore, ~50% of 2 nt excision was observed when reaction of 3′-^{32}P-C2-AP containing ternary complex (200 nM) was analyzed.

Identification of Pol β modification by DOB

The original report of Pol β inhibition by DOB suggested that covalent modification of the protein by the lesion containing oligonucleotide was the major pathway, but β-elimination product but-2-ene-1,4-dial released upon the lyase reaction accounted for as much as 10% of

the enzyme inactivation (7). The lyase active site of Pol β contains multiple Lys residues, which have previously been implicated in dRP excision. Of these Lys72 is believed to be the principal nucleophile involved in Schiff base formation with dRP (33,36,37). However, some reactivity with Lys84 has also been proposed. We sought to determine the position(s) of Pol β modification by combining peptide digestion and mass spectrometry. Pol β was incubated with 5 equivalents of ternary complex **1** at 37 °C for 1 h. The resulting material was digested with GluC peptidase, which selectively cleaves peptides at glutamic acid residues. The crude mixture of digested peptide fragments was analyzed by MALDI-TOF MS and the peptide fragment pattern was compared with that from digested Pol β that was not treated with DOB. Several expected GluC incision peptide fragments were observed in the Pol β digest (See Supporting Information), including one (residues 76-86) that contains Lys84 and Lys81. This fragment is replaced by one that is 66 Da higher when Pol β is incubated with DOB (See Supporting Information). This molecular ion is consistent with formal condensation between but-2-ene-1,4-dial and a Lys amino group, followed by loss of water (38). The fragment containing Lys72 was not detected in our hands in either the control digest or that following reaction with DOB (1). An alternative approach was carried out using trypsin to digest Pol β. Trypsin selectively cleaves peptide bonds C-terminal to lysine (and arginine) residues, but typically is inhibited by lysine alkylation (37). A peptide containing residues 73-81 (*m/z* = 994.1) was identified by MALDI-TOF MS, following trypsin digestion of Pol β and separation by reverse phase UPLC (See Supporting Information). This peptide fragment was not observed when Pol β was incubated with 5 equivalents of **1**, which is consistent with alkylation of Lys72 and/or Lys81. However, a longer peptide containing this modified peptide and an adjacent sequence was not detected.

Pol β inhibition by DOB analogues

Ternary complexes containing monoaldehyde analogues of DOB (**4**, **5**) were previously used to examine the driving force for DNA interstrand cross-link formation by the lesion (18). In the current study we used these probes to examine the source of Pol β inactivation by DOB. Radiolabeled dRP containing ternary complex (3′-^{32}P-**2**, 500 nM) was incubated with Pol β (7.5 nM) in the absence of any probe or in the presence of **1**, **4**, or **5** (60 nM) and the amount of dRPase product was measured as a function of time (Figure 2). No discernible difference was observed between the reaction containing **4** and that without any probe. In contrast, **5** inhibited the lyase activity of Pol β, although to a lesser extent than did DOB (**1**).

DOB inhibition of Pol λ

Pol λ contains an 8-kDa domain, which like that of Pol β possesses dRPase activity (39). The enzyme is believed to carry out the lyase reaction via Schiff base formation. Given the similarities between the enzymes, we qualitatively examined Pol λ inhibition by DOB (**1**). Incubation of 3′-^{32}P-**2** (200 nM) with Pol λ (10 nM) yielded ~43% of the dRPase product after 15 min, and reaction continued over the entire 40 min monitoring period (Figure 3A). In contrast, excision of a comparable substrate containing DOB (3′-^{32}P-**1**) under the same conditions ceased after 15 min and produced ~19% excised product (Figure 3A). The extent of reaction suggests that on average Pol λ carries out ~4 turnovers of DOB before being inactivated. This is consistent with a titration experiment in which aliquots (1 pmol) of Pol λ are sequentially added to 3′-^{32}P-**1** (200 nM, 20 pmol) (Figure 3B). Each addition of Pol λ yielded approximately 4 equivalents of product, after which the reaction slowed significantly. These observations are qualitatively similar to what was observed when DOB reacted with Pol β under comparable conditions (7). However, the reactions of Pol λ with DOB and dRP were slower.

Discussion

Although DOB unequivocally irreversibly inhibits DNA polymerase β, it was uncertain at the outset of the initial studies whether it would be a substrate for Pol β (7). DOB lacks a 5′-phosphate, which is important in the enzyme's recognition of dRP (32). Indeed, the importance of the phosphate group may be reflected in the observed rate constant for Pol β excision of DOB, which is 100 × slower than that for dRP. Although the presence of dRP does not affect Pol β extension of a primer, we anticipated that inactivation of the enzyme's lyase site via chemical modification would also inhibit polymerization activity (40). Under comparable reaction conditions extension in 5′-^{32}P-**1** and the lyase reaction cease in approximately the same amount of time but a greater number of turnovers are observed for extension than for the lyase reaction. This is consistent with the belief that the lyase reaction of dRP is the rate determining step in AP repair (40,41). In contrast, the disparity in the number of nucleotides (1) inserted by Pol β and the number excised by FEN1 (2) was unusual. For instance, in our hands strand displacement synthesis of C2-AP yields 2-nt extension and FEN1 incision of the same fragment length (35). In addition, the inefficiency of long patch BER of the DOB lesion differs from a previous report on 2-deoxyribonolactone (L) repair by this same pathway (42). However, Ape1 incised L forms very low yields of DNA-protein cross-links with Pol β and the lesion's inhibition (cross-linking) of the enzyme is inefficient (29,43). Hence, it is likely that active Pol β remains after cross-link formation, whereas the polymerase is the limiting reagent in the experiments with DOB.

The experiments above validate the hypothesis that the efficiency of DOB's inhibition of Pol β is attributable to the 1,4-dialdehyde's facile reaction with alkyl amines (19). GluC digestion and subsequent MALDI-TOF MS analysis provided direct evidence for Lys modification in the peptide fragment containing residues 76-86. Although two Lys residues are present in this fragment, only Lys84 is present in the lyase active site and is presumably the position that is derivatized (33). The modified fragment could result from liberated but-2-ene-1,4-dial or trapping by DOB and subsequent decomposition. Although Lys84 may play an active role in the lyase reaction, Lys72 is believed to be responsible for the majority of Schiff base formation en route to dRP excision (36,44,45). We did not detect a modified peptide fragment by MS containing this residue but inferential support for the Lys72 modification during DOB inactivation was obtained via trypsin digest. Disappearance of the amino acid 73-81 fragment is consistent with modification of Lys72 (37). Previous radioisotope experiments indicated that the major inhibition pathway involved covalent trapping of Pol β by DNA containing DOB (7). We speculate that the disappearance of the 73-81 amino acid fragment is due to formation of such a DNA-protein conjugate.

Incubations of Pol β with the monoaldehyde analogues further illustrate the importance of the 1,4-dialdehyde moiety in DOB (Scheme 2) and the lesion's dual reactivity mode. Analogue **4** does not inhibit Pol β at all, and **5** is a less potent inhibitor than DOB. This is consistent with these molecules' and AP's overall lower reactivity than DOB with amines in DNA (18,22). The dicarbonyl group is crucial for the facile formation of stable adducts between the DNA and Pol β (23,24,46). Furthermore, the difference in inhibition between **4** and **5** is consistent with our proposal that a portion of Pol β inactivation by DOB involves release of but-2-ene-1,4-dial. Analogue **4** cannot undergo β-elimination and does not inhibit Pol β, whereas **5** inactivates Pol β and can release the corresponding α, β-unsaturated aldehyde (crotonaldehyde), which forms adducts with Lys groups (47).

Although it was not investigated as thoroughly as Pol β inactivation, DOB exerts a similar effect on Pol λ. Pol λ reacts more slowly with dRP and DOB than does Pol β (Figure 3A) but the inactivation efficiency (~4 turnovers before being inactivated) by the latter is similar for

the two enzymes (Figure 3B). We predict that DOB inactivates other enzymes that possess dRPase activity, such as Pol IV, Pol γ, and Pol Θ (48–50). In addition, proteins that bind damaged DNA that possess juxtaposed nucleophilic residues are also likely to be cross-linked and/or modified by DOB. For instance, Ku was recently proposed to excise dRP and would be a likely candidate to react with DOB, as would the Lys rich side chains of histone proteins in nucleosomes (51).

Conclusions

The DOB lesion is produced by a variety of agents that oxidatively damage DNA via the intermediacy of 2′-deoxyribose centered radicals, specifically, the C5′-radical (52,53). The formation of other forms of damage, most notably double strand breaks by antitumor antibiotics has overshadowed DOB. However, not all cytotoxic agents that produce DOB also generate double strand breaks. DOB is the most efficient irreversible inhibitor of Pol β, an enzyme that plays a crucial role in BER and protection against cytotoxic agents that target DNA (11). The inhibition of Pol β and its possible back up, Pol λ, as well as the known overproduction of Pol β in some cancers suggest that DOB contributes significantly to the cytotoxicity of the agents that produce it (54,55). DOB's resistance to short and long patch BER also suggests that it may be the source of persistent oxidized abasic sites produced in cells exposed to reactive oxygen species (56).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Dr. Aaron Jacobs for providing the DNA used to prepare dRP (**2**) and for assistance with the Pol β digestion and single turnover kinetic experiments.

Abbreviations

AP	apurinic/apyrimidinic site
L	2-deoxyribonolactone
C2-AP	C2′-oxidized abasic site
C4-AP	C4′-oxidized abasic site
BER	base excision repair
DOB	5′-(2-phosphoryl-1,4-dioxobutane)
dsb	double strand break
dRP	5′-deoxyribose phosphate
Pol β	DNA polymerase β
Pol λ	DNA polymerase λ
ssb	single strand break
FEN1	flap endonuclease 1
nt	nucleotide

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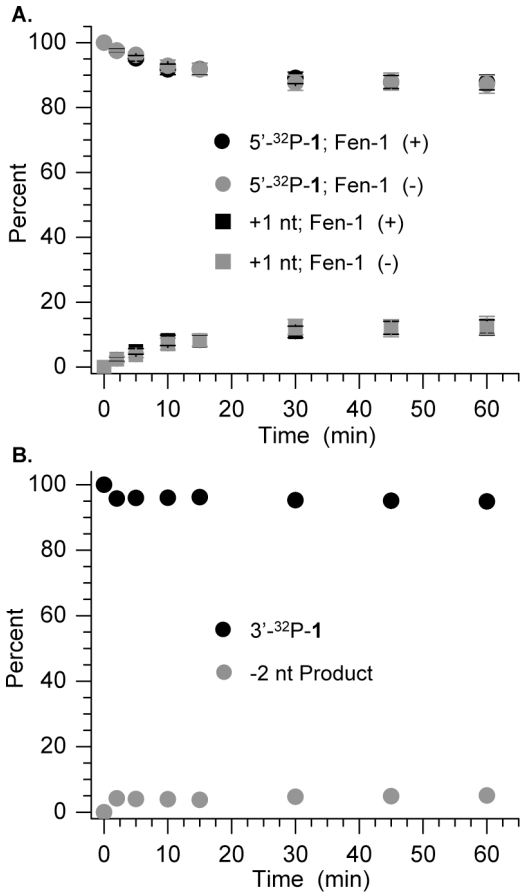


Figure 1.

Effect of DOB on long patch repair by Pol β. (A.) Extension of 5′-^{32}P-**1** in the absence and presence of FEN1. (B.) FEN1 incision of 3′-^{32}P-**1**.

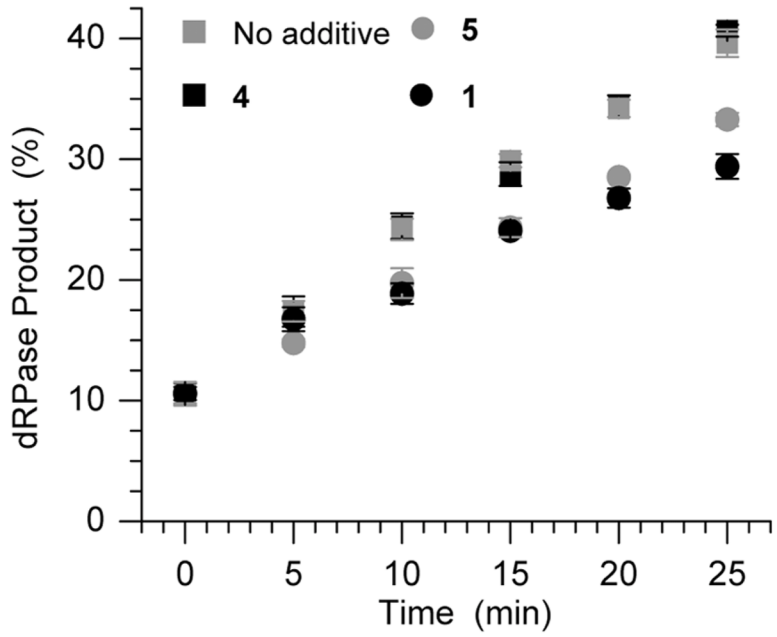


Figure 2.
Effect of DOB (**1**) and its monoaldehyde analogues (**4**, **5**) (60 nM) on Pol β (7.5 nM) dRPase reaction of 3′-^{32}P-**2** (500 nM).

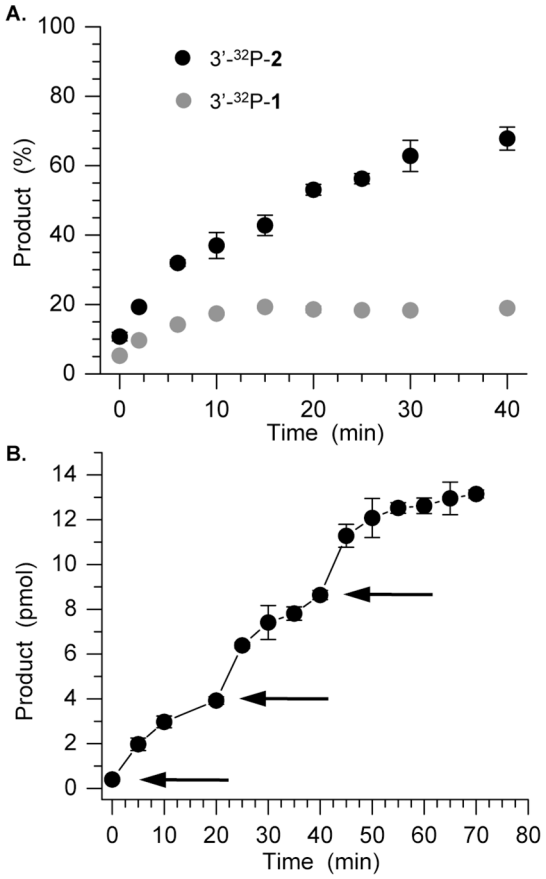
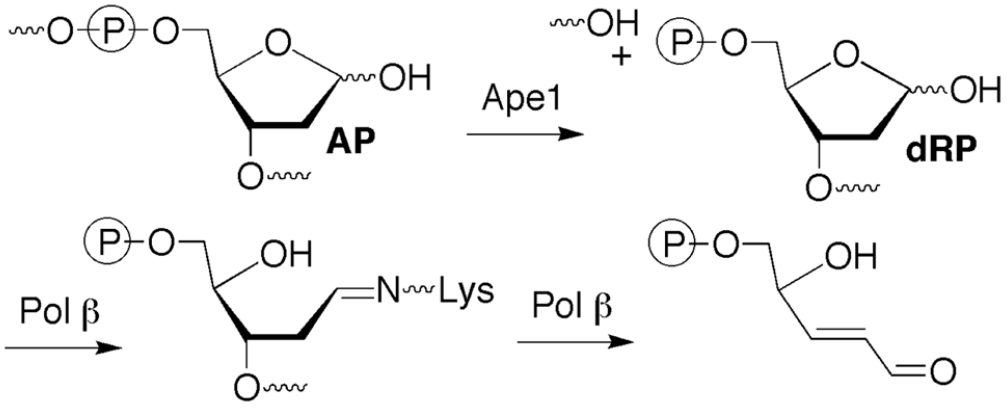
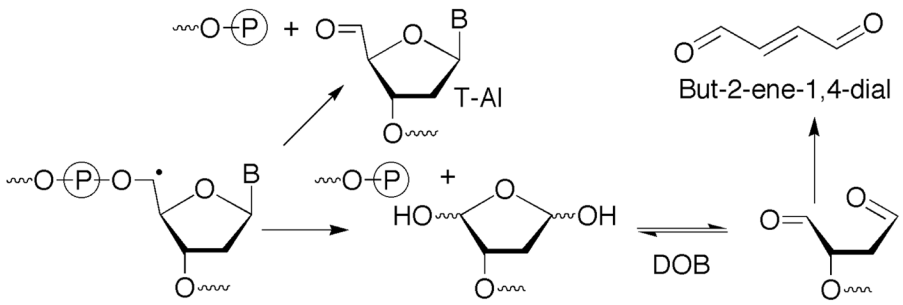


Figure 3.
DOB inhibition of Pol λ. (A.) Lyase reaction of dRP (3′-^{32}P-**2**) and DOB (3′-^{32}P-**1**). (B.) Lyase reaction of 3′-^{32}P-**1**. Each arrow indicates the addition of 1 pmol of Pol λ.



Scheme 1.
Role of Pol β in AP site repair.



Scheme 2.
Formation of DOB and T-Al from a C5′-nucleotide radical.