Repair of Oxidized Abasic Sites by Exonuclease III, Endonuclease IV, and Endonuclease III[†]

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ABSTRACT: 2-Deoxyribonolactone (L) and the C4′-oxidized abasic site (C4-AP) are produced by a variety of DNA-damaging agents. If not repaired, these lesions can be mutagenic. Exonuclease III and endonuclease IV are the major enzymes in *E. coli* responsible for 5′-incision of abasic sites (APs), the first steps in AP repair. Endonuclease III efficiently excises AP lesions via intermediate Schiff-base formation. Incision of L and C4-AP lesions by exonuclease III and endonuclease IV was determined under steady-state conditions using oligonucleotide duplexes containing the lesions at defined sites. An abasic lesion (AP) in an otherwise identical DNA sequence was incised by exonuclease III or endonuclease IV approximately 6-fold more efficiently than either of the oxidized abasic sites (L, C4-AP). Endonuclease IV incision efficiency of 2-deoxyribonolactone or C4-AP was independent of whether the lesion was opposite dA or dG. 2-Deoxyribonolactone is known to cross-link to endonuclease III (Hashimoto, M. (2001) *J. Am. Chem. Soc. 123*, 3161.). However, the C4-AP lesion is efficiently excised by endonuclease III. Oxidized abasic site repair by endonuclease IV and endonuclease III (C4-AP only) is ∼100-fold less efficient than repair by exonuclease III. These results suggest that the first step of C4-AP and L oxidized abasic site repair will be the same as that of regular AP lesions in *E. coli*.

Significant amounts of abasic lesions (APs),[1] which are mutagenic, are formed in cells via spontaneous depurination, as well as from damaged nucleobases which exhibit increased deglycosylation rates and can be substrates for glycosylases (*1*−*3*). Consequently, the interactions of AP lesions (or their tetrahydrofuran analogue F) with repair enzymes have been examined by many investigators (*4*−*7*). Oxidized abasic lesions such as 2-deoxyribonolactone (L) and the C4′-oxidized abasic lesion (C4-AP) are also formed as a result of endogenous reactive oxygen species and from exogenous agents that damage DNA (Figure 1). Evidence suggesting that these lesions are mutagenic and cytotoxic raises the level of importance of their repair (*8*−*10*). In this paper we report the repair of C4-AP and L lesions in synthetic oligonucleotides under steady-state conditions by repair enzymes found in *E. coli*. The oxidized abasic lesions (L, C4-AP) are efficiently incised by exonuclease III and endonuclease IV, albeit less so than an AP site. In addition, the C4′-oxidized abasic site is excised efficiently by endonuclease III.

C4-AP results from formal hydrogen atom abstraction from the C4′-position, and is produced in DNA by a variety of chemical pathways. For instance, C4-AP accounts for approximately 40% of the products formed following reaction of bleomycin with DNA (*11*). Other antitumor antibiotics, as well as diffusible species, produce the C4-AP lesion (*12*, *13*). The propensity for DNA-damaging molecules to react at the C4′-position is attributed to the accessibility of this atom, which lies on the outer edge of the minor grove to the oxidizing agents, and the relatively weak C4′-carbon−hydrogen bond (*14*, *15*). These physical features also provide support for model studies that suggest the C4-AP lesion is generated from initial oxidation of the phosphate group, as is postulated to occur as a result of the direct effects of γ-radiolysis (*16*).

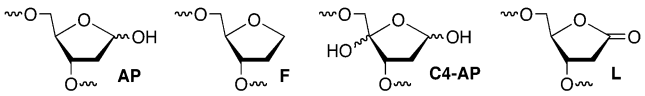

FIGURE 1: Structures of abasic lesions: "regular" abasic lesions (AP), the tetrahydrofuran analogue of an abasic site (F), C4′-oxidized abasic site (C4-AP), and 2-deoxyribonolactone (L).

L is produced from formal C1′-oxidiation, which requires cleavage of the relatively weak C1′-carbon−hydrogen bond that lies deep in the minor groove (*14*, *15*). Many DNA-damaging agents overcome the inaccessibility of the C1′-hydrogen by binding in the minor groove or through radical transfer in DNA (*13*, *17*−*19*). Increasing examples of the latter that emanate from nucleobase radicals, which are the major family of reactive species resulting from γ-irradiation of DNA, have been identified recently. The lactone lesion (L) is formed as the 5′-component of tandem lesions that are formed from the nucleobase radicals (*20*, *21*). Despite the common occurrence of the C4-AP and L lesions, with but one exception, quantitative studies on their repair have not been reported (*22*).

Synthetic oligonucleotides containing an AP lesion, or its tetrahydrofuran analogue F, are incised by Exo III and Endo

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[1] Abbreviations: C4′-oxidized abasic site, C4-AP; abasic site, AP; 2-deoxyribonolactone, L; tetrahydrofuran abasic site, F; polyacrylamide gel electrophoresis, PAGE; exonuclease III, Exo III; endonuclease IV, Endo IV; endonuclease III, Endo III; uracil DNA glycosylase, UDG.

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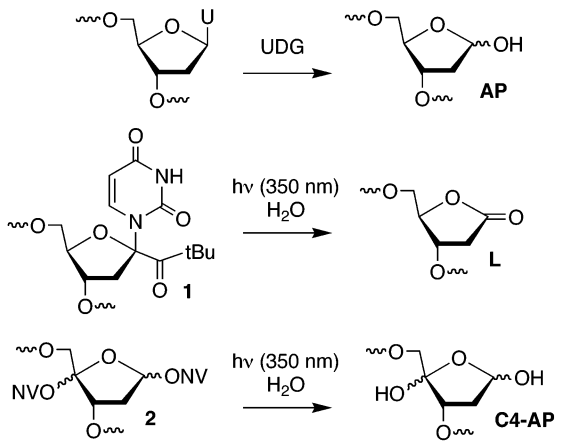

FIGURE 2: Methods for generating the AP, L, and C4-AP lesions.

IV at nanomolar enzyme concentrations (*7*). In the only side by side example in which L and AP lesions were compared quantitatively, Ape1 incised the latter ~5-fold more efficiently (*22*). Semiquantitative studies on Exo III and Endo IV incision of C4-AP and L have relied upon using substrates containing lesions generated randomly in DNA (*23*). Bleomycin or copper phenanthroline ($Cu(OP)_2$) was used to produce C4-AP and L, respectively. Repair of these DNA substrates was compared to that of AP sites produced by acidic hydrolysis. These studies led to the suggestion that C4-AP lesions were repaired considerably less efficiently than AP sites by Exo III, but slightly more efficiently by Endo IV. In contrast, both Exo III and Endo IV were slightly less effective at incising L than an AP lesion. Efficient incision of L is important in *E. coli* because this lesion cross-links Endo III (*24*, *25*). In contrast, excision of C4-AP by Endo III was more than 100 times less efficient than repair of an AP site (*23*).

The quantitative analyses of L and AP incisions by Ape1 were facilitated by chemical and enzymatic methods, respectively, for their independent generation at defined sites in oligonucleotides. AP sites are efficiently produced by the action of uracil DNA glycosylase (UDG) on oligonucleotides containing a 2′-deoxyuridine (Figure 2) (*26*). L is generated from the C1′-ketone derivative of deoxyuridine, which is one of three photochemical precursors of this lesion (*27*−*30*) (**1**, Figure 2). The studies described here concerning the C4-AP lesion were made possible by a recently reported method for synthesizing oligonucleotides containing a C4′-oxidized abasic site at a defined position from **2** (Figure 2) (*31*). The photochemical transformation utilizes the well-known *o*-nitrobenzyl photoredox reaction, proceeds very efficiently, and produces the C4-AP site in high yield.

MATERIAL AND METHODS

General Procedures. Oligonucleotide synthesis was carried out on an Applied Biosystems Inc. 394 DNA synthesizer. Oligonucleotides containing C4-AP or L were synthesized as described (*29*, *31*). Oligonucleotides containing AP sites were generated from 2′-deoxyuridine by UDG treatment (*26*). Purified oligonucleotides were characterized by ESI-MS or MALDI-TOF MS. Electrospray and MALDI-TOF mass spectrometry samples were prepared by ethanol precipitating from NH_4OAc. Electrospray mass spectra were obtained on a Finnigan/Thermoquest LCQ-Deca. MALDI-TOF mass spectra were obtained on a Kratos Seq 5. The oligonucleotides used in these experiments are shown in Table 1. Commercially available oligonucleotide synthesis reagents were obtained from Glen Research or Dharmacon Research. DNA manipulation, including enzymatic labeling, was carried out using standard procedures (*32*). Preparative oligonucleotide separations and kinetic analyses were carried out on 20% or 12% polyacrylamide denaturing gel electrophoresis (5% cross-link, 45% urea (by weight)). T4 polynucleotide kinase and uracil DNA glycosylase were obtained from New England Biolabs. Endonuclease IV was from Trevigen. Exonuclease III was obtained from Promega. Endonuclease III was isolated as its hexa-His-tagged analogue. [γ-^{32}P]ATP was purchased from Amersham Pharmacia Biotech. Radioactive samples were quantitated by Cerenkov counting using a Beckman LS6500 liquid scintillation counter. Quantification of radiolabeled oligonucleotides was carried out using a Molecular Dynamics Storm 840 PhosphorImager equipped with ImageQuant Version 5.1 software. Oligonucleotide photolyses were carried out in Pyrex tubes (0.25 in. i.d.) using a Rayonet photoreactor (RPR-100) equipped with 16 lamps having a maximum output at 350 nm.

Table 1: Oligonucleotides Employed in Polymerase Experiments

5′-d(GTC ACG TGC TGC A**X**A CGA CGT GCT GAG CCT)

a X = 1
b X = C4-AP
c X = 2
d X = L
e X = dU
f X = AP

5′-d(AGG CTC AGC ACG TCG TXT GCA GCA CGT GAC)

g X = A
h X = G
i X = T

5′-d(GTC ACG TGC TGC A**X**A CGA CGT GCT GAG CCT)
3′-d(CAG TGC ACG ACG T**Y**T GCT GCA CGA CTC GGA)

3a X = dU; Y = A
3b X = AP; Y = A
4a X = L; Y = A
4b X = L; Y = G
4c X = 1; Y =A
5a X = 2; Y = A
5b X = C4-AP; Y = A
5c X = 2; Y = G
5d X = C4-AP; Y = G

General Preparation of Duplexes Containing AP, C4-AP, or L Lesions. Procedures for preparing duplexes containing these lesions have been reported previously. Duplexes containing AP or C4-AP lesions were prepared by hybridizing oligonucleotides containing the respective precursor (2 μM) with 1.5 equiv of the appropriate complement, followed by UDG treatment or photolysis, respectively, as previously described ([duplex] = 1 μM). Duplexes containing L or **2** were hybridized at 55 °C for 5 min, and allowed to cool to room temperature over 2 h, whereas **3a** was hybridized at 90 °C for 5 min. The oligonucleotide containing **2** (c, Table 1) was photolyzed (2 μM) prior to hybridization (1 μM).

Freshly prepared stock solutions of duplexes containing a specific lesion were prepared at 1 μM and diluted accordingly for the experiments below.

Incision of L, C4-AP, and AP by Endonuclease IV. All experiments were carried out in triplicate. Two controls (three replicates each) were carried out for each experiment. The extent of conversion to the respective lesion was determined by treating the duplex with NaOH (0.1 M) for 20 min at 37 °C. Background cleavage was determined in samples to which enzyme was not added. The ability of Endo IV to incise C4-AP sites was investigated using **5b**,**d** as substrates. An (2×) enzyme solution was prepared containing 100 mM Hepes−KOH buffer (pH 7.6), 100 mM KCl, 2 mM DTT, 20% glycerol (w/v), and 4 nM endonuclease IV. The enzyme solution (5 μL) was added to 5 μL of a 2× concentration of DNA (20−500 nM). The reaction was allowed to run for 3.5 min at room temperature and then quenched with 95% formamide loading buffer (10 μL). The samples were denatured by heating at 55 °C for 3 min, and immediately placed on ice before the sample (4 μL) was loaded onto a PAGE gel.

Incision of L by Endo IV was carried out under the same conditions as described above using duplexes **4a**,**b** as substrates (10−400 nM) and 3 nM Endo IV (reaction time 3.5 min). Using duplex **3b** (20−125 nM) as substrate, a reaction for the incision of AP by Endo IV was carried out under the same conditions as above at 0.3125 nM Endo IV for 3 min.

Control experiments using the unphotolyzed precursors of C4-AP (**5a**) and 2-deoxyribonolactone (**4c**) were carried out as described above under the same conditions as for the respective photolyzed substrates.

Incision of C4-AP, L, and AP by Exonuclease III. Exonuclease III activity was assayed by measuring the rate of cleavage of duplexes **3b**, **4a**, and **5b**. Reaction mixtures contained 66 mM Tris−HCl, pH 8.0, 0.66 mM $MgCl_2$, 12.5 pM Exo III, and varying concentrations of substrate (**3b**, **4a**, or **5b**) in a total reaction volume of 10 μL. The reaction was allowed to run for a fixed period of time at room temperature and then quenched with 95% formamide loading buffer containing EDTA (20 μL). The samples were denatured by heating at 55 °C for 3 min, and immediately placed on ice before the sample (4 μL) was loaded onto a PAGE gel. The concentration range and reaction time for Exo III reactions were as follows: **5b** (C4-AP), 3−250 nM, 2 min; **4a** (L), 3−150 nM, 2 min; **3b** (AP), 0.5−30 nM, 1 min.

Control experiments using **4d** and **5a** were carried out under identical conditions to which the respective lesions were subjected. In addition, the amount of incision (cleavage) was corrected for background cleavage and extent of conversion of the precursor to the lesion as described above for the Endo IV experiments.

Excision of C4-AP and AP by Endonuclease III. The ability of Endo III to recognize C4-AP in duplex DNA was investigated using duplex **5b** as the substrate. An (2×) enzyme solution was prepared containing 20 mM Hepes−KOH buffer (pH 7.4), 200 mM KCl, 20 mM EDTA, and 6 nM endonuclease III. The enzyme solution (5 μL) was added to 5 μL of a 2× concentration of DNA (20−600 nM). The reaction was run for 2 min at room temperature and then quenched with 95% formamide loading buffer (10 μL). The samples were denatured by heating at 55 °C for 3 min, and immediately placed on ice before the sample (4 μL) was loaded onto a 20% PAGE gel.

Excision of AP in duplex DNA by Endo III was investigated under the same conditions as described above using duplex **3b** as the substrate (final concentration 1−15 nM) and 0.1 nM Endo III for a reaction time of 7 min.

Control experiments using **5b** were carried out under identical conditions to which the respective lesion was subjected. The amount of excision (cleavage) was corrected for background cleavage and extent of conversion of the precursor to the lesion as described above for the Endo IV experiments.

RESULTS

Substrate Preparation. As described previously, duplexes containing the alkali-labile lesions (AP, C4-AP, L) were prepared immediately prior to enzyme experiments. Duplexes (30 nucleotides, Table 1) containing AP or C4-AP sites were prepared by hybridizing oligonucleotides containing the respective precursor, followed by applying the appropriate method for lesion generation (Figure 2). The oligonucleotide containing the C4-AP precursor was prepared and purified by gel electrophoresis, as previously described (*31*). The precursor-containing oligonucleotide was hybridized to the appropriate complement and photolyzed as previously reported immediately prior to each experiment. Photochemical conversion was determined by treating a duplex in which the lesion-containing strand was radiolabeled with NaOH (0.1 M, 37 °C, 20 min). These conditions cleave the C4-AP site, but not its photochemical precursor (**2**) (*31*). Greater than 90% conversion to the C4-AP lesion was achieved. Template−primer duplexes containing "regular" abasic (AP) or L sites in otherwise identical sequences were prepared immediately prior to enzyme experiments using published procedures (*26*, *29*). The lactone lesion (L) was produced from **1** in an oligonucleotide (d, Table 1) prior to hybridization because the photochemical transformation is more efficient in single-stranded DNA (*29*). Hybridization was carried out at 55 °C to guard against cleaving L.

Kinetic Analysis of Exo III, Endo IV, and Endo III Digestion. All experiments were carried out in triplicate. For the L and C4-AP lesions, each experiment was in turn repeated three times. Because incision of an AP lesion by Exo III or Endo IV is well established, single experiments (three replicates) were carried out to provide a benchmark for this study (*7*). Duplexes were subjected to cleavage under Michaelis−Menten conditions. Abidance to these conditions was verified at the lowest concentration of each DNA substrate by measuring product formation as a function of time. Kinetic parameters were extracted from determinations of velocity as a function of DNA concentration. The amount of incision (cleavage) was calculated by correcting the observed cleavage for the extent of conversion and background cleavage. Background cleavage and conversion were determined in triplicate, and the average value was used to correct the observed cleavage when reaction velocity was determined. The extent of conversion was determined by hydrolyzing photolyzed or UDG-treated substrate with NaOH (0.1 M) for 20 min at 37 °C. UDG-treated samples typically showed greater than 95% conversion. L and C4-AP conver-

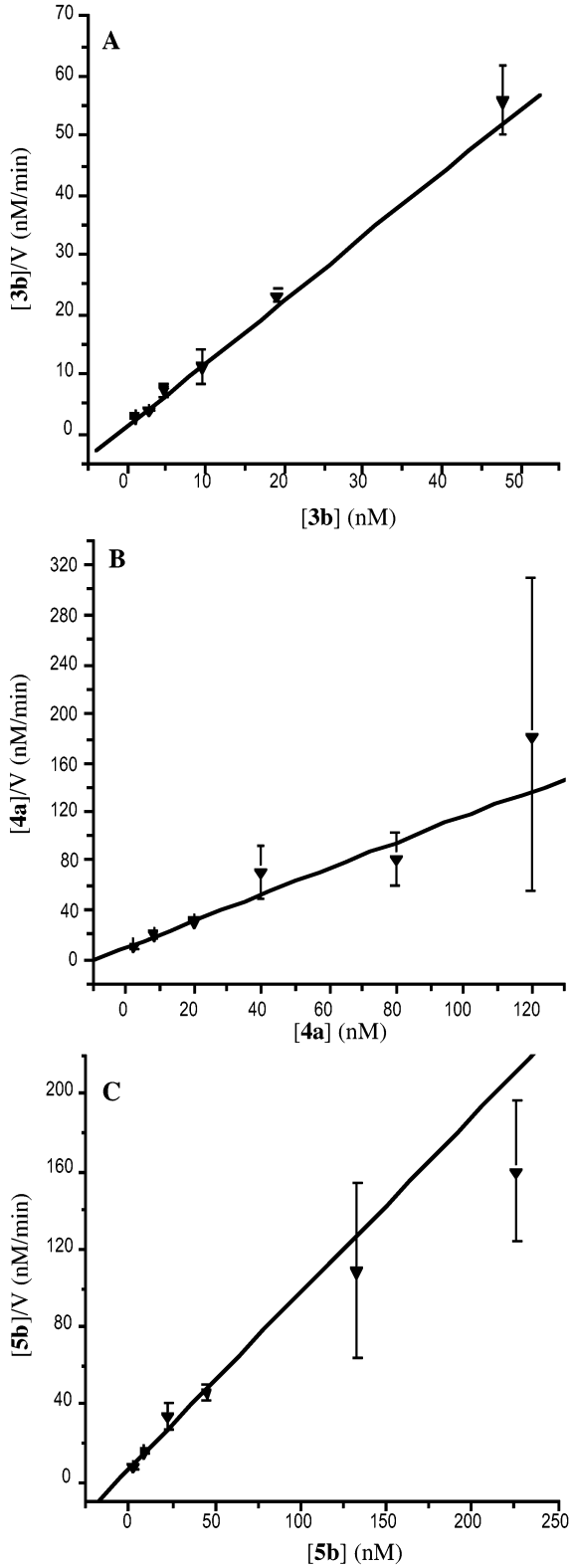

FIGURE 3: Hanes−Woolf plots describing incision of lesions by Exo III: (A) AP (**3b**), (B) L (**4a**), (C) C4-AP (**5b**).

sion was typically 85−90% and 90−95%, respectively. Background cleavage for the photochemically generated lesions (L, C4-AP) was typically 2−12%, whereas that for the AP lesions was <3%. The larger amounts of background cleavage in L and C4-AP reactions gave rise to larger errors in velocity determinations at the high end of the DNA concentrations used in kinetic experiments, because it was more difficult to accurately measure the small percentages of cleavage at these concentrations. Sample Hanes−Woolf plots, which illustrate this for the incision of the lesions by Exo III, are shown in Figure 3. Control experiments showed that duplexes containing the precursors for C4-AP (**2**) or L (**1**) lesions were not cleaved by the enzymes under the reaction conditions used to study the lesions.

Table 2: Kinetic Constants Describing Endonuclease IV Incision of Abasic Sites

lesion	base pair	K_m (nM)	k_{cat} (min^{-1})	k_{cat}/K_m (min^{-1}·nM^{-1}) × 10^2
L (**4a**)	L:dA	47.9 ± 15.1	2.8 ± 1.1	6.0 ± 3.0
L (**4b**)	L:dG	59.5 ± 17.6	4.5 ± 0.8	7.7 ± 2.7
C4-AP (**5b**)	C4-AP:dA	37.6 ± 4.5	2.3 ± 0.7	6.0 ± 1.9
C4-AP (**5d**)	C4-AP:dG	53.2 ± 12.8	2.4 ± 0.2	4.7 ± 1.2
AP (**3b**)	AP:dA	38.2	10.5	27.5

Table 3: Kinetic Constants Describing Exonuclease III Incision of Abasic Sites Opposite dA

lesion	K_m (nM)	k_{cat} (min^{-1})	k_{cat}/K_m (min^{-1}·nM^{-1})
C4-AP (**5b**)	6.8 ± 1.8	74.1 ± 17.3	10.8 ± 4.0
L (**4a**)	5.8 ± 2.7	79.3 ± 17.0	15.2 ± 8.0
AP (**3b**)	1.7 ± 0.7	75.4 ± 5.3	52.4 ± 21.9

Although the kinetics of AP incision by Exo III and Endo IV were reported previously, limited studies were carried out in the same duplex environment as the L and C4-AP lesions to provide calibration (*7*). The substrate used in this study (30 nucleotides) was slightly longer than that employed previously (23 nucleotides). The efficiency of Endo IV incision of AP when the lesion is opposite dA was ~80% of the value reported. The ability of Endo IV to incise L was found to be independent of whether dA or dG was opposite the lesion (Table 2). Furthermore, the L:dA duplex was incised 4.5 times less efficiently than the comparable substrate containing a typical AP lesion. Similar results were obtained for Endo IV incision of the C4′-oxidized abasic site opposite either dA or dG (C4-AP, Table 2). When compared to that of the AP lesion, incision of either oxidized abasic site by Endo IV proceeded with a higher K_m and lower k_{cat}, although the decrease in the latter was typically slightly larger than the relative effect on the Michaelis−Menten constant. The oxidized abasic lesions were also incised by Exo III 3.5−5-fold less efficiently than an AP site (Table 3). However, the decrease in this system was attributable to an increase in K_m for L and C4-AP.

Unlike that by Exo III and Endo IV, we are unaware of kinetic analysis of the excision of an AP site from an oligonucleotide containing the lesion at a defined site by endonuclease III (Endo III). The lyase activity of Endo III had been examined using base-damaged or depurinated DNA in which the lesions were randomly generated (*33*). Furthermore, previous studies in our laboratory established that L was not a substrate for Endo III (*24*, *25*). The AP lesion was excised approximately 6 times more efficiently than the C4′-oxidized abasic site (Table 4). Excision of AP and C4-AP sites by Endo III proceeded with efficiency comparable to that of their incision by Endo IV (Table 2). The relative efficiency of cleavage of these two lesions by Endo III was comparable to that of incision by Endo IV (AP/C4-AP ≈

Table 4: Kinetic Constants Describing Endonuclease III Excision of Abasic Sites Opposite dA

lesion	K_m (nM)	k_{cat} (min^{-1})	k_{cat}/K_m (min^{-1}•nM^{-1}) × 10^2
C4-AP (**5b**)	29.1 ± 4.3	2.0 ± 0.1	6.7 ± 1.0
AP (**3b**)	6.5 ± 2.1	2.5 ± 0.8	38.9 ± 18.0

4.5). However, the difference in cleavage efficiency by Endo III stems almost exclusively from a lower K_m for the AP substrate (**5b**). The k_{cat} values for excision of AP or C4-AP lesions by Endo III were within experimental error of one another.

DISCUSSION

Oxidized abasic lesions such as L and C4-AP are produced by a variety of DNA-damaging agents. Studies using agents that generate these lesions with low sequence specificity, along with other types of damage, suggest that they are cytotoxic and mutagenic (*8*, *9*, *34*, *35*). Experiments utilizing an L site specifically incorporated in a single-stranded shuttle vector revealed that the oxidized abasic site is bypassed in *E. coli*, and that dG and dA are preferentially incorporated opposite the lesion (*36*). Replication of L in *E. coli* also produces −1 frame shifts when the downstream nucleotide is dC. In addition, 2-deoxyribonolactone irreversibly inhibits base excision repair in vitro by forming a DNA−protein cross-link with endonuclease III (*24*, *25*). DNA pol β is also cross-linked by L, following the lesion's incision by Ape1 (*37*).

The above in vitro and in vivo studies indicate that efficient repair of C4-AP and L lesions is required to protect genomic DNA. To our knowledge, the only reported quantitative study concerning repair of an oxidized abasic site showed that L is incised ~5-fold less efficiently than an AP site by Ape1 (*22*). The more efficient incision of an AP lesion compared to L is attributable to a lower K_m. However, as mentioned above the subsequent step involving deoxyribonolactone excision by pol β is irreversibly inhibited (*37*). More qualitative studies indicate that Ape1 and pol β are able to process the C4-AP site (*38*). Qualitative studies on oxidized abasic site repair by bacterial repair enzymes found that most enzymes repair the C4-AP and L lesions less efficiently than typical AP sites (*23*). Only Endo IV incised an oxidized abasic site (C4-AP) more efficiently than an AP lesion. These valuable experiments were carried out using DNA treated with damaging agents known to produce the lesion of interest, along with other products.

The experiments described above were carried out using substrates containing independently generated abasic lesions, which remove any ambiguity regarding the type or concentration of lesion in the DNA. Homogeneous substrates made it possible to carry out experiments under Michaelis−Menten conditions. AP repair by Endo IV and Exo III had previously been quantitatively analyzed (*7*). These activities were measured in this study to provide direct comparisons between lesions independent of any sequence changes. AP incision opposite dA by Endo IV was remarkably similar to that reported when the lesion is opposite dC. However, in our hands the K_m for AP incision by Exo III when opposite dA was ~15-fold lower than that reported for an AP:dC duplex. It is already known that L is not a substrate for Endo III. However, this oxidized abasic lesion is a substrate for the type II repair enzymes Exo III and Endo IV. The C4′-oxidized abasic site is a substrate for all three enzymes studied. The oxidized abasic sites (C4-AP, L) were never more than ~6-fold poorer substrates than an AP site in all instances where the lesions were substrates. The reduced efficiency of C4-AP and L repair by Endo IV compared to a typical AP was mostly due to a lower k_{cat}. However, the diminished capacity of Exo III to incise these lesions and the ability of Endo III to excise C4-AP were attributed to higher K_m values. We do not know why one enzyme exercises selectivity in k_{cat} and the other in the Michaelis−Menten constant.

Quantitative examination of Endo IV and Exo III incision of AP sites did not reveal any dependence on the nucleotide opposite the lesion. A less complete study on Endo IV incision of L and C4-AP lesions revealed a similar lack of an effect. Incision opposite purines was examined, because antitumor agents that produce C4-AP and L show a preference for oxidizing pyrimidines (*11*, *13*, *17*, *34*). Hence, the lesions are likely to be produced opposite purines by these damaging agents. Furthermore, replication of these lesions in vitro and in vivo preferentially results in translesion incorporation of purines (*8*, *36*, *39*, *40*). All experiments in this study were carried out on substrates containing identical flanking sequences (5′-dAXA; X = C4-AP, AP, L). However, given the length of the substrate duplexes (30 nucleotides), we did not expect the flanking sequence to play a significant role in incision efficiency (*6*).

Conclusions. Despite significant structural differences, oxidized abasic lesions are repaired with ease comparable to that of typical AP sites by type II repair enzymes (Exo III, Endo IV). Furthermore, C4-AP is efficiently excised by Endo III, a type I repair enzyme with relatively high lyase activity. Incision of each respective oxidized abasic site by Exo III (Table 3) was almost 200-fold more efficient than that by Endo IV (Table 2), and excision of C4-AP by Endo III (Table 4). These data suggest that Exo III will form the first line of defense against L and C4-AP. Furthermore, Exo III will present a stalwart defense comparable in strength to that used against a typical AP.

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