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An apyrimidinic site kinks DNA and triggers incision by endonuclease VII of phage T4

Beate Greger and Börries Kemper*

Institute for Genetics of the University of Cologne, Zülpicher Straße 47, 50674 Köln, Germany

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ABSTRACT

Apurinic/apyrimidinic lesions (AP-sites) occur frequently in DNA, generated by physically and chemically induced or spontaneous loss of bases. Repair mechanisms have evolved in organisms to deal efficiently with AP-sites by first incising the DNA at the lesion, followed by excision and resynthesis of the damaged strand. Here we report that endonuclease VII (endo VII) of phage T4, which was originally classified as a debranching and Holliday structure resolving enzyme, also recognizes AP-sites with high efficiency. The enzyme cleaves both strands of double-stranded DNA in a stepwise fashion a few nucleotides 3′ of the lesion. In a search for a recognition signal shared by all known endo VII substrates, kinking of DNA has earlier been suggested as such a signal. In support of this hypothesis, we demonstrate here that AP-sites induce distinct kinks in synthetic oligonucleotides allowing efficient intramolecular ring closure by ligation.

INTRODUCTION

Genetic information is under constant threat by chemical and physical factors damaging the genetic material. Loss of single bases resulting in apyrimidinic/apurinic sites (AP-sites) is among the most frequently occurring lesions in DNA. AP-sites are non-instructional for DNA polymerases and inhibit DNA replication or cause base substitution mutations by allowing incorporation of incorrect nucleotides. Organisms have evolved powerful repair mechanisms to protect the structural integrity of their DNA.

Repair of AP-sites is initiated by AP-endonucleases or lyases marking the lesions with 5′ or 3′ incisions for further repair by other enzymes (1). AP-endonucleases have been identified in many prokaryotic and eukaryotic cells (for a review see 2). AP-endonucleases in *Escherichia coli* are, for example, exonuclease III (exo III) and endonuclease IV (endo IV). In addition, there are several enzymes which cleave at AP-sites via β-elimination (lyases). These enzymes include endonuclease III (endo III) (3), endonuclease VIII (4) and FPG protein (5). Phage T4, multiplying in *E.coli*, uses the host's enzymes and contributes the thymine dimer processing enzyme endonuclease V (endo V) for repair of AP-sites (6).

Endonuclease VII (endo VII) of phage T4 was originally described as a Holliday structure resolvase. Later it was shown to be a versatile repair enzyme that reacts with many lesions in DNA. These include branched DNAs, single-strand overhangs, nicks, gaps, base mismatches, heteroduplex loops, bulky adducts and even curves in DNA (7). The enzyme always introduces staggered nicks flanking the target on the 3′-side in a time-delayed nick and counter-nick fashion.

We report here that endo VII also recognizes AP sites with high efficiency, introducing staggered nicks a few nucleotides 3′ of the lesion, thus repeating the cleavage pattern observed with other substrates. We also confirm that AP-sites kink duplex DNA, which will be discussed as a universal recognition signal for endo VII.

MATERIALS AND METHODS

Chemicals, radiochemicals and oligonucleotides

Standard chemicals were purchased from Merck (Darmstadt, Germany). Polyacrylamide (*N*,*N*-methylene bis-acrylamide/bis-acrylamide, 19:1) and bis-acrylamide (5%) were purchased from Serva (Heidelberg, Germany). [γ-^{32}P]ATP (300 mCi/mMol) was purchased from Amersham (Braunschweig, Germany). Oligonucleotides were custom designed and purchased from Pharmacia (Freiburg, Germany). Crude oligonucleotides were purified by electrophoresis over a 20% denaturing polyacrylamide gel followed by elution overnight in TE buffer at 56°C. The sequence of the oligonucleotide used was 5′-GCC**T**CGAGGT C**C**GAC**T**CTAG AGG-CCTCTAG AGTCGGACCT CGAGGC-3′ (Bea-46). The oligonucleotide forms hairpins due to intramolecular complementarity (Fig. 1). The site of the turn is indicated by a hyphen. Residues used for generating AP-sites after replacement by dU residues (see Results for a further explanation) are in bold and underlined. Restriction sites for *Ava*I and *Xba*I were incorporated into the construct for quality control testing for double-strandedness. Routinely, *Ava*I and *Xba*I cleaved up to 80 and 90%, respectively, of purified ready-to-use Bea-46.

Uracil-containing oligonucleotides (U-DNAs) were: Bea-46U^{M}, 5′-GCCTCGAGGT C**U**GACTCTAG AGG-CCTCTAG AGTCAGACCT CGAGGC-3′; Bea-46U^{L}, 5′-GCC**U**CGAGGT CCGACTCTAG AGG-CCTCTA GAGTCGGACC TCGAGGC-3′; Bea-46U^{R}, 5′-GCCTCGAGGT CCGACTC**U**AG AGG-CCTCTA GAGTCGGAC CTCGAGGC-3′ (U residues in bold and underlined). Mismatch C/C-containing oligonucleotides were: Bea-46MM^{M}, 5′-GCCTCGAGGT C**C**GACTCTAG AGG-CCTCTAG AGTC**C**GACCT CGAGGC-3′; Bea-46MM^{L}, 5′-GCC**C**CGAGGT CCGACTCTAG AGG-CCTCTA GAGTCGGAC**C** TCGAGGC-3′; Bea-46MM^{R}, 5′-GCCTCGAGGT CCGACTC**C**AG AGG-CCTCT**C**G AGTCGGACCT CGAGGC-3′ (mismatched nucleotides in bold

*To whom correspondence should be addressed. Tel: +49 221 470 5287; Fax: +49 221 470 5172; Email: b.kemper@uni-koeln.de

and underlined); pseudo Y-junction oligonucleotide Bea-46YJ^L, 5′-**<u>GCCT</u>**CGAGGT CCGACTCTAG AGG-CCTCTAG AGTCGGACCT CG**<u>CAAT</u>**-3′ (non-pairing nucleotides in bold and underlined); loop oligonucleotide Bea-46LO^R, 5′-GCCTCGAGGT CCGACTCT**<u>AG</u>** **<u>AGG</u>**-**<u>TTCTG</u>**AGA GTCGGACCTC GAGGC-3′ (looping nucleotides in bold and underlined); Bea-103AP^M (top strand), 5′-GAATTCGCTA CATCGATCGG AGCCGCTAGG CCTACATGCC TGCAGGTC**<u>U</u>**G ACTCTAGAGG ATCCGGAATT CACTGGCCGT CAAAGAATTC CGGATTAGGG TTC-3′; Bea-103AP^M (bottom strand), 3′-AAGCGAT GTAGCTAGCC TCGGCGATCC GGATGTACGG ACGTCCAGAC TGAGATCTCC TAGGCCTTAA GTGACCGGCA GTTTCTTAAG GCCTAATCCC AACCTT-5′ (U residue in bold and underlined).

Enzymes

Restriction enzymes, T4 polynucleotide kinase, T4 DNA ligase and uracil glycosylase (UDG) from calf thymus were purchased from Boehringer (Mannheim, Germany) and used as recommended by the manufacturer. λ-Exonuclease was purchased from Pharmacia (Heidelberg, Germany) and was used as recommended by the manufacturer. Endo III from *E.coli* was a generous gift from Rick Cunningham (Albany, NY). Endo VII was purified in our laboratory from overexpressing bacteria following previously described procedures (8). HU protein was purified in our laboratory and kindly provided by Stefan Golz. Briefly, the protein was isolated from the cleared supernatant of crude extracts obtained after sonication of cells of *E.coli* BL21(DE3) by column chromatography over heparin–agarose at pH 6.0 in buffer C followed by Mono S at pH 6.0 in the same buffer.

Buffers

TE buffer contained 10 mM Tris–HCl, pH 8.0, and 1 mM EDTA. TBE buffer contained 89 mM boric acid and 2 mM EDTA. EMSA binding buffer contained 50 mM Tris–HCl, pH 8.0, 5 mM EDTA, pH 8.0, and 1 mM DTT. EMSA loading buffer contained 20 mM Tris–acetate, pH 7.4, 5 mM EDTA, pH 8.0, 1 mM DTT and 0.1 µg/µl BSA. EMSA electrophoresis buffer contained 67 mM Tris–HCl, pH 8.0, 33 mM sodium acetate and 20 mM EDTA. Endo III reaction buffer contained 10 mM Tris–HCl, pH 8.0, 10 mM EDTA, 10 mM β-mercaptoethanol and 40 mM KCl. Endo VII reaction buffer contained 50 mM Tris–HCl, pH 8.0, 1 mM $MgCl_2$,1 mM β-mercaptoethanol and 250 µg/ml BSA (nuclease-free). M&G stop mixture contained 90% (v/v) formamide, 0.1% (w/v) xylene xyanol and 0.1% (w/v) bromphenol blue in TBE buffer. Stop mixture for native gel electrophoresis contained 90% (v/v) glycerol, 0.1% (w/v) xylene xyanol, 0.1% (w/v) bromphenol blue, 10 mM Tris–HCl, pH 8.0, and 50 mM EDTA. Buffer C, used for purification of HU protein, contained 10 mM KPO_4, pH 6.0, 1 mM EDTA, 1 mM β-mercaptoethanol and 10% (v/v) glycerol.

Enzyme reactions

Glycosylase reactions were performed for 1 h at 37°C in 40 µl reaction mixture containing 4 pmol DNA and 1 U glycosylase. Reaction products were separated on a 10% native polyacrylamide gel. Endo III reactions were performed for 30 min at 37°C in 20 µl reaction mixture containing 25 fmol DNA. Endo VII reactions were performed for 15 min at 37°C in 10 µl reaction mixture containing 25 fmol DNA. Reaction products were precipitated with ethanol and analysed on a 15% denaturing polyacrylamide gel.

Regular ligation reactions were performed for 2 h at 30°C in 20 µl ligation buffer containing 100 fmol DNA. Ligation reactions in the presence of HU protein using 100 fmol DNA Bea-103U^M were done after pre-incubation of DNA with 5 ng HU protein for 30 min at 30°C without ligase in 20 µl ligation buffer. Ligation experiments using AP-DNA Bea-103AP^M were done in the same way but omitting the pre-incubation. Reactions were terminated by ethanol precipitation and the resulting products were analysed on a 6% denaturing polyacrylamide gel. Digestion of ligation products with λ-exonuclease was performed for 45 min at 37°C in 10 µl λ-exonuclease buffer with ethanol-precipitated ligation products. After the reaction, ethanol-precipitated DNA was further purified by phenol–chloroform extraction before analysis on a 6% denaturing polyacrylamide gel.

Electrophoreses

Denaturing polyacrylamide gels were run at 60 W in 1× TBE buffer for 2 h at room temperature. Native polyacrylamide gels were run at 100 V in 1× TBE buffer at 4°C for at least 48 h. The temperature of the gel should never exceed 10°C. Addition of 12 mM $MgCl_2$ to native gels and to the running buffer sharpens the bands and facilitates cutting out of gel slices for elution of DNA.

Quantifications

Quantitative results were obtained by phosphorimaging appropriate gels using a Fuji Bas1000 PhosphorImager. Results were averaged from three independent experiments.

RESULTS

Generation of substrates with AP-sites

AP-sites were introduced into synthetic double-stranded (ds) oligonucleotides by removing unique built-in uracil residues (dU residues) with UDG. Starting from the progenitor snap-back DNA Bea-46 (Fig. 1), three constructs, each carrying a unique dU residue replacing one of the top strand residues, were made (U-DNAs). These substrates were Bea-46U^M, Bea46-U^L and Bea46-U^R, each with one dU residue in the middle (M), at the left (L) or the right end (R) of the molecule. dU residues were removed by UDG treatment, giving substrates Bea-46AP^M, Bea-46AP^L and Bea-46AP^R (AP-DNAs).

The procedure for generating U-DNAs was optimized using Bea-46U^M as the model substrate. Purified 5′-end-labelled oligonucleotide Bea-46U^M was treated with UDG as described in Materials and Methods. When the reaction products were separated on a denaturing polyacrylamide gel, UDG-treated and untreated control DNA showed the same mobilities (Fig. 2, lanes 1 and 2). On a native polyacrylamide gel, however, two bands appeared in the UDG-treated sample. One of these bands co-migrated with untreated control DNA, the second band migrated markedly slower (Fig. 2, lanes 3 and 4). This band contained AP-DNA, as shown by its sensitivity to digestion with endo III from *E.coli* (9). Treatment of DNA from this band with endo III gave one major fragment of 12 nt, indicating incision 5′ of the presumed AP-site (Fig. 3a, lanes 10–18). A second faster migrating band reflects minor incisions (<5% of total incisions) 1 nt further towards the 5′-end of the DNA. DNA from the second band was resistant to cleavage by

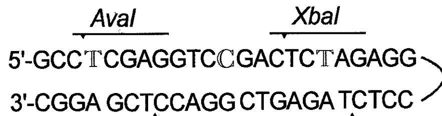

Figure 1. DNA Bea-46. The sketch shows the sequence of snap-back oligonucleotide Bea-46 containing 46 nt forming a perfect hairpin through built-in self-complementarity. Bea-46 is the progenitor DNA for the AP constructs described in this communication. Unique uracil residues were added into this sequence replacing the two thymine residues (open letters T) one at a time or the central C residue (open letter C), giving three uracil containing U-DNAs. Uracil was then removed by UDG treatment as described in the text, creating unique AP-sites in AP-DNAs. Restriction sites for *Ava*I and *Xba*I were incorporated into the construct for quality control testing for double-strandedness as described in Materials and Methods.

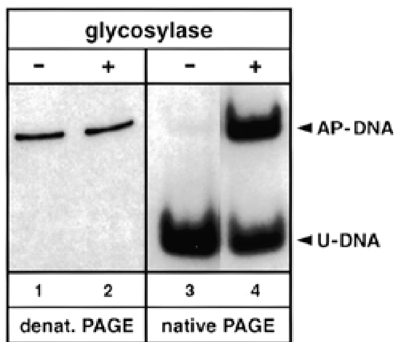

Figure 2. Electrophoretic mobility of UDG-treated U-DNA. UDG-treated (+) substrate Bea-46U^M and untreated (–) control DNA were electrophoresed on denaturing polyacrylamide gels (lanes 1 and 2) or native polyacrylamide gels (lanes 3 and 4) as described in Materials and Methods. AP-DNA and U-DNA to the right of the figure denote the positions of the respective DNAs in the native gel.

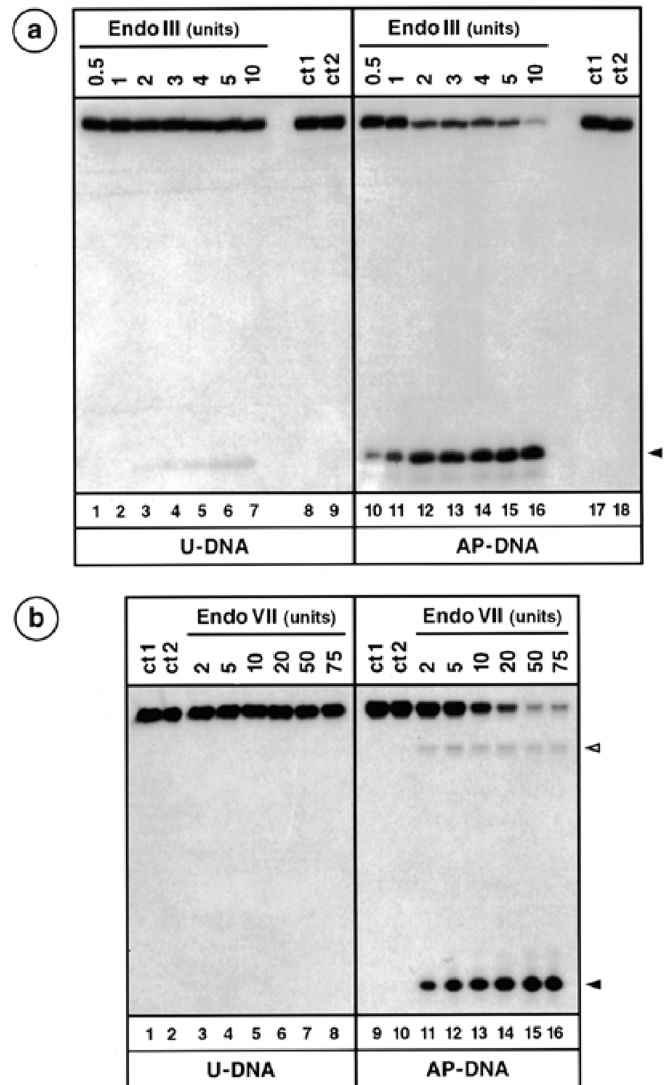

Figure 3. Reactions of U-DNAs and AP-DNAs with endo III and endo VII. Aliquots of samples containing U-DNA Bea-46U^M or AP-DNA Bea-46AP^M were treated with endo III or endo VII as described in Materials and Methods and then separated on a denaturing polyacrylamide gel. ct1 and ct2 denote control samples which were not treated with enzyme. (**a**) Equimolar amounts (25 fmol) U-DNA Bea-46U^M and AP-DNA Bea-46AP^M were treated with increasing amounts of endo III as indicated below the figure. (**b**) Equimolar amounts of U-DNA Bea-46U^M and AP-DNA Bea-46AP^M were treated with increasing amounts of endo VII.

endo III, as expected for unreacted U-DNA (Fig. 3a, lanes 1–9). AP-DNAs were purified on native polyacrylamide gels before they were used in experiments. Very faint incision bands visible in the experiment shown in Figure 3a (lanes 3–7) derive from variable small amounts of AP-DNA contaminating U-DNA due to incomplete separation on the purification gel.

The highest yield of AP-DNA after UDG treatment was 70–80% of the input DNA and was similar for all substrates used in these studies, as judged by digestions with endo III and endo VII (see below).

Cleavage of AP-DNA by endo VII

AP-DNAs were highly sensitive to cleavage by endo VII and in a limit digest of Bea-46AP^M ~80% of the DNA was cleaved with 50 U enzyme (Fig. 3b, lanes 9–16). The relative efficiency of cleavage was about the same as with cruciform DNA, reported earlier as one of the most sensitive substrates for endo VII (10).

Two classes of cleavage products were obtained after treatment with endo VII. The major class contained five fragments differing in size by 1 nt. The filled arrow in Figure 3b points to the major fragment of 14 nt; the minor fragments are not visible in this figure. The minor class contained two fragments also differing in size by 1 nt (open arrow in Fig. 3b). The major class of fragments originated from incisions in the top strand of the construct 3′ adjacent to the AP-site. The minor class fragments originated from incisions in the bottom strand of the construct, again 3′ of the AP-site. Both classes contained one major and several minor bands and reflect a typical endo VII cleavage pattern as frequently observed with other substrates (7,11). It should be noted that the extent of bottom strand cleavage is underestimated in these experiments since in hairpin constructs bottom strand incisions are overshadowed by concomitant top strand incisions. Unmodified U-DNA was completely resistant to endo VII cleavage (Fig. 3b, lanes 1–9).

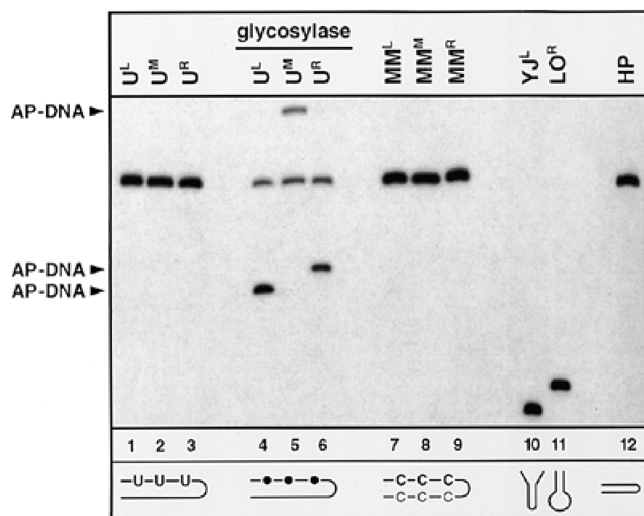

Figure 4. Comparison of electrophoretic mobilities of snap-back constructs. Samples containing 2 pmol 5′-end-labelled constructs were each electrophoresed through native polyacrylamide gels. Lanes 1–6, labels U^L, U^M and U^R in the headline of the figure denote U-DNAs Bea-46U^L, Bea-46U^M and Bea-46U^R; lanes 7–9, labels MM^L, MM^M and MM^R in the headline of the figure denote C/C mismatch-containing substrates Bea-46MM^L, Bea-46MM^M and Bea-46MM^R; lanes 10–12, labels YJ^L, LO^R and HP in the headline of the gel denote substrates Bea-46YJ^L, Bea-46LO^R and Bea-46, respectively. The label glycosylase denotes samples treated with 1 U UDG. The AP-DNA at the left side of the figure marks the positions of bands containing AP-DNA in lanes 4–6. For further explanations see Results.

AP-sites induce kinks in DNA

We pursued the question of why AP-DNAs migrate slower on a native gel than U-DNAs. It has been shown before that AP-sites can induce kinks in oligonucleotides (12). It has also been shown that kinks, curves and bends in DNA can markedly influence the electrophoretic mobility of short DNA fragments and that the extent of mobility shift varied with the location of the respective structural alteration in a linear molecule (13).

To investigate whether the observed AP-site-induced mobility shift in Bea-46AP^M was dependent on the location of the AP-site in the construct or not, substrates Bea-46AP^L and Bea-46AP^R containing off-centre AP-sites were made and their migration behaviour was compared with that of Bea-46AP^M.

To create the required AP-DNAs, substrates Bea-46U^M, Bea-46U^L and Bea-46U^R were treated in side-by-side reactions with UDG to remove the uracil. When the reaction products were analysed on native polyacrylamide gels each substrate gave rise to two product bands, one co-migrating with untreated progenitor U-DNA, the other migrating slower for Bea-46AP^M or faster for Bea-46AP^L and Bea-46AP^R (Fig. 4, lanes 1–6). This is a unique feature of AP-DNAs, since the same DNAs carrying C/C mismatches in exactly the same locations as the respective AP-DNAs carry their AP-sites did not show an altered migration behaviour. Treatment of the resulting DNAs Bea-MM^M, Bea-MM^L and Bea-MM^R with UDG using the same experimental set-up did not give rise to products with altered migration behaviour (Fig. 4, lanes 7–9). Together, these results indicate that the oligonucleotides are kinked by AP-sites, since molecules with a kink in the centre

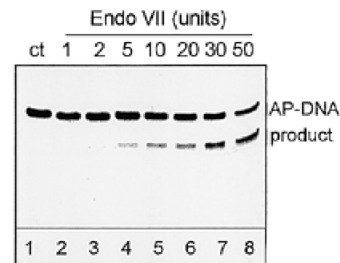

Figure 5. Reaction of Bea-103AP^M with endo VII. Samples containing 25 fmol 5′-end-labelled Bea-103AP^M were incubated with various amounts of endo VII as indicated above the figure and then separated on a 10% denaturing polyacrylamide gel. AP-DNA denotes input DNA; product denotes the major cleavage product in the gel.

migrated more slowly in an acrylamide gel than molecules with kinks located off-centre (13).

Since the AP-sites in constructs BeaAP46^L and BeaAP-46^R are located 4 nt from the left (open) end and 5 nt from the right (looping) end of the substrates, respectively, partial denaturation of the constructs was anticipated as another possible reason for the altered migration behaviour. This possibility was excluded by using two more control substrates, each mimicking one of the anticipated partially denatured DNAs. One was a hairpin with four non-pairing nucleotides at the open end of the molecule (Bea-46YJ^L) and one contained 10 non-pairing nucleotides at the looping end of the molecule (Bea-46LO^R). Both constructs migrated considerably faster than any of the AP-constructs (Fig. 4, lanes 10 and 11). The same results were also obtained after treatment with UDG. From these experiments we conclude that AP-DNAs are fully base-paired and that their unusual migration behaviour is indeed caused by the AP-site kinking the DNA.

AP-sites facilitate intramolecular circularisation of linear DNA

As shown by others, kinks or bends in DNA facilitate intramolecular circularisation. This was demonstrated, for example, with DNA bent by protein HU (14). Similarly, it was reasoned that AP-site-induced kinks should facilitate circularisation of linear molecules without addition of bending proteins. Following the published procedure for HU protein-induced bending, this was applied to a linear double-stranded substrate, Bea-AP103^M, which was obtained after UDG treatment of Bea-U103^M, which was made by hybridisation of two synthetic oligonucleotides. The construct contained one U residue in the middle (nt 55) of the molecule and two terminal *Eco*RI half-sites providing sticky ends for ligation. After removal of the U residue by UDG, substrate Bea-AP103^M was obtained. Bea-AP103^M migrated considerably more slowly on native polyacrylamide gels than Bea-U103^M, as was described above for construct Bea-AP46^M (results not shown). DNA Bea-AP103^M was sensitive to endo VII as expected and a major reaction product indicative of cleavage at the AP-site was obtained (Fig. 5).

The behaviour of DNAs Bea-U103^M and Bea-AP103^M was compared in side-by-side ligation reactions. The results are summarised in Figure 6. Ligation of Bea-U103^M gave rise to two slower migrating product bands which were identified by size measurements as linear dimers and trimers. The same two

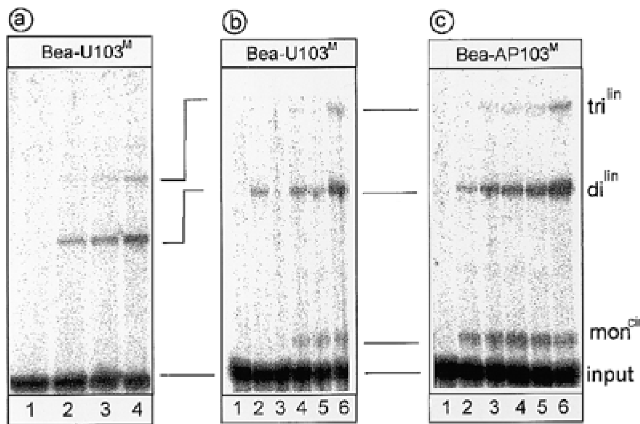

Figure 6. Ligation of DNAs Bea-U103M and Bea-AP103M. Samples containing 100 fmol 5′-end-labelled Bea-U103M or Bea-AP103M were treated with DNA ligase, as described in Materials and Methods. After the reaction, aliquots of each sample were analysed on a 6% denaturing polyacrylamide gel. (**a**) Ligation of uracil-containing progenitor DNA Bea-U103M. Lane 1, control without ligase; lanes 2–4, samples with 2, 4 and 8 U DNA ligase, respectively. (**b**) Ligation of uracil-containing DNA Bea-U103M in the presence of HU protein. Lane 1, control without ligase and HU protein; lane 2, control with 8 U ligase without HU protein; lane 3, control without ligase but with 20 ng HU protein; lanes 4–6, samples containing 1, 2 and 5 ng, respectively, HU protein in the presence of 8 U ligase. (**c**) Ligation of AP-DNA Bea-AP103M. Lane 1, control without ligase; lanes 2–6, ligation with 1, 2, 4, 8 and 16 U ligase, respectively. Labels to the right of the figure: tri^{lin}, linear trimers; di^{lin}, linear dimers; mon^{cir}, circular monomeric DNA; input, unreacted DNA.

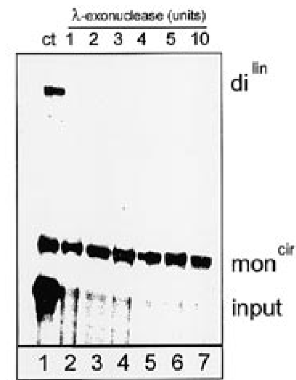

Figure 7. Digestion of ligation products of DNA Bea-103APM with λ-exonuclease. Ligation reactions were performed with 100 fmol 5′-end-labelled DNA and 8 U ligase as described in Materials and Methods. Then different amounts of λ-exonuclease were added to these reactions and the products were analysed on a 6% denaturing polyacrylamide gel as described in Materials and Methods. ct, control reaction without ligase. Labels to the right of the figure: di^{lin}, linear dimers; mon^{cir}, monomeric circular DNA; input, unreacted input DNA.

product bands were obtained with Bea-AP103M. In addition to these bands, however, another product band was found migrating slightly slower than input DNA. The same band occurred when Bea-U103M was treated with ligase in the presence of HU protein (Fig. 6b). This band contained exclusively circular DNA molecules, as was proven by their resistance to degradation by λ-exonuclease. As shown in Figure 7, treatment of the DNA in the complete ligation reaction mixture with increasing amounts of λ-exonuclease caused degradation of unreacted monomeric and linear dimeric DNA. A fraction containing the presumed circular monomers proved completely resistant to the action of λ-exonuclease.

Relative cleavage efficiencies of AP-sites by endo III and endo VII

The sensitivity of the reactions between AP-sites and endo III or endo VII was determined by measuring the relative cleavage efficiencies in reactions with AP-DNAs. The results are summarised in Figure 8. Endo III was unable to cleave the left, off-centre AP-site in Bea-46APL. Up to 20 U of the enzyme did not produce measurable amounts of cleavage products (Fig. 8a). In contrast, the same substrate showed considerable cleavage reactivity with endo VII (Fig. 8b). The right off-centre AP-site in Bea-46APR was a poor substrate for endo III (Fig. 8a) and a moderately good substrate for endo VII (Fig. 8b).

It is worth noting that all AP-DNAs were at least 3–4-fold better substrates for endo VII than any of the three sequence identical mismatch DNAs containing a C/C mismatch replacing the AP-sites (Fig. 8c). Earlier comparative experiments had shown that C/C mismatches were among the most reactive mismatch substrates for endo VII (15). This leads to the conclusion that AP-sites are more reactive targets for endo VII than any of the mismatches.

DISCUSSION

Spontaneous loss of bases from DNA occurs frequently under physiological conditions. The resulting AP-sites are potential hazards to living systems, since they are non-instructional for DNA polymerase and inhibit DNA replication or cause base substitution mutations due to incorporation of inappropriate nucleotides. Efficient repair mechanisms have been evolved by organisms specialised to prevent accumulation of AP-sites. For repair, AP-sites are recognized by AP-endonucleases or lyases, which have been isolated from numerous prokaryotic and eukaryotic sources (1). The majority of AP-endonucleases are specific for AP-sites; some are also associated with *N*-glycosylase activities. True AP-endonucleases incise the DNA backbone 5′ of the AP-site by hydrolysis of phosphodiester bonds, preparing the site for further repair reactions by other enzymes (16).

Endo VII of phage T4, which is a versatile repair enzyme, was shown here to also react with AP-sites by incising both strands 3′ flanking the lesion. Endo VII reacts with many targets in DNA, such as branched DNAs, Holliday structures and Y-junctions, single-strand overhangs, nicks, gaps, mismatches, heteroduplex loops, bulky adducts and curved DNA (7). Incisions are always placed 3′ flanking the target, which in the case of a Holliday structure results in precise resolution (17). In the case of mismatches and heteroduplex loops, incisions were shown to propagate excision repair *in vitro* by the combined action of the 3′→5′ exonuclease activity of DNA polymerase, which removes the unpaired nucleotide, and the polymerising activity, which resynthesizes the erased sequences across the formerly unpaired site (15,18).

The broad profile of recognizable targets in dsDNA suggests that endo VII is a versatile repair initiator which triggers repair functions of phage T4, rather than being specific for AP-sites. By current functional classifications endo VII is neither a true AP-endonuclease, incising DNA 5′ of AP-sites by cleavage of phosphodiester bonds, nor a lyase, which incises the DNA 3′ of

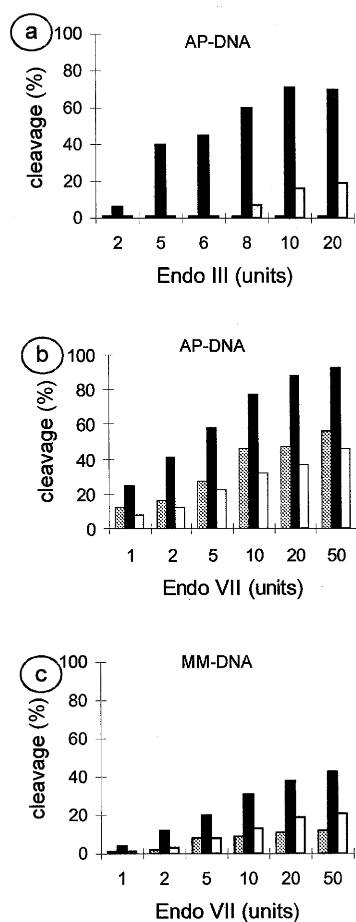

Figure 8. Relative cleavage efficiencies determined with (**a** and **b**) AP-DNAs and (**c**) MM-DNAs and (a) endo III and (b and c) endo VII. Samples with equimolar amounts (25 fmol) of AP-DNAs or MM-DNAs were treated with increasing amounts of enzymes endo III or endo VII as described in Materials and Methods. Aliquots of the samples were separated on denaturing 15% polyacrylamide gels and the total amount of cleavage products was quantitated by phosphorimaging. Units of the enzyme added to the reactions are indicated below each panel. Relative cleavage efficiencies are expressed by the amounts of cleavage products measured per total amount of input DNA, given in per cent. Substrates and their symbols are: Bea-AP46^{M}, black bars; Bea-46AP^{R}, white bars; Bea-46AP^{L}, grey bars.

AP-sites by β-elimination. The enzyme is a true endonuclease, hydrolysing exclusively internal phosphodiester bonds yielding ligatable 3′-OH and 5′-PO_4 termini, always incising 3′ of the respective targets, including AP-sites (15,17). A recognition signal common to all endo VII substrates has not been elucidated with certainty. However, the defects in DNA known as substrates for endo VII have in common the potential to distort the DNA locally. They can introduce sharp bends or kinks, as is most obvious for branched DNAs. Endo VII, acting as a dimer with two DNA binding sites (19), is envisaged here to contact two flanking arms extending from a kinked site or a junction (Fig. 9). The finding that AP-sites kink DNA is interpreted in favour of this model. Endo VII is therefore not an enzyme which reacts directly with missing bases, as true AP-endonucleases do (20), but reacts to the kink as a structural feature following base loss.

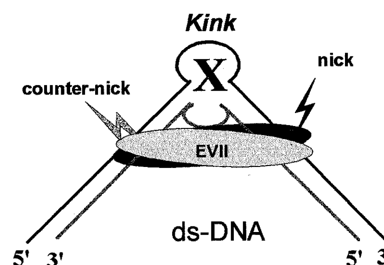

Figure 9. Model of endo VII binding to kinked DNA. The diagram shows endo VII binding as a dimer to two arms of a linear kinked DNA molecule. The X in the centre symbolizes any feature introducing a kink in dsDNA, as for example an AP-site.

When the same procedure for detecting kinks in AP-DNA was applied to mismatch C/C-containing DNAs of otherwise identical sequence no indications of kinking were found. Since mismatches in general and C/C mismatches in particular are good substrates for endo VII, one has to assume that kinks at mismatching bases are too shallow to be discovered by the procedure used here. Thus, it is not surprising that mismatches provide relatively weak signals and are cleaved by endo VII 3–4-fold less efficiently than is DNA containing AP-sites.

Finally, it should be considered that a shallow kink or bend may become enhanced after first contact with the enzyme, thereby stabilizing an initially weak DNA–protein complex. Indeed, evidence was presented recently that binding of endo VII to cruciform DNA alters the conformation of the DNA structure markedly (21).

Furthermore, endo VII reacts as efficiently with AP-sites as it does with cruciform DNA, which is among the best substrates for the enzyme. Endo VII has been shown to bend DNA (22). The specific activity of endo VII was comparable with that of endo III, which is an AP-specific lyase. Substrates with terminal off-centre AP-sites, which were poor or unreactive substrates for endo III, turned out to be remarkably good substrates for endo VII (Fig. 8). This is presumably due to differences in the requirements of the two enzymes for productive binding, with endo VII being more adaptable to short arms flanking the targets than endo III.

In summary, AP-sites enlarge the number of DNA substrates recognized and cleaved by endo VII, emphasising the importance of the enzyme as a potential and versatile 'all-round' repair tool. However, we want to point out that true repair of an AP-site, meaning re-insertion of the missing base, can only be successful when the incision by endo VII occurs in the same strand as the missing base. Incisions in the other strand are unproductive and may cause insertion of random nucleotides due to lack of information opposite an AP-site, failing to correct the AP-site itself.

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