Lesion selectivity in blockage of lambda exonuclease by DNA damage

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ABSTRACT

Various kinds of DNA damage block the 3′ to 5′ exonuclease action of both *E.coli* exonuclease III and T4 DNA polymerase. This study shows that a variety of DNA damage likewise inhibits DNA digestion by lambda exonuclease, a 5′ to 3′ exonuclease. The processive degradation of DNA by the enzyme is blocked if the substrate DNA is treated with ultraviolet irradiation, anthramycin, distamycin, or benzo[a]-pyrene diol epoxide. Furthermore, as with the 3′ to 5′ exonucleases, the enzyme stops at discrete sites which are different for different DNA damaging agents. On the other hand, digestion of treated DNA by lambda exonuclease is only tran-siently inhibited at guanine residues alkylated with the acridine mustard ICR-170. The enzyme does not bypass benzo[a]-pyrene diol epoxide or anthramycin lesions even after extensive incubation. While both benzo[a]-pyrene diol epoxide and ICR-170 alkylate the guanine N-7 position, only benzo[a]-pyrene diol epoxide also reacts with the guanine N-2 position in the minor groove of DNA. Anthramycin and distamycin bind exclusively to sites in the minor groove of DNA. Thus lambda exonuclease may be particularly sensitive to obstructions in the minor groove of DNA; alternatively, the enzyme may be blocked by some local helix distortion caused by these adducts, but not by alkylation at guanine N-7 sites.

INTRODUCTION

Nucleolytic enzymes provide tools for the analysis and manipulation of nucleic acids in the laboratory. Thus, exonuclease III of *E. coli* and the 3′ to 5′ exonuclease of T4 DNA polymerase are powerful tools for examining the DNA sequence positions of stable DNA adducts induced by various reactive molecules (1,2,3,4,5,6,7). The procedure makes use of the fact that these exonucleases are blocked at sites of many stable DNA lesions. If DNA labeled at its 5′ end and containing such lesions is used as the substrate the length of a labeled fragment after exonuclease digestion indicates the position of the lesion.

This paper investigates the use of the highly processive 5′ to 3′ exonuclease induced by bacteriophage lambda (8, 9) as a similar tool. Digestion of DNA by lambda exonuclease is blocked by some, but not all, types of DNA damage. Lambda exonuclease is strongly inhibited by anthramycin-induced DNA damage and to a lesser extent by distamycin A binding to DNA. Both compounds bind exclusively to the minor groove of DNA yet do not grossly alter DNA structure, although only the former produces covalently bound adducts. Agents, such as cisplatin and ultraviolet irradiation, whose interaction with DNA results in an altered DNA helix (e.g., a 'kinked' site) also render the DNA somewhat resistant to lambda exonuclease digestion. Lambda exonuclease seems to be blocked at DNA sites where these agents would be expected to induce damage, although the precise relationship between the stop sites and the lesion site remains to be determined. In contrast, agents that alkylate the guanine N7 position in the DNA major groove, yet do not alter the DNA helix, do not substantially reduce the digestion of DNA by lambda exonuclease. These studies suggest that lambda exonuclease may be primarily sensitive to damage in the minor groove of DNA.

MATERIALS AND METHODS

Materials

Reagents were obtained from the following sources: *cis*-dichlorodiammineplatinum(II) and dimethyl sulfate, Aldrich Chemical Company; distamycin A, Sigma Chemical Company; pBR322 DNA, ProMega Biotec; lambda exonuclease, Bethesda Research Laboratories; [α-^{32}P]dCTP (6000 Ci/mmol), New England Nuclear; quinacrine mustard and ICR-170 (2-methoxy-6-chloro-9-[3-(N-β-chloroethyl-N-ethyl)amino-propylamino]acridine), Serva Chemicals; chloroethyl-nitosoura (CNU) and anthramycin, Drug Development Branch, National Cancer Institute; nitrogen mustard (HN2) and anti (+)-*r*-7,*t*-8-Dihydroxy-*t*-9,10-epoxy-7,8,9,10-tetrahydro benzo[a]pyrene (*anti*-benzo-[a]pyrene diol epoxide, BPDE), Midwest Research Institute, NCI Chemical Carcinogen Reference Standard Repository. Other enzymes were obtained from Bethesda Research Laboratories, New England Biolabs, or Promega Biotec. A bacterial strain carrying pbc-N1 (an insert of the human c-Ha-*ras* gene in the Bam HI site of pBR322 (10)) was kindly provided by Dr. Steve Tronick, National Cancer Institute. Plasmid pBMras10 was constructed by inserting the 206-bp BamHI-SacI fragment of pbc-N1 into the BamHI-SacI sites of a pUC19-derived vector (pGEM-3z(tm), ProMega); this fragment is in the 5′ flanking region of the *ras* gene, 1460 bp from the first coding sequence on pbc-N1.

Preparation of damaged DNA

DNA fragments labeled at their 3′ ends with Klenow fragment of *E. coli* DNA polymerase I and [α-^{32}P]dCTP were prepared

as described by Maniatis el al (11). Labeled DNA was incubated with damaging agents as indicated in the Figure legends in a buffer of 1 m*M* EDTA and 50 m*M* triethanolamine HCl, pH 7.2, usually to a total volume of 50 μl. DNA with a low level of depurination sites was created by incubating labeled DNA in 9% formic acid for 60 min at 22°C (12). Reactions were terminated and DNA recovered by ethanol precipitation. For experiments with chemicals that do not covalently bind to DNA (i.e. distamycin and ultraviolet irradiation) the reaction mixtures were diluted with lambda exonuclease reaction buffer (see below) and directly treated with lambda exonuclease.

Sites of N7 guanine alkylation (commonly, but incorrectly, referred to as alkalai-labile lesions) were quantitatively converted to breaks by piperidine treatment at 90°C for 15 min as previously described (13). Depurination sites created by formic acid are also converted to strand breaks by piperidine treatment.

Labeled DNA was reacted with *cis*-dichlorodiammine-platinum(II) essentially as described by (2) in a buffer of 5 m*M* NaCl, 1 m*M* sodium phosphate, pH 7.0, for 3 hr at 37°C. Lambda DNA was included in the reaction to allow for accurate DNA phosphate/Pt ratios. The reaction was terminated by the addition of NaCl to 0.1 *M* and DNA recovered by ethanol precipitation. After treatment with lambda exonuclease bound Pt was removed from the DNA by treatment with 0.2 *M* NaCN for 3 hr at 37°C (2).

Lambda Exonuclease Treatment

DNA was incubated with lambda exonuclease in a 25 μl reaction mixture that included 1.68 μmoles glycine-KOH, pH 9.4, 0.063 μmoles $MgCl_2$ and lambda exonuclease (generally about 0.8 units). The amount of exonuclease was chosen to give about 10 pmol enzyme per pmol 5′ end, assuming a maximum specific activity of 49,000 units/mg protein and a molecular weight of 80,000 (9). Enzyme treatment was stopped by the addition of EDTA and SDS (final concentration equivalent to 15 m*M* and 0.15%, respectively). DNA was recovered by precipitation with three volumes of ethanol.

Gel Electrophoresis

6% denaturing polyacrylamide (5.7% acrylamide:0.3% N,N′-methylenebisacrylamide) gels are essentially those described by Maxam and Gilbert (12). All samples were heated at 90°C for 60 s, and then chilled in an ice-bath before loading. In the case of exonuclease-digested samples it is not practical to load equal amounts of radioactivity into each lane, as exonuclease treatment of control DNA significantly reduces the amount of DNA recovered after ethanol precipitation (presumably by digesting the DNA down to ethanol soluble fragments). Rather, experimental samples contained equal amounts of radioactivity prior to exonuclease treatment.

RESULTS

Lambda exonuclease does not appreciably digest DNA modified by benzo[a]-pyrene diol epoxide

At the concentration employed, lambda exonuclease almost completely digests untreated DNA after 60 min at 37°C (see Fig. 1, lane 6). However, at shorter digestion times some prominent fragments are observed. Note that digestion of control DNA at 20°C produces a very strong band corresponding to digestion of about 50% of the DNA fragment (Fig. 1, lanes 2 and 3). At this point the DNA becomes single-stranded and the rate of digestion decreases by 100 fold (9).

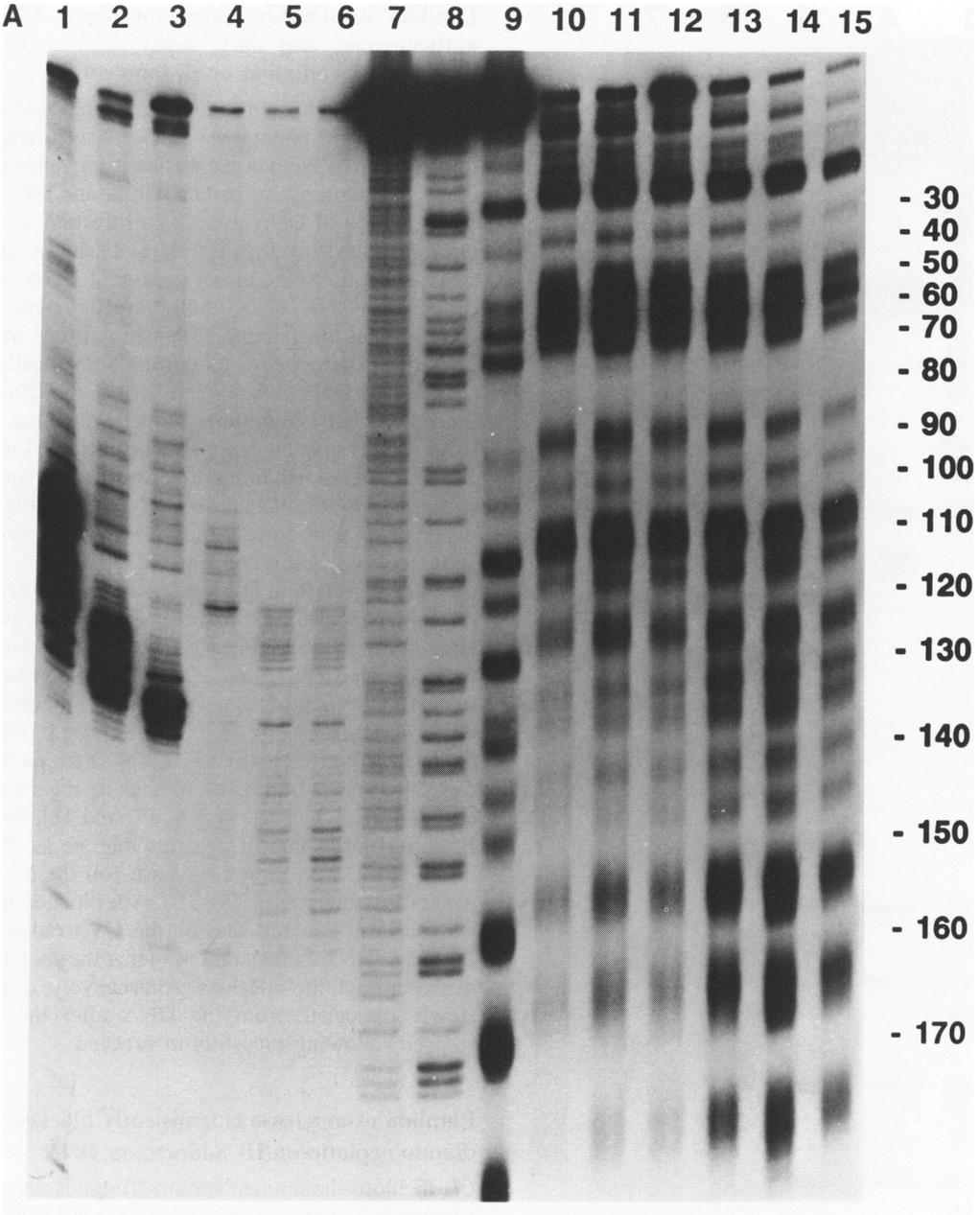
On the other hand, lambda exonuclease digests the BPDE-modified DNA (Fig. 1, lanes 10−15) at a very slow rate, if at all. Instead, a ladder of fragments is produced; the intensities of these fragments match those in the ladder produced after piperidine treatment of BPDE-modified DNA (Fig. 1, lane 9). Piperidine treatment creates breaks at sites of guanine N7-BPDE adducts (14,13). The ladder of fragments generated after piperidine treatment of BPDE-modified DNA resembled a Maxam-Gilbert G ladder (Fig. 1, lane 8) but displaced upward by 2−3 nucleotides and often smeared. The ladder of fragments produced after lambda exonuclease digestion of BPDE-modified DNA was even further displaced upward of the Maxam-Gilbert G ladder, generally about 6 nucleotides.

BPDE modifies both the N2 (15) and to a lesser extent the N7 position of guanine (15,16), although Sage and Haseltine have reported that the ratio of the two adducts may be closer to 1:1 (14). Hogan et al. (17) found that BPDE adduction of DNA alters DNA electro-phoretic mobility under non-denaturing conditions, but not under denaturing conditions. However, under the denaturing gel electrophoresis conditions used here the fragments resulting from DNA modified with BPDE and treated with piperidine run as smeared bands and do not, as a rule, comigrate with the Maxam-Gilbert G sequencing lane. The smearing of the bands from piperidine-treated BPDE-modified DNA (Fig. 1, lane 9) is probably caused by the presence of a heterogeneous population of molecules in each band having different amounts (or positions) of guanine N2-BPDE adducts. In a similar fashion the smearing of the bands after lambda exonuclease digestion of BPDE-modified DNA (Fig. 1, lanes 10−15) may be caused by the presence of residual BPDE adducts. Thus in this case it may be hard to determine the precise location of the enzyme stop site relative to the guanine site modified by BPDE. Not only might the enzyme stop a certain number of nucleotides from the modified guanine, but also the presence of residual BPDE adducts may alter the electrophoretic mobility of the fragment.

Lambda exonuclease will digest DNA modified by ICR-170

ICR-170 is an acridine mustard that can alkylate guanine N7 positions. The acridine moiety remains bound to DNA after alkylation, leaving a rather bulky group attached to the guanine N7 site; it does not intercalate into the DNA after alkylation (unpublished observations). Piperidine treatment demonstrates that treatment with 250 n*M* ICR-170 leads to extensive modification of the DNA at guanine N7 positions (Fig. 2, lane 5). Nonetheless, there are no visible lambda exonuclease stop sites on this modified DNA after 20 min of treatment at 37°C (Fig. 2, lane 7). In addition, lambda exonuclease will digest DNA modified by several other agents alkylating the N7 position of guanine: quinacrine mustard, nitrogen mustard, and chloroethylnitrosourea (data not shown). If the exonuclease digestion temperature is reduced to 20°C some bands are observed in the digest of DNA modified by ICR-170 (Fig. 2, lane 6) that are not seen in the digest of control DNA (Fig. 2, lane 1). These band do migrate in the gel at positions close to those of fragments produced by piperidine-treatment of ICR-170-modified DNA; thus, under these conditions lambda exonuclease might 'pause' at sites of ICR-170 adducts.

While both ICR-170 and benzo[a]-pyrene diol epoxide create bulky adducts on DNA, only the latter creates both guanine N2 and guanine N7 adducts. In this perspective the hypothesis



B

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          10         20         30         40         50         60
AATTCTCATGTTTGACAGCTTATCATCGATAAGCTTTAATGCGGTAGTTTATCACAGTTAAA

          70         80         90        100        110        120
TTGCTAACGCAGTCAGGCACCGTGTATGAAATCTAACAATGCGCTCATCGTCATCCTCGG

         130        140        150        160        170        180
CACCGTCACCCTGGATGCTGTAGGCATAGGCTTGGTTATGCCGGTACTGCCGGGCCTCTT

         190        200        210        220        230
GCGGGATATCGTCCATTCCGACAGCATCGCCAGTCACTATGGCGTGCTGC
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Fig. 1. Digestion of BPDE-modified DNA by Lambda Exonuclease. A: The 3′-labeled, 231-bp Nhe I-Eco RI fragment of pBR322 was used as a substrate for alkylation by benzo[a]-pyrene diol epoxide (BPDE). Lanes 1−6, control DNA; lanes 9−15, DNA treated with BPDE at 7.16 μM (2.16 μg/ml) for 60 min 37°C; lane 7, DNA mildly depurinated with formic acid; lane 8, DNA alkylated with dimethyl sulfate. DNA in lanes 7−9 were treated with piperidine as described in *Materials and Methods*. DNA in other lanes was treated with lambda exonuclease for 20 min at 20°C (lanes 1 and 10), 1 hr at 20°C (lanes 2 and 11), 2 hr at 20°C (lanes 3 and 12), 10 min at 37°C (lanes 4 and 13), 20 min at 37°C (lanes 5 and 14), or 1 hr at 37°C (lanes 6 and 15). Formic acid-treated and dimethyl sulfate-treated DNAs represent marker lanes for A+G and G sites, respectively (12). B: Sequence of the 231-bp Nhe I-Eco RI fragment of pBR322, 3′ labeled at the Nhe I site. The labeled base is italicized.

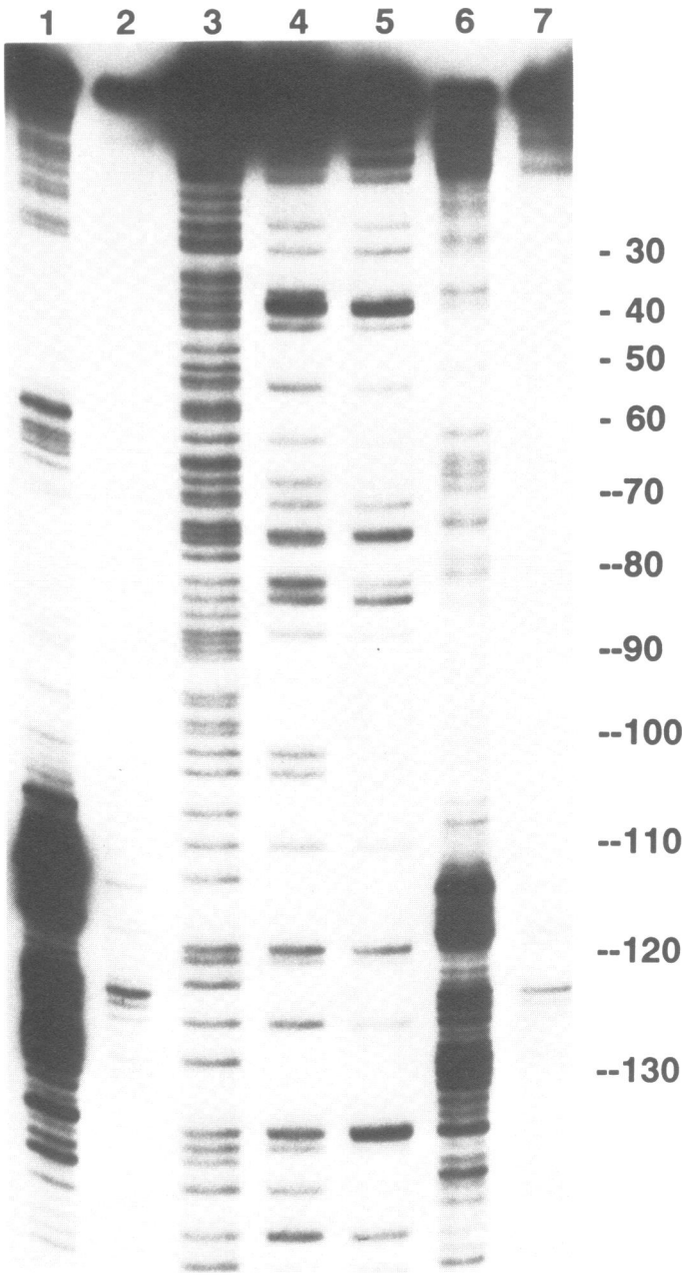

Fig. 2. Digestion of ICR-170-modified DNA. The 3'-labeled, 231-bp Nhe I-Eco RI fragment of pBR322 was used as a substrate for alkylation by ICR-170. Lanes 1 & 2, control DNA; lanes 5–7, DNA treated with at 250 n*M* for 60 min at 20°C;lane 3, DNA mildly depurinated with formic acid; lane 4, DNA alkylated with dimethyl sulfate. DNA in lanes 3–5 were treated with piperidine as described in *Materials and Methods*. DNA in other lanes was treated with lambda exonuclease for 20 min at 20°C (lanes 1 and 6), or 20 min at 37°C (lanes 2 and 7). Formic acid-treated and dimethyl sulfate-treated DNAs represent marker lanes for A+G and G sites, respectively (12).

evolved that lambda exonuclease will digest DNA regardless of features in the major groove of DNA (guanine N7 adducts), but is blocked by obstructions in the minor groove of DNA (guanine N2 adducts). On the other hand, benzo[a]-pyrene diol epoxide adducts in DNA profoundly alter DNA structure (17), whereas bound ICR-170 does not even unwind DNA, indicating rather little alteration of DNA structure (unpublished observations). Thus, alteration of DNA structure might play an important role in inhibition of lambda exonuclease.

Lambda exonuclease does not digest DNA modified by anthramycin, and only slowly digests DNA modified by ultraviolet irradiation or distamycin A

To test the hypothesis that damage or adducts in the minor groove of DNA and/or alteration of DNA structure provides a strong impediment to lambda exonuclease movement and action, the effect of anthramycin, distamycin A and ultraviolet irradiation on the ability of DNA to act as a substrate for the enzyme was examined. Distamycin A is well established as an agent that binds non-covalently to the minor groove of DNA (18), yet does not distort the DNA helix. Anthramycin forms stable, covalent adducts with the guanine N2 position in the minor groove (19, 20). Like distamycin A, it does not dramatically alter the helical structure of DNA, but instead stiffens the DNA helix (21). On the other hand, pyrimidine dimers resulting from ultraviolet irradiation distort the helix structure and unwind DNA 22, 23).

The results shown in Figure 3 show that whereas control DNA is digested to fragments about 72 bp long (Fig. 3, lanes 1–3), digestion of anthramycin-modified DNA is strongly blocked at sites between bases 2530 and 2550 (Fig 3, lanes 4–6). By contrast, digestion of DNA extensively modified by ICR-170 (as shown by piperidine treatment, Fig 3, lane 10) is digested by lambda exonuclease (Fig 3, lanes 7–9). It should be noted that for both control DNA and ICR-170 no fragments were observed in the (lower) part of the gel not included in the figure. Digestion of UV-irradiated or distamycin-treated DNA by lambda exonuclease gives a more complicated picture. After short digestion times fragments unique to the modified DNA are observed (Fig 3, lanes 14, 15, 17, and 18). In the case of UV-irradiated DNA these fragments migrate as if they correspond to a sequence position 1–2 bases on the 5' side of potential pyrimidine dimer sites (Fig 3B). After longer digestion times the intensities of the stop sites on the UV-irradiated or distamycin A-treated DNA fade, suggesting that the enzyme can eventually digest through these lesions. Alternatively, distamycin A might slowly dissociate from the DNA after the exonuclease has stopped, allowing digestion to proceed.

Lambda exonuclease is transiently blocked by *cis*-dichloro-diammineplatinum(II) adducts on DNA

Cis-dichloro-diammineplatinum(II) binds to guanine N7 and adenine N7 groups in the major groove of DNA (24) although the major adduct is an intra-strand crosslink between the N7 positions of two adjacent guanine residues (25). Adducts at these sites are also blocks to digestion of DNA by exonuclease III of *E. coli* (1, 2). In addition to simply binding in the major groove of DNA cis-dichloro-diammineplatinum(II) unwinds and shortens the DNA helix (26). The GG intra-strand crosslink introduces a 'kink' in the DNA (27). Thus this agent, while binding entirely in the major groove of DNA, can dramatically alter DNA structure.

Lambda exonuclease digestion of DNA treated with *cis*-dichloro-diammineplatinum(II) is inhibited at sites (Fig. 4, lanes 3–6) that correspond to GG sequences (see Fig. 4, lane 7, the marker lane for guanine sites). The sites of strongest inhibition (Fig. 4, lanes 3,4 and 6) are approximately at two GGGG sequences at positions 17 and 52; oligo-guanine sequences are the strongest sites of *cis*-dichloro-diammineplatinum(II) binding as determined by exonuclease III digestion of DNA (2). Lambda exonuclease seems to bypass some of the lesions if the efficacy of the digestion is increased by elevating the temperature (i.e.

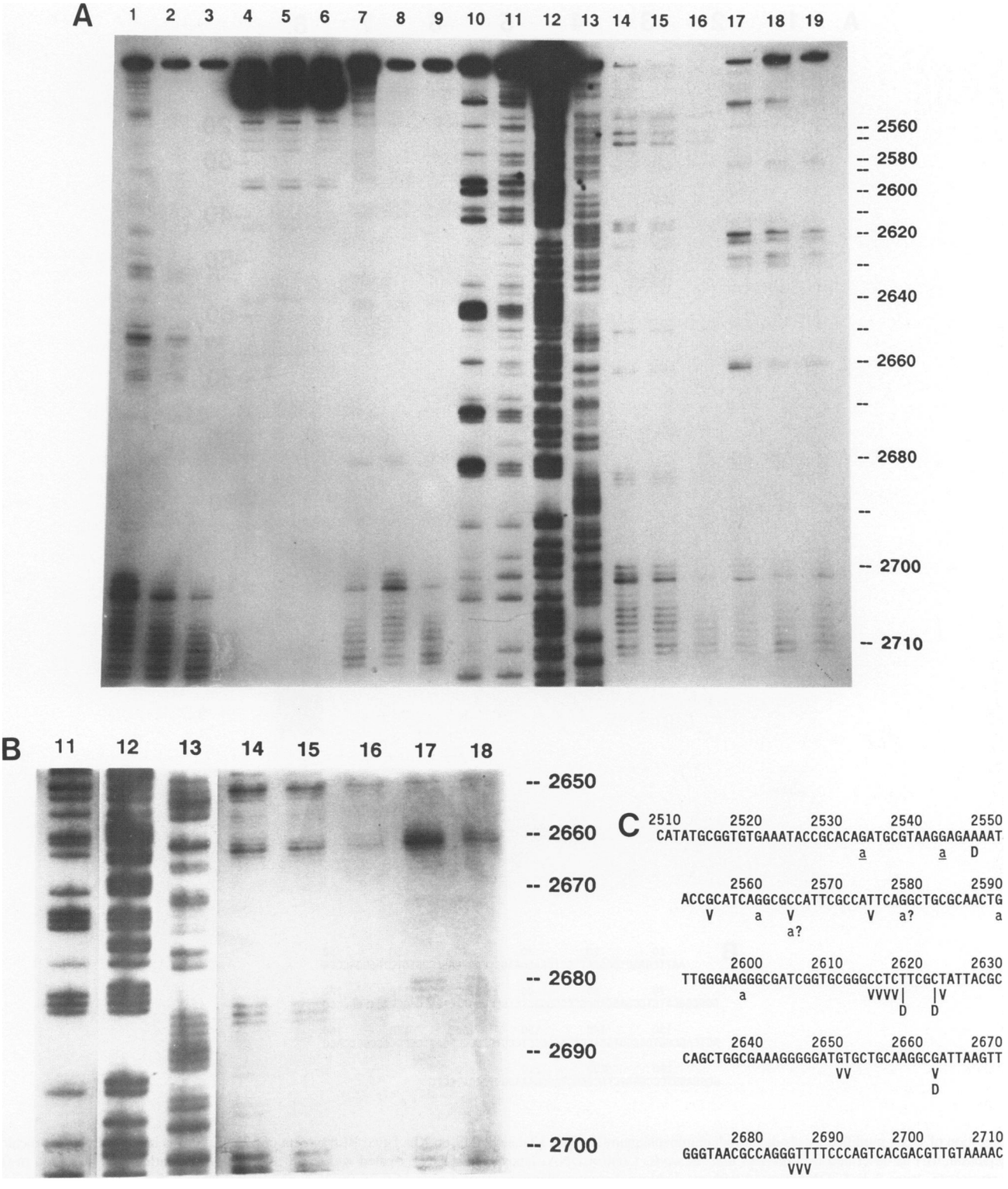

Fig. 3. Digestion of DNA modified by anthramycin, distamycin, ICR-170, or UV-irradiation. A: The 3′-labeled, 267-bp Xba I-Nde I fragment of pGEM-3z(tm) was treated with 2 μM anthramycin for 60 min at 22°C (lanes 4–6), 0.5 μM ICR-170 for 60 min at 22°C (lanes 7–10), 50 μM distamycin A for 30 min at 22°C (lanes 17–19), or with 500 J/m ultraviolet light (lanes 14–16). Lanes 1, 2, and 3 contained control DNA. Lane 11 contained DNA alkylated with dimethyl sulfate; lane 12, DNA depurinated with formic acid; lane 13, DNA treated with hydrazine. DNA in lanes 10 through 13 was treated with piperidine. DNA in other lanes was treated with lambda exonuclease at 37°C for 30 min (lanes 1, 4, 7, 14, 17), 60 min (lanes 2, 5, 8, 15, 18), or 90 min (lanes 3, 6, 9, 16, 19). Hydrazine-treated, formic acid-treated and dimethyl sulfate-treated DNAs represent marker lanes for C+T, A+G and G sites, respectively (12). B: A close-up of a region of the gel showing exonuclease stop sites on ultraviolet-irradiated and distamycin treated DNA. Lanes are labeled as for A. C: The sequence of the 3′-labeled, 267-bp Xba I-Nde I fragment of pGEM-3z(tm). *Approximate* positions are indicated underneath the sequence for stop sites of lambda exonuclease on anthramycin-modified DNA (a), distamycin-modified DNA (D), and ultraviolet-irradiated DNA (V).

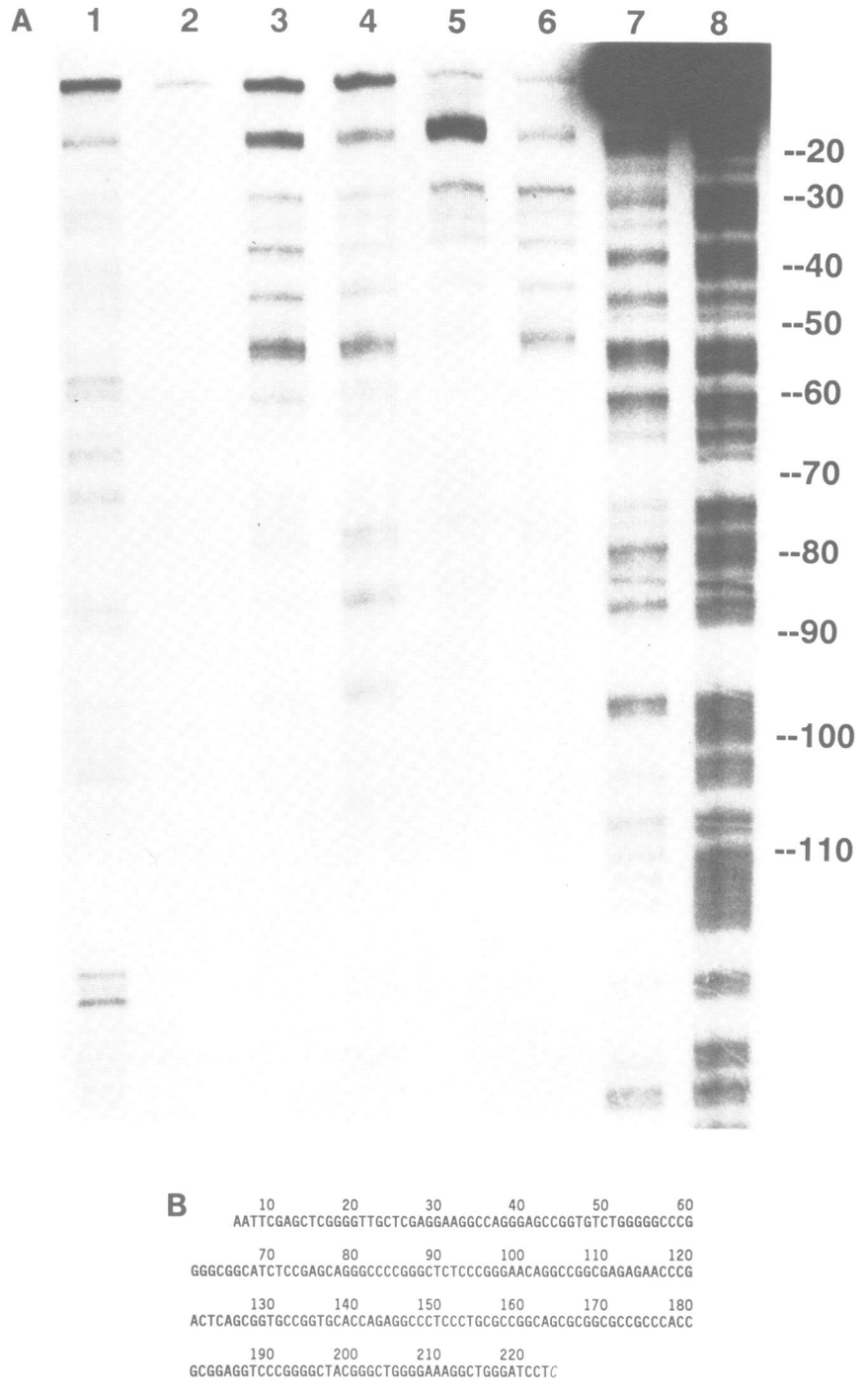

Fig. 4. Digestion of DNA modified by *cis*-dichlorodiammineplatinum(II). The 3′labeled, 219-bp Xba I-Eco RI fragment of pBMras10 was incubated with *cis*-dichlorodiammineplatinum(II) as described in *Materials and Methods*. Control DNA, lanes 1 & 2; DNA treated with *cis*-dichlorodiammineplatinum(II) at a ratio of 0.05 Pt/DNA phosphate, lanes 3 & 4; DNA treated with *cis*-dichloro-diammineplatinum(II) at a ratio of 0.25 Pt/DNA phosphate, lanes 5 & 6; Dimethyl Sulfate, lane 7; Formic Acid, lane 8. DNA in lanes 7 and 8 were treated with piperidine. DNA was treated with lambda exonuclease for 60 min at either 20°C (lanes 1, 3, and 5) or 37°C (lanes 2, 4, and 6). Formic acid-treated and dimethyl sulfate-treated DNAs represent marker lanes for A+G and G sites, respectively (12). B: Sequence of the 219-bp Xba I−Eco RI fragment of pBMras10, 3′ labeled at the Xba I site. The labeled base is italicized.

20° to 37°): note that the strong inhibition site seen at position 17 in lane 5 of Figure 4 is reduced in intensity in lane 6. Furthermore, bands of lower molecular weight are seen at the higher temperature. Thus, on molecules with more than one adduct the lambda exonuclease stops at the first adduct it encounters, i.e. the one closest to the 5′ end, but if the enzyme can bypass that adduct it will stop at adducts closer to the 3′ end. Accordingly, this major groove adduct seems to provide a weaker block to lambda exonuclease digestion than covalently bound minor groove adducts such as anthramycin.

Table 1. Effects of DNA damage on lambda exonuclease digestion

Agent	Modification	DNA Groove	Blocks Lambda Exonuclease ?
BPDE	guanine N-7,guanine N-2	major, minor	Yes
ICR-170	guanine N-7	major	No
HN2, CNU	guanine N-7	major	No
Anthramycin	guanine N-2	minor	Yes
UV	pyrimidine dimer	?, kinks DNA	Yes
Cisplatin	guanine N-7	major, kinks DNA	Yes

DISCUSSION

These experiments show that the digestion of DNA by lambda exonuclease is blocked by agents known to covalently bind to the minor groove of DNA (Table I). Alteration of DNA structure induced by ultraviolet irradiation or by *cis*-dichloro-diammineplatinum(II) adducts also seem to inhibit the progress of the enzyme, although digestion appears to proceed through these sites. Distamycin A, non-covalently bound to the minor groove, also seems to impede lambda exonuclease digestion. As with UV-induced lesions, distamycin A-induced lesions appear to be only a transient block to the enzyme, either because the the drug dissociates from the DNA or because the enzyme can actually proceed through the damage. DNA modified by agents such as ICR-170, other nitrogen mustards, and chloro-ethyl-nitroso-urea is not appreciably altered in its susceptibility to lambda exonuclease digestion. Such agents predominantly alkylate the N7 position of guanine in the major groove of DNA without dramatically altering DNA helix structure.

The simplest model accounting for the response of lambda exonuclease to DNA damage is one wherein the enzyme has a significant contact with the minor groove of DNA. If the minor groove is obstructed, progress of the enzyme is also obstructed. If DNA helix structure is altered, and the width of the minor groove is altered (narrowed?), by binding of an agent to the major groove, then the progress of the enzyme would still be blocked. In fact, a model of a nuclease with a moiety binding to the DNA minor groove is not novel, but appears to be embodied by DNase I, an enzyme with a small exposed loop fitting tightly into the minor groove of DNA (28). The sequence-dependent variation of cleavage by DNase I may partially be explained by a variation of the width of the minor groove with sequence (29). Lambda exonuclease digestion of unmodified DNA also shows sequence-dependent 'pause' sites that are bypassed after further incubation, an effect that may also be due to sequence-dependent variations in minor groove width. Despite these similarities in DNA binding behavior, no local similarities in amino acid sequence exist between the lambda exonuclease protein, as predicted by its GenBank(tm) DNA sequence, and DNase I (30) as determined with the FASTA program (31).

On the other hand, lambda exonuclease may indeed 'read' the major groove of DNA, but there may be a particular local helix distortion induced by those adducts that block lambda exonuclease that is not induced by guanine N7 alkylations. Pertinent to this point is the observation that T6 DNA, containing bulky glucosyl residues in the major groove, is not digested by lambda exonuclease (9). T6 DNA contains hydroxymethyl cytosine instead of cytosine and 72% of these bases are modified with β-glucosyl-α-glucosyl residues (32 p.325). One assumption would be that the glucosyl residues themselves are blocking the lambda exonuclease, and that the enzyme is indeed sensitive to major groove features. However, T2 DNA, also containing glucosylated hydroxy-methyl-cytosine, can assume a structure quite distinct from that of B-DNA (33). Thus, T6 DNA may also have an altered structure, with altered minor groove dimensions, and this may explain its resistance to lambda exonuclease. Yet in the absence of data actually identifying physical contacts between the enzyme and DNA one cannot definitively state that lambda exonuclease binds only to the major or minor groove of DNA.

Lambda exonuclease has also been used to map 5-methylisopsoralen photo-induced binding sites on SV40 DNA (34). In those experiments the enzyme paused at 5'-TA sites; with increasing times modified DNA was completely digested. Since other psoralen derivatives alter DNA structure after photo-induced binding (35), one might assume that the the 5-methylisopsoralen adducts could also disrupt DNA structure, leading to inhibition of the lambda exonuclease.

While exonuclease III and the 3' to 5' exonuclease of T4 DNA polymerase have been used for their ability to stop at sites of DNA adducts, precedent exists for the bypassing of certain types of adducts, at least by the latter enzyme. Bichara and Fuchs found that the 3' to 5' exonuclease activity of T4 DNA polymerase, though blocked by acetyl-amino-fluorene (AAF) adducts, is not 'efficiently' blocked by amino-fluorene (AF) adducts (36). These workers attribute the difference to the fact that AAF adducts induce a major conformational change, whereas AF adducts do not significantly affect DNA conformation. Panigrahi and Walker (37) have recently found that N2-guanine adducts of 4-nitroquinoline 1-oxide, but not C8-guanine adducts, block this exonuclease. They suggest that the former adduct might induce a conformational change, yet the difference in the DNA groove that the two adducts are found might also be considered. Similarly, *cis*-dichloro-diammineplatinum(II) adducts only slow down this enzyme (38,39), reminiscent of the pause sites for lambda exonuclease created by such adducts. By contrast, exonuclease III seems to be thoroughly stopped by *cis*-dichloro-diammineplatinum(II) adducts (1, 2). However, in the absence of further data there is no reason to assume that either exonuclease III or the 3' to 5' exonuclease of T4 DNA polymerase is sensitive to changes only in the minor groove.

The present findings suggest that lambda exonuclease may be used as a tool for examining the DNA binding sites of small molecules. The enzyme has already been used as a reagent to map protein-binding sites (40) and 5-methylisopsoralen binding sites (34). Lambda exonclease appears to 'pause' on ultraviolet-irradiated DNA at sites 1−2 bases on the 5' side of sites expected to have pyrimidine dimers. For DNA treated with benzo[a]-pyrene diol epoxide the pattern of stop sites corresponds to a pattern of guanine residues, and the intensity of the stop site

bands correspond approximately to the intensity of bands generated by piperidine-cleavage. Lambda exonuclease stop sites, or perhaps more properly pause sites, on DNA modified by *cis*-dichloro-diammineplatinum(II) also correspond with the known preference of this compound for GG sequences (25). It should be noted that for these agents there was no observed stop site that could conclusively be identified with DNA damage on the strand complementary to the labeled strand. Further work is required to accurately determine the precise position of the enyzmatic stop sites relative to the sites of damage for these and other agents such as anthramycin and distamycin.

With a knowledge of the DNA sequences preferentially attacked by a given xenobiotic agent, the genes or genomic sites favored by the agent might be predicted (see ref. 41). Conversely, knowledge of the interactions between a chemical and DNA that determine sequence preferences should allow rational alteration of the structure of the chemical so as to enhance or decrease the preference for a specific sequence. In all of these cases, well characterized tools for investigating DNA-xenobiotic interactions are needed: for several agents lambda exonuclease may provide just such a tool.

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