α-Deoxyadenosine, a Major Anoxic Radiolysis Product of Adenine in DNA, Is a Substrate for *Escherichia coli* Endonuclease IV†

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ABSTRACT: Oligonucleotides containing a unique α-deoxyadenosine or tetrahydrofuran (a model abasic site) were synthesized using phosphoramidite chemistry. Repair enzymes from *Escherichia coli,* including endonucleases III, IV, and VIII, exonuclease III, formamidopyrimidine *N*-glycosylase, and deoxyinosine 3′-endonuclease, as well as UV dimer *N*-glycosylases from T4 (den V) and *Micrococcus luteus,* were examined for their ability to recognize α-deoxyadenosine and tetrahydrofuran. In agreement with prior studies, a tetrahydrofuran-containing oligonucleotide was a substrate for endonuclease IV and exonuclease III, but not for the other repair enzymes. However, an oligonucleotide containing α-deoxyadenine was a substrate only for endonuclease IV. Competitive inhibition studies with both substrates confirmed that the activity recognizing α-deoxyadenine was endonuclease IV and not a possible contaminant in the endonuclease IV preparation. Using *E. coli* extracts, the activity that recognized α-deoxyadenine was dependent on *nfo,* the structural gene of endonuclease IV, further substantiating that endonuclease IV is the enzyme that recognized α-deoxyadenine. Kinetic measurements indicated that α-deoxyadenosine was as good a substrate for endonuclease IV as tetrahydrofuran; the K_m and V_{max} values for both substrates were similar. Using substrates that were labeled at either the 3′- or 5′-terminus, endonuclease IV was shown to hydrolyze the phosphodiester bond 5′ to either α-deoxyadenosine or tetrahydrofuran, leaving the lesion, α-deoxyadenosine or tetrahydrofuran, on the 5′-terminus of the nicked site. The ability of endonuclease IV to recognize α-deoxyadenosine suggests that endonuclease IV is able to recognize a new class of DNA base lesions that is not recognized by other DNA *N*-glycosylases and AP endonucleases.

The α-anomer of deoxyadenosine (αdA) is produced by abstraction of the anomeric hydrogen atom at C1′ by hydroxyl radicals (Mariaggi et al., 1979; von Sonntag, 1987). α-Deoxyadenosine constitutes a major adenine lesion detected in DNA, poly(dA–dT), and poly(dA) γ-irradiated under anoxic conditions (Lesiak & Wheeler, 1990). Recently, αdA was incorporated site-specifically into oligonucleotide templates using phosphoramidite chemistry (Ide et al., 1993), and its effect on DNA replication was assessed *in vitro* (Ide et al., manuscript in preparation). The results showed that DNA polymerases temporarily paused at the lesion and then bypassed the site. More importantly, αdA in the template directs the misincorporation of nucleotides, dC and dA, as well as the incorporation of the correct nucleotide, dT, opposite the lesion, suggesting that αdA constitutes a premutagenic lesion *in vivo.*

Radiation-induced base lesions have been shown to be repaired predominantly through the base excision repair pathway (Sancar & Sancar, 1988; Wallace, 1988). In base excision repair, damaged bases are released by DNA *N*-glycosylases, which hydrolyze the *N*-glycosylic bond between the damaged base and the sugar moiety. The resulting abasic sites are then acted upon by 5′-AP endonucleases, which cleave the phosphodiester bond 5′ to the missing base. Alternatively, certain DNA glycosylases, such as endonucleases III (Sancar & Sancar, 1988; Wallace, 1988) and VIII (Melamede et al., 1987, 1994) and formamidopyrimidine DNA *N*-glycosylase (Sancar & Sancar, 1988; Wallace, 1988; Boiteux et al., 1990), have an associated AP lyase activity, which cleaves 3′ to the lesion. One of the substrates for endonucleases III and VIII is 5,6-dihydrothymine which, like αdA, is an anoxic radiolysis product (Demple & Linn; 1980; Kow & Wallace, 1987; Wallace, 1988; Dizdaroglu et al., 1993; Melamede et al., 1994). Since a number of the *Escherichia coli* base excision repair enzymes have been purified to homogeneity, it was of interest to determine whether any of these recognized αdA in DNA. In this article, we report that αdA in DNA is recognized by *E. coli* endonuclease IV.

MATERIALS AND METHODS

Bacterial Strains. E. coli strains BW554 [BW554: AB1157 + Δ(*xth-pncA*)90 *nth-1::kan* +pBR322-*nfo*], BW434 [BW434: AB1157 + Δ(*xth-pncA*)90 *nth-1::kan*], and BW527 [BW527 = AB1157 + *nfo-1::kan*] were kindly supplied by Dr. B. Weiss (University of Michigan). *E. coli* AB 1157 [*leuB6 thr-1* Δ(*gpt-proA*)*62 hisG4 argE3 lacY1 galK2 ara-14 mtl-1 xyl-5 thi-1 tsx-33 rpsL31 supE44 rac*] is a laboratory strain.

Oligonucleotides. Three oligonucleotides (17-mer), containing either dA (5′-AGCATTCGAGACTGGGT-3′), tetrahydrofuran (5′-AGCATTCGFGACTGGGT-3′, **F** = tetrahydrofuran), or αdA (5′-AGCATTCGαGACTGGGT-3′, α = αdA) at position 9 from the 5′ end, were synthesized using an automated DNA synthesizer (Figure 1). The phosphoramidite monomers of αdA (Ide et al., 1993) or tetrahydrofuran (Ide et al., 1992) were prepared as reported.

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Oligonucleotides	Sequence
top strand	
A	5'-AGCATTCGAGACTGGGT-3'
αdA	----------α----------
F	----------F----------
bottom strand	
dA	3'-TCGTAAGCACTGACCCA-5'
dG	----------G---------
dC	----------C---------
dT	----------T---------

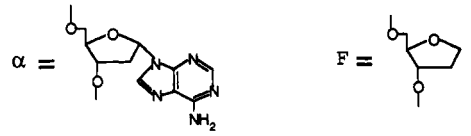

FIGURE 1: Nucleotide sequences of oligonucleotides used in this study.

Four complementary bottom strands containing either dA, dG, dC, or dT at position 9 from the 5′ end were also synthesized (Figure 1). The oligonucleotides synthesized were purified by reversed-phase HPLC (Alltech Absorbsphere C18) followed by 20% polyacrylamide gel electrophoresis under denaturing conditions (8 M urea). The concentration of oligonucleotides was determined by the absorbance at 260 nm using molecular absorption coefficients (ϵ_{260}) calculated on the basis of the method of Puglisi and Tinocco (1989).

End-Labeling. T4 polynucleotide kinase (PNK) and terminal deoxynucleotidyl transferase (TdT) were purchased from United States Biochemical. [γ-^{32}P]ATP (222 TBq/mmol) and [α-^{32}P]cordycepin 5′-triphosphate (185 TBq/mmol) were obtained from New England Nuclear. 5′-End-labeling of oligonucleotides using PNK and 3′-end-labeling with TdT and [α-^{32}P]cordycepin 5′-triphosphate were performed as recommended by the manufacturer. End-labeled oligonucleotides were purified by a Sep-Pak cartridge (Maniatis et al., 1989).

Repair Enzymes. A homogeneous preparation of *E. coli* exonuclease III (65 μg/mL) was obtained from Gibco-BRL. A homogeneous preparation of formamidopyrimidine *N*-glycosylase was a gift from Dr. Lois Rabow (University of Vermont) and was purified from overproducing strain TT101/pFPG2 (J. Miller, UCLA) according to Boiteux et al. (1990). Endonuclease VIII was a gift from Dongyan Jiang (University of Vermont) and was purified from an *E. coli* strain lacking both endonucleases III and IV (BW327, B. Weiss, University of Michigan) according to Melamede et al. (1987, 1994). Deoxyinosine 3′-endonuclease was a gift from Min Yao and was purified from *E. coli* strain BW327 according to Yao et al. (1994). *Micrococcus luteus* pyrimidine dimer DNA *N*-glycosylase was prepared from wild-type *M. luteus* according to Carrier and Setlow (1970). Endonuclease IV was prepared from *E. coli* strain BW554 harboring overproducing plasmid pBR322-*nfo* (B. Weiss, University of Michigan) after induction with paraquat, according to Ljungquist (1977). Endonuclease III and T4 den V were gifts from Dr. Richard Cunningham (SUNY at Albany) and Dr. Steven Lloyd (University of Texas Medical Branch), respectively.

Enzyme Reactions. Duplex oligonucleotide substrates were prepared as follows. An end-labeled top strand containing αdA, dA, or F (0.4 pmol) and a complementary strand (0.8 pmol, 2 × molar excess over the top strand to ensure duplex formation) in 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, and 10 mM NaCl (total, 20 μL) were heated at 90 °C and cooled to room temperature.

Typically, the annealed 17-mer duplex (20 nM) in a reaction buffer (10 μL; for compositions see below) was incubated with a repair enzyme for an appropriate period at 37 °C. The reaction was terminated by adding loading buffer consisting of 0.05% bromophenol blue, 0.05% xylene cyanol, and 95% formamide. The reaction buffers used were 100 mM NaCl, 10 mM Tris-HCl (pH 7.5), and 1 mM EDTA for endonuclease III, *M. luteus* pyrimidine dimer glycosylase, formamidopyrimidine DNA *N*-glycosylase, and endonuclease VIII; 50 mM NaCl, 10 mM Tris-HCl (pH 7.5), and 1 mM EDTA for endonuclease IV and T4 den V; 2 mM $CaCl_2$, 10 mM Tris-HCl (pH 7.5), and 1 mM EDTA for exonuclease III; and 2 mM $MgCl_2$, 10 mM Tris-HCl (pH 7.5), and 1 mM EDTA for deoxyinosine 3′-endonuclease.

To obtain kinetic parameters for endonuclease IV, 5′-end-labeled substrates were mixed with cold substrates to the appropriate final concentrations (0.5–6.5 nM) and incubated with 2 μL of 10^4-fold-diluted endonuclease IV (ca. 0.2 nM) at 37 °C for 5 min (final volume, 10 μL). The reaction was terminated by adding loading buffer. Reaction products were separated on a 20% denaturing gel, and the gel was autoradiographed. The radioactive substrates and products of the reactions were localized by overlaying the autoradiogram on the radioactive gel. The radioactive bands, i.e., the substrates and products, were then cut out from the gel. The radioactivity of both substrates and products was measured by Cerenkov counting using a Beckman LS7500 liquid scintillation counter.

Competition Studies. To show substrate competition between αdA–dT and F–dT in endonuclease IV reactions, a 5′-^{32}P-labeled duplex oligonucleotide containing an αdA–dT pair was incubated for 15 min at 37 °C in a reaction buffer (10 μL) containing 0.5 nM endonuclease IV and different concentrations (0, 50, 100, 250 and 500 nM) of cold duplex oligonucleotide containing an F–dT pair. For the control experiments, a 5′-^{32}P-labeled duplex containing an αdA–dT pair was incubated under similar conditions with a cold duplex containing a normal dA–dT pair. The products of the endonuclease IV reactions were analyzed by 20% denaturing polyacrylamide gel electrophoresis.

RESULTS

Screening for Enzymes That Recognize αdA in DNA. Four different types of substrates containing either a dA–dT, αdA–dT, αdA–dA, or F–dT base pair in the middle of a 17-mer duplex were used, where αdA and F denote α-deoxyadenosine and a model abasic site, respectively. 3′-End-labeled substrates (2 nM) were incubated with 1 μL of each of the undiluted enzyme preparations (endonuclease III = 2.5 μg; endonuclease VIII = 5 ng; formamidopyrimidine *N*-glycosylase = 0.12 μg; exonuclease III = 1 μg; deoxyinosine 3′-endonuclease = 70 ng; T4 den V = 2 μg; *M. luteus* pyrimidine dimer *N*-glycosylase = 10 ng; endonuclease IV = 65 ng) in the appropriate reaction buffer, and the reaction products were analyzed by 20% denaturing PAGE. No incision of the control substrate containing a dA–dT base pair or of substrates containing αdA or the model apurinic site, tetrahydrofuran, was observed with endonuclease III, formamidopyrimidine *N*-glycosylase, T4 den V, or *M. luteus* UV endonuclease (data not shown). With deoxyinosine 3′-endonuclease, specific nicking at the model abasic site, tetrahydrofuran, was observed over the weak background of nonspecific nicking (data not shown).

Endonuclease IV, which is the second most abundant class II AP endonuclease in *E. coli*, specifically cleaved oligonucleotides containing either αdA (Figure 2, lanes 4 and 6)

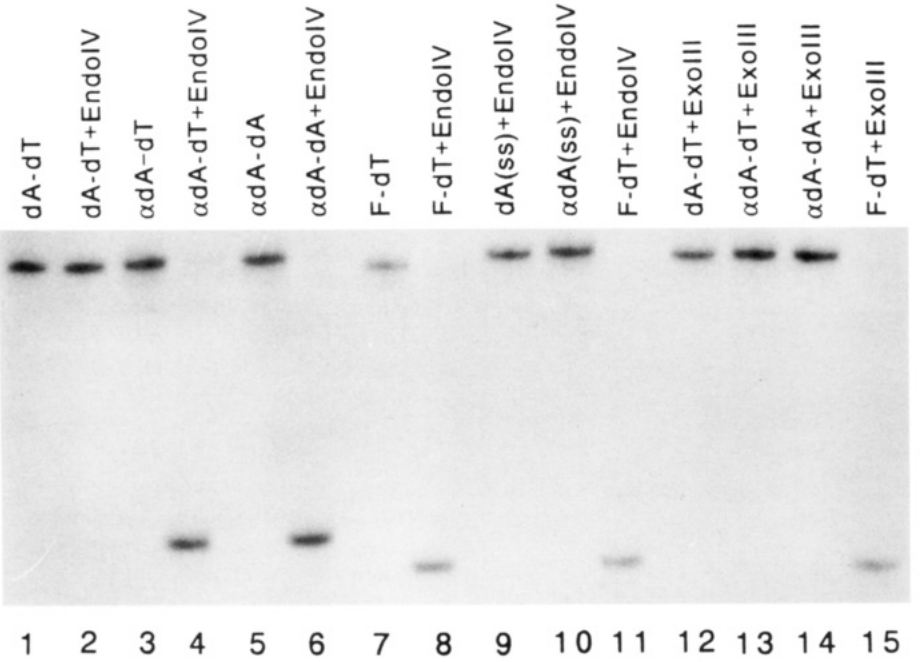

FIGURE 2: DNA duplexes containing α-deoxyadenosine (αdA) and tetrahydrofuran (F) as substrates for endonuclease IV and exonuclease III. 3′-End-labeled top strands containing deoxyadenosine (dA), α-deoxyadenosine (αdA), or tetrahydrofuran (F) were annealed to complementary bottom strands, and the duplex substrates formed (20 fmol) were incubated with endonuclease IV (70 pg, lanes 2, 4, 6, and 8) or exonuclease III (65 pg, lanes 12–15) at 37 °C for 10 min, as described in Materials and Methods. Single-stranded oligonucleotides dA and αdA were also treated with endonuclease IV in a similar manner (lanes 9 and 10). The reaction products were analyzed by 20% denaturing polyacrylamide gel electrophoresis.

or the tetrahydrofuran residue (Figure 2, lane 8). The latter lesion was previously shown to be a good substrate for endonuclease IV (Gossard & Verly, 1976; Takeshita et al., 1987; Levin & Demple, 1990). The nicking activity of endonuclease IV on αdA required duplex DNA, since a single-stranded oligonucleotide containing αdA was not cleaved by the enzyme (Figure 2, lane 10). However, duplex DNA containing αdA was not a substrate for exonuclease III, which is the major class II AP endonuclease in *E. coli* (lanes 13 and 14). In agreement with previously published reports (Takeshita et al., 1987; Levin & Demple, 1990), DNA containing a tetrahydrofuran residue was also specifically cleaved by exonuclease III.

The product derived from a 3′-end-labeled substrate containing a tetrahydrofuran residue (Figure 2, lanes 8 and 11) migrated faster in the gel than the product derived from the duplex oligonucleotide containing αdA (Figure 2, lanes 4 and 6). Since endonuclease IV hydrolyzes the phosphodiester bond 5′ to an AP site, it is likely that endonuclease IV hydrolyzed the phosphodiester bond 5′ to either αdA or tetrahydrofuran (shown below). Thus, the difference in migration of the products is probably due to the lack of a base moiety at the 5′-terminus of the product derived from the tetrahydrofuran-containing substrate.

Incision of the duplex DNA containing αdA was independent of the base opposite the lesion since, using 5′-end-labeled substrates, all four possible combinations, including αdA–dA, αdA–dG, αdA–dC, and αdA–dT, were cleaved (Figure 3, lanes 2–5). This was also the case for tetrahydrofuran (Figure 3, lanes 7–10). Further, products derived from duplexes containing αdA or the tetrahydrofuran residue showed the same mobility in a denaturing sequencing gel (Figure 3), indicating that endonuclease IV hydrolyzed the same phosphodiester bond, generating the same 5′-end-labeled product. The 5′-end-labeled product migrated as an authentic 8-mer with an identical sequence (data not shown). These

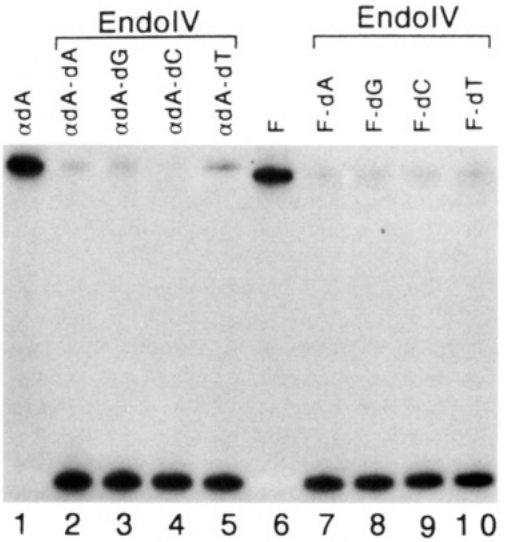

FIGURE 3: Endonuclease IV recognizes both αdA and tetrahydrofuran opposite all four bases in a duplex DNA. 5′-End-labeled top strands containing αdA (lanes 2–5) or F (lanes 7–9) annealed to complementary bottom strands (dA, dG, dC, or dT) to form duplex substrates (2 nM) were treated with endonuclease IV (0.2 nM) at 37 °C for 10 min. Lanes 1 and 6 show the original 17-mer oligonucleotides αdA and F without endonuclease IV treatment, respectively. The reaction products were analyzed by 20% denaturing polyacrylamide gel electrophoresis.

data, together with the experiments using the 3′-end-labeled substrates (Figure 2), suggest that endonuclease IV hydrolyzed the phosphodiester bond 5′ to αdA.

Competitive Inhibition Studies. In order to show that the activity that recognized αdA was not due to a contaminating activity in the endonuclease IV preparation, an enzyme dilution study was performed. Figure 4 shows that with both duplexes, containing either αdA or tetrahydrofuran, endonuclease IV activity was observed even at a 10 000-fold dilution (0.1 nM final concentration) of the stock enzyme preparation.

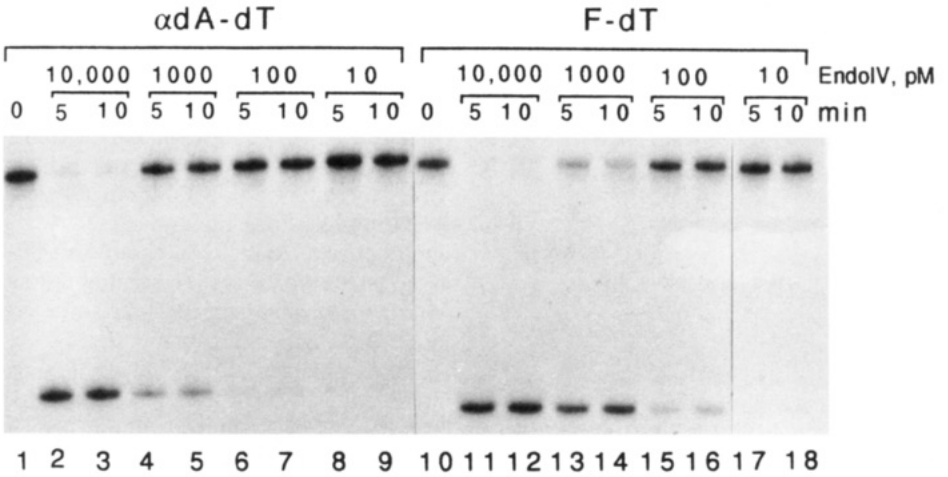

FIGURE 4: Enzyme dilution study of endonuclease IV on duplexes containing αdA or tetrahydrofuran. Duplex oligonucleotide substrates containing an αdA–dT pair (lanes 11–18) were treated with serially diluted endonuclease IV at 37 °C for 5 min. Top strand oligonucleotides containing αdA and F were 3′-end-labeled. The amount of endonuclease IV and the incubation times used are indicated at the top. The reaction products were analyzed by 20% denaturing polyacrylamide gel electrophoresis.

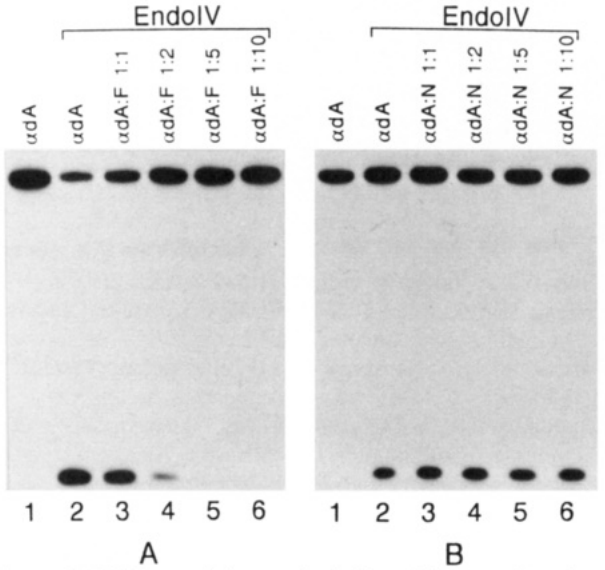

FIGURE 5: DNA containing tetrahydrofuran inhibits endonuclease IV activity when DNA containing αdA was used as substrate. A duplex oligonucleotide substrate containing an αdA–dT pair (50 nM) was incubated with endonuclease IV in the absence (panels A and B, lane 2) or presence of cold competitor duplex substrate containing an F–dT pair (panel A, lanes 3–6) or a dA–dT pair (panel B, lanes 3–6). The molar ratios of the labeled to cold competitor duplexes are indicated at the top, where αdA, F, and N denote duplexes containing αdA–dT, F–dT, and dA–dT pairs, respectively. Lanes 1 in panels A and B show the original oligonucleotide αdA without endonuclease IV treatment. Products were analyzed by 20% denaturing polyacrylamide gel electrophoresis.

In order to prove that it was indeed endonuclease IV that recognized αdA, a competitive inhibition study was also performed. In this case, duplex DNA containing αdA was used as a substrate, and the rate of cleavage was measured in the presence of either an oligonucleotide containing a tetrahydrofuran residue or a control DNA with no lesion (i.e., dA at the site of lesion). A 50% inhibition of endonuclease IV activity on αdA was observed when an equal amount of cold duplex containing tetrahydrofuran was present, and inhibition increased in proportion to the amount of cold competing substrate present (Figure 5, panel A). However, no inhibition was observed by control duplexes, even at a DNA concentration 30-fold higher than the substrate. Finally, the endonuclease IV preparation used in these studies showed a single band in a silver-stained polyacrylamide gel (data not shown). Taken together, these data show that αdA is indeed a substrate for endonuclease IV.

Identification of Endonuclease IV as an Endonuclease That Recognizes αdA. Competitive inhibition studies strongly suggest that endonuclease IV was the enzyme that recognized DNA containing αdA. However, to confirm that this activity was due to endonuclease IV and not to some contaminant in the preparation, a genetic approach was taken to address this point. A series of *E. coli* strains containing the wild-type endonuclease IV gene (*nfo*$^+$) or a disrupted version of the gene (*nfo::kan*) was examined for the level of endonucleolytic activities against duplex oligonucleotide containing αdA (Figure 6). Endonucleolytic activity against αdA was easily detected in crude extracts prepared from wild-type strains (Figure 6, lanes 3 and 4; BW434, 0.3 pmol nick min^{-1} (mg of protein)$^{-1}$; BW1157, 0.45 pmol nick min^{-1} (mg of protein)$^{-1}$), but not from strains carrying the disrupted endonuclease IV structural gene (Figure 6, lane 5; BW527, <2 fmol nick min^{-1} (mg of protein)$^{-1}$). The activity was overproduced by more than 10-fold in strains carrying multicopy *nfo*$^+$ plasmid (Figure 6, lane 2; BW554, 8.4 pmol nicks min^{-1} (mg of protein)$^{-1}$). These data demonstrated that endonuclease IV is indeed the enzyme that recognized αdA.

Kinetic Parameters. Kinetic parameters (K_m and V_{max}) for endonuclease IV incision of substrates containing αdA or tetrahydrofuran were determined by the Lineweaver–Burk analysis of substrate concentration and initial velocity. The kinetic parameters of endonuclease IV for oligonucleotide substrates containing either an αdA–dT or F–dT pair were calculated from the initial velocity analysis (data not shown). The apparent affinity (K_m), 8.6 nM, and turnover number, 1.2 min^{-1}, calculated from the V_{max} for αdA are comparable to those of the model abasic site, tetrahydrofuran (7.7 nM, 1.7 min^{-1}). The apparent K_m values for these substrates are also similar to those reported for the release of phosphoglyceraldehyde, phosphate, and deoxyribose 5-phosphate from the 3′-end of DNA (5–10 nM) (Levin et al., 1988). The turnover numbers of endonuclease IV for the incision of αdA or tetrahydrofuran are also within the same order as those reported (Levin et al., 1988) for phosphoglyceraldehyde (4.6 min^{-1}) and deoxyribose 5-phosphate (4.5 min^{-1}).

DISCUSSION

Endonuclease IV is a class II AP endonuclease that hydrolyzes the phosphodiester bond 5′ to an abasic site, leaving

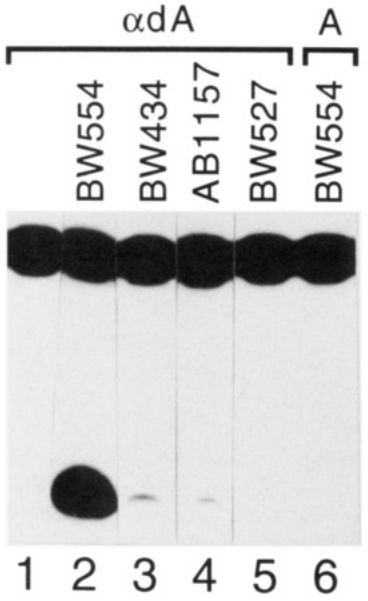

FIGURE 6: Identification of endonuclease IV as an endonuclease that recognizes αdA. Cell-free extracts were prepared from actively growing cells (OD_{600nm} = 0.5) by lysozyme treatment as described in Maniatis et al. (1989). One microliter of each of the extracts was then incubated with duplex oligonucleotide (1 pmol) containing an αdA–dT pair in a standard reaction mixture at 37 °C for 5 min. The endonucleolytic activity reported in the text was calculated by normalizing the amount of protein added to each reaction. Lane 1, control duplex containing αdA; lane 2, duplex containing αdA + BW554 (Δ*xth nth::kan nfo*$^+$*/pnfo*); lane 3, duplex containing αdA + BW434 (Δ*xth nth::kan nfo*$^+$); lane 4, duplex containing αdA + AB 1157 (*xth*$^+$ *nth*$^+$ *nfo*$^+$); lane 5, duplex containing αdA + BW527 (*xth*$^+$ *nth*$^+$ *nfo::kan*); lane 6, duplex containing dA + BW554.

behind 3′-OH and 5′-phosphate termini at the nicked site. However, endonuclease IV appears to play a subordinate role in the repair of abasic sites in *E. coli,* since the majority (ca. 90%) of the AP endonuclease activity in *E. coli* is accounted for by another class II AP endonuclease, exonuclease III (Ljungquist et al., 1976; Weiss, 1981). In addition to their AP endonuclease activities, endonuclease IV and exonuclease III share other overlapping substrate specificities, including the removal of 3′-blocking damages such as 3′-phosphoglyceraldehyde, 3′-phosphate, and 3′-deoxyribose 5-phosphate (Demple et al., 1986), as well as incision of the phosphodiester bond 5′ to a urea residue derived from base fragmentation (Kow & Van Houten, 1990). However, endonuclease IV and exonuclease III appear to have unique specificities since, *in vivo*, endonuclease IV mutants are hypersensitive to tetrabutyl hydroperoxide (Cunningham et al., 1980), while exonuclease III mutants are hypersensitive to hydrogen peroxide (Cunningham et al., 1980). In this study, we show that endonuclease IV has a novel activity that hydrolyzes the phosphodiester bond 5′ to α-deoxyadenosine. Unlike the other repair activities common to endonuclease IV and exonuclease III, the activity against αdA is unique to endonuclease IV. Furthermore, these results suggest that endonuclease IV may be responsible for the repair of a new class of DNA damages that are not recognized by the broad spectrum *N*-glycosylases/AP lyases such as endonuclease III and formamidopyrimidine *N*-glycosylase.

The present results may also help to explain how DNA lesions such as abasic sites, 3′-blocking lesions, urea residues, and αdA are all recognized by endonuclease IV. One common feature shared by these lesions is the lack of an intact base projecting in the β-configuration, which creates an empty space (abasic sites and 3′-blocking lesions) or a deeper major groove (urea and αdA) in duplex DNA. If endonuclease IV uses this feature for the recognition of DNA lesions, the mechanism resembles that proposed for exonuclease III (Weiss, 1976). However, unlike endonuclease IV, exonuclease III does not recognize αdA. The difference in the abilities of endonuclease IV and exonuclease III to recognize αdA might be accounted for by the following observations (Ide et al., manuscript in preparation). First, molecular modeling studies of a 9-mer duplex oligonucleotide containing a single αdA in the middle of the duplex show that the 6-amino group and the C8 hydrogen atom in the adenine heterocycle protrude into the minor groove, producing a "shallow" minor groove. If exonuclease III uses the minor groove for binding or sensing the damage, the interaction between the enzyme and the DNA in the minor groove would be disrupted by the base moiety projecting into the minor groove, rendering exonuclease III unable to recognize αdA. Alternatively, local melting or breathing of duplex DNA surrounding the lesion might be important for damage recognition by exonuclease III, but not by endonuclease IV. For example, the T_m values of 9-mer duplexes containing a single αdA range between 29 and 40 °C, whereas those for the corresponding duplexes containing tetrahydrofuran are between 13 and 18 °C, which are much lower than those of αdA (H. Ide, unpublished data). Thus, only the duplex containing tetrahydrofuran might exhibit local melting or breathing in the vicinity of the lesion, which may explain why exonuclease III can recognize tetrahydrofuran but not αdA.

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