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A Mutant Endonuclease IV of *Escherichia coli* Loses the Ability To Repair Lethal DNA Damage Induced by Hydrogen Peroxide but Not That Induced by Methyl Methanesulfonate

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A mutant allele of the *Escherichia coli nfo* gene encoding endonuclease IV, *nfo-186*, was cloned into plasmid pUC18. When introduced into an *E. coli xthA nfo* mutant, the gene product of *nfo-186* complemented the hypersensitivity of the mutant to methyl methanesulfonate (MMS) but not to hydrogen peroxide (H_2O_2) and bleomycin. These results suggest that the mutant endonuclease IV has normal activity for repairing DNA damages induced by MMS but not those induced by H_2O_2 and bleomycin. A missense mutation in the cloned *nfo-186* gene, in which the wild-type glycine 149 was replaced by aspartic acid, was detected by DNA sequencing. The wild-type and mutant endonuclease IV were purified to near homogeneity, and their apurinic (AP) endonuclease and 3′-phosphatase activities were determined. No difference was observed in the AP endonuclease activities of the wild-type and mutant proteins. However, 3′-phosphatase activity was dramatically reduced in the mutant protein. From these results, it is concluded that the endonuclease IV186 protein is specifically deficient in the ability to remove 3′-terminus-blocking damage, which is required for DNA repair synthesis, and it is possible that the lethal DNA damage by H_2O_2 is 3′-blocking damage and not AP-site damage.

Partially reduced oxygen species, such as superoxide anion radical, hydrogen peroxide (H_2O_2), and hydroxyl radical, are toxic to organisms. They produce various types of damage in DNA that are major causes of their toxicities (1, 9–11). It has been shown that strand breaks with the 3′ termini phosphorylated are produced by H_2O_2 treatment in vivo (7). Because they lack 3′-OH termini, these broken ends cannot be repaired by DNA polymerases. Hence, this type of lesion is called 3′-blocking damage.

Escherichia coli exonuclease III and endonuclease IV, encoded by *xthA* and *nfo* genes, respectively, are major enzymes which repair such strand breaks through exonucleolytic removal of 3′-blocking damages (12, 22, 24). *xthA* and *xthA nfo* strains are highly sensitive to H_2O_2 (6), suggesting that the 3′-blocking damages are lethal.

Exonuclease III and endonuclease IV also have class II apurinic (AP) endonuclease activity, which cleaves endonucleolytically at AP sites in DNA (14). Exonuclease III is responsible for about 90% of the AP endonuclease activity in *E. coli* (14). Endonuclease IV, on the other hand, contributes only a minor portion of the activity (14). Redox cycling agents such as methyl viologen (paraquat) induce endonuclease IV activity (4), and *nfo* mutants show higher sensitivity to bleomycin and *t*-butyl hydroperoxide than do wild-type and *xthA* strains (6). Thus, it is certain that endonuclease IV plays a critical role in cellular protection against active oxygen species.

In addition to being hypersensitive to H_2O_2, the *xthA nfo* mutant is also sensitive to methyl methanesulfonate (MMS), which alkylates mainly purine bases in DNA and also generates AP sites (6). In the double mutant, AP sites may be lethal to DNA (6). Hence, considering that H_2O_2 produces strand breaks in DNA, it is possible that lethal damages induced by H_2O_2 differ in quality from those induced by MMS. In order to test this possibility, an attempt could be made to isolate exonuclease III or endonuclease IV mutants which show incomplete complementation; that is, they would have normal activity for repairing DNA damages generated either by H_2O_2 or by MMS but not by both. The *nfo* gene is a better candidate than the *xthA* gene for this approach, because endonuclease IV has activities to repair oxidative damages only, whereas exonuclease III carries 3′-exonuclease and RNase H activities as well (19, 25). Furthermore, the important role of endonuclease IV in the repair of bleomycin-induced DNA damage as described above stimulated us to examine the function of this enzyme.

In this paper, we report that the wild-type *nfo* gene cloned into pUC18 showed a complete complementation with both H_2O_2 and MMS sensitivity of the *xthA nfo* mutant of *E. coli*, but a mutant *nfo* allele in the recombinant plasmid can complement hypersensitivity of the *xthA nfo* strain only to MMS and not to H_2O_2 and bleomycin.

MATERIALS AND METHODS

Bacteria and plasmids. Bacterial strains and plasmids used are listed in Table 1. RPC500 and UM196 were generous gifts from R. P. Cunningham and P. C. Loewen, respectively. All strains are derivatives of *E. coli* K-12. *nfo*::*kan* and *katG*::Tn*10* were transferred by transduction with bacteriophage P1 according to the method of Miller (17). The *nfo*::*kan* genotype of DE882 and DE916 was confirmed by increased sensitivity to MMS and X rays. The *katG*::Tn*10* genotype of DE916 was confirmed by enzyme assay (15). Plasmid pNT180 was constructed by inserting the *Hpa*I-*Mfl*I fragment (21) of pHI901 into the pUC18 *Hin*cII-*Bam*HI site. Plasmid pHI901 is a derivative of pUC19 containing a 3.9-kb DNA fragment that includes the *nfo* gene (8a). Plasmids pNOP190 and pNOP196 were constructed by transferring

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TABLE 1. *E. coli* strains and plasmids used

Strain or plasmid	Relevant genotype[a]	Source or reference(s)[b]
Strains		
AB1157	*leuB6 thr-1 Δ(gpt-proA2) hisG4 argE3 lacY1 galK2 ara-14 mtl-1 xyl-5 thi-1 tsx-33 rpsL31 supE44 rac*	CGSC
CSH7	*lacY rpsL thi-1*	CGSC
BW9109	As AB1157 but Δ(*xth-pncA*)	B. Weiss (25)
RPC500	As AB1157 but *nfo*::*kan*	R. P. Cunningham (22)
UM196	As CSH7 but *katE katG*::Tn*10*	P. C. Loewen (15)
DE882	As BW9109 but *nfo-1*::*kan*	This study
DE916	As DE882 but *katG*::Tn*10*	This study
Plasmids		
pLC38-27	ColE1 *nfo*$^+$	J. Carbon (5, 6)
pHI901	pUC19 *nfo*$^+$	H. Ikehata et al. (8a)
pNT180, pNOP180	pUC18 *nfo*$^+$	This study
pNT186, pNOP186	pUC18 *nfo-186*	This study

[a] All strains are F$^-$ λ$^-$ derivatives of *E. coli* K-12.
[b] CGSC, *E. coli* Genetic Stock Center, Yale University School of Medicine, New Haven, Conn.

the *Pst*I-*Hpa*I fragment of pHI901 into pNT180 and pNT186, respectively.

Mutagenesis and mutant screening. For mutagenesis, pNT180 was treated with 0.3 M hydroxylamine at 37°C for 30 h, dialyzed against Tris-EDTA (TE) buffer, and then precipitated by ethanol (3). The plasmid DNA was digested with *Hin*dIII and *Kpn*I, and the resulting 930-bp fragment was isolated from agarose gels and religated into the same site of pUC18. *E. coli* DE916 was then transformed with hydroxylamine-treated pNT180. Ampicillin-resistant colonies were tested for their sensitivities to MMS and H_2O_2 by replica plating on Luria-Bertani (LB) agar containing either 2 mM MMS or 1 mM H_2O_2. About 10% of the colonies had the phenotype of high sensitivity to either MMS or H_2O_2. These clones were further selected by screening on LB agar containing either 2 mM MMS or 0.5 mM H_2O_2 (see Fig. 1B).

Survival curves. Overnight bacterial cultures were diluted 100-fold in LB broth and incubated at 37°C until the optical density at 600 nm reached about 0.6. The cells were collected by centrifugation, washed once, and suspended in phosphate-buffered saline (PBS). MMS or H_2O_2 was added to the cell suspensions, which were then incubated at 37°C for 1 h with shaking. The cell suspensions were then diluted with PBS and plated on LB agar (1.5%). For bleomycin sensitivity, exponentially growing cells were mixed with the drug in LB soft agar (0.6%), and the mixtures were poured onto LB agar plates. After 2 days of incubation at 30°C, the viable colonies were counted to estimate survival.

Purification procedure. The protein concentration was determined by using the Bio-Rad Protein Assay (Bio-Rad Laboratories) with bovine serum albumin as a standard. The *E. coli* strains for preparing crude cell extracts were DE882 carrying pNOP190 (wild-type *nfo*$^+$) or pNOP196 (*nfo-186*$^+$). Wild-type endonuclease IV was purified as previously described (12, 13) except that crude cell extract (fraction I) was prepared by sonication. Briefly, cells from 41 LB cultures were harvested, suspended in 40 ml of 50 mM Tris-HCl (pH 8.0) containing 1 mM dithiothreitol (DTT), and sonicated three times for 30 s each time at 30-s intervals with cooling on ice. Since endonuclease IV186 was found to be heat unstable (see Results), an altered purification method was employed for the mutant protein. All buffers used contained 1 mM DTT. Fraction II [$(NH_4)_2SO_4$ precipitation] was dialyzed with 50 mM potassium phosphate (pH 7.4) and then loaded onto a column of DEAE cellulose (Sigma Chemical Co.) equilibrated with 50 mM potassium phosphate (pH 7.4). After the column had been washed with 10 times the volume of the same buffer, endonuclease IV186 was eluted with 50 mM potassium phosphate (pH 7.4) containing 100 mM NaCl (fraction III). After concentration by Amicon YM10 ultrafiltration, fraction III was loaded onto a column of Sephadex G75 as described elsewhere (13) (fraction IV). Fraction IV was then applied to a column of single-stranded-DNA agarose (Bethesda Research Laboratories) as previously described (12). Endonuclease IV186 was then eluted with 20 mM Tris-HCl (pH 8.0) containing 5% glycerol and 400 mM NaCl. Active fractions were collected, dialyzed successively against 50 mM Tris-HCl (pH 8.0)–25% polyethylene glycol 6000 and 20 mM Tris-HCl (pH 8.0)–50% glycerol–200 mM NaCl, and then concentrated by ultrafiltration with Suprec-02 (Takara Shuzo) (fraction V).

Assays for enzyme activities. (i) EDTA-resistant AP endonuclease activity. Covalently closed circular (form I) plasmid DNA (pBluescript; Stratagene) was partially depurinated at 70°C for 20 min in 20 mM sodium acetate buffer (pH 4.8) containing 0.1 M NaCl (6), resulting in an average of two AP sites per DNA molecule. The reaction mixture contained the substrate DNA thus prepared and purified endonuclease IV (up to 30 ng) in 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid)-KOH (pH 8.2) containing 1 mM EDTA and 1 mM DTT. After incubation at 37°C for up to 32 min, the reaction was stopped by adding 4 μl of stop solution (40% glycerol, 0.6% sodium dodecyl sulfate [SDS], 60 mM EDTA, 2.0 mg of bromophenol blue per ml). DNA was subjected to agarose gel electrophoresis, stained by ethidium bromide, and then photographed. The bands of forms I and II were measured by densitometry, and the average number of strand breaks was calculated by Poisson analysis.

(ii) 3′-Phosphatase activity. 3′-Phosphatase activity associated with endonuclease IV was determined by its activity stimulating nick translation of DNA containing 3′-phosphate termini. The substrate for this assay was prepared as previously described (16, 18). Briefly, 50 nmol of pBluescript DNA was incubated with 1.7 mU of micrococcal nuclease at 37°C for 20 min in 50 mM Tris-HCl (pH 9.0) containing 1 mM $CaCl_2$. The treatment caused strand breaks, with the 3′-termini phosphorylated. The reaction was terminated by addition of phenol-chloroform (1:1; volume equal to volume of reaction mixture) followed by ethanol precipitation. The

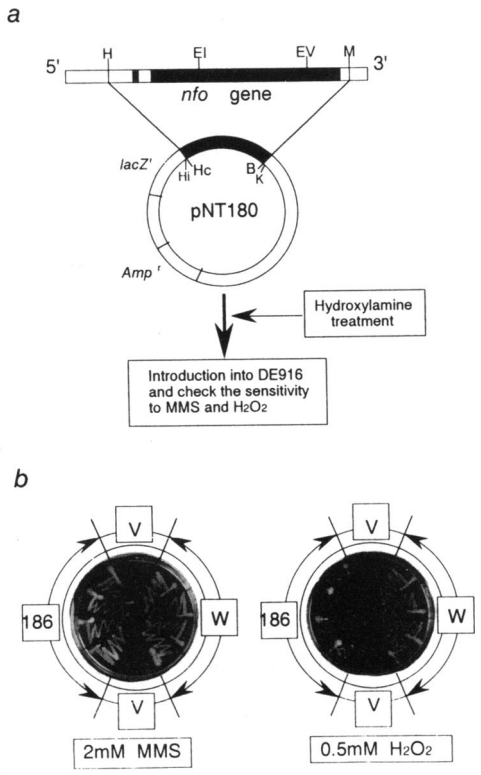

FIG. 1. Strategy for isolating *nfo* alleles. (a) *Hpa*I-*Mfl*I fragment, including the *nfo* structural gene, was inserted into a pUC18 multicloning site (*Hin*cII-*Bam*HI). Mutagenesis and screening were carried out as described in Materials and Methods. Solid and shaded boxes indicate the *nfo* structural gene and the ribosome-binding site, respectively. Abbreviations: B, *Bam*HI; EI, *Eco*RI; EV, *Eco*RV; H, *Hpa*I; Hc, *Hin*cII; Hi, *Hin*dIII; K, *Kpn*I; M, *Mfl*I. (b) DE916 carrying pUC18 (V), pNT180 (W), or pNT186 (186) was spread on LB-agar plates containing 2 mM MMS or 0.5 mM H_2O_2.

primer activation reaction was carried out by incubation of the substrate DNA with purified endonuclease IV at 37°C in 50 mM HEPES-KOH (pH 7.6) containing 100 mM KCl and 1 mM DTT followed by phenol-chloroform extraction and ethanol precipitation. The nick translation reaction was carried out by incubating the mixture of 15 nM endonuclease IV-treated DNA and *E. coli* DNA polymerase I at 37°C in the presence of 10 μM dATP, dGTP, dTTP, and [α-^{32}P]dCTP in 50 mM Tris-HCl (pH 7.8) containing 10 mM $MgCl_2$ and 1 mM DTT. The reaction was terminated by adding 50 μl of 2-mg/ml bovine serum albumin and 1 ml of ice-cold 5% trichloroacetic acid. The radioactivity of ^{32}P in the acid-insoluble fractions was counted by a liquid scintillation counter (Aloka LSC-3500) in a DPO-POPOP [2,5-diphenyloxazole–1,4-bis(5-phenyloxazolyl)benzene] system.

DNA sequencing. The 930-bp DNA fragments of the wild-type and mutant *nfo* genes were subcloned into M13mp18. Single-stranded DNA was sequenced by the dideoxy chain termination method (20) with a nucleotide sequencing kit using Sequenase version 2.0 (Toyobo), M4 universal primer (Takara Shuzo), or synthetic oligonucleotide primers complementary to the *nfo* gene.

Other reagents and enzymes. *E. coli* DNA polymerase I was purchased from Toyobo. Restriction enzymes were the products of Toyobo and Takara Shuzo. Micrococcal nuclease was purchased from Worthington Biochemical Co. Deoxynucleoside triphosphates were purchased from Pharmacia, Inc. Other chemicals and reagents were the products of Sigma.

RESULTS

Isolation of mutant *nfo* gene in a recombinant plasmid. In order to isolate *nfo* mutants, a 930-bp fragment containing the wild-type *nfo* gene was cloned into the multi cloning site of pUC18 (Fig. 1). The resulting plasmid, pNT180, could complement *E. coli* DE882 (*xthA nfo*) for resistance to MMS and H_2O_2 (Fig. 2). For mutagenesis, pNT180 DNA was treated with 0.3 M hydroxylamine, which induces the conversion of cytosine to thymine (3). To exclude any mutations in the vector sequence that affect the *nfo* phenotype, the *Hin*dIII-*Kpn*I fragment was isolated from pNT180 (Fig. 1) and then religated into unmutagenized pUC18. Since the *nfo* fragment did not contain an endogenous promoter region (21), any mutations in the promoter sequence could also be excluded. DE916, a *xthA nfo katG* triple mutant, was transformed with the mutagenized plasmid and screened for sensitivity to MMS and H_2O_2. The triple-mutant strain was used because of its extremely high sensitivity to both MMS and H_2O_2.

DE916(pNT180) and DE916(pUC18) were used as positive and negative controls, respectively, for testing sensitivity to MMS and H_2O_2. Among about 9,000 transformants, 20 clones were identified as sensitive to both MMS and H_2O_2, and 2 were resistant to MMS but highly sensitive to H_2O_2. The last two plasmids were named pNT186 (*nfo-186*) (Fig. 1B) and pNT1053 (*nfo-1053*). No clone sensitive to MMS but resistant to H_2O_2 was obtained. The *nfo-1053* strain showed a less striking phenotype than the *nfo-186* strain. Therefore, only the *nfo-186* allele was used for the following experiments.

Survival curves. Figure 2 shows survival curves for DE882(pNT186) after MMS and H_2O_2 treatment compared with curves for the positive and negative controls. It was evident that pNT186 conferred resistance to MMS in DE882. DE882(pNT186) showed the same resistance as DE882 (pNT180) (wild *nfo*$^+$). In contrast, DE882(pNT186) showed a sensitivity to H_2O_2 similar to that of DE882(pUC18). Thus, endonuclease IV186 has an almost normal ability to repair

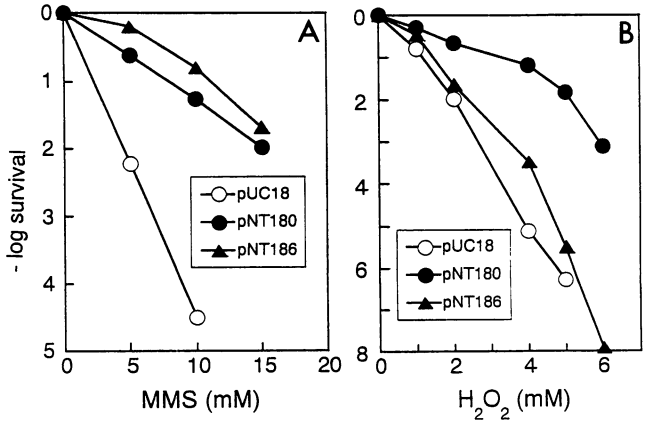

FIG. 2. Survival curves for DE882 carrying pUC18, pNT180 (*nfo*$^+$), or pNT186 (*nfo-186*$^+$). Exponentially growing cells were challenged with MMS (A) or H_2O_2 (B) as described in Materials and Methods.

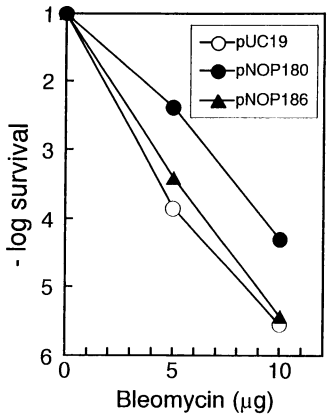

FIG. 3. Survival curves for DE882 exposed to bleomycin. pUC19, pNOP180 (*nfo*$^+$), and pNOP186 (*nfo-186*$^+$) were used. Cells were mixed with various concentrations of bleomycin in 0.6% LB soft agar and immediately poured into solid-LB plates.

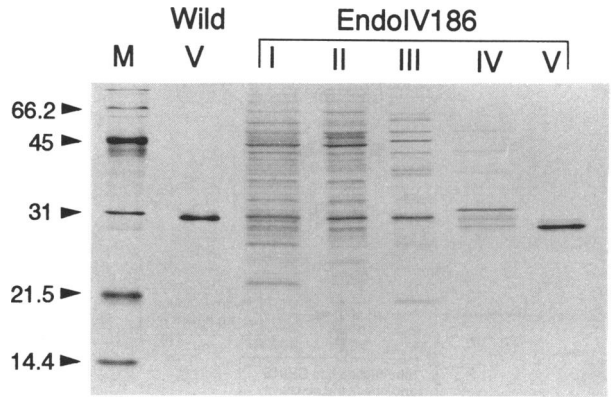

FIG. 5. SDS-polyacrylamide gel electrophoresis of endonuclease IV and endonuclease IV186. Lanes: M, molecular weight standards (in thousands); wild V, fraction V of wild-type endonuclease IV; EndoIV186 I through V, endonuclease IV186 fractions I through V. The gel consisted of 12% acrylamide and 0.4% bisacrylamide and was stained by the silver staining method.

DNA damages induced by MMS but not those induced by H_2O_2.

The *E. coli xthA nfo* mutant is also sensitive to bleomycin (6). The mechanism for producing DNA damages by bleomycin has been well characterized in vitro (2). Bleomycin attacks DNA to produce strand breaks whose 3′ termini are phosphoglycolated (2). The strand breaks cannot be directly repaired by DNA polymerases and DNA ligase because they lack the 3′-OH ends essential for repair synthesis by DNA polymerases. Thus, it was important to examine the sensitivity to bleomycin of the *xthA nfo* mutant carrying the *nfo* allele. As shown in Fig. 3, DE882(pNT180) was more resistant to bleomycin than DE882(pNT186), which was as sensitive as DE882(pUC19) (Fig. 3).

Nucleotide sequence of the *nfo-186* gene. The *nfo-186* gene in pNT186 was sequenced and compared with the wild-type gene. Only one difference in the nucleotide sequences was detected (Fig. 4). Guanine in position 446 in the wild type was substituted for adenine. This mutation resulted in changing glycine 149 to aspartic acid. In order to confirm further that the *nfo-186* gene does not carry any other mutations, we constructed two recombinants between pNT180 and pNT186

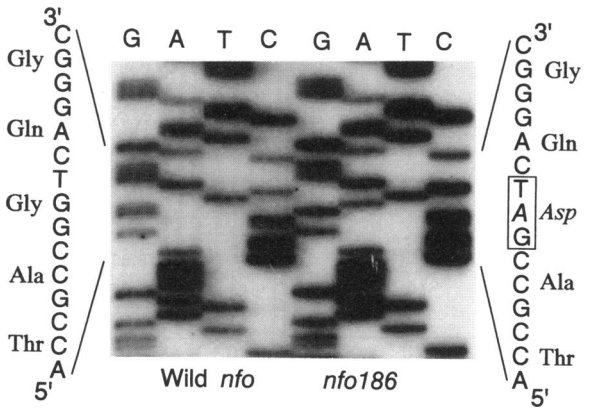

FIG. 4. Amino acid substitution in endonuclease IV186. The *nfo* and *nfo-186* sequences were determined by the dideoxy method. Nucleotide sequences of the genes (439 to 453 from translation start site) and amino acid sequences (codons 147 to 151) are shown.

by swapping *Eco*RI and *Hin*dIII-*Eco*RV fragments (Fig. 1). The recombinant plasmids containing the mutant *nfo* sequence showed the same phenotype as pNT186 in regard to sensitivity to MMS and H_2O_2. In addition, the reciprocal recombinant showed the phenotype of the wild-type *nfo* (data not shown). Thus, the missense mutation at glycine 149 is responsible for the mutant phenotype of endonuclease IV, which is coded for by *nfo-186*.

Enzymatic activities of endonuclease IV and endonuclease IV186. In order to biochemically characterize the mutant endonuclease IV, wild-type endonuclease IV and endonuclease IV186 were purified and their enzymatic activities were examined. Purification of the wild-type endonuclease IV was carried out as previously reported (12). However, since heat treatment at 65°C caused aggregation of the mutant enzyme (data not shown), the heat treatment was omitted, and DEAE-cellulose column chromatography was used to further purify the enzyme. The protein preparations were nearly homogeneous, as shown in Fig. 5.

The purified enzymes were tested for AP endonuclease activity with a partially depurinated plasmid DNA as a substrate. Figure 6A shows the time course of conversion of form I to form II DNA due to single-strand breaks by the endonuclease activity. Figure 6B shows the reaction rate as a function of the amount of protein. From these results, it was concluded that endonuclease IV186 has about the same level of AP endonuclease activity as the wild-type endonuclease IV.

Next, 3′-phosphatase activities associated with the wild-type and mutant endonuclease IV were assayed. The activity was indirectly measured by its ability to render the 3′-phosphate ends of a nicked DNA template effective primers for nick translation by *E. coli* DNA polymerase I. From the results in Fig. 7, it was evident that the amount of ^{32}P incorporated into DNA by nick translation did not increase after endonuclease IV186 reaction, while the wild-type endonuclease IV was quite effective in stimulating incorporation by nick translation. These results supported our conclusion that the mutant endonuclease IV186 was defective in 3′-phosphatase activity while retaining AP endonuclease activity.

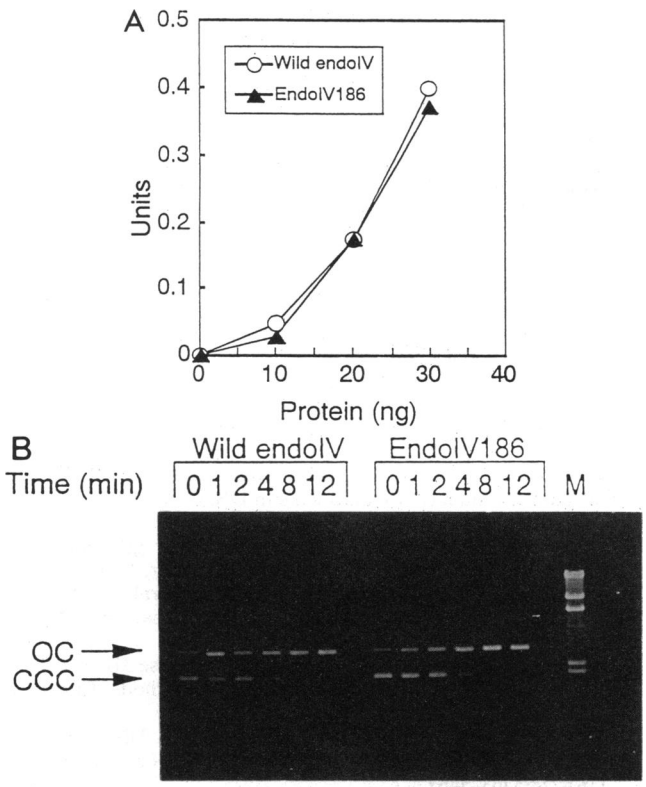

FIG. 6. AP endonuclease activities of endonuclease IV and endonuclease IV186. (A) Activity was determined as described in Materials and Methods. One unit of enzyme activity was defined as 1 pmol of nicking events per min (6). (B) Plasmids containing AP sites were incubated at 37°C with 20 ng of fraction V of endonuclease IV or endonuclease IV186. OC, open circular DNA; CCC, covalently closed circular DNA.

DISCUSSION

In this paper, we report the first successful isolation and characterization of a mutant *nfo* allele with an incomplete-complementation phenotype. The sensitivity of DE882 (*xthA nfo*)(pNT186) to MMS and H_2O_2 suggests that DNA damages caused by MMS but not those caused by H_2O_2 can be repaired by this mutant enzyme, endonuclease IV186. In order to understand the biochemical basis of this striking difference between the wild-type and mutant genes, we identified the mutation by direct sequencing of the *nfo-186* gene and found only one base change, a transition mutation of cytosine to thymine that was expected with hydroxylamine treatment. Thus, the phenotypic change is not due to an alteration in the plasmid copy number or in the regulation of expression of the endonuclease IV gene.

As previously described (6, 7), lethal damages induced by MMS and H_2O_2 require the endonuclease IV to be repaired. However, the damages may be quite distinct from each other, and different activities of the same protein may be involved. MMS generates AP sites, which inactivate *xthA nfo* cells of *E. coli* (6). H_2O_2, on the other hand, seems to kill the cells by generation of DNA strand breaks which cannot be repaired by DNA polymerase I unless primer activation by exonuclease III or endonuclease IV occurs (7). Thus, it is proposed that the AP endonuclease activity of endonuclease IV186 is normal but that endonuclease IV186 does not remove 3′-blocking damages.

The sensitivity of DE882 to bleomycin was also tested. Almost no increased resistance to bleomycin was observed in DE882(pNT186) compared with DE882(pNT180) (the wild-type *nfo* gene). Since bleomycin produces DNA strand breaks with their 3′ termini phosphoglycolated, one of the 3′-blocking damages (2), these results support the conclusion mentioned above.

To confirm the conclusion, the two enzymatic activities associated with the wild-type and mutant endonucleases were compared. Both of the enzymes were purified to near homogeneity, and their AP endonuclease and 3′-phosphatase activities were measured. Endonuclease IV186 was found to have a level of AP endonuclease activity similar to that of the wild-type endonuclease IV but not that of 3′-phosphatase.

These experimental results also provided some insights into the identification of lethal DNA damages induced by H_2O_2. *nfo-186* could not complement the sensitivity of DE882 to H_2O_2 (Fig. 2), and endonuclease IV186 had a normal AP endonuclease activity. Therefore, it is possible that lethal DNA damage caused by H_2O_2 does not involve AP sites. In contrast, since endonuclease IV186 cannot remove 3′-blocking damages, it is possible that some single-strand breaks produced by H_2O_2 contain blocked 3′ termini and that the blocks need to be removed before repair synthesis can restore the integrity of duplex DNA.

Asp-149 was deduced to be substituted for glycine in the endonuclease IV186 polypeptide (Fig. 4). Endonuclease IV186 migrated slightly faster than the wild-type enzyme (Fig. 5). The Asp-149 substitution for glycine probably caused the change in mobility, which probably resulted from a conformational alteration under SDS-polyacrylamide gel electrophoresis conditions (8, 23).

Although the effect of the amino acid substitution on the enzyme activities is not yet understood, we have some insights about it. Endonuclease IV186 seems to lack both a phosphodiesterase activity, which removes 3′-phosphoglycolates (Fig. 3), and a phosphomonoesterase activity, which removes 3′-phosphoryl groups (Fig. 7). However, the mutant enzyme may still retain the ability to hydrolyze the phoshoester bond, because it has a normal AP endonuclease activity. Thus, it is unlikely that the inability to remove

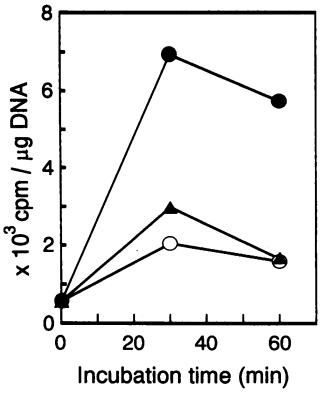

FIG. 7. Primer activation activities of endonuclease IV and endonuclease IV186. The assay was carried out as described in Materials and Methods. Symbols: ○, with no enzymes; ●, fraction V of endonuclease IV; ▲, fraction V of endonuclease IV186. The enzymes used in this experiment were the same as those used for the experiments of Fig. 6. Each value represents the mean of at least three experiments.

3′-blocking damages is due to loss of the esterase activity. Rather, endonuclease IV186 may have less ability to recognize or access 3′-blocking damage than does the wild-type endonuclease IV. More-detailed analyses of the purified enzymes are required to clarify the problems.

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