denV Gene of Bacteriophage T4 Determines a DNA Glycosylase Specific for Pyrimidine Dimers in DNA

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Endonuclease V of bacteriophage T4 has been described as an enzyme, coded for by the *denV* gene, that incises UV-irradiated DNA. It has recently been proposed that incision of irradiated DNA by this enzyme and the analogous "correndonucleases" I and II of *Micrococcus luteus* requires the sequential action of a pyrimidine dimer-specific DNA glycosylase and an apyrimidinic/apurinic endonuclease. In support of this two-step mechanism, we found that our preparations of T4 endonuclease V contained a DNA glycosylase activity that produced alkali-labile sites in irradiated DNA and an apyrimidinic/apurinic endonuclease activity that converted these sites to nicks. Both activities could be detected in the presence of 10 mM EDTA. In experiments designed to determine which of the activities is coded by the *denV* gene, we found that the glycosylase was more heat labile in extracts of *Escherichia coli* infected with either of two thermosensitive *denV* mutants than in extracts of cells infected with wild-type T4. In contrast, apyrimidinic/apurinic endonuclease activity was no more heat labile in extracts of the former than in extracts of the latter. Our results indicate that the *denV* gene codes for a DNA glycosylase specific for pyrimidine dimers.

Until recently the *denV* gene of bacteriophage T4 was thought to code for an enzyme, designated endonuclease V, that specifically incised UV-irradiated DNA by cleaving a phosphodiester bond on the 5' side of each pyrimidine dimer (13, 17, 21, 23). However, a new mechanism of action has recently been proposed for this enzyme and for analogous enzymes from *Micrococcus luteus* based upon the properties of fragments produced by treating UV-irradiated DNA of known nucleotide sequence with these enzymes (8, 8a). Comparison of the electrophoretic mobilities of these fragments with those of fragments generated by chemical reactions that cleave DNA at specific bases indicated that strand scission at the site of a dimer entails cleaving two bonds: the glycosylic bond of the 5' pyrimidine of the dimer and the phosphodiester bond between the two pyrimidines comprising the dimer. We have obtained evidence that supports the proposed scheme and indicates that the *denV* gene of T4 codes for the glycosylase activity.

MATERIALS AND METHODS

Bacteria and bacteriophage. Bacteriophage T4D and T4 *denV1* were obtained from E. C. Friedberg (Stanford University). M. Sekiguchi (Kyushu University) kindly provided the thermosensitive mutants of T4: F431, F794, and F821 (17). The phage were propagated from single plaques on *Escherichia coli* B (12).

Extracts of phage-infected cells. Extracts were

prepared from infected cells of an endonuclease I-deficient derivative of *E. coli* B, B41, as previously described (18) except that the cells were lysed in 50 mM Tris-hydrochloride (pH 7.5) by sonication (P. C. Seawell, E. C. Friedberg, A. K. Ganesan, and P. C. Hanawalt, in E. C. Friedberg and P. C. Hanawalt, ed., *DNA Repair: a Laboratory Manual of Research Procedures*, in press).

Protein concentrations of the extracts were determined by the Bio-Rad protein assay (Bio-Rad technical bulletin 1051).

Enzymes. Endonuclease V from T4-infected *E. coli* was purified through DEAE and phosphocellulose columns as described by Seawell et al. (in press). The preparation used in these experiments contained 410 U/ μ g of protein when assayed by nicking of the UV-irradiated superhelical DNA. No activity against unirradiated, undamaged DNA was detected in the presence of 10 mM EDTA; however, nicking of undamaged DNA occurred in the presence of 5 mM MgCl₂ without EDTA. Electrophoresis in sodium dodecyl sulfate-polyacrylamide gels revealed several protein bands of various intensities.

E. coli exonuclease III (50,000 U per unit of absorbancy at 280 nm), micrococcal nuclease (EC 3.1.4.7), and DNase I (EC 3.1.4.5) were purchased from Worthington Biochemicals Corp.

Enzyme assays. Binding of T4 endonuclease V to UV-irradiated DNA was measured as described by Seawell et al. (18; in press). The assay depends upon the retention by nitrocellulose filters of DNA bound to protein at 0°C. Each reaction contained ¹⁴C-labeled ColE1 DNA (approximately 100 ng, 5,000 cpm) irradiated with 200 J/m² at 254 nm.

Nicking of DNA was assayed with superhelical ¹⁴C-

labeled ColE1 or ^3H -labeled pBR322 DNA analyzed by electrophoresis in agarose gels (P. C. Seawell and A. K. Ganesan, in E. C. Friedberg and P. C. Hanawalt, ed., *DNA Repair: a Laboratory Manual of Research Procedures*, in press). For T4 endonuclease V the reactions contained approximately 100 ng (5,000 cpm) of DNA in 50 μl of 10 mM Tris-hydrochloride (pH 8.0 at 20°C)–100 mM NaCl–10 mM EDTA. For *E. coli* exonuclease III the reactions contained the same amount of DNA in 50 μl of 50 mM Tris-hydrochloride (pH 8.0 at 20°C)–5 mM MgCl_2 –100 μg of bovine serum albumin per ml.

The production of alkali-labile (AL) sites was assessed with superhelical ^{14}C -labeled ColE1 or ^3H -labeled pBR322 DNA by comparing the number of nicks per molecule present in DNA analyzed on alkaline sucrose gradients with the number of nicks per molecule in the same DNA analyzed on neutral agarose gels. The linear gradients contained 5 to 20% (wt/vol) sucrose in 0.2 N NaOH and were centrifuged at 45,000 rpm for 110 min at 20°C in a Beckman SW50.1 rotor. The DNA was incubated on the gradients for 30 min in the dark at approximately 22°C before centrifugation. The conditions for analysis of DNA on agarose gels were the same as those used for the nicking assay (Seawell and Ganesan, in press). In both cases the average number of nicks per molecule was calculated from the proportion of DNA remaining superhelical, using the Poisson expression. The number of AL sites was calculated by subtracting the number of nicks observed in neutral conditions from the number observed in alkali (total sites).

Micrococcal nuclease and DNase I were assayed by the release of cold acid-soluble radioactivity from *E. coli* DNA labeled with ^3H thymine. For micrococcal nuclease the reactions contained approximately 22,000 cpm of ^3H DNA in 100 μl of 10 mM Tris-hydrochloride (pH 8.0 at 20°C)–1 mM CaCl_2 . For DNase I the reactions contained approximately 60,000 cpm of ^3H DNA in 100 μl of 4 mM Tris-hydrochloride (pH 8.0 at 20°C)–20 mM MgCl_2 –125 mM NaCl.

DNA. ^{14}C -labeled ColE1 and ^3H -labeled pBR322 DNA were amplified and isolated as described elsewhere (Seawell and Ganesan, in press). The cells were lysed with detergent at room temperature (22°C).

DNA containing apurinic sites (AP DNA) was prepared by heating ^{14}C -labeled ColE1 DNA at 60°C for 45 min at pH 5 in 10 mM Tris-hydrochloride–10 mM sodium acetate–100 mM NaCl (10, 11). This treatment produced an average of approximately 1.7 AP sites per ColE1 molecule as judged by the number of nicks observed when the treated DNA was sedimented in alkaline sucrose gradients or treated with *E. coli* exonuclease III.

UV-irradiated DNA containing AL sites (AL DNA) was prepared by incubating ^{14}C -labeled ColE1 DNA irradiated with 20 J/m² at 254 nm (approximately 1.8 pyrimidine dimers per molecule) with T4 endonuclease V for 12 min at 0°C. These conditions were chosen to maximize the ratio of AL sites to nicks rather than to ensure that all pyrimidine dimers had been converted to AL sites or nicks.

Irradiation. DNA was irradiated at 254 nm with an unfiltered germicidal bulb under conditions described elsewhere (18). The fraction of thymine resi-

dues converted to pyrimidine dimers was 4.2×10^{-5} per J per m², corresponding to approximately 0.09 dimer per ColE1 molecule per J per m².

DNA was irradiated at 313 nm with a xenon short-arc lamp (X-75, Illumination Industries, Inc., Sunnyvale, Calif.) filtered through a plastic tissue culture flask filled with water (2-cm light path), a glass diffusing filter (1.9 to 2.1 mm thick), and a Corning 0-54 filter (1.9 to 2.1 mm thick) to remove wavelengths below 300 nm. Solutions containing 4.7 μg of DNA per ml, 10 μM AgNO_3 , and 3 mM acetophenone in 10 mM sodium phosphate buffer (pH 6.8) were irradiated in a quartz cuvette (1-cm light path). Nitrogen (Liquid Carbonic Hi-Pure) was bubbled through water and then through the DNA solution for 15 min before irradiation and during irradiation (15, 16). Because we had no satisfactory way to determine the incident dose rate at 313 nm, doses are expressed as time of exposure.

Pyrimidine dimer assays. The pyrimidine dimer content of DNA irradiated at 313 nm (approximately 200,000 cpm per sample) was determined by one-dimensional thin-layer chromatography on silica gel (1; R. J. Reynolds, K. H. Cook, and E. C. Friedberg, in E. C. Friedberg and P. C. Hanawalt, ed., *DNA Repair: a Laboratory Manual of Research Procedures*, in press). The number of thymine dimers per molecule of pBR322 DNA was calculated by multiplying the fraction of thymine converted to dimers by 1,010 (2 thymines per dimer; 2,019 thymines per pBR322 molecule [19]).

Sodium borohydride treatment. To a solution of DNA (3 to 7 $\mu\text{g}/\text{ml}$ in 10 mM sodium phosphate, pH 7) at 0°C an equal volume of 0.2 M sodium borate (pH 9.5) was added. Then NaBH_4 (2 M in 50 mM NaOH) was added to produce a final concentration of 0.18 M. After 120 min on ice, the reaction was terminated by the addition of 0.08 volume of 1 M H_3PO_4 (7). The DNA was dialyzed against 0.1 mM EDTA–10 mM Tris-hydrochloride (pH 8).

RESULTS

Evidence that preparations of T4 endonuclease V contains DNA glycosylase specific for pyrimidine dimers and an AP endonuclease. DNA glycosylases cleave the *N*-glycosylic bonds between specific bases and deoxyribose in DNA (9). The resulting AP sites can be detected as nicks after alkaline hydrolysis or digestion with AP endonuclease. At 0°C our preparation of T4 endonuclease V produced sites in UV-irradiated DNA that could be converted to nicks in alkali (Fig. 1). The number of AL sites produced in 1 min under these conditions was directly proportional to the enzyme concentration at values below 3.6 μg of protein per ml (Fig. 2).

The number of AL sites observed was consistent with the idea that they were produced at or near pyrimidine dimers and that most of them could be converted to nicks by further incubation with T4 endonuclease V. AL sites were not detected in un-irradiated, undamaged DNA incubated with the enzyme preparation, or in UV-

FIG. 1. Production of AL sites in UV-irradiated DNA by T4 endonuclease V. Superhelical ^{14}C -labeled ColE1 DNA (850 ng; 56,100 cpm), irradiated with 20 J/m^2 at 254 nm, was incubated with T4 endonuclease V (Δ , 4.4 μg of protein; $\circ$, 0.44 μg of protein) in 250 μl of 100 mM NaCl–10 mM EDTA–10 mM Tris-hydrochloride (pH 8.0 at 20°C) containing 100 μg of bovine serum albumin per ml. Samples were taken at the times indicated and analyzed for nicks by electrophoresis on agarose gels (---) and for total sites (AL sites and nicks) by sedimentation on alkaline sucrose gradients (—). Calculated numbers of events per molecule were corrected for the number of events observed in un-irradiated DNA treated with endonuclease (0.06 nicks, 0.2 total sites). A total of 3.0 sites per molecule was produced when the substrate DNA was incubated for 15 min at 37°C with the higher enzyme concentration (4.4 μg of protein).

FIG. 2. Effect of enzyme concentration on the production of AL sites and nicks in UV-irradiated DNA. ^{14}C -labeled ColE1 (200 ng; 2,220 cpm) irradiated with 10 J/m^2 was incubated in 50- μl reactions for 1 min at 0°C with the indicated amounts of T4 endonuclease V and then analyzed for nicks ($\square$) and total sites (AL sites and nicks; $\circ$). The difference represents AL sites (Δ).

irradiated DNA before enzyme treatment. To assess the relative yields of pyrimidine dimers, AL sites, and nicks as accurately as possible, we irradiated pBR322 DNA with 313-nm light in the presence of acetophenone and AgNO_3 to produce only thymine-thymine dimers (15, 16).

From the proportion of thymine converted to dimers we calculated the number of dimers produced and compared this value with the numbers of AL sites and nicks observed after the irradiated DNA had been incubated with a high concentration of T4 endonuclease V (Table 1). When reactions were incubated for 1 min at 0°C (Table 1A), the total number of AL sites and nicks approximated the number of dimers; about 90% of the total sites were AL. When the reactions were reincubated for 15 min at 37°C (Table 1B), the number of nicks increased to a value approximating the number of dimers in the DNA. Concomitantly, the number of AL sites decreased. However, some AL sites could still be detected under these conditions, an observation that we cannot presently explain.

By several criteria, the AL sites produced in UV-irradiated DNA by T4 endonuclease V resembled AP sites produced in DNA by acid depurination. DNA containing either AL or AP sites showed more nicks when analyzed on alkaline sucrose gradients than when analyzed on neutral agarose gels (Table 2), indicating that alkaline hydrolysis occurred on the gradients and converted both AL and AP sites to nicks. Reduction with NaBH_4 decreased the sensitivity of both AP and AL sites to alkali (Table 2). In addition, most AL sites, like AP sites, could be converted to nicks by treatment with *E. coli* exonuclease III. The endonuclease activity of this enzyme (also called *E. coli* endonuclease VI) specifically incises AP DNA (7, 9, 20). Under

TABLE 1. Yields of pyrimidine dimers, AL sites, and nicks in DNA^a

Irradiation (s)	No. of dimers	Events observed per molecule of DNA			
		A		B	
		Nicks	AL sites	Nicks	AL sites
0	0	0	0	0	0
45	0.61	0.05	0.44	0.64	0.07
90	1.21	0.14	0.95	1.36	0.16
135	1.54	0.17	1.58	1.78	0.33
180	2.22	0.24	2.27	2.57	0.96

^a Samples of ^3H -labeled pBR322 DNA, irradiated at 313 nm in the presence of acetophenone and AgNO_3 , were analyzed for their thymine dimer content and for the numbers of AL sites and nicks per molecule produced by incubation with T4 endonuclease V for 1 min at 0°C (A) followed by 15 min at 37°C (B). Enzyme reactions (200 μl) contained 156 ng (20,000 cpm) of DNA and 44 μg of T4 endonuclease V. The number of AL sites per molecule was calculated by subtracting the number of nicks per molecule observed under neutral conditions from the number of nicks per molecule observed under alkaline conditions.

conditions chosen to avoid detectable exonuclease or RNase H activity (20), this enzyme produced 93 and 75% as many nicks in AL and AP DNAs, respectively, as did alkali (Table 3). Similarly, T4 endonuclease V nicked both AL and AP DNAs (Table 3), producing 119 and 95% as many nicks, respectively, as did alkali. Some of the nicks produced in the AL DNA by T4 en-

donuclease V probably resulted from pyrimidine dimers that had not been converted to AL sites during the preparation of AL DNA. These would be sites for incision by T4 endonuclease V but not by *E. coli* exonuclease III or alkali.

The activity of T4 endonuclease V on AP DNA indicated that it contained AP endonuclease in addition to DNA glycosylase. This AP endonuclease differed from *E. coli* exonuclease III in that it was active in the presence of 10 mM EDTA and was less active against AL or AP DNA reduced with NaBH₄ (Table 4). Gossard and Verly (7) reported that *E. coli* exonuclease III incised AP DNA reduced with NaBH₄ nearly as efficiently as unreduced DNA. We obtained similar results with exonuclease III and AL DNA (data not shown). In contrast, the AP endonuclease in our T4 endonuclease V preparation produced significantly fewer nicks in AL or AP DNA treated with NaBH₄ than in untreated DNA (Table 4).

The presence of a DNA glycosylase and an AP endonuclease in preparations of T4 endonuclease V is consistent with the proposal (8a) that the first step in the incision of UV-irradiated DNA by this enzyme is the cleavage of a glycosylic bond at the site of a pyrimidine dimer. Complete incision requires the subsequent cleavage of a phosphodiester bond, a reaction that may, in principle, be catalyzed by any AP endonuclease. In an effort to determine whether the two activities reside in more than one protein, we tried to separate them by physical procedures. However, we found that they cochromatographed on phosphocellulose (data not shown). The ratio of glycosylase to AP endonuclease activity was approximately 5 in the leading edge, the center, and the trailing edge of the peak. The two activities cosedimented in neutral sucrose gradients (Fig. 3) and were not resolved

TABLE 2. Conversion of AL and AP sites to nicks by alkali after reduction by sodium borohydride^a

DNA	NaBH ₄ reduction	Conditions of analysis	Nicks per molecule	Sites per molecule converted to nicks
AL	—	Neutral	0.10	
	—	Alkali	1.81	1.71
	+	Neutral	0.11	
	+	Alkali	0.32	0.21
AP	—	Neutral	0.38	
	—	Alkali	1.22	0.84
	+	Neutral	0.41	
	+	Alkali	0.52	0.11

^a ¹⁴C-labeled ColE1 DNA (45 to 68 ng; 3,000 to 4,500 cpm) containing either AL or AP sites was either treated with NaBH₄ or untreated before being analyzed for nicks on alkaline sucrose gradients and neutral agarose gels. —, No reduction; +, reduction.

TABLE 3. Nicking of AL and AP sites by *E. coli* exonuclease III and T4 endonuclease V^a

DNA	Enzyme	Conditions of analysis	Nicks per molecule	Sites per molecule converted to nicks
AL	—	Neutral	0.10	
	—	Alkali	1.81	1.71
	Exo III	Neutral	1.69	1.59
	Endo V	Neutral	2.14	2.04 ^b
AP	—	Neutral	0.38	
	—	Alkali	1.22	0.84
	Exo III	Neutral	1.01	0.63
	Endo V	Neutral	1.18	0.80

^a The same DNA was used for these reactions as for those described in the footnote to Table 2. It was incubated for 15 min at 37°C with either *E. coli* exonuclease III or our T4 endonuclease V (44 µg/ml) before being analyzed for nicks on alkaline sucrose gradients or neutral agarose gels. The amount of exonuclease III used (0.75 U per 50-µl reaction) did not incise undamaged or UV-irradiated DNA or cause significant loss of radioactivity due to exonuclease activity. Exo, Exonuclease; endo, endonuclease; —, no enzyme.

^b The difference between the number of sites in AL DNA nicked by alkali and the number nicked by T4 endonuclease V probably reflects the number of dimers left intact during the preparation of the AL DNA. These could be nicked by T4 endonuclease V but not by alkali or exonuclease III.

TABLE 4. Nicking by T4 endonuclease V of AL and AP sites reduced with sodium borohydride^a

DNA	Endonuclease V	Nicks per molecule	Sites per molecule converted to nicks
AL	—	0.10	
	+	2.14	2.04
Reduced AL	—	0.11	
	+	0.71	0.60
AP	—	0.38	
	+	1.18	0.80
Reduced AP	—	0.41	
	+	0.59	0.18

^a The DNA and incubation conditions used for these reactions were the same as those described in footnote a of Table 3. DNA was analyzed for nicks on neutral agarose gels. —, No enzyme added; +, enzyme added.

by high-pressure liquid chromatography (data not shown). These results indicate that, if the two activities reside in different proteins, the proteins are similar in size.

Evidence that the *denV* gene of bacteriophage T4 codes for a DNA glycosylase specific for pyrimidine dimers. To determine whether the *denV* gene codes for the DNA glycosylase or the AP endonuclease, we tested four different *denV* mutants. One of them, *denV1*, has been described as being deficient in endonuclease V (4). The others, F431, F794, and F821, have been characterized as producing thermostable endonuclease V (17). Extracts prepared from cultures of *E. coli* B41 infected with each of the mutants or with the wild type, T4D, were assayed for binding to UV-irradiated DNA, production of AL sites in UV-irradiated DNA, nicking of UV-irradiated DNA, and nicking of AP DNA.

All of these extracts, and one from uninfected cells, contained similar amounts of an activity that nicked AP DNA in the presence of 10 mM EDTA (Table 5). None of the extracts showed a significant reduction in this activity when incubated for 2 min at 43°C before assay (Table 5).

In contrast, extracts of cells infected with any of the *denV* mutants nicked UV-irradiated DNA

TABLE 5. Nicking of AP DNA by extracts of *E. coli* infected with T4 *denV* mutants^a

Extract	Protein in reaction (ng)	Incubation (°C)	Nicks per molecule
T4D	64	30	1.14
		43	1.05
<i>denV1</i>	87	30	1.03
F821	73	30	0.69
		43	0.73
F431	107	30	1.02
		43	1.00
F794	93	30	0.98
		43	0.96
Uninfected cells	160	30	1.42

^a Extracts were incubated for 2 min at 30 or 43°C, after which a limiting amount of each extract was added to reaction mixtures containing 85 ng of ¹⁴C-labeled ColE1 DNA (5,610 cpm) in 100 mM NaCl-10 mM EDTA-10 mM Tris-hydrochloride (pH 8.0 at 20°C). After 15 min at 30°C, the reactions were terminated, and the DNA was analyzed by electrophoresis on agarose gels. The substrate DNA contained 1.6 AP sites per molecule as determined by sedimentation in alkaline sucrose gradients. The data have been corrected for the number of nicks present in DNA not exposed to the enzyme (0.17 nick per molecule).

FIG. 3. Sedimentation of T4 endonuclease V in neutral sucrose. The enzyme (110 µg of protein) was centrifuged in a neutral sucrose gradient (5 to 20% [wt/vol] in 100 mM NaCl-10 mM Tris-hydrochloride [pH 8.0]-0.1 mM EDTA) at 59,000 rpm for 24 h at 4°C in a Beckman SW60 Ti rotor. A total of 36 fractions (0.1 ml each) were collected in tubes containing 10 µg of bovine serum albumin. The fractions indicated were assayed for the following: (○) binding to UV-irradiated DNA (100% = 3,465 cpm); (□) production of AL sites in UV-irradiated DNA (100% = 2.6 sites per molecule); (△) nicking of UV-irradiated DNA (100% = 2.6 nicks per molecule); and (▲) nicking of AP DNA (100% = 3.0 nicks per molecule). The arrows indicate the peak positions of micrococcal nuclease, *M*, 16,807 ①, and DNase I, *M*, 31,000 ②, sedimented in separate gradients.

less efficiently than did the extract of cells infected with T4D (Table 6). The deficiency was particularly severe in the *denV1* and F821 extracts. Although the F431 and F794 extracts contained more activity than did the *denV* and F821 extracts, it was significantly reduced by incubation for 2 min at 43°C (Table 6). Similar results were obtained when the extracts were tested for binding to UV-irradiated DNA (Table 7). As previously reported (18), no significant binding was observed with an extract of uninfected cells. When tested for the production of AL sites in UV-irradiated DNA, the *denV* extracts, particularly the *denV1* and F821 extracts, were less active than the T4D extract (Table 8). Furthermore, the activity in the F431 and F794 extracts was more thermostable than that in the T4D extract. These results indicate that the *denV* gene codes for a DNA glycosylase specific for pyrimidine dimers and that this activity is a prerequisite for binding or nicking of UV-irradiated DNA under the conditions that we have used.

DISCUSSION

The enzyme originally designated as T4 endonuclease V (22) now appears to contain two activities, a DNA glycosylase specific for pyrimidine dimers and an AP endonuclease. By acting sequentially, the two activities can incise UV-

irradiated DNA (8a). First, AL sites are produced (Fig. 1 and Table 1). These sites appear to be AP sites in that they can be converted to nicks by alkali or by incubation with an endonuclease specific for AP sites, such as *E. coli*

TABLE 6. Nicking of UV-irradiated DNA by extracts of *E. coli* infected with T4 denV mutants^a

Extract	Protein in reaction (μg)	Incubation (°C)	Nicks per molecule	Activity remaining (%)
T4D	0.25	30	1.55	100
		43	1.39	90
denV1	17.40	30	0.11	—
		43	0.05	—
F821	14.60	30	0.03	—
		43	0.05	—
F431	5.35	30	1.22	100
		43	0.41	34
F794	9.30	30	1.45	100
		43	0.48	33

^a Extracts were incubated for 2 min at 30 or 43°C, after which a limiting amount of each extract was added to reaction mixtures (50 μl) containing 57 ng of ¹⁴C-labeled ColE1 DNA (3,740 cpm) irradiated with 20 J/m² at 254 nm, 100 mM NaCl-10 mM EDTA-10 mM Tris-hydrochloride (pH 8.0 at 20°C). After 15 min at 30°C, the reactions were terminated, and the DNA was analyzed on neutral agarose gels. The data have been corrected for the number of nicks present in un-irradiated DNA treated with T4 endonuclease V (0.08 nick per molecule). Complete incision of the substrate DNA produced 2.8 nicks per molecule (5, 6).

TABLE 7. Binding of UV-irradiated DNA by extracts of *E. coli* infected with T4 denV mutants^a

Extract	Protein in reaction (μg)	Incubation (°C)	cpm retained	Activity remaining (%)
T4D	6.4	30	2,230	100
		43	1,520	68
denV1	26.1	None	167	—
F821	21.9	None	141	—
F431	10.7	30	1,340	100
		43	340	25
F794	18.6	30	1,300	100
		43	270	21
Uninfected cells	48.0	None	230	—

^a Extracts were incubated for 2 min at 30 or 43°C or tested without prior incubation. A limiting amount of each extract was added to reaction mixtures containing 67 ng of ¹⁴C-labeled ColE1 DNA (4,400 cpm) in 100 mM NaCl-10 mM EDTA-10 mM Tris-hydrochloride (pH 8.0 at 20°C) containing 10% (vol/vol) ethylene glycol. After 3 min at 0°C, the reactions were terminated and filtered through nitrocellulose filters. The substrate DNA had been irradiated with 200 J/m² at 254 nm and contained approximately 18 pyrimidine dimers per molecule. Saturating amounts of purified T4 endonuclease V caused the retention of 3,810 cpm.

TABLE 8. Production of AL sites in UV-irradiated DNA by extracts of *E. coli* infected with T4 denV mutants^a

Extract	Protein in reaction (μg)	Incubation (°C)	Events per molecule of DNA			Activity remaining (%)
			Total sites	Nicks	AL sites	
T4D	2.5	30	0.57	0.07	0.50	100
		43	0.29	0.06	0.23	46
denV1	8.7	30	0	0.01	0	—
F821	7.3	30	0.03	0.02	0.01	—
		43	0.01	0.03	0	—
F431	3.2	30	0.28	0.05	0.23	100
		43	0.08	0.01	0.07	30
F794	4.7	30	0.31	0.02	0.29	100
		43	0.08	0.01	0.07	24

^a Extracts were incubated for 2 min at 30 or 43°C, after which a limiting amount of each extract was added to reaction mixtures (50 μl) containing 113 ng of ¹⁴C-labeled ColE1 DNA (7,500 cpm) irradiated with 20 J/m² at 254 nm, 100 mM NaCl-10 mM EDTA-10 mM Tris-hydrochloride (pH 8.0 at 20°C). After 3 min at 0°C, the reactions were terminated. Half of each reaction mixture was analyzed on alkaline sucrose gradients (total sites) and half was analyzed on agarose gels (nicks). Complete incision of the substrate DNA produced 3.2 nicks per molecule (5, 6).

exonuclease III (Table 3). Furthermore, like AP sites, they become stable to alkali after reduction by NaBH₄ (Table 2). The sites do not appear in un-irradiated, undamaged DNA treated with T4 endonuclease V or in irradiated DNA not exposed to the enzyme. Under conditions permitting a direct comparison, the frequency of AL sites in UV-irradiated DNA treated with the enzyme is approximately the same as the frequency of pyrimidine dimers (Table 1). Evidence that these sites result from the cleavage of glycosylic bonds between deoxyribose and the 5' pyrimidine of each dimer has come from studies of the electrophoretic mobility on sequencing gels of UV-irradiated DNA treated with the enzyme and from the observation that free thymine can be released from enzyme-treated DNA by enzymatic photoreactivation or by photoreversal of the dimers [8a; E. H. Radany and E. C. Friedberg, *Nature* (London), in press].

The AL sites produced by T4 endonuclease V in UV-irradiated DNA can be converted to nicks by an AP endonuclease. The AP endonuclease in our preparation of T4 endonuclease V nicked acid-depurinated DNA even in the presence of 10 mM EDTA (Table 3). The rate of nicking (data not shown) was identical to that of AL DNA (Fig. 1). Studies of fragments produced by treating UV-irradiated DNA of known nucleotide sequence with the enzyme indicate that, unlike *E. coli* exonuclease III, it cleaves the

phosphodiester bond on the 5' side of the AP site, leaving a base-free deoxyribose at the 3' terminus of the nick (8a). Subsequent examination of the ability of the termini to act as primers for DNA polymerase I has supported this interpretation (H. R. Warner, B. F. Demple, W. A. Deutsch, C. M. Kane, and S. Linn, Proc. Natl. Acad. Sci. U.S.A., in press).

Studies of mutants of T4 indicated that the *denV* gene (formerly designated the *u* or *v* gene) codes for T4 endonuclease V (see reference 3 for review; 17, 21). To determine whether this gene codes for the DNA glycosylase or the AP endonuclease (or both), we examined several *denV* mutants. We found that extracts of cells infected with the *denV1* mutant, previously described as being deficient in the production of endonuclease V, contained a normal amount of AP endonuclease (Table 5) but little or no DNA glycosylase activity (Tables 6, 7, and 8). Extracts of cells infected with F431 or F794, previously described as producing thermosensitive endonuclease V, also contained normal levels of AP endonuclease (Table 5). In contrast, their DNA glycosylase activities were low even at the permissive temperature (30°C) and were further reduced by incubation at 43°C for 2 min before assay (Tables 6, 7, and 8). Extracts of cells infected with the F821 mutant, which had also been described as a thermosensitive mutant, behaved similarly to those of the *denV1* mutant, showing a normal amount of AP endonuclease and little or no DNA glycosylase. Because our phage stocks had been propagated from single plaques, the difference between the behavior of F821 and that of F431 and F794 might have resulted from our choosing a variant that carried a *denV* mutation different from the original one. The simplest interpretation of our data is that the *denV* gene codes for the DNA glycosylase but not for the AP endonuclease. From studies of the substrate specificity of T4 endonuclease V (2, 14), we expect this glycosylase to be specific for pyrimidine dimers in DNA.

An extract of uninfected cells of *E. coli* contained about the same amount of AP endonuclease active in 10 mM EDTA as did extracts of cells infected with wild-type T4 (Table 5). This enzyme, of bacterial origin, might persist through the procedures used for purifying T4 endonuclease V. However, we have not yet ruled out the possibility that an AP endonuclease of phage origin is present in preparations of T4 endonuclease V and might even reside in the same protein as the DNA glycosylase.

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