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Dark Repair of Ultraviolet-Irradiated Deoxyribonucleic Acid by Bacteriophage T4: Purification and Characterization of a Dimer-Specific Phage-Induced Endonuclease

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The purification and properties of an ultraviolet (UV) repair endonuclease are described. The enzyme is induced by infection of cells of *Escherichia coli* with phage T4 and is missing from extracts of cells infected with the UV-sensitive and excision-defective mutant $T4V_1$. The enzyme attacks UV-irradiated deoxyribonucleic acid (DNA) containing either hydroxymethylcytosine or cytosine, but does not affect native DNA. The specific substrate in UV-irradiated DNA appears to be pyrimidine dimer sites. The purified enzyme alone does not excise pyrimidine dimers from UV-irradiated DNA. However, dimer excision does occur in the presence of the purified endonuclease plus crude extract of cells infected with the mutant $T4V_1$.

Bacteriophage T4 possesses at least three genes that affect its recovery from ultraviolet (UV) radiation damage (1, 4, 17). One of these has been named the "V" gene and has been shown to be related to the dark repair of deoxyribonucleic acid (DNA) (15, 20). We previously reported that a mutant $T4V_1$ defective in the V gene is deficient in an endonuclease activity that degrades UV-irradiated DNA (3). The present communication describes the purification, characterization, and dimer specificity of this enzyme. An endonuclease with similar properties has been isolated from *Micrococcus luteus* (2, 7).

MATERIALS AND METHODS

Bacteria and bacteriophage. Bacteriophage T4D with a wild-type V gene was used for induction of endonuclease. Phage $T4V_1$ was obtained from W. Harm. *Escherichia coli* B-3 (*thy*$^-$) was used as the host bacterium. Phage T7 was obtained from J. Wohlheiter.

Preparation of DNA. ^{3}H-labeled and nonradioactive T4 and T7 DNA was prepared by phenol extraction from phage grown on *E. coli* B-3 (11). ^{3}H-labeled *E. coli* DNA was prepared by the procedure of Thomas et al. (18). UV irradiation of DNA was carried out using a low-pressure mercury vapor UV germicidal lamp. DNA samples with various dimer contents were prepared by UV irradiation of DNA in the presence of proflavine (16). DNA containing 0.5% thymine dimers was prepared by irradiating ^{3}H-labeled DNA *E. coli* (62 μg/ml) at 10^5 ergs/mm^2 in the presence of proflavine at a molar ratio of proflavine-DNA-P of 1:3. DNA containing 1.9% dimers was prepared by preirradiation at 5×10^4 ergs/mm^2 followed by further irradiation at 5×10^4 ergs/mm^2 in the presence of proflavine. DNA species containing 3.9% and 5.4% dimers were prepared by irradiation at 5×10^4 and 10^5 ergs/mm^2, respectively, after which proflavine was added to the DNA. All DNA-proflavine samples were dialyzed extensively after irradiation to remove the dye.

Sedimentation velocity of DNA. Sedimentation velocity was measured in density gradients of 5 to 20% sucrose at *p*H 7.0 and containing 1.0 N NaCl for neutral gradients and 0.8 N NaCl-0.2 N NaOH for alkaline gradients. Generally, 0.25 to 0.5 μg of ^{3}H-labeled DNA in 0.05 ml was layered onto each gradient. Centrifugation was in a Spinco type T56 rotor at 32,000 rev/min for 180 min (T4 DNA) or 35,000 rev/min for 410 min (T7 DNA) at 20 C. Ten-drop fractions were collected and 1.0 ml of NCS reagent (Amersham Searle Co.) was added. Radioactivity was measured in a liquid scintillation spectrometer.

Calculation of single- and double-strand breaks. Single- and double-strand DNA breaks were measured by using the nomogram constucted by Litwin, Shahn, and Kozinski (10).

Analytical disc gel electrophoresis. Stacking gel consisted of 2.5% acrylamide, 0.65% N,N^2-methylene bis-acrylamide in tris(hydroxymethyl)aminomethane (Tris)-H_3PO_4 buffer (*p*H 9.65). Separating gel consisted of 12% acrylamide, 0.4% N,N^2-methylene bis-acrylamide

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in Tris-hydrochloride (*p*H 10.2). Upper reservoir buffer was Tris-hydrochloride (*p*H 7.51). A total of 36 μg of protein was applied in 0.1 ml of Tris-glycine buffer (*p*H 8.9) with glycerol. Electrophoresis was at 100 v (2.5 ma/tube) for 70 min and then at 200 v (5.0 ma/tube) for 30 min. Gels were stained with naphthol blue-black. Destaining was carried out electrophoretically in 7% acetic acid.

DNA-cellulose. T4 DNA cellulose was prepared by the procedure of Litman (9). T4 DNA (0.5 mg/ml) was irradiated at 10^5 ergs/mm^2, and 75.0 ml was mixed with 9.0 g of acid-washed cellulose powder (Munktell's no. 410).

Extracts of T4V$_1$-infected cells. Crude extracts of *E. coli* B-3 infected with phage T4V$_1$ were prepared identically to those of cells infected with wild-type phage. The procedure is described fully in the section on enzyme purification.

Measurement of dimer content of DNA. UV-irradiated ^{3}H-labeled DNA (20 μg) was precipitated with cold 5% trichloroacetic acid in thick-walled hydrolysis vials. Samples were centrifuged in the cold at 5,000 × *g*, and the supernatant fluid was collected. To each trichloroacetic acid precipitate, 0.2 ml of 97% formic acid was added. Tubes were sealed and hydrolyzed at 176 C for 30 min. After evaporation of formic acid to dryness, the residue was redissolved in 0.005 ml of water. Approximately 0.001 ml was subjected to thin-layer chromatography on Brinkman MN-Polygram Cel 300 thin-layer plates. Chromatograms were developed with a mixture of *n*-butanol-water (14:86, v/v). Developed and dried chromatograms were overlaid with strips of cellophane tape and divided into 0.5-cm sections. Each section was placed in a liquid scintillation vial, and radioactivity was eluted in 0.5 ml of water. A 10.0 ml amount of Permafluor in a toluene-cellosolve mixture was added, and ^{3}H radioactivity was measured in a liquid scintillation spectrometer. The radioactivity in the dimer peak was corrected for trailing thymine monomer. The dimer content was then expressed as the radioactivity in the dimer peak as a percentage of the total radioactivity measured.

Assay of T4 endonuclease activity. We previously demonstrated that crude extracts of T4-infected cells degrade UV-irradiated T4 DNA at a significantly greater rate than do extracts of either uninfected cells or cells infected with the mutant T4V$_1$ (3). This rate difference is a function of a phage-induced UV-specific endonuclease. The endonuclease itself does not result in the formation of acid-soluble nucleotide product (*see below*). Thus, the increased rate of degradation of UV-irradiated DNA presumably results from attack by other nucleases at the sites of initial endonucleolytic cleavage. This observation has facilitated the assay of endonuclease activity by measuring the formation of acid-soluble nucleotide under conditions where the amount of endonuclease is the rate-limiting step in the degradation of UV-irradiated DNA. These conditions are met when the reaction mixture contains saturating amounts of a standard preparation of endonuclease-deficient extract obtained from cells infected with the mutant T4V$_1$.

Assay A. Incubation mixtures (1.0 ml) contained 10 μg of ^{3}H-labeled T4 or *E. coli* DNA irradiated at 10^5 ergs/mm^2, 50 mM Tris (*p*H 8.0), 1.0 mM $MgCl_2$, 50.0 mM KCl, 100 μg of *E. coli* transfer ribonucleic acid, (tRNA), 2.0 to 45.0 units of endonuclease, and 1.5 to 2.0 mg of crude extract of T4V$_1$-infected cells. Reactions were started by the addition of the crude extract and endonuclease in quick succession. Incubations were at 37 C for 15 min. Reactions were terminated by placing tubes into ice and adding 0.5 ml of cold 1% carrier bovine serum albumin (BSA) followed by 0.5 ml of cold 20% trichloroacetic acid. Samples were centrifuged at 5,000 × *g* for 5 min, and the radioactivity in 1.0 ml of supernatant fluid was determined in a liquid scintillation spectrometer. Samples containing no endonuclease were included in every experiment. The amount of labeled acid-soluble nucleotide formed in the absence of added endonuclease was subtracted from the amount formed in the presence of endonuclease. This procedure was used as the standard assay of UV-endonuclease activity. All in vitro studies were done under laboratory illumination by yellow fluorescent lamps (above 500 nm) to preclude photoreactivation.

An alternative method was used in experiments designed to test the effect of activators or inhibitors on the endonuclease specifically. In this procedure, a two-step incubation was performed, thereby separating the reaction with endonuclease from the presence of other nucleases.

Assay B. Incubation mixtures (0.5 ml) contained 10 μg of ^{3}H-labeled UV-irradiated DNA, 50 mM Tris (*p*H 8.0), 1.0 mM $MgCl_2$, 100 μg of *E. coli* tRNA, and 2 to 45 units of endonuclease. Incubation was at 37 C for 15 min. Reactions were terminated as previously described by adding 0.1 ml of cold 1% BSA and 0.4 ml of cold 12.5% trichloroacetic acid. Samples were centrifuged at 5,000 × *g*, and the supernatant fluid was discarded. The pellets were redissolved at room temperature in 0.5 ml of 0.1 N NaOH, after which 0.05 ml of 2.0 M Tris (*p*H 6.45) was added to restore the *p*H of the mixture to 8.0. To the mixture was added 1.0 mM $MgCl_2$, 1.5 to 2.0 mg of crude extract of T4V$_1$-infected cells, and water to a volume of 1.0 ml. Incubation was repeated for 15 min at 37 C, and the reactions were completed as previously described.

Relative S value determination of endonuclease. Determination of the relative sedimentation coefficient of the enzyme was done by sedimentation of fraction 5 in 5 to 20% sucrose in 0.05 M Tris (*p*H 8.0). Centrifugation was carried out in a Spinco type SW T65 rotor at 45,000 rev/min for 22 hr. Purified *E. coli* alkaline phosphatase (Worthington Biochemical Corp.) was used as a marker. Ten-drop fractions were collected after centrifugation and assayed for alkaline phosphatase and endonuclease activity. Alkaline phosphatase was assayed by using 0.2 mg of *p*-nitrophenyl phosphate per ml in 1.0 M Tris-hydrochloride (*p*H 8.0) as substrate. To 1.0-ml of the mixture, 0.01 ml of the enzyme fraction was added, and the absorbancy at 410 nm was measured at 30 sec.

Units. One unit of endonuclease activity is defined for purification procedures in this paper as the amount of enzyme which, in the presence of saturating amounts of crude extract of T4V$_1$-infected cells, results in the formation of 1 nmole of acid-soluble nucleotide product per hour by using the standard assay procedure.

Enzyme purification: growth of cells. *E. coli* B-3

(*thy*$^-$) was grown to exponential phase in nutrient broth with 0.5% NaCl. Phage infection was carried out at a multiplicity of infection of 3 to 5 in the presence of DL-tryptophan (8 μg/ml). Cells were incubated for a further 15 min at 37 C, at which time chloramphenicol was added to a final concentration of 150 μg/ml. Cultures were quickly cooled to 4 C in a liquid nitrogen bath, and cells were harvested by centrifugation at 7,000 × *g*. Cells were washed once in 0.05 M Tris (*p*H 8.0) and stored at −20 C.

Crude extract (fraction 1): Twenty-four grams of phage-infected cells were thawed slowly in 75.0 ml of 5.0 M NaCl, 20 mM $MgCl_2$, and 50 mM Tris (*p*H 8.0) at 4 C. The suspension was treated in a Branson model W140D sonic oscillator.

Dextran-polyethyleneglycol fractionation: (fraction 2). To 93.0 ml of crude extract, 3.720 g of Dextran T 500 (40 mg/ml) and 5.580 g of polyethyleneglycol 6000 (60 mg/ml) were added in the cold. The mixture was stirred gently until complete solution was obtained. After centrifugation at 7,000 × *g*, the top clear phase was decanted and saved. This fraction was dialyzed against three changes (each of 2 liters) of 50 mM Tris (*p*H 8.0) with 3% polyethyleneglycol.

DEAE cellulose chromatography: (fraction 3). Fraction 2 (75.0 ml) was applied to a diethylaminoethyl (DEAE) cellulose column (20.0 by 2.5 cm) equilibrated in 50 mM Tris (*p*H 8.0) with 3% polyethyleneglycol. Elution was carried out with the equilibrating buffer. Enzyme activity was recovered in the void volume of the column. The pooled enzyme fraction was dialyzed against 50 mM phosphate buffer (*p*H 7.0) with 3% polyethyleneglycol.

Phosphocellulose chromatography: (fraction 4). A column (20 by 2.5 cm) was packed with phosphocellulose equilibrated with 50 mM phosphate buffer (*p*H 7.0) with 3% polyethyleneglycol, and 80.0 ml of fraction 3 was applied to the column. Elution was carried out with a linear gradient of 0.05 to 0.5 M phosphate at *p*H 7.0 with 3% polyethyleneglycol. A total of 1 liter of buffer was used for elution. Fractions (9.0 ml) were collected. Fractions containing UV endonuclease activity were pooled and dialyzed against 50 mM phosphate buffer (*p*H 7.0) with 3% polyethyleneglycol. The enzyme was eluted at about 0.4 M phosphate.

DNA cellulose chromatography: (fraction 5). Dry T4 DNA-cellulose powder was equilibrated in 50 mM phosphate buffer (*p*H 7.0). A column (5.0 by 3.0 cm) was prepared, and 180.0 ml of fraction 4 was applied. Elution was carried out in 250 ml of 50 mM phosphate buffer (*p*H 7.0) containing a linear gradient of 0 to 1.5 M NaCl. Fractions (4.0 ml) were collected into tubes containing 25 μg of calf thymus DNA per ml. Fractions were assayed, and those containing endonuclease activity were pooled and dialyzed against 50 mM Tris (*p*H 8.0). The purified enzyme was stored in the presence of calf thymus DNA at 4 C.

RESULTS

Assay conditions. Figure 1 shows the saturation kinetics of the degradation of irradiated endonuclease-treated DNA by crude extract of $T4V_1$-infected cells. About 1.0 ng/ml of a standard preparation of crude extract saturates the endonucleolytic cleavage sites produced by 44.0 units of purified endonuclease in a 15-min incubation at 37 C.

The crude extract of $T4V_1$-infected cells requires magnesium ion at about 10^{-3} M and is stimulated by the presence of 0.05 M KCl or NaCl. *E. coli* tRNA is included to inhibit nonspecific endonuclease I activity (8). Figure 2 demonstrates the linear dependence of the assay on the amount of endonuclease-containing fraction added. Assays were linear over a period of 20 min at 37 C.

Purification and properties. The T4-induced UV-endonuclease was purified about 800-fold (Table 1). Analytical disc gel electrophoresis in polyacrylamide gel of concentrated preparations of fraction 5 reveals a single identifiable band with an R_m of 0.43 relative to the migration of bromphenol blue. Fraction 5 does not result in the formation of detectable acid-soluble nucleotide product when incubated with either native or UV-irradiated T4 DNA and is judged as being free of contaminating exonucleases. This fraction does not cause endonucleolytic cleavage of native T4 DNA but does degrade UV-irradiated DNA (Fig. 3). UV-irradiated *E. coli* DNA and T7 DNA are also degraded endonucleolytically by the enzyme.

Fractions 3 to 5 are unstable when frozen and are stored at 3 C in the presence of either 3% polyethylene glycol or DNA (20 μg/ml). About 80% of the activity is lost by heating the enzyme to 45 C for 10 min.

The enzyme has a broad *p*H optimum between 7.0 and 8.0. Activity is not inhibited by addition of *p*-chloromercuribenzoate at 10^{-4} M, *E. coli* tRNA at 100 μg/ml, caffeine at 10 mg/ml, or ethylenediaminetetraacetic acid at 10^{-3} M. There is no requirement for added divalent cation. However, the addition of either $MgCl_2$ or $MnCl_2$ results in approximately 20% stimulation at an optimal concentration of 5.0×10^{-3} M. $CaCl_2$ at this concentration causes about 15% inhibition. Monovalent cations do not affect the activity of the endonuclease. Some inhibition of endonuclease activity is observed when the reaction is carried out in the presence of actinomycin D at 10^{-3} M. A similar result was obtained by Reiter et al. (13) by using crude extracts of *B. subtilis*. Since actinomycin D binds specifically to DNA, it is likely that the observed inhibition is the result of the blocking of substrate sites by the antibiotic.

The endonuclease is induced early after infection of sensitive cells with bacteriophage T4. The activity, initially detected in crude extracts 2 min postinfection, continues to increase for at least 20 min after infection (Fig. 4).

Sedimentation in 5 to 20% sucrose at *p*H 8.0

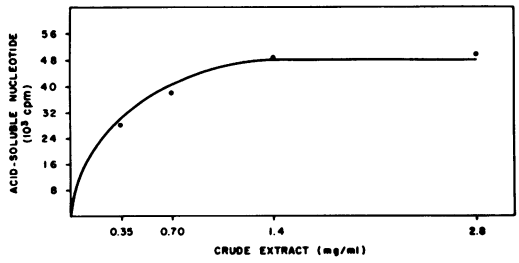

FIG. 1. *Kinetics of saturation of endonuclease assay by crude extract of cells infected with T4V$_1$ phage. Crude extract was prepared at a concentration of 7.0 mg/ml. Endonuclease (44.7 units; fraction 5) was present in each tube. Substrate was ^{3}H-labeled T4 DNA (specific radioactivity = 4,300 counts per min per nmole) irradiated at 10^5 ergs/mm^2.*

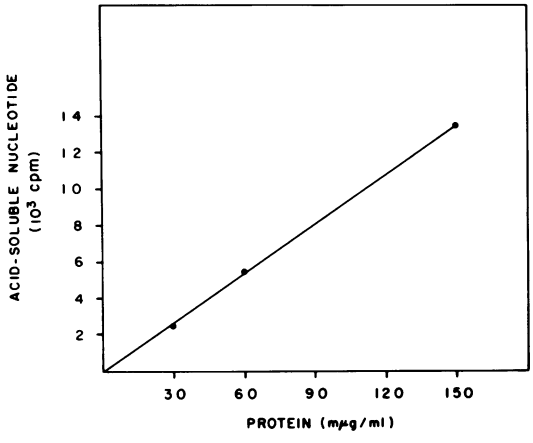

FIG. 2. *Release of acid-soluble nucleotide as a function of the concentration of added endonuclease. Endonuclease (2.5 to 12.5 units; fraction 5) was tested, using the standard assay procedure.*

reveals an *S* value of 3.0 when using *E. coli* alkaline phosphatase as a marker.

Dimer specificity of the endonuclease. An attempt has been made to demonstrate the pyrimidine dimer specificity of the endonuclease by two experiments. (i) A comparison was made of the relative rates of degradation of DNA samples irradiated with the same dose of UV light but containing different amounts of dimer. By irradiating DNA at 10^5 ergs/mm^2 in the presence and absence of proflavine (which blocks dimer formation; 16), DNA samples were prepared containing 0.5 and 6.5% thymine dimers, respectively. Although both samples were degraded at equal rates by extracts of uninfected cells, cells infected with wild-type phage degraded the DNA of higher dimer content at a faster rate (Fig. 5). The amount of acid-soluble nucleotide product formed during 10 min of incubation of crude extract from T4-infected cells with a series of DNA

TABLE 1. *Purification of T4 ultraviolet endonuclease*

Fraction no.	Step	Units	Specific activity	Purification
1	Crude	57,313	10.4	
2	Polyethyleneglycol-Dextran	40,418	50.6	4.8
3	Diethylaminoethyl cellulose	10,843	637.2	61.2
4	Phosphocellulose .	13,436	3,245.4	312.0
5	DNA cellulose ...	3,802	8,372.0	805.0

samples containing 0.5, 1.9, 3.9, and 5.4% dimers showed a linear relationship to the dimer content of the DNA. On the other hand, extracts of uninfected or T4V$_1$-infected cells resulted in the same rate of degradation irrespective of the dimer content of the DNA.

(ii) The observed relation between the kinetics of degradation of UV-irradiated DNA and the dimer content was examined in greater detail by using purified endonuclease and lightly irradiated DNA. T4 and T7 DNA were irradiated at doses between 20 and 350 ergs/mm^2 and incubated with enzyme under conditions that resulted in complete reaction; i.e., no further degradation of DNA was observed with the addition of more enzyme or with longer incubation. In both instances, the measured number of single-strand breaks showed a linear relationship to the UV dose (Fig. 6). Furthermore, the number of single-strand breaks observed at any given dose closely approximated the number of dimers calculated for that dose (Table 2).

Single- and double-strand breaks. Figure 7 shows the sedimentation profile of both T4 and T7 DNA treated with enzyme and sedimented in neutral and alkaline sucrose density gradients. It is clear that the endonuclease makes predominantly single-strand breaks in UV-irradiated DNA. However, in both substrates, some double-strand breakage is observed. The ratio of single to double strand breaks is approximately 20:1 for both T4 and T7 UV-irradiated DNA.

In vitro dimer excision. Incubation of UV-irradiated T4 DNA with the purified endonuclease did not result in a reduction of the dimer content of the acid-insoluble fraction of the DNA (Table 3, column 3). Incubation with crude extract of T4V$_1$-infected cells gave similar results (column 4). The use of a crude extract from cells infected with phage T4V$_1$ resulted in some nonspecific degradation of UV-irradiated DNA to form acid-soluble nucleotide. However, the ratio of thymine dimers to monomers in the acid-insoluble fraction remained constant under these conditions. On the other hand, preincubation with the endonuclease followed by the addition of

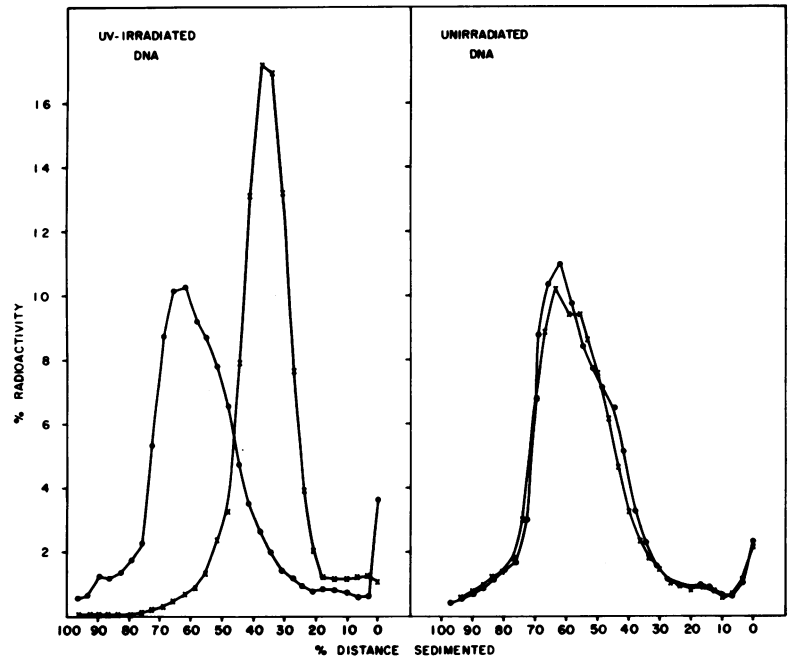

FIG. 3. *Effect of endonuclease on unirradiated and UV-irradiated T4 DNA. DNA was irradiated at 5,000 ergs/mm². Incubation mixtures (0.8 ml) contained 5 μg of T4 DNA, 50 mM Tris (pH 8.0), and 5.0 units of endonuclease (fraction 5). Incubation was at 37 C for 30 min. DNA was sedimented in 5 to 20% alkaline sucrose gradients. Symbols: ●, DNA without enzyme incubation; ×, DNA after enzyme incubation.*

endonuclease-deficient crude extract did result in a significant reduction of the dimer content of the acid-insoluble fraction of DNA (column 5).

DISCUSSION

It was demonstrated both in vivo (15) and in vitro (14) that cells infected with wild-type phage T4 can excise pyrimidine dimers from phage DNA. On the other hand, phage T4V$_1$, which is mutated in the V gene, and phage T2, which does

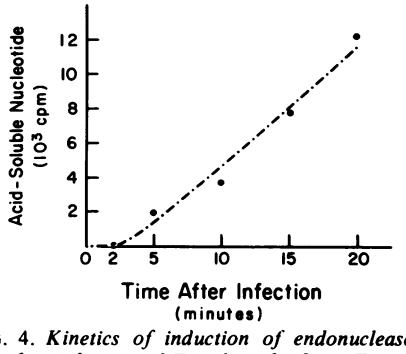

FIG. 4. *Kinetics of induction of endonuclease activity after infection of E. coli with phage T4. E. coli (1.5 liters) was grown to exponential phase and infected with phage T4 at a multiplicity of infection of 3. At 0, 2, 5, 10, 15, and 20 min, 200-ml samples were removed, and chloramphenicol was added to a final concentration of 100 μg/ml. Cultures were cooled in liquid nitrogen, and the cells were harvested and sonically disrupted in 50 mM Tris (pH 8.0). A 0.1-ml amount of each extract (0.5 mg of protein) was assayed by using the standard procedure.*

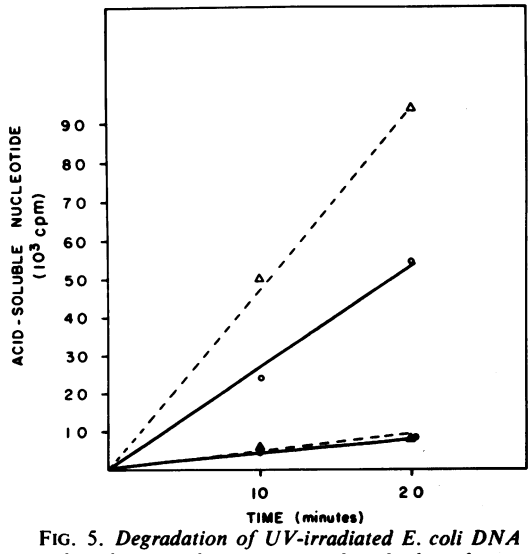

FIG. 5. *Degradation of UV-irradiated E. coli DNA samples of various dimer contents. Standard incubation mixtures contained 0.7 mg of crude extract of uninfected or T4-infected cells. Symbols: (Δ) T4-infected extract, 6.5% dimers; (○) T4-infected extract, 0.5% dimers; (▲) uninfected extract, 6.5% dimers; (●) uninfected extract, 0.5% dimers.*

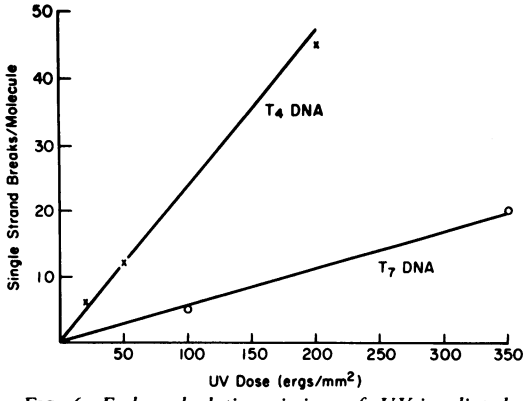

FIG. 6. *Endonucleolytic scission of UV-irradiated T4 and T7 DNA as a function of UV dose. DNA (10 μg) was incubated with 15 units of endonuclease (fraction 5) for 15 min at 37 C. E. coli tRNA (100 μg) was added to inhibit any endonuclease I. After incubation, 0.05 ml containing 0.5 μg of DNA was layered on alkaline sucrose gradients and sedimented.*

TABLE 2. *Correlation between number of dimers and DNA nicks*

Source of DNA	Molecular weight	UV dose (ergs/mm²)	Measured no. of single-strand breaks	Calculated no. of dimers[a]
T4	130×10^6	20	6	5
T4	130×10^6	50	12	13
T4	130×10^6	200	45	50
T7	25×10^6	100	5	5
T7	25×10^6	350	20	18

[a] Our measurements of the dimer content of T4 DNA UV-irradiated at doses between 1,000 to 20,000 ergs/mm² yield a figure of 0.23 dimers per erg per mm². This correlates well with the data quoted by Witkin (19) that the *E. coli* genome (molecular weight = 3×10^9) contains approximately 6 dimers per erg per mm². The latter figure has been used to calculate the number of dimers quoted in the table.

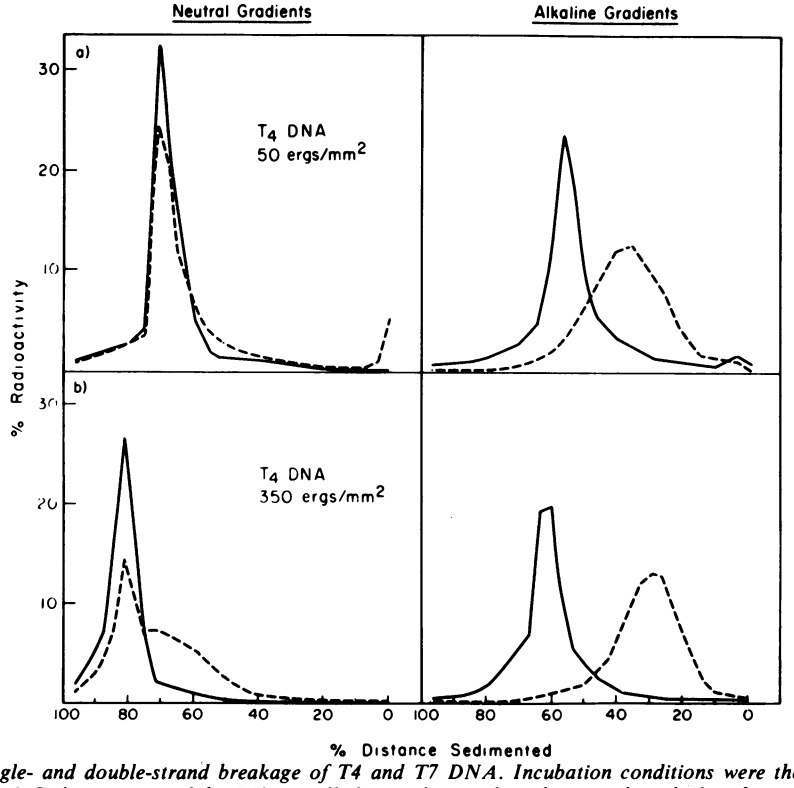

FIG. 7. *Single- and double-strand breakage of T4 and T7 DNA. Incubation conditions were the same as described in Fig. 6. Sedimentation of the DNA in alkaline and neutral gradients is described in the text. Continuous lines, no enzyme present; interrupted lines, enzymes added.*

not possess a V gene, are unable to effect dimer excision (14, 20). These studies strongly implicate the V gene as having a role in the dark repair of UV-irradiated DNA.

The present studies resulted in the purification of a phage-induced enzyme from T4-infected cells which is not detectable in extracts of cells infected with phage $T4V_1$. The enzyme degrades

TABLE 3. *Dimer excision from T4 DNA*[a]

Expt. no.	No enzyme addition	Incubation with endonuclease	Incubation with crude extract of $T4V_1$-infected cells	Preincubation with endonuclease plus addition of $T4V_1$ crude extract
1	3.9	3.8	—	3.1
	4.0	4.0	—	3.2
2	3.6	3.5	3.8	3.2
	—	3.7	3.9	3.0
3	3.4	3.2	3.4	2.8
	—	—	3.5	2.7
	—	—	—	2.7
Mean	3.5	3.6	3.6	3.0

[a] ^3H-labeled T^4 DNA was irradiated with 2×10^4 ergs/mm^2. Reactions in which endonuclease was used contained 40 units of fraction 5 of the enzyme, and incubations were at 37 C for 60 min. Where crude extract of $T4V_1$-infected cells was used, 100 μg of extract was added, and incubation was for 10 min at 37 C. Reactions were terminated by the addition of trichloroacetic acid (5% final concentration). The numbers quoted in the table refer to the percentage of thymine dimer in the acid-insoluble fraction of the DNA. An analysis of variance indicated that column 5 is highly significantly different from columns 2, 3, and 4 which do not differ significantly from each other.

UV-irradiated DNA endonucleolytically since it causes a reduction in the sedimentation velocity of the DNA without the formation of acid-soluble nucleotide. Degradation of UV-irradiated DNA occurs predominantly by single-strand breakage. The endonuclease is a relatively small protein with an *S* value of about 3.0 and does not require added divalent cation for activity. It, thus, very closely resembles the endonuclease isolated from *M. luteus* which appears to play a role in the dark repair of UV-irradiated damage to DNA in that organism (2, 7).

The endonuclease is not specific for DNA containing glucosyl groups and hydroxymethylcytosine since it degrades *E. coli* and T7 DNA in addition to T4 DNA. This observation supports the demonstration by Harm of V-gene reactivation of UV-irradiated mutants of *E. coli* defective in dark repair (5). Unirradiated DNA is not affected by the enzyme.

Varying the dimer content of DNA without altering the total dose of UV irradiation results in a corresponding variation in the rate of degradation of the DNA by the endonuclease. More significantly, over a series of different UV doses, all endonucleolytic phosphodiester scission observed can be quantitatively accounted for by dimers alone, in both T4 and T7 DNA. It is possible that UV-irradiated DNA contains other as yet unidentified photoproducts, the concentration of which are also affected by irradiation in the presence of proflavine. Nonetheless, the results of both experiments are consistent with the view that the endonuclease is specific for pyrimidine dimers in DNA. Patrick (12) demonstrated that the UV endonuclease from *M. luteus* is also apparently dimer specific. He showed that the kinetics of activity of the endonuclease are essentially identical between DNA irradiated in the presence of acetophenone (which contains only thymine dimers as photoproducts) and DNA irradiated directly.

Our observations that the T4-induced endonuclease is (i) defective in extracts of cells infected with a UV-sensitive mutant, (ii) degrades UV-irradiated and not native DNA, and (iii) is specific for dimers in UV-irradiated DNA suggest very strongly that it is indeed the endonuclease involved in the dark repair of UV radiation damage in T4-infected cells.

The genetics of dark repair in *E. coli* (6) and the results of in vitro experiments with purified proteins from *M. luteus* (7) suggest that pyrimidine dimer excision is a multienzyme process. The results of experiments shown in Table 3 demonstrate that the purified T4 endonuclease does not cause dimer excision in vitro. However, preincubation of UV-irradiated DNA with endonuclease, followed by the addition of crude extract of $T4V_1$-infected cells, reduces the dimer content of the acid-soluble fraction of the DNA. These results suggest that other as yet unidentified enzymes are required to complete dimer excision. The question as to whether these are phage-induced or are preexisting host enzymes is currently being studied.

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