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# Excision of Thymine Dimers In Vitro by Extracts of Bacteriophage-Infected *Escherichia coli*

E. C. FRIEDBERG, K. MINTON, G. PAWL, AND P. VERZOLA

*Laboratory of Experimental Oncology, Department of Pathology, Stanford University School of Medicine, Stanford, California 94305*

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Extracts of DNA polymerase I defective *Escherichia coli* infected with phage T4 contain an exonuclease activity that removes thymine dimers from UV-irradiated DNA previously nicked with T4 UV endonuclease. This activity is not expressed if cells are infected in the presence of chloramphenicol. The enzyme has a requirement for divalent cation and is not affected by caffeine, but excision is inhibited in the presence of proflavine. The enzyme is present in all phage T4 mutants thus far examined, including 25 UV-sensitive mutants isolated during the course of the experiments, all of which are defective in the *v* gene. A similar activity can be detected in cells infected with phages T2, T3, and T6, but not in cells infected with phage T7.

Bacteriophage T4 is unique among the coliphages studied thus far, in that it carries genetic information coding for DNA repair-specific enzymes (3). After infection of a sensitive host, bacteriophage T4 expresses an endonuclease coded for by the *v* gene (7, 23) that is specific for pyrimidine dimers and does not attack either unirradiated double-stranded DNA or DNA that has been treated with radiomimetic chemicals (5, 6, 22). The enzyme readily attacks UV-irradiated *Escherichia coli*, T7 and SV40 DNA in addition to T4 DNA (6, 8). Its role in excision repair in vivo has been clearly established, since mutants of T4 defective in the *v* gene are abnormally sensitive to UV radiation and are unable to excise pyrimidine dimers from their DNA (3, 18).

The mechanism of dimer excision in vivo after endonucleolytic incision of UV-irradiated T4 DNA has not been elucidated. *E. coli* DNA polymerase I has been shown to excise thymine dimers from UV-irradiated DNA randomly nicked with pancreatic DNase (12). These results have been confirmed with DNA specifically nicked with the T4 UV endonuclease (E. C. Friedberg and I. R. Lehman, manuscript in preparation). In addition, a host cell enzyme termed exonuclease VII of *E. coli* has been shown to excise thymine dimers in vitro from UV-irradiated DNA specifically nicked with the *Micrococcus luteus* UV endonuclease (J. W. Chase and C. C. Richardson, Fed. Proc., **32:** 619, 1973). It has also been reported that after infection with phage T4 a phage-coded nuclease activity is expressed that excises thymine dimers from UV-irradiated nicked T4 DNA (16; E. C. Friedberg et al., Biophys. Soc., p. 218a, 1973). The results presented in this paper demonstrate that this nuclease is expressed after infection of *E. coli* with a number of other phages in the T-series, even though these apparently do not code for a UV endonuclease activity. Evidence is presented that the T4-induced enzyme is distinct from previously described nuclease activities and the results of a search for mutants defective in this activity are described.

## MATERIALS AND METHODS

**Bacteriophage.** Table 1 indicates the phages used in these experiments. Phage stocks were prepared by infecting either *E. coli* H560 (*pol* A1, *endo* I), *E. coli* OK 2002 (*res*-1) DNA polymerase I defective, or *E. coli* CR63 ($SU^+$) (for amber mutants) at 37 C in brain heart infusion broth. Incubation was continued until satisfactory lysis was achieved. The lysates were centrifuged at 13,000 rpm for 90 min at 4 C by using a type 15 rotor in a Spinco model L3-50 ultracentrifuge. The resulting pellet was suspended in a solution containing 0.1 M NaCl, 1.0 mM $MgCl_2$, and 0.1 mM $CaCl_2$, and was centrifuged at 6,000 × *g* for 15 min at 4 C. The supernatant fraction containing the bacteriophage was recentrifuged at 27,000 × *g* for 60 to 120 min (depending on the phage type) at 4 C. The pellet was resuspended in 50 mM Tris-hydrochloride buffer, pH 8.0, and was stored over a small volume of chloroform. Phage titers were measured by the standard soft-agar overlay method.

**Preparation of cell-free extracts of uninfected and phage-infected cells.** Cells were grown in brain

TABLE 1. *Bacteriophage strains and mutants*

| Strain | Relevant genotype | Phenotype | Obtained from |
|---|---|---|---|
| T4D | Wild-type | | W. Harm |
| T4$v_1$ | *v* | UV sensitive. Defective in UV endonuclease. | W. Harm |
| T4*x* | *x* | UV sensitive. Recombination defective. | W. Harm |
| T4 del (39-56) | Deletion in genes 39-56 | Defective in exonuclease A. | |
| T4 46 *am* | Gene 46 amber mutation | Defective in degradation of host DNA. UV sensitive. | J. F. Koerner |
| T4 44 *am* | Gene 44 amber mutation | Defective DNA replication. | I. R. Lehman |
| T4 61 *am* | Gene 61 amber mutation | Defective DNA replication. | I. R. Lehman |
| T4 62 *am* | Gene 62 amber mutation | Defective DNA replication. | I. R. Lehman |
| T2 | Wild-type | | Miles Laboratories |
| T3 | Wild-type | | R. Davis |
| T6 | Wild-type | | D. Korn |
| T7 | Wild-type | | R. Davis |

heart infusion broth (Difco) to early log phase. Extracts of cells infected with phages T2, T4, and T6 were prepared by using either *E. coli* H560 or *E. coli* 2002. Extracts of cells infected with phages T3 and T7 were prepared by using *E. coli* 2002. Cells were infected with a multiplicity of approximately 5 phage/cell. Incubation at 37 C was continued with vigorous shaking for a further 15 to 18 min, at which time chloramphenicol was added at a final concentration of 150 μg/ml, and the cultures were cooled to 4 C in a dry-ice ethanol bath. In some experiments chloramphenicol was added to the culture at the same time as the phage.

Phage-infected cells were harvested by centrifugation at 6,000 × *g* for 15 min at 4 C. In the case of the T-odd phage-infected cells, it was necessary to carry out this procedure almost immediately after the chloramphenicol addition, otherwise the culture lysed. The pellets were washed once in 50 mM Tris-hydrochloride buffer, pH 8.0. Sonic treatment and the preparation of polyethylene glycol (PEG) fractions of phage-infected cells were carried out as described previously (8).

**T4 UV endonuclease.** The preparation and characterization of the T4-induced UV endonuclease is described previously (8).

**Other enzymes.** Purified exonuclease A of T4 was obtained as a gift from James F. Koerner of the University of Minnesota. The enzyme preparation contained 0.117 mg of protein per ml and had a specific activity of 35,555 units/mg. When assayed by the procedure of Oleson and Koerner (17) with $^3$H-labeled *E. coli* DNA (degraded to 5% acid-soluble nucleotide product with pancreatic DNase) as substrate, 208 units of exonuclease A produced 17.6% acid-soluble nucleotide in 20 min at 37 C.

**DNA substrates.** The preparation of $^3$H-labeled T4, *E. coli* and T7 DNA are described previously (8). UV-irradiated nicked DNA was prepared as follows. UV-irradiation of DNA was carried out using a 15-W GE low-pressure mercury-vapor ultraviolet germicidal lamp. UV fluence was measured with an International light germicidal photometer, model IL 254. Nicked DNA was prepared by incubating DNA UV-irradiated at doses between 1,500 to 3,000 ergs/mm$^2$, with purified T4 UV endonuclease. Reaction mixtures contained 1.5 μmol of *E. coli* DNA or 150 to 375 nmol of T7 DNA; EDTA, 10.0 mM; T4 UV endonuclease, 300 to 1,500 units. Incubation was at 37 C for 90 min at which time reactions were terminated by the addition of an equal volume of cold buffered-distilled phenol. The DNA was extracted with phenol twice and then dialyzed extensively against 50 mM Tris-hydrochloride buffer, pH 8.0. Evidence is presented in the section of Results, to indicate that in all cases about 90% of the thymine dimer sites were nicked by preincubation with T4 UV endonuclease.

**Thymine dimer excision.** Since excision of thymine dimers by extracts of phage-infected cells occurs with equal efficiency by using T4, T7, or *E. coli* DNA, all three were used as substrates in various experiments. Incubations (0.4 to 0.6 ml) contained nicked DNA, (60 to 95 nmol of *E. coli* DNA, or 6.75 nmol of T7 DNA, or 20 nmol of T4 DNA). The specific radioactivity of the DNA varied between 25,000 and 250,000 counts per min per μg, accounting for the variations in DNA concentrations used. Also included in the incubations were $MgCl_2$, 1.0 mM; *p*-chloromercuriphenylsulfonic acid (PCMPSA), 1.0 mM; Tris-hydrochloride buffer, pH 8.0, 50 mM, and the PEG fraction of phage-infected cell extract, 0.05 to 0.2 mg of protein. For testing excision with exonuclease A, 208 units of enzyme were used both with and without the inclusion of PCMPSA (1.0 mM) in the incubation. In some cases the control consisted of nicked UV-irradiated DNA without enzyme addition; in others the PEG fraction of extracts of uninfected DNA polymerase I defective cells was used.

After incubation at 37 C, reactions were terminated by the successive addition of 0.1% bovine serum albumin and 10% cold trichloroacetic acid. All incubations were carried out under yellow light to preclude photoreactivation. Separation of the acid-solu-

ble products from acid-precipitable DNA and measurement of the thymine dimer concentration in the latter fraction were carried out as described previously (9).

**Isolation of UV-sensitive mutants of phage T4.** Bacteriophage T4D was suspended in 50 mM acetate buffer, pH 4.5, and treated with 21 mM $NaNO_2$ for 40 min at 37 C. The phage were harvested by centrifugation at 32,000 × *g* for 90 min and resuspended in 50 mM Tris-hydrochloride, pH 8.0. Treated and untreated phage were titered on *E. coli* strain B. and a survival value of $4 \times 10^{-7}$ was determined for this treatment. *E. coli* $B_{s-1}$ in mid-log phase was infected with treated phage and incubation was continued until lysis had occurred. The phage were harvested by differential centrifugation and plated on agar plates with *E. coli* $B_{s-1}$ as the indicator. Individual plaques were picked with Pasteur pipettes and resuspended in 10.0 ml of 50 mM Tris-hydrochloride buffer, pH 8.0. Each cloned phage population was subjected to UV-irradiation at 180 ergs/mm² and plated out on *E. coli* $B_{s-1}$ in initial experiments, and on *E. coli* H560 (*pol* A1, *endo* I) or *E. coli* OK 2004 (*res*-1) in later experiments. An identical sample was plated without irradiation and mutants showing UV sensitivity were selected. Each mutant so isolated was carefully retested, and complete UV survival curves were performed on each. All procedures with UV-irradiated phage were carried out under yellow light to prevent photoreactivation. UV-sensitive mutants were grown in large quantities and used to infect either *E. coli* H560 or OK 2004 for the production of PEG fractions of phage-infected cells. All extracts were assayed for both T4 UV endonuclease as previously described (8) and for dimer excising activity as described above.

## RESULTS

Experiment 1 of Table 2 demonstrates control conditions under which no significant excision of dimers from UV-irradiated DNA is observed. These include the use of nicked UV-irradiated DNA alone, DNA treated with endonuclease plus extracts of uninfected *E. coli* defective in DNA polymerase I activity, and unnicked DNA treated with an extract of DNA polymerase I defective cells infected with a T4 mutant defective in UV endonuclease activity. In contrast, the results of experiment 2 of Table 2 show that

TABLE 2. *Excision of thymine dimers by extracts of T4-infected cells*

| Expt[a] | Substrate | Source of extract | Source of DNA | Thymine dimer content of acid-insoluble fraction of DNA |
|---|---|---|---|---|
| 1 ... | UV-irradiated DNA (not nicked). | | *E. coli* | 100.0 |
| 1 ... | UV-irradiated DNA (not nicked). | T4$v_1$-infected *E. coli* H560 (*pol* A1) | *E. coli* | 100.0 |
| 1 ... | UV-irradiated DNA nicked with T4 UV endonuclease. | | *E. coli* | 97.3 |
| 1 ... | UV-irradiated DNA nicked with T4 UV endonuclease. | *E. coli* H560 (*pol* A1) | *E. coli* | 93.6 |
| 2 ... | UV-irradiated DNA nicked with T4 UV endonuclease. | T4$v_1$-infected *E. coli* H560 (*pol* A1) | *E. coli* | 10.7 |
| 2 ... | UV-irradiated DNA nicked with T4 UV endonuclease. | T4-infected *E. coli* H560 (*pol* A1) | *E. coli* | 20.0 |
| 2 ... | UV-irradiated DNA nicked with T4 UV endonuclease. | T4-infected *E. coli* H560 (*pol* A1). Chloramphamicol addition at time of infection. | *E. coli* | 103.2 |
| 3 ... | UV-irradiated DNA nicked with T4 UV endonuclease. | T4$v_1$-infected *E. coli* H560 (*pol* A1) | T4 | 25.4 |
| 3 ... | UV-irradiated DNA nicked with T4 UV endonuclease. | T4-infected *E. coli* OK 2002 (*res*-1) | T7 | 17.2 |

[a] $^3$H-labeled *E. coli* DNA (60 nmol), UV-irradiated at 1,500 ergs/mm², was used in experiments 1 and 2. In experiment 3, T4 DNA (27 nmol) and T7 DNA (6.75 nmol) were irradiated at 1,500 and 3,000 ergs/mm², respectively. At these fluences of UV-irradiation 0.6% and 1.0%, respectively, of the total radioactivity of the DNA is in thymine dimers. The DNA used in experiments 2 and 3 had been previously nicked with T4 UV endonuclease as described in the text. Incubations with cell-free extract were carried out for 45 min at 37 C in thick-walled hydrolysis tubes. The concentration of $MgCl_2$, PCMPSA, and Tris-hydrochloride buffer are included in the text. The amounts of protein added were as follows: T4$v_1$-infected *E. coli* H560, 150 μg; T4-infected *E. coli*, 116 μg; *E. coli* H560, 264 μg; T4-infected *E. coli* OK 2002, 163 μg. The final volume of the incubation was 0.4 ml. After incubation, 0.05 ml of 0.1% bovine serum albumin was added to all tubes, followed by 0.45 ml of 10% cold trichloroacetic acid. Tubes were centrifuged at 5,000 × *g* for 10 min and the acid-soluble and acid-precipitable fractions were separated as described in the text. Thymine dimer content was calculated as the fraction of the total radioactivity in the thymine monomer fraction. This value was normalized to 100% in the experiment in which the incubation mixture contained unnicked UV-irradiated DNA. All other data are expressed relative to this value.

if the UV-irradiated DNA has been specifically nicked, extracts of either T4$v_1$ or T4-infected cells can remove a significant fraction of the thymine dimers from *E. coli* DNA. The results of the particular experiment shown in the table indicate that under optimal conditions about 91% of the thymine dimers are excised by an extract of T4$v_1$-infected cells. Since this extract is shown in experiment 1 of the table to be incapable of dimer excision unless the UV-irradiated DNA is previously nicked with T4 UV endonuclease, these data indicate that at least 90% of the thymine dimer sites in UV-irradiated DNA are accessible for excision after incubation with T4 UV endonuclease. This does not necessarily imply that 90% of thymine dimer sites were nicked since clustering of dimers may facilitate excision of more than one dimer by degradation at a single nicked site. We have prepared extracts of T4-infected cells on approximately 20 different occasions and have found that such extracts excise from 50 to 95% of thymine dimers from either *E. coli*, T4, or T7 DNA. The reasons for this variation are not clear, but may reflect differences in the specific activity of the dimer excision enzyme(s) in various preparations. Under the conditions used in these experiments, particularly the use of PEG fractions and the inclusion of 1.0 mM PCMPSA in the incubation, a significant degree of nonspecific DNA degradation is eliminated, and excision of 50 to 90% of the thymine dimers by extracts of phage-infected cells is accompanied by the degradation of only 2 to 5% of T4 DNA to acid-soluble nucleotide.

Experiments have been carried out in which the acid-soluble nucleotide containing excised thymine dimers was exhaustively incubated with bacterial alkaline phosphatase and spleen phosphodiesterase. The limit products of oligonucleotide digestion by these two enzymes are nucleosides and inorganic phosphate. The former have a very low binding affinity for DEAE cellulose. Oligonucleotides containing pyrimidine dimers, however, are resistant to degradation by spleen phosphodiesterase, and bind strongly to DEAE cellulose. The details of these experiments will be reported elsewhere (G. Pawl and E. C. Friedberg, manuscript in preparation), but the results show that thymine dimers are excised as oligonucleotides which average eight nucleotides in length in the acid-soluble fraction.

Table 3 shows that the phage-induced excision activity is detected after infection with a number of other T phages, T7 being a unique exception. Also shown in Table 3 are the results of studies with a series of mutants of T4 isolated by others. Mutations in genes 46, 47, x, and 43 have been reported to be associated with increased UV sensitivity of phage T4 (1, 3). The deletion in genes 39 to 56 includes the gene for exonuclease A of T4 (21); thus the excision activity is clearly distinct from exonuclease A. Furthermore, nicked UV-irradiated T4 DNA was incubated with partially purified exonuclease A, but no loss of dimers from the acid-insoluble fraction was observed. Phages mutant in genes 44, 61, and 62, all of which are required for normal DNA replication in T4, were found to code for active excision nuclease activity.

TABLE 3. *Excision of thymine dimers by extracts of cells infected by other phages*

| Extract added to nicked UV-irradiated DNA[a] | Thymine dimer content of acid-insoluble fraction of DNA |
|---|---|
| T2-infected *E. coli* H560 (*pol* A1) | 17.9 |
| T3-infected *E. coli* OK 2002 (*res*-1) | 44.0 |
| T6-infected *E. coli* OK 2002 (*res*-1) | 39.0 |
| T7-infected *E. coli* OK 2002 (*res*-1) | 102.3 |
| T4 *am* gene 46-infected *E. coli* H560 | 15.1 |
| T4 *am* gene 47-infected *E. coli* H560 | 30.2 |
| T4 *x*-infected *E. coli* H560 | 26.4 |
| T4 *am* 43-infected *E. coli* OK 2002 | 25.2 |
| T4 del (39-56)-infected *E. coli* H560 | 17.9 |

[a] The conditions of the incubation of nicked UV-irradiated DNA with extracts of phage-infected cells are identical to those described in the footnote to Table 2. UV-irradiated (1,500 ergs/mm$^2$) T4 DNA nicked with T4 UV endonuclease was used in all experiments except those with T3-infected *E. coli* OK 2002, T6-infected *E. coli* OK 2002, and T7-infected *E. coli* 2002, for which UV-irradiated T7 DNA (3,000 ergs/mm$^2$ was used). The volume of the incubation mixture in all instances was 0.4 ml and contained between 176 and 280 μg of protein. Details of the growth of phage and preparation of cell-free extracts of phage-infected cells are included in the text.

We have isolated a total of 25 UV-sensitive mutants from a population of phage treated with nitrous acid, by using either wild-type or DNA polymerase I defective hosts. UV survival curves were determined for all the mutants and were found to be identical within expected experimental error. The slope of this curve is 2.1 relative to that of T4D, the parental strain. This survival might be expected of mutants defective in either the *v*-gene (3) or in the gene coding for the excision enzyme. All extracts of *E. coli* H560 infected with these mutants were found to be defective in the UV endonuclease, the product of the *v* gene. No double mutants of the phenotype (endonuclease$^-$, excision nuclease$^-$) were detected.

As shown in Table 4, the excision activity expressed in T4-infected cells is not inhibited by caffeine at concentrations as high as 0.024 M

but is inhibited by proflavine at concentrations of $5 \times 10^{-5}$ M or more.

The kinetics of dimer excision from UV-irradiated *E. coli* DNA by extracts of T4-infected *E. coli* H560 (*pol* A1, *endo* I) are shown in Fig. 1. There is an initial rapid rate of excision that decreases during longer incubation times. By 30 min nearly all thymine dimers have been removed from the DNA. All of the experiments quoted in Tables 2 to 4 were done by using incubation times of 45 min and do not represent initial rates of excision.

## DISCUSSION

Based on the known in vitro properties of a number of nucleases, it is clear that after endonucleolytic incision of UV-irradiated T4 DNA in vivo, dimers may conceivably be excised by more than one pathway. *E. coli* DNA polymerase possesses a 5′ → 3′ exonuclease which can excise dimers from UV-irradiated DNA nicked with the T4 UV endonuclease (E. C. Friedberg and I. R. Lehman, manuscript in preparation). In addition, Chase and Richardson have described a nuclease activity in *E. coli* that is distinct from DNA polymerase I, called exonuclease VIII (Fed. Proc., **38**: 619, 1973). This enzyme has properties very similar to the UV exonuclease of *M. luteus* (11, 13). Both activities degrade single-stranded UV-irradiated and unirradiated DNA at the same rate, from both the 3′ and 5′ ends, and can excise thymine dimers in the 5′ → 3′ direction from UV-irradiated DNA nicked with the *M. luteus* UV endonuclease.

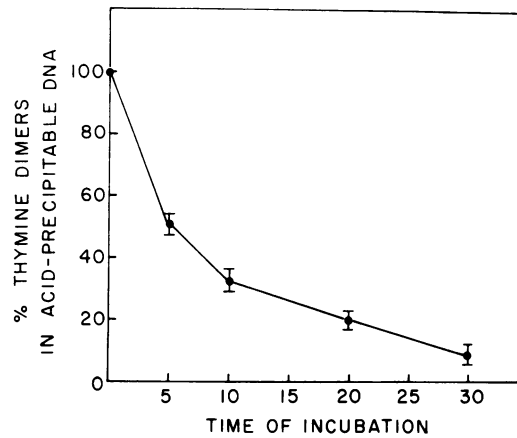


FIG. 1. *Kinetics of excision of thymine dimers by extracts of T4-infected cells. A PEG fraction of T4-infected E. coli H560 (pol A1, endo I⁻) was prepared as indicated in the text. Incubation mixtures (0.5 ml) contained E. coli DNA (60 nmol) UV-irradiated at 1,000 ergs/mm² and previously nicked with T4 UV endonuclease; $MgCl_2$, 1.0 mM; PCMPSA, 1.0 mM potassium phosphate, 20 mM; Tris-hydrochloride buffer, 50 mM, pH 8.0; and 53 μg of extract. Incubation was at 37 C for the times indicated at which point 0.5 ml of 10% cold trichloroacetic acid was added to each tube. The separation of acid-soluble and acid-precipitable DNA fractions and measurement of thymine dimers in the latter fraction was carried out as described previously (12).*

TABLE 4. *Effect of caffeine and proflavine on thymine dimer excision*

| Expt | Incubation conditions | Thymine dimer content of acid-insoluble DNA |
|---|---|---|
| 1 .... | Extract of T4-infected *E. coli* H560 (*pol* A1) | 26.6 |
| 1 .... | Extract plus caffeine (0.008 M) | 20.0 |
| 1 .... | Extract plus caffeine (0.024 M) | 26.6 |
| 2 .... | Extract plus proflavine ($10^{-6}$ M) | 29.7 |
| 2 .... | Extract plus proflavine ($10^{-5}$ M) | 29.7 |
| 2 .... | Extract plus proflavine ($5 \times 10^{-5}$ M) | 94.8 |
| 2 .... | Extract plus proflavine ($10^{-4}$ M) | 87.0 |

[a] The experimental conditions are essentially those described in the text and in the footnote to Table 2. For experiment 1, incubation mixtures (0.6 ml) contained nicked UV-irradiated (1,000 ergs/mm²) *E. coli* DNA, 60 nmol; $MgCl_2$, 1.0 mM; PCMPSA, 1.0 mM; Tris-hydrochloride buffer, 50 mM, caffeine (8 to 24 mM) and extract of T4-infected *E. coli* H560, 160 μg of protein. For experiment 2, the incubation volume was 0.5 ml and contained proflavine at the final concentration indicated, incubation was at 37 C for 45 min. The thymine dimer content of the acid-precipitable fraction of DNA was determined as described previously (12).

Under the conditions in which our extracts of uninfected cells are prepared and incubated, we do not observe any significant dimer excising activity in extracts of *pol* A1 or *res*-1 cells. However, after infection with phage T4, a new nuclease activity is expressed which, in the presence of the SH inhibitor PCMPSA, releases thymine dimers into the acid-soluble phase. Similar findings were previously obtained by Ohshima and Sekiguchi (16).

In the present studies we have shown that this activity represents a new nuclease expressed by phage T4. Since the *E. coli* mutants defective in DNA polymerase I activity have been shown to possess significant 5′ → 3′ exonuclease activity in vitro (14), we considered the possibility that T4 DNA polymerase (which does not possess 5′ → 3′ exonuclease activity) (10), may form some kind of functional association with the 5′ → 3′ exonuclease fragment of *E. coli* DNA polymerase I, thereby mimicking the expression of a phage-coded nuclease. The demonstration of excision nuclease activity in extracts of *E. coli* infected with a gene 43 phage mutant mitigates against this possibility. This dimer excising

activity is also expressed in extracts of cells infected with a mutant in the gene for exonuclease A (21), and partially purified exonuclease A did not release dimers into the acid-soluble phase during incubation with nicked UV-irradiated DNA. Finally, the activity is clearly distinct from the T4 UV endonuclease, since it is present in extracts of cells infected with UV endonuclease-defective mutants (see Table 2); moreover, the purified endonuclease does not excise thymine dimers from UV-irradiated DNA in vitro (8).

The substrate specificity of this enzyme has not been fully determined. The fractions possessing dimer excision activity also degrade unirradiated and UV-irradiated denatured DNA at the same rate, suggesting a strong resemblance to exonuclease VII of *E. coli* and the UV exonuclease of *M. luteus*. However, until the enzyme is purified, it is not possible to be sure that these catalytic activities are a function of a single enzyme. The extracts excise dimers as oligonucleotides, which, when harvested in the acid-soluble fraction, average eight nucleotides in length. Calculations based on the number of thymine dimers excised and the fraction of T4 DNA rendered acid-soluble indicate that between 10 to 20 nucleotides are removed for every thymine dimer excised. Despite this mode of degradation of nicked double-stranded DNA, the requirement for free ends in the DNA characterizes this activity as an exonuclease. Since the $3' \rightarrow 5'$ exonuclease function of T4 DNA polymerase (10) and exonuclease A (20) are the only two other phage-coded exonucleases thus far identified, we tentatively designate this activity as exonuclease B of T4.

A complete characterization of this enzyme is in progress and will be presented at a later date. In the meantime we wish to note that the activity is stable to freezing in crude extract and demonstrates an absolute requirement for $MgCl_2$. In crude extracts the activity is not inhibited by caffeine, a known inhibitor of dimer excision repair in bacteria (15), but is inhibited by proflavine. It is not known from the present studies whether the proflavine acts by direct inhibition of the enzyme or by altering the substrate. The kinetics of dimer excision indicate that an initial rapid rate slows significantly with continued incubation. This observation may reflect the clustering of dimers in pyrimidine-rich areas reported by others; (2, 19) i.e., with a nonrandom distribution of dimers, multiple dimers existing in a single cluster might be excised as rapidly as a single dimer in another portion of the DNA molecule.

These studies demonstrate that, although bacteriophage T4 is the only member of the T series that codes for a pyrimidine dimer-specific endonuclease, phages T2, T3, and T6 do code for a UV exonuclease activity. Studies by others (4) have shown that an exonuclease that initiates hydrolysis specifically at the 5'-ends of either single- or double-stranded DNA is expressed after infection with phage T5. This enzyme has been shown to degrade UV-irradiated DNA as efficiently as unirradiated DNA. It is possible that the enzymes expressed by different phages are not identical; meaningful comparisons will have to await purification of the individual enzymes.

The activity is present in all of the T4 phage mutants we have thus far studied, and we have not been successful in isolating a phage mutant of the desired type by nitrous acid mutagenesis. The observation that none of the 25 UV-sensitive mutants isolated in our laboratory is defective in the ability to excise dimers from nicked, UV-irradiated DNA, coupled with the observation that the activity is expressed by other T phages, raises the possibility that we are dealing with an enzyme required for some vital function in DNA metabolism. In this respect it is probable that the exonuclease itself has no specificity for pyrimidine dimers. The specificity for excision repair is provided by the UV endonuclease, which recognizes dimers in DNA and creates incisions in very close proximity to them. Since the exonuclease can initiate DNA degradation at free ends in duplex DNA and can remove oligonucleotide sequences, dimers are excised with a minimum of general DNA degradation. Thus it is possible that this exonuclease subserves other functions in DNA metabolism in vivo, some of which may be vital for phage DNA replication. We have not initiated a search for conditional lethal mutants defective in exonuclease B activity. Alternatively, it is possible that more than a single excision pathway exists in T4-infected cells in vivo.

In conclusion, the demonstration of the expression of both a UV endonuclease and an exonuclease activity in T4-infected cells provides a satisfactory biochemical explanation of a mechanism of dimer excision repair in this system in vivo. Further studies are aimed at purifying and characterizing the UV exonuclease activity and attempting the complete repair of UV-irradiated T4 DNA in vitro.

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