Two enzyme activities which release nucleotides preferentially from the 5'-termini of DNA were found in T4-infected Escherichia coli. Since no corresponding activities were found in uninfected cells, the activities appear to be induced by T4. Both activities are capable of excising pyrimidine dimers from ultraviolet-irradiated DNA which has been treated with T4 endonuclease V.

One of the activities, referred to as T4 exonuclease B, was purified 400-fold from an extract of T4UV-infected cells. The enzyme initiates hydrolysis of DNA specifically at the 5'-termini to yield products which are mainly oligonucleotides of varying length. The hydrolysis reaction proceeds in a limited manner. The enzyme shows optimal activity at pH 7.0 and absolutely requires Mg2+. The molecular weight of the enzyme, as estimated by gel filtration, is approximately 35,000.

Another activity, referred to as T4 exonuclease C, was purified 240-fold from the extract. This activity also excises pyrimidine dimers from ultraviolet-irradiated, incised DNA and releases nucleotides at 5'-termini. It has a pH optimum at 7.5 and requires Mg2+. The molecular weight of the enzyme is approximately 20,000.

To characterize the enzyme reaction, we have purified the dimer-excising activity from T4-infected cells. In this paper we present evidence that the enzyme initiates hydrolysis of DNA specifically at the 5'-termini to yield products which are mainly mono- and oligonucleotides; thus, the enzyme is indeed a T4-induced 5'-3'-exonuclease. In addition, we found another exonuclease activity that is capable of excising dimers from irradiated DNA. Since the two activities represent hitherto unknown T4-induced exonuclease, we designate the enzymes T4 exonuclease B and C.

EXPERIMENTAL PROCEDURE

Materials

Enzymes—Escherichia coli alkaline phosphatase was purchased from Sigma Chemical Co. and further purified by DEAE-cellulose chromatography. Pancreatic deoxyribonuclease I was also obtained from Sigma. E. coli exonuclease III was purchased from Boehringer Mannheim. T4 DNA polymerase and polynucleotide kinase were prepared as described by Goulain et al. (11) and Richardson (12), respectively. T4 endonuclease V, prepared from T4-infected E. coli 1100 by a modification of the procedure previously described (2), was provided by Dr. S. Yasuda of this laboratory. The purification procedure, details of which will be published elsewhere, includes phase partition, column chromatography on CM-Sephadex C-25 and on hydroxylapatite, and final DNA-cellulose chromatography. The enzyme was purified about 3600-fold and is free of other endo- and exonucleases.

Nucleic Acids—Salmon sperm DNA was purchased from Sigma. T4 [14C]DNA (8 to 10 x 10^8 cpm per pmol) was prepared with [14C]hypoxanthine as previously described (13) except that E. coli P3478 was used as a host in place of E. coli B.

Short and Koerner (8) proposed that phage-induced exonucleases be designated by a capital letter in an alphabetical order approximating the order of discovery. Since the enzymes described here are the second and third T4-induced exonucleases which are not associated with other enzymes (e.g. polymerase-associated exonuclease), we adopt the names exonuclease B and C. Recently, Friedberg et al. (9) suggested that the dimer excision enzyme found by Ohshima and Sekiguchi (7) be named T4 exonuclease B. Since the dimer excision enzyme described previously corresponds to Peak b activity described here, this accords with the present proposal. On the other hand, designation by Roman numerals has been used for T4-induced exonucleases (10), as is the case with endo- and exonucleases of Escherichia coli.
a host instead of *E. coli* B8. Concentrations of DNA are expressed as micrograms of DNA.

**Bacteria and Bacteriophages—**E. coli 1100 (Su+, endo I-) was provided by Dr. T. Hoffman-Berling of the Max-Planck Institute für medizinische Forschung at Heidelberg. E. coli P3478 (polA-1, thy-) was furnished by Dr. J. Cairns of the Cold Spring Harbor Laboratory. Bacteriophage T4D and its UV-sensitive mutant, T4lul, were provided by Dr. W. Harn of the University of Delaware, and T4dnd100 (dexA) and amC218 (gene 43) were obtained from Dr. H. R. Warner of the University of Minnesota and Dr. R. S. Edgar of the California Institute of Technology, respectively.

**Radioisotopes—**Carrier-free [*3H]*orthophosphate was purchased from the Japan Radioisotope Association. [*14C]*Thymine was obtained from Daiichi Pure Chemicals Co., Ltd. [*3H]*dTTP and four types of unlabeled deoxyribonucleoside triphosphates were obtained from Radiochemical Centre (Amersham, England) and from Schwarz BioResearch, Inc., respectively.

**Other Materials—**DEAE-Sepharose A-25 and Sephadex G-100 were purchased from Pharmacia Fine Chemicals. Dowex 1-X8 was obtained from Dow Chemical Co.

**Methods**

**Preparation of UV-irradiated, Incised T4 [*14C]*DNA—**T4 [*14C]*DNA 

Preparation of [*5'-*2P*]DNA—Sonomically treated T4 DNA was dephosphorylated with alkaline phosphatase and then incubated with polynucleotide kinase and [*5'-*3P*]ATP to label the 5'-termini with *3P* according to the procedure of Frenkel and Richardson (15).

**Preparation of T4 [*3'-*2P*]**—Sonomically treated T4 DNA was dephosphorylated with alkaline phosphatase and then incubated with polynucleotide kinase and [*5'-*3P*]ATP to label the 5'-termini with *3P* according to the procedure of Frenkel and Richardson (15).

**Assay of Activity to Release 5'-P (Assay B)—**The assay measures the conversion of acid-insoluble [*3P*] into acid-soluble form in 30 min at 37°C. A solution containing 0 to 0.6 M linear gradient of NaCl (total volume 240 ml) at a flow rate of 4 ml per hour. Fractions of 2 ml were collected, an aliquot (0.4 ml) was mixed with 10 ml of Kinard’s scintillation fluid (17), and the radioactivity was determined in a liquid scintillation spectrometer (Packard). The peak position was identified by referring to the pattern of absorbance at 260 nm.

**RESULTS**

**Induction of Two Dimer-excising Activities by T4**

It was previously reported that the dimer-excising activity increases about 5-fold after infection with T4, and the increase is inhibited by chloramphenicol (7). To characterize the activity we have analyzed enzyme fractions from normal and T4-infected cells by DEAE-cellulose chromatography, the details of which are described in the next section.

As shown in Fig. 1A, one distinct peak (Peak a) is found in normal cells. The dimer-excising activity is eluted at 0.17 M NaCl, together with DNA polymerase activity. Since the polymerase activity is relatively resistant to *N*-ethylmaleimide, it seems that the activity represents DNA polymerase I of *Escherichia coli*. Thus, Peak a may be ascribed, at least in part, to the 5'→3' exonuclease activity associated with DNA polymerase I. E. coli exonuclease VII, which can excise pyrimidine dimers, is eluted at 0.17 M KCl from DEAE-cellulose (19), and it might overlap with Peak a in our chromatogram.

In addition to Peak a, two distinct peaks appear in T4-infected cells (Fig. 1B). The first activity, designated Peak b, is eluted at 0.07 M NaCl and comprises about 40% of the total dimer-excising activity recovered from the DEAE-cellulose column. The second activity, designated Peak c, is eluted at 0.12 M NaCl and comprises about 30% of the total activity. The chromatogram also shows the existence of two DNA polymerase peaks, the one corresponding to *E. coli* DNA polymerase I and the other being identified as T4 DNA polymerase by its chromatographic position and greater sensitivity to *N*-ethylmaleimide. The T4 polymerase is eluted a little ahead of Peak c from the DEAE-cellulose column and is also separable from Peak b and c activities by phosphocellulose chromatography.
in an ultrasonic disintegrator for two repeats of a 3-min period. The paste (77 g) was stored at -20°C. The T4-induced exonuclease, A, has been shown to be eluted from DEAE-cellulose at higher than 0.25 M NaCl (8), suggesting that the Peak b and c enzymes differ from exonuclease A.

Until now only two exonuclease activities, 3'-\rightarrow 5' exonuclease associated with T4 DNA polymerase and T4 exonuclease A, both of which initiate hydrolysis at the 3'-termini, have been shown to be induced by infection with T4 (8, 11, 20). The notion that neither Peak b nor c is related to these enzymes was supported by experiments with mutants. Extracts of E. coli B infected with \( T4amC218 \) (defective in DNA polymerase) or E. coli 1100 infected with \( T4nd100 \) (defective in exonuclease A) contained about normal levels of dimer-excising activity (96% of the wild type for \( amC218 \) and 93% of the wild type for \( nd100 \)). Moreover, when an ammonium sulfate fraction derived from \( nd100 \)-infected cells was analyzed by DEAE-cellulose chromatography, three distinct peaks, corresponding to Peaks a, b, and c, were found (data not shown). From these results it seems that Peaks b and c represent new exonucleases induced by T4.

**Purification of Enzymes**

The results of a typical purification are summarized in Table I. Unless otherwise indicated, all operations were carried out at 0-4°C.

**Growth of Infected Cells**—E. coli 1100 was grown at 37°C under forced aeration in 20 liters of nutrient broth. At a cell density of \( 1 \times 10^9 \) per ml, T4\( \alpha \) phase was added at a multiplicity of five. Seventy minutes after infection the culture was chilled and the cells were harvested in a Kubota refrigerated continuous flow centrifuge at a flow rate of 0.4 liter per min. The paste (77 g) was stored at -20°C.

**Preparation of Extract**—Frozen cells (22 g) were suspended in 90 ml of 50 mM Tris-HCl (pH 7.5)/2 mM GSH/1 mM EDTA. Three ml-portions of cell suspension were sonically treated in an ultrasonic disintegrator for two repeats of a 3-min period of irradiation. After centrifugation for 20 min at 15,000 \( \times \) g, the supernatant fluid was taken as the extract (Fraction I).

**Phase Separation**—To 100 ml of Fraction I were added with stirring 11.6 ml of 20% (w/w) Dextran 500 and 32.2 ml of 30% (w/w) polyethylene glycol 6000 (recrystallized) (21). Then 33.3 g of NaCl were added with stirring over a 30-min period to give a final concentration of about 4 M, and stirring was continued for an additional hour. The mixture was then centrifuged at 1000 \( \times \) g for 10 min, and the upper phase was pooled. The lower phase was reextracted by stirring for 1 hour with another upper phase, which had been prepared in the same manner from 50 ml of 50 mM Tris-HCl (pH 7.5)/2 mM GSH/1 mM EDTA, 5.8 ml of 20% Dextran-500, 16.1 ml of 30% polyethylene glycol-6000, and 16.6 g of NaCl. After centrifugation at 2000 \( \times \) g for 10 min, the upper phase was taken and combined with the first upper phase. The combined fluid was then dialyzed for 16 hours against 3 liters of 10 mM Tris-HCl (pH 7.5)/5 mM 2-mercaptoethanol/1 mM EDTA (Buffer A) with two changes of buffer. The volume after dialysis was 294 ml (Fraction II), and it contained 86% of the activity and less than 5% of the UV-absorbing materials at 260 nm originally present in the extract.

**Ammonium Sulfate Fractionation**—To 290 ml of Fraction II were added 47.6 g of solid ammonium sulfate with stirring at 0°C during a 30-min period. After an additional 1-hour stirring period, the mixture was centrifuged at 2,500 \( \times \) g for 20 min, and a lower phase containing the activity was separated from the upper polyethylene glycol phase. To 265 ml of Fraction III were added 47.6 g of solid ammonium sulfate with stirring at 0°C during a 30-min period. After an additional hour, the precipitate was recovered by centrifugation at 15,000 \( \times \) g for 20 min and dissolved in 25 ml of Buffer A (Fraction III).

**DEAE-cellulose Chromatography**—A column of DEAE-cellulose (3 cm x 30 cm) was prepared and washed with 2 liters of Buffer A. Fraction III was dialyzed against 2 liters of Buffer A. After washing the column with 200 ml of Buffer A, the enzyme activity was eluted with a 700-ml linear gradient from 0 to 0.30 M NaCl in Buffer A. The flow rate was 30 ml per hour, and 20-ml fractions were collected. The dimer-excising activity of the effluent was confined to three distinct regions of the
chromatogram. As shown in Fig. 1B, the T4-induced activities, Peaks b and c, are eluted at about 0.07 M and 0.12 M NaCl, respectively, while the E. coli activity, Peak a, is eluted at 0.17 M. Active fractions (tubes 13 to 16 for Fraction IVb, tube 25 for Fraction IVc) were pooled. Approximately 37% of the activity applied to the column was recovered in Fraction IVb and IVc.

Phosphocellulose Chromatography of Peak b Enzyme—A column of phosphocellulose (1.0 cm x 15 cm) was prepared and washed with 1 liter of 20 mM potassium phosphate buffer (pH 7.5/0 mM 2-mercaptoethanol/1 mM EDTA (Buffer B). Forty milliliters of Fraction IVb were dialyzed against 3 liters of Buffer B for 6 hours and applied to the column. After washing the column with 30 ml of Buffer B, the enzyme activity was eluted with a 200-ml linear gradient from 0 to 0.8 M KCl in Buffer B and fractions of 5 ml were collected. The major portion of Peak b activity eluted at about 0.4 M KCl.

Since T4 endonuclease V induces a break to the 5'-side of a pyrimidine dimer, it was supposed that the dimer-excising enzyme hydrolyzes DNA in a 5' → 3' direction (2, 4, 7). Accordingly, the activity which releases 32P esterified at the 5'-termini of T4 DNA was determined with phosphocellulose columns. As shown in Fig. 2, the activity for release of 32P-32P (Assay B) coincides well with the activity for excision of dimers (Assay A). Assay B is simpler than Assay A and, thus, with purified preparations (beyond the phosphocellulose step), the activity was measured mostly by Assay B. With less purified preparations, however, Assay A was used since Assay B measures also phosphatases and other enzymes.

Fractions containing the major portion of the Peak b activity were pooled and concentrated by vacuum dialysis with a collodion bag (Sartorius) against 1 liter of 2-fold concentrated Buffer A containing 2 mM GSH. Concentrated solution (3 ml) was diluted twice with glycerol and stored at -20° (Fraction V). The fraction contained 49% of the enzyme activity originally applied to the phosphocellulose column.

Fraction V of Peak b enzyme was purified about 400-fold over the extract. In Fraction V, no DNA polymerase and 3' → 5' exonuclease activities were detected when the enzyme activities were measured using 0.9 μg and 0.2 μg of the protein, respectively. The fraction does not inactivate the infectivity of circular single-stranded DNA or double-stranded replicative form of φX174, indicating that it contains no detectable contamination with the polymerase. Combined fractions were concentrated as described above. The resulting preparation (Fraction V) contained 35% of the activity applied to the column. Fraction V of Peak b enzyme was purified 240-fold over the extract.

Comparison of Peak b and c Enzymes

Chromatographic Behavior—Peak b enzyme elutes from DEAE-cellulose and phosphocellulose columns at about 0.07 M NaCl and 0.40 M KCl, respectively. Peak c enzyme, on the other hand, elutes from these columns at about 0.12 M NaCl and 0.27 M KCl, respectively. Thus, Peak b enzyme is more positively charged at neutral pH than is Peak c enzyme.

Stability—Peak b enzyme (Fraction IV) has been stored for 3 months at 0° with only slight loss of activity (10%), whereas Peak c enzyme (Fraction IV) lost 80% of the activity after 2 weeks storage at 0°. However, when kept at -20° in Buffer A containing 50% glycerol, Peak b and c enzymes (Fraction V) exhibit 70% and more than 90%, respectively, of the original activity after 1 month of storage.

There is a considerable difference in the heat stability of the two enzymes. Peak b enzyme is more readily inactivated by incubation at 42° than is Peak c enzyme (Fig. 3).

Requirements for Reaction—Both enzymes require Mg2+ for their activity. The optimal concentrations for Peak b and Peak c were 10 mM and 20 mM, respectively. In the absence of Mg2+, there is no activity for Peak b enzyme and less than 10% of maximal activity for Peak c enzyme.

Sensitivity Against Sulfhydryl Blocking Reagent—Peak c enzyme appears resistant to N-ethylmaleimide, whereas Peak b enzyme is partially sensitive to the reagent (35% inhibition at 1 mM N-ethylmaleimide). The addition of 2-mercaptoethanol (10 mM) to the reaction mixture, however, resulted in only 10% stimulation for Peak b enzyme.

Estimation of Molecular Weights—In order to verify the dissimilarity of these enzymes more clearly, molecular weights of both enzymes were estimated by gel filtration studies. As

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1 S. Ohshima and M. Sekiguchi, unpublished observations.
shown in Fig. 4, Peak b and Peak c enzymes, without heat treatment, release 2.45 and 2.81 pmol of 5'-s2P, respectively. Relative activity is expressed as percentage of the activity without heat treatment. O—O, Peak b enzyme; O—O, Peak c enzyme.

FIG. 4 (right). Gel filtration of T4 exonuclease. Molecular weights were estimated by gel filtration on a column (1 x 60 cm) of Sephadex G-100. Approximately 100 units of Peak b or Peak c enzyme (Fraction V) were applied with marker proteins to the column. The column was run in 20 mM Tris-HCl (pH 7.5), 0.5 M NaCl, 5 mM 2-mercaptoethanol, and 1 mM EDTA. Fractions (1 ml) were collected at 1 ml per hour. 5'-Exonuclease activity was measured with T4 [5'-32P]DNA containing 3.0 pmol of 32P (7 x 10^5 cpm per pmol of 5'-P) and 50 μl of each fraction. Although separate experiments were carried out for Peak b and c enzymes, the results are presented in the same figure. O, Peak b enzyme; ☐, Peak c enzyme; △, cytochrome c; ●, chymotrypsinogen A; ■, bovine serum albumin (monomer).

shown in Fig. 4, Peak b and c enzymes elute from Sephadex G-100 in such a manner that, if both were spherical proteins, they would have molecular weights of 35,000 and 20,000, respectively. For both Peak b and c enzymes, the activity which releases 5'-P from DNA coincides well with the dimer-excision activity.

Mode of Action of Peak b Enzyme

Direction of Hydrolysis—Purified Peak b enzyme (Fraction V) was incubated with T4 [3'-3H]DNA or T4 [5'-3P]DNA for varying periods and the amount of acid-soluble radioactivity was determined. As shown in Fig. 5, an amount of Peak b enzyme sufficient to release a large amount of the radioactivity from the 5'-end of the molecule does not release any radioactivity from the 3'-end. Thus, Peak b enzyme initiates hydrolysis specifically at the 5'-termini of the DNA strands. Peak c enzyme also hydrolyzes DNA preferentially from the 5'-termini of the DNA strands. In addition, the enzyme released a considerable amount of 3'-H during the incubation. Although the Peak c enzyme preparation (Fraction V) contained no detectable DNA polymerase activity, there still remains the possibility that the preparation is contaminated with polymerase-associated exonuclease or other 3'→5'-exonuclease activity. Thus, the answer to the question whether Peak c enzyme possesses 5'→3'-exonuclease activity per se must await further purification to a homogeneous preparation.

Excision of Thymine-containing Dimers—When purified Peak b enzyme was incubated with UV-irradiated DNA, no detectable pyrimidine dimers were released. When, however, irradiated DNA is exposed to T4 endonuclease V before exonuclease reaction, pyrimidine dimers are preferentially released. Typical reaction kinetics of excision of thymine-containing dimers from UV-irradiated, incised [14C]DNA are illustrated in Fig. 6. After 60 min of incubation, 55% of the dimers originally present in the DNA become acid-soluble, while only 1.8% of the thymine is released from the DNA. Even though additional enzyme was added after 60 min of the incubation, no enhancement of degradation of DNA was observed.

This limited reaction was confirmed by a test for the substrate specificity of the enzyme. As shown in Table II, the purified Peak b enzyme catalyzes only little release of nucleotides, less than 2% of total, from various DNA substrates, even though a large proportion of dimers or 3P at the 5'-ends of T4 DNA molecules is liberated by the same amount of the enzyme. In addition, it appears that Peak b enzyme is able to hydrolyze both single- and double-stranded DNA, and the former is degraded about 2 times more rapidly than the latter. These results indicate that Peak b enzyme catalyzes hydrolysis of single- or double-stranded DNA from the 5'-termini in a limited manner. We can estimate that approximately 14 nucleotides are released for every pyrimidine dimer by Peak b enzyme.

Peak c enzyme, in contrast with Peak b, seems to catalyze a more progressive hydrolysis of DNA, still indicating a significant preference for release of pyrimidine dimers; 59% of the dimers are made acid-soluble while 27% of the thymine is released from the UV-irradiated, incised DNA.

Products of Reaction—Acid-soluble products resulting from hydrolysis of T4 [5'-3P]DNA by Peak b and c enzymes contained no detectable charcoal-nonadsorbable 32P. This indicates that both enzyme preparations possess no phosphomonoesterase activity.

The size of the products formed by the action of Peak b enzyme on UV-irradiated, incised T4 [14C]DNA was analyzed by DEAE-Sephadex A-25 chromatography (see "Methods"). The percentages of 14C in mono-, di-, tri-, tetra-, and larger oligonucleotides are 10, 26, 20, 13, and 32%, respectively. Thus, the maximum extent of release of dimers depends on the degree of treatment of irradiated DNA with T4 endonuclease V. With DNA preparations that have been treated exhaustively with T4 endonuclease V, more than 90% of dimers were released by incubation with Peak b enzyme.
**T4 5'-Exonucleases**

**Fig. 6.** Kinetics of dimer excision reaction by Peak b enzyme. Reaction was performed with 4.8 units of Peak b enzyme under the standard assay conditions using 5.2 nmol of UV-irradiated, incised T4 ["C]DNA containing 10.8 pmol of thymine dimers (33.7 cpm per pmol) as substrate. At the times indicated, samples were withdrawn and radioactivity of acid-soluble thymine and its dimers was determined (see “Methods”). At 60 min of incubation, additional Peak b enzyme (4.8 units) was added (arrows). O—O, thymine released; •—•, thymine-containing dimers released. Thymine (Δ) and dimers (▲) released after addition of fresh enzyme are shown by dotted lines.

The acid-soluble products formed by the action of Peak b enzyme are mainly small oligonucleotides of average size about three.

**Discussion**

Our results demonstrate the existence in T4-infected *Escherichia coli* of two enzymes that initiate hydrolysis at the 5'-end of a DNA strand. One enzyme, referred to as Peak b, is identical with the dimer excision enzyme previously reported (7). Although the acid-soluble products formed by the action of Peak b enzyme are oligonucleotides for the most part, the fact that a DNA terminus is required for the activity indicates that the mode of hydrolysis is exonucleolytic. No endonucleolytic activity against single- or double-stranded DNA has been detected in the enzyme preparation. Another enzyme, referred to as Peak c, is distinct from the Peak b enzyme in many respects, such as chromatographic behavior and physical and enzymic properties (see “Results”). Since a large proportion of the terminal regions of the substrate DNA is made acid-soluble by the action of Peak c enzyme, this enzyme acts also in an exonucleolytic manner.

There are two known exonucleases induced by T4, namely exonucleolytic activity associated with T4 DNA polymerase (11) and exonuclease A (8). The Peak b and c enzymes differ from these enzymes in the following respects.

1. Peak b and c enzymes are separable from T4 DNA polymerase and exonuclease A by ion exchange chromatography.
2. Peak b and c enzymes can initiate hydrolysis at the 5'-terminus of a DNA molecule, whereas the other exonucleases initiate hydrolysis only at the 3'-terminus of a DNA strand (8, 11).
3. Molecular weights of Peak b and c enzymes are approximately 35,000 and 20,000, respectively. These values are smaller than those of T4 DNA polymerase (112,000) (11) and exonuclease A (40,000) (8).
4. Peak b and c enzymes are found in cells infected with a T4 mutant defective in exonuclease A.

Since the Peak b enzyme hydrolyzes DNA exclusively in the 5' to 3' direction, it appears to be a T4-induced 5' → 3' exonuclease and may be called T4 exonuclease B. The enzyme resembles *E. coli* DNA polymerase I-associated 5' → 3'-exonuclease (exonuclease VII) (22, 23) in many respects except that the T4 enzyme catalyzes a limited hydrolysis of DNA. Since T4 DNA polymerase possesses no 5' → 3'-exonuclease activity (24), it is inferred that T4 exonuclease B functions to fulfill the roles which are played by the polymerase-associated 5' → 3' exonuclease in *E. coli*. It has been shown that a similar enzyme activity is induced by bacteriophage T5 (15).

For the Peak c enzyme, we propose that it be named T4 exonuclease C. The enzyme appears to be similar to exonuclease VII of *E. coli* (19) in some respects but is clearly distinct in other properties; exonuclease VII does not require Mg**2+** and exhibits a larger molecular weight (88,000) (19). However, more purification is necessary to characterize the enzyme.

T4 5' → 3'-exonuclease (exonuclease B) catalyzes the preferential release of pyrimidine dimers from UV-irradiated DNA that has been nicked at the 5'-side of dimers by T4 endonuclease V. Its limited manner of hydrolysis from the 5'-termini at the nicks may be advantageous to an efficient repair; it prevents an extensive degradation of DNA and may facilitate repair-replication. The molecular mechanism for the limited hydrolysis remains to be solved. T4 exonuclease C is also able to excise pyrimidine dimers from irradiated, incised DNA. Although direct evidence that these enzymes function in *vivo* has not yet been obtained, there is the possibility that either or both of the exonucleases are involved in the excision of dimers in T4-infected cells.

It is recalled that at least four enzymes of *E. coli*, 5' → 3'-exonucleases associated with DNA polymerase I and with DNA polymerase III, exonuclease VII, and the recBC enzyme (exonuclease V), are capable of exciting pyrimidine dimers

**Table II**

<table>
<thead>
<tr>
<th>DNA</th>
<th>Amount of substrate</th>
<th>Acid-soluble products pmol</th>
<th>% release</th>
</tr>
</thead>
<tbody>
<tr>
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<td>265</td>
<td>0.80</td>
</tr>
<tr>
<td>T4 [&quot;P]DNA, denatured</td>
<td>10,000</td>
<td>213</td>
<td>2.13</td>
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<tr>
<td>T4 [&quot;P]DNA, irradiated and incised</td>
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<td>70</td>
<td>0.70</td>
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<td>0.71</td>
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<tr>
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<tr>
<td>T4 [&quot;C]DNA, native</td>
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<td>1.0</td>
<td>36.7</td>
</tr>
</tbody>
</table>

* Amount of thymine-containing dimers.
* Amount of "P esterified at 5'-termini.
from irradiated, incised DNA (25–29). In *Micrococcus luteus*, UV-exonuclease and DNA polymerase-associated exonuclease catalyze excision of dimers in vitro (30, 31). In neither case, however, has a mutant which exhibits a decreased rate of dimer excision due to a defect of exonuclease been found.

There is evidence that 5′ → 3′-exonucleases may play important roles not only in excision repair but also in replication and recombination of DNA. The enzyme may be necessary to remove primer RNA from newly replicated DNA (32, 33), and it was recently shown that *E. coli* mutants defective in polymerase I-associated 5′ → 3′-exonuclease are conditionally lethal (34, 35). A bacteriophage T5 mutant defective in 5′ - 3′-exonuclease activity is unable to carry out normal DNA replication (36). Furthermore, it has been shown that 5′ → 3′-exonucleases induced by bacteriophage λ and T7 are essential for their genetic recombination (37–39).

To elucidate the roles of T4 exonucleases B and C in these processes, it is necessary to isolate mutants defective in either or both of the enzymes. This is also necessary to establish that the enzymes are coded by the T4 genome, excluding the possibility that the activities are due to an alteration of preexisting enzymes. Although attempts have been made in this and other studies (40), no mutant defective in either one of the enzyme activities has been found. The difficulty may be ascribed to the existence of more than one 5′ → 3′-exonuclease in T4-infected cells, and studies are currently undertaken to isolate such mutants by means of direct enzyme assay.

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