Bacteriophage T7 Endonuclease I

PROPERTIES OF THE ENZYME PURIFIED FROM T7 PHAGE-INFECTED ESCHERICHIA COLI B*

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SUMMARY

An endonuclease activity present in extracts from Escherichia coli B infected with T7+ phage has been purified 2500-fold. This enzyme, T7 endonuclease I, is the product of gene 3, and its activity is maximal 7.5 min after infection at 37°. The activity is at least 100 times greater with single stranded DNA as substrate than with duplex DNA. The limit product obtained by digestion of single stranded DNA with the endonuclease is about 50% acid soluble and consists of oligonucleotides with a chain length of about 10. The enzyme produces single strand and double strand breaks in duplex DNA, and the limit product obtained when double stranded DNA is digested with the endonuclease consists of fragments of duplex DNA whose strands are about 125 nucleotides long. T7 endonuclease I produces breaks with 5′-phosphate and 3′-hydroxyl termini. Although all four deoxynucleoside monophosphates are found at the 5′-terminus, the enzyme makes fragments with predominantly pyrimidine-containing nucleotides at their 5′-termini. The enzyme acts equally well on E. coli DNA and T4, T7, and λ phage DNA.

The T-even and T-odd bacteriophage have long been known to cause degradation of the host genome (1-10). After infection with T4 and T7 phages, the host DNA undergoes endonucleolytic cleavage to yield fragments of DNA with a molecular weight of about 10^6 (11-14). These fragments are then reduced directly to acid-soluble products.

The T4 induced enzymes endonuclease II and endonuclease IV have been implicated in the endonucleolytic breakdown of cellular DNA (15, 16). Indeed, T4 mutants have been described previously (14, 15). λt68, a mutant of λ defective in the S function necessary for lysis was obtained from Dr. H. Eisen, University of California Medical Center, San Francisco, California. The phage was lysogenized into E. coli CR 34 (thy-, B1- three-, leu-).

Media—GC medium has been described previously (15). GCA medium was the glycerol-casamino acids medium of Fraser and Jerrel (24). LBP medium contained 10 g of tryptone (Fisher), 5 g of yeast extract (General Biochemicals), 5 g of NaCl, and 0.025 M potassium phosphate buffer, pH 7.4, per liter.

Enzymes—Exonuclease I was purified from E. coli strain 1100, E. coli BBW/1, T7+ and T4+ phages, and the T7 amber mutants have been described previously (14, 15). λt68, a mutant of λ defective in the S function necessary for lysis was obtained from Dr. H. Eisen, University of California Medical Center, San Francisco, California. The phage was lysogenized into E. coli CR 34 (thy-, B1- three-, leu-).

The T7-induced enzymes III and IV have been implicated in the endonucleolytic breakdown of cellular DNA (15, 16). Indeed, T4 mutants have been isolated which are defective in the endonucleolytic breakdown of host DNA (17, 18). These mutants fail to induce normal amounts of T4 endonuclease II after infection.

Because of the extensive genetic and physiologic studies recently conducted with phage T7 (19, 20), there has been renewed interest in this system. Hausmann and Gomez (21) reported preliminary evidence for a T7-induced deoxyribonuclease involved in the breakdown of host DNA. Sadowski and Hurwitz (15, 16) also noted the presence of an endonuclease activity in crude extracts from T7-infected Escherichia coli cells. Center, Studier, and Richardson (22) have identified gene 3 as the structural gene for a T7-induced endonuclease involved in the breakdown of cellular DNA. Finally, Sadowski and Kerr (14) showed that gene 3 mutants of T7 failed to cause the endonucleolytic breakdown of the host genome.

In this paper, I report the purification and properties of the gene 3 endonuclease from T7-infected E. coli B. Because of the likelihood that additional T7-induced endonucleases will be discovered, I propose to call this enzyme T7 endonuclease I. Similar studies of this enzyme have recently been reported (23).

EXPERIMENTAL PROCEDURE

Materials

Bacterial and Phage Strains—E. coli B, E. coli 1100, E. coli BBW/1, T7+ and T4+ phages, and the T7 amber mutants have been described previously (14, 15). λt68, a mutant of λ defective in the S function necessary for lysis was obtained from Dr. H. Eisen, University of California Medical Center, San Francisco, California. The phage was lysogenized into E. coli CR 34 (thy-, B1- three-, leu-).

Media—GC medium has been described previously (15). GCA medium was the glycerol-casamino acids medium of Fraser and Jerrel (24). LBP medium contained 10 g of tryptone (Fisher), 5 g of yeast extract (General Biochemicals), 5 g of NaCl, and 0.025 M potassium phosphate buffer, pH 7.4, per liter.

Enzymes—Exonuclease I was purified from E. coli strain 1100, by the procedure of Lehman (25). Bacterial alkaline phosphatase (Worthington) was freed of contaminating nuclease activity by the procedure of Weiss, Live, and Richardson (26). Venom phosphodiesterase was obtained from Worthington and was dissolved in 0.05 M Tris buffer, pH 8.8, at a concentration of 5 mg per ml. Crystalline pancreatic RNase and DNase were obtained from Worthington. Polynucleotide kinase was purified from T4+ infected cells by the procedure of Richardson (27).

Radioactive Compounds—Thymine-2-14C (59 mCi per mmole) was from Amersham Searle, Toronto, Ontario. 32P-orthophosphate was from Atomic Energy of Canada, Ottawa, Ontario.

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‡ Scholar of the Medical Research Council of Canada.
**Methods**

**Preparation of Phage Stocks**—T<sub>7</sub><sup>+</sup> phage was grown on E. coli BBW/1. An overnight culture was diluted 50-fold into LBP medium and grown in a gyratory shaker at 37°. When the cell concentration reached 7 × 10<sup>8</sup> per ml, T<sub>7</sub><sup>+</sup> phage was added at a multiplicity of infection of 0.2. The culture was shaken vigorously until lysis occurred about 40 min later. Chloroform was added, and the culture was centrifuged at 16,000 × g for 10 min. Solid sodium chloride was added to give a concentration of 1.0 m.

T<sub>4</sub> phages were grown for 18 hours at 37°, harvested by centrifugation, and washed in 0.15 M NaCl. The wet weight of packed cells was 0.65 g. The cells were resuspended in 4.5 ml of standard saline citrate, and the reaction was incubated for 30 min at 38°. Acid-soluble radioactivity was measured as described previously (15). The unit of activity is the release of 1 pmole of acid-soluble radioactivity.

**Preparation of DNA**—Phage DNA was extracted with phenol as described previously (15). E. coli DNA labeled with <sup>32</sup>P was prepared by the following modification of Lehman's procedure (25). An overnight culture of E. coli B was diluted 100-fold into 200 ml of GC medium containing 2.5 mCi of <sup>32</sup>P-diphosphate (final specific activity, 34 μCi per μmole of phosphorus). The cells were grown for 18 hours at 37°, harvested by centrifugation, and washed in 0.15 m NaCl. The wet weight of packed cells was 0.65 g. The cells were resuspended in 4.5 ml of standard saline citrate (0.15 m NaCl-0.015 m sodium citrate), and 320 mg of solid sodium dodecyl sulfate was added. The mixture was stirred for 1 hour at room temperature. Two volumes of 95% ethanol were added, and the stringy precipitate obtained by centrifugation was extracted with 5 ml of 1.4 m NaCl containing 0.015 m sodium citrate by stirring in the cold room overnight. The precipitate obtained by centrifugation was re-extracted with 5 ml of 1.4 m NaCl-0.015 m sodium citrate. The two extracts were pooled, and the DNA was precipitated with 2 volumes of 95% ethanol. The precipitate was sedimented and dissolved in 5 ml of standard saline citrate by stirring 24 hours at 4°. The DNA was treated with 20 μg per ml of pancreatic RNase at 37° for 20 min and then extracted with an equal volume of phenol saturated with 0.05 m Tris buffer, pH 7.5, standard saline citrate, and 10<sup>-3</sup> m EDTA. After centrifugation the aqueous phase was re-extracted with an equal volume of phenol and the mixture centrifuged. Each phenol phase was washed sequentially with 0.5 volume of equilibrating buffer, and the aqueous phases were combined. They were extracted several times with ether to remove the phenol and then dialyzed against 0.01 m Tris buffer, pH 7.5, containing 0.05 m NaCl and 10<sup>-3</sup> m EDTA (two changes of 1 liter). The DNA was treated with 1 ml of a 20% Norit suspension, and the Norit was removed by centrifugation. The Norit treatment was repeated, and the supernatant was centrifuged for 15 min at 40,000 rpm in the IEC A270 rotor (International Equipment Company, Needham Heights, Massachusetts). This procedure yielded 5 ml of DNA with an optical density at 260 μm of 4.3 and a specific activity of 6800 cpm per nmole of nucleotide (as determined by liquid scintillation counting in Bray's solution). Unlabeled DNA was prepared in the same way.

**Growth of Phage-infected Cells**—LBP medium (100 liters) was inoculated with 3 liters of an overnight culture of E. coli B in a New Brunswick fermentor (New Brunswick Scientific, New Brunswick, New Jersey). The cells were grown with forced aeration (2 cu ft per min) at 30° until the cell density reached 1 to 2 × 10<sup>9</sup> per ml (optical density at 650 μm, 1.45). Wild type T<sub>7</sub> phage was added to a multiplicity of 5 to 10 and 10 min later cooling was begun with liquid nitrogen. After 10 min the temperature had reached 9°, and the cells were collected in a continuous flow centrifuge. The yield of cells was 325 g. Small scale infections were done in 2-liter flasks at 37°. Infection was terminated by pouring the culture over frozen 0.15 m NaCl.

**Assay of T<sub>7</sub> Endonuclease I**—The assay was identical with that used for the purification of T<sub>4</sub> endonuclease IV (16). <sup>14</sup>C-labeled fd DNA was exposed to the T<sub>7</sub> endonuclease for 10 min, and the reaction was terminated by heating. Exonuclease I was added, and the amount of acid-soluble radioactivity released after 30 min was measured. Since exonuclease I acts specifically at 3'-hydroxyl termini (28), the assay measures the number of 3'-hydroxyl termini produced by the action of the endonuclease. The reaction mixture (0.1 ml) contained 5 μmoles of Tris buffer, pH 8.4, 1.0 μmole of MgCl<sub>2</sub>, 0.1 μmole of 2-mercaptoethanol, 20 μmoles of E. coli tRNA, 7.5 μmoles of <sup>14</sup>C-labeled fd DNA (1000 to 2000 cpm per nmole), and 0.0003 to 0.003 unit of T<sub>7</sub> endonuclease I. After 10 min at 38°, 0.05 ml of 1.0 m sodium-glycine buffer, pH 9.2, was added, and the reaction was heated 3 min at 100° and cooled on ice. Exonuclease I (0.35 unit) was added, and the reaction was incubated for 30 min at 38°. Acid-soluble radioactivity was measured as described previously (15). The unit of activity is the release of 1 μmole of acid-soluble nucleotide after sequential treatment with endonuclease and exonuclease I. The activity was linearly related to enzyme concentration in the range of 0.0003 to 0.003 unit. Enzyme fractions were diluted in the endonuclease diluent used previously (15).

**Identification of 5'-Terminal Nucleotides Produced by action of T<sub>7</sub> Endonuclease I**—Generally the methods of Weiss et al. (26) were followed. Unlabeled fd DNA was first degraded by T<sub>7</sub>
endonuclease I in the reactions which contained per ml, 50 μmoles of Tris buffer, pH 8.4, 10 μmoles of MgCl₂, 250 μmoles of fd DNA and 0.15 to 0.75 unit of endonuclease. The reactions were incubated at 37°C for 30 min and terminated by heating to 65°C for 5 min. A blank was run simultaneously in which endonuclease was omitted. These levels of endonuclease released no detectable acid-soluble material.

Alkaline phosphatase, 6.25 units per ml was added, and the reactions were incubated at 37°C for 30 min. An additional 6.25 units of phosphatase per ml were added, and incubation was continued at 65°C for 30 min. The reactions were heated at 100°C for 10 min and cooled.

The dephosphorylated DNA was then phosphorylated with 5'-hydroxyl polynucleotide kinase in the presence of γ-32P-ATP. Each reaction mixture (0.6 ml) contained 0.3 ml of dephosphorylated fd DNA, 0.6 μmole of potassium phosphate buffer, pH 7.4, 20 μmoles of Tris buffer, pH 7.5, 3.0 μmoles of MgCl₂, 10 μmoles of 2-mercaptoethanol, 30 μmoles of γ-32P-ATP (1200 cpm per pmole), and 20 units of polynucleotide kinase. After 30 min at 37°C, an additional 20 units of polynucleotide kinase were added, and the incubation was continued for 30 min. The reaction was terminated by addition of 20 μmoles of sodium pyrophosphate, 0.75 mg of bovine serum albumin, and 0.1 ml of 100% trichloroacetic acid. After centrifugation, the supernatant was aspirated, and the pellet was dissolved in 0.2 ml of 0.2 M NH₄OH. Sodium pyrophosphate (20 μmoles), 2.0 ml of water, and 0.05 ml of 100% trichloroacetic acid were added. After centrifugation the pellet was dissolved in 0.2 M NH₄OH, and the washing procedure was repeated. The pellet was taken up in 0.2 ml of 0.02 M NH₄OH containing 30 μmoles of potassium phosphate buffer, pH 7.4.3 MgCl₂ (3 μmoles) and 50 μg of pancreatic DNase were added, and the mixture was incubated at 37°C for 60 min. Then 15 μmoles of sodium glycine buffer, pH 9.2, were added and degradation to 5'-mononucleotides was carried out by addition of 50 μg of snake venom phosphodiesterase and incubation for 60 min at 37°C. The reactions were heated 5 min at 100°C, cooled, and the precipitate was removed by centrifugation. The supernatants were concentrated by evaporation and the 5'-mononucleotides were separated by chromatography on Whatman No. 1 paper in the saturated ammonium sulfate-acetate buffer-isopropanol solvent of Markham and Smith (29). Mononucleotides were located by chromatography on Whatman No. 1 paper in the saturated ammonium phosphate buffer, pH 7.4. 2 MgCl₂ (3 mmoles) and 50 pg of pancreatic DNase, were added, and the incubation was continued at 37°C and infection was terminated at the times indicated by pouring the culture over frozen 0.15 M sodium chloride solution. Where indicated 100 μg per ml of chloramphenicol were added prior to addition of the phage and the infection was terminated 10 min later. Crude extracts were prepared and assayed using the standard assay.

Puri$cattin of T7 Endonuclease I from T7+ Infected Cells—A summary of the purification procedure is given in Table II.

### Table I

<table>
<thead>
<tr>
<th>Phage used</th>
<th>Gene</th>
<th>Activity (units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T7+</td>
<td>Wild type</td>
<td>0.4</td>
</tr>
<tr>
<td>am 64</td>
<td>2</td>
<td>5.0</td>
</tr>
<tr>
<td>am 20</td>
<td>4</td>
<td>5.2</td>
</tr>
<tr>
<td>am 28</td>
<td>5</td>
<td>4.5</td>
</tr>
<tr>
<td>am 147</td>
<td>6</td>
<td>7.3</td>
</tr>
<tr>
<td>am H 280</td>
<td>1</td>
<td>3.3</td>
</tr>
<tr>
<td>am 29</td>
<td>3</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Fig. 1. Appearance of T7 endonuclease I after T7+ phage infection. E. coli B at 1 x 10⁹ cells per ml was infected with T7+ phage at a multiplicity of infection of 10. The cultures were shaken at 37°C and infection was terminated at the times indicated by pouring the culture over frozen 0.15 M sodium chloride solution. Where indicated 100 μg per ml of chloramphenicol were added prior to addition of the phage and the infection was terminated 10 min later. Crude extracts were prepared and assayed using the standard assay.

Analysis of the limit product obtained following digestion of single stranded DNA by T7 endonuclease was done on DEAE-cellulose paper in 7 M urea as described by Laskey and Tener (33).
Fraction was dialyzed against two changes of 0.02 M potassium phosphate buffer, pH 7.5, and 10% glycerol. The DEAE fraction was dialyzed against two changes of 4 liters of equilibrating buffer, each change lasting 2 hours. The dialyzed DEAE fraction (32 ml) was applied to the column under gravity, and the column was eluted with a 200-ml linear gradient of 0.05 M to 0.5 M potassium phosphate buffer, pH 6.5, containing 10% glycerol. Fractions of 17 ml were collected, and the enzyme was eluted in fraction numbers 6 to 8. The recovery of the activity applied was 78% with an average purification of about 9-fold.

**Phosphocellulose Chromatography**—A P-11 column (1 x 11 cm) was equilibrated with 0.02 M potassium phosphate buffer, pH 6.5, containing 10% glycerol. The phosphocellulose fraction was dialyzed for 4 hours against 5 liters of 0.02 M potassium phosphate buffer, pH 6.5, containing 20% glycerol. The dialyzed fraction (40 ml) was applied to the column at a rate of 0.5 ml per min and was eluted with a linear gradient of 0.1 M to 1.0 M ammonium sulfate in equilibrating buffer. The total volume was 100 ml, and the flow rate was 0.5 ml per min. The peak of the enzyme activity was eluted in the fifth fraction. All of the activity applied to the column was recovered, and the purification in the peak fraction was about 3.5-fold. The phosphocellulose fraction represented a 1250-fold purification over the activity in the crude extract with an overall yield of 20%. It could be further purified on hydroxylapatite.

**Hydroxylapatite Chromatography**—A column of Hypatite-C (1 x 8 cm) was equilibrated with 0.02 M potassium phosphate buffer, pH 6.5, containing 10% glycerol. The hydroxylapatite fraction was dialyzed against 2 liters of the same buffer containing 20% glycerol for 3 hours and applied to the column. The elution was done with a linear gradient (70 ml in volume) of 0.1 M to 1.0 M ammonium sulfate in equilibrating buffer. Fractions of 3 ml were collected. The peak of enzyme activity was eluted in the fifth and sixth fractions. A 2-fold purification was obtained, and all of the activity applied was recovered.

**Comments on Purification Procedure**—All fractions were stable for limited periods of time when stored at 0°C (no detectable loss in activity in 1 week). However, the hydroxylapatite fraction lost considerable activity when stored under these conditions for greater than 1 week, i.e., approximately half of its activity was lost after 12 days at 0°C, and only 20% of the activity remained after 5 weeks. The phosphocellulose fraction retained 75% of its original activity after 6 weeks at 0°C. Both the phosphocellulose and hydroxylapatite fractions were stable if they were concentrated by dialysis against 50% glycerol containing 0.02 M potassium phosphate buffer, pH 6.5, and stored at 20°C. The experiments to be described were done with hydroxylapatite or phosphocellulose enzyme. These fractions did not differ detectably in their properties.

**Requirements of T7 Endonuclease I**—The requirements for endonuclease I activity are shown in Table III. Omission of MgCl₂ completely abolished the activity of the endonuclease. The optimal magnesium ion concentration was 0.01 M, and no activity was detectable when CoCl₂, MnCl₂, or CaCl₂ were substituted for MgCl₂. The enzyme had a broad pH optimum in the alkaline range from 8.1 to 8.8 (Tris-Cl buffer) and 9.2 to 9.5 (sodium glycine buffer). The activity at pH 7.5 (Tris-Cl

### Table II

**Purification of T7 endonuclease I from T7-infected cells**

| Fraction | Volume (ml) | Protein (mg/ml) | Total activity (units/ml) | Concentration of activity (units/mg) | Specific activity (units/mg) | Yield (%) | Purification-

| 1. Crude extract | 175 | 0.5 | 18,400 | 105 | 11,100 | 1 |
| 2. Protamine eluate | 150 | 0.9 | 12,900 | 86 | 96 | 70 | 8.7 |
| 3. Ammonium sulfate | 8 | 10.4 | 9,150 | 1,140 | 107 | 50 | 9.7 |
| 4. DEAE-cellulose (dialyzed) | 32 | 0.33 | 4,600 | 14 | 440 | 25 | 44 |
| 5. Carboxymethylcellulose | 31 | 0.018 | 3,570 | 70 | 3,580 | 19.5 | 352 |
| 6. Phosphocellulose | 10 | 0.027 | 3,700 | 370 | 13,700 | 20 | 1,245 |
| 7. Hydroxylapatite | 26 | <0.005 | 3,620 | 140 >28,000 | 20 | 2,500 |

* Values are corrected for the fact that only 60% of the phosphocellulose fraction was chromatographed on hydroxylapatite.
The complete reaction mixture had the same composition as used in the standard assay. Reactions were incubated with 0.0017 unit of phosphocellulose enzyme for 10 min at 37°. Acid-soluble material released by exonuclease I was determined.

Table III

**Requirements of T7 endonuclease I**

<table>
<thead>
<tr>
<th>Reaction mixture</th>
<th>Acid-soluble radioactivity (nmoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>1.7</td>
</tr>
<tr>
<td>2-Mercaptoethanol omitted</td>
<td>1.8</td>
</tr>
<tr>
<td>Mg++ omitted</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>tRNA omitted</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Fig. 2. a, inhibition of T7 endonuclease I by p-chloromercuribenzoate (PCMB). Mixtures had the same composition as the standard assay except 2-mercaptoethanol was omitted and varying concentrations of p-chloromercuribenzoate were added. The reactions were incubated with 0.0019 unit of endonuclease for 10 min at 37°. After termination of the reaction by heating, 2 nmole of 2-mercaptoethanol were added and the acid-soluble material released by exonuclease I was determined. b, inhibition of T7 endonuclease I by tRNA. The reactions had the usual composition except that the concentration of E. coli tRNA was varied from 0 to 1000 nmoles per ml. Activity was determined in the presence of 0.0016 unit of enzyme.

buffer) was 50% of that at the pH optimum. Omission of 2-mercaptoethanol from the reaction caused no inhibition of the activity whereas the activity was completely inhibited by 10-5 M p-chloromercuribenzoate (Fig. 2a). The enzyme was partially inhibited by E. coli tRNA (Fig. 2b). The concentrations of tRNA necessary to inhibit T7 endonuclease I were many times higher than those which inhibit endonuclease I of E. coli (26). For this reason 20 nmoles of tRNA were included in the routine assay to inhibit the E. coli enzyme.

**Action of Endonuclease on Native and Denatured DNA**—The T7 endonuclease I showed a strong preference for denatured DNA, showing at least a 100-fold greater activity on fd DNA than on native λ DNA. Assays of the various fractions during the purification procedure with fd and λ DNA showed that the ratio of activity on fd versus λ DNA remained relatively constant (Table IV), suggesting that the two activities are attributable to the same enzyme. Also the two activities showed similar rates of heat inactivation, i.e. after heating a phosphocellulose fraction for 20 min at 56° the enzyme retained 32% of its original activity on fd DNA and 35% of its original activity toward native λ DNA.

The T7 endonuclease I exhibited similar activities on all phage DNA's tested (T4, T7, λ, fd) whether native or denatured (Table V). As discussed below the enzyme also degrades E. coli DNA to the same extent as λ DNA.

**Nature of Product, Native DNA**—When native λ DNA was treated with T7 endonuclease I the presence of single strand breaks was detected before the appearance of double strand breaks. As seen in Fig. 3, about two breaks per strand of DNA were detectable on sedimentation of the product in an alkaline sucrose gradient (Fig. 3b) whereas the DNA sedimented at the same rate as intact DNA in a neutral sucrose gradient (Fig. 3a). Double strand breakage became evident in neutral sucrose (Fig. 3c) when about three breaks per strand had been introduced as detected by sedimentation in alkaline sucrose (Fig. 3d). With a higher level of enzyme, all of the molecules showed double strand breakage in neutral sucrose (Fig. 3c), and the strands had suffered about four breaks each as shown by alkaline sucrose gradient electrophoresis (Fig. 3d).
The size of the limit product obtained by the action in vitro of the enzyme is at least 10-fold smaller than the smallest size of degraded host DNA observed in vivo (14). The reason for this discrepancy is not known.

Finally, it should be noted that in contrast to its action on single stranded DNA (see below), the enzyme produces negligible amounts of acid-soluble material with native DNA as the substrate.

**Nature of Product, Single Stranded DNA**—As has already been noted, T7 endonuclease I has a marked preference for single stranded DNA. When either 14C-labeled fd DNA or heat-denatured 32P-labeled E. coli DNA was digested with an excess of the endonuclease about 50 to 60% of the radioactivity became acid soluble (Table VI). When this product was chromatographed on DEAE-cellulose paper in 7 M urea-0.2 M ammonium acetate, less than 2% of the radioactivity chromatographed on DEAE-cellulose paper as 5'-mononucleotides whereas the bulk of the mononucleotides whereas the bulk of the radioactivity remained at the origin (Table VI). This result indicates that the endonuclease preparation is essentially free of exonuclease activity. Furthermore, it shows that the product is composed of predominantly oligonucleotides of chain length greater than five (32).

The limit product was then analyzed on a DEAE-cellulose column in 7 M urea and the pattern obtained with 32P-labeled E. coli DNA is shown in Fig. 4. This system was capable of resolving the mononucleotide to pentanucleotide peaks clearly. Oligonucleotides with a chain length greater than six were not clearly separated, probably because of the heterogeneity of the base composition. It can be seen that the bulk of the radioactivity eluted at a salt concentration between 0.25 and 0.35 M NaCl. The peaks of radioactivity were pooled as shown, urea

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**Table VI**

<table>
<thead>
<tr>
<th>DNA</th>
<th>14C-labeled fd</th>
<th>32P-labeled</th>
<th>E. coli (heat denatured)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radioactivity made acid soluble</td>
<td>48</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>Radioactivity in mononucleotide region</td>
<td>1.99</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Radioactivity remaining at origin</td>
<td>57</td>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>
50 pmoles of Tris buffer, pH 8.4, 10 pmoles of MgCl₂, 400 nmoles of 8.4, containing 10⁻⁴ M EDTA was denatured by heating 10 min at 100° and cooling on ice. The reaction mixture (1 ml) contained 50 µmoles of Tris buffer, pH 8.4, 10 µmoles of MgCl₂, 400 µmoles of denatured ³²P-labeled E. coli DNA (3000 cpm per µmole), and 15.5 units of T7 endonuclease I. The reaction was incubated at 37° for 30 min and terminated by addition of 4 ml of 0.02 M Tris buffer, pH 7.5, containing 7 µm urea. After addition of 5 µmoles of each of the 4 deoxynucleoside monophosphates the entire sample was applied to a DEAE-cellulose column (0.5 × 2 cm) which was equilibrated in the same buffer. The sample was washed on with 5 ml of buffer and the column was eluted with a 600 ml of gradient of 0 to 0.4 M NaCl in 0.01 M Tris buffer, pH 7.5, containing 7 µm urea. The flow rate was maintained at 15 ml per hour and 3-ml fractions were collected. Aliquots were counted in Bray’s solution. The average chain length was determined from the proportion of ³²P radioactivity which was sensitive to alkaline phosphatase (30) and is shown by the numbers over the peaks.

**TABLE VII**

Identification of 5’-terminal nucleotides produced by action of T7 endonuclease I

<table>
<thead>
<tr>
<th>5’-Deoxynucleoside monophosphate</th>
<th>³²P-labeled radioactivity, 0.15 unit</th>
<th>³²P-labeled radioactivity, 0.25 unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>dAMP</td>
<td>203</td>
<td>5</td>
</tr>
<tr>
<td>dGMP</td>
<td>611</td>
<td>11</td>
</tr>
<tr>
<td>dTMP</td>
<td>1,225</td>
<td>47</td>
</tr>
<tr>
<td>dCMP</td>
<td>1,365</td>
<td>33</td>
</tr>
</tbody>
</table>

* Blank values subtracted.

and salt were removed by the method of Tomlinson and Tener (33), and the average chain length was determined by measuring the proportion of ³²P released by alkaline phosphatase (30). Values over this peak of from seven to 12 nucleotides were obtained (Fig. 4). Virtually identical patterns were obtained with ¹⁴C-labeled fd DNA as the substrate.

Specificity of Reaction—T7 endonuclease I produces fragments of DNA bearing 5’-hydroxyl and 5’-phosphate termini as deduced from two observations. First, the enzyme renders fd DNA sensitive to exonuclease I, an enzyme which has an absolute requirement for 3’-hydroxyl termini. Secondly, no phosphorylation of endonuclease-treated DNA was detectable in the presence of 5’-hydroxylpolynucleotide kinase and γ-³²P-ATP unless the DNA was first dephosphorylated by alkaline phosphatase.

The 5’-termini produced by the action of the endonuclease on fd DNA were dephosphorylated by alkaline phosphatase and labeled with 5’-hydroxyl polynucleotide kinase and γ-³²P-ATP. Label was detected in all four 5’-deoxynucleotides although the enzyme exhibited a distinct preference for pyrimidine-containing mononucleotides (Table VII). The same distribution of nucleotides was observed with levels of enzyme which introduced as few as one or as many as 60 breaks per molecule of fd DNA.

**DISCUSSION**

T7-Induced endonuclease I is the product of gene 3 and is responsible for the endonucleolytic degradation of host DNA which follows T7 infection (14, 22). Cellular DNA undergoes breakage with the introduction of single and double strand breaks and is reduced to fragments with a molecular weight of about 1 × 10⁴ (14). The purified enzyme causes single strand and double strand breaks in native DNA although the size of the limit product obtained in vitro is at least 10-fold smaller than that observed in vivo. The enzyme produces negligible amounts of acid-soluble material when it acts on duplex DNA in vitro. This is consistent with the observations in vivo which showed that T7 mutants in gene 6 did not degrade cellular DNA to acid-soluble products although the endonucleolytic breakdown catalyzed by the gene 3 endonuclease still occurred (14). The enzyme degrades mature T7 phage DNA as well as all other types of DNA tested making it unclear why replicating T7 DNA is not degraded by this enzyme in vivo.

T7 endonuclease I differs in several respects from endonuclease I of uninfected E. coli (30). Although both enzymes produce double strand breaks in duplex DNA, the T7 enzyme produces fragments whose chain length is greater than 100 nucleotides whereas the E. coli enzyme produces fragments with a chain length of about seven nucleotides. The T7 enzyme has a strong preference for single stranded DNA whereas the bacterial enzyme acts preferentially on native DNA. Transfer RNA is a potent inhibitor of the E. coli enzyme (36) but inhibits the phage enzyme only weakly. While both enzymes produce breaks containing 3’-hydroxyl and 5’-phosphate termini, the T7 enzyme produces fragments bearing predominantly pyridine-containing nucleotides at their 5’-termini whereas the fragments produced by the bacterial enzyme contain a predominance of guanosine and cytosine-containing nucleotides at their 5’-termini.

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