Gene 6 Exonuclease of Bacteriophage T7

I. PURIFICATION AND PROPERTIES OF THE ENZYME*

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SUMMARY

An exonuclease activity was detectable in crude extracts from Escherichia coli 1200 cells (Endo I−, su−) infected with T7 phage bearing amber mutations in gene 3 (T7 endonuclease I) and gene 5 (T7 DNA polymerase). The activity was not detectable if the infecting phage also contained an amber mutation in gene 6. That gene 6 is the structural gene for this enzyme was shown by the fact that phage bearing a temperature-sensitive mutation in gene 6 induced an exonuclease activity which was more heat labile than the wild type enzyme.

The enzyme has been purified 500-fold, and the purified preparations are essentially free of ribonuclease, endonuclease, DNA polymerase, and exonuclease I activity although they do contain measurable 3'-phosphatase activity, likely the reflection of residual E. coli exonuclease III. The enzyme has an absolute requirement for divalent cations and a sulfhydryl reagent and is stimulated greatly by potassium ions. The enzyme shows a marked preference for duplex DNA and liberates 5'-mononucleotides as the sole acid-soluble product.

The gene 6 exonuclease is involved in the degradation of cellular DNA to acid-soluble products after T7 phage infection.

The degradation of the host DNA following infection with bacteriophage T7 occurs in three stages (1). The DNA is first released from a membrane-like structure (1, 2), and this process requires the action of gene 1 (1), the structural gene for a T7-specific RNA polymerase (2). The release from the membrane is thought to be catalyzed by a class 2A protein (2) which is in turn synthesized under the control of the T7 RNA polymerase. The released DNA is then cleaved endonucleolytically to yield fragments with a molecular weight of about 1 × 10⁶. This stage is catalyzed by T7-induced endonuclease I (4-6), the product of gene 3 (7). These fragments of DNA are then converted directly to acid-soluble nucleotides, a process which requires the action of gene 6 (1).

It has been suggested that gene 6 codes for an exonuclease involved in the degradation of cellular DNA (1, 7). This report shows that indeed gene 6 is the structural gene for an exonuclease which liberates 5'-mononucleotides as the sole acid-soluble product. The purification of the enzyme and requirements of the reaction are described in this paper. In the accompanying paper we describe studies of the mechanism of action of the enzyme.

EXPERIMENTAL PROCEDURE

Materials

Bacterial and Phage Strains—Escherichia coli 1200 (ATCC 27196) is an Endo I−, su− host and was obtained from Dr. W. Summers, Yale University, New Haven, Connecticut. The T7 amber mutants in genes 1 to 6 were those described previously (1, 4). The double mutants T7 am 29, am 28 which has amber mutations in genes 3 and 5, respectively, and T7 am 29, am 147 which has amber mutations in genes 3 and 6, respectively, were obtained from Dr. F. W. Studier, Brookhaven National Laboratory, Upton, New York. T7 ts 136 which bears a temperature-sensitive mutation in gene 6 was isolated by F. W. Studier and obtained from Dr. W. Summers. The triple amber mutant T7 am 29, am 28, am 147 (genes 3 + 5 + 6) was constructed by crossing the double mutant T7 am 29, am 28 (genes 3 and 5) against T7 am 147 (gene 6). In the case of the triple mutant T7 am 29, am 28, ts 136 (genes 3 + 5 + 6 ts) the double mutant T7 am 28, ts 136 (genes 5 + 6 ts) was first obtained by crossing T7 am 28 against the T7 mutant ts 136. This double mutant was then crossed against T7 am 29 (gene 3) to obtain the triple mutant. Phages bearing multiple mutations are named according to the gene number containing the mutation, e. g. “T7 am 3 + 5” bears amber mutations in genes 3 and 5. Crosses were made according to the method of Studier (8). Phage PM 2 and its host Ps. BAL 31 was obtained from Dr. Romilio Espejo, University of Chile, Santiago, Chile. Other bacterial and phage strains have been described previously (1, 4).

Media—LB medium contained 10 g of Difco Bactotryptone, 5 g of Difco yeast extract per liter, and was brought to pH 7.0 with 10 N NaOH. This medium was used for making phage stocks.

LBP medium (4) was used to make phage-infected cells. GCA

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medium (1) was used for preparing DNA of both bacterial and phage origin.

**Enzymes**—Pancreatic RNase, micrococcal nuclease, and bacterial alkaline phosphatase were obtained from Worthington. The alkaline phosphatase was further purified by the procedure of Weiss, Live, and Richardson (9). Venom 5'-nucleotidase was purchased from Sigma; the enzyme had no detectable 3'-nucleotidase activity (less than 1% of its 5'-nucleotidase activity).

**Radioactive Compounds**—[3H]Thymidine (23 Ci per mmole) was from Amersham-Searle, Toronto, Ontario. [32P]Orthophosphate (carrier free) was from Atomic Energy of Canada, Ottawa, Ontario.

**Other Materials**—Streptomycin sulfate was purchased from Chas. Pfizer, Toronto, Canada, and dithiothreitol was obtained from Calbiochem, Los Angeles, California. Crystalline bovine serum albumin was purchased from Pentex Incorporated, Kan-kakee, Illinois. Other materials were those described in previous papers (1, 4).

**Methods**

**Preparation of Phage Stocks**—Stocks of amber mutants were grown on the permissive host E. coli BBW/1. An overnight culture was diluted 50-fold in LB medium and grown in a gyratory shaker at 30° until the cell concentration reached 5 x 10^8 per ml. The appropriate T7 phage was then added at a multiplicity of infection of 0.1, and the culture was shaken vigorously until lysis occurred about 1 hour later. Chloroform was added, and the centrifugation was centrifuged at 16,000 × g for 10 min. Titters of 5 x 10^8 to 2 x 10^9 per ml were obtained. Reversion frequencies were never greater than 1 x 10^-3 for single T7 mutants, whereas multiple mutants had reversion frequencies of less than 1 x 10^-4. Phage PM 2 was grown and purified by the method of Espejo and Canedo (10).

**Preparation of Nucleic Acids**—Phage DNA's were prepared by phenol extraction (11, 12). E. coli DNA was labeled with [3H]-thymidine in the following way. An overnight culture of E. coli B was diluted 50-fold into 500 ml of GCA medium containing 250 µg per ml of deoxyadenosine, 0.4 µg per ml of thymidine, and 0.5 µCi per ml of [3H]thymidine (23 Ci per mmole). The cells were grown for 7 hours at 37°, harvested by centrifugation at 16,000 × g for 10 min and washed in 100 ml of GCA medium. E. coli B DNA was labeled with [32P]Orthophosphate as described previously (4) and the [3H]- or [32P]DNA was purified as described in a previous paper (4). 3'-Phosphoryl terminated [32P]DNA was made by partial digestion of E. coli [32P]DNA with micrococcal nuclease (13). E. coli RNA labeled with [3H]orthophosphate was extracted from [32P]-labeled E. coli B by the method of Littauer and Eisenberg (14). Residual contamination by DNA (0.1%) was removed by treatment with pancreatic DNase.

**Growth of Phage-infected Cells**—The procedure is generally the same as already described (4). LB medium (100 liters) was inoculated with 3 liters of an overnight culture of E. coli 1200. The cells were grown to a density of 1 x 10^8 per ml at 30° and infected with T7 am 29, 28 (genes 3 + 5) at a multiplicity of infection of 10. After 15 min cooling was begun with liquid nitrogen and 10 min later the cells were collected in a continuous flow centrifuge. The yield of cells was 242 g. Small scale infections were done at 30° in 2-liter flasks. The percentage of infected cells was always greater than 98% as determined by viable cell titers. However, it was found that with triple mutants it was necessary to use a multiplicity of 20 phage per cell in order to infect greater than 98% of the cells.

**Assay of Exonuclease**—The assay measures the release of acid-soluble radioactivity from E. coli [3H]DNA in the presence of the exonuclease. The reaction mixture (0.1 ml) contained 5 µmoles of Tris-chloride buffer, pH 8.1, 0.5 µmole of MgCl2, 0.1 µmole of dithiothreitol, 2.0 µmoles of KCl, 10 µmoles of E. coli [3H]DNA (1000 to 2000 cpm per nmole), and 0.1 to 4.0 units of enzyme. After incubation for 15 min at 37° the reaction was terminated by addition of 300 µg of bovine serum albumin and 0.01 ml of 100% trichloroacetic acid (w/v). After centrifugation, the supernatant was counted in Bray's solution in a liquid scintillation counter. The blank values were less than 1% of the radioactivity. One unit is the release of 1.0 µmole of acid-soluble nucleotide after 15 min at 37°. The activity was linearly related to enzyme concentration in the range of 0.1 to 4.0 units.

Enzyme fractions were diluted in a solution containing 0.05 M Tris-chloride buffer, pH 7.5, 0.001 M dithiothreitol, and 100 µg per ml of crystalline bovine serum albumin. A stock solution of dithiothreitol was prepared each day since it was found to deteriorate if stored frozen for prolonged periods of time. Potassium chloride was omitted from assays of crude extracts as it was not required for activity in such fractions. Different DNA preparations gave up to 4-fold variation in activities in this assay possibly because of variation in the chain length of the DNA.

**Other Methods**—Analytical band centrifugation of PM 2 DNA was performed in 2.5 M CaCl2 containing 0.1 M NaOH. Greater than 90% of the DNA was in the supercoiled form with an uncorrected sedimentation coefficient of 62 S.

**RESULTS**

**Identification of T7 gene 6 as Structural Gene for Exonuclease**

E. coli 1200 was infected with various T7 phages and the exonuclease activity in crude extracts was determined (Table 1). Following infection with T7 am 3 + 5, the exonuclease activity rose greater than 12-fold, whereas a rise of 5-fold was found when the cells were infected with T7 am 3 + 6 phage. No rise in activity was detected when T7 am 3 + 5 + 6 phage were used. We conclude that both gene 6 and gene 5 control nuclease activities. Since gene 5 is said to code for a phage-induced DNA polymerase (7), the nuclease activity seen after infection with T7 am 3 + 6 phage is probably a polymerase-associated exonuclease. The activity induced by wild type phage was greater than that in-
induced by T7 am 3 + 5 phage (Fig. 1). Thus, it is likely that the enzyme makes no acid-soluble nucleotides from native DNA (4, 5). Although this enzyme appears to be sensitive to thermal inactivation, it does introduce single and double strand breaks which might provide sites for the action of exonuclease(s).

A 4-fold increase in exonuclease activity was detected following infection with T7 am 3 + 5 + 6 ts, a phage bearing a temperature-sensitive mutation in gene 6 (Table I). The activity induced by this phage was more sensitive to thermal inactivation than that induced by T7 am 3 + 5 phage (Fig. 1). Thus, it is likely that gene 6 is the structural gene for an exonuclease activity which we have called the T7 gene 6 exonuclease.

Appearance of Gene 6 Exonuclease Activity after Infection of E. coli 1200

E. coli cells were infected with T7 am 3 + 5 + 6 ts phage, and exonuclease activity was determined at varying times after infection (Fig. 2). The activity was barely detectable after 5 min, reached a maximum after 20 min. The maximum activity was increased 20-fold over that found in uninfected cells. The rise in activity could be prevented by addition of chloramphenicol (100 μg per ml) at the time of infection. These kinetics of induction suggest that the enzyme is produced in response to the presence of the infected cells.

Purification of Gene 6 Exonuclease from T7 am 3 + 5 Phage-infected E. coli 1200

A summary of the purification procedure is given in Table II. The procedure was devised for disrupting the cells. The cell suspension was divided into two equal volumes and sonication was carried out for 15-set intervals interspersed by cooling in an ice-salt bath. The output setting was set at 50% of maximum and the sonication time was 60 sec. Small quantities of cells (less than 1 g) were disrupted in the same manner except the cell suspension was divided into two equal volumes and sonication was done in 50-ml polypropylene tubes. The 1/4-inch diameter probe of a Branson model 140 D sonicator (Heat Systems Ultrasonics, Plainview, New York) was used, and sonication was carried out for 15-sec intervals interspersed by cooling in an ice-salt bath. The output setting was set at 50% of maximum and the total sonication time was 60 sec. Small quantities of cells (less than 1 g) were disrupted in the same manner except the micro-tip was used. The sonically disrupted cells were centrifuged at 40,000 rpm in the IEC A-25/ rotor for 30 min at 5°. The optical density at 260 nm of the supernatant was adjusted to 150 by addition of Buffer B. This fraction was the crude extract (70 ml).

Streptomycin Precipitation and Nucleic Acid Digestion

To 70 ml of crude extract were added 17.5 ml of a 5% solution of streptomycin sulfate. The mixture was incubated at 0° for 10 min and centrifuged at 18,000 × g for 10 min. The sediment was suspended in 70 ml of 0.1 M potassium phosphate buffer, pH

### Table II

| Fraction                | Protein mg/ml | Total activity units | Yield % | Specific activity units/mg | Purification fold |
|-------------------------|--------------|----------------------|---------|-----------------------------|------------------|
| 1. Crude extract        | 9.6          | 182,000              | 100     | 272                         | 1                |
| 2. RNase-treated streptomycin autolysate | 0.7          | 227,500              | 125     | 4,640                       | 17               |
| 3. DEAE-cellulose (dialyzed) | 0.08         | 102,500              | 56      | 25,800                       | 95               |
| 4. Phosphocellulose      | 0.007        | 45,000               | 24      | 140,200                      | 518              |

*Methods are described in the text.*
7.4, containing 0.001 M dithiothreitol. (In this and succeeding steps, all buffers contained this concentration of dithiothreitol.) A solution of 1.0 M McCl2 (0.35 ml) was added to bring the magnesium concentration to 0.005 M and the mixture was incubated at 30°. After 2 hours 18% of the ultraviolet-absorbing material at 260 nm had become acid-soluble as measured by the procedure of Richardson et al. (16). To the autolysate was added 0.525 mmole of EDTA and 17 μg per ml of pancreatic RNase. After 30 min at 30°, 96% of the ultraviolet-absorbing material at 260 nm had become acid soluble. The mixture was chilled on ice and centrifuged at 16,000 x g for 10 min. The supernatant (70 ml) was subjected to DEAE-cellulose chromatography.

**DEAE-Cellulose Chromatography**

A DEAE-cellulose column (2 x 10 cm) was equilibrated in 0.1 M potassium phosphate buffer, pH 7.4, containing 10^{-3} M EDTA (equilibrating buffer). The RNase treated streptomycin autolysate was applied to the column under gravity. The column was washed with 50 ml of equilibrating buffer and eluted with a 300-ml linear gradient of 0.1 M to 0.4 M ammonium sulfate in equilibrating buffer. The flow rate was maintained at 1 ml per min, and fractions of 15 ml were collected. The exonuclease activity was eluted between 0.18 and 0.22 M ammonium sulfate (Fractions 6 to 8) with an average purification of about 5-fold.

**Phosphocellulose Chromatography**

A phosphocellulose (Whatman P-11) column (2 x 8 cm) was equilibrated with 0.02 M potassium phosphate buffer, pH 6.5, containing 10^{-4} M EDTA (equilibrating buffer). The DEAE-cellulose eluate was dialyzed against 2 liters of the same buffer for 1 hour. The buffer was changed and dialysis was continued for an additional hour. The dialyzed DEAE-cellulose eluate (50 ml) was applied to the column under gravity, and the column was washed with 25 ml of equilibrating buffer. The column was eluted with a linear 250-ml gradient of 0.05 to 0.5 M ammonium sulfate in the same buffer at a flow rate of 2 ml per min. Fractions of 12.5 ml were collected, and the exonuclease was eluted at 0.13 to 0.15 M ammonium sulfate (Fractions 3 and 4). The recovery of exonuclease in these fractions was about 40% of the activity applied with a 5-fold purification. The overall recovery was 24%, with a 500-fold purification. Since the enzyme was extremely unstable at this point the fraction was made 40% with glycerol, and 1 mg per ml of crystalline bovine serum albumin was added. The enzyme was stored at -20° and has been stable for 6 months.

**Comments on Purification Procedure**

With the exception of the phosphocellulose eluate, all fractions were stable for short periods of time when stored at 0° (24 to 48 hours). The crude extract and the streptomycin autolysate retained about 60% of the original activity after 3 weeks whereas the DEAE-cellulose eluate lost 70% of its original activity in this time. The stability of crude extracts was considerably enhanced by the use of dithiothreitol. The activity was much more stable in the presence of 2-mercaptoethanol. All experiments described were performed with the phosphocellulose eluate.

**Presence of Other Enzymatic Activities in Purified Enzyme**

Ribonuclease—When 10 nmole of E. coli [3P]RNA (19,000 cpm per nmole) were incubated with 10 units of exonuclease for 15 min under standard assay conditions, there was no detectable release of acid-soluble material. If the incubation was prolonged for 5 hours, 0.06 mmole of acid-soluble nucleotide was released. However, the crystalline bovine serum albumin contained in the final enzyme preparation was found to contain an equivalent amount of ribonuclease when assayed by itself. Thus it is probable that the exonuclease contains no ribonuclease activity. In any case the RNase detected in the final enzyme preparations constituted less than 0.5% of its activity toward native DNA.

**Endonuclease—**When 10 nmole of the closed circular duplex DNA of phage 2 were incubated with 5 units of gene 6 exonuclease for 30 min at 37° there was no conversion of the supercoiled form to the open circular form as analyzed by band centrifugation in alkaline cesium chloride. (The reaction mixtures (0.05 ml) had the same composition as the standard assay except the reduced glutathione (10 mM) was substituted for dithiothreitol.

The latter reagent introduced approximately twice as many single strand breaks into PM 2 DNA as reduced glutathione.)

Prolongation of the incubation time to 60 min resulted in a detectable conversion of the supercoiled form to the open circular form of the DNA indicating the introduction of single strand breaks. That this was in part due to the presence of a sulfhydryl reagent (glutathione) was shown by the fact that if the sulfhydryl reagent was omitted from the reaction there was no detectable breakage of the DNA by 5 units of exonuclease. Thus although the final enzyme preparations may be contaminated by a sulfhydryl-requiring endonuclease, we estimate that the exonuclease introduced less than one endonucleolytic break per 10,000 phosphodiester bonds broken exonucleolytically.

**Exonuclease I—**When the gene 6 exonuclease was incubated with 10 nmole of heat-denatured E. coli DNA the activity was 0.5% of that obtained with native DNA. Thus the enzyme has a marked preference for native DNA. We are unable to determine whether the residual activity toward denatured DNA reflects contamination with E. coli exonuclease I, an enzyme highly specific for single stranded DNA (18), or is an inherent property of the gene 6 exonuclease.

**E. coli DNA Polymerase—**Ten units of gene 6 exonuclease caused no detectable incorporation of [3H]dATP (20,000 cpm per nmole) into acid-insoluble polyoxyadenylatethymidylate copolymer as measured in the standard assay for E. coli DNA polymerase (16). (This amount of exonuclease inhibited authentic DNA polymerase about 74%.) The ratio of exonuclease activity to DNA polymerase activity was greater than 1000 and we thus conclude that the gene 6 exonuclease preparations are not significantly contaminated by the 3'- or 5'-exonuclease activities of E. coli DNA polymerase.

**E. coli Exonuclease III—**The presence of exonuclease III contamination was detected by measuring the 3'-phosphatase activity (13) of the preparations using 3'-phosphoryl-[3P]DNA (average chain length, 70 nucleotides as determined by sensitivity of the 3P to bacterial alkaline phosphatase). One unit of gene 6 exonuclease liberated 0.012 nmole of Norit nonadsorbable 3P in 15 min indicating the presence of a 3'-phosphatase activity in the final preparations. That this activity was not attributable to the gene 6 exonuclease was shown by the fact that no 3'-phosphatase activity was induced by the various T7 phage tested (Table III).

Thus, after infection with T7 am + 5 phage the exonuclease activity increased 10-fold relative to the 3'-phosphatase activity.

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1 The introduction of single strand breaks into duplex DNA by reducing reagents has been observed by Bode (17).
TABLE III

Exonuclease activity and 3′-phosphatase activity induced after infection with T7 phages

Reaction mixtures had the same composition as the standard assay but contained 3 nmoles of E. coli 3′-phosphoryl-[32P]DNA (50,000 cpm per n mole). After incubation with 1.5 to 3.0 μg of crude extract protein for 15 min the reaction was terminated with albumin and triphenyltetrazolium acid in the usual manner. An aliquot of the acid-soluble supernatant was counted for the determination of exonuclease activity, and the remainder was twice treated with Norit for the determination of 3′-phosphatase activity (13). One unit of exonuclease activity is the release of 1 n mole of acid-soluble nucleotide in 15 min, and one unit of 3′-phosphatase activity is the release of 1 n mole of Norit nonadsorbable 32P in 15 min.

| Phage used | Gene | Exonuclease activity | 3′-Phosphatase activity | Ratio of exonuclease activity to 3′-phosphatase activity |
|------------|------|----------------------|-------------------------|----------------------------------------------------------|
| Uninfected E. coli 1200 | 60 | 63 | 20 | 3.2 |
| T7 am 28, am 29 | 60 | 600 | 20 | 30 |
| T7 am 28, am 29, am 147 | 60 | 56 | 13 | 4.3 |

TABLE IV

Requirements of T7 gene 6 exonuclease

The complete reaction mixture was the same as that used in the standard assay (see under "Methods"). Where indicated, 0.1 μmole of MnCl2 was added in place of MgCl2. Acid-soluble radioactivity released by 3 units of gene 6 exonuclease was measured.

| Reaction mixture | Acid-soluble radioactivity (nmoles per mg protein) |
|------------------|--------------------------------------------------|
| Complete         | 3.0                                              |
| MgCl2 omitted    | <0.1                                             |
| MgCl2 omitted, MnCl2 added | 4.5                                         |
| Dithiothreitol omitted | <0.1                                          |
| KCl omitted      | 1.0                                              |

The final preparations of gene 6 exonuclease had a ratio of exonuclease activity to 3′-phosphatase activity of 60, whereas authentic exonuclease III when assayed under the same conditions had a ratio of 1.4. Thus about 2.3% of the exonuclease activity of the gene 6 exonuclease preparations is attributable to exonuclease III contamination. This level was not appreciable since we show in the accompanying paper that the gene 6 exonuclease begins its attack at the 5′-terminus of DNA whereas exonuclease III starts its attack at the 3′-terminus (19).

Requirements of T7 gene 6 Exonuclease

The requirements of the gene 6 exonuclease are shown in Table IV. The enzyme has an absolute requirement for a divalent cation, the activity was completely abolished by omission of MgCl2. The optimal magnesium ion concentration was 0.005 M, the activity was 28% of maximum at a magnesium ion concentration of 0.002 M and 63% of maximum at a concentration of 0.015 M. Manganese ions gave a greater activity than magnesium ions; the optimal manganese concentration was 0.001 M. At a manganese ion concentration of 10−4 M, the activity was 82% of maximal and at a concentration of 0.003 M the activity was 40% of maximal. When CaCl2 or CoCl2 (0.005 M) were used as the divalent metal the activity was 5% and 10%, respectively, of that obtained with magnesium ions.

The enzyme had an absolute requirement for a sulfhydryl reagent (dithiothreitol) (Table IV); no activity was detectable in its absence. Potassium ions stimulated the activity about 3-fold and the optimal potassium ion concentration was 0.02 M. The enzyme was not stimulated by addition of equivalent concentration of NH4Cl or NaCl in place of KCl. The pH optimum for the enzyme is 8.1 (Tris-chloride buffer); the activity at pH 7.5 (Tris-chloride buffer) was 60% of that obtained at pH 8.1. The activity at pH 9.0 (sodium glycine buffer) was 28% of that obtained with Tris-chloride buffer at pH 8.1 and the activity at pH 7.4 (potassium phosphate buffer) was 28% of that found at pH 8.1 (Tris chloride buffer).

Manganese ions were able to substitute at least partially for potassium chloride and dithiothreitol (Table V). No activity was detectable when MnCl2, KCl, and dithiothreitol were omitted; however, when Mn2+ ions were included but KCl and dithiothreitol were omitted, the activity was 15% of that observed in
the complete system. If dithiothreitol was omitted but KCl and MnCl₂ were added, the activity was 45% of that obtained with the complete system and when KCl was omitted but MnCl₂ and dithiothreitol were added the activity was 75% of that obtained with the complete system.

**Nature of Acid-soluble Product**

When double stranded *E. coli* DNA was digested with the enzyme, 43% of the radioactivity became acid-soluble. Greater than 95% of this label was rendered irreversibly to Norit by incubation with bacterial alkaline phosphatase or venom 5'-nucleotidase (Table VI). The acid-soluble product was also analyzed chromatographically on DEAE-cellulose paper in 7 M urea (4) and found to chromatograph as mononucleotides. Furthermore, no acid-soluble oligonucleotides could be detected chromatographically when 10%, 21%, 29%, or 38% of the input radioactivity had been rendered acid soluble. Thus the acid-soluble product is predominantly if not entirely 5'-mononucleotides and we conclude that the enzyme acts exonucleolytically.

**DISCUSSION**

In this paper we report the purification and partial characterization of a potent exonuclease from *E. coli* 1200 cells infected with T7 phage bearing amber mutations in genes 3 and 5. This phage was chosen so as to eliminate T7 endonuclease I (4-7) and was enhanced and stabilized in the presence of dithiothreitol, and is greatly stimulated by potassium ions. The final preparation was essentially free of ribonuclease, endonuclease, that the enzyme was inactivated by too vigorous sonication, and that the activity was not induced by a phage bearing an amber mutation in gene 6 and that the activity induced by a phage bearing a temperature-sensitive mutation in gene 6 was more heat labile than the wild type enzyme. Since gene 6 mutants fail to degrade host DNA to acid-soluble fragments, it seems likely that the gene 6 exonuclease is involved in this function *in vivo*. The mechanism of action of the enzyme in this process will be discussed in the accompanying paper.

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