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A Novel Oligoribonuclease of *Escherichia coli*

I. ISOLATION AND PROPERTIES

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A new ribonuclease has been isolated from *Escherichia coli*. The enzyme is present in the 100,000 × *g* supernatant fraction and has been purified over 200-fold. Studies of the enzyme reveal that:

1. The enzyme shows a marked preference for oligoribonucleotides; indeed, the reaction rate is inversely proportional to the chain length of the substrate. The enzyme does not attack polynucleotides even at high concentrations of enzyme and has no detectable DNase activity.
2. The enzyme is stimulated strongly by Mn^{2+}, less strongly by Mg^{2+}, and not at all by Ca^{2+} and monovalent cations.
3. The enzyme is purified free of RNase I, RNase II, RNase III, polynucleotide phosphorylase, and other known ribonucleases of *E. coli*. The enzyme displays identical properties when isolated from mutants of *E. coli* that are deficient in the above ribonucleases.
4. The enzyme has a marked thermostability, a point of further distinction from RNase II.

A number of ribonucleases capable of degrading polyribonucleotides have been found in *Escherichia coli* (1–12) (Table I). Some of these are endonucleases, while others are exonucleases and have widely different substrate specificities. Although the physiological roles of most of the enzymes are still relatively unknown, RNases II and III have been implicated in messenger RNA degradation (13, 14), and RNase III has also been shown to be active in rRNA processing (15). While testing oligoribonucleotides for priming activity with RNA polymerase, Niyogi and Stevens (16) observed that crude enzyme preparations from *E. coli* B showed no priming activity, but when purified preparations of RNA polymerase were used, the stimulation with oligomers was observed. This result indicated that there must be certain factors in the crude extract which specifically interfere with the stimulatory effect of oligonucleotides on RNA synthesis. Exploring this idea, Stevens and Niyogi (17) detected an enzyme which rapidly degrades short oligoribonucleotides. In this paper we will report the purification of this enzyme from the 100,000 × *g* supernatant fraction of *E. coli* Q13 (a mutant which is deficient in RNase I and polynucleotide phosphorylase) and show some of the properties that make it different from other known ribonucleases. In addition, we will show that this enzyme is also present in *E. coli* AB105, an RNase I- and RNase III-deficient strain, and *E. coli* N464, which has a thermolabile RNase II activity. Because of its unique specificity in hydrolyzing short oligoribonucleotide chains, we will call the enzyme "oligoribonuclease."

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MATERIALS AND METHODS

Escherichia coli Q13 ATCC 29079, a multiple mutant deficient in both RNase I and polynucleotide phosphorylase, was isolated in Dr. Walter Gilbert's laboratory (18) and obtained through the courtesy of Dr. Raymond Gesteland. *E. coli* AB105, an RNase III-deficient strain isolated from A19, was obtained from Dr. F. W. Studier (19). *E. coli* N464, which has a thermolabile activity, was provided by Dr. D. Schlessinger (20).

The *Azotobacter agilis* endonuclease, which was used to degrade poly(A) to oligonucleotides containing phosphate monoesterified to the terminal 5′-OH group, was the gift of Dr. Audrey Stevens (21). Alkaline phosphatase was obtained from Worthington Biochemicals Corp. Polynucleotide phosphorylase was purchased from P-L Biochemicals.

Poly(A), poly(U), and poly(C) were purchased from Miles Laboratories. Sephadex G-200 was obtained from Pharmacia Fine Chemicals. DE52 cellulose was obtained from Whatman Biochemicals Ltd.

Growth of Cells—E. coli Q13 was grown in enriched medium as described by Canellakis *et al.* (22). *E. coli* AB105 was grown in 25 liters of medium containing 250 g of bactotryptone, 125 g of NaCl, 250 g of yeast extract, 50 g of casamino acids, 500 ml of 50% glucose, and 2.5 liters of 1 M Tris-HCl (pH 7.5). *E. coli* N464 was grown at 30° in a medium containing 8 g per liter of nutrient broth and 8 g per liter of casamino acids.

Preparation of Substrates—^{14}C- or ^{3}H-labeled poly(A) was prepared from the corresponding nucleoside diphosphate according to the method of Singer and O'Brien (23) using polynucleotide phosphorylase from *Micrococcus luteus*.

Preparation of Oligoribonucleotides—^{14}C-Labeled oligonucleotides of the general structure $(pA)_n$, when $n = 2$ to 5, were prepared by digestion of [^{14}C]poly(A) (5.5×10^5 cpm per μmol of adenosine) with the *Azotobacter* endonuclease following the method of Stevens and Hilmoe (21). Oligonucleotides of the type $(Ap)_n$ were prepared by controlled alkaline hydrolysis of [^{14}C]poly(A). The poly(A) was incu-

TABLE I

Ribonucleases of Escherichia coli

Enzyme	Substrate specificity	Requirements	Mode of action	Product	References
RNase I	Single-stranded RNAs		Endonuclease producing cyclic 2′,3′-nucleotides as intermediates	3′-nucleotides	1,2
RNase II	Single-stranded RNAs	K^+, Mg^{2+}	Exonuclease 3′→5′-direction	5′-nucleotides, including short resistant oligonucleotides	3,4
RNase III	Double-stranded RNAs	K^+, Mg^{2+}	Endonuclease	Produces molecules of 10 to 25 nucleotide chain length	5–7
RNase H	Hydrolyzes the RNA strand of DNA-RNA hybrids	K^+, Mg^{2+}	Endonuclease	Mono- as well as oligonucleotides	8–10
RNase IV	R17 RNA		Endonuclease cleaves at a specific position	15 S fragment carrying 5′-terminus of the original molecule and 21 S fragment lacking 5′-end	11
RNase P	Precursor tRNA	Mg^{2+}, Mn^{2+} K^+, NH_4^+	Endonuclease cleaves at a specific position		12
Oligoribonuclease	Specific for short ribooligonucleotides	Mn^{2+}	Exonuclease 3′→5′-direction	5′-mononucleotides	17, 29, and this paper

bated in 1 N KOH at room temperature (23°) for 16 hours or in 0.3 N KOH for 30 to 40 min at 37° to obtain oligomers in the desired length range. After the incubation, the pH of the mixture was adjusted to between 7 and 7.5 by addition of perchloric acid, and the precipitated potassium perchlorate was removed by centrifugation. Oligonucleotides of the type $(Ap)_nA$ were obtained by the action of *E. coli* alkaline phosphatase on either $(pA)_{n+1}$ or $(Ap)_{n+1}$. The oligonucleotides of different chain lengths were separated and purified by paper chromatography on Whatman No. 3MM paper using 1-propanol/concentrated NH_4OH/H_2O (55/10/35, v/v/v) as the solvent.

Assay of Enzyme—The activity of the enzyme at various purification steps was determined by following the hydrolysis of oligonucleotides. The incubation mixture in 0.05 ml contained 0.25 μmol of $MnCl_2$, 5 μmol of Tris (pH 7.8), 3 nmol of [^{14}C]$(pA)_3$ (specific activity 550 cpm per nmol of adenosine), 5 μg of alkaline phosphatase, and required amounts of enzyme protein. The reaction was carried out for 10 min at 37°, then stopped by the addition of 1 μmol of EDTA (pH 7.5). The incubation containing no enzyme served as a blank. The whole reaction mixture was then applied to a Whatman DE81 disc and dried under an infrared lamp; the dried paper was soaked in 2 ml of 70% ethanol. Under these conditions all the nucleosides are released from the DE81 disc, but nucleotides and oligonucleotides having phosphate groups remain bound to the disc. One milliliter of the eluted solution containing [^{14}C]adenosine was counted in a Packard Tri-Carb liquid scintillation spectrometer using a scintillation fluid containing 20 g of 2,5-diphenyloxazole (PPO) and 0.5 g of 1,4-bis[2-(4-methyl-5′-phenyloxazolyl)]benzene (dimethyl POPOP) in 3.5 liters of toluene, and 1.5 liters of methyl Cellosolve.

One unit of enzyme was defined as that amount which produced 1 nmol of adenosine from the hydrolysis of [^{14}C]$(pA)_3$ under the standard assay conditions described. The specific activity was defined as units per mg of protein.

The reaction mixture for RNase II was essentially similar to that described by Singer and Tolbert (24) except that alkaline phosphatase (5 μg) was included in the incubation mixture (0.05 ml) and the reaction was carried out at 37° for 10 min. The rest of the procedure and the definition of unit for RNase II were the same as described above for the oligoribonuclease assay. These modifications enabled us to measure only the exonuclease activity of RNase II in the presence of small amounts of contaminating endonucleases.

Protein was estimated by the method of Lowry *et al.* (25). Hydroxylapatite was prepared according to Tiselius et al. (26).

RESULTS

Purification

All the operations were carried out at 0–4° unless otherwise stated.

Preparation of Crude Extract—Frozen *Escherichia coli* Q13 cells (50 g wet weight) were thoroughly ground with 125 g of alumina in a mortar and pestle. The alumina paste was extracted with 250 ml of buffer containing 10 mM Tris·HCl (pH 7.8), 10 mM $MgCl_2$, and 20 mM NH_4Cl. The crude suspension was centrifuged for 30 min at 15,000 rpm in a Sorvall SS-34 rotor to remove alumina and cell debris. The protein content of the solution varied from 30 to 35 mg per ml from batch to batch.

Ultracentrifugation of Crude Extract—The crude supernatant obtained was then centrifuged for 4 hours at 28,000 rpm (30 rotor, Spinco model L) to remove the ribosomes. The supernatant obtained was referred to as the 100,000 × *g* supernatant.

Streptomycin Sulfate Precipitation—To the 100,000 × *g* supernatant, 0.1 volume of freshly prepared 10% streptomycin sulfate was added slowly with constant stirring. The mixture was stirred for another 30 min, and the precipitate formed was removed by centrifugation at 10,000 rpm (SS-34 rotor) for 15 min in a Sorvall centrifuge. This treatment led to the removal of a considerable amount of nucleic acids. The A_{280}:A_{260} ratio of the sample was around 0.7.

Ammonium Sulfate Fractionation—The streptomycin sulfate-treated supernatant was then subjected to ammonium sulfate fractionation. It was found that addition of ammonium sulfate at this stage led to inactivation of the enzyme; therefore prior to ammonium sulfate fractionation, to the supernatant glycerol was added to a final concentration of 25% to help retain the enzyme activity during the ammonium sulfate fractionation. To this fraction was added, slowly and with constant stirring, cold saturated ammonium sulfate solution (pH adjusted to 8.5 with concentrated NH_4OH) to 55% saturation. The mixture was stirred for 30 min longer and then centrifuged at 15,000 rpm (GSA rotor) for 30 min in a Sorvall centrifuge to remove the precipitate. The supernatant was adjusted to 75% saturation by further addition of cold saturated ammonium sulfate as before and stirred for an additional 30 min; the precipitate formed was collected by centrifugation as before. The precipitate was dissolved in 10 ml of buffer containing 10 mM Tris (pH 7.8), 5 mM $MgCl_2$, 50 mM KCl, and

10% glycerol (Buffer A), and the ammonium sulfate fraction was dialyzed overnight against the same buffer. The protein content of the fraction was around 28 mg per ml.

DEAE-Cellulose Column Chromatography—The dialyzed enzyme fraction was further purified through a DEAE-cellulose column. DE52 cellulose suspended in Buffer A was deaerated and packed into a chromatographic column (2.7 × 30 cm). After equilibration with Buffer A, the column was charged with the sample (diluted with an equal volume of Buffer A), slowly over a period of about 60 min. The elution was carried out with a linear gradient of KCl from 0.05 to 0.30 M containing 10 mM Tris (pH 7.8), 5 mM $MgCl_2$, and 10% glycerol (Buffer B). Each 15-ml fraction was collected over an interval of 20 min; approximately 70 fractions were collected. All the fractions were assayed for protein by measuring absorption at 280 nm in a Zeiss ultraviolet spectrophotometer (PMQII) and for the enzyme activity by the assay method already described. Fig. 1 shows the elution pattern and the enzyme activity of the fractions. One major absorbance was obtained at 280 nm, and the enzyme activity coincided with the major protein peak. To avoid contamination with other proteins, only tubes numbering 30 to 45 were pooled. This represented most of the recoverable enzyme activity. It can be seen that RNase II activity also elutes at the same position. Though there was not much purification of the enzyme in this step, there was considerable removal of nucleic acids. The A_{280}:A_{260} ratio went up from 0.7 to 1.1 in this step.

Ammonium Sulfate Fractionation—The pooled fraction containing activity from the DE52 cellulose column was subjected to ammonium sulfate fractionation after the eluate was made to 25% glycerol. The protein fraction precipitating between 55 to 75% saturation with ammonium sulfate (pH 8.5) was collected in the same way as in the first ammonium sulfate fractionation step (described above). The protein fraction was dissolved in 20 ml of Buffer B and dialyzed overnight against the same buffer with two changes. The protein concentration of the fraction was about 7 mg per ml.

Treatment with Hydroxylapatite—The dialyzed DE52 cellulose fraction was further purified through a hydroxylapatite column equilibrated with Buffer B. When a gradient of 0 to 0.1 M potassium phosphate (pH 7.5) in Buffer B was used, the oligoribonuclease activity was eluted at about 0.02 M potassium phosphate concentration, whereas RNase II was eluted at about 0.08 M potassium phosphate concentration (Fig. 2). It was found, however, that on prolonged exposure to hydroxylapatite, the enzyme became inactivated. Therefore the enzyme was treated batchwise with hydroxylapatite to reduce prolonged contact. In this procedure the enzyme fraction was allowed to adsorb to hydroxylapatite previously equilibrated with Buffer B. After adsorption of the enzyme (with stirring for 10 min), the hydroxylapatite slurry was centrifuged for 15 min at 15,000 rpm in a Sorvall SS-34 rotor and the supernatant was discarded. The hydroxylapatite was washed once more with 40 ml of Buffer B, and the oligoribonuclease activity was eluted from hydroyxlapatite by the addition of 50 ml of Buffer B containing 0.025 M potassium phosphate (pH 7.5). After centrifugation as above, the oligoribonuclease activity was found in the supernatant fraction, leaving the RNase II activity still adsorbed to the hydroxylapatite. About 40% recovery was achieved by this procedure, with an almost 10-fold purification from the DE52 cellulose step. The fraction was dialyzed against Buffer B, then further concentrated and purified on a small hydroxylapatite column (1 × 3 cm). After loading on the column the fraction was eluted with 4 ml of Buffer B containing 0.025 M potassium phosphate (pH 7.5). A further 2-fold purification was achieved by this procedure, with 100% recovery of the enzyme from the previous step. All the hydroxylapatite fractions were assayed in the presence of Mg^{2+} instead of Mn^{2+}, because Mn^{2+} reacts with the phosphate of hydroxylapatite fractions causing precipitation of manganous phosphate.

Gel Filtration through Sephadex G-200—The hydroxylapatite fraction was further purified by Sephadex G-200 gel filtration. Two milliliters of the hydroxylapatite fraction were layered on top of the column (1.8 × 75 cm) previously equilibrated with 0.05 M Tris (pH 7.8) containing 5 mM $MgCl_2$, 50 mM KCl, and 10% glycerol. Elution was carried out with the same buffer. Fractions (3 ml) were collected at 30-min intervals and assayed both for protein by measuring absorbancy at 280 nm in a Zeiss ultraviolet spectrophotometer (PMQII) and for enzyme activity. The elution pattern, shown in Fig. 3, has only one protein peak, and the enzyme activity comes out just after the protein peak. The fraction (No. 29) having the highest

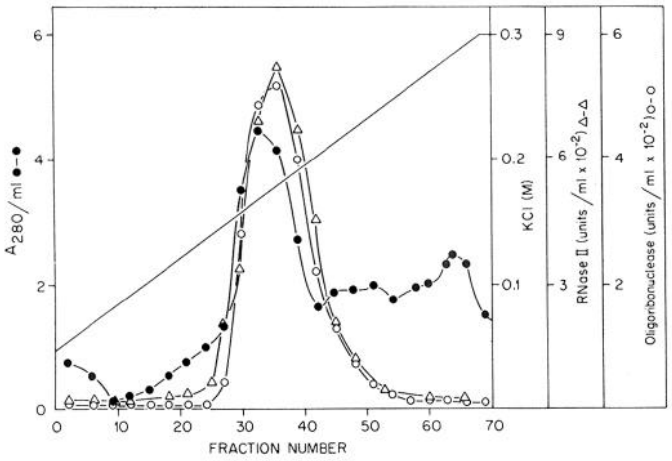

FIG. 1. Chromatography of $(NH_4)_2SO_4$ (55 to 75% saturation) fraction on DE52 cellulose. A column (2.7 × 30 cm) of DEAE-cellulose was packed and equilibrated with 10 mM Tris (pH 7.8), 5 mM $MgCl_2$, 0.05 M KCl, and 10% glycerol. After dialysis the $(NH_4)_2SO_4$ (55 to 75% saturation) fraction was applied and the column was washed with one bed volume of equilibrating buffer. A linear gradient elution was carried out with increasing concentration of KCl from 0.05 to 0.3 M containing 10 mM Tris (pH 7.8), 5 mM $MgCl_2$, and 10% glycerol. Fractions of 15 ml were collected over 20-min periods.

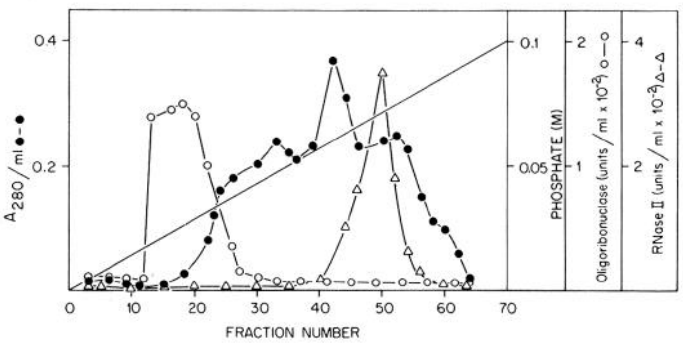

FIG. 2. Hydroxylapatite column chromatography of DEAE-cellulose fraction. Dialyzed fraction after DEAE-cellulose was loaded on a hydroxylapatite column (1.5 × 25 cm) equilibrated with 10 mM Tris (pH 7.8), 5 mM $MgCl_2$, and 10% glycerol. A linear gradient elution with increasing concentration of potassium phosphate (pH 7.5) from 0 to 0.10 M in the above buffer was carried out. Fractions (4 ml) were collected over 30-min periods.

specific activity of the enzyme was used for studying the properties. At this stage the enzyme was purified about 215-fold compared with the crude extract. This fraction was stored at −25° in 50% glycerol. The purification and recovery of the enzyme activity at different stages are shown in Table II.

Purification from Other Strains—The enzyme has also been purified from *E. coli* AB105, a strain deficient in both RNase I and RNase III, and from *E. coli* N464, a strain having a thermolabile RNase II (20). The purification gave results as above—the properties of the enzyme were invariably the same in all three strains tested.

Polyacrylamide Gel Electrophoresis—Fraction 29, which had the highest specific activity, was analyzed by polyacrylamide gel electrophoresis to determine with which protein band the oligoribonuclease activity coincided and to determine the purity of the enzyme in this fraction (Fig. 4). Only one protein band, corresponding to the first major band in the stained gel, had the enzyme activity. The stained gel was scanned in a microdensitometer to determine the relative amount of protein in each band. From this analysis it was estimated that about 40% of the protein in Fraction 29 is the oligoribonuclease.

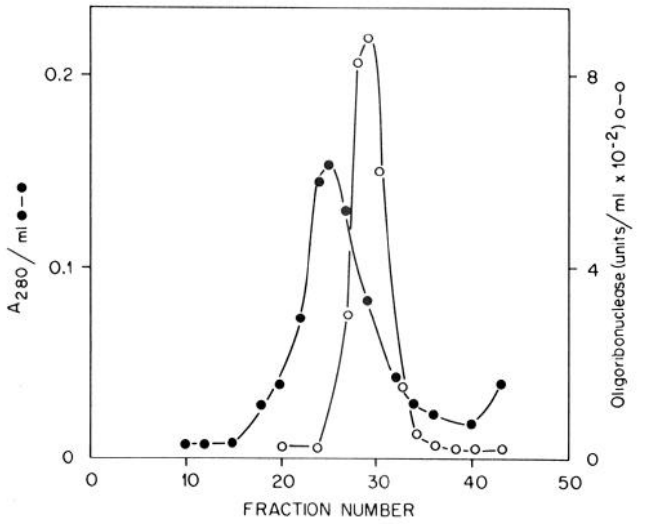

FIG. 3. Gel filtration through Sephadex G-200. Concentrated hydroxylapatite fraction (2 ml) was layered on a Sephadex G-200 column (1.8 × 75 cm) previously equilibrated with 50 mM Tris (pH 7.8) containing 5 mM $MgCl_2$, 0.05 M KCl, and 10% glycerol. Elution was carried out with the same buffer. Fractions (3 ml) were collected over 30-min periods.

TABLE II

Purification of enzyme from extracts of Escherichia coli Q13

Specific activity has been expressed as the units of the enzyme per mg of protein.

Fraction	Volume	Protein	Total	Specific activity
	ml	*mg/ml*	*units*	
Crude extract	220	33	363,000	50
100,000 × *g* supernatant	192	20	345,000	90
Streptomycin sulfate fraction	204	18	362,000	100
Ammonium sulfate (55 to 75% saturation)	29	28	87,000	103
Ammonium sulfate (55 to 75% saturation) after DE52 column	41	7	82,000	285
Hydroxylapatite (batchwise)	45	0.25	28,000	2,500
Hydroxylapatite (stepwise)	4.0	1.3	28,800	5,500
Sephadex G-200	7	0.08	6,000	10,750[a]

[a] The values for the peak tube of the Sephadex G-200 column are given.

Testing for Contaminating Activities—The purified fraction was tested to determine the number of contaminating enzymes, if any. RNase I activity was measured according to the procedure of Datta and Burma (28). The assay method for RNase II has already been described under "Materials and Methods." RNase III activity was followed according to the method of Robertson *et al.* (6). Polynucleotide phosphorylase was assayed according to the procedure of Singer and O'Brien (23). DNase activity was also measured using ^{32}P-labeled T7 DNA. There was no detectable activity of any of these enzymes.

Properties of Purified Enzyme

Requirement of Divalent Cations—Fig. 5 shows the dependence of the enzyme activity upon divalent cations. In the presence of Mn^{2+}, the rate of hydrolysis of $[^3H](pA)_3$ by the dialyzed Sephadex G-200 eluate increases almost linearly up to 2 mM concentration and then more slowly up to a concentration of 10 mM. But Mg^{2+} is not as effective as Mn^{2+}, as shown in the figure. Even in the presence of 50 mM Mg^{2+}, the rate of hydrolysis is only 20% of the rate with 10 mM Mn^{2+}. Neither Ca^{2+} nor any monovalent cations like K^+ or NH_4^+ have any effect on the activity of the enzyme (results not presented).

Effect of pH—The variation of the rate of degradation of $[^3H](pA)_2$ at different pHs is shown in Fig. 6. A number of buffers was used to study the effect of varying pH on the activity of oligoribonuclease. The enzyme has a broad pH

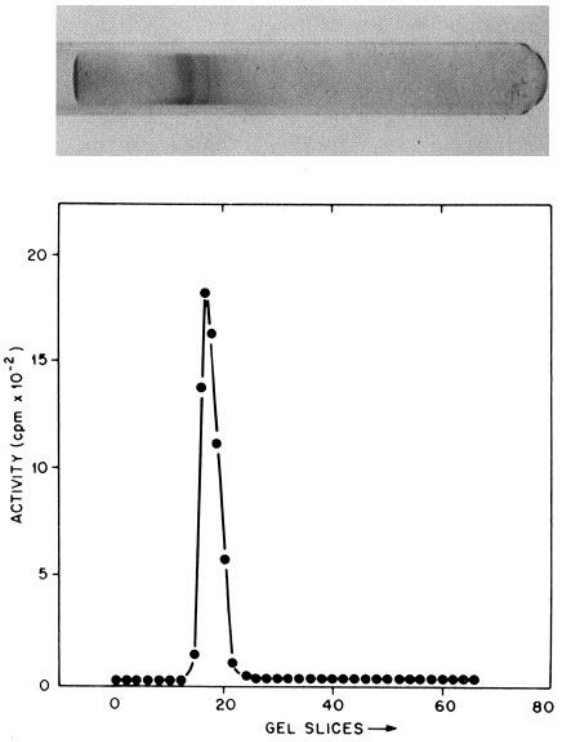

FIG. 4. Polyacrylamide gel electrophoresis of Sephadex G-200 fraction. Two polyacrylamide gels were prepared according to the procedure of Davis (27), and 0.1 ml of Fraction 29 (of the Sephadex G-200 fraction) was layered on each gel. Electrophoresis was carried out for 5 hours at 2.5 ma per gel. One gel was stained with Coomassie blue, destained by diffusion, and scanned in a microdensitometer. The other gel was sliced and the slices were eluted with 0.1 ml of incubation mixture without substrate for 30 min at 0°, then incubated with substrate for 1 hour at 37°. The rest of the procedure is the same as the usual assay described under "Materials and Methods."

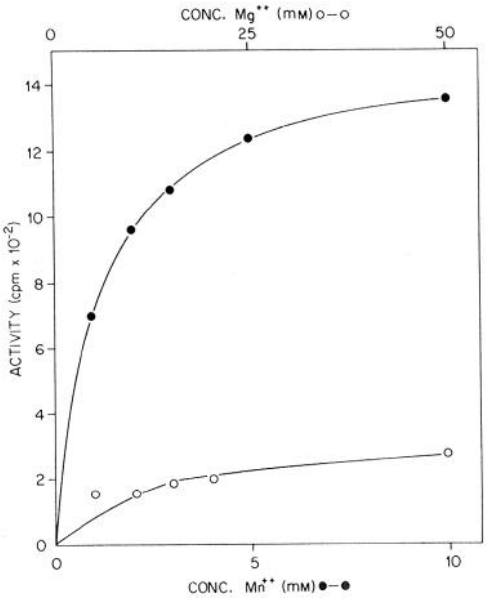

FIG. 5. Effect of divalent cations on the activity of the enzyme. The conditions of incubation were the same as described under "Materials and Methods" except that varying amounts of Mn^{2+} and Mg^{2+} were added to the incubation mixture. Purified dialyzed enzyme (0.15 μg of protein) was used in each assay.

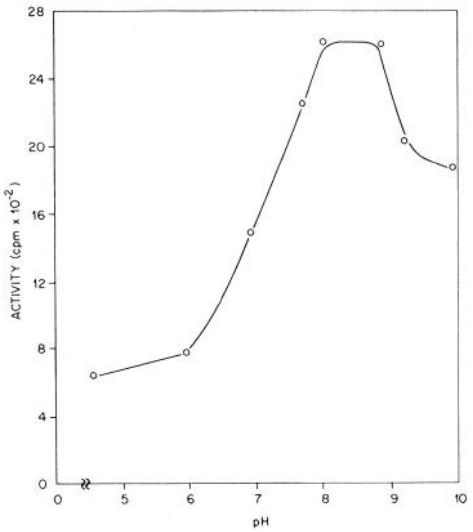

FIG. 6. Effect of pH on the oligoribonuclease activity. The buffers used were succinate-KOH (pH 4.6), citrate-KOH (pH 6.0), Tris-HCl (pH 7.1 to 9.0), and glycine-KOH (pH 10). Incubation conditions were the same as described in Fig. 5 except that $(pA)_2$ was used as substrate, and that when Mg^{2+} was used, enzyme concentration was 10-fold higher. See text for details.

optimum between 8 and 9. At pH 7.0 and 9.5, 50 and 70%, respectively, of the optimum rates were obtained. The effect of pH was studied in the presence of 50 mM Mg^{2+}, because $MnCl_2$ at pH 9 causes precipitation of $Mn(OH)_2$ which interferes with the reaction.

Proportionality of Activity with Amount of Enzyme Protein—From Fig. 7 it is clear that the rate of the enzyme reaction is proportional to the amount of enzyme added, even up to 60 to 70% hydrolysis of the substrate. With substrates of higher chain length, the rate of reaction gradually increases with increasing protein concentration. This phenomenon will be discussed in detail in the accompanying paper (29).

Thermostability of Enzyme—The purified enzyme is quite stable to heat treatment, which makes it unique from other

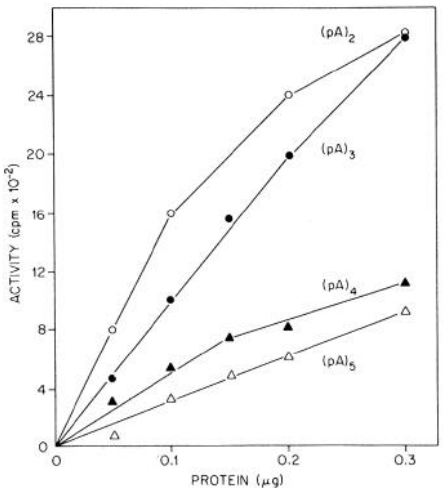

FIG. 7. Proportionality of the reaction. The assay conditions were as described under "Materials and Methods" except that increasing amounts of enzyme were added and different substrates were used, as indicated in the figure.

existing ribonucleases of *E. coli*. As shown in Fig. 8, incubation of the enzyme even at 100° for 5 to 10 min is not sufficient for complete inactivation. Heat inactivation curves at 65°, 70°, and 100° show that the half-life of the enzyme at these temperatures is around 60, 10, and 2.5 min, respectively. In contrast, RNase II of *E. coli* is completely inactivated in 5 min at 45° (24).

Temperature Optimum of Enzyme—The unusual thermostability of the enzyme led us to check the optimum temperature of the reaction. As expected, the temperature optimum of the enzyme is quite high—around 50° (Fig. 9).

DISCUSSION

A nuclease that degrades oligoribonucleotides has been isolated from *Escherichia coli*. This nuclease, which we have called oligoribonuclease, is most probably in the cytoplasm; it appears to be highly specific for short oligoribonucleotides. The enzyme has been purified over 200-fold. Polyacrylamide gel electrophoresis at pH 8.3 shows three bands in the purified fraction of the enzyme preparation (Fig. 4). The molecular weight of the enzyme has been determined to be around 38,000.[1]

This oligoribonuclease has several novel properties which distinguish it from other nucleases of *E. coli*, including its mode of cleavage and specificity.

RNase I (an *E. coli* endonuclease) is absent from the purified fraction of this enzyme, as shown by this preparation's inability to solubilize single-stranded RNAs including poly(A), poly(U), and poly(C), which are susceptible to RNase I digestion. In addition RNase I does not have a requirement for divalent cations, whereas this enzyme *in vitro* needs Mn^{2+} for its optimum activity.

The enzyme activity described here differs from that of RNase II, since purified RNase II does not attack short oligoribonucleotides such as $(pA)_2$ and $(pA)_3$ (30). Nossal and Singer have shown that oligonucleotides of the type $(pA)_n$ are hydrolyzed if n is 7 or greater (30). They have also demonstrated that oligonucleotides of chain length shorter than 7 can be hydrolyzed by RNase II provided the concentration of the oligoribonucleotides in the incubation mixture is sufficiently high (220 μM and higher). The concentration of oligoribonu-

[1] S. K. Niyogi and A. K. Datta, unpublished results.

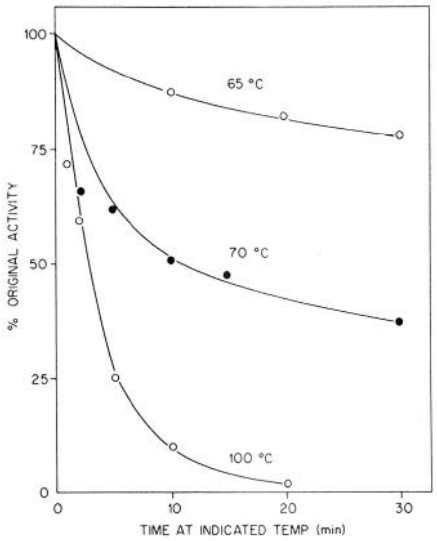

FIG. 8. Effect of temperature on the stability of the enzyme. One-tenth milliliter of Fraction 29 from Sephadex G-200 was heated at indicated temperatures in different batches. Samples were removed and assayed for enzyme activity in the standard incubation mixture described under "Materials and Methods."

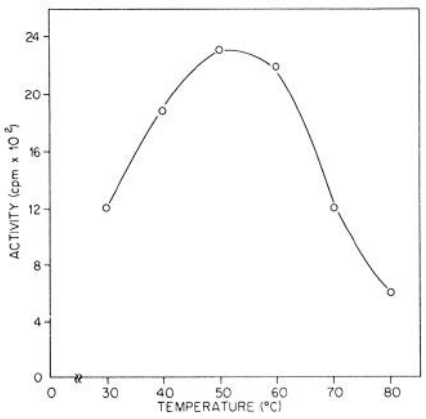

FIG. 9. Temperature optimum of the enzyme. Incubations were carried out at the indicated temperature. The other conditions of incubation were the same as described under "Materials and Methods." Purified enzyme (0.25 μg of protein) was used in each incubation.

cleotides used in the present studies was considerably less (60 μM) than that used by Nossal and Singer (30). The oligoribonuclease activity can be detected even at oligoribonucleotide concentrations of 10 to 20 μM. There are several other properties that make the enzyme different from RNase II. One of them is the requirement of cations. It is well known (4) that RNase II has a strong dependence on Mg^{2+} and K^{+} for its activity. But the oligoribonuclease prefers Mn^{2+} over Mg^{2+} in the divalent cation requirement, as illustrated in Fig. 5. In addition, the enzyme does not require monovalent cation for its activity, and the addition of monovalent cation does not affect its activity. There is another difference, which is perhaps the most convincing, and that is the effect of high temperature on the activity of the enzyme. Singer and Tolbert (24) have demonstrated that RNase II is very sensitive to heat treatment; incubation of the enzyme at 45° for 5 min almost completely destroys its activity. We have also observed the same phenomenon with RNase II. But from Fig. 8 it is quite evident that the oligoribonuclease is not at all inactivated at this temperature. On the contrary, the activity of the oligoribonuclease is remarkably thermostable even at 65° (Fig. 8), and the optimum temperature is found to be about 50° (Fig. 9). The enzyme can also be separated from RNase II by using hydroxylapatite, and we have already taken advantage of this step to separate and purify our enzyme from RNase II (see "Purification Procedure").

The oligoribonuclease can also be differentiated from RNase III and RNase H, as it will be shown in the accompanying paper (29) that double-stranded structures are inhibitory to the activity of the enzyme.

The specific mode of action of this enzyme rules out its identity with other ribonucleases like RNase P (12), RNase IV (11) or polynucleotide phosphorylase (31).

In the accompanying paper, detailed substrate specificities, product analysis, and mode of action of the enzyme are presented.

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