Purification of a Potassium Ion–Activated RNA, 5′-Phosphodiesterase from *Lactobacillus casei*

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The ribosome fraction of disrupted *Lactobacillus casei* cells contained enzyme activity which degraded RNA to nucleoside-5′-monophosphate. This activity was stimulated by potassium ions. Purification of the enzyme activity was achieved by its extraction into solution at *p*H 8.0, ammonium sulfate fractionation, and adsorption and elution from alumina. The purified preparation required potassium ions, exhibited both endonuclease and exonuclease functions, and was free of enzymes which degraded DNA, nucleoside-3′-phosphates, and glycerophosphate. A trace of 5′-nucleotidase was present. Kinetic studies showed that a polynucleotide fraction of 12–24 nucleotide chain length accumulated more rapidly than nucleoside-5′-monophosphates in the early stages of degradation of RNA by the purified enzyme.

Nucleases and phosphodiesterases in the ribosomal fractions of bacteria have been shown to degrade endogenous RNA to nucleoside-3′-, and -5′-phosphates (Elson, 1959; Sekiguchi and Cohen, 1963; Wade and Lovett, 1961). *Lactobacillum casei* ribosomes contain enzyme activity which degrades RNA exclusively to nucleoside-5′-monophosphates, as reported in this paper—a reaction that is stimulated by potassium ions in the crude state, and that is dependent on potassium ions in the freshly prepared purified enzyme. A similar activity, in a soluble fraction prepared from *E. coli* has been briefly described recently (Spahr and Schlessinger, 1963).

Materials and Methods

L. casei 7469 was obtained from the American Type Culture Collection, and was grown in the folic acid assay medium supplied by Baltimore Biological Laboratories. Cultures were incubated at 37° for 16 hours and the cells were collected by centrifugation in a Sharples centrifuge. Bacterial cells were disrupted with glass beads in the Nossal apparatus manufactured by the machine shop of Western Reserve University, and by sonic disintegration in the Branson Sonifier (Branson Instruments Co., Stamford, Conn.). Sonic disintegration of cells resulted in ribosome fractions of low particle weight, as evidenced by ultracentrifugal analysis and in the extensive solubilization of RNA and phosphodiesterase activity. Therefore, to characterize the distribution of RNA-5′-phosphodiesterase activity in the cell, the glass-bead disruption method was employed. Because of the ease of processing large amounts of cells by sonic disintegration, this technique was used to prepare extracts for purification of enzyme activity.

Glass-Bead Method.—Two-g portions of freshly harvested cells were placed in stainless steel capsules with 7.0 ml of Superbrite 120 glass beads (Minnesota Mining and Manufacturing Co.) and 9.0 ml of 0.001 M Tris buffer, *p*H 7.4, 0.001 M magnesium acetate. The capsules were chilled in ice and then shaken in the Nossal apparatus for three 20-second periods, with cooling to 0° between each operation. The preparation was then centrifuged three times at 3000 × *g* for 10 minutes, and the supernatant fractions were collected. Particles in the supernatant fraction were separated by centrifugation at 10,000 × *g* for 30 minutes, 30,000 × *g* for 30 minutes, and 105,000 × *g* for 60 minutes. Each particulate fraction was suspended in 2 ml of 0.001 M Tris buffer, *p*H 7.4, 0.001 M magnesium acetate. The *p*H of the particle suspension was adjusted to 8 by the addition of a small amount of 1 N NaOH and the suspension was allowed to stand at 4° for 15 minutes. Each suspension was then centrifuged at 105,000 × *g* for 60 minutes and the pellet was re-extracted at *p*H 8.0 in the same manner. The extracts of the 30,000 and 105,000 × *g* particles contained over 80% of the total particulate-RNA and phosphodiesterase activity.

Sonic Disintegration.—Twelve g of cells was disrupted in 2-g portions, each of which was suspended in 20 ml of 0.03 M potassium phosphate buffer at *p*H 8.0, containing 0.001 M mercaptoethanol. The suspension was placed in an ice-ethanol bath and subjected to four 2-minute periods of sonication at 6 amp with intermittent cooling to 0°–2°. The combined extracts were centrifuged at 6000 × *g* at 4° for 15 minutes. The supernatant fraction was adjusted to *p*H 8.0 with 1 N KOH, and the mixture was incubated at 37° for about 2 hours. The *p*H of the mixture was maintained by addition of small amounts of KOH, and aliquots were assayed for production of acid-soluble nucleotide by ultraviolet absorption at 260 mμ. When the production of acid-soluble nucleotides had ceased, the autolysate was centrifuged at 78,410 × *g* for 60 minutes at 4°. All subsequent operations were conducted at 4° unless otherwise stated. The supernatant fraction was adjusted to *p*H 7.5 with 1 N KOH, and ammonium sulfate was added in small portions with stirring to achieve a final concentration of 39 g/100 ml. After centrifugation at 34,000 × *g* for 20 minutes, the supernatant fraction was discarded and the precipitate was dissolved in 120 ml of 0.005 M sodium acetate buffer, *p*H 5.0, containing 0.001 M mercaptoethanol. A 40-ml portion of this solution was brought to 0.3 saturation by the addition of 7.04 g of ammonium sulfate, and was centrifuged at 34,000 × *g* for 20 minutes. The precipitate was discarded. The supernatant fraction was brought to 0.4 saturation by the addition of 2.48 g of ammonium sulfate over a 10-minute period, and then centrifuged at 34,000 × *g* for 20 minutes. The precipitate, which contained most of the phosphodiesterase activity, was dissolved in 5 ml of 0.005 M sodium acetate, *p*H 5.0, 0.001 M mercaptoethanol. This solution was immersed in a water bath at 50° for 5 minutes. The tube was agitated gently at 1, 3, and 5

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TABLE I
PURIFICATION OF *L. casei* PHOSPHODIESTERASE

Step	Volume (ml)	Activity (units/ml)	Specific Activity (units/mg protein)	Purification (-fold)	Yield (%)
(1) Extract	92.5	284.8	13.7	1	100
(2) Centrifugation	100.0	182.4	18.0	1.3	68.2
(3) $(NH_4)_2SO_4$ fractionation I	40.0	524.0	25.0	1.8	78.0
(4) $(NH_4)_2SO_4$ fractionation II	5.0	2024.0	41.4	3.0	38.4
(5) Heat treatment	4.0	1500.0	135.2	9.9	25.8
(6) Alumina Cγ gel	5.0	384.0	391.8	28.6	7.3[a]

[a] Actually 13.0% of the original activity was obtained since 1500 units were utilized during assays and protein determinations.

minutes, removed, and cooled in ice for 10 minutes. The precipitate, which was removed by centrifugation at 34,000 × *g* for 20 minutes, was discarded. Heat treatment resulted in a 9% loss of phosphodiesterase activity and a 3-fold increase in activity per mg of protein in the supernatant fraction. Further purification of the enzyme activity was achieved by the addition of one volume of alumina Cγ gel (Sigma) in an amount equivalent to 50 mg wet wt of gel per ml of solution. The *p*H of the mixture was adjusted to 5.0 with 1 M acetic acid, and the solution was gently stirred. The gel was separated by centrifugation at 18,000 × *g* for 10 minutes, and the supernatant fraction was discarded. The enzyme activity was eluted from the gel when the gel had remained for a period of 15 minutes at 0° in a solution of 5.0 ml of 1 M Tris buffer, *p*H 8.0, which contained 0.6 M ammonium sulfate. Centrifugation at 18,000 × *g* for 15 minutes yielded a supernatant fraction which contained the enzyme. The purified enzyme was stored at −10° in 1.5-ml aliquots. The purification of the 5′-phosphodiesterase activity resulted in preparations which retained 7–20% of the activity of the original extract, contained about 1 mg of protein per ml, and yielded an overall purification in several preparations of 25- to 30-fold. The preparation had an $A_{280/260}$ ratio of 1.54. The purification procedure, which is summarized in Table I, yielded a preparation which could be stored for over 6 months at 10° with only a small decrease of activity. However, as described below, the potassium-ion requirement for activity was replaced by NH_4^+ on storage in the presence of ammonium sulfate.

Preparation of Substrates.—*Escherichia coli* and *L. casei* RNA were prepared by the phenol extraction method (Hoagland *et al.*, 1958) from ribosomal and soluble fractions of the bacterial cells. For routine studies of enzyme activity, total RNA was prepared by the phenol method (Hoagland *et al.*, 1958) from the 15,000 × *g* supernatant fraction of sonically disrupted *E. coli* cells. Preparations of RNA were dialyzed to remove low-molecular-weight components. (As shown in Fig. 3, ribosomal and soluble RNA's of *E. coli* and *L. casei* were degraded at comparable rates.) Mammalian RNA was isolated from rat liver by the procedure of Harshaw *et al.* (1962). Yeast RNA was obtained from the Worthington Biochemical Corp.

Assay of Phosphodiesterase Activity.—Phosphodiesterase activity was assayed spectrophotometrically by the formation of acid-soluble components from RNA, and by identification of the mononucleotides which were produced. When purified phosphodiesterase preparations were employed, the assay mixture contained 0.05 ml of enzyme, 1.0 ml 0.1 M Tris buffer, *p*H 8.0, 0.15 ml of RNA which contained 25 A_{260} units per ml, 0.2 ml of 2.5 M KCl, and distilled water sufficient to give a final volume of 2.5 ml. Incubations were conducted at 37°, and 0.2-ml aliquots were removed from the mixture at intervals, cooled in ice, and precipitated with 3.0 ml 1 M $HClO_4$ at 0°. After centrifugation at 18,000 × *g* for 10 minutes the supernatant fluid was transferred to a cuvet, and the absorption of ultraviolet light at 260 mμ was measured in the Beckman spectrophotometer. As shown in Figure 3, the rate of formation of acid-soluble products from RNA was fairly linear over the first 20 minutes of incubation when *E. coli* RNA was the substrate. Phosphodiesterase activity was determined as the increment of absorption at 260 mμ × 10^3 in a 20-minute period of incubation. Specific

TABLE II
END PRODUCTS OF ENZYMIC DEGRADATION OF RNA

Substrate	Per Cent Conversion Acid-Soluble Fraction[a]	Per Cent Recovered as 5′-Nucleotide[b]	Molar Ratio[c]	
L. casei RNA[d] in ribosome	86	91	CMP	0.67
			AMP	1.00
			GMP	1.40
			UMP	0.84
Purified *L. casei*[e] ribosomal RNA	94	92	CMP	0.88
			AMP	1.00
			GMP	1.90
			UMP	1.20
Purified *E. coli*[f] ribosomal RNA	94	90	CMP	1.04
			AMP	1.00
			GMP	1.25
			UMP	0.70
E. coli DNA[g]	0	0		

[a] Based on A_{260} of alkaline hydrolysate. [b] Chromatographic analysis on Dowex-1 (formate). [c] Ratio of *L. casei* RNA on alkaline hydrolysis, C, A, G, U: 0.93, 1.0, 1.7, 1.3; *E. coli* RNA, C, A, G, U: 0.98, 1.0, 1.30, 0.80. [d] An *L. casei* ribosomal suspension (16.9 ml) incubated for 40 minutes at 37° in 42.5 ml 0.05 M potassium phosphate buffer, *p*H 7.4. [e] Ribosomal phosphodiesterase (6.0 ml) (2.46 mg protein/ml) incubated for 90 minutes at 37° with 5.0 ml purified *L. casei* ribosomal RNA (120 A_{260} units) in 50.0 ml 0.1 M Tris buffer, *p*H 8.0, containing 3 mmoles of KCl. Volume of reaction mixture = 63.0 ml. [f] Purified ribosomal phosphodiesterase (1.25 ml) (specific activity 391 units/mg protein, 1 mg protein/ml) incubated for 360 minutes at 37° with 1.5 ml purified *E. coli* ribosomal RNA (316 A_{260} units) in 5.0 ml 0.1 M Tris buffer, *p*H 8.0, containing 1.2 mmoles of KCl. Volume of reaction mixture = 12.5 ml. [g] Crude ribosomal phosphodiesterase (5.0 ml) (2.46 mg protein/ml) incubated for 120 minutes at 37° with 5.0 ml of *E. coli* DNA (112 A_{260} units) in 50.0 ml 0.1 M Tris buffer, *p*H 8.0, containing 3 mmoles of KCl. Volume of reaction mixture = 63.0 ml. No acid-soluble product was formed when the DNA was heated to 100° for 5 minutes and rapidly cooled to 4° before a similar incubation.

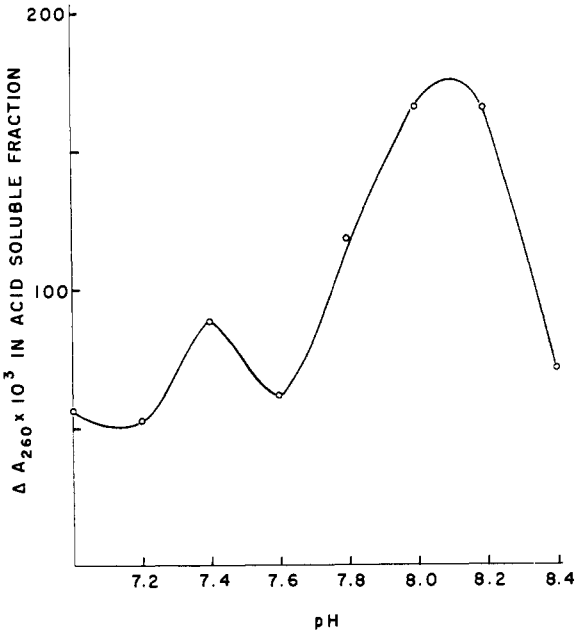

FIG. 1.—Phosphodiesterase activity as a function of *p*H The reaction mixture contained 0.05 ml of purified phosphodiesterase (specific activity of 452.4 units/mg of protein, 2.2 mg protein/ml), 0.15 ml of *E. coli* RNA (160 A_{260}/ml), 0.20 ml of 2.5 M KCl, and 1.0 ml of 0.1 M Tris-HCl buffer at indicated *p*H. Final volume, 2.5 ml. Incubation was at 37° for 20 minutes. The *p*H-activity curve sharply decreased below *p*H 7.0 in acetate and phosphate buffers.

NH_4Cl at 0°, adsorbed nucleotides were eluted from the charcoal with 60% (v/v) ethanol containing 2% (v/v) concentrated NH_4OH (specific gravity 0.90) in the proportions 3 ml ammoniacal ethanol to 20 mg Norit A. Ethanol and ammonia were removed *in vacuo* at 30°. The migration of the nucleotides was determined on Whatman No. 1 paper after descending chromatography in the following solvents: (a) 7 volumes ethanol–3 volumes 1.0 M ammonium acetate, *p*H 7.5, containing 0.001 M EDTA (Pabst Laboratories, 1956), (b) 7 volumes ethanol–3 volumes 1.0 M ammonium acetate, *p*H 9.0, saturated with $Na_2B_4O_7$ (Klenow and Lichtler, 1957) and 0.001 M with respect to EDTA; and (c) 100 ml isobutyric acid–55.8 ml water–4.2 ml concd ammonium hydroxide (specific gravity 0.90)–1.6 ml 0.1 M EDTA (Keir and Smellie, 1959). Finally, the position of the phosphomonoester group was established by ability of 5′-nucleotidase prepared from bull seminal plasma to remove the nucleotide phosphorus (Heppel and Hilmoe, 1955; Lehman and Pratt, 1960), and by the reaction of nucleotides on paper chromatograms with the periodate-Schiff's spray reagent (Baddiley, *et al.*, 1956).

Protein was estimated by the method of Lowry *et al.* (1951), and ribose by the method of Mejbaum (1939). Inorganic phosphate was determined by the techniques of Dryer *et al.* (1957), Kolb *et al.* (1963), and Fiske and Subbarow (1925).

Purified alkaline phosphatase of *E. coli* was purchased from Worthington Chemical Corp. and was employed to remove phosphomonoester end groups as described by Fiers and Khorana (1963).

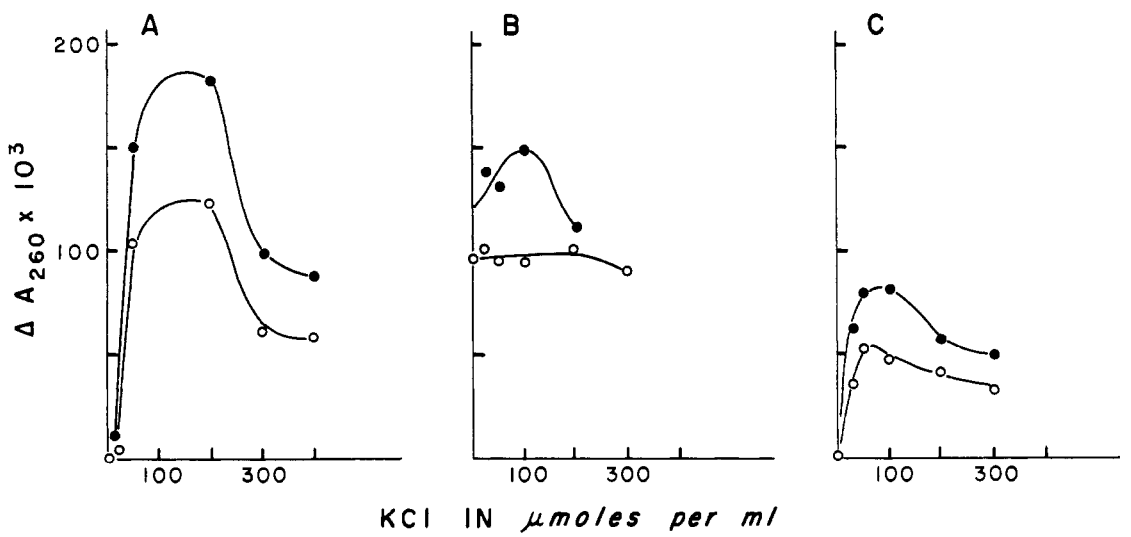

FIG. 2.—The effect of potassium-ion stimulation at *p*H 8.0 (●) and *p*H 7.4 (○) on purified phosphodiesterase activity. (A) Freshly prepared enzyme stored for 24 hours at −10° in 0.6 M $(NH_4)_2SO_4$; (B) stored under the same conditions for 7 days; and (C) after Sephadex G-25 treatment to remove ammonium sulfate from the enzyme preparation which had been stored for 7 days. The reaction mixture contained 0.10 ml of purified phosphodiesterase (2.2 mg protein/ml, specific activity 452 units/mg protein), 0.15 ml of *E. coli* RNA (160.5 A_{260}/ml), 0.20 ml of KCl, and 1.0 ml 0.1 M Tris-HCl buffer *p*H 8.0 or *p*H 7.4. Final volume, 2.5 ml. Incubation was at 37° for 20 minutes.

activity of the purified enzyme was expressed as $A_{260} \times 10^3$ per mg of protein under the foregoing conditions.

Identification of End Products of the Enzyme Activity.—Acid-soluble products of the enzymatic degradation of RNA were fractionated on columns of Dowex-1 resin, 20 cm long by 1 cm diameter, in the formate form (Pabst Laboratories, 1956). The nucleotides were identified as follows: The ultraviolet-absorption spectra were measured at *p*H 1 and 13 (Harshaw *et al.*, 1962; Beaven *et al.*, 1955) before and after treatment with activated charcoal. After three washes with 3 ml 0.1 M

RESULTS

The ribosome fractions prepared from *L. casei* cells by glass-bead disruption exhibited a slow formation of acid soluble components from endogenous RNA when incubated at 37° in 0.05 M Tris buffer, *p*H 7.45. The addition of potassium ions to the incubation mixture greatly accelerated the formation of acid-soluble products. Upon extracting the particles with dilute NaOH at *p*H 8.0 and 4°, the RNA was solubilized and most of the enzymatic activity which degraded RNA appeared

in the soluble fraction. The hydrolysis of RNA in this soluble fraction was also stimulated by K^+, the maximum effect being observed at 0.16 M. Both the particulate and the solubilized fractions exhibited two *p*H optima, a major peak at *p*H 8.0 and a smaller peak at *p*H 7.4. Other cations, including Mg^{2+}, Ca^{2+}, Na^+, Co^{2+}, Fe^{3+}, and NH_4^+ were without effect, except that Mg^{2+} at concentrations over 0.01 M inhibited the potassium-stimulated enzyme activity in the solubilized fraction. The presence of phosphate ions did not alter the course of the reactions.

The purified enzyme exhibited the characteristics of the ribosomal and crude solubilized preparations. As shown in Figure 1, the purified-phosphodiesterase activity, measured by formation of acid-soluble products from *E. coli* RNA, had a principal *p*H optimum at 8.0–8.2 and a minor peak at *p*H 7.4. The ratio of the two peaks was the same in the crude and purified preparations. The purified-enzyme activity required potassium ions, and the maximum concentration for activation was approximately 150 μmoles/ml at both *p*H 7.4 and 8.0 (Fig. 2). In contrast to the crude preparations of phosphodiesterase activity, the potassium-ion requirement of the purified enzyme could be replaced by NH_4^+ upon storage in 0.6 M ammonium sulfate at −10° for several days, as shown in part B of Figure 2. Removal of ammonium sulfate by Sephadex treatment decreased the specific activity of the enzyme, but restored the potassium-ion requirement (Fig. 2C).

Purified or crude ribosomal phosphodiesterase hydrolyzed *L. casei* or *E. coli* RNA to nucleoside-5′-monophosphates, and the conversion was 85–95% complete on prolonged incubation (Table II). The *p*H of the medium did not significantly influence the molar composition of the product, or the extent of degradation, when incubation at *p*H 7.4 and 8.0 were compared. Crude preparations of the enzyme did not degrade nucleotides, as shown by the recovery of nucleoside-5′-monophosphates and lack of formation of nucleosides after chromatographic separation of incubation mixtures on Dowex-1 (formate) columns. The amount of phosphatase activity for several substrates incubated with the purified enzyme preparation is shown in Table III. A small amount of AMP-5′-nucleotidase was found in the purified preparation, but other monophosphoesters which were tested as substrates were not degraded to a significant extent.

TABLE III

PHOSPHATASE ACTIVITY IN *L. casei* PHOSPHODIESTERASE

Substrate	Enzyme[a]	Inorganic Phosphorus (μg) 30 min	60 min
3′AMP	*E. coli* alkaline phosphatase	385.0	523.0
3′AMP	*L. casei* phosphodiesterase	0.6	1.8
5′AMP	*L. casei* phosphodiesterase	6.0	12.5
p-Nitrophenylphosphate	*L. casei* phosphodiesterase	0.0	0.0
Glycerophosphate[b]	*L. casei* phosphodiesterase	0.5	1.3

[a] The reaction mixture contained 1.0 ml of 0.1 M Tris-HCl, *p*H 8.0, 10 μmoles of substrate, and 0.25 ml of a 1:100 dilution of *E. coli* alkaline phosphatase (Worthington, 12 units/mg protein) or 0.05 ml of purified *L. casei* phosphodiesterase (452 units/mg protein, 2.2 mg protein/ml) in 2.5 ml. After incubation at 37°, aliquots were removed at indicated times and inorganic phosphorus was determined by the technique of Kolb *et al.* (1963). The presence of 0.005 M Mg^{2+} did not increase the nucleotidase or glycerophosphatase activity. [b] DL-α-Disodium salt; 6 H_2O.

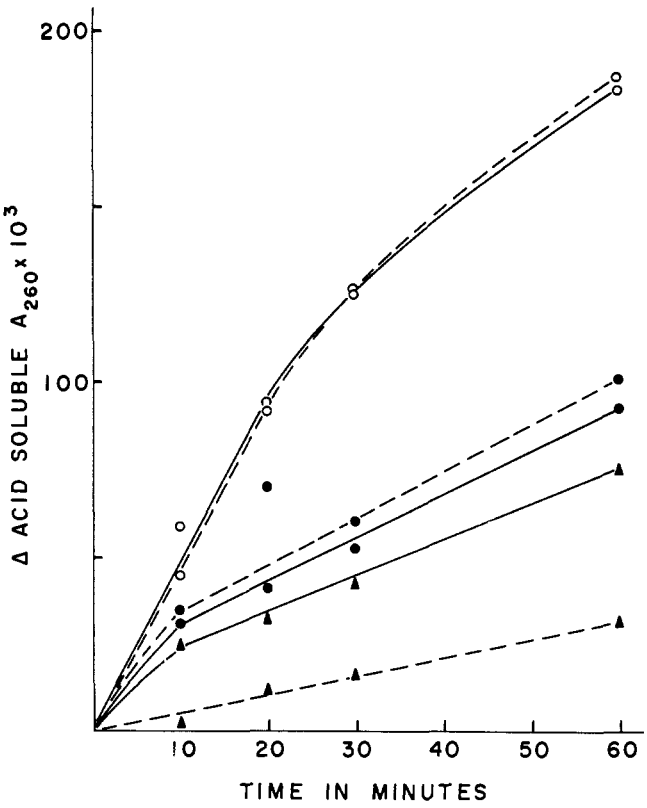

FIG. 3.—Degradation of ribosomal and soluble RNA by purified phosphodiesterase. The reaction mixture contained 0.05 ml of phosphodiesterase (specific activity of 452 units/mg protein, 12.2 mg protein/ml), 0.20 ml 2.5 M KCl, 1.0 ml of 0.1 M Tris buffer, *p*H 8.0, and 10.25 A_{260} units of RNA. *E. coli* ribosomal RNA, O—O; *E. coli* soluble RNA, O- - - -O; *L. casei* ribosomal RNA, ●—●; *L. casei* soluble RNA, ●- - - -●; rat liver ribosomal RNA, ▲—▲; rat liver soluble RNA, ▲- - - -▲. Total volume of incubation mixture was 2.5 ml; incubation was at 37°.

The phosphodiesterase preparations did not degrade native or heat-denatured *E. coli* or thymus DNA to to mononucleotides or acid-soluble products. Treatment of DNA with DNAase and the purified phosphodiesterase enzyme did not lead to the formation of deoxynucleoside monophosphates. There was no evidence for RNA polynucleotide phosphorylase in the crude or purified phosphodiesterase preparation assayed by the method of Grunberg-Manago *et al.* (1956).

Although a definitive mechanism of action for *L. casei* ribosomal phosphodiesterase has not been established, some unique characteristics of its action on RNA have been determined. A comparison of the rates of degradation of RNA from several sources indicated that *E. coli* RNA was attacked most rapidly (Fig. 3). Soluble and ribosomal RNAs were degraded at equal rates in the case of samples prepared from *E. coli* and *L. casei*. Liver soluble RNA was degraded much less rapidly than the high-molecular-weight RNA prepared from liver by the streptomycin precipitation method (Harshaw *et al.*, 1962). In the case of *E. coli* RNA, the maximum rate of degradation to acid-soluble components was achieved at a substrate concentration of 587 A_{260} units per mg of purified enzyme protein under the conditions described in the legend of Figure 3, while for rat liver RNA the comparable value was 30 A_{260} units.

Yeast RNA, which was obtained from Worthington Chemical Corp. (lot 6154), was not degraded to mono-

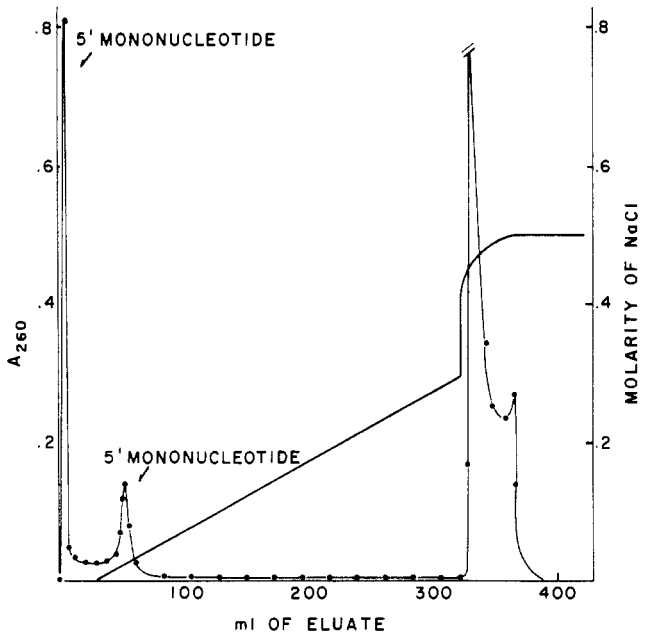

FIG. 4.—DEAE-cellulose chromatography of a 10-minute period of phosphodiesterase degradation of *E. coli* RNA. The column (10.0 cm × 1.0 cm, diameter) was loaded with a mixture containing 1.0 ml 0.1 M Tris-HCl, *p*H 8.0, 0.40 ml of RNA (161.0 A_{260} units), 0.20 ml of 2.5 M KCl, and 0.05 ml phosphodiesterase (specific activity 452.4 units/mg protein, 2.2 mg protein/ml). Total volume was 2.5 mls. A 3.0-ml fraction was collected every 5 minutes. Recovery of ultraviolet-absorbing material from the column was 22.1 A_{260} units.

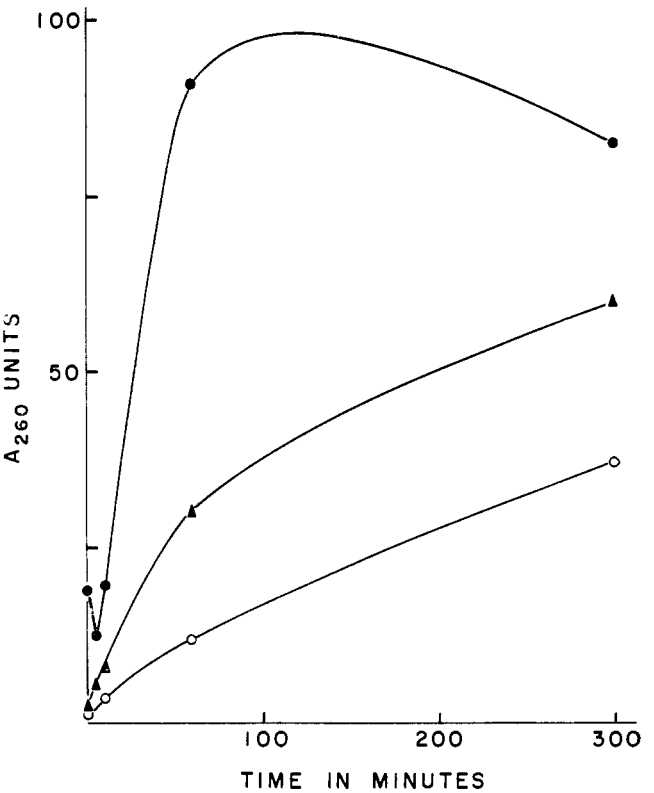

FIG. 5.—Analysis of products of *E. coli* RNA degradation by purified phosphodiesterase after various periods of incubation. The incubation mixture contained 1.0 ml of 0.1 M Tris-HCl buffer at *p*H 8.0, 0.40 ml of *E. coli* RNA (A_{260} = 161), 0.20 ml of 2.5 ml of 2.5 M KCl, and 0.05 ml of phosphodiesterase (2.2 mg protein per ml, specific activity of 452.4 units/mg protein), and 1 ml of H_2O. The mononucleotide product (O) and the 0.3–0.5 M NaCl polynucleotide fraction (●) were determined by the DEAE-cellulose chromatographic procedure. The acid-soluble ultraviolet absorbing material in the reaction mixture (△) was measured as total A_{260} based on a 0.2-ml aliquot which was removed at each period of incubation. Separate incubation mixtures of the above composition were analyzed at 0, 2, 10, 60, and 300 minutes.

nucleotides by the purified phosphodiesterase. This preparation of yeast RNA contained a high proportion of low-molecular-weight oligonucleotides when analyzed by the DEAE-cellulose-column chromatographic procedure of Tomlinson and Tener (1963). The oligonucleotide fraction containing three to five nucleotide residues was concentrated by adsorption on charcoal and elution with ethanol containing ammonium hydroxide. After removal of the solvent, the oligonucleotide fraction and the unfractionated yeast RNA were treated with purified *E. coli* phosphomonoesterase in order to remove phosphomonoester end groups, as described by Fiers and Khorana (1963). Prolonged incubation of the oligonucleotides and yeast RNA, either before or after phosphomonoesterase treatment, with the purified phosphodiesterase in the presence of potassium ions did not yield nucleoside monophosphates. The addition of the oligonucleotide fraction or the heterogeneous yeast RNA, either before or after phosphomonoesterase treatment, did not inhibit the formation of nucleoside-5′-monophosphates from *E. coli* RNA by purified phosphodiesterase when equal amounts (A_{260}) of *E. coli*–RNA and yeast-RNA fractions were employed.

The rate of formation of mononucleotides and low-molecular-weight polynucleotides during incubation of *E. coli* RNA with the purified phosphodiesterase was determined by the DEAE-cellulose chromatographic procedure of Tomlinson and Tener (1960). Rate-limiting concentrations of enzyme were employed and portions of the incubation mixture were analyzed by column chromatography at various times. The elution diagram for the 10-minute incubation period is shown in Figure 4. During this period nucleoside-5′-monophosphates were formed and a large amount of polynucleotide fraction corresponding to the region of 12–24 nucleotide residues was recovered. No intermediate oligonucleotide fractions (2–12 residues) were detected in this period, nor were they found in similar analyses conducted after periods of incubation up to 300 minutes. When the formation of nucleoside-5′-monophosphates, acid-soluble components, and the polynucleotide fraction was determined at various times, the data illustrated in Figure 5 were obtained. Analysis of the zero-time mixture showed that *E. coli* RNA contained a small amount of the 12–24-residue polynucleotide fraction which decreased in the early incubation period and then increased to a maximum amount at 60 minutes. The mononucleotide fraction increased throughout the incubation period at a rate slower than that observed for the acid-soluble fraction. The amount of polynucleotide formed greatly exceeded the formation of acid-soluble products and nucleoside monophosphates during the first hour of incubation. Subsequently, the polynucleotide fraction decreased while the mononucleotide and acid-soluble fractions continued to rise. These findings are consistent with the formulation that the 12–24-residue polynucleotide fraction is the first product of the degradation of *E. coli* RNA by the purified phosphodiesterase, and that the subsequent formation of mononucleotides proceeds without the intermediary formation of lower-molecular-weight oligonucleotides. The data of the experiments illustrated in Figures 4 and 5 were obtained from incuba-

tion mixtures at *p*H 8.0. At *p*H 7.4 similar findings were obtained but the rate of formation of products was decreased. The formation of the 12–24-residue polynucleotide fraction, as well as acid-soluble products, did not occur if potassium ions were omitted from the incubation mixture.

Discussion

The characteristic features of the *L. casei* RNA-5′-phosphodiesterase are the requirements for potassium ions, the ability of the preparation to perform both exonuclease and endonuclease functions, and the formation of nucleoside-5′-monophosphates as the terminal product of the degradation of RNA. Although the purification of the enzyme which has been achieved makes it suitable for preparative and analytical use, a more extensive purification would be necessary to determine whether both the exo- and endonuclease activities are performed by a single enzyme. Further purification of the phosphodiesterase was restricted by the instability of the purified enzyme in low-ionic-strength buffers, a finding which limited the application of cellulose-column chromatography to fractionation of the enzyme activity. The two peaks in the *p*H-activity curve of the purified enzyme were found in constant ratio throughout purification procedures and are not considered to be sufficient evidence of two enzyme activities. The evidence available indicates that the reactions at *p*H 7.4 and 8.0 are both dependent on K^+ and both catalyze endo- and exonuclease activity. However, the question will remain unresolved until a homogeneous preparation of the enzyme is available, or until separation of the endonuclease and exonuclease activities is obtained. In general, this is the situation with respect to other bacterial nucleases which have been described.

A possible role of RNA-5′-phosphodiesterase in the degradation of messenger RNA was suggested by Spahr and Schlessinger (1963). The purified *L. casei* enzyme activity degraded both ribosomal and soluble RNA of *E. coli* and *L. casei* to mononucleotides. If the enzyme functions in destruction of messenger RNA, this activity apparently does not depend upon substrate specificity. Other conditions which involve association of enzyme and RNA, activation of the enzyme at particular intracellular sites, or the dissociation of RNA from protecting protein may be invoked to explain a specific function of the enzyme.

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