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PURIFICATION OF AN ALKALINE RIBONUCLEASE FROM SOLUBLE FRACTION OF BEEF BRAIN

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Summary

A ribonuclease (ribonucleate 3'-pyrimidine-oligonucleotidohydrolase, EC 3.1.4.22) was purified 8300-fold from soluble fraction of beef brain and its properties were investigated. The enzyme is an endonuclease capable of hydrolyzing tRNA, rRNA, poly(C), but shows no activity towards poly(U), poly(A), and poly(G). The preparation is free of deoxyribonuclease, non-specific phosphodiesterase and phosphomonoesterase activity. The enzyme has a pH optimum of 7.6, is not heat stable, has a molecular weight of 25 000, and has a K_m of 134 μg rRNA and K_m of 1600 μg poly(C) per ml.

Introduction

A variety of enzymes hydrolyzing RNA exist in all tissues and it seems that the activity, rather than its presence, varies from one tissue to another. The properties and specificity of several of these ribonucleases have been established in some detail.

Two more apparently distinct enzymes from various tissues that hydrolyze RNA have been described by Zytko, de Lamirande, Allard and Cantero [1], Roth [2], Stevens and Reid [3], and Maver and Greco [4]. Activities of these enzymes have been differentiated on the basis of pH optimum and stability to heat and acid, one enzyme being termed alkaline RNAase and the other acid RNAase. Studies on the alkaline RNAase have shown that it is frequently associated with a natural inhibitor which may be destroyed by acid treatment [5], mercuribenzoate [6] or heat [7,8]. The inhibitor has been partially purified [7,8] and appears to be a highly labile protein [9]. For the cytoplasmic

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alkaline RNAase of brain tissue, a 60-fold purification was reported to date. This enzymatic activity was increased by destruction of the natural inhibitor by sulfuric acid [10].

Previously, we reported the purification and properties of beef brain nuclear alkaline RNAase [11]. In this paper we report a purification procedure of a cytoplasmic alkaline RNAase from beef brain which results in high recovery of homogeneous enzyme and purifications of the order of 8300-fold.

Experimental procedure

Chemicals

^{32}P-labeled rRNA was prepared from mice plasmocytoma tumors by extraction with acetate buffer and phenol according to the procedure of Kempf and Mandel [12]. tRNA of mice plasmocytoma tumors was a gift from Dr M. Wintzerith. Homopolyribonucleotides were products of the Sigma Chemical Co. or the Miles Laboratories (U.S.A.). Sephadex was bought from Pharmacia (Sweden), and phosphocellulose and glass fiber paper from Whatman (U.K.), silica gel F_{254} was the product of Merck (G.F.R.). Ampholine was the product of LKB (Sweden).

Assay of enzyme activity

Ribonuclease activity was determined as follows. The reaction mixture contained 60 mM sodium phosphate buffer, pH 7.5, 0.6 mM β-mercaptoethanol, 20% glycerol (v/v), 20 μg rRNA, and an appropriate amount of enzyme in a total volume of 0.25 ml. After incubation at 40° C for 10 min, 2 ml of cold 10% trichloroacetic acid containing 0.02 M sodium pyrophosphate, and 0.1 ml of 1% bovine serum albumin were added. The mixture was kept on ice for 20 min and was filtered on Whatman glass fiber paper and washed 5 times with cold 5% trichloroacetic acid containing 0.02 M sodium pyrophosphate. The filters were then dried in the oven at 110° C for 30 min. The radioactivity of the acid-insoluble fraction was measured in a Packard liquid scintillation spectrometer. One unit of enzyme activity is defined as the amount of enzyme which hydrolyzes 1 μg rRNA to acid-soluble mono- and oligonucleotides in 1 h at 40° C. Specific activity is expressed in units per mg protein. Under the above assay condition, the specific activity of crystalline commercial bovine pancreas RNAase was $12 \cdot 10^6$.

Phosphomonoesterase activity was tested with *p*-nitrophenyl phosphate as substrate according to the method of Bessey, Lowry and Brock [13] in citrate phosphate buffer (pH 5.8), in sodium phosphate buffer (pH 7.0), and in Tris · HCl buffer (pH 8.0). The reaction mixture was incubated for 2.5 h at 40° C.

Phosphodiesterase activity was tested by the method of Sinsheimer and Koerner [14]. Incubation time was 5 h at 40° C.

Deoxyribonuclease activity was assayed by measuring at 260 nm the acid-soluble material liberated from native or denatured DNA according to the method of Bacova, Zelinkova and Zelinka [15].

Acrylamide gel electrophoresis

Electrophoresis was performed by the method of Reisfeld et al. [16] on

10% polyacrylamide gels in β-alanine/acetic acid buffer (pH 4.5). The localization of ribonuclease activity was established by the standard RNAase assay after elution of enzymes from gel slices with 0.05 ml of 0.1 M sodium phosphate buffer (pH 7.5) containing 44% glycerol and 1 mM β-mercaptoethanol for 18 h at 4° C [11].

Isoelectric focusing

To determine the isoelectric point, the method of Catsimpoolas [17] using microisoelectric focusing in a polyacrylamide gel column containing ampholine (LKB) pH 3—10, was employed.

Identification of digestion products of poly(C)

The products of poly(C) digested with purified enzyme were analysed with ascending thin-layer chromatography on Merck silica gel F_{254} using the following solvent system: 0.1 M H_3BO_3/28% NH_4OH/isopropanol (3 : 1 : 6, by vol). The following R_F values are observed in this solvent: 5'-CMP, 0.36; 3'-CMP, 0.43; 2'-CMP, 0.48; cytidine, 0.65; 2',3'-cyclic CMP, 0.73.

Determination of molecular weight

The molecular weight of the enzyme was determined by gel filtration on Sephadex and by polyacrylamide gel electrophoresis with sodium dodecylsulphate. The column of sephadex was calibrated with bovine serum albumin (M_r = 68 000), ovalbumin (M_r = 45 000), chymotrypsinogen (M_r = 25 000), and pancreas ribonuclease (M_r = 13 700) according to the method of Andrews [18]. The polyacrylamide gel electrophoresis with sodium dodecylsulphate was used to determine the molecular weight of the enzyme as well as its subunit structure according to the method of Weber and Osborn [19] and Laemmli [20].

Protein

Protein was determined by the method of Lowry et al. [21] on the 15% trichloroacetic acid precipitate of the samples.

Purification of ribonuclease

Crude extract. All operations were performed at 4° C. Beef brain was immersed in ice immediately on removal from the animal. After removing the meninges, the brains were homogenized in a mixer with 1 l (per brain) of 0.25 M sucrose containing 1 mM EDTA and 10 mM Tris · HCl buffer, pH 7.5 for 2 min. The homogenate was centrifuged either 12 000 × *g* for 90 min in order to remove cell debris, nuclei and mitochondria or at 105 000 × *g* to remove microsomes as well. In both cases the enzyme was present in the supernatant in the same quantity.

$(NH_4)_2SO_4$ fractionation and heating. The supernatant solution was decanted and brought to 50% saturation with solid $(NH_4)_2SO_4$. The mixture was stirred for 60 min and then was centrifuged at 12 000 × *g* for 90 min. After centrifugation, the 50% $(NH_4)_2SO_4$ precipitate was suspended in 200 ml (per brain) of distilled water and stirred overnight. The suspension was heated in a water bath at 70° C with continuous stirring, and the temperature of the sus-

pension was maintained at 70° C for 10 min. After cooling in ice, the turbid preparation was centrifuged at 36 000 × *g* for 180 min, and the precipitate was discarded. The supernatant fraction was dialysed against 1 mM phosphate buffer, pH 6.8, containing 1 mM β-mercaptoethanol and concentrated in visking tubes with solid sucrose. This procedure was successful in eliminating the RNAase inhibitor and increased the specific activity up to 20-fold.

Cellulose phosphate chromatography. A 3.6 × 50 cm cellulose phosphate (Whatman P-11) column was equilibrated with 0.05 M Tris · HCl buffer (pH 7.2) containing 1 mM β-mercaptoethanol and 0.2 M KCl. 582 ml of the heat treated fraction were applied to the column and eluted with a 3000 ml linear KCl gradient (0.2—1.5 M) in equilibrating buffer as shown in Fig. 1. The eluant was collected in 10 ml fractions. Tubes 270—300 (300 ml) which contain the nuclease activity were pooled, and the pooled enzyme was dialyzed for 40 h against 6 l of 1 mM sodium phosphate buffer (pH 6.8) containing 1 mM β-mercaptoethanol with several changes and once with bidistilled water, and lyophilized. Lyophilized enzyme was dissolved in 1.20 ml of 0.1 M sodium phosphate buffer (pH 7.5) containing 44% glycerol and 1 mM β-mercaptoethanol. Insoluble materials, if present, were centrifuged out.

Gel filtration. 1.10 ml of fractions of the cellulose phosphate were applied to a Sephadex G-100 column (2.6 × 44 cm), and eluted with 0.05 M Tris · HCl buffer (pH 7.4) containing 0.5 M KCl, 20% glycerol (by vol.), and 1 mM β-mercaptoethanol as shown in Fig. 2. Fractions 34—44 (33 ml), which contained the

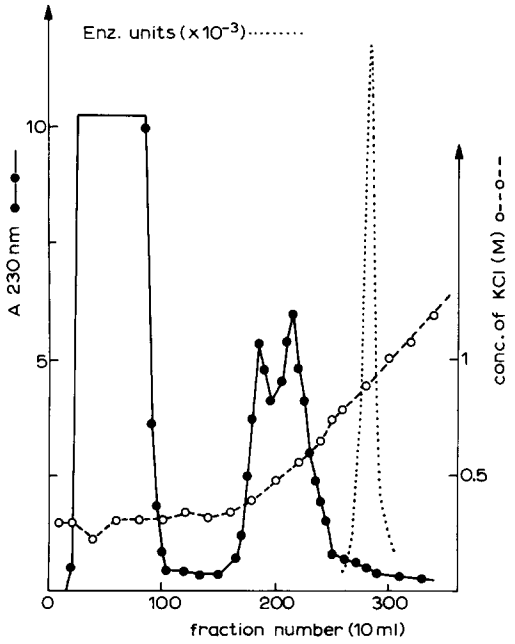

Fig. 1. Cellulose phosphate chromatography of beef brain ribonuclease. Column (3.6 cm × 50 cm for enzyme preparation from 20 beef brains) was equilibrated with 0.05 M Tris · HCl buffer (pH 7.2) containing 0.2 M KCl and 1 mM β-mercaptoethanol. After sample application, the enzyme was eluted with a 0.2—1.5 M KCl gradient. See text for details of sample preparation. The flow rate was 40 ml/h and each fraction contained 10 ml. Protein was measured by absorption at 230 nm. - - - - - -, ribonuclease activity; ●———●, absorbance at 230 nm; o- - - - -o, concentration of KCl.

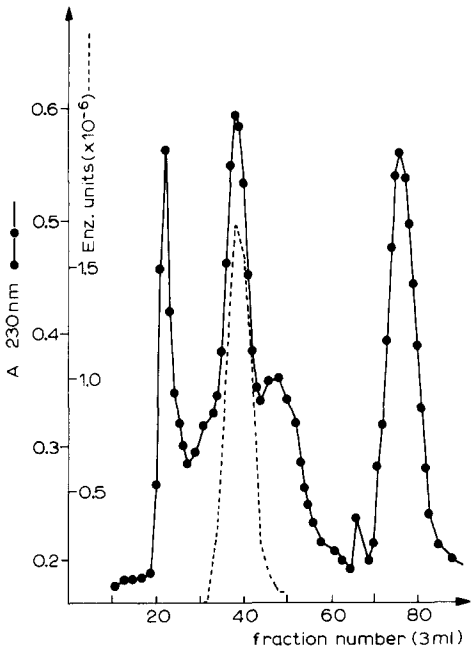

Fig. 2. Sephadex G-100 (I) chromatography of beef brain ribonuclease. Column (2.6 × 44 cm) was equilibrated with 0.05 M Tris · HCl buffer (pH 7.4) containing 20% glycerol (by vol.), 1 mM β-mercaptoethanol and 0.5 M KCl. The flow rate was 15 ml/h and each fraction contained 3 ml : protein was measured by absorption at 230 nm. - - - - - -, ribonuclease activity; ●———●, absorbance at 230 nm.

nuclease activity, were pooled, dialyzed and lyophilized as described above. Rechromatography on Sephadex G-100 was performed under the same conditions. A third filtration was performed in order to eliminate completely the trace contamination. Lyophilized enzyme from the second gel filtration was dissolved in buffer (0.1 M sodium phosphate buffer, pH 7.5, containing 44% glycerol and 1 mM β-mercaptoethanol), applied to a Sephadex G-100 (superfine) column (1.2 × 73 cm), and eluted with 0.05 M Tris · HCl buffer (pH 7.4) containing 0.5 M KCl, 20% (v/v) glycerol and 0.5 mM dithiothreitol. The fractions with ribonuclease activity were pooled and lyophilized after dialysis as described above.

Results

Enzyme purification

The results of a typical purification of ribonuclease from beef brain soluble fraction are summarized in Table I, which is an average of 5 purifications. The data are given for 10 beef brains (about 3 kg of fresh tissue). This procedure gives a 8300-fold purification or more.

Alkaline phosphatase, phosphodiesterase and deoxyribonuclease activities are not detectable in the purified enzyme preparations. These activities disappear after phosphocellulose column chromatography. Purified enzyme stored for several months at −20° C in a solution containing 0.1 M phosphate buffer, 44% glycerol and 1 mM β-mercaptoethanol retained 100% of its activity. Gener-

TABLE I

PURIFICATION OF BEEF BRAIN CYTOPLASMIC ALKALINE RNAase

Fraction	Ribonuclease activity			
	Protein (mg)	Total (units [a] $\times 10^{-3}$)	Specific (units/mg protein $\times 10^{-3}$)	Yield (%)
Cytosol [b]	45 000	120 700	2.6	100
$(NH_4)_2SO_4$, heat	1 230	62 400	50.7	52
Phosphocellulose	20.3	21 220	1 045	18
Sephadex G-100 (I)	2.41	17 160	7 100	14
Sephadex G-100 (II)	0.30	6 560	21 850	5.4
Sephadex G-100 (III)	0.03	1 650	55 000 [c]	1.4

[a] μg RNA hydrolyzed for 1 h at 40° C at pH 7.5.

[b] Only in this fraction, 1.6 mM *p*-chloromercuribenzoate was added in the incubation medium. In the absence of *p*-chloromercuribenzoate, no activity was obtained.

[c] Because of a feeble quantity of enzyme preparation, specific activity at this stage may be not exact.

ally purified enzyme was stored in the lyophilized state in tightly closed tubes at −20° C.

Criteria of homogeneity

Disc electrophoresis at pH 4.5 without sodium dodecylsulphate and localization of RNAase activity. Purified enzyme was examined by disc electrophoresis. Ribonuclease activity was found at the same position of staining band as shown in Fig. 3A.

Disc electrophoresis with sodium dodecylsulphate. A clear band was obtained with gel polyacrylamide containing 0.1% sodium dodecylsulphate with or without dithiothreitol (Fig. 3B).

These results strongly suggest that our enzyme preparation is homogeneous and contains no trace of contamination.

Molecular weight

After gel filtration of standard proteins and of the enzyme on a Sephadex G-100 column, the molecular weight of the ribonuclease was obtained from a plot of elution volume or K_{av} vs log M_r as suggested by Andrews [18], and was found to be 37 000. In contrast to this result, the molecular weight of the purified enzyme by disc gel electrophoresis containing sodium dodecylsulphate (Fig. 3B) was found to be about 25 000 with a continuous [19] or discontinuous [20] buffer system. This difference in molecular weight will be discussed later. It should be noted that there are no differences between preheated or non-preheated sample in sodium dodecylsulphate and with or without dithiothreitol before gel electrophoresis. We could not detect the band of 37 000 or small fragments in 15% gel which permits the detection of cytochrome *c* (M_r = 12 000) and insulin (M_r = 5700). It may be possible that the fragment is further broken down to undetectable sizes, or is a molecule of non protein nature.

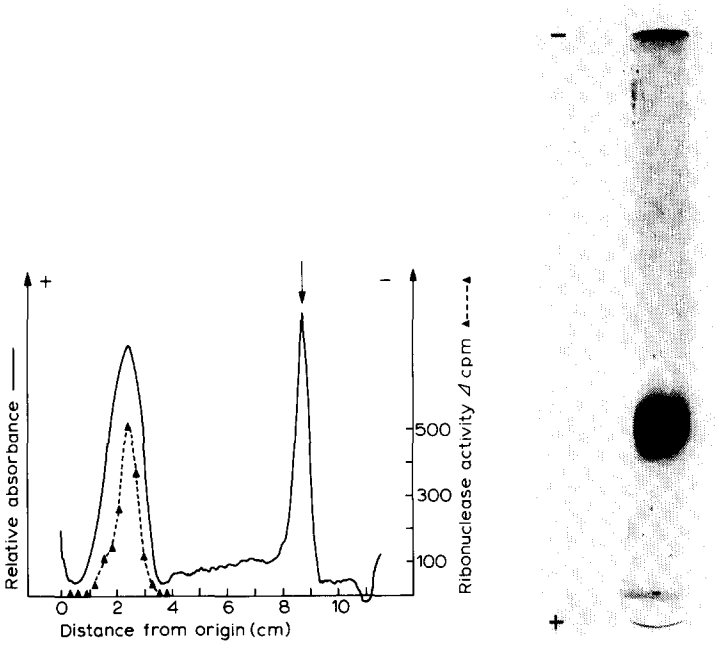

Fig. 3. Disc electrophoresis of purified beef brain ribonuclease. (A) Densitometric profile of analytical polyacrylamide gel of purified beef brain RNAase. Purified RNAase, 5 μg obtained after chromatography on Sephadex G-100 superfine was applied on two identical 10% polyacrylamide gels in β-alanine/acetic acid buffer (pH 4.5). Electrophoresis was carried out for 6 h at 0.4 mA/gel. In the extract of slices of the unstained gel RNAase activity was found to coincide with the unique band. The arrow indicates the position of cytochrome *c*. (B) Resolution of purified beef brain RNAase by gel electrophoresis on a 10% polyacrylamide gel (pH 7) containing 0.1% sodium dodecylsulfphate [19]. Purified beef brain RNAase was heated for 2 min at 100° C in the presence of 0.1% sodium dodecylsulphate and 0.1% β-mercaptoethanol before electrophoresis. 6 μg of protein was applied to the gel.

Isoelectric point

Purified cytoplasmic ribonuclease was electrofocused according to the method of Catsimpoolas [17]. The stained gel column showed no band. In the same manner pancreatic ribonuclease A showed one band in the alkaline region. It is well known that the isoelectric point of RNAase A is 9.5. These results suggest that the isoelectric point of the purified cytoplasmic RNAase must be more alkaline than 9.5 and that the protein has migrated out of the gel column, since our enzyme was adsorbed strongly in cellulose phosphate column and was a basic protein.

General enzymic properties

Effect of pH. The pH activity profile for purified beef brain RNAase is shown in Fig. 4. For Tris · HCl and carbonate buffers, ionic strength was adjusted to 0.1 with KCl. Tris buffer gave lower rates than did phosphate. The pH optimum is also different in Tris · HCl and phosphate buffers, the optimum being at 7.5 in phosphate and 8.5 in Tris buffer.

Effect of temperature. Optimal temperatures for enzyme activity are 50° C and 40° C in the presence of rRNA and poly(C) as substrates respectively.

Purified enzyme was relatively heat labile without substrate and almost all

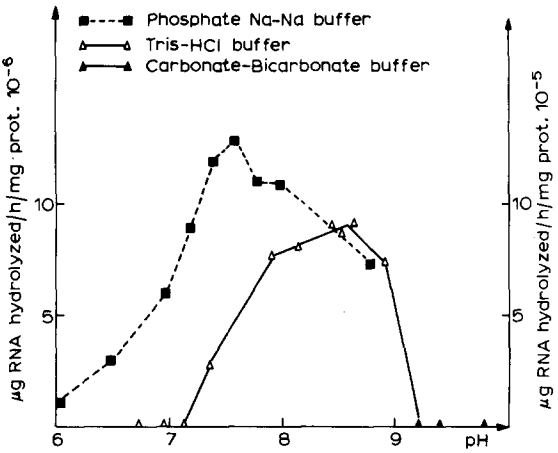

Fig. 4. pH curve of beef brain ribonuclease. Each reaction contained 40 units of enzyme. See text for assay procedure. ■------■, 60 mM sodium phosphate buffer; △———△, 60 mM Tris · HCl buffer; ▲———▲, 60 mM carbonate-bicarbonate buffer.

activity was lost after a 10 min preincubation at 50° C. However RNAase activity in crude homogenate was heat resistant even in the absence of substrate. Thus heat treatment was used successfully in order to destroy RNAase inhibitor which is heat labile [7,8].

Effects of ions and reducing agents. The effects of changes in ionic strength on enzyme activity are shown in Fig. 5. Increasing phosphate buffer concentration up to 0.08 M or salt (KCl or NaCl) concentration up to 0.10 M results in stimulation of enzyme activity. None of the divalent ions tested at 1 mM concentration was stimulatory. The most marked effects were produced

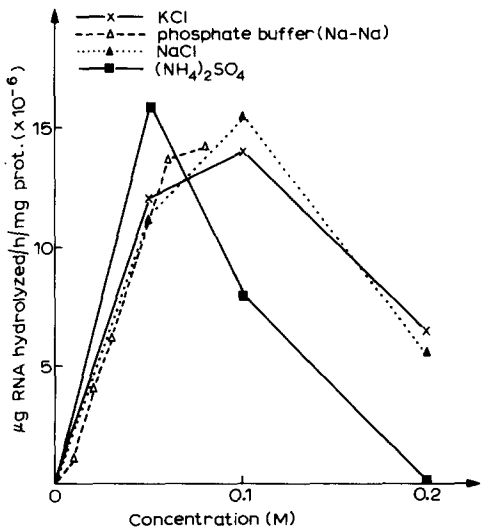

Fig. 5. Effect of ionic strength on brain ribonuclease activity. 50 units of enzyme were used per assay. Concentration of sodium phosphate buffer (pH 7.5) (△- - - - -△), varied as indicated. Salt concentration was varied by addition of KCl (★———★), NaCl (▲· · · · ·▲) or $(NH_4)_2SO_4$ ■———■), as indicated in figure, with buffer concentration being maintained at 0.03 M.

TABLE II

EFFECT OF DIVALENT CATIONS ON RIBONUCLEASE ACTIVITY

Assays were performed as described in Experimental Procedure, using 60 units of enzyme per 0.25 ml. Divalent ions were present in 1 mM concentration.

Addition	Relative activity (%)
None (or 1 mM EDTA)	100
Ca^{2+}	102
Mg^{2+}	96
Mn^{2+}	73
Zn^{2+}	5
Cu^{2+}	0

by Zn^{2+}, Cu^{2+} which inhibited ribonuclease activity (Table II). The sulfhydryl reagents β-mercaptoethanol, dithiothreitol, and *p*-chloromercuribenzoate had no striking effect on enzyme activity at a concentration of 0.4 mM.

Specificity of beef brain cytoplasmic alkaline ribonuclease. The purified enzyme was tested for its activity against a variety of synthetic polymers and rRNA, and the results are shown in Fig. 6. Activities against poly(C) and rRNA were found but less activity for rRNA was seen. No activity was found against poly(A), poly(U), poly(G), and poly(I).

Kinetic measurements. Using rRNA or poly(C) concentrations ranging from 10 to 160 μg per ml, measurements of the rate of hydrolysis were made at pH 7.5 at 40° C. Brain cytoplasmic alkaline ribonuclease obeyed Michaelis-Menten kinetics (Fig. 7). K_m values are given in Table III, which also includes some values for brain nuclear alkaline RNAase taken from ref. 11. Table III shows that the cytoplasmic RNAase is different from nuclear RNAase in its kinetic properties.

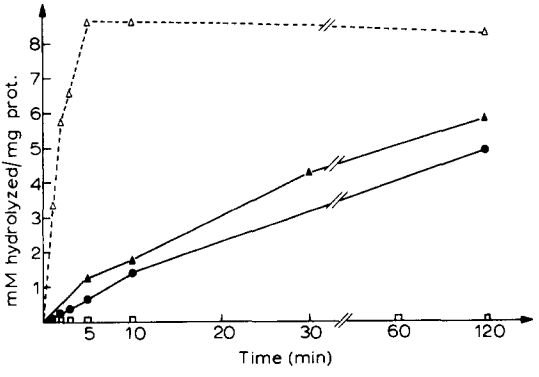

Fig. 6. Action of purified beef brain ribonuclease on synthetic polyribonucleotides and rRNA. Incubation mixture contained 50 mM citrate phosphate buffer (pH 7.1), 1 mM β-mercaptoethanol, 22% (v/v) glycerol, 25 μg homopolyribonucleotides, tRNA or rRNA and 0.003 μg of purified RNAase in a final volume of 200 μl. Incubations with poly(U), poly(A), poly(G) and poly(I) were performed with 0.06 μg of purified RNAase. The reactions were stopped by addition of $HClO_4$ at a final concentration of 0.6 M. Incubations with poly(U) were stopped by addition of 300 μl solution of 1 vol., 0.75% sodium uranyl acetate, 2.5 M $HClO_4$ and 10 vol. 30% alcohol/0.6 M HCl. △- - - - -△, poly(C); ▲———▲, tRNA; ●———●, rRNA; □———□, poly(U), poly(G), poly(A), or poly(I).

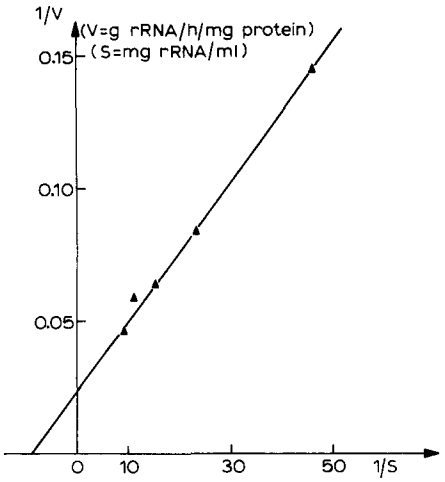

Fig. 7. Lineweaver-Burk plot for beef brain ribonuclease, pH 7.5 (40° C). Reaction mixture : 60 mM sodium phosphate buffer, 1 mM β-mercaptoethanol, 22% glycerol, 10—160 μg RNA per ml and 120 units enzyme per ml. Incubation time was 10 min.

Mode of action. The degradation products were separated on a column of Sephadex G-100 as described by Niedergang et al. [11]. The RNA which has an intermediate molecular weight was detected [23]. Furthermore, the separation of the digestion products of rRNA on DEAE-cellulose, according to the method of Kelley et al. [25] showed a typical pattern of an endoribonuclease. This enzyme produces, as the sole product of poly(C), 2′,3′-cyclic cytidine monophosphate, which was identified by its chromatographic behavior as shown in Fig. 8. No CMP (2′-, 3′- and 5′-) or cytidine are produced under the conditions used. When 2′,3′-cyclic CMP was used as substrate, only negligible amounts of the non-cyclic mononucleotides resulting from enzymatic hydrolysis were found, even after 23 h of incubation. It is unlikely that our highly purified enzyme contains an inhibitor of the cyclic phosphodiesterase activity described by Beard and Razzell [24].

TABLE III

KINETIC PARAMETERS OF RIBONUCLEASE FROM BEEF BRAIN NUCLEAR AND CYTOPLASMIC FRACTIONS, MEASURED AT pH 7.5 (40° C)

The data for cytoplasmic alkaline RNAase were obtained in the manner described in Fig. 7. The reaction mixture contained 60 mM sodium phosphate buffer, 1 mM β-mercaptoethanol, 22% glycerol, 10—160 μg rRNA per ml and 120 units per ml. For homopolynucleotides, the reaction mixture contained 50 mM citrate phosphate buffer, pH 7.15, 1 mM β-mercaptoethanol, 22% glycerol, 10—40 μg homopolynucleotides and 115 units enzyme. Final volume was 0.2 ml and incubation was carried out for 5 min at 40° C. Coefficients of variation for K_m measurements were about 10%. The data for the nuclear preparation are taken from Niedergang et al. [11].

Enzyme	K_m (μg/ml)		
	rRNA	poly (C)	poly (U)
Nuclear enzyme	45	122	555
Cytoplasmic enzyme	134	1 600	—

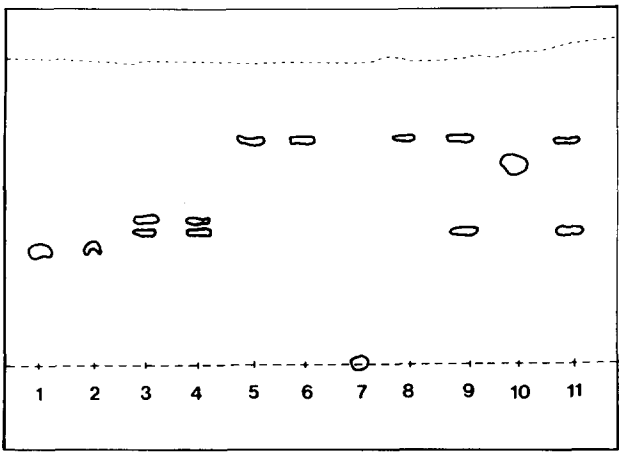

Fig. 8. Analysis of the products of hydrolysis of poly(C) by beef brain ribonuclease on thin layer chromatography. Markers were : 5′-CMP (1), 2′-, + 3′-CMP (3), 2′,3′-cyclic CMP (5), poly(C) (7) and cytidine (10). Spot 2, 4, 6, 8 was from beef brain RNAase hydrolysis of 1, 3, 5, 7 respectively. Spot 9 and 11 was from RNAase A hydrolysis of 7 and 5. Reaction mixture: 0.04 M citrate phosphate buffer (pH 7.17), 20 μg of substrate, 0.5 μg of enzyme (7500 units for beef brain RNAase, 6000 units for RNAase A), final volume 50 μl. Incubation time was 60 min at 40° C.

Discussion

Beef brain alkaline ribonuclease has been purified 8300-fold from a soluble fraction. At this stage of purification the preparation is homogeneous and contains no nucleotidase or nucleosidase activity, since even after prolonged hydrolysis cyclic nucleotides are the only products. Further, no deoxyribonuclease, alkaline phosphatase, or phosphodiesterase activities were detectable.

The enzyme digests rRNA to a mixture of mono-and oligonucleotides and poly(C) to 2′,3′-cyclic cytidine monophosphate. Only a negligible amount of 3′-CMP could be detected even after prolonged hydrolysis. On the basis of the digestion products, the enzyme is a cyclizing endonuclease, and has an extreme preference for poly(C). It should be noted that the analysis of terminal nucleotides of rRNA shows that the major product is CMP but there is also UMP (Ittel et al., unpublished data).

The enzyme has a pH optimum in the alkaline range, and no detectable divalent cation requirement. Some divalent cations tested, Zn^{2+}, Cu^{2+}, produced significant inhibition at a concentration of 1 mM.

The purified enzyme has a molecular weight of approx. 37 000 obtained by filtration on Sephadex G-100. In contrast to this result, sodium dodecylsulphate-acrylamide gel (10% and 15%) electrophoresis of the purified enzyme revealed a single sharp band of molecular weight about 25 000, when compared to bovine serum albumin, ovalbumin, chymotrypsinogen.

These results showed that our estimate for the molecular weight of this enzyme, 37 000, obtained by filtration on Sephadex G-100, may represent that of an aggregate formed between the ribonuclease protein, and a small molecule (perhaps of non-protein nature, such as polysaccharide) present in the enzyme preparation. It is well known that Sephadex does contain acid groups and basic proteins might be retarded. If this is the case, observed molecular weight must

be smaller than expected. Since obtained molecular weight was more larger than expected, this possibility is unlikely and we could not detect a dimeric form of the enzyme in any conditions of polyacrylamide gel electrophoresis.

It is interesting to note that many differences were found between nuclear [11] and cytoplasmic alkaline ribonuclease from beef brain. The molecular weight of the nuclear enzyme and the cytoplasmic one are 39 000 and 25 000, respectively, by sodium dodecylsulphate-polyacrylamide gel electrophoresis. The nuclear enzyme can hydrolyze polyuridylic acid (poly(U)), but the cytoplasmic enzyme cannot hydrolyze this substrate. These two enzymes also showed different kinetic properties. These results strongly suggest that cytoplasmic ribonuclease differs from the nuclear enzyme and that these two ribonucleases may play different roles in brain tissue.

Acknowledgement

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