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RIBONUCLEASES OF BEEF BRAIN NUCLEI. PURIFICATION AND CHARACTERIZATION OF AN ALKALINE RNAase

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SUMMARY

Two ribonucleases were separated from beef brain nuclei by hydroxyapatite column chromatography. One was an alkaline (RNAase BN1), another was an acid ribonuclease (RNAase BN2). This paper describes the purification and characterization of the alkaline ribonuclease.

RNAase BN1 was purified around 2400-fold with a yield of 13% by chromatography on phosphocellulose and Sephadex G-200. Purity was checked by polyacrylamide gel electrophoresis.

The pH optimum was 7.6, the molecular weight was 39 000, and the effect of ions and K_m on the RNAase BN1 were determined. The ribonuclease had a net positive charge. RNAase BN1 was an endonuclease, hydrolyzing the phosphodiester bounds of pyrimidine ribonucleotides, preferentially those of polyC, to give the 2',3'-cyclic nucleotides as final products.

INTRODUCTION

It has been shown that only a fraction of the RNA synthesized in the nucleus is transferred to the cytoplasm [1–5]. Moreover the 45 S pre-rRNA of the mammalian preribosome is cleaved into discrete intermediate pre-rRNA molecules which are shaped into mature 18 S and 28 S rRNA [6, 7]. There is also growing evidence that mRNA is processed from high molecular weight heterogeneous nuclear RNA [8], even though there is no clear demonstration whether transcription in eucaryotic cells is mono- or polycistronic. The details of all these processes are not known, but it is very probable that ribonucleases are involved both in pre-rRNA maturation and in giant mRNA cleavage. Few studies have been devoted on nuclear ribonucleases, and these enzymes have either only been detected [9–13] or, at best, partially purified and characterized [14–18].

This paper describes the separation of two beef brain nuclear ribonucleases,

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and the purification and characterization of one of them: the alkaline brain nuclear ribonuclease (RNAase BN1). The acidic nuclear ribonuclease (RNAase BN2) will be described in detail elsewhere (Okazaki, H., Ittel, M. E., Niedergang, C. and Mandel, P., unpublished). A short communication has been already presented [19].

MATERIALS AND METHODS

Measurement of ribonuclease activity

For standard ribonuclease activity determination, the incubation mixture contained 60 mM of sodium phosphate buffer (pH 7.6), 1 mM β-mercaptoethanol, 22% v/v glycerol, 20 μg ^{32}P-labelled rRNA (spec. act. about 1000 counts/min per μg rRNA) extracted from mouse plasmocytoma as described by Kempf and Mandel [20], and 5–10 units of the enzyme preparation in 250 μl final volume. The incubation was performed at 40 °C for 10 min and was stopped by addition of 2 ml of cold 10% trichloroacetic acid containing 20 mM sodium pyrophosphate and 0.2 ml of 1% serum albumin. After 30 min at 0 °C the precipitate was collected on a Whatman GF/B glass paper (diameter 24 mm) and washed six times with 6 ml of cold 5% trichloroacetic acid and once with 6 ml of ether. After drying (30 min, 100 °C) the filter was counted with a Packard Tri-Carb scintillation counter in a vial containing 10 ml of 0.4% omnifluor (NEN Chemicals, U.S.A.) in toluol. To determine hydrolyzed RNA the value obtained was subtracted from the blank assay (which contained no enzyme). The incubation assays were always made in triplicate.

In early experiments the incubations were carried out in citrate phosphate buffer at pH 7.15. One unit of the enzyme was defined as the amount of protein which hydrolysed 1 μg of RNA per hour at 40 °C. The specific activity was expressed as units per mg of protein.

Hydrolysis of homopolyribonucleotides was determined by absorbance measurements at 260 nm of the acid soluble products obtained after enzymatic digestion. The extinction coefficients used to calculate the number of mM of polyribonucleotides hydrolyzed were those of Cohn (see ref. 21). Details of incubation conditions are given in the legend to Fig. 9.

Protein determination

Proteins were determined by the method of Lowry et al. [22] using crystalline bovine serum albumin as standard. The proteins (including serum albumin) were first precipitated in 15% trichloroacetic acid to avoid the effect of interfering substances such as thiol compounds and sucrose.

Nuclei preparation

All steps involved were performed at 0–4 °C. Brain nuclei were prepared from fresh beef brain according to Chauveau et al. [23] with slight modifications. Brains were freed from meninges and blotted with filter paper. The brain tissue was homogenized with a mixer in 1.3 M sucrose containing 1 mM $MgCl_2$ and 10 mM potassium succinate (pH 6.3) (1-l/brain). The homogenate was centrifuged for 10 min at 3000 $\times$ g. The crude nuclear pellet was then suspended in 2.4 M sucrose containing 1 mM $MgCl_2$ and 10 mM potassium succinate (70 ml/brain). The final concentration of sucrose of the homogenized nuclei was 2.2 M. This homogenate was filtered through a double

sieve (300 μ) and centrifuged for 60 min at 105 000 $\times$ g. The nuclei pellets were resuspended in 2.2 M sucrose containing 1 mM $MgCl_2$ and 10 mM potassium succinate (12 ml/brain), filtered through a double sieve and centrifuged again in the same manner as described above.

In some cases the nuclear pellet was treated with 0.1% Triton X-100.

RESULTS

The nuclear fraction examined by phase and electron microscopy did not show cytoplasmic contaminations. Moreover, subcellular contamination was estimated with marker enzymes: lactate dehydrogenase (EC 1.1.1.27) for the soluble fraction [24], monoamine oxidase (EC 1.4.3.4) for mitochondria [25], and 2',3'-cyclic-nucleoside monophosphate phosphodiesterase (EC 3.1.4.16) for myelin [26]. From these measurements the contamination appeared to be below 0.02% in the nuclear preparation when the activity found in total cellular homogenate was taken as 100%.

Control experiments were performed with nuclei treated by 0.1% Triton X-100 which removed the major part of the external nuclear membrane. The same results were obtained for the nuclear RNAase activity with or without Triton X-100 treatment. These results strongly suggested that the RNAases were indeed nuclear.

Enzyme solubilization

Nuclei of three beef brains were suspended in 100 ml distilled water homogenized with a Potter-Thomas apparatus. The homogenate was made 2 M in KCl and stirred for 1 h, then sonicated with a Branson sonifier ten times for 30 s in a double-walled beaker and cooled with circulating methanol at -20 °C. This decreased the viscosity of the homogenate and solubilized the enzymes. The temperature was continuously controlled during sonication and was maintained at 3–6 °C. The homogenate was then centrifuged at 105 000 $\times$ g for 30 min and the supernatant was dialysed for 24 h against several changes of 1 mM phosphate buffer (pH 6.8), containing 1 mM β-mercaptoethanol. The dialysed enzyme solution was centrifuged again at 105 000 $\times$ g for 30 min and the pellet discarded. The supernatant contained half the protein and 70–80% of the RNAase activity of the original homogenate (Table I).

Separation of two different RNAases on hydroxyapatite

The dialysed supernatant was applied to a hydroxyapatite column, equilibrated with 1 mM potassium phosphate buffer (pH 6.8)–1 mM β-mercaptoethanol. Elution was carried out with a stepwise gradient of potassium phosphate buffer containing KCl. The elution pattern is shown in Fig. 1. Eight peaks were obtained. Each peak was assayed for protein and enzyme activity (Table I).

Ribonuclease activities were eluted in three peaks (1, 3 and 5). The first peak (eluted with 5 mM phosphate buffer (pH 6.8)) was not adsorbed to hydroxyapatite. Peak 3 (RNAase BN1) eluted with 5 mM phosphate buffer (pH 6.8) containing 3 M KCl contained most of the total enzymatic units, it is an alkaline RNAase. Peak 5 (RNAase BN2) eluted with 50 mM phosphate buffer pH 6.8 containing 3 M KCl is an acidic RNAase. Its purification and properties will be related elsewhere (Okazaki, H., Ittel, M. E., Niedergang, C. and Mandel, P., unpublished).

TABLE I

SEPARATION OF TWO NUCLEAR RNAases. CHROMATOGRAPHY ON HYDROXYAPATITE

The incubation medium contained 30 mM phosphate–citrate buffer, pH 7.15; 1 mM β-mercaptoethanol; 20 μg ^{32}P-labelled rRNA; aliquot of enzyme protein final volume 250 μl.

Purification steps	Total proteins (mg)	Specific activity at pH 7.15 (units/mg protein)	Total units
Nuclear lysate (crude enzyme)	327	122	39 900
Supernatant	170.5	190	32 400
Hydroxyapatite chromatography			
Peak 1	37	92	3 400
2	0.32	-	--
3*	6.4	2920	18 700
4	35.5	--	—
5**	5.9	(189)***	(1120)***
6	3.4	—	--
7	3.3	--	-
8	0.87	-	-

* RNAase BN1; **RNAase BN2; ***RNAase activity measured at pH 6.2 in phosphate–citrate buffer (Okazaki, H., Ittel, M .E., Niedergang, C. and Mandel, P., unpublished).

The present study deals with the purification and some properties of the RNAase BN1.

Purification of RNAase BN1 obtained by chromatography on a hydroxyapatite column

Step 1: Chromatography on phosphocellulose column I. Peak 3 eluted from the hydroxyapatite column was dialysed against 1 mM phosphate buffer (pH 6.8)–1 mM

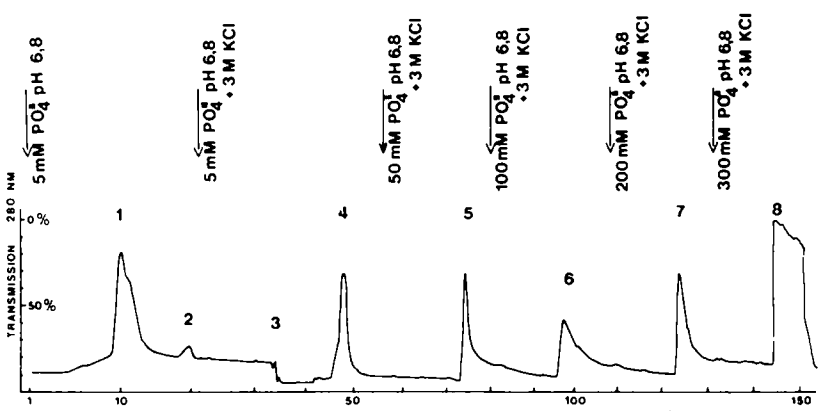

Fig. 1. Chromatography of the supernatant of sonicated nuclei on a hydroxyapatite column. The column (2.1 cm × 30 cm) was equilibrated with 1 mM potassium phosphate buffer (pH 6.8) containing 1 mM β-mercaptoethanol. The load of the column was 150 mg of proteins in 50 ml phosphate buffer. The elution was performed by a stepwise gradient of KCl and potassium phosphate buffer (pH 6.8). The flow rate was 60 ml/h.

β-mercaptoethanol, concentrated by dialysing against crystalline sucrose and then submitted to column chromatography on phosphocellulose (Whatman P11) equilibrated with 50 mM Tris–HCl (pH 7.2)–1 mM β-mercaptoethanol. About ten preparations of Peak 3 (90 mg/30 ml) from hydroxyapatite columns were used for one phosphocellulose column (2 cm × 29 cm). The column was eluted with the same buffer and a linear gradient from 0.2–1.5 M of KCl (600 ml). The active protein was eluted by a concentration of 0.5 M–0.75 M KCl. The RNAase fractions obtained from the first phosphocellulose column were collected, dialysed against 1 mM phosphate buffer (pH 6.8)–1 mM β-mercaptoethanol and concentrated with sucrose.

Step 2: *Phosphocellulose column II.* RNAase fractions from three preparations of phosphocellulose column I (6 mg proteins, 170 000 enzyme units) were rechromatographed in a similar manner to Step 1 (Fig. 2). The RNAase fractions were dialysed and concentrated as described above.

Step 3: *Sephadex G-200.* The concentrated RNAase fractions were then fractionated on a column (1.5 cm × 61 cm) of Sephadex G-200 equilibrated with 0.05 M

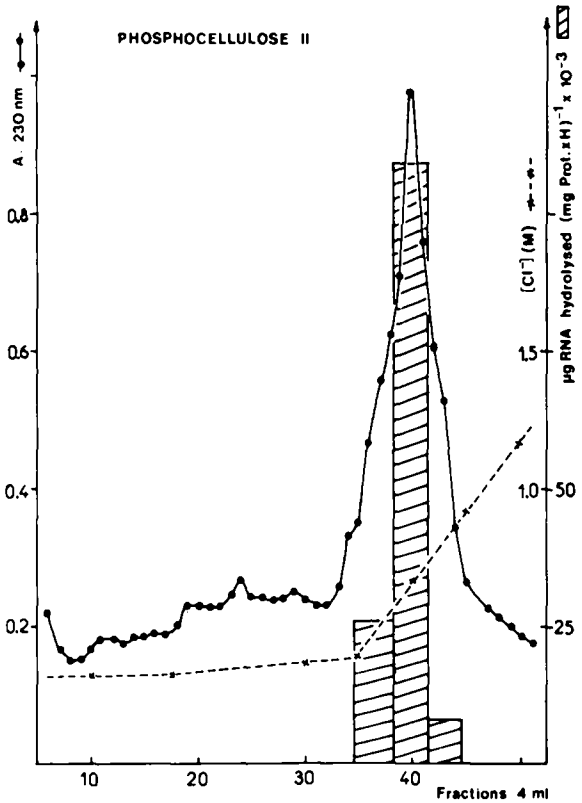

Fig. 2. Chromatography of RNAase BN1 on phosphocellulose column II. The column (1.5 cm × 16 cm) was equilibrated with 0.05 M Tris–HCl buffer (pH 7.2) containing 1 mM β-mercaptoethanol. The enzyme solution (6 mg proteins in 62 ml of equilibrating buffer) was applied on the column. The elution was carried out with a linear gradient 0.2–1.5 M KCl in 100 ml of equilibrating buffer. The flow rate was 30 ml/h.

Tris–HCl (pH 7.2) and 0.5 M KCl containing 1 mM β-mercaptoethanol. Fig. 7 shows the elution profile of RNAase BN1.

A typical purification scheme is summarized in Table II. All the following experiments were performed with enzyme in the final purification step where the purification was approximately 2400-fold.

TABLE II

PURIFICATION OF RNAase BN1

The figures given correspond to nuclei prepared from 80 adult beef brains. The incubation mixture was 30 mM phosphate–citrate buffer pH 7.15; 1 mM β-mercaptoethanol; 20 μg ^{32}P-labelled rRNA; aliquot of enzyme protein final volume 250 μl.

Purification steps	Total proteins (mg)	Specific activity at pH 7.15 (units/mg protein)	Total units	Purification factor	Yield[a] (%)
Nuclear lysate (crude enzyme)	8330	147	1 221 000	1	100
Hydroxyapatite chromatography (Peak 3 = enzyme A)	202	2 220	448 000	15	37
P-cellulose I	6.07	28 100	170 000	192	13.9
P-cellulose II	1.58	109 000	172 000	740	14.1
Sephadex G-200	0.46	350 000	160 000	2380	13.1

[a] Total enzymatic activity of the nuclear lysate was taken as 100% yield.

Purity of the enzyme

Electrophoresis was performed by the method of Reisfeld et al. [27] on 12.5% polyacrylamide gel (3 mm × 40 mm or 3 mm × 50 mm) in β-alanine–acetic acid buffer (pH 4.5). The purified ribonuclease preparation (after chromatography on Sephadex G-200) showed two bands present in approximately equal amounts (Fig. 3).

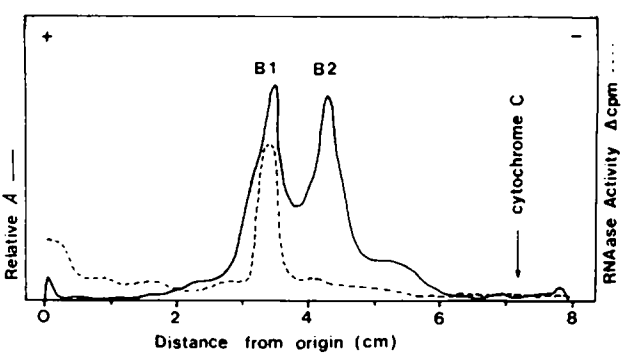

Fig. 3. Densitometric profile of analytical polyacrylamide gel of RNAase BN1. Purified RNAase BN1, 9 μg, obtained after chromatography on Sephadex G200 was applied on two identical 12.5% polyacrylamide gels in β-alanine–acetic acid buffer (pH 4.5). Electrophoresis was carried out for 4 h at 2.0 mA/gel. In the extract of slices of the unstained gel RNAase activity was found to coincide with the first band.

To relate the position of protein bands to that of ribonuclease activity, two identical gels were subjected to electrophoresis under the same conditions. One gel was stained immediately after electrophoresis while the other was cut into 1-mm sections. The ribonuclease activity was localized by the standard ribonuclease assay after elution of the protein from gel slices (1-mm) with 0.2 ml 0.1 M sodium phosphate buffer (pH 7.6) for 18 h at 4 °C. Ribonuclease activity corresponded to the first band (B_1). The nature of the second band has not yet been determined. It could be a contaminating protein which copurifies with the RNAase BN1, or it could be an inactive subunit of this enzyme.

Band B_1 was separated by preparative disc gel electrophoresis in the gel system described above. The protein appeared homogeneous on analytical polyacrylamide gels (pH 4.5) (Fig. 4).

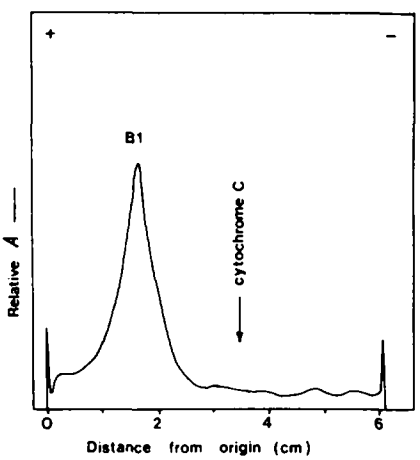

Fig. 4. Densitometric profile of analytical polyacrylamide gel of the protein B1 (see Fig. 3) separated by preparative electrophoresis. 6 μg of protein was applied to the analytical gel. The electrophoresis was carried out as described in the legend of Fig. 3.

The protein separated by preparative disc gel electrophoresis, was treated for 2 min at 100 °C with 0.1% final concentration of sodium dodecylsulfate containing 0.1% β-mercaptoethanol, and electrophoresed in 10% polyacrylamide gels (pH 7) in the presence of 0.1% sodium dodecylsulfate [28] (Fig. 5). Under these conditions the protein had a molecular weight of 37 000 determined relative to standard protein markers. This result agrees with the Sephadex G-200 gel filtration experiments (Fig. 7).

Properties of the nuclear RNAase A

pH optimum. The ribonuclease activity at various pH values was measured in sodium phosphate buffer (Fig. 6). The pH optimum was found to be 7.6. The same result was obtained in Tris–HCl buffer. No activity was observed in acid solutions.

Metal ions. The RNAase activity was affected neither by monovalent cations (K^+, Na^+) at a concentration of 50 mM, nor by divalent cations (Mn^{2+}, Mg^{2+}) at a concentration of 1 mM. A slight activation was observed in the presence of Ca^{2+}. At the concentration of 1 mM, Cu^{2+} and Zn^{2+} reduced activity by 80% (Table III).

Fig. 5. Resolution of protein B1 (see Fig. 3) by gel electrophoresis on a 10% polyacrylamide gel (pH 7) containing 0.1% sodium dodecylsulfate. Protein B1 was heated for 2 min at 100 °C in the presence of 0.1% sodium dodecylsulfate and 0.1% β-mercaptoethanol before electrophoresis. 8 μg of protein was applied to the gel.

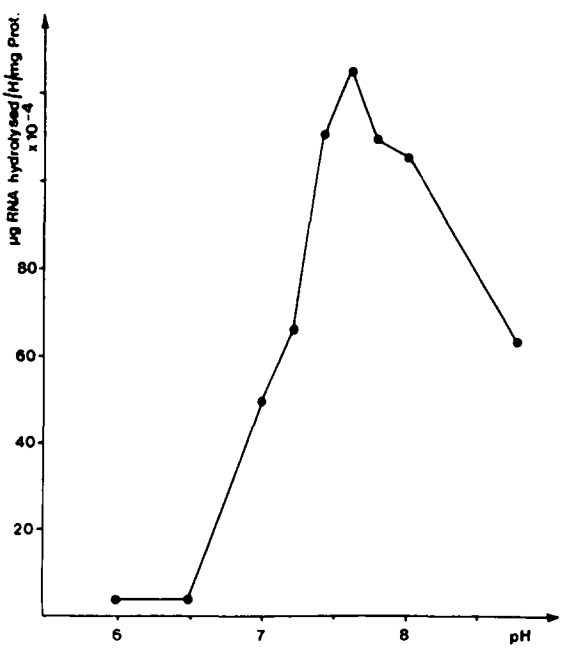

Fig. 6. Effect of pH on hydrolysis of rRNA by RNAase BN1. The reaction mixture and procedure were as described for the standard assay (see Materials and Methods) in 60 mM sodium phosphate buffer at 40 °C.

TABLE III

EFFECT OF IONS ON RNAase BN1

Standard RNAase assay (see Materials and Methods) was used without and with addition of ions as indicated. The results are expressed relative to the value obtained under standard conditions (without supplementary addition) equal to 100.

Addition	Final concentration (M)	Relative activity
None	-	100
$CaCl_2$	$1 \cdot 10^{-3}$	126
$MnSO_4$	$1 \cdot 10^{-3}$	109
$MgCl_2$	$1 \cdot 10^{-3}$	97
$ZnSO_4$	$1 \cdot 10^{-3}$	21
$CuSO_4$	$1 \cdot 10^{-3}$	15
KCl	$50 \cdot 10^{-3}$	114
NaCl	$50 \cdot 10^{-3}$	109
$(NH_4)_2SO_4$	$50 \cdot 10^{-3}$	102

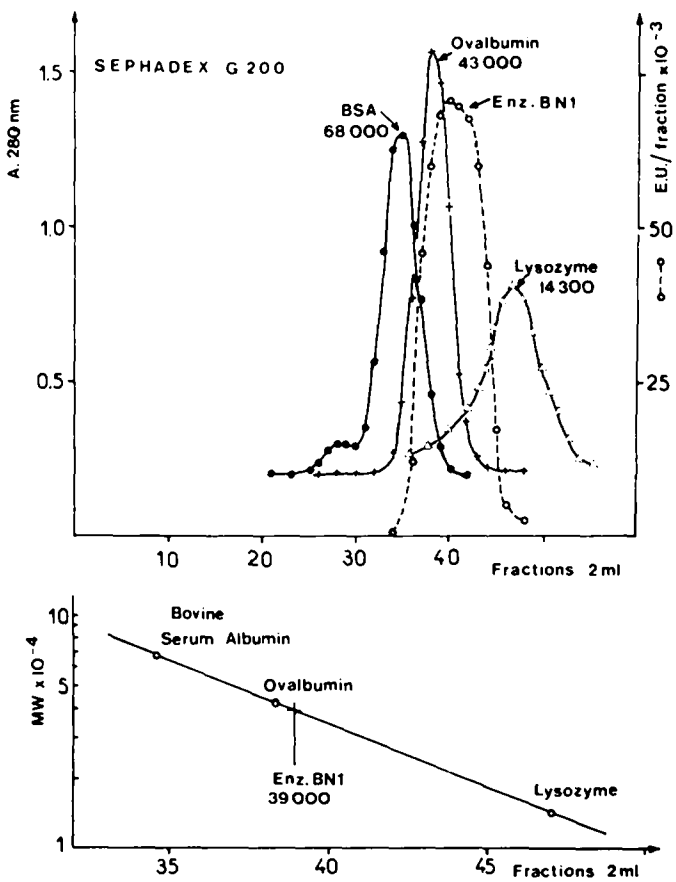

Fig. 7. Molecular weight determination on Sephadex G200 column. The upper graph shows the elution pattern of RNAase BN1 and of several marker proteins. The column (1.5 cm × 61 cm) was equilibrated and developed with 0.05 M Tris–HCl buffer (pH 7.2) and 0.5 M KCl. The flow rate was 4 ml/h. The lower graph gives the molecular weight of RNAase BN1 relative to that of the markers. E.U., enzyme units.

Molecular weight. The molecular weight of RNAase BN1 was determined according to the method of Andrews [29]. Several marker proteins (serum albumin, ovalbumin, lysozyme) and RNAase BN1 were applied separately to a column of Sephadex G-200 and eluted with 0.05 M Tris–HCl buffer pH 7.5 containing 0.5 M KCl and 1 mM β-mercaptoethanol. From the relation between molecular weight and elution volume, the molecular weight of the RNAase BN1 was estimated to be about 39 000 (Fig. 7).

Isoelectric point. It was established by the method of Catsimpoolas [30] using micro-isoelectrofocusing in polyacrylamide gel column, containing ampholine (LKB) pH 3–10, that the RNAase BN1 was a basic protein; its isoelectric point was about 9. The same result was obtained with the technique of Vesterberg and Svensson [31] using a sucrose gradient with ampholine.

Mode of action. After incubation of rRNA with the enzyme, successive formation of oligonucleotides decreasing in molecular weight were observed. This has been shown by Sephadex G-100 column chromatography of the digestion products as indicated in Fig. 8. This result is typical of an endonuclease [32].

Specificity. The substrate specificity of RNAase BN1 was tested on different

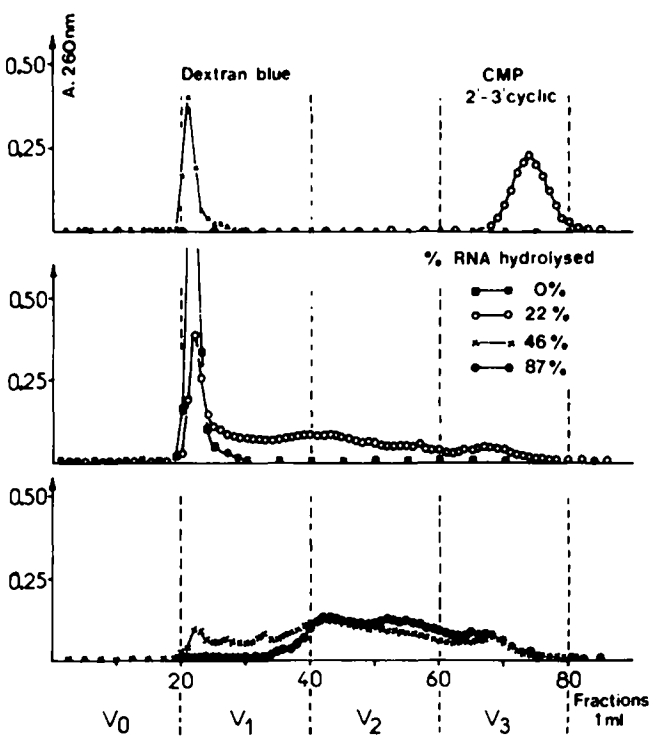

Fig. 8. Gel filtration on Sephadex G100 of rRNA and digestion products at different stages of hydrolysis by RNAase BN1. The incubation medium contained 30 mM phosphate citrate buffer pH 7.15; 1 mM β-mercaptoethanol 100 μg rRNA and 50 enzymatic units of RNAase BN1. The final volume was 0.6 ml. The reaction was stopped by 3.33% (v/v) diethyl pyrocarbonate after different times of hydrolysis and the mixtures were chromatographed on Sephadex G100 column (1 cm × 60 cm) equilibrated and developed with 10 mM sodium acetate buffer (pH 5.6).

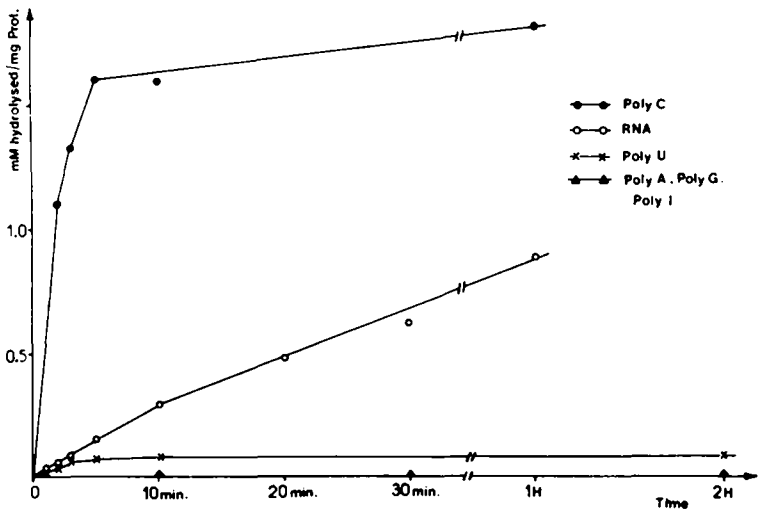

Fig. 9. Hydrolysis of RNA and synthetic homopolyribonucleotides by purified RNAase BN1. Incubation mixture contained 60 mM sodium phosphate (pH 7.6); 1 mM β-mercaptoethanol; 22% (v/v) glycerol; 25 μg homopolyribonucleotides or RNA and 8 enzyme units of RNAase BN1 in a final volume of 200 μl. Incubations with polyU, polyA, polyG and polyI were performed with 320 enzyme units. The reactions were stopped by addition of $HClO_4$ at a final concentration of 0.6 M. Incubations with polyU 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.

homopolyribonucleotides. Under the conditions indicated in Fig. 9 a kinetic study was performed. When polyC, polyU and rRNA were used as substrates, the polynucleotides were degraded to acid-soluble nucleotides, while polyA, polyG and polyI were not degraded.

Effect of substrate concentration. RNAase BN1 was assayed under the conditions described above, with varying amounts of rRNA, polyC or polyU. The K_m values calculated according to Lineweaver and Burk [33] were respectively $2.02 \cdot 10^{-4}$ M for rRNA (Fig. 10), $3.78 \cdot 10^{-4}$ M for polyC and $1.71 \cdot 10^{-3}$ M for polyU. The maxi-

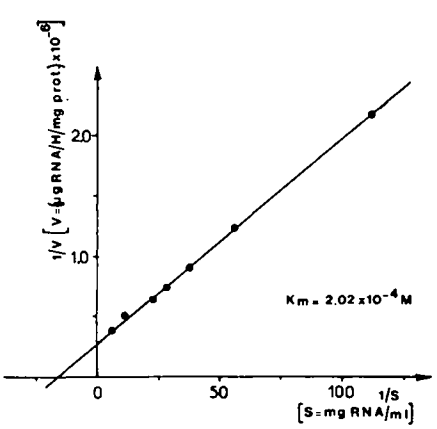

Fig. 10. Lineweaver–Burk plot of hydrolysis of rRNA by RNAase BN1. Standard assay procedure (see Materials and Methods) was employed with varying amount of rRNA substrate (9 μg–160 μg).

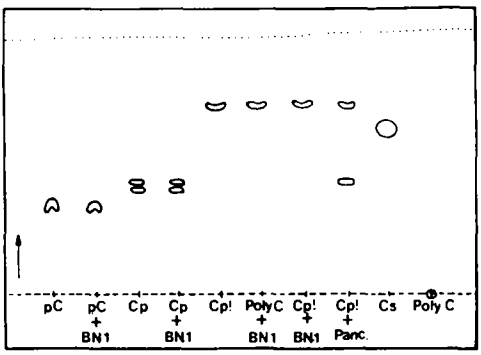

Fig. 11. Thin-layer chromatographic separation of cytidine compounds. BN1, Brain nuclear RNAase BN1; panc., pancreatic RNAase; pC, CMP 5′; Cp, CMP 2′,3′; Cp!, CMP 2′,3′-cyclic; Cs, cytidine. The incubation mixture contained 20 μg of substrate in 40 μl 30 mM citrate phosphate buffer (pH 7.15) and 180 enzyme units. The reaction was run for 24 h at 40 °C. The thin-layer chromatography was performed on silica gel F 254 (Merck). The solvent used was 0.1 M H_3BO_3 (30 vol), isopropanol (60 vol.) conc. NH_4OH (10 vol.).

mal velocities (mmoles hydrolysed/h per mg protein) for the same substrates were respectively 11.6 with rRNA, 44.2 with polyC and 8.3 with polyU. Thus RNAase BN1 has a higher affinity for the substrate polyC than for the substrate polyU and rRNA.

Identification of digestion products. In order to clearly identify the digestion products, polyC was used as substrate, in view of its sensitivity to RNAase BN1. Using thin-layer chromatography as indicated in Fig. 11, 2′,3′-cyclic CMP was found to be the only product released from polyC after extensive action of the enzyme.

DISCUSSION

Two different RNAases were separated from purified beef brain nuclei. We have described some properties of the RNAase BN1 which was purified around 2400-fold. This enzyme is an alkaline endoribonuclease with high affinity for polyC and smaller affinity for polyU. PolyA, polyG and polyI were not hydrolyzed by this enzyme. The end products of hydrolysis of polyC were 2′,3′-cyclic cytidine monophosphates.

Heppel [16] has described a 80-fold purified endonuclease from pig liver nuclei. But this enzyme cleaved phosphodiester bonds at the 3′-position, whereas the brain nuclear RNAase described in this report gave 2′,3′-cyclic nucleotides.

Sporn and Lazarus [14, 34] have separated an endonuclease and an exonuclease from normal liver and Ehrlich ascite tumor cell nuclear extracts. These enzymes attacked polyribonucleotides to produce 5′-nucleotides. These authors studied more particularly the exonuclease which was purified 23-fold.

Sierralta and Minguell [18] have purified a ribonuclease from rat bone marrow nuclei 7-fold. This ribonuclease attacked the phosphodiester bond attached to the 3′-OH group of a pyrimidine nucleotide and produced the respective 2′,3′-cyclic nucleotide. Some properties of this enzyme were similar to those of RNAase BN1. However, the rat bone marrow nuclear RNAase was active over a pH range from 6.2–7.2, whereas the RNAase BN1 has an optimum pH at 7.6 and no activity at pH 6.2.

Niessing and Sekeris [11] found that the proteins from 30-S nuclear particles included an endonuclease which in vivo might be involved in the cleavage of high molecular weight DNA-like RNA. Liau et al. [9] isolated nucleoli from L cells to convert 45 S RNA in vivo to 28 S and 18 S ribosomal RNA. They suggested a tentative model for the conversion process whereby 45 S RNA underwent a single splitting with an endonuclease followed by sequential trimming with exonuclease. Mirrault and Scherrer [17] also reported the isolation from HeLa cells of a nucleolar endoribonuclease which they purified 4-fold. Recently, Perry and Kelley [15] have shown that the exonuclease described by Sporn and Lazarus [14, 34] hydrolyzed the methyl-deficient segments at the 3'-OH ends of both 45-S and 32-S molecules. In a recent paper, Prestayko et al. [35] described a nucleolar endoribonuclease from Novikoff hepatoma. This enzyme, purified 200-fold, also hydrolyzed most rapidly polyC and more slowly polyU. Other poly-ribonucleotides were not hydrolyzed. In contrast to the enzyme described in the present paper, the RNAase reported by Prestayko et al. [35] has a pH optimum of 7.0 and the molecular weight was 7000.

Until now only low factors of purification and partial characterizations of the nuclear ribonucleases have been reported. The alkaline RNAase from beef brain nuclei, isolated and studied in this paper, has been purified about 2400-fold and some of the physico-chemical properties were determined. This highly purified enzyme will no doubt be useful in defining the role of the nuclear RNAases in maturation of messenger and ribosomal RNAs.

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