Characterization of a Ribonuclease from Bovine Brain†

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ABSTRACT: An alkaline ribonuclease (pH optimum near 8) has been purified from whole beef brains and found to have a base specificity like that of bovine pancreatic ribonuclease, but in most other respects to be distinguishable from the enzymes of bovine pancreas, semen, or brain nuclei. The preparation appears homogeneous in sedimentation equilibrium and probably so in polyacrylamide gel electrophoresis under normal or dissociating conditions. Sedimentation equilibrium and SDS gel electrophoresis both indicate a molecular weight of 2.4–2.6 × 10^4, and tryptic and chymotryptic peptide patterns are consistent with a protein of this size. No dissociation into subunits has been attained. The enzyme is not precipitated by antiserum to pancreatic ribonuclease, although its activity is inhibited by this antiserum with low efficiency. In comparisons of the hydrolysis of RNA the brain enzyme was found to have a similar specificity to pancreatic RNase, but to have a lower K_m for RNA and to produce significantly different oligonucleotides upon partial hydrolysis of bacteriophage RNA, suggesting differences in the mechanism of substrate recognition. In contrast, nuclease inactivation by iodoacetate at pH 5.5 is indistinguishable for pancreatic or purified brain RNase.

The brain is active in protein synthesis, and shows a significant level of RNA turnover (e.g., see Satake, 1972) and so it should be expected that enzymes which degrade RNA would be present in reasonable quantities. Ribonucleases with acid and alkaline pH optima are present in many mammalian tissues including brain (Barnard, 1969; Datta et al., 1964; Roth and Milstein, 1952), and efforts have been directed at enzyme localization and purification (e.g., Aksenova and Nechaeva, 1971; Houck, 1958; Nechaeva, 1972; Niedergang et al., 1974).

Some relationship between the alkaline brain RNase[1] and bovine pancreatic ribonuclease is apparent, since both enzymes are reversibly inactivated by an inhibitory protein found in many tissues, including the brain (Roth, 1956). Takahashi et al. (1970) have purified the inhibitor from pig brains, and the interactions of inhibitors with nucleases have been observed in a number of studies (e.g., Gagnon et al., 1974). However, in many cases it was considered necessary to use impure inhibitor preparations or enzyme from a different tissue or animal; e.g., Roth and Hurley (1966) measured the inhibition of a series of derivatives of bovine pancreatic ribonuclease by rat liver RNase inhibitor. Thus, relatively little is yet known about the interactions of RNase and inhibitor within a given tissue.

As part of a study of this enzyme–inhibitor system, we have begun isolation of the ribonucleases of bovine brain. In this paper we present results which indicate that the alkaline ribonuclease of beef brain is related to bovine pancreatic ribonuclease, but that it is a distinct protein with significant individual character. A preliminary report of some of our observations has been made (Glitz and Elson, 1974).

Materials and Methods

Assays. Nuclease activity was measured in an assay simplified from that of Woodroof and Glitz (1971). The reaction mixture (1.0 ml) contained 0.1 *M* NaCl, 0.05 *M* Tris, 1 mg of bovine serum albumin (Pentex, fraction V), and 1 mg of wheat germ high molecular weight RNA (Glitz and Dekker, 1963); the pH was adjusted to 8.0 with HCl. If nuclease inhibitor was expected to be present, 4 × 10^{-4} *M* lead acetate and/or *p*-hydroxymercuribenzoate (Sigma Chemical Co.) were added (Roth, 1956; Shortman, 1962). The reaction mixture (minus RNA) was equilibrated at 37°, and hydrolysis was started by the addition of RNA (0.1 ml of a 1% solution). After 15 min the reaction was halted by addition of 3.0 ml of cold 10% (w/v) perchloric acid with vigorous mixing. After 15–30 min on ice, the samples were centrifuged at 4° for 10 min at 4400*g* (Sorvall SM24 rotor), and the absorbance of the supernatants was measured at 260 mμ. One unit of RNase activity is defined as that amount of enzyme which results in an absorbance of 1.0 greater than that of a blank sample incubated in the absence of added nuclease. The assay is linear over a range of 0.2–1.5 units, and is normally reproducible within limits of 5% (except in initial extracts).

Nuclease in chromatographic eluents was detected qualitatively with a spot-plate assay modified from Glitz and Dekker (1964). Protein was measured by a modification of the method of Lowry et al. (1951) using bovine serum albumin as the standard, or appromixated from absorbance at 280 mμ.

Enzyme Purification. Fresh beef brains were collected on ice from a local slaughterhouse and frozen in ca. 2-kg portions. As needed, they were thawed overnight in a refrigerator or for a few hours at room temperature. All operations were carried out at 0–4°. A preparation from 10–12 kg of brain is described.

The thawed or partially frozen tissue was cut into approximately 1-in. cubes, and 500-g portions were homogenized with 750 ml of cold 0.02 *M* Tris-HCl (pH 7.2). The homogenate was centrifuged for 15 min at 13,700*g* (Sorvall GS-3 rotor) and the clear red supernatants decanted. The

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[1] Abbreviations used are: RNase, ribonuclease; DNase, deoxyribonuclease; SDS, sodium dodecyl sulfate.

pellets and the creamy pink semisolid layer were resuspended in ca. 75 ml of buffer per centrifuge bottle and sedimented as above, and the clear supernatants combined with the initial supernatants[2] (step 1).

Sulfuric acid (5 *M*) was added slowly with stirring to bring the supernatants to 0.25 *M* (Kunitz and McDonald, 1953). The brown suspension was stirred overnight and centrifuged as above, and the supernatant (step 2) saved. Ammonium sulfate (243 g/l.) was added over a 20-min period to 2-l. portions of the preparation, and after 15 min of gentle stirring the suspension was centrifuged as before and the supernatants retained. Ammonium sulfate was added to these at a level of 420 g/l., and the mixture was slowly stirred for at least 3–5 days and centrifuged. The precipitate was dissolved in ca. 1 l. of 0.1 *M* Tris-HCl (pH 9) and dialyzed against 18 l. of 0.05 *M* sodium acetate (pH 4.0), followed by 18 l. of 0.01 *M* sodium acetate (pH 4.0). In each case 10^{-3} *M* 2-mercaptoethanol was present and Spectrapore 1 dialysis tubing (Spectrum Medical Industries, Los Angeles, Calif.; approximate molecular weight cutoff $6–8 \times 10^3$) was used[3] (step 3).

A 2.5 × 75 cm column of phosphocellulose (Schleicher and Schuell, 0.7 mequiv/g) was equilibrated with 0.02 *M* sodium acetate buffer (pH 4.0) and the preparation above was applied at a flow rate of 0.5 ml/min. The column was eluted with 1 l. of equilibration buffer, followed by a linear gradient of 0–1.2 *M* NaCl in 5 l. of the same buffer. RNase was eluted as a broad peak centered at 0.8 *M* NaCl; fractions with a specific activity greater than 2×10^3 units/A_{280} were pooled and dialyzed against two 18-l. changes of 0.02 *M* sodium acetate–10^{-3} *M* 2-mercaptoethanol (pH 5.5) (step 4).

A 5-ml column of agarose-5′-(4′-aminophenyl)uridine 2′(3′)-phosphate (Agarose pUp, Wilchek and Gorecki, 1969; purchased from Miles Laboratories) was equilibrated with 0.02 *M* sodium acetate buffer (pH 5.5). The enzyme was applied at ca. 1 ml/min, and the column was then washed with 1 l. of buffer. Nuclease was eluted with 0.1 *M* sodium pyrophosphate (pH 5.5); it was dialyzed for 24 hr against three 1-l. portions of 10^{-3} *M* 2-mercaptoethanol and lyophilized (step 5). This preparation was applied to a 1.5 × 25 cm column of phosphocellulose equilibrated with 0.02 *M* sodium phosphate (pH 6.0) and eluted with a linear gradient of 0–0.75 *M* NaCl in 4 l. of the same buffer. RNase was eluted as a sharp peak midway in the gradient, and fractions with a specific activity greater than 2×10^4 units A_{280} were pooled, dialyzed 24 hr against 10^{-3} *M* 2-mercaptoethanol (three changes of 4 l.), lyophilized, and dissolved in a minimal volume of distilled water (step 6).

The preparation above was applied to a 3 × 60 cm column of Sephadex G-100 equilibrated with 0.005 *M* sodium phosphate (pH 6.0) and eluted with the same buffer. A small peak of nuclease activity emerged at void volume, and a major symmetrical peak at about 1.5 × void volume. Fractions from the major peak were pooled, dialyzed against two 4-l. portions of distilled water, and lyophilized (step 7).

In an alternative procedure which avoids acid treatment the extract (step 1) was fractionated in 1-l. portions with acetone (40–60% v/v cut), followed by chromatography on phosphocellulose at pH 4 as described and then on a similar column equilibrated at pH 8.0 and 0.01 *M* Tris-HCl. In some cases such preparations were successfully further purified by affinity chromatography as in step 5, but frequently the nuclease was poorly retained by the adsorbent.

Chromatography on carboxymethylcellulose (Whatman) as described by Becker et al. (1973) was sometimes used in place of gel filtration (step 7) with similar results.

Determination of RNase Specificity. *Escherichia coli* (ATCC strain 15766) was cultured overnight in 100 ml of 1% tryptone supplemented with 30 mCi of ^{32}P (as inorganic phosphate, purchased from I.C.N.). RNA was extracted as described by Sanger et al. (1965) and dissolved in 1 ml of a buffer of 0.05 *M* Tris-HCl (pH 8.0)–8 *M* urea, plus 10% sucrose. Aliquots of 0.5 ml were fractionated by electrophoresis according to Peacock and Dingman (1967). A single sample well was formed in a 10% slab of Cyanogum 41 (E.-C. Apparatus Co.), and electrophoresis was conducted at 200 V for 6 hr in an E.-C. Co. cell. The 5S RNA band was located by autoradiography and eluted from the gel. Hydrolysis and fractionation procedures were those of Sanger et al. (1965) and Brownlee and Sanger (1967). Similar digests were made with ^{32}P labeled bacteriophage f2 RNA prepared as described for MS2 RNA (Glitz, 1968).

Other Methods. Sedimentation equilibrium was done at 32,000 rpm in a Spinco Model E centrifuge at 20° for up to 48 hr. The protein concentration was 0.3–1.2 mg/ml in 0.05 *M* Tris-HCl (pH 7.0), sometimes supplemented with 6 *M* guanidine · HCl plus 0.5% 2-mercaptoethanol. Molecular weights were determined from a plot of log *C* vs. X^2 according to Van Holde and Baldwin (1958); the partial specific volume was calculated from the amino acid composition (Smith et al., 1954).

Polyacrylamide gel electrophoresis of proteins was conducted using Eastman Kodak electrophoresis grade materials; at pH 4.0 the buffer was 0.05 *M* sodium acetate, and the gels contained 7.5% acrylamide and 0.375% bisacrylamide. Gels for SDS-polyacrylamide gel electrophoresis contained 10% acrylamide and 0.25% bisacrylamide, and conditions were derived from Weber et al. (1972). Staining was with 1% Amido Blue-Black (Matheson Co.) in 7% acetic acid or with 0.05% Coomassie Brilliant Blue R (Sigma Chemical Co.) in 50% methanol plus 10% acetic acid. Slab gel electrophoresis of oligonucleotides was done as previously described (Glitz et al., 1972) except that gel and buffer contained 8 *M* urea.

Amino acid composition was determined in duplicate by the method of Moore and Stein (1963) with a Spinco Model 120B automatic analyzer; 0.5-mg samples were hydrolyzed in vacuo in 6 *N* HCl at 110° for 24 hr. Tryptophan was estimated spectrophotometrically (Beaven and Holiday, 1952). Protein reduction and carboxymethylation followed the method for pancreatic ribonuclease described by Hirs (1967), and hydrolysis to peptides was done for 2 hr at 37° in 0.1 *M* ammonium bicarbonate buffer (pH 8.0) using 2% by weight (relative to substrate) of chymotrypsin (Calbiochem) or triphenyl chloromethyl ketone trypsin (Worthington Biochemical Co.). Peptide electrophoresis was on Whatman 3MM paper for 75 min at 3000 V (35 V/cm); the buffer was acetic acid–pyridine–water (100:10:890, v/v) (pH 3.5), and peptides were visualized by dipping the papers in 0.2% ninhydrin plus 5% collidine in 95% ethanol.

The immunochemical procedures used have been de-

[2] Re-suspension as described results in the recovery of 10–15% more RNase activity than if this step is eliminated.

[3] In the absence of 2-mercaptoethanol a heavy precipitate normally formed during dialysis, and significant amounts of nuclease activity were often found in the precipitate. In a few cases RNase was lost upon dialysis when using ungraded dialysis tubing, but we have no evidence that enzyme passed through the membrane.

Table I: Purification of Ribonuclease from Bovine Brain.

Step	Vol (ml)	RNase (Units/ml)	Protein (mg/ml)[a]	Sp Act. (Units/mg of Protein)[a]	Yield (%)
(1) Extract	18,700	101	7.8	13.0	100
(2) Acid supernatant	20,000	90	6.6	13.4	95
(3) Ammonium sulfate	1,200	1365	8.9	153	87
(4) P-cellulose I (pH 4)	3,250	472	0.37	1275	81
(5) Affinity chromatography	6.3	1.73×10^5	12.5	1.38×10^4	58
(6) P-cellulose II (pH 6)	1.0	9.1×10^5	14.1	6.45×10^4	48
(7) Sephadex G-100[b]	8.7	9.05×10^4	1.1	8.3×10^4	41.5

[a] Based on a bovine serum albumin standard in the assay of Lowry et al. (1951). [b] Average values from three preparations.

scribed (Eichler and Glitz, 1974). Pancreatic ribonuclease was either the 5 × crystallized material or Hirs component A and RNase T_1 was the Sankyo product (all supplied by Calbiochem).

Inactivation of RNases with iodoacetate at pH 5.5 was based on the work of Crestfield et al. (1963). At appropriate times 25-μl samples were diluted to 0.5 ml with 0.1 *M* sodium phosphate and 1 mg/ml of bovine serum albumin (pH 8) and assayed. All unspecified chemicals were of reagent grade or the nearest equivalent.

Results

Enzyme Purification. The purification of nuclease from 12 kg of bovine brain is summarized in Table I. RNase activity at pH 8 was initially almost undetectable unless assayed in the presence of lead acetate or *p*-hydroxymercuribenzoate; after acid treatment these supplements had little or no effect on activity measurements. The nuclease was stable throughout purification, but precipitation of other proteins (e.g., during dialysis) sometimes resulted in partial precipitation of RNase activity.

Incomplete purification of the enzyme was also attained by a method which avoided acid treatment. These preparations were often stimulated by the presence of lead acetate or *p*-hydroxymercuribenzoate in the assay mixture, and resisted further purification; their specific activity never was greater than 3×10^4 units/mg of protein.

Criteria of Purity. The best preparations described above appear to be homogeneous or nearly so by several criteria. Polyacrylamide gel electrophoresis at pH 4 (Figure 1) shows a single band with a poorly defined leading edge; nuclease activity in gel slices corresponds to the position of the stained band. Bovine pancreatic RNase migrates well ahead of the brain enzyme, and is separated from it in a mixture. SDS–polyacrylamide gel electrophoresis of the brain enzyme gives a single slightly fuzzy band (Figure 1 significantly behind pancreatic RNase; heating of the brain nuclease for 3 min at 100° in 8 *M* urea plus 1% 2-mercaptoethanol did not alter the electrophoretic pattern.

Sedimentation equilibrium of the preparation resulted in a linear plot of log *C* vs. X^2, indicative of homogeneity; sedimentation in 6 *M* guanidine hydrocholoride also gave a straight-line graph. In each case the molecular weight was calculated to be $(2.55 \pm 0.08) \times 10^4$; a value of 0.72 was calculated for the partial specific volume. Approximations of the molecular weight from SDS–polyacrylamide gel electrophoresis gave values of $(2.4 \pm 0.1) \times 10^4$ (based on standards of pancreatic RNase, lysozyme, pancreatic DNase, chymotrypsin, and bovine serum albumin).

The specific activity of our best preparations is 8.3×10^4 units/mg of protein. Commercial samples of bovine pancreatic RNase in the same assay give a range of values, the highest of which was 3.9×10^5 units/mg of protein (Hirs component A).

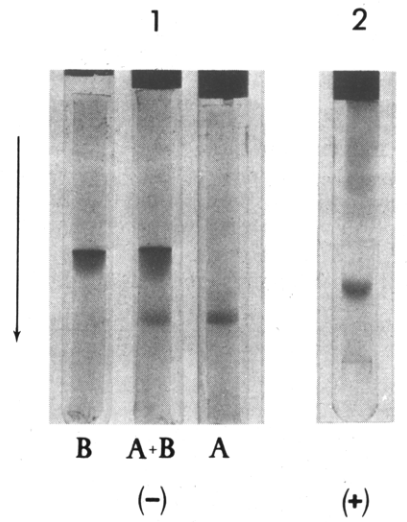

FIGURE 1: Polyacrylamide gel electrophoresis of nucleases: (1) electrophoresis in 7.875% gels at pH 4.0; gels labeled A, bovine pancreatic RNase, Hirs component A; and B, brain RNase; (2) SDS–polyacrylamide gel electrophoresis in a 10.25% gel.

Composition. The results of amino acid analysis of 24-hr hydrolysates of the brain ribonuclease are presented in Table II; for comparison, the composition of bovine pancreatic ribonuclease is also included. A sample of pancreatic RNase was also analyzed, and, exept for the sulfur-containing amino acids, agreed (within one residue) with known values (Moore and Stein, 1973). Both RNases were reduced and carboxymethylated, and then subjected to hydrolysis by trypsin or chymotrypsin. Electrophoretic analysis of the resulting peptides is shown in Figure 2.

Immunology. Antibodies induced by pancreatic RNase have never been seen to precipitate brain nuclease, whether purified or relatively crude, and regardless of the method of preparation (Figure 3). Figure 4 shows that the brain enzyme is inhibited by antibodies to pancreatic RNase, but with much less efficiency than is seen with the pancreatic enzyme. Ribonuclease T_1, used as an unrelated control enzyme, was consistently stimulated by the addition of antiserum, even after correcting for a small amount of hydrolysis of the RNA substrate by serum nucleases. In reciprocal experiments, antibodies to the brain ribonuclease precipitated the brain enzyme but not pancreatic ribonuclease.

Table II: Comparative Composition of Ribonucleases.[a]

Component	Brain Ribonuclease[b]	Pancreatic RNase[c]
Lysine	9.7	8.1
Histidine	4.5	3.2
Arginine	6.4	3.2
Aspartic acid	10.1	12.1
Threonine	6.6[d]	8.1
Serine	9.9[e]	12.1
Glutamic acid	8.8	9.7
Proline	8.2	3.2
Glycine	4.8	2.4
Alanine	5.8	9.7
Half-cystine	3.0[f]	6.5
Valine	6.3	7.3
Methionine	2.3	3.2
Isoleucine	2.1	2.4
Leucine	4.0	1.6
Tyrosine	3.9	4.8
Phenylalanine	2.4	2.4
Tryptophan	1.2[g]	0.0

[a] Data are expressed in mole percent. [b] Based on duplicate analysis of 24-hr hydrolysates. [c] Calculated from Moore and Stein (1973). [d] Corrected for an estimated 5% loss in hydrolysis. [e] Corrected for an estimated 10% loss in hydrolysis. [f] No precaution was taken to prevent destruction of sulfur-containing residues. [g] Estimated spectrophotometrically.

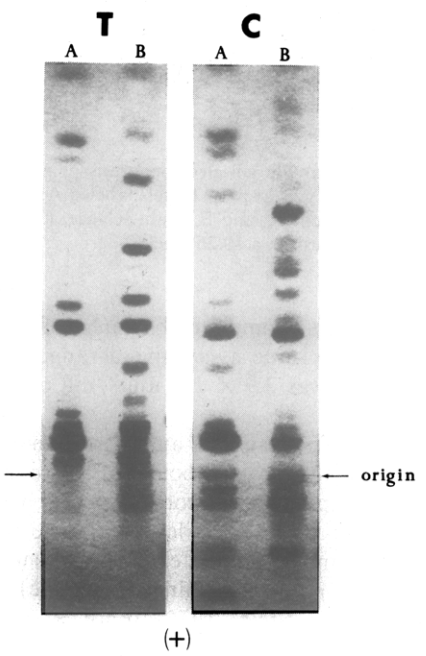

FIGURE 2: Tryptic and chymotryptic peptides of nucleases. Reduced and carboxymethylated pancreatic ribonuclease (A) and brain ribonuclease (B) were hydrolyzed with 2% (w/w) triphenyl chloromethyl ketone trypsin (T) or chymotrypsin (C) and the digests subjected to electrophoris at pH 3.5.

Action on RNA. The effect of pH on the hydrolysis of RNA by the brain enzyme was compared with pancreatic RNase. In each case maximum activity was found near pH 8, but the curve obtained with the brain RNase was skewed toward more alkaline values.

The specificity of the brain enzyme is like that of pancreatic RNase, as judged by fractionation of ^{32}P-labed *E. coli* 5S RNA degradation products as shown in Figure 5. Similar patterns were obtained using brain enzyme purified without acid treatment. Electrophoresis of labeled bacteriophage f2 RNA hydrolysates gave more complex but still similar patterns.

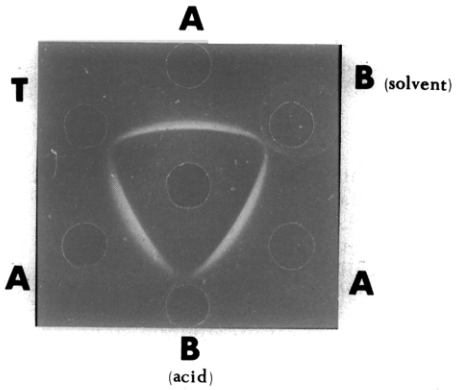

FIGURE 3: Immunodiffusion of nucleases. The center well contained antiserum to bovine pancreatic ribonuclease, while peripheral wells contained ca. 0.5 mg/ml of pancreatic RNase (A), ribonuclease T_1 (T), or brain ribonuclease (B) prepared either in the normal fashion (acid) or by the procedure including acetone fractionation (solvent).

Figure 6 illustrates slab polyacrylamide gel electrophoresis patterns of oligonucleotides generated from bacteriophage f2 RNA by partial hydrolysis with brain or pancreatic RNases. In contrast to the dissimilar patterns shown here, closely related nucleases (e.g., the guanine specific RNase T_1 and an enzyme from *Aspergillus fumigatus*) produce nearly identical oligonucleotide patterns over a wide extent of hydrolysis (Glitz et al., 1972, 1974). The effect of RNA concentration on reaction velocity was measured under conditions of our assay. Values of K_m were calculated to be 0.18 mg of RNA/ml for pancreatic RNase and 0.04 mg of RNA/ml for the brain enzyme.

Iodoacetate reactivity of brain and pancreatic ribonucleases was compared at pH 5.5. The inactivation rates were indistinguishable if purified brain nuclease was used, but the less pure acetone-fractionated preparation was inactivated at only about 10% of the rate above.

Discussion

Purification of the Enzyme. The preparation of the brain enzyme is without major difficulties, and is only slightly complicated by the lipids in the extract and by the need to remove or inhibit the protein ribonuclease inhibitor for successful assay and purification. The final product appears pure by a number of criteria: it is homogeneous in sedimentation equilibrium and in SDS–polyacrylamide gel electrophoresis, and shows a single (although fuzzy) band in gel electrophoresis under nondissociating conditions. Both amino acid analysis and peptide patterns appear consistent with degradation of a single protein of this size. It is likely that the preparation contains glycoprotein components, since amino sugars were apparent in amino acid analysis. The situation may be analogous to the carbohydrate-substituted forms of bovine (Plummer and Hirs, 1963), ovine (Becker et al., 1973), equine (Scheffer and Beintema, 1974) or porcine (Reinhold et al., 1968) pancreatic ribonucleases; carbohydrate components could affect both the homogeneity and molecular weight of the enzyme preparation. In addition, the purification includes treatment with acid, which Reinhold et al. (1968) have shown causes degradation of the carbohydrate of pig pancreatic RNase.

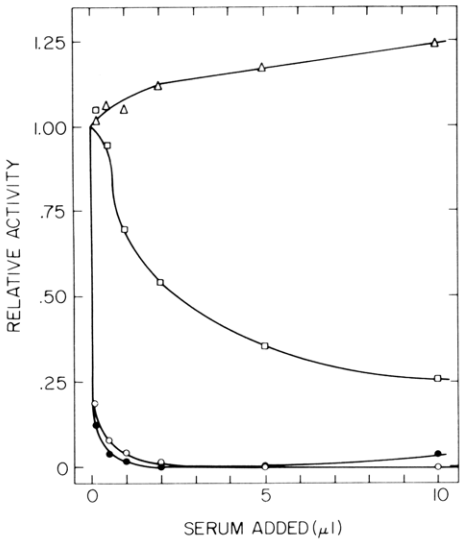

FIGURE 4: Inhibition of nucleases by antiserum to bovine pancreatic RNase. The indicated quantities of antiserum were included in 1.0-ml reaction mixtures in the normal assay: (○) pancreatic RNase (pH 7.0); (●) pancreatic RNase (pH 8.0); (□) brain RNase; (△) ribonuclease T_1.

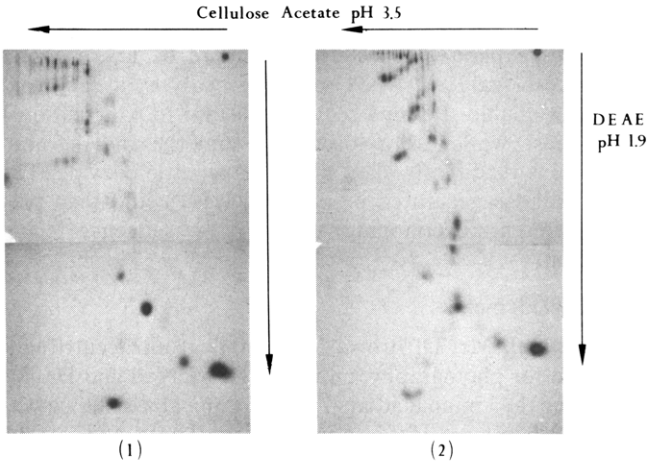

FIGURE 5: Two-dimensional electrophoresis of nuclease hydrolysates of *E. coli* 5S RNA according to Sanger et al. (1965): pattern 1, pancreatic RNase; pattern 2, brain RNase.

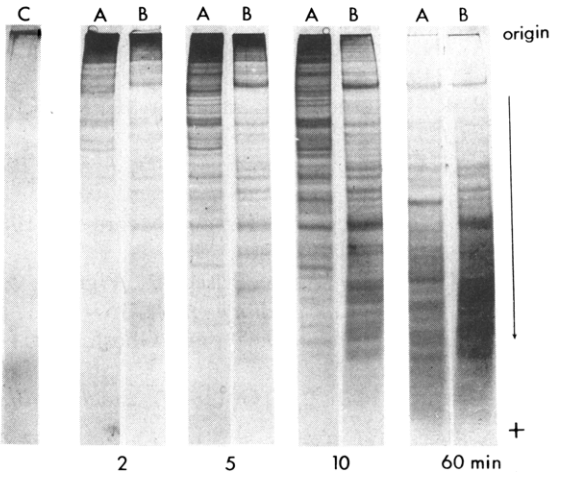

FIGURE 6: Polyacrylamide gel electrophoresis of nuclease hydrolysates of bacteriophage f2 RNA. Digestion was carried out at 30° with 0.6 mg of RNA in 0.3-ml assay buffer (pH 8.0) containing 0.6 unit of bovine pancreatic ribonuclease (A) or brain ribonuclease (B). At the indicated times 50-μl samples were withdrawn and extracted at room temperature with an equal volume of buffer-saturated phenol, and the aqueous phase was withdrawn and lyophilized. About 40 μg of digest was applied to each slot of a 10% polyacrylamide gel, and electrophoresis at 15° was continued for 5.5 hr at 200 V.

The specific activity of the enzyme is lower than the highest values obtained with pancreatic ribonuclease. However, many properties of the proteins are clearly dissimilar, including their interactions with RNA. Thus, the low specific activity may not indicate incomplete purification.

Molecular Weight. The size of this brain ribonuclease is estimated to be about 2.5×10^4 on the basis of SDS–polyacrylamide gel electrophoresis and sedimentation equilibrium studies. These estimates ignore the probability that the preparation is at least partly glycoprotein, which could alter SDS binding (Weber et al., 1972) and the partial specific volume.

Comparison with Other Nucleases. The base specificity of this brain ribonuclease is similar to that of bovine pancreatic RNase (Figure 5), the major ribonuclease of bovine seminal plasma (Floridi et al., 1972), and probably the nuclease of beef brain nuclei (Niedergang et al., 1974), as well as the pancreatic RNases of other species. We are particularly interested in the relationship of the brain enzyme to other bovine ribonucleases.

Many characteristics are shared by the brain and pancreatic ribonucleases. Purification of the brain enzyme was partly based on techniques used with pancreatic RNases. Specificity, pH optima, and the rate of iodoacetate inactivation at pH 5.5 are similar. However, other data indicate that the brain ribonuclease is a distinct entity. It is electrophoretically separable from pancreatic RNase (Figure 1) whether judged by gel staining or nuclease assay, and has a higher apparent molecular weight. The amino acid and peptide composition also indicate that this is a separate protein. The interactions of the brain RNase with antiserum to pancreatic ribonuclease (Figures 3 and 4) suggest that some relationship exists, since the brain enzyme can be inhibited (and thus in some way recognized) by these antibodies. But the inability to precipitate the brain RNase also indicates significant dissimilarity in structure. Finally, the interactions of the nucleases with RNA are clearly in part differentiable (see below).

The brain RNase also shows some similarity to the pancreatic like RNase of bull semen, which occurs as a dimer of about 26,000 mol wt (D'Alessio et al., 1972; Floridi et al., 1972), but we have been unable to dissociate the brain enzyme, and the peptide patterns of Figure 2 suggest it is a larger protein. This brain enzyme may also be related to the beef brain nuclear RNase described by Niedergang et al. (1974). While little is yet known about the nuclear RNase, it appears to be larger (39,000 mol wt), shows a different pH profile, and behaves differently during purification. It thus appears possible that the same animal has four related but distinctive ribonucleases with pyrimidine specificity. The relationship of this brain RNase with at least the pancreatic and seminal RNases could probably best be seen through amino acid sequence analysis; the quantities of enzyme in bovine brain make such a project conceivable.

Action on RNA. The oligonucleotide patterns generated from partial hydrolysis of bacteriophage RNA by the brain and pancreatic ribonucleases are not identical (Figure 6). The seminal RNase has been found to act significantly on double-stranded RNA (Libonati and Floridi, 1969); a similar action on the part of the brain RNase could lessen the

stability of double-stranded oligonucleotide intermediates and thus explain the patterns of Figure 6. The enzymes could be of value in RNA sequence analysis, if they split RNA in double-stranded regions resistant to pancreatic ribonuclease, while less rapidly hydrolyzing the single-stranded regions readily attacked by the pancreatic enzyme. The intermediates generated by each nuclease could then provide different (overlapping) elements for sequence reconstruction.

Acknowledgments

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