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Purification and Characterization of Human Pancreatic Ribonuclease†

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ABSTRACT: A ribonuclease (RNase) has been isolated from normal human pancreas obtained upon autopsy. About 5 mg of RNase is normally recovered per kilogram of pancreas, equivalent to ca. 70% of the total activity and a 700-fold purification from the initial acidified extract. The specific activity of the purified enzyme is identical with that of bovine pancreatic ribonuclease, and a single component is found in sodium dodecyl sulfate–polyacrylamide gel electrophoresis, gel filtration, and reversed-phase high-pressure liquid chromatography. Aggregation of the protein is found upon ultracentrifugation under native and denaturing conditions, and several bands of equal specific activity are seen in polyacrylamide gel electrophoresis of the native protein. At least two components are glycoproteins. A molecular weight of 15 000 is estimated from sodium dodecyl sulfate gel electrophoresis, gel filtration, and amino acid and peptide analyses. The enzyme is related to bovine pancreatic RNase, but distinguishable by amino acid analysis, tryptic peptide maps, and low cross-reactivity of antibodies with the heterologous enzymes. The human enzyme is also inactivated by treatment with iodoacetic acid at pH 5.5 and is essentially identical with bovine RNase in its far-ultraviolet circular dichroism spectrum. The human RNase is like bovine pancreatic RNase catalytically; RNA is cleaved at pyrimidine residues, and activity against poly(cytidylic acid) is high.

Bovine pancreatic ribonuclease is a very well-characterized enzyme. In part because of its easy availability and unusual stability, it frequently serves as a model in biochemical investigations; as a result, much is known about both the protein and its actions [reviewed in Richards & Wyckoff (1971) and Sierakowska & Shugar (1977)]. Investigations of pancreatic ribonucleases from other species have been of interest to workers studying protein structure and function as well as biochemical evolution [e.g., Beintema (1980) and references cited therein, Lenstra et al. (1977), and Welling et al. (1975)].

Human pancreatic ribonuclease has not played a role in these studies. Human tissues are frequently difficult to obtain in large quantities, and the physiological function of the human enzyme is questionable. Bernard (1969) suggests that pancreatic ribonuclease is vestigial in nonruminant vertebrates where it is not needed for salvage of phosphorus from microbial RNA. The enzyme is thus present only at a very low level [less than 10 μg/g of tissue, relative to 1200 μg/g of bovine pancreas (Barnard, 1969)]. Nevertheless, some attempts to purify the human RNase[1] have been reported (Delaney, 1963; Ukita et al., 1964; Bardon et al., 1976; Neuwelt et al., 1977). Our interest in this enzyme was stimulated by the suggestion of Reddi & Holland (1976) that serum ribonuclease, presumably of pancreatic origin, could serve as a specific marker for pancreatic carcinoma. Peterson (1979) has since questioned the diagnostic value of measurement of serum RNase. This disagreement may result from measurements which were limited to enzymatic activity, since several RNases are known to be present in serum (Akagi et al., 1976; Blank & Dekker, 1977). In part to help clarify this question, we have undertaken the biochemical and immunological characterization of some human ribonucleases. Here, we describe the purification and properties of human pancreatic ribonuclease.

Materials and Methods

Assays. Ribonuclease activity was measured by the formation of perchloric acid soluble nucleotides from wheat germ ribosomal RNA (Glitz & Dekker, 1963) or poly(cytidylic acid)

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[1] Abbreviations used: RNase, ribonuclease; $NaDodSO_4$, sodium dodecyl sulfate; poly(C), poly(cytidylic acid); CM-cysteine, carboxymethylcysteine; BSA, bovine serum albumin; Mes, 2-(*N*-morpholino)-ethanesulfonic acid.

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(Calbiochem). The assay of Elson & Glitz (1975) was used, except that the reaction buffer (1.0 mL) included 0.05 M Tris-HCl, 0.15 M NaCl, 0.5 mM $MgCl_2$, and 1 mg/mL bovine serum albumin (Pentex, crystalline), pH 8.2. The assay was linear from 0.2 to 1.5 units, and repeatable within 0.02 unit. In experiments in which the RNA concentration was varied, 4-mL reaction mixtures were used, samples were withdrawn at intermediate reaction times, and initial velocities were determined graphically. Hydrolysis of cytidine cyclic 2',3'-phosphate (Sigma) was measured spectrophotometrically (Blackburn, 1979). Protein was measured by a variation of the method of Lowry et al. (1951) with crystalline bovine pancreatic RNase (Calbiochem) as a standard.

Enzyme Purification. Human pancreas, obtained at autopsy through the Los Angeles County Coroner's Office, was immediately frozen on dry ice and stored in ca. 100-g quantities in a Kelvinator Series 100 ultracold freezer at −70 °C. As needed, tissue was partially thawed at room temperature and processed in ca. 100-g amounts; all work was done in the cold (0–5 °C).

Step 1: Tissue was diced and homogenized in 220 mL of 0.25 M H_2SO_4 and 1 mM $MgCl_2$. A Sorvall Omni-Mixer was used at a power setting of 10, and eight 30-s periods of homogenization were interspersed with chilling on ice. After centrifugation for 20 min at 39000*g* (Sorvall SS-34 rotor, 18 000 rpm), the supernatant was adjusted to pH 8 with concentrated NH_4OH, and the suspension was centrifuged as above.

Step 2: The supernatant (ca. 200 mL) was made 33% in acetone by the addition of 0.5 volume over 20–30 min, followed by a 30-min period unstirred. The precipitate was removed by centrifugation as above, and the stirred supernatant was brought to 60% acetone (v/v). The precipitate was allowed to settle out by gravity, and collected by centrifugation for 5 min at ca. 500*g* (SS-34 rotor, 2000 rpm). The supernatant and an oil layer were decanted, and the sticky precipitate was dissolved in ca. 20 mL of 0.5 M sodium phosphate and 1 mM $MgCl_2$, pH 6.0. The preparation was then dialyzed overnight against three 1-L portions of 0.02 M sodium phosphate and 1 mM $MgCl_2$, pH 6.0.

Step 3: The dialyzed sample was centrifuged for 10 min at 39000*g* and the supernatant applied to a 1.5 × 15 cm column of phosphocellulose (Schleicher & Schuell) which had been equilibrated with 0.02 M sodium phosphate and 1 mM $MgCl_2$, pH 6.0. The column was eluted at 1 mL/min with equilibrating buffer until the A_{280} of the effluent returned to the initial level. Enzyme was eluted with 400 mL of a linear NaCl gradient (0–1.0 M) combined with a pH gradient of 6.0 → 4.9 in 0.02 M sodium phosphate and 1 mM $MgCl_2$. Fractions (6 mL) were collected, and both RNase activity and A_{280} were measured.

Step 4: Fractions containing RNase were pooled, dialyzed overnight against 1 L of 20 mM sodium acetate and 1 mM $MgCl_2$, pH 5.2, and applied to a 5-mL column of agarose–5'-[(4-aminophenyl)phosphoryl]uridine 2'(3')-phosphate (Wilchek & Gorecki, 1969; purchased from Miles Biochemicals) equilibrated with the same buffer. The column was washed with 20 mL of buffer and then with 20 mL of 1% (v/v) acetic acid and 1 mM $MgCl_2$. RNase was eluted with 0.1 M sodium pyrophosphate and 10% (v/v) acetic acid adjusted to pH 5.5 with NaOH. Fractions containing RNase were dialyzed against water, lyophilized, and stored in water or 0.3 M NaCl at −20 °C.

Polyacrylamide Gel Electrophoresis. Native proteins were analyzed at pH 4.5 and 4–6 °C in a β-alanine acetate buffer (Reisfeld et al., 1962); the resolving gel contained 15% acrylamide and 0.1% methylenebis(acrylamide) (Bio-Rad Laboratories), and the spacer gel included 2.5% acrylamide and 0.625% methylenebis(acrylamide) (w/v). $NaDodSO_4$–polyacrylamide gel electrophoresis was done in 0.05 M sodium phosphate and 1 mg/mL $NaDodSO_4$, pH 7.0; gels included 7.5% acrylamide and 0.375% methylenebis(acrylamide). Standard proteins included 5 times crystallized RNase A and crystalline α-chymotrypsinogen (Calbiochem), RNase B and cytochrome *c* (Sigma), and 5 times crystallized ovalbumin (Worthington). Protein reduction and carboxymethylation were described by Hirs (1967).

Gels were stained for protein by using 0.05% Coomassie brilliant blue R in methanol–acetic acid–water, 7:3:30 (v/v). Glycoproteins were detected according to the procedure of Racusen (1979). RNase activity in gels was visualized as described by Wilson (1969), except that the buffer was 0.1 M Tris-HCl and 0.15 M NaCl, pH 8.2. $NaDodSO_4$ was removed from all gels by washing in 25% (v/v) 2-propanol (Blank et al., 1980) in buffer. Estimation of molecular weight followed the procedure of Weber et al. (1972). Slab gel electrophoresis of bacteriophage f2 RNA was described by Glitz et al. (1974).

Amino Acid Analysis. Amino acid composition was determined in duplicate by the method of Moore & Stein (1963) with a Spinco Model 120B automatic analyzer. Samples containing ca. 14 nmol of reduced carboxymethylated RNase were hydrolyzed in vacuo for 24 h at 110 °C in 1 mL of constant-boiling HCl (5.7 M) containing 50 μL of 10% phenol and 2 μL of thioglycolic acid.

Peptide Mapping. Samples of reduced carboxymethylated ribonuclease (ca. 150 μg) were dissolved in 50 μL of 0.05 M ammonium bicarbonate, pH 8.4, and 5–7 μg of triphenyl chloromethyl ketone trypsin (Worthington) was added. After 12 h at 37 °C, the solution was lyophilized; the sample was dissolved in 10 μL of electrophoresis buffer and spotted on a plastic-backed cellulose thin-layer plate (Eastman 13255, 20 × 20 cm) for fractionation as described by Pierce et al. (1976). Electrophoresis (50 min, 10 mA) at pH 1.9 in formic acid–acetic acid–H_2O (1:3.5:37, v/v) was followed by ascending chromatography in butanol–pyridine–acetic acid–water (6:4:1:4). Peptides were detected by spraying with ninhydrin.

Immunology. Antibodies were induced in young adult male New Zealand rabbits by an initial intradermal injection of 0.5 mg of RNase, emulsified with 4 volumes of complete Freund's adjuvant in a total volume of 1 mL, at multiple sites along the back. Booster injections of 0.1 mg of RNase, emulsified with an equal volume of adjuvant, were given at 15, 22, and 29 days. Blood was collected weekly from day 21. Immunoglobulins were purified from serum by two rounds of precipitation from 45%-saturated ammonium sulfate solution.

Other Methods. Two-dimensional fractionation of ^{32}P-labeled oligonucleotides was done according to Sanger et al. (1965). Sedimentation equilibrium was done at (34–40) × 10^3 rpm in a Spinco Model E ultracentrifuge, and circular dichroism spectra were recorded on a Beckman CD spectrophotometer. Neuraminidase treatment used enzyme purchased from Sigma and followed the procedure of Paulson et al. (1977). Inactivation of RNases with iodoacetic acid was carried out at 37 °C in 0.2 M Mes buffer, pH 5.5 (Plapp, 1973), containing 0.1 mg/mL BSA. The reaction was initiated by the addition of RNase, and aliquots were removed at intervals, and the reaction was quenched by dilution into cold 0.1 M Tris-HCl, pH 8.2, containing 2 mg/mL BSA. Half-lives of the modified enzymes were estimated graphically.

Table I: Purification of Ribonuclease from 100 g of Human Pancreas

step	volume (mL)	RNase (units/mL)	protein (mg/mL)[a]	sp act. (units/mg of protein)[a]	yield (%)
1, homogenate	220	1550	2.8	560	100
2, acetone	26	10400	4.4	2360	79
3, phosphocellulose	160	1580	0.096	15000	68
4, affinity chromatography	11	17100	0.042	391000	53

[a] Protein measurements based on a bovine RNase A standard.

Reversed-phase high-pressure liquid chromatography was done with a Beckman-Altex instrument with a Microbondapak C18 column and a gradient of 5 mM trifluoroacetic acid to 65% (v/v) acetonitrile. A column (0.9 × 60 cm) of Sephadex G-100 was used at 4 °C for gel filtration studies. The elution buffer contained 0.2 M NaCl in 0.02 M sodium phosphate, pH 6.0.

Results

Enzyme Purification. Autopsy human pancreas was obtained at times ranging from a few hours to several days after death. Some preference was shown for tissue from young adults. Pancreas from individuals with infectious diseases or known histories of alcohol or drug abuse as well as obviously fatty tissue was avoided. Otherwise, no selection was made based on sex, race, or cause of death.

The specific activity of crude preparations ranged from ca. 1750 to 3500 enzyme units/g of pancreas, or 450–700 units/mg of protein in the initial extract. We found no correlation of activity with any identified characteristic of the sample, including the period (up to 5 days) between death and autopsy. Therefore, samples were pooled and processed in 100–125-g lots. The purification of a typical preparation is summarized in Table I. Overall yields ranged from 50 to 75% of initial activity, with purifications of 500–1000-fold from the acidified extract.

Criteria of Purity. The final specific activity of the enzyme preparation, 3.9×10^5 units/mg of protein, is equal to or greater than that of several commercial samples of bovine pancreatic RNase in the same assay. Reversed-phase high-pressure liquid chromatography showed a single sharp absorbance peak, and gel filtration on Sephadex G-100 also gave a single, slightly trailing protein peak which was eluted at a volume nearly identical with that of bovine pancreatic ribonuclease. In each instance, the protein and enzymatic activity were coincident.

Polyacrylamide gel electrophoresis in the presence of $NaDodSO_4$ gave a single band of protein corresponding directly to RNase activity. Electrophoresis of native enzyme at neutral or slightly acidic pH in 7.5% polyacrylamide also gave a single component. In contrast, electrophoresis at pH 4.5 in 15% acrylamide gave evidence for at least three protein bands, all moving somewhat behind bovine pancreatic RNase. Each band of protein corresponded directly with bands detected by staining for RNase activity. Assay of ribonuclease activity in slices of unstained gels also showed a direct and quantitative correspondence of RNase and protein. Nearly identical observations have been reported by Scheffer & Beintema (1974) in their sequence analysis of horse pancreatic ribonuclease. Gels stained for carbohydrate showed that the two slowest moving components were glycoproteins, but treatment with neuraminidase did not alter the electrophoretic pattern. Electrophoresis of crystalline bovine pancreatic RNase A under the same conditions showed a similar single band in $NaDodSO_4$ gels, and two active components in native gels, and RNase B showed three active bands in native gels. Electrophoretic results are shown in Figure 1.

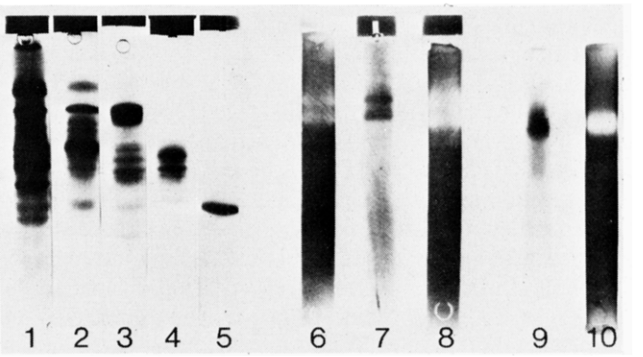

FIGURE 1: Polyacrylamide gel electrophoresis of RNases. Gels 1–5 show electrophoresis in 15% acrylamide at pH 4.5 for ca. 5 h. Gels 1–4 show purification of the human RNase at steps 1–4 of Table I. Gel 5 shows bovine pancreatic RNase. Gels 6–8 show electrophoresis of purified human RNase in 15% acrylamide at pH 4.5 for ca. 3 h. Gels 6 (2 μg of RNase) and 8 (4 μg of RNase) were stained for activity; gel 7 (12 μg of RNase) was stained with Coomassie blue. Gels 9 and 10 display electrophoresis of purified human RNase in 7.5% polyacrylamide gels containing 0.1% $NaDodSO_4$. Gel 9 was stained with Coomassie blue, and gel 10 was stained for RNase activity. In gels 1–8, migration was toward the cathode (bottom), and in gels 9 and 10 toward the anode (bottom).

Aggregation of the protein was observed in several instances. An initially clear enzyme preparation became cloudy after a few days at 4 °C or upon freezing and thawing. Centrifugation [$(1.89 \times 10^5)g$, 12 h] gave an enzymatically active precipitate and a clear (active) supernatant which still showed significant light scattering in the ultraviolet. Storage in buffers containing 0.3 M NaCl reduced the extent of aggregation but did not eliminate the problem. Sedimentation equilibrium of the native enzyme also showed aggregation of the protein; after 24 h of centrifugation, there was a nearly linear plot of log C vs. X^2, but continued centrifugation for 48 and 72 h showed two and then several larger components. Sedimentation equilibrium in 6 M guanidinium hydrochloride was also confused by aggregation of the protein upon extended centrifugation. The tendency of human RNases to aggregate has been previously noted by Neuwelt et al. (1977).

Properties of the Protein. The molecular weight of the human RNase is estimated to be about 15 000. This value is based on comparison with known proteins in gel filtration (RNase = 14 500 ± 1500 daltons) and $NaDodSO_4$–polyacrylamide gel electrophoresis (Figure 1; RNase = 15 000 ± 1000 daltons). Amino acid analysis of the enzyme, compared with that of bovine RNase in Table II, is consistent with a protein of 14–15 000 daltons. Two-dimensional maps of tryptic peptides, shown in Figure 2, reveal 16–19 components; this number is consistent with the number of lysine plus arginine residues in the analysis of Table II and thus with the estimated molecular weight. Both the amino acid composition and the peptide patterns are significantly different from those of bovine RNase. In contrast, circular dichroism at 250–330 nm was essentially indistinguishable when the human and bovine RNases were compared.

Antibodies directed against either the human or the bovine pancreatic RNase show the proteins to be different but related. Each antibody preparation effectively precipitated the homologous enzyme, but failed to show cross-reactivity with the heterologous enzyme in immunodiffusion. Results are shown in Figure 3. But immunoglobulin inhibition of RNase activity did reveal some cross-reaction, as shown in Figure 4.

Table II: Composition of Ribonucleases

component	human pancreatic RNase nmol[a]	human pancreatic RNase nearest integer[b]	bovine RNase A residues[c]
lysine	40.9	11	10
histidine	19.5	5	4
arginine	29.0	7	4
CM-cysteine	21.6	6	8
aspartic acid	60.8	15	15
threonine	27.3	7	10
serine	69.9	18	15
glutamic acid	53.1	13	12
proline	16.5	4	4
glycine	45.1	11	3
alanine	24.3	6	12
valine	33.0	8	9
methionine	8.9	2	4
isoleucine	10.8	3	3
leucine	12.3	3	2
tyrosine	15.8	4	6
phenylalanine	13.6	3	3
tryptophan	nd		0
glucosamine	present		

[a] Values obtained in duplicate 24-h hydrolysates. No corrections for losses or incomplete hydrolysis have been made; nd, not determined. [b] Calculated on the basis of five histidine residues per molecule. [c] From Moore & Stein (1963).

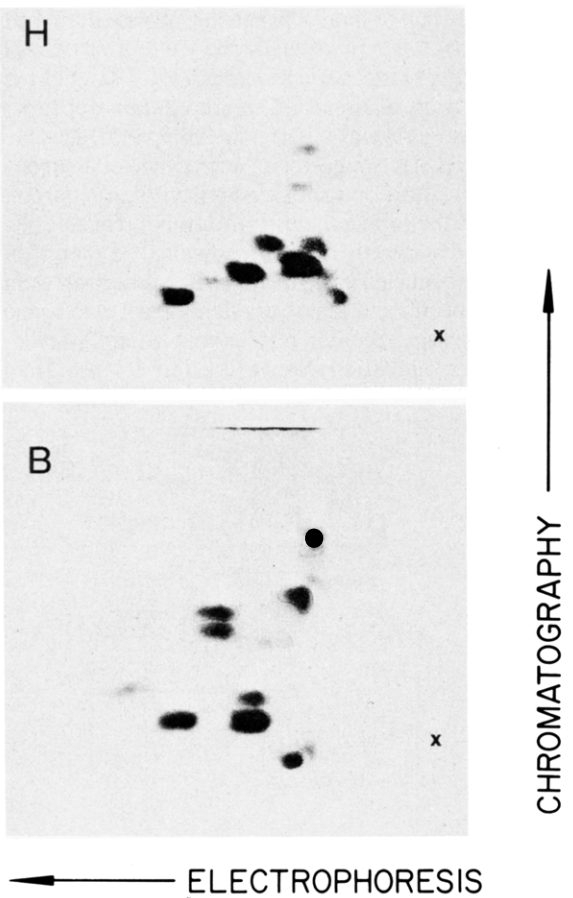

FIGURE 2: Tryptic peptides of pancreatic ribonucleases: (H) human; (B) bovine RNase A. Separation in the first dimension was by electrophoresis at pH 1.9, and in the second dimension by ascending chromatography.

Cross-reactivity of the anti-RNase A immunoglobulins is very slight; ca. 10^5 times as much antibody is needed to attain inhibition of human RNase equivalent to that seen with RNase A. In contrast, the lower titer antihuman RNase immunoglobulin preparation gives inhibition of RNase A at levels only ca. 30-fold higher than are needed to inhibit human RNase. (In each case, immunoglobulins were prepared from serum from a single bleeding of one rabbit.)

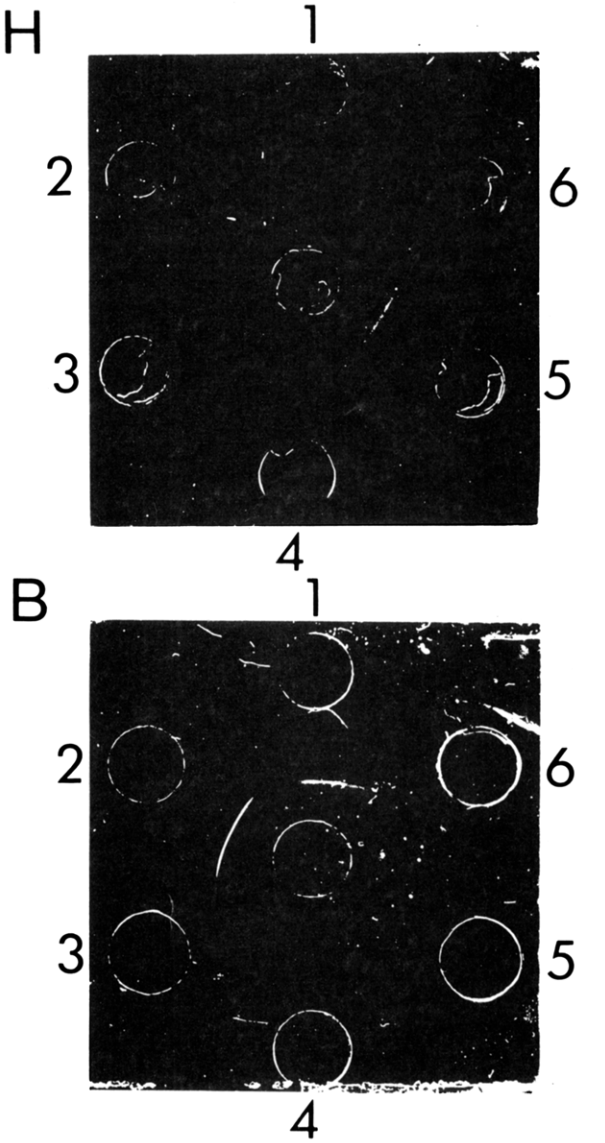

FIGURE 3: Immunodiffusion of ribonucleases. The center wells contained immunoglobulins to (H) human pancreatic RNase and (B) bovine pancreatic RNase A. Peripheral wells 1–3 contained RNase A at 0.5, 0.25, and 0.125 mg/mL. Well 4 contained bovine brain RNase, 1 mg/mL. Well 5 contained 1.8 mg/mL purified human RNase (not aggregated), and well 6 contained 0.8 mg/mL visibly aggregated human RNase.

Catalytic Properties. Assay conditions (pH, ionic strength) were varied to determine conditions for optimal activity of crude or partially purified RNase. The enzyme was active at neutral and alkaline pH values with an optimum near pH 8.2. Addition of NaCl to the assay mixture stimulated RNase activity, with a broad optimum of 0.1–0.25 M; higher levels of NaCl resulted in enzyme inhibition. In contrast, bovine pancreatic RNase showed optimal activity in this assay in the absence of added NaCl.

Hydrolysis of ^{32}P-labeled 23S ribosomal RNA from *E. coli* followed by two-dimensional electrophoretic fractionation of the oligonucleotide products gave the maps of Figure 5; human and bovine paincreatic ribonucleases produced essentially identical patterns. Partial hydrolysis of bacteriophage f2 RNA with each RNase also gave closely related product patterns,

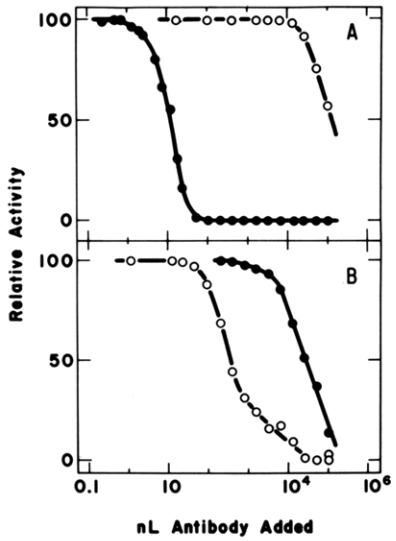

FIGURE 4: Antibody inhibition of ribonuclease activity. Immunoglobulins were added to the standard RNase assay as shown. (A) Antibovine RNase; (B) antihuman RNase: (●) bovine RNase A, (○) human RNase.

as judged by one-dimensional polyacrylamide gel electrophoresis shown in Figure 6. But some products of partial hydrolysis, shown by the arrows in Figure 6, appear characteristic of either the human or the bovine RNase, but not of both.

The hydrolysis of poly(C) was measured in comparison with the results obtained with bovine pancreatic and brain ribonucleases, as summarized in Table III. These data show the human pancreatic enzyme to be much more like the (secretory) bovine pancreatic enzyme than like the (cellular) brain RNase. The effect of substrate RNA concentration on reaction velocity was also compared, as shown in Figure 7. The K_m of the human RNase, 0.05 mg/mL RNA, equivalent to ca. 0.15 mM total nucleotides or 0.07 mM pyrimidine nucleotides in the wheat germ RNA substrate, is much lower than the K_m value of 0.15 mg/mL RNA found for bovine pancreatic RNase. When hydrolysis of cytidine cyclic 2′,3′-phosphate was measured, K_m values of 0.46 mM (bovine RNase A) and ca. 1 mM

Table III: Substrate Dependence of Ribonuclease Activity[a]

enzyme	substrate	wavelength (nm)	activity (units/mg) × 10^{-6}	activity mmol min^{-1} mg^{-1}
human RNase	poly(C)	260	0.83	35.0
		280	1.72	
	RNA	260	0.34	10.3
bovine RNase A	poly(C)	260	0.57	22.6
		280	1.05	
	RNA	260	0.37	11.3
bovine brain RNase	poly(C)	260	0.027	1.1
		280	0.049	
	RNA	260	0.083	2.4

[a] The assay of Elson & Glitz (1975) was modified to include measurement of perchlorate-soluble products of poly(C) hydrolysis at 280 nm, without change in the definition of an enzyme unit.

(human pancreatic RNase) were determined by extrapolation of $[S]/V_i$ vs. [S] plots.

Inactivation of the human and bovine RNases by iodoacetic acid is shown in Figure 8. In each instance, 0.1 mg/mL bovine albumin was present in the reaction mixture. In the absence of added albumin, the activity of human RNase was quickly lost, regardless of the presence of iodoacetate.

Discussion

The purification of human pancreatic RNase described here is relatively simple and gives a good yield of protein (Table I); from 12 preparations, we have obtained 3–6 μg of purified RNase per gram of tissue. The preparation appears to be homogeneous by several criteria, including $NaDodSO_4$–polyacrylamide gel electrophoresis, the presence of a single component in gel filtration and high-pressure liquid chromatography, consistent stoichiometry in amino acid and peptide analyses, and a specific activity equivalent to that of bovine pancreatic ribonuclease. Heterogeneity is seen in sedimentation equilibrium and in polyacrylamide gel electrophoresis of native enzyme. Similar results have been observed with several pure pancreatic RNases [e.g., pig RNase, Reinhold et al. (1968), and horse RNase, Scheffer & Beintema (1974)].

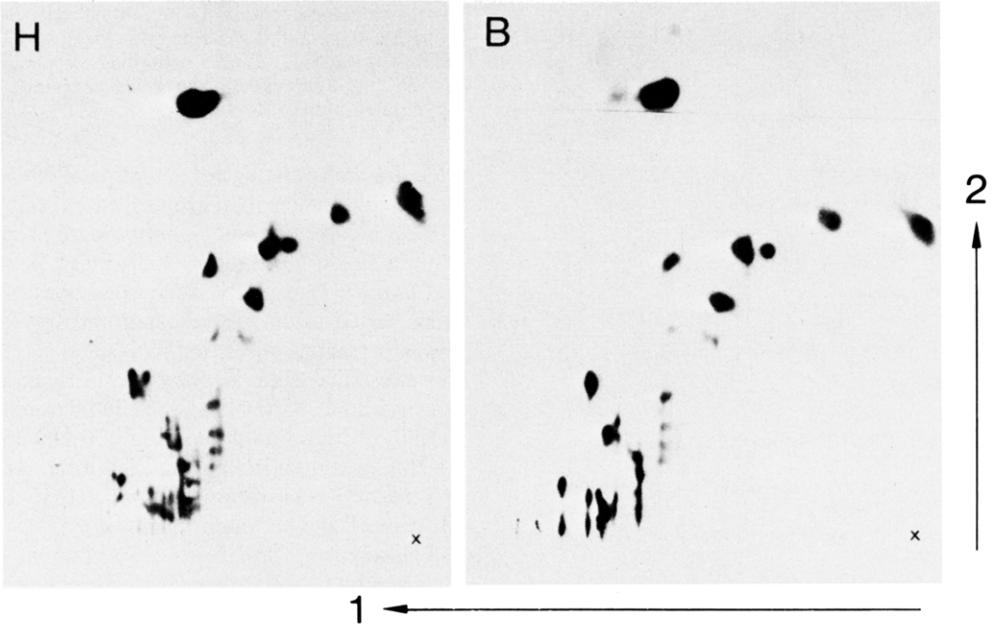

FIGURE 5: Two-dimensional separation of RNase hydrolysates of *E. coli* ribosomal RNA according to Sanger et al. (1965). (H) Human pancreatic RNase; (B) RNase A. First dimension: electrophoresis on cellulose acetate at pH 3.5. Second dimension: electrophoresis on DEAE paper at pH 1.9.

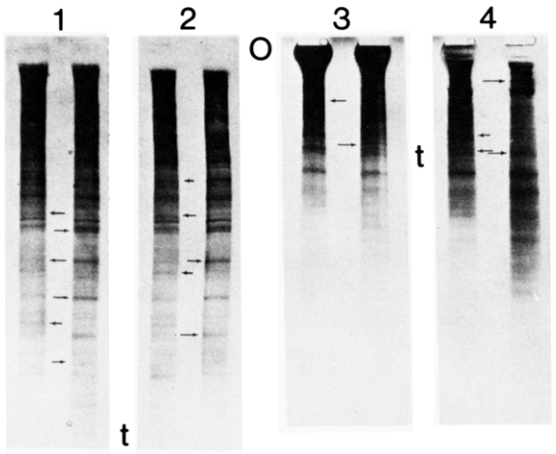

FIGURE 6: Polyacrylamide gel electrophoresis of RNase hydrolysates of bacteriophage f2 RNA. In each panel, the left-hand track is a bovine RNase A hydrolysate and the right-hand track a human RNase hydrolysate. The origin is marked with O, and the position of a tRNA marker with t. Panels 1 and 2: 5% polyacrylamide, 10- and 20-min hydrolyses, respectively. Panels 3 and 4: 12.5% polyacrylamide, 45- and 120-min hydrolyses.

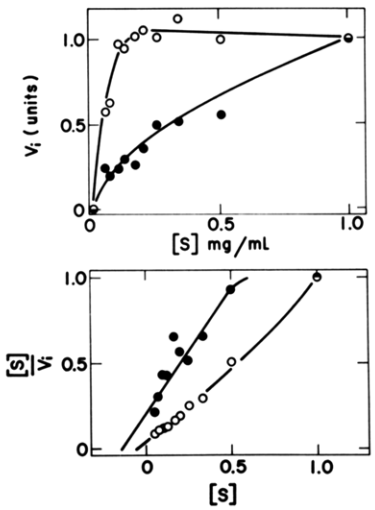

FIGURE 7: Effect of substrate concentration on RNase activity. Initial rates of formation of perchloric acid soluble nucleotides from wheat germ RNA were measured for (O) human pancreatic RNase and (●) bovine RNase A.

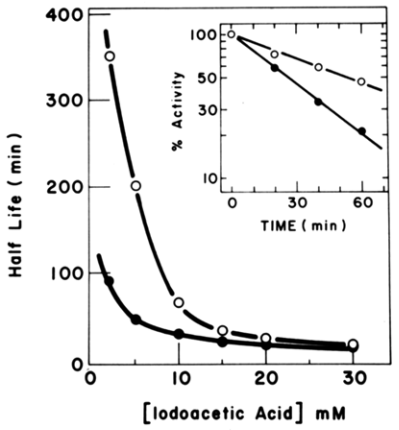

FIGURE 8: Inactivation of RNases by iodoacetic acid at pH 5.5. Half-lives were estimated graphically (inset: 10 mM iodoacetic acid) for (O) human pancreatic RNase and (●) bovine RNase A.

These enzymes and other RNases show heterogeneity because of glycosylation (Beintema et al., 1976), and the human enzyme is at least partially glycosylated. However, in contrast to the findings of Thomas & Hodes (1979) with urinary RNases, neuraminidase treatment did not alter the electrophoretic pattern. Finally, Neuwelt et al. (1977) attribute multiple banding of human RNases to enzyme aggregation.

We believe this enzyme preparation to be more highly purified than any reported in earlier studies. Delaney (1963) lost all activity after a few steps, and his urinary RNase P shares few properties with this enzyme. Ukita et al. (1964) attained a ca. 500-fold purification of one component, but they were limited to paper electrophoresis and activity measurements to judge purity. Bardon et al. (1976) also purified the enzyme about 500-fold, but they started with a neutral extract which included more soluble protein than the acid extracts used here and by Ukita et al. (1964). Although it appeared homogeneous upon electrophoresis, the preparation of Bardon et al. (1976) had less than one-fourth the specific activity of bovine pancreatic RNase in RNA hydrolysis. Neuwelt et al. (1977) also started with a neutral extraction procedure; their yield of 300 μg of enzyme from 24 g of pancreas is not consistent with the enzyme amounts in human pancreas found by Barnard (1969) or in this work, and no attempts to demonstrate purity were reported. In each instance above, limitations imposed by the difficulty of obtaining human pancreas plus the low level of RNase in the tissue undoubtedly contributed to the problems of enzyme purification and characterization.

As a protein, the human RNase is most easily differentiated from the bovine enzyme by its amino acid composition (Table II), tryptic peptide maps (Figure 2), and its immunological properties (Figures 3 and 4). Yet, it is obviously like other pancreatic ribonucleases. Structural studies of pancreatic RNases (Lenstra et al., 1977; Beintema, 1980) show considerable variation in those amino acid residues which are not involved in the catalytic site or overall folding of the protein, and lead to the conclusion that the enzyme has evolved very rapidly (Welling et al., 1975; Beintema, 1980). The data in Table II are consistent with this view, especially if our recovery of CM-cysteine was incomplete and the usual four disulfide bridges are present in the human enzyme. Similar problems are apparent in the cysteine analyses of, e.g., bovine seminal vesicle (D'Alessio et al., 1972; Irie et al., 1973) and brain (Elson & Glitz, 1975) RNases or whale pancreatic RNase (Yamada et al., 1974).

Antibody inhibition of RNase activity (Figure 4) indicates some interference in bovine RNase action by antihuman RNase immunoglobulins, but much less cross-reactivity in the interaction of antibovine RNase antibodies with the human enzyme. These results are almost totally opposite to the precipitation results of Neuwelt et al. (1977), although in better agreement with their blocking immunoassay. Since different antibody preparations were used in each laboratory, the inconsistency is easily rationalized, and each result indicates some immunological relatedness. Such a conclusion would be expected from the immunological cross-reactivity of several (nonhuman) pancreatic RNases (Welling et al., 1976; Prager et al., 1978).

The catalytic specificity of the human RNase is very similar to that of bovine pancreatic ribonuclease (Figures 5 and 6; Table III). Its susceptibility to iodoacetate inactivation (Figure 8) further suggests a related catalytic mechanism (Crestfield et al., 1963; Heinrikson, 1966). But the human enzyme differs from bovine RNase in secondary aspects of specificity, as judged by the oligonucleotide patterns of Figure 6 (Glitz et al., 1974), and in its primary interaction with an RNA substrate (Figure 7). The high content of basic amino acids (Table II) and migration in polyacrylamide gels (Figure 1) at pH 4.5 are each consistent with the pK_a value of 10.3

reported by Ukita et al. (1964); the very high affinity of the enzyme for RNA relative to that for cytidine cyclic phosphate may thus reflect ionic interactions of this very basic protein with the acidic polynucleotide.

Reddi & Holland (1976) reported that circulating levels of pancreatic ribonuclease serve as a marker for pancreatic cancer. Their conclusion is supported by Nakane et al. (1979), Renner et al. (1978), and Warshaw et al. (1980), but not by Isaacs (1977) or Peterson (1979). Maor & Mardiney (1979) suggest that much of the confusion is due to the normal multiplicity of ribonucleases in serum. The use of antibodies to well-defined RNase proteins, as opposed to measurement of RNase activities, should allow resolution of this conflict.

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