NATURE AND POSSIBLE ORIGIN OF HUMAN SERUM RIBONUCLEASE

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SUMMARY: Human Serum contains an acidic RNase which is glycoprotein in nature. It is thermostable at pH 4.2 and thermolabile at pH 8.5. It has a pH optimum at 6.5. Its activity either on poly (C) or RNA is endonucleolytic and is absolutely dependent on citrate or phosphate. It exhibits highest preference for the secondary phosphate esters of cytidine 3'-phosphates. It has no action on cytidine 2':3'-cyclic phosphate. Poly (A) and poly (G) are not only refractory to its action, but also inhibit its action on poly (C). Its rate of hydrolysis of Poly (U) is about 2% of that of poly (C). It differs from bovine pancreatic RNase. It is, however, similar to human pancreatic RNase suggesting that its primary source is pancreas.

Ribonucleases are widely distributed in nature. They all possess one common property, namely the cleavage of RNA via intramolecular transphosphorylation, which results in the production of ribonucleoside 2':3'-cyclic phosphates in the first stage of reaction. Some RNases exhibit base specificity and some do not. Thus, for instance, bovine pancreatic RNase is highly specific to secondary phosphate esters of cytidine 2':3'-cyclic phosphates and uridine 2':3'-cyclic phosphates (1); RNase T_1 specifically cleaves secondary phosphate esters of guanosine 2':3'-cyclic phosphates (2,3); and others of bacterial (4) and plant (5) origin do not show such base specificity and hydrolyse all the diester bonds in RNA. In this paper is presented evidence showing that human serum RNase has the highest preference for the secondary phosphate esters of cytidine 2':3'-cyclic phosphates and is similar to human pancreatic RNase. Furthermore, human serum RNase could serve as a biochemical marker in certain pancreatic disorders because of its pancreatic origin and unique specificity.

MATERIALS AND METHODS

Materials: Highly polymerized yeast RNA and cytidine 2':3'-cyclic phosphate were purchased from Schwarz/Mann; poly (C), poly (G), poly (A), and poly (U) were purchased from Miles Laboratories, Inc.; highly polymerized calf thymus DNA, bis-p-nitrophenyl phosphate and p-nitrophenyl phosphate were obtained

from Sigma Chemical Co. All other reagents used in this investigation were of reagent grade.

Serum RNase Preparation: Blood was drawn from volunteer laboratory workers, allowed to clot for one hour at room temperature and centrifuged at 3000 rpm for 15 min. Serum was removed with a capillary pipette. To 10 ml of serum were added with mixing 10 ml of 7% $HClO_4$. After 10 min at room temperature, the mixture was centrifuged in the cold at 12,000 x g for 30 min. The clear supernatant was removed and saved. The precipitate was suspended in 5 ml of 3.5% $HClO_4$ and centrifuged as above. The supernatant was combined with the first and dialysed against two changes of 2 liters of H_2O with gentle stirring at 4° for 24 hours. This preparation will be referred to as serum RNase.

Pancreatic Extract: Human pancreas obtained at autopsy was placed in dry ice and was powdered in a frozen state. Fifty g of powder and 50 g of sand were transferred to a porcelain mortar. To this mixture were added 100 ml of H_2O. It was ground with a pestle until a slurry was attained. This was strained through three layers of cheese cloth. The filtrate was centrifuged at 12,100 x g in the cold for 20 min. The clear supernatant was tested for RNase activity. Since it was the objective to compare all forms of RNase present in pancreas with human serum RNase, no attempt was made to fractionate or purify the supernatant.

Other Methods: Protein was determined according to the colorimetric procedure of Lowry et al. (6).

RESULTS: Precipitation of the bulk of serum proteins with 3.5% $HClO_4$ and dialysis of acid-soluble fraction resulted in 100-fold purification of serum RNase (Table 1). It has an absorption maximum and minimum at 278 nm and 250 nm

Table 1. ACTION OF WHOLE SERUM, SERUM RNase AND PANCREATIC RNase ON HOMOPOLYRIBONUCLEOTIDES

Preparation	µg of protein in 0.05 ml	Polymer hydrolysed (µg) Poly (C)	Poly (U)	Poly (A)	Poly (G)	Serum RNase purification factor
Whole serum	350	180	3.8	0	0	
Serum RNase	2.8	150	3.0	0	0	105
Pancreatic RNase	85	175	5.4	0	0	

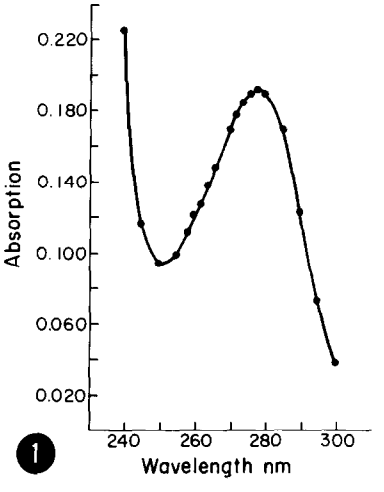
Reaction mixtures consisting of 0.05 ml of polymer (200 µg), 0.15 ml of 0.10 M phosphate-borate buffer, pH 6.5, and 0.05 ml of preparation were incubated at 37° for 15 min. Enzyme and reagent blanks were run side by side. At the end of the incubation period, the tubes were transferred to an ice bath. To each tube was added wtih mixing 0.25 ml of 12% $HClO_4$ containing 20 mM lanthanum nitrate. After 20 min at 0°, the precipitates were removed by centrifugation in the cold at 12,100 x g for 30 min. The supernatants were diluted with H_2O and their absorption was measured. The supernatants containing poly (C) reaction products were measured at 278 nm and the others were measured at 260 nm.

respectively. The ratio of light absorption at 280 nm and 260 nm is 1.57 (Fig. 1).

Electrophoresis of the serum RNase preparation in polyacrylamide gel at pH 8.3 and staining the gel with periodic acid-Schiff reagent revealed that it has two protein components migrating toward the anode. All the RNase activity is confined to the major component while the minor component is free from it (Fig. 2). It is evident from these results that the serum RNase is an acidic glycoprotein.

The serum RNase preparation is free from DNase, phosphodiesterase and phosphomonoesterase activities when tested with DNA, bis-p-nitrophenylphosphate, and p-nitrophenylphosphate respectively as substrates.

Action on Homopolyribonucleotides: Serum RNase, like the whole serum from which it is isolated and human pancreatic RNase, has the highest activity toward poly (C), has no action on poly (A) or poly (G) and has only a slight



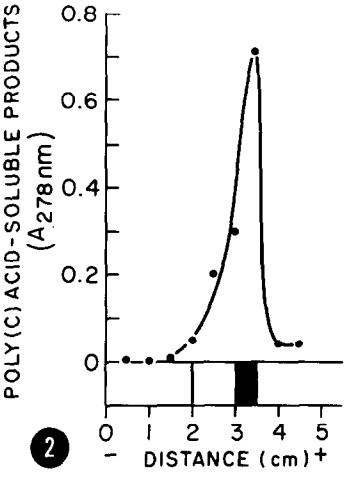
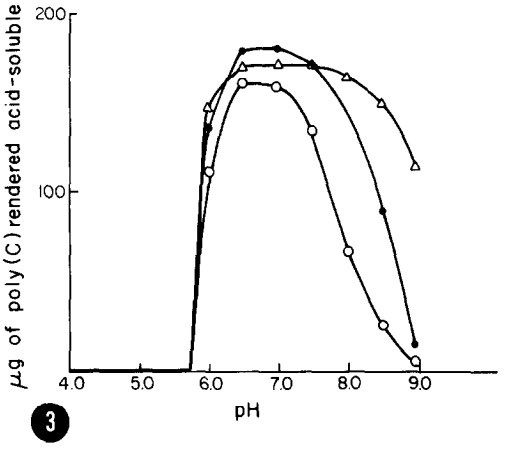


Figure 1. Absorption spectrum of serum RNase (180 µg protein per ml) in H_2O.

Figure 2. Polyacrylamide gel electrophoresis of serum RNase, Electrophoresis in polyacrylamide gels was done at pH 8.3 in Tris-glycine buffer (12). Each gel contained 18 µg of protein. The gels were run at 4° for 20 min at 2.0 mA per tube and for additional 60 min at 4.0 mA per tube. The gels were stained with the periodic acid-Schiff reagent (13) specific to glycoproteins. The unstained gels were cut and the gel slices were eluted in 0.5 ml of phosphate borate buffer at pH 6.5. The RNase activity of the eluates was determined as described in the legend to Table 1 with poly (C) as a substrate.



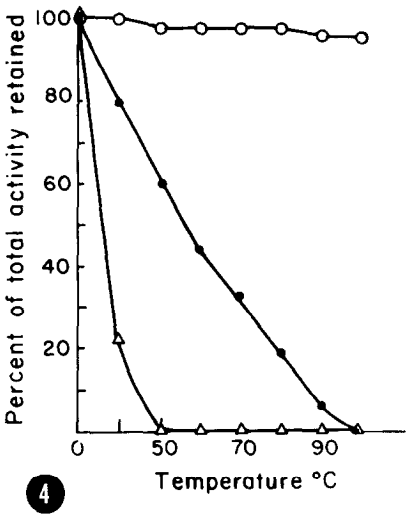


Figure 3. The effect of pH on the rate of hydrolysis of poly (C) by the whole serum, serum RNase and pancreatic RNase. The buffers used were 0.1 M succinate-borate for pH values ranging from 4.0-5.7 and 0.1 M phosphate-borate buffer for pH values ranging from 6.0-9.0. Activities were determined as described in the legend to Table 1. O——O, whole serum; ●——●, serum RNase; Δ——Δ, pancreatic RNase.

Figure 4. Stability of serum RNase and pancreatic RNase. Four-tenths ml of enzyme samples in succinate-borate buffer, pH 4.2, or in phosphate-borate buffer, pH 8.5 were held for 10 min at temperatures ranging from 40°-100° and transferred to an ice bath. After appropriate dilution with 0.1 M phosphate-borate buffer, pH 6.5, their activities were determined as described in the legend to Table 1 with poly (C) as a substrate. O——O, serum RNase at pH 8.5; Δ——Δ, pancreatic RNase at pH 8.5.

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activity toward poly (U). The rate of hydrolysis of poly (U) by the serum RNase is about 2% of that of poly (C) (Table 1).

pH Effect: Serum RNase exhibits maximal activity at pH 6.5. The whole serum and human pancreatic RNase likewise have their optimal activity at pH 6.5 (Fig. 3). However, with RNA as a substrate, all three have their pH optimum at 8.5.

Stability: The results presented in Fig. 4 show that both the serum RNase and the pancreatic RNase are remarkably stable at pH 4.2, but their activities are readily lost at pH 8.5. The pancreatic RNase lost its activity completely when it was held at 50° for 10 min at pH 8.5, while the serum RNase lost about 40% of its activity under the same conditions. The difference in the stability

of the serum RNase and pancreatic RNase at pH 8.5 could be attributed to the presence of proteolytic enzymes in the crude pancreatic extract.

Activators and Inhibitors: The results presented in Table 2 show the effect of phosphate and citrate on the activities of serum RNase and pancreatic RNase. Both the enzymes are absolutely dependent on 0.1 M phosphate or 0.02 M citrate for their activity.

The results presented in Table 3 show that poly (G) completely inhibits the action of serum RNase and pancreatic RNase on poly (C), while poly (A) under the same conditions inhibits 93% of the serum RNase activity and about 70% of pancreatic RNase activity.

Table 2. EFFECT OF PHOSPHATE AND CITRATE ON THE HYDROLYSIS OF POLY (C) BY SERUM RNase AND PANCREATIC RNase

Additions	Absorbance at 278 nm	
	Serum RNase	Pancreatic RNase
0.05 ml H_2O	0	0
0.05 ml of 0.5 M sodium phosphate, pH 6.5	0.420	0.490
0.05 ml of 0.1 M sodium citrate, pH 6.5	0.470	0.455

Reaction mixtures consisting of 0.05 ml of poly (C) (200 μg), 0.10 ml of 0.1 M borate buffer, pH 6.8, 0.05 ml of addition given in the Table and 0.05 ml of enzyme (serum RNase 2.8 μg; pancreatic RNase 85 μg), were incubated at 37° for 15 min. The rest of the procedure was the same as given in the legend to Table 1.

Table 3. EFFECT OF POLY (G) AND POLY (A) ON THE HYDROLYSIS OF POLY (C) BY SERUM RNase AND PANCREATIC RNase

Additions	Activity %	
	Serum RNase	Pancreatic RNase
0.05 ml H_2O	100	100
0.05 ml poly (A) 200 μg	7	32
0.05 ml poly (G) 200 μg	0	0

Reaction mixtures consisting 0.05 ml of poly (C) (200 μg), 0.10 ml of 0.10 M phosphate-borate buffer, pH 6.5, 0.05 ml of addition given in the Table, and 0.05 ml of enzyme (serum RNase 2.8 μg; pancreatic RNase 85 μg) were incubated at 37° for 15 min. The rest of the procedure was the same as given in the legend to Table 1.

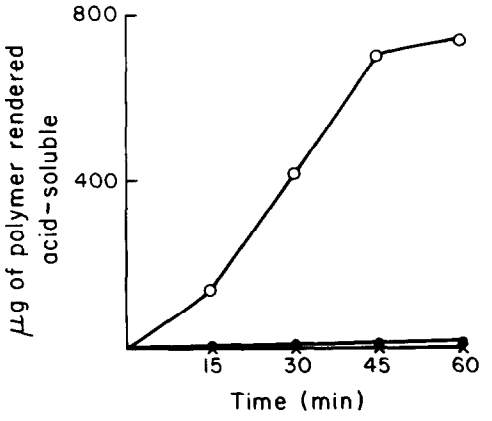

Figure 5. Time course of hydrolysis of homopolyribonucleotides by serum RNase. Reaction mixtures consisting of 0.5 ml of 0.1 M phosphate-borate buffer, pH 6.5, 0.3 ml of polyribonucleotides (1200 μg) and 0.2 ml of serum RNase (11 μg protein) were incubated at 37° and at intervals of time 0.2 ml aliquots were pipetted into test tubes at 0° containing 0.2 ml of lanthanum nitrate-$HClO_4$ solution. Rest of the procedure is the same as described in the legend to Table 1. O——O, poly (C); ●——●, poly (U); X——X, poly (A) and poly (G).

Time Course of Hydrolysis of Homopolyribonucleotides by Serum RNase: The results presented in Fig. 5 show that poly (A) and poly (G) were unaffected by the serum RNase even after prolonged digestion; only about 16 μg or about 2% of poly (U) was hydrolysed in 2 hours; and about 800 μg of poly (C) was hydrolysed under the same conditions. It is evident from these results that the serum RNase has the highest preference for poly (C).

Mode of Action of Serum RNase: If the serum RNase is an exonuclease and cleaves poly (C) stepwise, mononucleotides should predominate in the reaction products. On the other hand, if it functions as an endonuclease and cleaves poly (C) at random, oligonucleotides should predominate in the reaction products. These two types of reaction products could be distinguished by employing (1) perchloric acid in which mononucleotides and oligonucleotides containing less than five nucleotides are soluble and (2) perchloric acid plus uranyl-acetate in which only the mononucleotides are soluble. The results presented in Fig. 6 show that as the digestion of poly (C) by the serum RNase progresses perchloric acid soluble material is more abundant in the reaction products

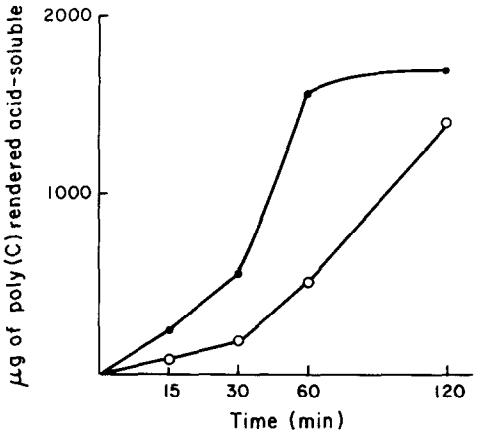

Figure 6. Time course of hydrolysis of poly (C) by serum RNase. The reaction mixture consisting of 0.5 ml of 0.1 M phosphate-borate buffer, pH 6.5, 0.3 ml of poly (C) (2000 μg) and 0.2 ml of serum RNase (11 μg protein) was incubated at 37°. At intervals of time, two 100 μl aliquots of the reaction mixture were withdrawn; one was mixed with 0.5 ml of cold 7% $HClO_4$ (v/v) and the other with 0.5 ml of cold 7% $HClO_4$ containing 0.25% (w/v) uranylacetate. After 15 min at 0°, the precipitates were removed by centrifugation in the cold. After suitable dilution, the absorbencies of the supernatants were measured at 278 nm. O——O, fraction soluble in 5.8% $HClO_4$; ●——●, fraction soluble in 5.8% $HClO_4$ plus 0.21% uranylacetate.

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than that soluble in perchloric acid plus uranylacetate, suggesting that the mode of action of serum RNase is endonucleolytic. The enzyme acts in similar manner on naturally occurring RNA's such as yeast RNA.

The digestion products of poly (C) contained mono cytidine 2':3'-cyclic phosphate, di and tri nucleotides terminated by cytidine 2':3'-cyclic phosphate, suggesting that the serum RNase cleaves poly (C) via intramolecular transphosphroylation. The enzyme has no action on cytidine 2':3'-cyclic phosphate or uridine 2':3'-cyclic phosphate.

DISCUSSION: Human serum RNase differs from bovine pancreatic RNase, which in addition to being highly specific to secondary phosphate esters of cytidine 2':3'-cyclic phosphate and uridine 2':3'-cyclic phosphate is also capable of hydrolysing poly (A) when a large excess of enzyme is used (7). Furthermore, cytidine 2':3'-cyclic phosphate and uridine 2':3'-cyclic phosphate are converted to their corresponding nucleoside 3'-phosphates by bovine pancreatic RNase, while these cyclic nucleotides are completely inert to the action of

human serum RNase. Bovine pancreatic RNase is a basic protein, while human serum RNase is acidic and a glycoprotein in nature.

As shown in this paper, there are striking similarities between human serum RNase and human pancreatic RNase. They both have their pH optimum at 6.5. They are thermostable at pH 4.2 and thermolabile at pH 8.5. Their activity is absolutely dependent on the presence of phosphate or citrate. They are inhibited by poly (A) and poly (G). They are highly specific to secondary phophate esters of cytidine 3'-phosphate. These similarities suggest that serum RNase could be of pancreatic origin. This conclusion is at variance with earlier studies (8,9) which suggested that the white cells are the source of serum RNase. We examined both the red and white cells for their ribonucleases. The red cells have very little RNase; the RNase present in the white cells has properties quite different from those of serum RNase (10). Hence the white cells could not be the source of the serum RNase.

Human serum RNase could have regulatory and defense functions. It could degrade infectious viral RNA's rendering them harmless to the organism. Such a defense function for blood RNases has already been suggested (11). Since there is evidence suggesting that the serum RNase might be of pancreatic origin, any deviation in its quantity and quality from the pattern in the normal serum could serve as an indicator in appraising the state of pancreas. Such data have been accumulated (Reddi and Holland) for cancer of pancreas and will be reported elsewhere.

Lastly, because of its specificity toward the secondary phosphate esters of cytidine 3'-phosphates, human serum RNase could be of help in elucidating the primary structure of RNA.

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