Human Platelet Ribonuclease

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SUMMARY: Human platelets contain an RNase which has a pH optimum at 5.0. It hydrolyzes the secondary phosphate esters of uridine 3'-phosphates. It slowly converts uridine 2':3'-and cytidine 2':3'-cyclic phosphates to their corresponding nucleoside 3'-phosphates. Poly (A), poly (G) and poly (C) are not only refractory to the action of this enzyme, but also inhibit its action on poly (U). It differs from human granulocyte RNase, human serum RNase and bovine pancreatic RNase. Because of its unique property, this enzyme could serve as a biochemical marker in disorders involving the platelet destruction.

INTRODUCTION

Human serum contains ribonuclease variants derived from blood elements (1), pancreas (2) and possibly other organs. Their amounts in the serum are dependent on the disease states of the individuals. Thus for instance, granulocyte specific RNase of the serum markedly increases in conditions of agranulocytosis (1), pancreatic specific RNase is elevated in pancreatic cancer, renal insufficiency and in cancers causing obstruction of the pancreatic ducts (3). Although the physiological significance of serum RNase variants is, at present, unknown, they could be helpful (i) in providing defense against RNA containing viruses, (ii) in hydrolyzing the messenger RNA's after their functions are completed and providing the building blocks for the synthesis of new messenger RNA's and (iii) in serving as biochemical markers in the assessment of the healthy and disease states.

Human platelets have been reported to contain a multitude of enzymes (4) but RNase is not one of them. The present communication deals with a platelet RNase, which is specific to secondary phosphate esters of uridine 3'-phosphates and thus contradicts the earlier findings (5) which showed that the platelets are free of RNase. Because of its unique properties, which differ significantly from those of human granulocyte RNase and serum RNase and its

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elevation in the serum of patients with thrombocytopenia, this enzyme could serve as a platelet marker. The elevation of this enzyme in the serum of patients with platelet destruction also suggests that it is indeed the integral part of the platelets and not simply adsorbed on to them.

MATERIALS AND METHODS

Materials: Poly (A), poly (G), poly (C) and poly (U) were purchased from Schwarz/Mann; uridine 2':3'- and cytidine 2':3'-cyclic phosphates and highly polymerized calf thymus DNA were purchased from Sigma Chemical Company. All other reagents used in this investigation were of reagent grade.

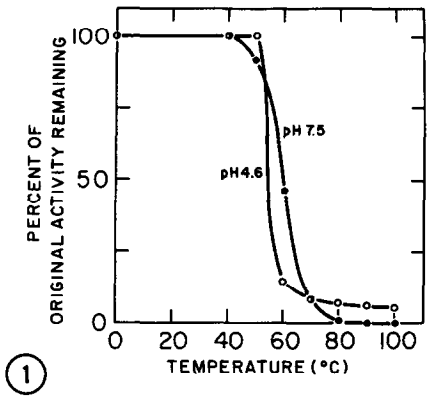
Human Platelets: Human platelets used in this investigation were collected by plateletphoresis from normal individuals. The preparation was freed of contaminating red cells and other blood elements as follows. The cell suspension was centrifuged at 160 g for 15 min at 15°. The colorless top layer was carefully removed and centrifuged at 5900 g for 30 min. The pellet was washed once by gently suspending in 0.9% NaCl. The microscopic examination revealed that the final preparation thus obtained consisted of only the platelets.

Platelet RNase preparation: The pellets containing 3.5×10^{11} platelets were suspended in 10 ml of 0.05 ml of 0.05 M phosphate-borate buffer, pH 7.2 containing 0.1% Triton x 100. The suspension was placed in dry ice-ethanol mixture. The frozen cell mass was quickly thawed at 37°. Five ml batches of the thawed material was then homogenized in a glass-teflon homogenizer for 2 min at 0°. The homogenates were combined and centrifuged at 12,100 g in the cold for 15 min. The supernatant was saved. The residue was suspended in 5 ml of above buffer, homogenized and centrifuged as above. The combined supernatants were centrifuged in the cold at 235,644 g in the rotor Ti 75 for 2 hr. The supernatant was dialyzed at 4° with gentle stirring against 2 liters of 0.5 M succinate-borate buffer, pH 4.6 for 15 hr. During this time, a heavy precipitate which had no RNase activity was formed. This was removed by centrifugation in the cold at 12,100 g for 30 min. The supernatant was distributed in 1 ml portions, held at 50° for 10 min, cooled and centrifuged in the cold for 15 min at 12,100 g. The supernatant, which will be referred to as platelet RNase was clear and colorless. The protein content as determined by the colorimetric proceedure (6) was 7.4 mg/ml.

RESULTS

There was no loss in the activity of platelet RNase when held at 50° for 10 min at pH 4.6, while under the same conditions at pH 7.5, about 54% of its activity was lost. When held at temperatures above 50°, it rapidly lost its activity both at pH 4.6 and pH 7.5 (Fig 1).

With poly (U) as a substrate, the platelet RNase activity was optimal at pH 5.0 (Fig 2). Results presented in Fig. 3 further show that while poly (U) was rapidly hydrolyzed by this enzyme, poly (A), poly (G), and poly (C) remained totally unaffected even after prolonged digestion. Poly (A), poly (G) and



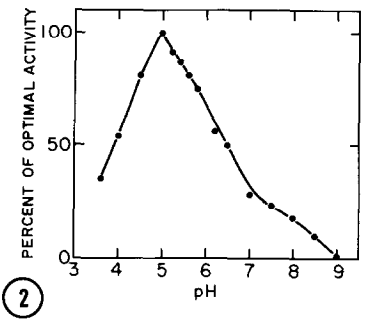
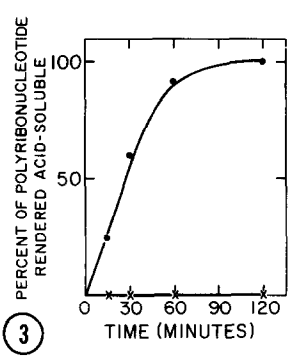


Fig. 1 - Stability of human platelet RNase. Thin-walled glass tubes containing 0.2 ml of enzyme preparation in 0.1 M succinate-borate buffer, pH 4.6 or in 0.1 M phosphate-borate buffer, pH 7.5 were placed at temperatures ranging from 40° to 100° for 10 min. They were then cooled and centrifuged at 12,100 g for 20 min in the cold. The RNase activities of the supernatants were determined as follows. Reaction mixtures containing 0.05 ml of poly (U)(100 µg), 0.15 ml of 0.1 M succinate-borate buffer and 0.5 ml of enzyme were incubated at 37° for 15 min. At the end of the incubation period, the tubes were transferred to an ice bath. To each tube was added with mixing 0.25 ml of cold 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 g for 30 min. The supernatants were diluted with H_2O and measured at 260 nm. Substrate blanks were run side by side.

Fig. 2 - Effect of pH on the rate of hydrolysis of poly (U) by human platelet RNase. The buffers used were, 0.1 M succinate-borate for pH values ranging from 3.5 to 5.7 and 0.1 M phosphate-borate buffer for pH values ranging from 5.8 to 8.0. Reaction mixtures consisting of 0.05 ml of poly (U) (100 µg), 0.15 ml of buffer and 0.05 ml of enzyme preparation (370 µg) were incubated at 37° for 15 min. Remainder of the procedure is the same as given in the legend to Fig. 1.

poly (C) were not only refractory to the action of this RNase but also inhibit its action on poly (U) and the degree of their inhibitory effect was poly (G) > poly (C) > poly (A) (Fig. 4). Mg^{++} at 0.01 M and 0.004 M concentrations respectively inhibited 30% and 10% of platelet RNase activity, while Ca^{++} at the above concentrations respectively inhibited 19% and 5% of its activity. Its activity was inhibited by Zn^{++} at all concentrations ranging from 0.01 M to 0.001 M. p-chloromercuriphenyl sulfonic acid at 0.002 M and 0.001 M concentrations respectively inhibited 84% and 68% of its activity, while iodoacetic acid at above concentrations had no effect. EDTA at 0.01 M concentration inhibited only about 4% of its activity (Table 1).



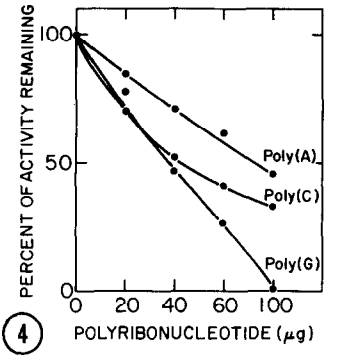


Fig. 3 - Time course of hydrolysis of polyribonucleotides by human platelet RNase. Reaction mixtures consisting of 0.3 ml of polyribonucleotide (600 μg), 0.9 ml of 0.1 M succinate-borate buffer, pH 5.0, and 0.3 ml of platelet enzyme (1.1 mg protein) were incubated at 37°. At intervals of time 0.2 ml of digest was pipetted into test tubes at 0° containing 0.2 ml of lathanum nitrate-$HClO_4$ solution. The remainder of the experimental conditions were the same as described in the legend to Fig. 1. (●) poly (U); (X) poly (C), poly (A) and poly (G).

Fig. 4 - Effect of poly (A), poly (G) and poly (C) on human platelet RNase activity. Reaction mixtures contained 0.05 ml of poly (U) (100 μg) in 0.1 M succinate-borate buffer, pH 5.0, 0.1 ml of 0.1 M succinate-borate buffer, pH 5.0, 0.05 ml of polyribonucleotide, the effect of which is to be tested in H_2O, and 0.05 ml of RNase (370 μg). The remainder of the conditions were the same as described in the legend to Fig. 1.

Uridine 2':3'-cyclic and cytidine 2':3'-cyclic phosphates were slowly converted to their corresponding nucleoside 3'-phosphates when they were incubated for a prolonged period with a large excess of enzyme (Table 2).

Exhaustive digestion of poly (U) by the platelet RNase resulted in the production of mono (uridine 2':3'-cyclic phosphate and uridine 3'-phosphate), di and tri nucleotides suggesting that the enzyme is an endonuclease and hydrolyzes poly (U) via intramolecular transphosphorylation. The enzyme has no action on DNA when tested with calf thymus DNA as a substrate at pH 5.0 and pH 7.5.

DISCUSSION

Human platelet RNase is specific to secondary phosphate esters of uridine 3'-phosphates and in that respect it is similar to human granulocyte RNase. But it differs from the latter in several other aspects. It is unstable when held above 50° at pH 4.6, while granulocyte RNase at this pH is remark-

Table 1. Effect of metal ions and other substances on the hydrolysis of poly (U) by human platelet RNase.

Substance	Percentage of Total Activity Remaining			
	10^{-2} M	4.10^{-3} M	2.10^{-3} M	10^{-3} M
Mg acetate	70	90	98	100
$CaCl_2$	81	95	100	100
Zn acetate	15	25	33	60
EDTA	96			
PCMPS			16	32
Iosoacetic acid			100	

EDTA, ethylenediamine tetraacetate; PCMPS, ρ-chloromercuriphenyl sulphonic aicd.

Reaction mixtures consisting of 0.05 ml of poly (U) (100 µg), 0.10 ml of 0.1 M succinate-borate buffer, pH 5.0, 0.05 ml of H_2O or the test substance and 0.05 ml of platelet RNase (370 µg protein) were incubated at 37° for 15 min. The remainder of the procedure was the same as that described in the legend to Fig. 1.

Table 2. Effect of Human platelet RNase on Uridine 2':3'-cyclic and cytidine 2':3'-cyclic phosphates.

Substance	Percent of total cyclic nucleotides hydrolyzed
Uridine 2':3'-cyclic phosphate	20.0
Cytidine 2':3'-cyclic phosphate	8.4

Reaction mixtures consisting of 0.1 ml of uridine 2':3'-cyclic or cytidine 2':3'-cyclic phosphates(200 µg) in 0.1 M succinate-borate buffer, pH 5.0 and 0.1 ml of platelet RNase (740 µg protein) were incubated at 37° for 24 hrs. Blanks consisted of cyclic nucleotides without enzyme. At the end of the incubation period, 0.05 ml of reaction mixtures and the blanks were placed on Whatman 3 mm filter paper and dried in a current of air at room temperature. The development of chromatogram and the identification of the reaction products were done according to the method described earlier(7).

ably stable at all temperatures ranging from 40-100°. The granulocyte RNase exhibits optimal activity at pH 6.5, while platelet RNase has its pH optimum at 5.0. The granulocyte RNase has no action on uridine 2':3'-cyclic and cytidine 2':3'-cyclic phosphates, while the platelet RNase slowly converts them to their corresponding nucleoside 3'-phosphates. The granulocyte RNase is inhibited by EDTA at all concentrations ranging from 0.01 M to 0.001 M while the platelet RNase is not effected at those concentrations. At 0.01 M Mg^{++} concentration about 52% of the granulocyte RNase activity is inhibited, while at the same concentration, about 30% of the platelet RNase activity is effected. At 0.01 M Ca^{++} concentration 70% of the granulocyte RNase is inhibited, while at the same concentration only about 19% of the platelet RNase activity is effected. Iodoacetic acid at 0.001 M concentration inhibited about 25% of the granulocyte RNase activity, while no activity of the platelet RNase was lost even at 0.002 M concentration. ρ-chloromercuriphenyl sulfonic acid at 0.001 concentration inhibited 98% of granulocyte RNase activity, while at the same concentration about 68% of the platelet RNase activity was effected (1). The platelet RNase also differs from human pancreatic RNase in its stability, pH optimum, specificity and in its action toward uridine 2':3'-cyclic and cytidine 2':3'-cyclic phosphates (2).

The *in vivo* function of the platelet RNase is unknown. In the sera of patients with thrombocytopenia, two-fold elevation of this enzyme was observed (Reddi, unpublished data). Thus, this enzyme might serve as a platelet marker in cases of platelet destruction resulting either from drug therapy or pathological conditions. Furthermore, the elevation of this enzyme in the serum of patients with platelet destruction indicates that it is an integral part of the platelets and not simply adsorbed on to them.

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