RNA Metabolism of Normal and Leukemic Leukocytes

II. Ribonuclease

By Robert Silber, Kenneth W. Unger, Joan Keller and Joseph R. Bertino

In the course of studies on the synthesis of RNA, it was noted that RNA isolated from normal leukocytes was more frequently degraded to oligonucleotides than RNA from leukemic leukocytes. Since it had been previously reported that ribonuclease is present in rabbit leukocytes, it appeared possible that this difference reflected the relative activities of ribonuclease in normal and leukemic cells. Inasmuch as information concerning the properties and level of human leukocyte ribonuclease was lacking, characterization and purification of the enzyme were undertaken. When optimal conditions for the assay of ribonuclease activity had been established, the levels in normal and leukemic cells were determined.

Materials and Methods

Yeast RNA was obtained from Schwarz Bioresearch. A 1 per cent solution of RNA in 0.01 M potassium phosphate buffer, pH 6.5, was dialyzed against 100 volumes of this buffer for 48 hours. Sephadex G75 was obtained from Pharmacia Fine Chemicals. Bentonite and uranyl acetate were purchased from Fisher Scientific. Leukocytes were isolated by a previously described procedure which yields a granulocyte rich preparation containing less than 15 per cent lymphocytes. Normal lymphocytes were isolated from peripheral blood by passage through a glass wool column and were contaminated by less than 10 per cent granulocytes. One-tenth to 0.5 ml. of packed cells were homogenized for 3 minutes in 2–10 volumes of 0.05 M potassium phosphate buffer, pH 6.5, in a Virtis homogenizer at 45,000 rpm. The homogenate was centrifuged at 10,900 g for 15 minutes; the supernatant was used for assays and for further purification studies. Ribonuclease was assayed by Anfinsen's method with the following minor modifications: The incubation mixture contained 0.6 ml. 0.05 M potassium phosphate buffer, pH 6.5; 1 mg. RNA in 0.6 ml. 0.05 M phosphate buffer, pH 6.5, and 0.02 to 0.4 mg. of homogenate protein. The final volume was brought to 1.8 ml. with 0.05 M phosphate buffer, pH 6.5. The reaction was carried out at room temperature.

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This work was done, in part, at the Summer Research Institute, Will Rogers Memorial Hospital, Saranac Lake, New York.

This work was supported by USPHS Grant CA 06657 and The American Cancer Society Grant P-360. Robert Silber is a Career Investigator of the New York City Health Research Council. Joseph R. Bertino is a recipient of a USPHS Career Development Award.

First submitted March 14, 1966; accepted for publication May 10, 1966.

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Fig. 1.—Effect of pH on leukocyte activity. The enzyme was isolated from the peripheral blood of normal subjects.

Three-tenths ml. aliquots were removed at 0, 2, 4, 6, and 8 minutes and deproteinized with 0.1 ml. of 0.75 per cent uranyl acetate in 25 per cent perchloric acid. After a 10-minute centrifugation at 2000 g, 0.1 ml. of supernatant was diluted to 1 ml. with water and its absorbancy at 260 μm determined. All assays were run at two more concentrations of homogenate to insure that the reaction measured was linear. Duplicate determinations agreed within 20 per cent. One enzyme unit is that activity resulting in an increase of one optical density unit per ml. per minute of incubation, measured at 260 μm. Specific activity is expressed in units per mg. of homogenate protein. Protein was determined by the biuret method with crystalline human albumin as a standard. "Normal" leukocytes were isolated from the blood of laboratory personnel. Patients with leukemia were studied before therapy or were in relapse and not currently on any chemotherapeutic regimen. The leukocyte count of all patients with chronic lymphocytic leukemia exceeded 50,000 per ml. with more than 85 per cent small lymphocytes. Only those chronic myelocytic leukemia patients with leukocyte counts above 40,000 per ml. and with more than 30 per cent immature granulocytic forms were studied. In patients with acute leukemia, the leukocyte count exceeded 25,000 per ml., with at least 75 per cent blast forms.

RESULTS

Tenfold purification of the enzyme from normal leukocytes was accomplished by a procedure similar to that described by Rushizky et al.6 Because of the small amount of normal leukocytes available as starting material, only limited purification was feasible. The enzyme in the final step had a specific activity of 200 units and had no acid or alkaline phosphomonoesterase activity with disodium p-nitrophenyl phosphate as substrate. Enzyme activity at this stage of purification was stable for at least 8 weeks at −4 C. A pH versus activity profile for the enzyme purified from a preparation containing 90 per cent granulocytes is shown in Figure 1. It can be seen that the enzyme activity has a pH optimum between 6 and 6.5, showing a rather sharp decrease in activity above 7.5 or below 6. The enzyme purified from lymphocytes had a similar pH optimum.

Increasing the concentration of substrate increased the velocity of the reac-
Fig. 2.—Effect of substrate concentration on rate of ribonuclease activity.

Fig. 3.—Fractionation of yeast RNA digested by pancreatic ribonuclease (A) and leukocyte ribonuclease (B). First dimension: Electrophoresis from left to right in 0.06 M formic acid adjusted to pH 2.7 with concentrated ammonium hydroxide. Second dimension: Paper chromatography in descent with 53:45 tertiary butanol: 0.02 M ammonium formate pH 3.7. The paper was saturated so that solvent could run off the paper without edge effects.

The substitution of leukocyte RNA or E. coli RNA for the yeast RNA had no effect on the reaction.

The site of enzymatic action on the substrate was investigated by separating and identifying the oligonucleotides produced by two-dimensional electrophoresis and chromatography. The nucleotide map obtained with purified leukocyte ribonuclease was similar to that obtained with pancreatic ribonuclease (Fig. 3), and differed markedly from that of T₁ ribonuclease, indicating that leukocyte ribonuclease acts on phosphodiester bonds adjacent to pyrimidines, as does pancreatic ribonuclease.
Table 1.—Inhibition of Ribonuclease by Bentonite

<table>
<thead>
<tr>
<th>Concentration of Bentonite in Reaction Mixture (Per Cent)</th>
<th>Inhibition Per Cent of Control</th>
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<tbody>
<tr>
<td>.001</td>
<td>0</td>
</tr>
<tr>
<td>.005</td>
<td>30</td>
</tr>
<tr>
<td>.01</td>
<td>92</td>
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<tr>
<td>.015</td>
<td>98</td>
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<tr>
<td>.025</td>
<td>100</td>
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The enzyme was purified from normal leukocytes. Bentonite was suspended in 0.001 M potassium acetate buffer pH 4.8.

Table 2.—Levels of Ribonuclease in Leukocytes

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Number of Subjects</th>
<th>Activity (Δ OD/min./mg.)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>mean ± S.E.</td>
</tr>
<tr>
<td>Normal—&quot;granulocyte rich&quot;</td>
<td>15</td>
<td>19.0 ± 3.2</td>
</tr>
<tr>
<td>Normal—&quot;lymphocyte rich&quot;</td>
<td>3</td>
<td>2.5</td>
</tr>
<tr>
<td>Acute leukemia</td>
<td>5</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>Chronic myelocytic leukemia</td>
<td>8</td>
<td>8.2 ± 2.5</td>
</tr>
<tr>
<td>Chronic lymphatic leukemia</td>
<td>12</td>
<td>6.2 ± 2.4</td>
</tr>
<tr>
<td>Leukocytosis secondary to infections</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Polycythemia vera</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Myeloid metaplasia</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Eosinophilia</td>
<td>3</td>
<td>68</td>
</tr>
</tbody>
</table>

The effect of an inhibitor of ribonuclease, bentonite, was tested with leukocyte ribonuclease (Table 1). Bentonite at a concentration of 0.015 per cent completely inhibited enzyme activity. In this respect the enzyme resembled ribonucleases present in other cells.

After the properties of ribonuclease had been defined under the above conditions, a comparative study of its activity in normal and leukemic cells was undertaken. The clinical and enzyme data are summarized in Table 2. In the granulocytic series the lowest levels were observed in myeloblastic leukemia and the highest in mature granulocytes. Among cells in the granulocytic series it seems likely that eosinophils are richest in this enzyme as shown by the elevated values found in three subjects with eosinophilia. There is a tenfold (p < 0.01) difference in the mean activity of leukocyte ribonuclease between patients with myeloblastic leukemia and normal subjects; intermediate values are observed in chronic myelocytic leukemia. The wide range of activities present in the leukocytes from patients with chronic myelocytic leukemia could not be accounted for by therapy or correlated with the visible granulation of the cell. The levels in chronic lymphatic leukemia were low, but comparable to those observed in normal lymphocytes isolated from peripheral blood.

The ribonuclease activity in leukocytes from a variety of hematologic and nonhematologic disorders manifesting leukocytosis was also determined. Low levels of ribonuclease activity were also encountered in polycythemia vera, myeloid metaplasia, infectious mononucleosis, Hodgkin's disease and infection.
DISCUSSION

Previous studies on the ribonuclease in rabbit polymorphonuclear granulocytes have suggested a predominantly lysosomal localization. Although a clear concept of the in vivo function of ribonuclease is lacking, its presence in organelles which are involved in phagocytosis would suggest a role for this enzyme in the degradation of ingested foreign bacteria to acid soluble nucleotides.

Although the presence of ribonuclease in the phagocytic, nonmitotic, mature granulocyte is not unexpected, low levels of this enzyme were also found in leukemic myeloblasts and lymphocytes, cells which have mitotic potential but no phagocytic activity. Specific activity in these cells, however, is only about a tenth that in mature granulocytes. Recently, the presence of a latent ribonuclease in the 30S component of ribosomes has been reported, and a role for the enzyme in biosynthetic processes has been suggested. It is conceivable that the ribonuclease found in “blast” cells falls into this category. It is of interest that while such biosynthetic enzymes as dihydrofolic reductase and thymidylate synthetase decline during leukocyte maturation, ribonuclease, which catalyzes the degradation of RNA, shows a tenfold increase.

The pH optimum of the leukocyte enzyme is somewhat lower than that of pancreatic ribonuclease; it appears, nevertheless, to act on the same phosphodiester bonds as the enzyme from pancreas (Fig. 3). In this respect it resembles the other ribonucleases isolated from mammalian sources. The detailed knowledge of the pH optimum and bentonite inhibition characteristics of leukocyte ribonuclease has been of help in the extraction of relatively undergraded RNA from normal and leukemic cells.

SUMMARY

Ribonuclease has been partially purified from human leukocytes and its properties investigated. The enzyme has a pH optimum between 6 and 6.5. It is equally active with RNA prepared from various sources, attacking phosphodiester bonds adjacent to pyrimidine bases. Activity in myeloblasts or lymphocytes is about one-tenth that of mature granulocytes.

SUMMARIO IN INTERLINGUA

Ribonuclease esseva purificate partialmente ab leucocytos human, e su proprietates esseva investigate. Le enzyma ha un pH optime de inter 6 e 6.5. Illo es equalmente active con acido ribonucleic preparate ab diverse fontes, attaccante ligamines phosphodiesteric adjacente al bases pyrimidinic. Le activitate in myeloblastos o lymphocytos es circa un decimo de illo in granulocytos matur.

REFERENCES


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