PURIFICATION AND PROPERTIES OF A RIBONUCLEASE FROM RAT LIVER POLYRIBOSOMES

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Received 7 July 1972

1. Introduction

It is a general experience that polyribosomes isolated from rat liver invariably contain ribonuclease activity. It is not clear whether this ribonuclease is an integral part of the polysomes or represents a contamination derived from other parts of the cell.

In the present paper we report on the purification of the ribonuclease associated with rat liver polyribosomes. The properties of the purified enzyme suggest that it is different from the previously described alkaline ribonuclease of rat liver.

2. Materials and methods

Polyribosomes were prepared as described by Earl and Morgan [1] from livers of male Wistar rats (250–300 g), and stored as pellets at −70°.

Ribonuclease activity was measured by incubating, at 37° for 2 hr, 200 μl of enzyme solution, 200 μl of substrate solution (1 mg of yeast RNA in distilled water) and 200 μl of 0.1 M Tris-HCl buffer, pH 8.5, containing 60 mM KCl. The reaction was terminated by rapid cooling to 0° and addition of 0.6 ml of 1 M HCl in 76% alcohol. After 15 min the precipitate was removed by centrifugation (10 000 *g*), the supernatant was diluted 10 times with distilled water and the absorbance was read at 260 nm. Blanks without enzyme were treated in the same way in order to correct for non-enzymatic depolymerization of RNA.

One unit of ribonuclease activity is defined as the amount of enzyme which gives an increase of 0.1 in optical density at 260 nm. The specific activity of ribonuclease is expressed as the number of enzyme units per mg of protein. Protein was determined by the method of Lowry et al. [2] with bovine serum albumin as reference standard.

Yeast RNA, sRNA from calf liver, poly A, poly G, poly I, poly C, poly U, calf thymus DNA, bovine serum albumin (M.W. 67 000) and cytochrome *c* (horse heart, M.W. 12 400) were obtained from Sigma. Yeast RNA and sRNA were purified as described by Utsunomiya and Roth [3]. Carboxypeptidase B (hog pancreas, M.W. 34 300), and chymotrypsinogen A (bovine pancreas, M.W. 25 000) were from Worthington. Carboxymethyl cellulose (CM 52) was obtained from Koch Light Laboratories.

3. Results and discussion

Previously we have obtained evidence that the ribonuclease associated with polysomes is present in a complex with an inhibitor which requires sulfhydryl groups for its activity [4], like the alkaline ribonuclease of the cell sap [5]. Thus, latent ribonuclease of rat liver polysomes could be strongly activated by the use of sulfhydryl blocking agents. In the present extraction and purification of the ribonuclease from polysomes the latent enzyme was therefore activated by treatment with *p*-chloromercuribenzoate.

Polysomes from 60 livers were suspended in 180 ml ice-cold TKM buffer (0.05 M Tris-HCl buffer, pH 7.6, 0.025 M KCl, 0.005 M $MgCl_2$), containing 1 mM *p*-chloromercuribenzoate, and gently dispersed in a Potter-Elvehjem teflon-glass homogenizer.

The suspension was centrifuged at 105 000 *g* for

Table 1
Summary of enzyme purification.

Step	Protein (mg/ml)	Ribonuclease activity (units/ml)	Ribonuclease activity Specific activity (units/mg protein)	Purification (fold)	Yield (%)
1 Suspension of polysomes	3.2	28.3	8.8	1	100
2 High speed supernatant	0.66	14.2	22	2.5	85
3 Ammonium sulphate precipitation	0.023	4.3	187	21	45
4 Heat-treatment	0.009	3.9	434	49	36
5 Acid-treatment	0.003	2.6	866	99	25
6 Carboxymethyl cellulose chromatography	0.002	10.1	5050	574	9

2 hr and the polysomes were subsequently washed in a small volume of TKM buffer. The enzyme of the pooled high-speed supernatants was then purified by ammonium sulphate precipitation, heat-treatment, acid treatment and chromatography on carboxymethyl cellulose (table 1).

The high-speed supernatant was brought to 30% saturation by addition of solid ammonium sulphate and stirred for 15 min. The precipitate was removed by centrifugation at 10 000 *g* and the supernatant was dialyzed for 3 hr against 20 vol of TKM buffer, containing 1 mM *p*-chloromercuribenzoate.

The dialysate was heat-treated for 15 min at 70°

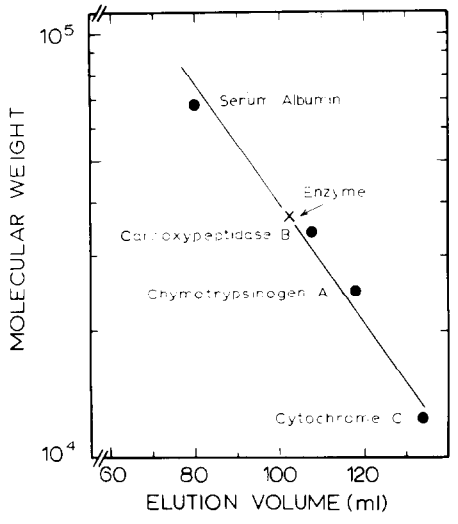

Fig. 1. Estimation of the molecular weight of polysome-associated ribonuclease by gel filtration [6]. The ribonuclease solution and protein markers of known molecular weight were passed independently through a Sephadex G-100 column (2.5 cm × 40 cm), equilibrated with 4 mM acetate buffer, pH 4.6, and eluted with the same solution at a flow rate of 55 ml per hour. Fractions (2.5 ml) were collected and the ribonuclease activity was determined as described under Materials and methods.

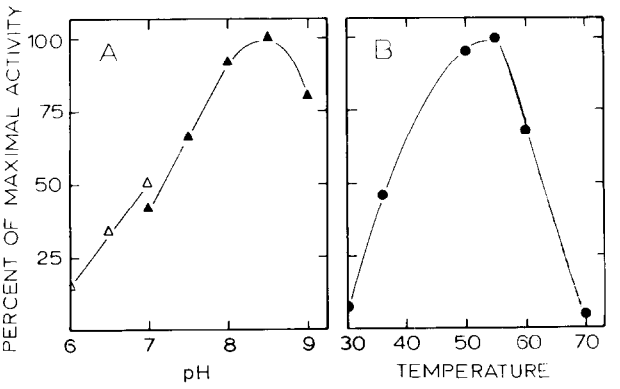

Fig. 2. Effect of pH and temperature on the purified polysome-associated ribonuclease activity. Enzyme assay was carried out as described under Materials and methods, except that the pH of the incubation mixture (A) and the temperature (B) were varied as indicated. The activity at different pH values was measured in 0.1 M phosphate buffer (pH 6.0 to 7.0) and 0.1 M Tris-HCl buffer (pH 7.0 to 9.0).

Table 2
Ribonuclease tested with different substrates.

Substrate	Relative activity
Yeast RNA	1.00
Calf liver sRNA	0.55
Poly C	0.17
Poly U	0
Poly A	0
Poly G	0
Poly I	0
DNA	0

The activity was measured as described under Materials and methods, except that the substrate concentration was 0.5 mg. The degradation of yeast RNA was set equal to 1.00.

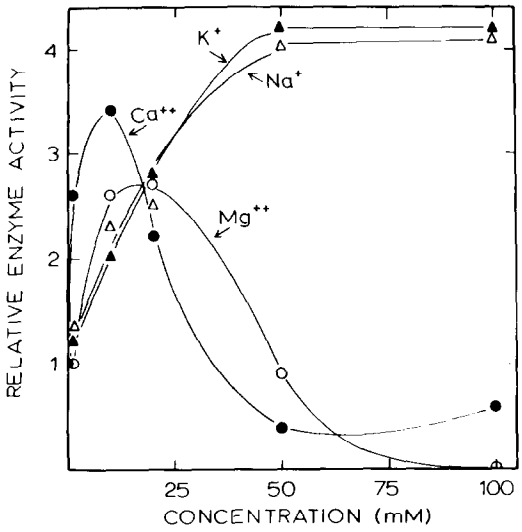

Fig. 3. Effect of monovalent and divalent cations on activity of the purified ribonuclease. Enzyme activity was measured as described under Materials and methods, except that the basic incubation mixture contained no KCl. The cations were present in the incubation mixture in the concentrations indicated.

and rapidly cooled to 0°. The precipitate was sedimented and the pH of the supernatant was adjusted to 3.8 with 5 N acetic acid. After stirring for 10 min, the precipitate was removed by centrifugation and the pH of the supernatant was readjusted to 7.6 by addition of 5 M NaOH. At this stage of the purification the enzyme was quite stable and could be stored for several months at 4° without detectable loss of activity.

The supernatant was desalted on a Sephadex G-25 column (2.5 × 40 cm) which had been equilibrated with 4 mM acetate buffer, pH 4.6. The solution was then applied to a carboxymethyl cellulose column (0.9 cm × 15 cm), equilibrated with 4 mM acetate buffer, pH 4.6. The enzyme was eluted with a linear gradient of sodium chloride (0–0.35 M) in 4 mM acetate buffer, pH 4.6. Fractions (10 ml) were collected at a flow rate of 40 ml per hour. The enzyme was eluted as a sharp peak at a sodium chloride concentration of 0.25 M, and the solution was desalted on a Sephadex G-25 column (2.5 × 40 cm), equilibrated with 10 mM Tris-HCl buffer, pH 8.5.

The results from a typical enzyme purification are summarized in table 1. It is seen that in this particular case a purification factor of 574 was obtained with an overall yield of 9%. The purified enzyme could only be stored for about 1 week at 4° without loss of activity. Thereafter it decreased rapidly and after about 10 days the enzyme was completely inactivated.

The molecular weight of the enzyme was estimated by gel filtration on Sephadex as described by Andrews [6]. From the results shown in fig. 1 the molecular weight was estimated to be 37 000 daltons. This value is considerably higher than those reported for other alkaline ribonucleases from rat liver [7].

The results in fig. 2A show that the enzyme exhibits optimal activity at pH 8.5. Fig. 2B demonstrates that the rate of degradation of yeast RNA was maximal at 55°. Exposure of the enzyme to higher temperatures led to rapid inactivation.

From the results in fig. 3 it appears that the enzyme exhibited a small, but definite activity in the absence of added divalent cations. Since the substrate had been extensively dialyzed against EDTA the data demonstrate that the enzyme does not require divalent cations for activity. However, addition of magnesium and calcium ions caused an apparent increase in activity with a maximum at a concentration of about 10 mM. At higher concentrations the activity again decreased. When either sodium or potassium ions were added the rate of degradation of the substrate increased with increasing concentrations up to about 4 times the initial activity.

It is not clear whether the increases in the activity here observed by the addition of cations represent a true activation of the enzyme, or are due to effects on the substrate [5]. However, the fact that a definite

activity was observed in the absence of added cations seems to favour the latter possibility. The cation effects here observed seem to distinguish clearly our enzyme preparation from the previously described alkaline ribonucleases from rat liver [8–10].

In attempts to characterize the base specificity of the purified enzyme its activity towards several synthetic homopolymers was studied. The results in table 2 show that the enzyme caused a small, but definite degradation of poly C, while it had no effect on poly U, poly A, poly G or poly I. Calf liver sRNA was degraded about half as fast as yeast RNA, the standard substrate used. These results also distinguish the present enzyme from the alkaline ribonuclease of rat liver previously described, since these are able to degrade poly U as well as poly C [5].

The data presented on the molecular weight, the sensitivity to cations and the base specificity of the ribonuclease associated with rat liver polyribosomes, suggest that this enzyme is different from the alkaline ribonucleases of the cell sap and mitochondria. The fact that, in spite of considerable efforts, it has not been possible to isolate polysomes free of ribonuclease activity, suggests that the bound ribonuclease may be an integral part of polysomes, and may be involved in their normal function. It is an interesting possibility that this ribonuclease may be involved in the degradation of messenger RNA and possibly play a role in control of protein biosynthesis.

Acknowledgement

O.C. Ingebretsen is supported by the Norwegian Research Council for the Sciences and Humanities.

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