A RIBONUCLEASE FROM THE DEBRIS OF ESCHERICHIA COLI

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Since the first demonstration of the existence of alkaline phosphatase activity at the surface of Escherichia coli (Malamy and Horecker,1961), evidence has been accumulating that several degradative enzymes are localized specifically at the cell surface and can be released when cells are converted to spheroplasts by means of EDTA-lysozyme (Neu and Heppel,1964a,b,c). Neu and Heppel reported that the latent RNase exists in an easily releasable form at the cell surface and cast some doubt on the localization previously proposed by Elson (1959), and Spahr and Hollingworth (1961).

We now find that the cell debris of E.coli B contains a considerable amount of RNase. This RNase activity was purified, compared with the ribosomal latent RNase and characterized as a new enzyme. The remarkable difference between the debris and ribosomal RNase was found in their chromatographic behavior and also in their mode of action.

EXPERIMENTAL

E.coli strain B was grown in the reported medium in which glucose was substituted for sodium lactate (Anraku, 1964). For determination of RNase activity the reaction mixture (1.0 ml) contained 5 mg of purified commercial

yeast RNA*, 50 μmoles of Tris, pH 8.2 and enzyme. After 20 min at 37°, 0.25 ml of 0.75 % uranyl acetate in 25 % perchloric acid was added in the cold and the mixture centrifuged; 0.1 ml of the clear supernatant was immediately added to 2.9 ml of water, and the absorbance of this solution was read at 260 mμ. One unit of RNase was defined as the amount of enzyme which, under the above conditions, gave an absorbancy of 0.1. To determine the localization of RNase, 16 g wet weight of freshly cultured cells (4 x 10^9/ml) was employed. The cells were mixed with 35 g of acid-washed sea sand (200 to 400 mesh) and 10 ml of Tris, 0.005 M, pH 7.4, containing 10 mM of $MgCl_2$, and ground in the cold for 30 min. The ground cell material was extracted by adding 50 ml of the same buffer, centrifuged and washed successively at 5,000 xg for 5 min and 20,000 xg for 20 min giving a "debris" fraction. The 20,000 xg supernatant was then spun at 105,000 xg for 90 min. The supernatant (soluble fraction) was carefully pipetted out and the pellet, after being suspended in the same buffer and recentrifuged, was designated the ribosome fraction.

RESULTS

A typical result on the localization of RNase activity was summarized in Table 1. Debris fraction was found to contain a considerable amount of enzyme activity. The ratios of RNase activity to RNA content in the ribosome fraction and in the debris fraction differed, indicating that the activity

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* Commercial yeast RNA was purified mainly according to the method (Crestfield, et al,1955). The purified RNA was free of highly polymerized material; thus none of it was precipitated by 1 M NaCl.

Table 1. Subcellular localization of RNase activity

Fraction	Total units of RNase	Per cent of total activity	Per cent of total RNA***
Debris	340	24	7
Ribosome	940	68	77
Soluble	110	8	16
Total	1390	100	100

*** The data were cited from Andoh and Mizuno (1963). Each fraction obtained by the method in text was made finally to 0.005 M Tris, pH 7.4, 0.05 M NaCl and 4 M urea, and dialyzed against the same buffer for 6 hr at 30° and then against running water for 24 hr in the cold. Clear dialysates obtained after centrifugation were used as enzyme preparations. Enzyme activity was measured under standard conditions.

in the debris was not due merely to a contamination by ribosomes.

Ribosome as well as debris fractions** were treated and purified in the same way as described by Spahr and Hollingworth (1961). Ribosomal RNase was purified by 1,300 fold in a good agreement with Spahr and Hollingworth (1961). All of the RNase activity from debris, however, was retained on the CG-50 column despite eluting with more than four resin bed volumes of 0.2 M potassium phosphate, pH 7.2. The enzyme was finally eluted when the salt concentration was raised to 0.4 M with NaCl, giving an over-all purification of 4,000 fold. The purified debris RNase did not hydrolyse DNA, uridine-5'-p-nitrophenylphosphate and di-p-nitrophenylphosphate (Anraku,

** For the preparation of enzymes, frozen cells after storage for 8 months at -15°were used (Anraku,1964).

1964), and was not contaminated with acid phosphatase (Spahr and Hollingworth,1961).

The debris RNase was incubated with Poly A (2 mg/ml) in Tris buffer, pH 8.2 for 3.5 and 24 hr at 37° and the products chromatographed (ascending method; solvent, isopropanol:ammonia:water,7:1:2). Each chromatogram gave only one spot of A-cyclic-p. Moreover, when yeast RNA was digested by the debris RNase in combination with cyclic phosphodiesterase (Anraku, 1964), release of Pi was observed. These results clearly in-

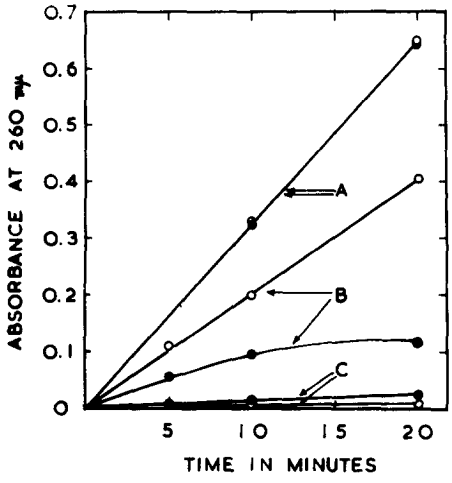

Fig.1. Selective digestion of RNA's by ribosomal and debris RNase. Activity was measured by standard condition. 6.5 units of each enzyme were used throughout the experiment. Substrate, A; yeast RNA, B; soluble RNA (E.coli), C; ribosomal RNA (E.coli); solid circle; ribosomal RNase, open circle; debris RNase.

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dicate that the debris RNase is similar in catalytic activity to the ribosomal latent RNase but differs in its chromatographic behavior. Further details on the purification and properties of these two kinds of RNase are to be described elsewhere (Anraku and Mizuno, in preparation), and we will now report briefly another difference on their mode of action.

As shown in Fig.1, selective digestion of RNA's was demonstrated from the experiment by using the same unit of enzymes. It is of interest that both enzymes prefer to use small molecules as substrates, acting faster on commercial yeast RNA than on highly polymerized ribosomal RNA. However, a difference was found in that the debris RNase hydrolyses soluble RNA about two times faster than does the ribosomal RNase. Another test which also revealed the difference of their mode of action is illustrated in Fig.2 where the substrate concentration is 100 times lower than that in the standard condition employed in Fig.1 (see the legend of Fig. 2). The reaction was followed by an increment of absorbance at 260 mμ derived from the depolymerization of RNA. Comparison of the initial velocity of depolimerization clearly shows a difference between the two RNases.

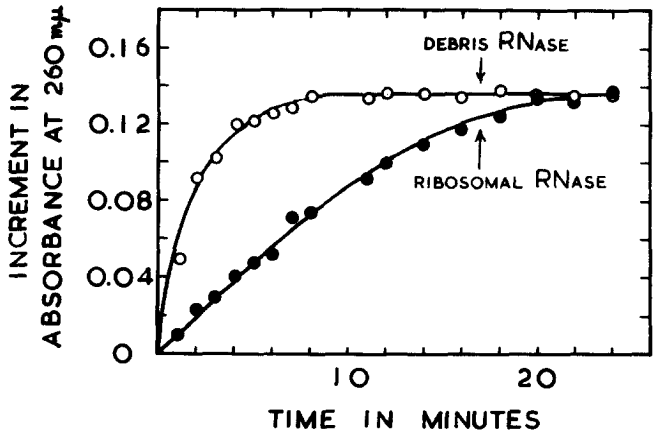

Fig.2. Comparison of initial velocity of depolymerization by ribosomal and debris RNase. Reaction mixture contained (2 ml) 100 μg of yeast RNA, 100 μmoles of Tris, pH 8.2 and the enzyme. Calibrated 1 cm quartz cuvettes containing the prewarmed reaction mixture without enzyme were placed in a Ito Chotanpa Spectrophotometer model QU equipped with thermospacer attachment at 37°. After 10 min of temperature equilibrium the enzyme (6.5 units) was introduced to the cuvettes to start the reaction and an increment in absorbance at 260 mμ was followed.

So far as the results mentioned above are concerned, it can be concluded that the RNase purified from cell debris has unique characteristics which are clearly distinguishable from the ribosomal latent RNase. According to the current view which has been advanced by Neu and Heppel (1964a,b), RNase of E.coli exists at the cell surface and is released rapidly when EDTA-lysozyme spheroplasts are made. They also have examined the "spheroplast medium-RNase", and found that it is different from the ribosomal RNase in its elution position on DEAE-cellulose but not in other enzymic properties(Neu and Heppel,1964a). On the other hand, it is also true that purified ribosomes bind RNase activity (Elson,1959; Spahr and Hollingworth,1961) and can adsorb a partially purified RNase additively (Neu and Heppel,1964b). In this connection, it is interesting that the ribosome fraction obtained by the present method has a negligible amount of debris RNase activity while the purified debris RNase can bind to ribosomes additively to the same extent as that of ribosomal RNase (Anraku and Mizuno, in preparation). The reason why the debris RNase was found only in the cell debris but not in the ribosomes or vice versa remains to be ascertained.

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