EFFECT OF RIBONUCLEASE ON ESCHERICHIA COLI RIBOSOMES*

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Summary

Partial digestion of Escherichia coli 70S ribosomes with pancreatic ribonuclease releases about 3% absorbance at 260 mμ. RNA isolated from the nuclease-treated particles consists of a large fraction in low molecular weight pieces, as judged by urea-Sephadex gel filtration patterns. These ribosomes, although containing RNA with several breaks, are as active as normal ribosomes in poly U-directed ^{14}C-polyphenylalanine synthesis. Thus the surface RNA removed by limited digestion with ribonuclease is of little importance in maintaining the ribosomes functional in in vitro polyphenylalanine synthesis.

Enzymatic digestion has yielded information on the susceptibility of Escherichia coli ribosomes to ribonuclease (RNase) (1,2). Little RNA can be removed from the particles with digestion at 10mM $MgCl_2$, when the particles are tightly folded. Although only a small quantity of RNA is removed from 70S ribosomes with partial nuclease digestion, this paper shows that the RNA from the particles is appreciably degraded. Also, these ribosomes can sustain ribonuclease-induced breaks in the high molecular weight RNA fraction and retain activity in poly U-directed polyphenylalanine synthesis.

Experimental

Escherichia coli strain Q13 (RNase I deficient), from Dr. B. Leichtling, or from strain Q13 supplied by General Biochemicals was used. Cells grown in 1.3% Tryptone, 0.7% NaCl at 37°C under aeration were harvested in early log phase. To extract ribosomes,

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cells were ground with alumina in buffer containing 10^{-2}M Tris-HCl, pH 7.8, 10^{-2}M $MgCl_2$, 3 x 10^{-2} M NH_4Cl, 5 x 10^{-3} M mercaptoethanol (TMA) and 5 μg/ml DNase (Worthington, RNase free). The extract was centrifuged in the Spinco Model L-2 preparative centrifuge at 30,000 rpm to sediment debris and then at 50,000 rpm for 90 minutes to sediment ribosomes. The pellet was resuspended in a small volume of TMA, clarified at low speed, and centrifuged through 30% sucrose in TMA several times (3). Ribosomes were washed by centrifugation at 50,000 rpm and stored in solid CO_2 until used. This constituted the 70S ribosome preparation. Ultracentrifuge analysis showed 70-75% of the ribosome preparation as 70S particles; the rest as 50S and 30S subunits.

The poly U-dependent phenylalanine incorporation system was essentially as described by Hosokawa et al. (4).

RNA was extracted from ribosomes by the phenol method and ethanol precipitation. Pancreatic ribonuclease A used for digestions was from Sigma. Sedimentation rates were measured with a Spinco Model E ultracentrifuge equipped with photoelectric scanner. S_{20} values are those corrected for viscosity to 20°.

Results and Discussion

70S ribosomes were digested in TMA-mercaptoethanol for 5 minutes with pancreatic ribonuclease (0.03 μg/A_{260}) at 0°C and then added to columns of Sephadex G-200. About 3% of the absorbance at 260 mμ was released from the ribosomes (Fig. 1). 70S ribosomes did not dissociate to subunits with digestion under these conditions. Particles that were passed through Sephadex were used for RNA extractions. Sedimentation analysis with the Spinco Model E analytical centrifuge showed 2 major RNA components sedimenting at 10-12S and 18S for the nuclease-treated ribosomes (Table 1). Passage through urea-Sephadex columns (Fig. 2) where secondary structure is disrupted, showed that a large fraction

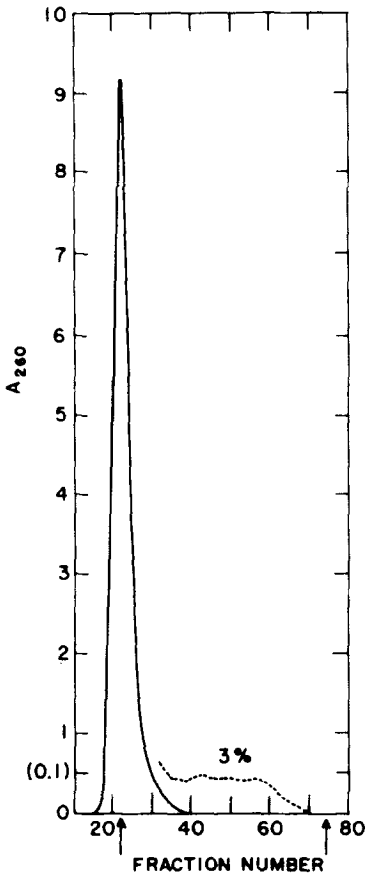

Fig. 1. Gel filtration with Sephadex G-200 of RNase-treated 70S ribosomes. The column was run at 5° and eluted with 10^{-2}M Tris-HCl, pH 7.8, 10^{-2}M $MgCl_2$, and 3 x 10^{-2}M NH_4Cl; 1.0 ml fractions were collected; column volume, 75 ml; arrows indicate the void and total volumes of the column. The dotted line is plotted in an expanded scale shown in parenthesis.

consisted of relatively low molecular weight fragments. About 50% of the RNA from digested ribosomes came off the column near the void volume (Fig. 2b). Approximately 30% eluted just before t-RNA elution with a distribution coefficient K_d = 0.4. From a calibration of K_d and molecular weight using t-RNA and 5S RNA, the molecular weight of the 30% fraction is estimated to be approximately 40,000. To account for the high percentage of smaller pieces, a lower limit of 5 breaks in either the 16S or 23S RNA is estimated.

TABLE I

RNA Fractions from Control
and RNASE-Treated Ribosomes

Sample	Components	% RNA
	S_{20}	
Control	22.5 S	62
	16.5 S	33
	low mol. wt. (less than 6S)	5
RNASE-Treated	18 S	45
	10-12 S	51
	low mol. wt. (less than 6S)	4

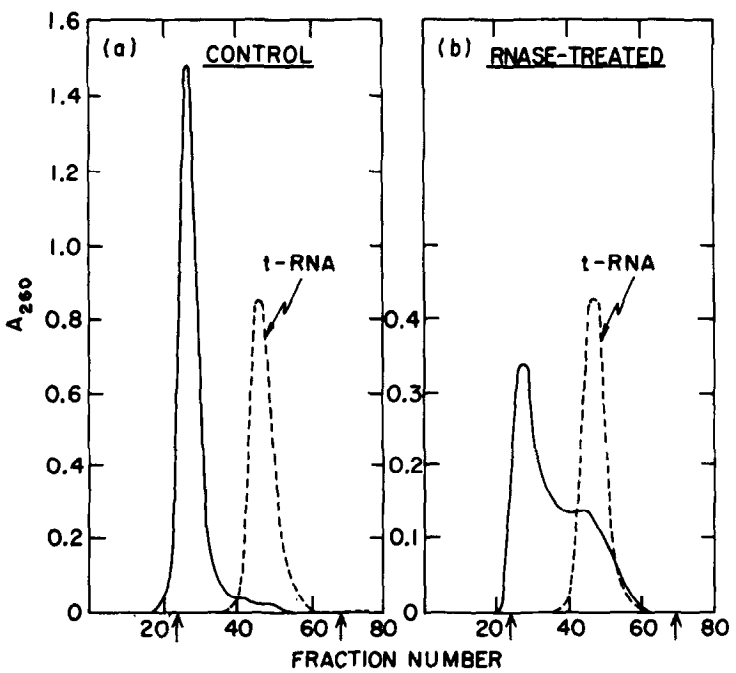

Fig. 2. Gel filtration with 6 M-urea-Sephadex G-200 of RNA from (a) control and (b) RNase-digested ribosomes. The columns were run at 25°C and eluted with 10^{-1}M Tris-HCl, pH 7.0, 6 M urea. Column volume, 65 ml; 1.0 ml fractions collected; arrows indicate void and total column volumes. RNA samples were dialyzed against 10^{-1}M Tris-HCl, pH 7.0 and 6 M urea, heated to 45°C, and then added to columns.

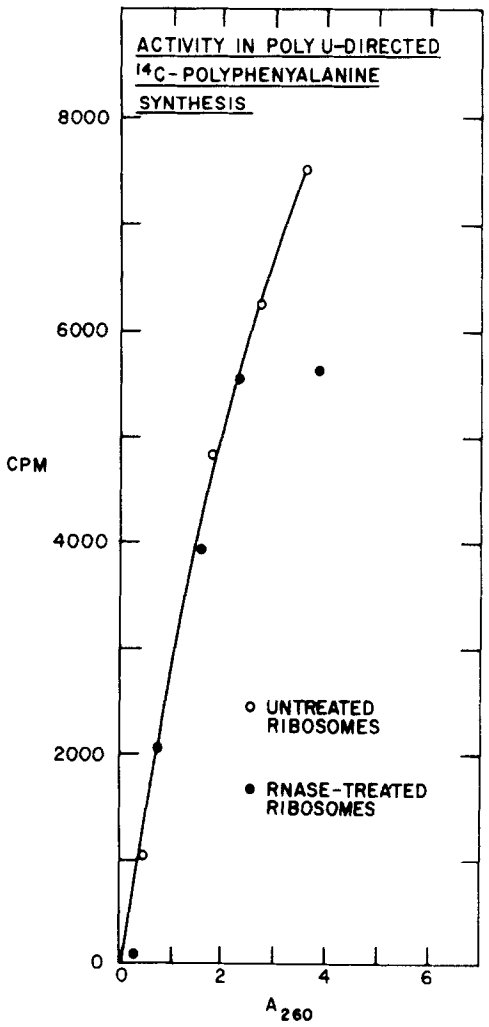

Fig. 3. Ribosome activity in poly U-directed ^{14}C-polyphenylalanine synthesis. Control or RNase-treated ribosomes (after passage through Sephadex G-200) were added in various amounts to the incorporation mixtures. The amounts are shown in absorbance units. After incubation at 37°C for 30 min., TCA was added. The precipitate was collected on Whatman glass fiber filters and counted for ^{14}C activity.

To test the functional capacity of the nuclease-treated ribosomes in poly U-directed ^{14}C-polyphenylalanine synthesis, various amounts of the ribosomes digested under the above conditions were added to the incorporation mixture (Fig. 3). The nuclease-treated ribosomes were as active as the control samples. Also, strong RNA-RNA and RNA-protein

interactions must exist for the ribosome to hold together with an appreciable number of breaks in the RNA. Ribonuclease may be removing single-stranded looped regions along with an RNA tail (2). Part of the surface RNA, the portion cleaved by RNase, is apparently not functional in in vitro synthesis of polyphenylalanine, since the nuclease-treated ribosomes appeared to function as well as the controls. How far the digestion can be carried or how many breaks can occur in the RNA before the ribosomes begin to lose their functional capacity has not been determined. Activity in other systems, such as f-2 virus RNA-directed protein synthesis, was not tested. Perhaps, functions in this system are altered with RNase-digested ribosomes.

In another study, Szer (5) showed ribosome activity in polyphenylalanine synthesis, but after breakage of RNA due to autodegradation.

Acknowledgments

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