A RIBONUCLEASE-LABILE "TAIL" ON THE 50S RIBOSOMES FROM ESCHERICHIA COLI *

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Viscosity studies on the 50S ribosomal subunit from Escherichia coli strain MRE600 indicate that a small portion of the RNA can be easily removed by pancreatic ribonuclease (RNase), with an accompanying large decrease in the intrinsic viscosity. Preliminary evidence indicates that this polymer is neither tRNA nor 5S RNA, but is probably a segment of the 23S RNA. No protein was found to be attached to this RNA.

MATERIALS AND METHODS

E. coli strain MRE600 (RNase I^-) were grown on rich medium under aeration at 37°C and then iced and harvested in early log phase. Ribosomes were obtained in a manner similar to that outline by Tissières, Watson, Schlessinger & Hollingworth (1959) except that the bacteria were ground and the ribosomes resuspended in 0.01 M $MgCl_2$, 0.5 M NH_4Cl, 0.01 M Tris, pH 7.4. These ribosomes were washed in this buffer overnight and then centrifuged at 25,000 g for 30 minutes. The solution was then centrifuged at 150,000 g for two hours and the pellet rinsed 3 times and resuspended in 0.004 M $MgCl_2$, 0.07 M KCl, 0.01 M Tris, pH 7.4. The solution was stirred 2 hours to dissociate the 70S ribosomes and then given four differential centrifugations at 150,000 g for 80 minutes each. The sample was then analyzed in the Spinco model E analytical ultracentrifuge to determine purity.

(a) Viscosities were determined in Ubbelohde viscometers, with flow times of approximately 250 seconds at 25.000 ± .002 °C. Corrections were made for solution densities.

(b) Nucleotide analysis was conducted by reducing the RNA to nucleotide monophosphates by means of a 12 hour hydrolysis in 0.3M KOH. The sample was then applied to a Dowex-1-x8, 200-400 mesh column and eluted at room temp-

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erature for 4 hours with HCOOH increasing from 0 to 0.2N.

(c) The protein content was determined by means of a microbiuret method as outlined by Itzhaki & Gill (1964). The Lowry method was not used due to the inhibition by the Cl^-ion.

(d) The RNA content of the ribosomes was determined by means of polyacrylamide gel analysis. Aliquots of 50 λ each of a ribosome solution of A_{260}=120 were extracted three times with a phenol-SDS solution and the last time with phenol only. The samples were then placed on a multiple-channel polyacrylamide gel slab (Richards & Coll, 1965) and run at a current of 3 ma/sample for 90 minutes. The gel was then stained one hour in a 0.1% toluene blue, 1% HAc solution. Destaining was performed by soaking overnight in a 1% acetic acid solution, changed several times.

RESULTS

The intrinsic viscosity of the 50S particles was found to be 7.1 ml/gm by extrapolation from data obtained at eight different concentrations using three separate preparations (Fig. 1). This value was considerably larger than the 5.4 ml/gm reported by Tissières et al. (1959). Since the earlier study was made using a RNase-active strain, it was felt that the difference in these values might be due to a piece of RNA projecting from the 50S subunit. Therefore, 2.5 µg. of pancreatic RNAase were added to the 20 ml of ribosome solution in the viscometer. The resultant flow times were immediately reduced, as shown by the arrows in Fig. 2. These values did not change further with time, nor when the amount of RNase was doubled. A dilution series of these solutions in the viscometer gave an extrapolated intrinsic viscosity of 5.5 ml/gm. This value is in excellent agreement with that reported by Tissières et al. (1959).

In order to determine the composition of the material removed, 50S ribosome solutions of concentrations 10 mg/ml were incubated for 10 minutes at 25 C° with 0.2 µg RNase per 10 mg ribosomes, and centrifuged for 5 hours at 150,000 g into a 40% sucrose bed. The supernatent fraction was found to contain approximately 5 A_{260} units/ml more UV absorbing material than the supernatent fraction of the control sample. This amounted to about 3% of the A_{260} units of the sample itself. This supernatent material was analyzed for nucleotides as noted in the Materials and Methods section. The resulting monophosphates were found to have the following relative concentrations:

A - 41% G - 18% C - 15% U - 26%

The relative values are not in agreement with known nucleotide contents of tRNA, 5S RNA or the whole 23S RNA.

In order to determine whether or not protein was released, we tested the

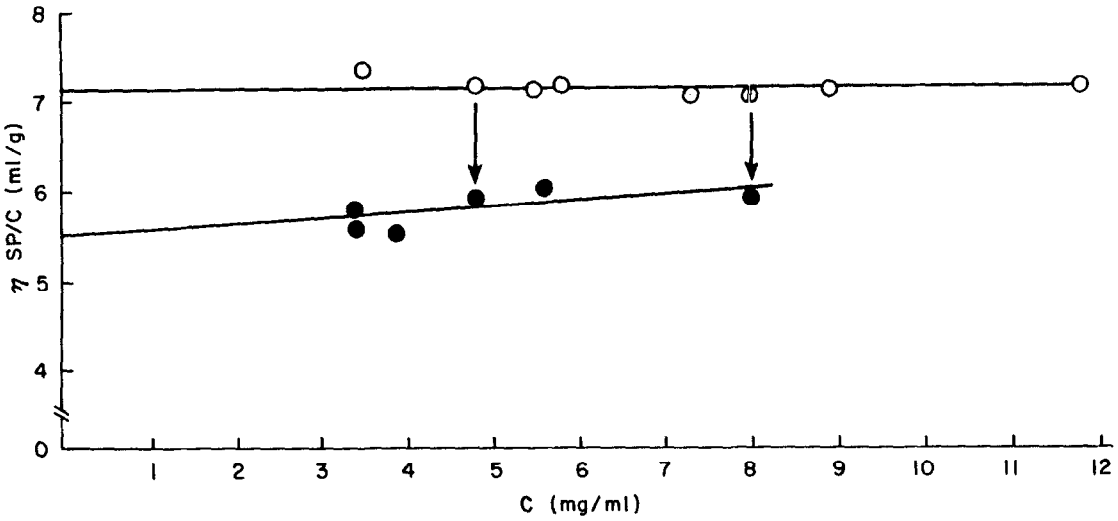

Fig. 1. Intrinsic viscosity values extrapolated to infinite dilution for 50S ribosomes (0) and 50S ribosomes treated with RNase (●). Arrows indicate change in values upon addition of RNase directly to solutions in the viscometer.

supernatents for protein content by the microbiuret method. It was found that the difference in absorption between equivalent fractions of the ribonuclease treated and control samples was less than 0.02 A_{310} units, which was within our experimental error. It was estimated that if one ribosomal protein were released per 50S subunit, a difference of about 0.2 A_{310} units would have been obtained.

The pellets of 50S ribosomes derived in the manner outlined above were analyzed for RNA content as described in the Materials and Methods section. The results of this analysis are shown in Fig. 2. This plate shows that no tRNA was present in either of the 50S samples, but was apparent in the 70S sample from which they were derived. In all cases, the 5S RNA doublet was present, indicating that this was not being removed by the RNase. This analysis also showed that the 23S RNA was also being degraded into discrete units. These units appear, from their positions on the gel, to have sedimentation coefficients of 6S and above, and are quite well defined.

DISCUSSION

The large decrease in intrinsic viscosity upon RNase treatment cannot be accounted for by any loosening or opening of the ribosome structure, since

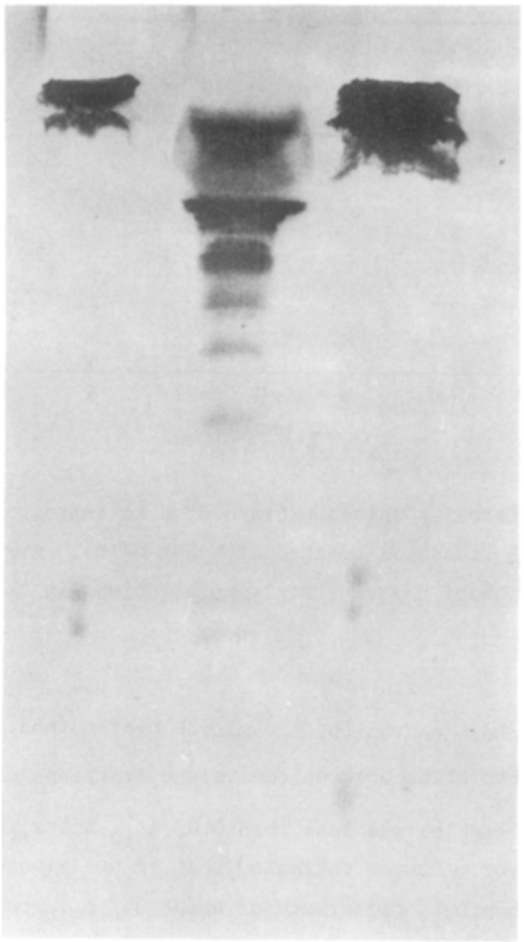

Fig. 2. Polyacrylamide gel patterns showing (a) 50S ribosomes, (b) 50S ribosomes treated with RNase, and (c) 70S ribosomes.

this would rather tend to increase the viscosity. Sedimentation velocity studies also indicated that the 50S particles that were treated with RNase remained quite homogeneous, having approximately the same sedimentation co-

efficient as the control sample. Therefore it was concluded that the RNase must be removing an RNA or RNA-protein tail on the 50S subunit. This conclusion has recently been substantiated by means of small-angle x-ray scattering results (Hill, Thompson & Anderegg, 1968). It was shown therein that the tail noticeably altered the scattering curve of the 50S subunit.

The results of the polyacrylamide gel electrophoresis as well as the nucleotide analysis indicate that the tail is neither tRNA nor 5S RNA. The microbiuret test indicates that there is no significant amount of protein attached to this RNA tail.

Ultraviolet absorption measurements indicate that the RNA being removed has an absorption of about 3% of the A_{260} units of the sample itself. This would indicate a piece of RNA corresponding to about 2% of the 50S particle, or roughly 30,000 molecular weight, with quite an unusual base composition.

It might be argued that the RNase is merely clipping several small RNA loops that are extending beyond the protein subunits. Indeed, the appearance of components with quite small sedimentation coefficients in Fig. 2 would indicate that this is happening. However, unless at least one of the loops being clipped was quite extensive, so as to cause the large drop in viscosity upon its removal, this hypothesis would not account for the data. It is possible that the nucleotide components observed may be a result of those in the tail as well as some from smaller segments being released. Answers to this and other questions will have to be obtained through further study of this phenomenon.

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