DISSOCIATION OF *ESCHERICHIA COLI* 70 S RIBOSOMES WITH ENZYMATIC DIGESTION*

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1. Introduction

Although RNA-RNA interactions through magnesium bridges are thought to hold *Escherichia coli* 50 S and 30 S ribosomal subunits together [1, 2], 70 S particles do not dissociate to subunits with ribonuclease (RNAase) even though some surface RNA is removed [3, 4]. Mild tryptic digestion, on the other hand, can dissociate particles into 50 S and 30 S subunits with loss of some functions [5]. Other non-RNA specific agents such as sulfhydryl reagents [6], can also dissociate 70 S particles. Data here shows that mild trypsin digestion, while inducing partial dissociation of 70 S ribosomes, renders the 70 S particles susceptible to dissociation into subunits by ribonuclease. In addition, trypsin, when inducing a small reduction in the 70 S ribosome level, produces several changes in the protein pattern from polyacrylamide gel electrophoresis separations.

2. Materials and methods

Escherichia coli strain Q13 (RNAase I deficient), from Dr. B.Leichtling, or strain Q 13 supplied by General Biochemicals was used. Cells grown in 1.3% Tryptone, 0.7% NaCl at 37° under aeration were harvested in early log phase. To extract ribosomes, cells were ground with alumina in buffer containing 10^{-2} M tris-HCl, pH 7.8, 10^{-2} M $MgCl_2$, 3×10^{-2} M NH_4Cl, 5×10^{-3} M mercaptoethanol (TMA) and 5 μg/ml DNAase (Worthington, RNAase free). The extract was centrifuged in the Spinco Model L-2 preparative centrifuge at 30,000 rpm for 10 min to sediment debris and then at 50,000 rpm for 90 min 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 [7]. Ribosomes were washed by centrifugation at 50,000 rpm and stored in solid CO_2 until used. This constituted the 70 S ribosome preparation.

The level of 70 S, 50 S and 30 S ribosomes in the stock ribosome preparations and the enzyme treated samples was measured with the Spinco Model E ultracentrifuge equipped with photoelectric scanner. Scans of absorbance at 265 nm vs. boundary distance in sedimentation velocity runs were used to measure the levels of 70 S ribosomes and subunits.

Ribosomal proteins were extracted by the method of Spitnik-Elson [8]. Polyacrylamide gel disc electrophoresis was similar to the modification [9] of the Reisfeld et al. [10] technique. Pancreatic RNAase was from Sigma; trypsin (twice crystallized) was from Worthington.

3. Results

70 S ribosomes in TMA without mercaptoethanol were treated with trypsin (0.03 $\mu g/A_{260}$) for 3 min at 35° and trypsin inhibitor (0.3 $\mu g/A_{260}$) was added. After passage through Sephadex G-200 at 5° using TMA for elution, the 70 S ribosome level was deter-

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Table 1
Dissociation of 70 S ribosomes and enzymatic digestion.

Sample	% A_{265}*		
	70 S	50 S	30 S
Control	69	19	12
RNAase (0.03 $\mu g/A_{260}$, 5 min, 0°)	71	21	8
Trypsin (0.03 $\mu g/A_{260}$, 3 min, 35°)	51	30	19
Trypsin and RNAase (0.03 $\mu g/A_{260}$, 5 min, 0°)	16	53	31

* Values determined with the Spinco Model E photoelectric scanner using the "stair-step" absorbance calibration and boundary heights.

Table 2
Polyacrylamide gel electrophoresis absorbance values.

Band number	% A_{600}* (Control)	% A_{600}* (Trypsin-treated)
1	4.20 ± 0.02	1.03 ± 0.32
2	1.85 ± 0.05	1.22 ± 0.19
3	3.27 ± 0.53	2.84 ± 0.23
4	3.65 ± 0.07	3.69 ± 0.20
5	2.14 ± 0.04	2.88 ± 0.09
6	6.24 ± 0.43	3.47 ± 0.12
7	17.35 ± 0.76	14.45 ± 0.25
8	2.85 ± 0.15	2.88 ± 0.20
9	4.14 ± 0.16	3.59 ± 0.24
10	6.55 ± 0.05	2.68 ± 0.15
10a		3.76 ± 0.30
11	5.45 ± 0.25	6.95 ± 0.07
12	2.95 ± 0.45	3.40 ± 0.20
13	11.35 ± 0.45	11.25 ± 0.40
14	7.15 ± 0.25	7.35 ± 0.55
15	9.60 ± 0.40	12.65 ± 0.05
16	2.30 ± 0.10	6.47 ± 0.04
17	2.61 ± 0.12	1.43 ± 0.14
18	3.93 ± 0.12	4.37 ± 0.17
19	1.65 ± 0.05	2.62 ± 0.24

* Values represent the average of duplicate experiments with average deviations. The percent absorbance was calculated from areas under the band curves.

mined with the Model E analytical centrifuge (table 1). Control ribosomes were treated in the same fashion, except for the addition of trypsin inhibitor before trypsin. Trypsin digestion produced a reduction in the 70 S particle level to 74% of the controls. The trypsin-treated ribosome preparation was then treated with pancreatic RNAase (0.03 $\mu g/A_{260}$) at 0° for 5 min, passed through Sephadex, and the 70 S ribosome level again was measured. Table 1 shows further reduction to 16%. These conditions of nuclease digestion release about 3% A_{260} from the ribosome and produce RNA fragments with sedimentation values about 10–12 S and 18 S. Approximately the same A_{260} was released with RNAase, with and without trypsin pretreatment.

To examine the effect of trypsin digestion on ribosomal proteins in 70 S ribosomes, proteins were extracted from control and trypsin-digested particles and analyzed by polyacrylamide gel electrophoresis. In this digestion, the 70 S ribosome level was reduced to 85% of the controls. A number of changes in the absorbance profiles are evident (table 2). Major decreases in intensities were seen in bands 1, 2, 6, 10 and 17 and a large increase in 16. A new mobility appeared (band 10a) in the treated sample. In another experiment, where digestion was carried further to the extent that 80% of the control 70 S ribosomes were dissociated, further decreases in intensities of bands 1, 6 and 7 and an increase in 16, were evident.

4. Discussion

In view of the ability of agents unrelated to RNA breakage [5, 6] to induce 70 S ribosome dissociation, it is possible that protein interactions also play an important role in subunit association and that these interactions are disturbed by tryptic digestion.

Two possible explanations can be given for the induced sensitivity of 70 S ribosomes to ribonuclease. a) Cleavage of some peptide chains in certain proteins along with breakage of RNA chains may produce enough instability to dissociate the 70 S particles. Trypsin, by breaking peptide bonds in certain proteins, may alter crucial protein interactions in 70 S ribosomes such that the two subunits cannot hold together. With partial dissociation, the remaining 70 S ribosomes may not have sufficient breaks in given

proteins to produce dissociation. After RNAase, RNA breakage (in segments of RNA not involved in subunit association), in addition to the existing peptide breaks, may produce enough instability to dissociate the 70 S ribosomes. b) RNA portions directly involved in subunit association may become exposed by mild tryptic digestion; these portions may then be cleaved by RNAase after trypsin treatment. With regard to the latter explanation, a marked change in the sensitivity to ribonuclease of RNA *in situ* was not seen with trypsin pre-treatment.

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