THE RELEASE OF RIBONUCLEASE INTO THE MEDIUM WHEN E. COLI CELLS ARE CONVERTED TO SPHEROPLASTS

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There have been several reports on enzymes at the bacterial surface (1-3). Malamy and Horecker (3) observed that the alkaline phosphatase of E. coli (4,5) is liberated quantitatively into the medium when cells are converted to spheroplasts with EDTA and lysozyme (6). This, with their other evidence, indicated that the enzyme is external to the cell membrane. We now find that a ribonuclease is liberated under the same conditions (Fig. 1) [1], namely, the "latent" ribosomal RNase (7,8).

EXPERIMENTAL

A variety of strains of E. coli and various media were used, with similar results. Either glycerol (0.5%) or glucose (0.5%) was used as the carbon source. Cells were grown at 37° on a rapid rotary shaker, harvested by centrifugation and washed with 0.01 M Tris-HCl, pH 8, at 0°. One gram (wet) was suspended in 80 ml of 20% sucrose-0.03 M Tris, pH 8, at 23°, to give 10^{10} cells per ml. After addition of Na-EDTA (to 0.001 M) and lysozyme (to 10 μg per ml), samples were removed at intervals from the gently agitated mixture. Lysability, an indicator of spheroplast formation, was followed by the decrease in O.D. at 600 mμ of samples diluted 10-fold in water. Other samples of the suspension were centrifuged with rapid acceleration to obtain "spheroplast medium;" the spheroplasts were packed in 2-3 minutes [2].

The pellet of spheroplasts was lysed by dilution in water or else it was suspended in 0.01 M Mg acetate-0.01 M Tris, pH 7.4. The suspen-

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[1] A deoxyribonuclease, also released, is being investigated by Dr. R. J. Hilmoe.

[2] Filtration through glass, asbestos, etc., cannot be used in these experiments because RNase is completely adsorbed. Treatment with powdered glass is useful in the removal of RNase from alkaline phosphatase preparations.

sion was ruptured by sonication or use of the French press and ribosomes were separated according to Tissières, et al. (9). Ribosomes were also prepared from intact cells.

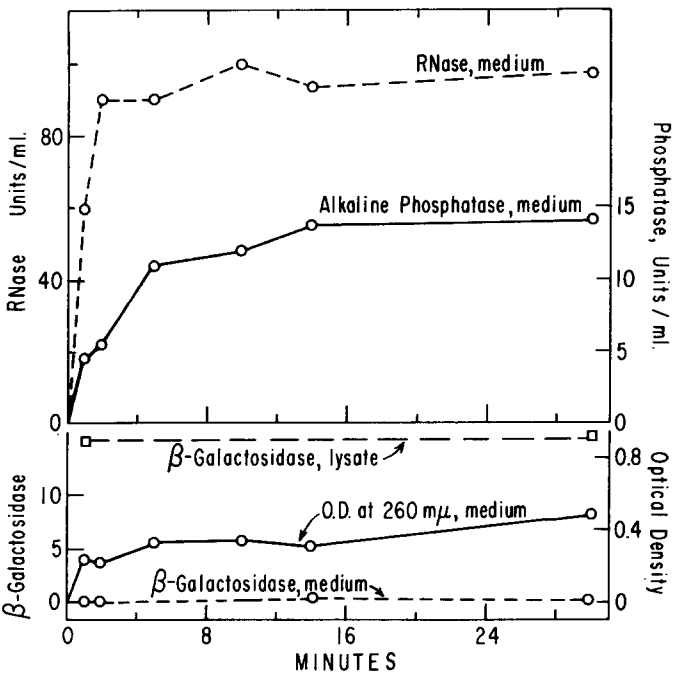

Fig. 1. Release of enzymes into surrounding medium on formation of spheroplasts from E. coli C4F1. Cells were grown to stationary phase in a medium with 0.5% glycerol and 0.04 M phosphate. A cell suspension in sucrose-tris medium was gently agitated and samples removed at intervals after addition of EDTA and lysozyme. A lysate prepared from spheroplasts sedimented after 14 minutes contained only 6% of the total alkaline phosphatase, but accounted for 42% of the total RNase, i.e., 94% of the alkaline phosphatase and 58% of the total RNase had been released into the medium. The assays for alkaline phosphatase and β-galactosidase were colorimetric (3) and one unit represents a change in O.D. at 410 mμ of 1.0 per minute, at 23°.

For RNase assays, the ribosomal endogenous RNA was first degraded by: 1), dialysis at 3° against 4 M urea (8), or 2), incubation at 37° in 0.01 M EDTA-0.1 M phosphate, pH 7, for 40 minutes. The two methods gave similar results. Samples were then incubated in a solution with 1 mg s-RNA and 10 μmoles EDTA per ml, and 260 mμ-absorbing material soluble in 3% perchloric acid was measured. Under these conditions neither polynucleotide phosphorylase nor phosphodiesterase (10) is able to attack s-RNA at an appreciable rate.

RESULTS

After removal of spheroplasts by centrifugation, the ribonuclease released into the sucrose-tris supernatant was partially purified by DEAE-cellulose chromatography. It resembles the "latent" ribosomal RNase (7,8) in the formation of cyclic-terminal intermediates and finally 3'-AMP from polyadenylic acid, in pH optimum, heat stability, activity in 0.01 M EDTA and degree of inhibition by Mg^{++}. It acts with equal rates on ribosomal RNA and s-RNA. Balance studies were carried out, in which RNase was measured in various fractions from intact cells and from spheroplasts. Ribosomes from spheroplasts show a deficit in their content of this RNase compared with intact cells (Table I).

TABLE I. Release of RNase on spheroplast formation

	Enzyme units/wet weight of cells, g	
	Stationary phase	Exponential phase
Ribosomes, intact cells	8,360	4,210
Supernatant *	45	90
Total	8,405	4,300
Ribosomes, spheroplasts	2,480 **	700
Supernatant *	50	0
Sucrose-tris medium	4,510	3,440
Total	7,040	4,140

*"Supernatant" represents the supernatant fluid obtained when ruptured cells were centrifuged to sediment ribosomes. Enzyme assays were carried out with s-RNA from E. coli.

**Direct assay of a water lysate of another aliquot of spheroplasts showed 2720 units per g wet weight of cells.

This deficit is approximately balanced by the amount of activity liberated into the sucrose-tris medium surrounding the spheroplasts. We conclude that ribosomal RNase is released into the medium when E. coli spheroplasts are made. Presumably some alteration occurs with liberation of the enzyme because "spheroplast medium-RNase" is eluted from DEAE-cellulose at a higher concentration of NaCl than enzyme prepared from ribosomes. Also, when the preparations are mixed and applied to the column, two peaks of activity are eluted.

Cells in exponential phase released 75-90% of their ribosomal RNase into the medium after only a few minutes of treatment with EDTA-lysozyme. When cells in stationary phase were converted to spheroplasts

only 50-60% of the RNase was found in the surrounding medium (Table I). In all cases, 96% of the alkaline phosphatase but only 4% of β-galactosidase was released. Polynucleotide phosphorylase and a recently described phosphodiesterase (10) were not liberated into the medium, as shown by the fact that prolonged incubation with polyadenylic acid in the presence of 0.01 M $MgCl_2$ resulted in no detectable adenosine, 5'-AMP or 5'-ADP. Total protein in the sucrose-tris medium surrounding spheroplasts reached 0.2 mg per ml in 10 minutes and increased only slightly with prolonged incubation. The release of 260 mμ-absorbing material (Fig. 1) (11) was due mostly to RNA breakdown products that are acid-soluble.

It is indeed surprising that brief treatment of exponential-phase cells with 0.001 M EDTA and lysozyme releases most of the "latent" RNase in a few minutes, especially since our studies and those of Malamy and Horecker (3) show no significant leakage of five internal enzymes. In view of recent reports concerning the occurrence of ribosomes associated with cell membranes (12), we may speculate that the bulk of the ribosomes containing RNase occur near or at the cell surface, and this facilitates release of enzyme when EDTA-lysozyme spheroplasts are made.

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