RIBOSOME DEGRADATION IN ESCHERICHIA COLI INDUCED BY COLICIN E_2

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SUMMARY

The mode of action of colicin E_2 on ribosomes in Escherichia coli cells was investigated by zonal centrifugation analysis. Ribosome particles, both 50S and 30S, were degraded to smaller contents with the lapse of time by the action of colicin E_2. Gradual reduction of S values of each particles could not be observed and degradative intermediates of possible RNA-protein complex were detected only at the position between 30S and 4S in the zonal centrifugation profile, which indicated the destruction of ribosome in burst-out attitude. 50S ribosome fraction influenced by colicin E_2 contained both 23S and half-sized RNA. From these data, the mode of action of colicin E_2 on ribosomes in E. coli was discussed.

INTRODUCTION

Colicin E_2, one of the bacteriocins produced by certain Enterobacteriaceae strains, is a simple protein (molecular weight 60,000) (1), and bactericidal only to certain strains of the same genus (2). The main feature of its biochemical action is the inhibition of cell division (3) and the induction of degradation of cellular DNA (2,4,5) and RNA (4,6).

As we reported previously (4), colicin E_2 - challenge induces degradation of 23S and 16S ribosomal RNA, but not of t-RNA in Escherichia coli Q13, a defective mutant for

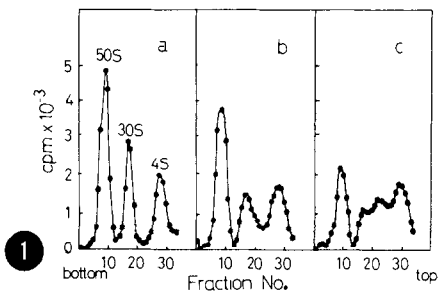
ribonuclease I. This suggests that RNaseI, which is the main degradative enzyme for RNA in E. coli cells, does not participate in the r-RNA degradation.

We investigated further the r-RNA degradation and the alteration of ribosome constitution during the colicin action, and followings were suggested. First, ribosome particles were degraded by the action of colicin E_2, and the contents of both 50S and 30S particles in the sensitive cells were decreased. Gradual reduction of S values of each particles could not be observed and degradative intermediates of possible RNA-protein complex were increased only at the position between 30S and 4S in the zonal centrifugation profile, which indicated the ribosome destruction in burst-out attitude. Second, in 50S ribosomes, their 23S RNA was first split into halves before particle destruction induced by colicin E_2, which suggested that 50S ribosomes could yet retain their structure with the damaged RNA.

MATERIALS AND METHODS

Colicin E_2 was prepared as reported before (7). E. coli Q13 was cultured with shaking at 37°C in 70ml. of 1% Penassay broth (Difco) supplemented with [^{14}C]uracil (0.2μCi/ml.) or [^{3}H]lysine (0.5μCi/ml.) in 500ml Sakaguchi flask to the cell concentration of 5.5×10^8/ml., and collected and washed by centrifugation (10,000xg, 15min.) with 1% Penassay broth at 37°C. After the washed cells resuspended in 70ml. of 1% Penassay broth resumed normal growing at 37°C, colicin E_2 was added at 6×10^8 cells/ml. (10μg/ml., multiplicity 50).

At intervals, 20ml. aliquots of the culture were centrifuged (10,000xg, 20min.) and the pellets were rapidly freezed by dryice-acetone and stored overnight at -75°C. The collected



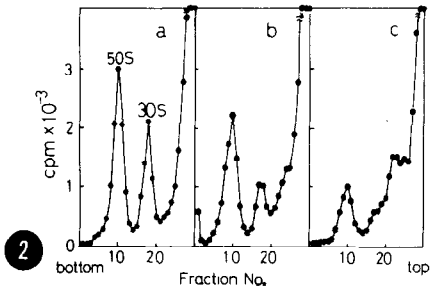


Fig.1 Effect of colicin E_2 on zonal centrifugation profile of ribosomes in E. coli Q13 labelled with [^{14}C]uracil. A: Omin., B: 30min., C: 70min. Detailes are shown in Materials and Methods.

Fig.2 Effect of colicin E_2 on zonal centrifugation profile of ribosomes in E. coli Q13 labelled with [^{3}H]lysine. A: Omin., B: 30min., C: 70min.

cells were suspended in 10ml. of 10mM Tris-HCl 0.1mM $MgCl_2$ buffer (pH 7.4) containing 30mM NH_4Cl-6mM 2-mercaptoethanol and sonically oscillated under constant disintegration condition (35W, 7min.). After centrifugation at 10,000xg, 20min., 0.2ml. of the supernatant fractions were charged on 4.8ml. linear sucrose density-gradients (5-20%w/w) in 10mM Tris-HCl-0.1mM $MgCl_2$ (pH 7.4) and centrifuged in Hitachi RPS40 rotor at 37,000 rpm, for 170min., at 4°C. Fractionation was performed by piercing a needle at the bottom of tubes and zonal centrifugation profiles were obtained by measuring the radioactivities of 0.1ml. of each fraction diluted with 0.4ml. of 0.1M NaCl-0.05M Na-acetate buffer (pH5.1) by liquid scintillation counting.

The diluted 50S and 30S peak fractions were incubated with 0.3% recrystallized SDS at 37°C, for 30min., according to R.F. Gesteland (8), and zonally centrifuged through linear sucrose density-gradients (1.5 - 15% sucrose in 0.1M NaCl-0.05M CH_3-COONa, pH5.1) in a Hitachi RPS40 rotor. 23S and 16S peaks of

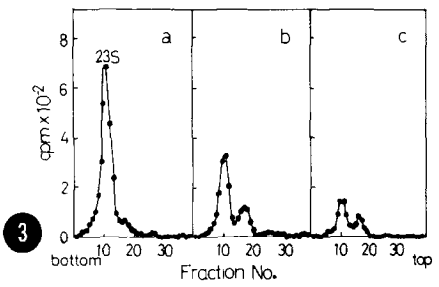
RNA were detected by measuring [^{14}C]radioactivities by liquid scintillation counting.

RESULTS AND DISCUSSION

In zonal centrifugation profile of RNAs extracted by SDS-phenol method from E. coli Q13 labelled with [^{14}C]uracil, there appeared three radioactive peaks, 23S r-RNA, 16S r-RNA and 4S t-RNA. We had already reported that the addition of colicin E_2 induced gradual decrease of both S-values and amounts of the two ribosomal RNAs except t-RNA (4). Structural change in ribosome particles during such RNA degradation by colicin E_2 was investigated here in zonal centrifugation profile.

Centrifugation of the supernatant fraction of E. coli sonicate through sucrose density-gradient showed the profile as in Fig.1-a possessing 50S and 30S peaks of ribosome particles. In the profile of colicin E_2-challenged E. coli cells (Fig.1-b, c), two radioactive peaks of 50S and 30S with decreased heights appeared but gradual decrease in their size (S-value) could not be observed with the lapse of colicin E_2 challenge. On the other hand, we could recognize the gradual increase of radioactivity in the peak between 30S and 4S peaks. In sucrose density-gradient profile of ribosomes labelled with [^{3}H]lysine (Fig.2), there also appeared a peak increased between 30S and 4S, the same position as in Fig.1-b, c, after the colicin E_2 challenge.

These data suggested that these increased substances between 30S and 4S were degradative intermediates of ribosome particles consisted of RNA-protein complex. Furthermore, the fact that degradative intermediates were not found between 50S and 30S suggested that ribosome particles, especially 50S particles, degraded abruptly into smaller fragments without producing gradually decreasing size of intermediates.



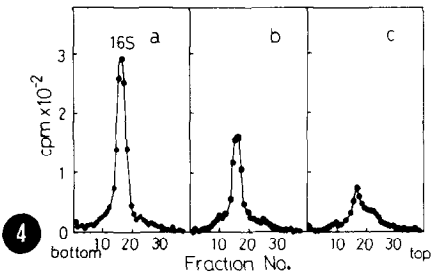


Fig.3 Zonal centrifugation profile of RNA in 50S ribosomes treated with colicin E_2. A: Omin., B: 30min., C: 70min.

Fig.4 Zonal centrifugation profile of RNA in 30S ribosomes treated with colicin E_2. A: Omin., B: 30min., C: 70min.

Possibility could not be excluded however, that the ribosome particles in the colicin-treated cells became unstable to the extracting operations and the "degradative intermediates" were artificial products by sonication.

In order to obtain further information on the degradation process of ribosome particles, 50S and 30S particles from the colicin-treated cells were isolated, extracted their RNAs respectively, and analyzed whether they contained intact RNAs or not.

As shown in Fig.3, only one peak of 23S RNA was found in 50S ribosome from the control cells(a), but smaller RNA of about 16S was found besides 23S RNA in the 50S particles of the colicin-treated cells(b, c). In contrast, each RNAs from 30S peaks in Fig.1 consisted of one 16S peak, as is shown in Fig.4. The shoulder shown in Fig.4-c might come from the contamination of degradative intermediates in the profile of Fig.1-c.

W. Szer reported in 1969 that storage of purified ribosome particles from E. coli Q13 at 0°C for a few weeks resulted in cleavage of r-RNA, both 23S and 16S, to the fragments with a half S-values, and endonuclease activity of RNase IV type in split

protein was assumed to be involved in this limited degradation (9).

It seems possible to account for the mode of ribosome degradation in E. coli Q13 cells by the action of colicin E_2 as follows. Adsorption of colicin E_2 to cell surface somehow influences ribosomes in the cells one by one to induce structural change, e.g., slight unfolding, in the particles. RNase activity of RNase IV type in the split protein(s) gets unmasked and makes a nick or nicks in the r-RNA. In 50S ribosome, 23S RNA is split at first into pieces with about half S-value.

Structural entities of the ribosomes can be retained even when this limited degradation of their RNA component has occurred. But ribosomes with a limitedly damaged r-RNA are loosened and made sensitive to cytoplasmic RNase distinct from RNase I. Then further enzymatic breakdown of r-RNA results in loss of particle conformation and degradation into small fragments. Investigation about the degradative intermediate may give some information on the ribosome structure.

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