INACTIVATION OF RIBOSOMES BY COLICIN E3 *IN VITRO*: REQUIREMENT FOR 50 S RIBOSOMAL SUBUNITS*

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1. Introduction

Colicin E3 has been shown to kill sensitive bacteria by primarily inhibiting protein synthesis, without affecting other macromolecular synthesis or energy metabolism [1, 2]. The effect of E3 on protein synthesis has been ascribed to a specific cleavage of 16 S ribosomal RNA [3, 4] which inactivates 30 S subunits, but does not affect the functional activity of 50 S subunits [5]. Recently, a cell-free system has been developed in which incubation of 70 S ribosomes with E3 results in ribosome inactivation and 16 S RNA cleavage, an identical result with that *in vivo* [6, 7]. Experiments reported here show that both 50 S and 30 S ribosomal subunits are required for inactivation *in vitro*, and that both subunits and E3 must be present simultaneously for the inactivation to occur. Independently, Boon has obtained similar results [8].

2. Materials and methods

Ribosomes were obtained from *E. coli* strain Q13, and were purified by pelleting through 1 M NH_4Cl in TMA I buffer (10^{-2} M Tris pH 7.6, 10^{-2} M $MgCl_2$, 3×10^{-2} M NH_4Cl, 6×10^{-3} M β-mercaptoethanol); 70 S ribosomes were then isolated from 10–30% sucrose-TMA I gradients. 30 S and 50 S subunits were isolated from the 70 S ribosomes by dissociation at 3×10^{-4} M Mg^{2+}, followed by centrifugation on similar sucrose gradients containing 3×10^{-4} M Mg^{2+}. Most of the small membrane fragments usually found in "S-30" crude cell extracts were removed from the ribosomes by this purification procedure. The colicin E3 preparation used has been described previously [7]. Anti-E3 antiserum was obtained from a New Zealand white rabbit bled one week after the last of 6 subcutaneous injections of 1.0 mg each of the E3 preparation emulsified in Freund's Complete Adjuvant (Perrin's modification, Calbiochem) administered over a 4-week period. IgG antibody was purified by column chromatography on DEAE-Sephadex [9] and then Sephadex G-200. Poly U-dependent polyphenylalanine synthesis under standard conditions [10, 11] for 15 min at 37° was used as the measure of ribosome activity.

3. Results and discussion

Ribosomes were mixed with E3 or buffer as described in table 1, and were incubated at 37° for 30 min. 5 μl aliquots of the reaction mixtures were removed and were immediately diluted 10-fold into cold TMA I buffer. Other components necessary for the activity assay were then added, making the final dilution a total of 30-fold compared to the incubation mixture. No significant E3-induced ribosome inactivation occurred during the protein synthesis assay under these conditions.

The results described in table 1 show that E3 does

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Table 1
Inactivation of ribosomes by colicin E3 *in vitro*.

Components in the incubation mixture		Incorporation activity	
Ribosome	E3	(cpm)	(%)
70 S	–	4687	100
	+	1509	32
50 S	–	860*	100
	+	865	101
30 S	–	1321*	100
	+	1035	78
50 S + 30 S	–	1376	100
	+	356	26

2.7 A_{260} units of 70 S, 1.8 A_{260} units of 50 S or 0.9 A_{260} units of 30 S ribosomal subunits were incubated at 37° in 20 μl TMA I, in the presence or absence of 2 μg of colicin E3. 5 μl samples were removed after 30 min and diluted for the protein synthesis assay. Samples lacking 30 S or 50 S subunits were supplemented in the assay with 0.45 A_{260} units of 50 S or 0.225 A_{260} units of 30 S subunits.

* Since the supplemented 30 S subunits had not been heat activated, the 50 S subunit activity appears decreased. Later identical experiments using heated 30 S subunits gave appropriately higher counts and identical conclusions.

not significantly inactivate 30 S subunits unless 50 S subunits are present. This result is of interest because E3 treatment causes inactivation of the 30 S subunit, but not the 50 S subunit [5–7]; therefore, 50 S subunits must somehow play a role in the inactivation of 30 S subunits. Two alternative possibilities exist. The first is that E3 interacts with 50 S subunits and causes a stable alteration; the altered 50 S subunits then cleave the 16 S RNA of the 30 S subunits. The second possibility is that E3 induces 16 S RNA cleavage only in the presence of both subunits, possibly by interacting with a 30 S–50 S complex.

In order to decide between these two alternatives, 30 S and 50 S subunits were separately treated with E3 at 37° for 30 min (table 2). As in the experiment described in table 1, the controls showed that E3 caused more than 70% inactivation when both subunits were present together under identical conditions (data not shown). Thus, if the first possibility is correct, the 50 S subunits incubated with E3 must have been altered during this incubation and would be able to inactivate 30 S subunits without E3. This was

Table 2
Requirement of the simultaneous presence of 30 S and 50 S subunits and E3 for ribosome inactivation.

	Preincubation conditions		Incorporation activity	
Tube no.	50 S	30 S	(cpm)	(%)
1	+ Buffer	+ Buffer	665	100
2	+ E3	+ Buffer	50	8
3	+ E3	+ Anti-E3	650	98
4	+ Buffer	+ E3	109	16
5	+ Anti-E3	+ E3	707	106

7.0 A_{260} units of 50 S and 3.5 A_{260} units of 30 S subunits were separately preincubated in 50 μl TMA I buffer in the presence or absence of colicin E3 (2.5 μg) or purified anti-E3 γ-globulin (100 μg). After preincubation for 30 min at 37°, 10 μl of each of the indicated 30 S and 50 S samples were combined as indicated and incubated at 37° for an additional 60 min. 5 μl aliquots were used to assay incorporation activity.

tested by then incubating such "activated" 50 S subunits with 30 S subunits in the presence of a sufficient amount of purified anti-E3 γ-globulin to stop further action of colicin E3 (tube no. 3 in table 2). No inactivation of 30 S subunits was observed after 60 min (cf., tubes no. 3 and 1). In the absence of the anti-E3 γ-globulin, the inactivation of 30 S subunits occurred during the subsequent incubation (tube no. 2). Table 2 also shows that there is no inactivation of 30 S subunits when treated with E3 in the absence of 50 S subunits (tube no. 5). This confirms the conclusion obtained from the experiment shown in table 1. In addition, the results show that in the absence of 50 S subunits E3 does not induce any stable alteration of 30 S subunit structure which later leads to inactivation in the presence of 50 S subunits. In separate experiments, it was shown that the anti-E3 γ-globulin used in these experiments neutralizes E3 activity nearly completely under the experimental conditions used and has no effect on the protein synthesizing ability of ribosomes.

These results show that 30 S and 50 S subunits and E3 must be present simultaneously for 30 S subunit inactivation to occur. The results rule out the occurrence of any stable, E3-induced alteration of either subunit which would allow later 30 S subunit inactivation in the absence of the E3 molecule.

The present study supports the previous conclusion that, *in vivo,* E3 inactivates 30 S ribosomal subunits by making direct contact with the ribosomes inside the cell [6, 7]. The general mechanism of action of colicins *in vivo* has been fully discussed in the previous paper of this series [7].

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