ROLE OF COMPONENTS OF THE 50 S RIBOSOMAL SUBUNIT IN COLICIN E3 ACTIVITY

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1. Introduction

The protein antibiotic (bacteriocin) colicin E3, produced by certain strains of *Escherichia coli*, inhibits bacterial protein synthesis by causing a specific cleavage in the 16 S RNA of the 30 S ribosomal subunit both in vivo [1,2] and in vitro [3–5]. The in vitro reaction requires the presence of the 50 S ribosomal subunit, although that subunit itself is unaffected by the bacteriocin [6,7]. The requirement for the 50 S subunit in the reaction suggests that components of this subunit may be directly involved in the colicin-induced cleavage. Previously, we have characterized three different 50 S CsCl cores that lack an increasing number of specific proteins but retain EF-T and EF-G GTPase activity to various extents, in the presence of added proteins L7, L12 and 30 S subunits [8]. The experiments described in this communication were designed to test the activity of these particles, as well as that of 50 S subunits treated with 2 M or 4 M LiCl and of 23 S RNA, in the colicin E3 reaction in vitro. Only the 50 S CsCl cores supported colicin E3 activity, which paralleled their ability to associate with 30 S subunits. The smallest, active 50 S CsCl core consisted of undamaged 23 S RNA and the 11–12 proteins most tightly associated with it.

2. Materials and methods

Electrophoretically homogeneous elongation factors G and T, ribosomal subunits prepared from NH_4Cl-washed ribosomes and ribosomal 50 S CsCl cores were the same as described [8]. The 50 S CsCl core a lacks proteins L1, L7, L8*, L10, L12, L16, L25, L33 and some L6 and L11; the core b additionally lacks protein L6, and the core c, also proteins L5, L15, L18, L27, L28, L30 as well as most of L9, L14, L19 and L21. Extraction of 50 S subunits with 2 M or 4 M LiCl was carried out according to Marcot-Queiroz and Monier [10] but in the presence of 5 mM $MgCl_2$. In both conditions, the resulting particles had a protein composition similar to that of CsCl core c, with the following exceptions: additional loss of protein L2; complete loss of L9, L11, L14, L21; proteins L1 and L5 only partially-extracted. In addition, the 23 S RNA of the LiCl-extracted 50 S particles was quantitatively degraded into a number of smaller fragments, as visualized on sodium dodecyl sulphate polyacrylamide gel electrophoresis [8]. The 23 S RNA was prepared according to standard procedures (e.g. [10]) using phenol extraction of the 23 S RNA peak obtained by centrifugation of unwashed ribosomes in sucrose gradients containing sodium dodecyl sulfate.

2.1. *Assays*

The role of the 50 S subparticles or 23 S RNA in colicin E3 activity was investigated by taking advantage of the property of the colicin, documented in a previous publication [11], to be inhibited in the presence of the components needed for poly(phenylalanine) synthesis. The effect of the colicin treatment on the 30 S subunit could therefore be tested by analyzing the activity of the latter in poly(phenylalanine) synthesis after addition of intact 50 S subunits, even in reaction mixtures containing the bacteriocin. The assay consisted therefore of two steps.

*Protein L8 has recently been identified as a complex of proteins L7, L10 and L12 [9]

2.1.1. Preincubation with colicin E3 (1 h at 30°C)

The system contained, in 50 μl 20 mM Tris–HCl (pH 7.8)–10 mM $MgCl_2$–40 mM KCl, 100 pmol 30 S subunits, 8 μg colicin E3 and 100 pmol either 50 S subunits or the component to be examined. After preincubation the tubes were cooled to 0°C. When the 50 S cores were tested, the 50 S subunits were added prior to the second step to allow polypeptide synthesis; inversely, in the control the respective core and/or colicin E3 were added to the tubes after preincubation with 50 S subunits or without colicin. All of the tubes thus contained colicin E3, 50 S subunits and one of the 50 S derived particles or 23 S RNA in final vol. 75 μl.

2.1.2. Assay for poly(U)-directed poly(phenylalanine) synthesis

Immediately after preincubation, 15 μl of the 75 μl reaction mixture, containing 20 pmol of ribosomes, were added to 60 μl prepared solution to yield the following final composition: 50 mM Tris-HCl (pH 7.8), 6.5 mM $MgCl_2$, 40 mM KCl, 0.4 mM ATP, 0.4 mM GTP, 1 mM dithiothreitol, 3 μg poly(U), 2 A_{260} units of total tRNA, 100 pmol L-[^{14}C]phenylalanine, 0.2 μg purified phenylalanyl-tRNA synthetase [12], 10 pmol EF-G and 25 pmol EF-T. 15 μl samples were taken after 0, 15 and 30 min of incubation at 30°C and assayed for incorporation of [^{14}C]phenylalanine into hot trichloroacetic acid-insoluble material. Radioactivity was measured using an Intertechnique liquid scintillation spectrometer model SL 32; the scintillation fluid contained 5 g 2,5-diphenyloxazole in 1 litre toluene.

3. Results and discussion

Colicin E3 is only active at Mg^{2+} concentrations that allow association of the two ribosomal subunits, i.e., at ⩾ 4 mM Mg^{2+} (fig.1). Previous work [8] has shown that the 50 S CsCl cores used here have a somewhat higher Mg^{2+} requirement for their efficient association with 30 S subunits (⩾ 10 mM). Preincubation of ribosomal subunits and/or cores with without colicin E3 was therefore carried out at 10 mM Mg^{2+}.

Figure 2 shows the results. The controls (panel A) confirm the requirement for both ribosomal subunits to allow colicin E3 action (filled circles), and show in addition that the preincubation does not noticeably damage the ribosomal subunits. The effect of the various 50 S CsCl cores on the nucleolytic activity of the colicin is shown in panel B. Both the 50 S CsCl cores a and b were as active as 50 S subunits in promoting colicin E3 activity (filled squares). The 50 S core a, which lacks (among others) proteins L7 and L12, has previously been found to be inactive in the EF-T-dependent GTPase reaction and about one-fourth as active as the 50 S subunit in the EF-G GTPase reaction [8]; replacement of the 50 S CsCl core a by the core b, which we found to cause a strong reduction of both EF-G and EF-T GTPase activities in the presence of added proteins L7, L12 [8], does not at all affect colicin E3 action. This shows that in addition to proteins L7 and L12, also protein L6 has no function in this particular reaction. Even the core c, which we have previously found to be totally inactive in both the EF-G and EF-T GTPase reactions in the absence of proteins L7, L12 [8] retained 90% of the capacity of 50 S subunits in the colicin reaction (filled circles). Therefore, none of the approx. 19 proteins missing from the 50 S CsCl core c plays an essential role in

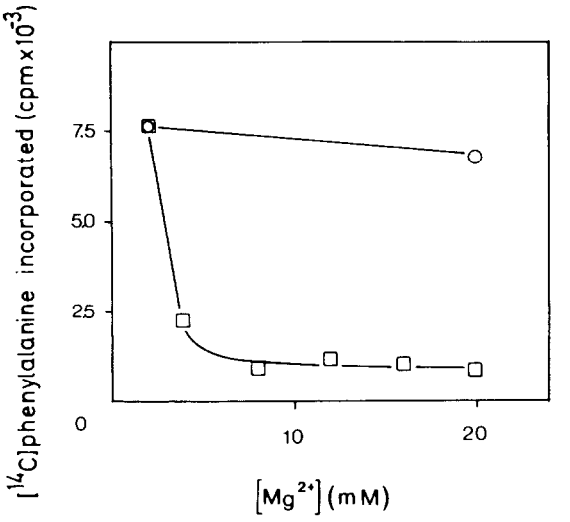

Fig.1. Dependence of colicin E3 activity on the Mg^{2+} concentration. 10 pmol 70 S ribosomes were incubated for 30 min at 30°C with 25 pmol colicin E3 at the [Mg^{2+}] indicated in the abscissa and assayed for formation of poly(phenylalanine) as described in Materials and methods. Incubation time: 10 min at 30°C. (○) Control without colicin. (□) Experiment with colicin E3.

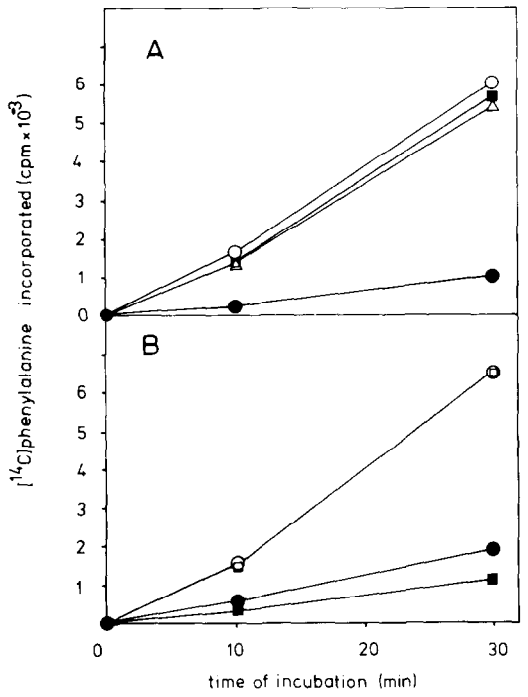

Fig.2. Ability of 50 S subunits and 50 S derived particles to promote colicin E3 action. Assays were carried out as detailed in Materials and methods. Controls are shown in panel A: (○) preincubation of 50 S and 30 S subunits without colicin E3; (■) preincubation of 30 S subunits alone with colicin E3; (△) preincubation of 30 S subunits alone; (●) preincubation of 50 S and 30 S subunits with colicin E3. Panel B: preincubation of 30 S subunits carried out in the presence of: 50 S CsCl core a or b without (□) and with (■) colicin E3, 50 S CsCl core c without (○) and with (●) colicin E3. Preincubation of 30 S subunits in the presence of either 50 S subunits treated with LiCl or of 23 S RNA yielded results similar to the control (○) both with and without colicin E3.

the activation of the colicin. Indeed, the somewhat lower inhibition caused by colicin E3 treatment in the presence of 50 S CsCl core c may be entirely due to its slightly lower ability to associate with 30 S subunits [8,13]. These findings raised the question whether the RNA backbone is sufficient to act as an effector for the colicin. This was tested by adding purified 23 S RNA to the preincubation mixtures, but no measureable effect was observed. 50 S cores prepared by treatment with 2 M or 4 M LiCl were equally unable to sustain colicin E3 activity; in this case the 23 S RNA was quantitatively degraded into a number of smaller fragments during preparation, and the particles were unable to associate with 30 S subunits at 10 mM Mg^{2+}.

The 50 S subunit may act in different ways in the colicin reaction. It may open the 30 S subunit, thereby rendering accessible to the colicin a normally hidden site, or its association with the 30 S subunit may create the binding site for the bacteriocin. The results presented here show that either or both of these functions can be carried out by 50 S particles having lost more than half their proteins but preserving the ability to associate with 30 S subunits. The 23 S RNA was itself unable to act as an effector in the colicin reaction; therefore the 11 major proteins remaining in the 50 S CsCl core c (L2, L3, L4, L13, L17, L21, L22, L23, L24, L29, L32) could act either by directly stimulating its association with the 30 S subunit and E3 activity, or by maintaining proper folding of the 23 S RNA, which indeed may itself be the primary active component in this particular function of the larger ribosomal subunit.

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