REGULATION OF ENZYME SYNTHESIS IN VITRO BY A FACTOR BOUND TO RIBOSOMES

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1. Introduction

Though the structure of ribosomes is known in great detail [1], many of their biological functions are not entirely understood. Thus it is not yet clear whether the activity of the ribosomes may be regulated by additional factors (besides initiation or elongation factors) or by some kind of conformational changes [2–4].

Here we show that the T3 and T7 DNA dependent syntheses of 3 enzymes and total protein in an in vitro *E. coli* system were differently regulated depending on the ribosome concentration and that this regulation was due to a factor attached to the ribosomes.

2. Materials and methods

The isolation of protein fraction and ribosomes as wel as the assay conditions for both the synthesis and the activity test of the enzymes have been described previously [5]. The following enzymes were studied: RNA polymerase (EC 2.7.7.6) and lysozyme (EC 3.2.1.17) of the bacteriophages T3 and T7 and *S*-adenosyl-methionine cleaving enzyme (EC 3.3.1.–) of T3. The systems were prepared from the *E. coli* strains Q13 [6], K12 Hfr [7] and 1200 [8].

3. Results and discussion

The in vitro syntheses of the 3 enzymes and of total protein were highly dependent on the ribosome concentration (fig.1A). The synthesis of the RNA polymerase through an optima and then decreased to background levels with increasing ribosome concentrations

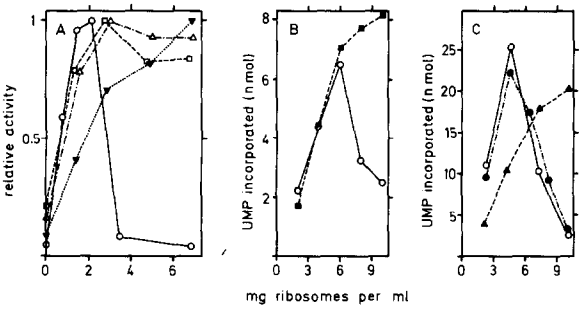

Fig.1. Synthesis of enzymes and total protein depending on the ribosome concentration in the presence of 8mM Mg^{2+}, 3 mM spermidine and 10 mM phosphoenolpyruvate. Ribosomes were from *E. coli* Q13. Incubation was for 25 min at 35°C followed by the activity tests of the enzymes [5]. (A) T3 DNA as template. The relative activity of 1 was equivalent to the following values for the synthesized enzymes and protein. (○—○) RNA polymerase, incorporation of 32.5 nmol UMP; (△– . – .△) SAM cleaving enzyme, production of 136 nmol thiomethyl-adenosine; (□- - -□) lysozyme, release of 3200 counts/min of [^{3}H] diaminopimelic acid; (▼ . . . . . ▼) total protein, incorporation of 15.3 nmol leucine [7]. (B) Synthesis of T7 RNA polymerase with (○—○) untreated ribosomes and (■- - -■) ribosomes preincubated for 10 min at 52°C in ribosome buffer (0.01 M Tris-acetate, 0.01 M Mg^{2+}, 0.02 M KCl and 0.001 M mercaptoethanol pH 7.3). (C) Synthesis of T7 RNA polymerase with (○—○) untreated ribosomes, with (▲- - -▲) ribosomes after washing (see below) and with (●– . – .●) washed ribosomes after readdition of partly fractionated ribosomal wash (the amount of which was obtained by titration of an assay with 10 mg of ribosomes/ml to background activity). The ribosomes were washed after treatment with 0.5 M NH_4Cl in ribosome buffer (see legend of fig.1B) for 4 h at 0°C by centrifugation for 4 h at 200 000 g_{av} through a sucrose cushion. The sediment was resuspended and dialysed against ribosome buffer. The supernatant (wash) was precipitated with 80% $(NH_4)_2SO_4$ and redissolved in 0.01 M Tris-acetate pH 7.4 and dialysed.

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when the synthesis of the cleaving enzyme and the lysozyme reached a plateau and total protein synthesis was still increasing. There was no difference within experimental error between the two templates studied. This inhibitory effect on the synthesis of the polymerase was shown by ribosomes from the *E. coli* strains Q13 (F^-) and K12 Hfr but not from 1200 (F^-). In the latter case the ribosome-dependence of the polymerase synthesis paralleled that of the other enzymes. Hence the effect was not due to the F factor.

Preincubation of the Q13 and K12 ribosomes at 52°C irreversibly abolished the inhibitory effect (fig.1B). This result could be due to either inactivation or destruction of a ribosome-bound factor or to an irreversible conformational change of the ribosomal structure. This question was answered by the following experiment: washing of the ribosomes reversibly abolished the inhibitory effect (fig.1C). Further, by addition of the ribosomal wash to *E. coli* 1200 ribosomes, the same inhibition of polymerase synthesis was obtained as with ribosomes of the two other *E. coli* strains. Neither preincubation nor washing of the ribosomes qualitatively influenced the syntheses of the other enzymes or of total protein. Hence the different regulation of the syntheses of the enzymes studied was due to a ribosome-bound factor which did not affect the activity test of these enzymes as was shown by additional control experiments. However, the effect was not specific for the synthesis of the polymerase since the syntheses of all proteins of a mol. wt. of more than about 60 000 were inhibited as indicated by gel electrophoresis of pre-labeled proteins (fig.2). This effect on the synthesis of large proteins was not influenced by varying concentrations of spermidine and/or Mg^{2+}, i.e. the mechanism seems to be different from that described by Atkins et al. [9] for a eukaryotic system. The inhibitory factor could not be found in the protein fraction but was always attached to the ribosomes, i.e. the factor must be different from that described by Kung et al. [4].

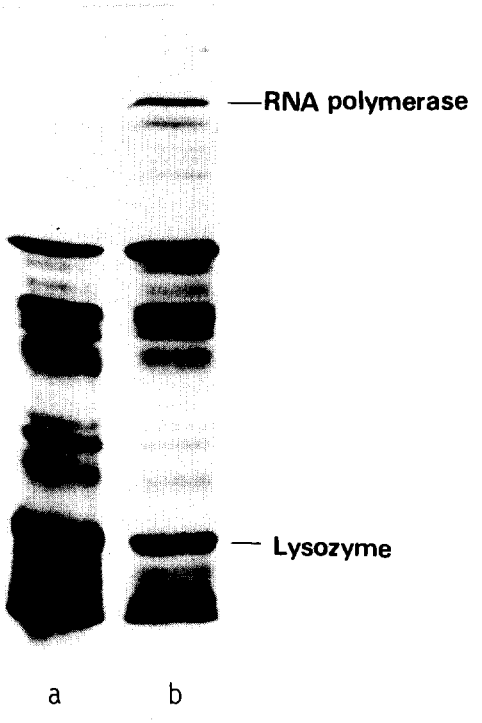

Fig.2. Electrophoretic pattern of proteins synthesized with T7 DNA as template and different concentrations of ribosomes of *E. coli* K12 Hfr. The proteins were labeled with [^{3}H]leucine (500 Ci/mole). Gel electrophoresis according to [10] in a 12.5% polyacrylamide gel. Visualisation of the pattern according to [11] Synthesis in the presence of (a) 10 mg and (b) 5 mg ribosomes/ml.

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