DEFECTIVE 30S RIBOSOMAL PARTICLES IN A POLYAMINE AUXOTROPH OF *ESCHERICHIA COLI*

G. Echandi and I. D. Algranati

Instituto de Investigaciones Bioquímicas "Fundación Campomar" and Facultad de Ciencias Exactas y Naturales, Obligado 2490, Buenos Aires (28), Argentina

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SUMMARY: Polypeptide synthesis directed by poly(U) or MS 2 phage RNA is several fold more active in cell-free systems prepared from polyamine supplemented bacteria than in extracts of polyamine depleted cells. This effect depends on the presence of defective 30S ribosomal subunits in the starved bacteria. It is concluded that polyamines play a role in the normal biosynthesis, maturation and/or assembly of the small ribosomal subparticles.

A great number of studies have indicated that polyamines are involved in the biosynthesis of macromolecules and in the stabilization of many subcellular structures (1-4).

The results obtained with cell-free systems have shown that putrescine, spermidine and spermine are able to stimulate or partially replace the Mg^{++} requirement in nearly all the steps of the translation process. The effects of polyamines on the charging of tRNA (5), the binding of aminoacyl-tRNA to ribosomes (6) and polypeptide synthesis *in vitro* with synthetic or natural messengers have already been described in bacteria and eukaryotic cell systems (7-12). In addition several *in vivo* studies with *E. coli* mutants unable to synthesize putrescine have indicated that polyamines play a physiological role in protein synthesis (13-15).

Our experiments with cell-free systems prepared from polyamine-deficient bacteria grown in the absence and presence of putrescine allowed us to investigate the translation process independently of RNA synthesis. We have been able to demonstrate that ribosomes of starved cells were less active in polyphenylalanine synthesis than those derived from polyamine supplemented bacteria (16). Moreover the equilibrium between 70S monomers and ribosomal subunits was shifted towards the subparticles in polyamine starved cells (17).

The present paper deals with structural and functional changes of 30S ribosomal subunits upon the addition of putrescine to starved cultures of polyamine requiring bacteria.

Abbreviations: MMO, minimal medium containing ornithine (100 μg/ml); MMOP, minimal medium with ornithine and putrescine (100 μg/ml, each).

MATERIALS AND METHODS

Putrescine dihydrochloride, spermidine trihydrochloride and poly(U) as K^+ salt were obtained from Sigma; sucrose (ribonuclease-free) was purchased from Schwarz-Mann; E. coli stripped tRNA from General Biochemicals; MS 2 phage RNA from Miles; [^{14}C] phenylalanine (464 Ci/mole) and [^{14}C] valine (280 Ci/mole) from New England Nuclear Corporation.

The polyamine auxotroph Escherichia coli MA 261, isolated by Dr. W.K. Maas and collaborators, was used in all the experiments.

The growth media and the procedure for obtaining polyamine depleted bacteria have already been described (16, 17). The starved cells were cultivated in the absence or presence of putrescine (MMO or MMOP media, respectively).

Cell-free systems, ribosomal suspensions and supernatant fractions S_{30} and S_{150} were prepared as previously reported (16).

Preparation of purified 30S and 50S subunits. The suspended ribosomes were dissociated into their subunits by dialysis for 12 h against a buffer solution containing 10 mM Tris-HCl, pH 7.8, 0.1 mM magnesium acetate, 60 mM NH_4Cl and 6 mM 2-mercaptoethanol. Purified 30S and 50S subparticles were isolated by centrifugation of 40 A_{260} units of dissociated ribosomes in a 12 ml linear sucrose gradient (10-30%) containing 10 mM Tris-HCl buffer, pH 7.8, 5 mM magnesium acetate and 50 mM KCl. After centrifugation in a Spinco SW 40 Ti rotor for 5 h at 35,000 rpm, the fractions corresponding to the 30S and 50S peaks were pooled and dialyzed for 12 h against a solution containing 10 mM Tris-HCl, pH 7.8, 5 mM magnesium acetate, 60 mM NH_4Cl and 6 mM 2-mercaptoethanol. The supernatant fractions and the ribosomal and subunit suspensions were stored at -70°C.

The ribosomal profiles were obtained as described previously (16) by centrifugation in linear sucrose gradients (15-40%) made up in 10 mM Tris-HCl buffer, pH 7.8, containing 50 mM KCl and the magnesium acetate concentration indicated in each case.

In vitro polypeptide synthesis induced by poly(U) or natural mRNA. The reaction mixture for polyphenylalanine synthesis was as already described (16), using 30S and 50S subunits instead of ribosomal suspensions. Spermidine was added in some experiments.

The assay for polypeptide synthesis with natural mRNA was carried out in a total volume of 0.05 ml and contained: Tris-HCl, pH 7.8, 50 mM; NH_4Cl, 60 mM; ATP, 1 mM; GTP, 0.02 mM; phosphoenolpyruvate, 5 mM; pyruvate kinase, 1.5 µg; 2-mercaptoethanol, 8 mM; [^{14}C] valine (spec. act. 166 Ci/mole), 0.06 mM; unlabelled amino acids excluding valine, 0.025 mM each; MS 2 phage RNA, 0.25 A_{260} units; preincubated S_{30} extracts from starved or unstarved cells, 1.0 A_{260} units; S_{150} supernatant fraction from bacteria grown in the presence of putrescine (7.5 µg of protein) and magnesium acetate as indicated in each case.

S_{30} extracts containing Tris-HCl, pH 7.8, 50 mM; ATP, 1 mM; GTP, 0.04 mM; phosphoenolpyruvate, 5 mM and pyruvate kinase, 30 µg/ml were preincubated for 7 min at 34°C just before the reaction, which was carried out at the same temperature for 20 min. The radioactivity of 5% trichloroacetic acid insoluble material after heating at 90°C for 15 min was measured as reported previously (16).

RESULTS AND DISCUSSION

Previous studies carried out in our laboratory have indicated that polyphenylalanine synthesis in cell-free systems derived from bacteria grown in the presence of putrescine was several fold higher than that obtained with extracts prepared from polyamine starved cells (16). We report here similar

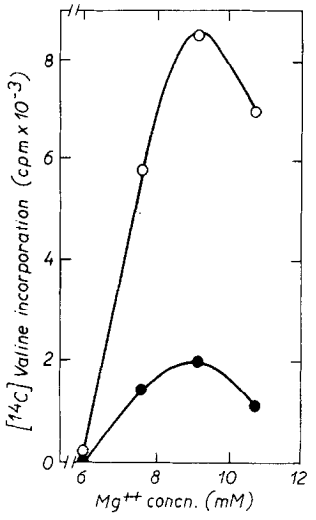

Fig. 1. Effect of Mg^{++} concentration on valine incorporation induced by MS 2 RNA in cell-free extracts of starved and unstarved E. coli MA 261. The reaction mixtures were as described in Materials and Methods. Symbols: ● and ○ correspond to extracts from bacteria grown in the absence and presence of putrescine, respectively.

Table I. Poly(U)-dependent phenylalanine incorporation catalyzed by reconstituted systems containing ribosomal subunits from starved or unstarved bacteria

The reaction mixtures contained subunits (0.05 A_{260} units of 30S and 0.10 A_{260} units of 50S) prepared from cells cultivated in MMO or MMOP media as indicated in each experiment. S150 supernatant fluid (15 μg of protein) obtained from polyamine supplemented bacteria was used in all cases. Mg^{++} concentration was 13 mM and the incubation was carried out for 30 min at 37°C. All other details as described in Materials and Methods. The blank values obtained in the absence or poly(U) were substracted in each case.

	30S subunit		50S subunit		Polypeptide synthesis (cpm)
	from bacteria grown in medium				
	MMO	MMOP	MMO	MMOP	
Expt. 1	+	-	+	-	1724
	+	-	-	+	1909
	-	+	+	-	4817
	-	+	-	+	5970
Expt. 2	+	-	+	-	1771
	+	-	-	+	1492
	-	+	+	-	7956
	-	+	-	+	7550

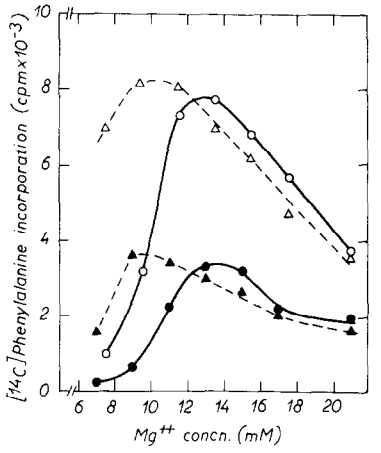

Fig. 2. Effect of Mg^{++} concentration and spermidine addition on polyphenylalanine synthesis in cell-free systems derived from starved and unstarved bacteria.
The reaction mixtures contained 0.05 A_{260} units of 30S particles, 0.10 A_{260} units of 50S subunits, S_{150} supernatant fluid (15 µg of protein) prepared from cells cultivated in MMOP medium and all other components as previously described (16). Symbols: ● and ○ correspond to mixtures containing ribosomal subparticles from bacteria grown in the absence and presence of putrescine, respectively; ▲ and △ correspond to the same systems with the addition of 0.5 mM spermidine.

results with polypeptide synthesis directed by natural mRNA. Fig. 1 shows the [^{14}C]valine incorporation induced by MS 2 phage RNA with S_{30} extracts of putrescine-starved and -supplemented bacteria. In this case the polypeptide synthesis was four-fold higher in cell-free systems prepared from bacteria cultivated in the presence of polyamine and the Mg^{++} level required for maximal activity was about 9 mM in both extracts.

We have already shown that most of the effect of polyamine starvation on polypeptide synthesis could be related to some deficiency at the level of ribosomal particles (16). In order to study this problem further we have purified the ribosomal subunits from putrescine-starved and -supplemented bacteria, and the polyphenylalanine synthesis was measured in reaction mixtures in which ribosomal subparticles from starved and unstarved cells were combined. In all these experiments the same S_{150} supernatant fraction was used. The values given in Table I demonstrate that the level of polyphenylalanine formed was dependent exclusively on the source of 30S particles. The incorporation was the same with both kinds of 50S subunits, but increased 3- to 5-fold when 30S subparticles from polyamine supplemented bacteria were used instead of the small subunits prepared from starved cells. Since the observed effect could be due to differences in the polyamine content of the ribosomal subunits ob-

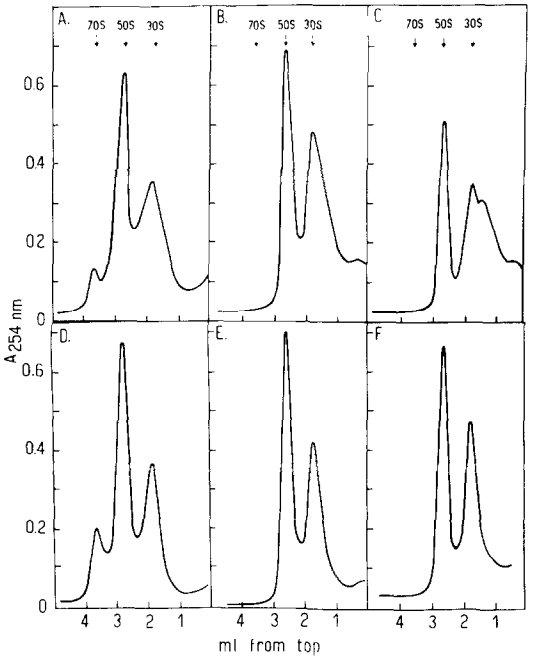

Fig. 3. Profiles at different Mg^{++} concentrations corresponding to ribosomes prepared from bacteria cultivated in the absence of putrescine (A, B and C), or in the presence of this polyamine (D, E and F). A and D, were obtained at 5 mM Mg^{++}; B and E, at 1 mM Mg^{++} and C and F, at 0.1 mM Mg^{++}.

tained under various conditions, the polyphenylalanine synthesis was measured at different Mg^{++} concentrations with and without the addition of spermidine. Fig. 2 shows that the presence of polyamine in the in vitro systems could not increase the efficiency of ribosomal particles derived from starved bacteria, although a similar decrease of the optimal Mg^{++} concentration required for polypeptide synthesis was observed with both kinds of ribosomes.

The results described indicate that ribosomal particles (especially the 30S subunits) obtained either from polyamine-starved or -unstarved bacteria were functionally different. They also behaved in a different way when submitted to sucrose gradient centrifugation in the presence of various Mg^{++} concentrations, as shown in Fig. 3. The ribosomal profiles obtained at 5, 1 and 0.1 mM Mg^{++} show that only the 30S subunits from bacteria grown in the absence of polyamine gave asymmetric or double peaks, indicating some heterogeneity in the population of these particles. The sucrose gradient analyses of the purified subunits (Fig. 4) confirmed these results since the 30S subparticles obtained from starved bacteria gave a double peak, one of them with sedimentation

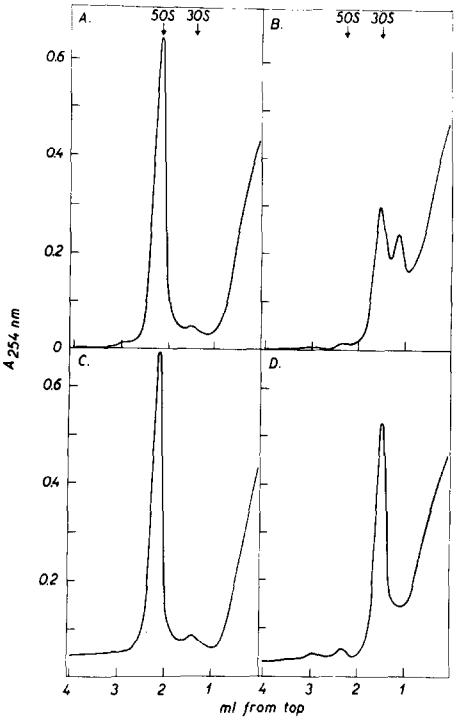

Fig. 4. Sucrose gradient analyses of purified subunits prepared from starved or unstarved bacteria.
The sucrose gradients contained 5 mM magnesium acetate. A and B are the profiles corresponding to 50S and 30S particles obtained from cells grown in the absence of putrescine; C and D are the patterns of subunits prepared from polyamine supplemented bacteria.

coefficient lower than 30S. This peak may correspond to a defective particle or to a precursor of mature 30S subunit.

Preliminary studies have indicated that the ribosomal RNA extracted from the abnormal 30S particles of polyamine starved bacteria was partially unfolded and/or destroyed.

The results described in this communication confirm previous conclusions of a direct role of polyamines in the translation process (13-16). Besides the well known effects of these organic cations in the activation of amino acids (5) and the decrease of optimal Mg^{++} concentrations for polypeptide synthesis (8, 9), our data strongly suggest that polyamines participate in the normal biosynthesis, maturation or assembly of 30S subunits, perhaps through the interaction with ribosomal RNA. The defective small particles formed in polyamine-depleted bacteria display a decreased polypeptide synthetic capacity. At the same time they show a low affinity for the 50S sub-

units, as indicated by the previously reported fact that the equilibrium between 70S monomers and subparticles is shifted towards dissociation under conditions of polyamine starvation (16, 17).

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