EFFECTS OF PUTRESCINE AND MAGNESIUM ON THE RIBOSOMES OF A PSEUDOMONAS

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Recently there has been considerable interest in the physiological functions of the polyamines. Ribosomes contain significant amounts of polyamines and these components appear to play some role in the formation of the ribonucleoprotein complexes[1-3]. We have isolated a *Pseudomonas* species which contains only putrescine as intracellular polyamine. In this communication, we wish to report on the effects of different concentrations of magnesium and putrescine on ribosomal aggregation and on the ability of these aggregates to catalyze poly U directed polyphenylalanine synthesis. Putrescine addition appears to favor the formation of high sedimenting ribosomes which are less effective in promoting protein synthesis than magnesium.

Materials and Methods

The *Pseudomonas* species was adapted to grow on a medium containing inorganic salts and putrescine as the sole source of carbon and nitrogen. The organisms were harvested during exponential growth and washed with fresh medium. Cell extracts were prepared by suspending the cells in "standard buffer" containing 0.01 M Tris buffer, pH 7.8; 0.001 M magnesium acetate and 0.004 M succinic acid. (2:1, w/v) and then disrupting the cells in a French pressure cell (American Instrument Co.). 100 γ DNase per gram of wet cells was added and the mixture was kept in ice for 5 minutes. Cellular debris was removed by centrifugation at 12,000 x g for 15 minutes. Ribosomes

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were sedimented by centrifugation at 105,000 x g for 120 minutes, resuspended in approximately one fourth the original volume and dialyzed 15 hours against the standard buffer, plus or minus putrescine or additional magnesium. Sedimentation analysis was carried out using a Spinco Model E ultracentrifuge at a speed of 42,040 rpm and a temperature of 4°C. Sedimentation coefficients thus obtained were corrected to $S_{20,w}$ standard buffer. If the cell extract was to be used for protein synthesis, the initial centrifugation was for 30 minutes at 31,000 x g. The ribosomes were again sedimented by centrifugation at 105,000 x g for 120 minutes and resuspended in one fourth the original volume of buffer. The ribosomes and the soluble fraction were dialyzed 15 hours against the standard buffer with or without additions. Protein synthesis was carried out essentially according to the procedure of Nirenberg[4]. Putrescine was estimated by the method of Dubin and Rosenthal[5].

Results and Discussion

The Pseudomonas species which we have been studying contains 5.6 μmoles of putrescine per gram wet weight cells whether the cells are grown on a glucose-ammonia medium or on putrescine as the sole carbon and nitrogen source. Approximately 85% of this putrescine is found in the soluble fraction of cell free extracts and 15% is associated with the ribosomal fraction.

A typical schlieren diagram of the ribosomes in standard buffer is shown in Figure 1a. The 50s particle is the predominant species. Lesser components sediment at 30s and 20s. There is also a component with a sedimentation coefficient of 7s which is composed of protein and soluble RNA. The status of this 20s particle is currently under investigation, as it is present in all preparations. It is worthy to note that this particle appears not to participate in either putrescine or magnesium induced ribosomal aggregation. Control experiments

with E. coli B gave rise to only 50s and 30s components.

As in other systems, changes in the magnesium and/or putrescine concentration cause association of dissociation of the ribosomes. Figure 1c shows the ribosomal aggregation pattern in the

Figure 1

Analytical ultracentrifuge patterns of Pseudomonas ribosomes in standard buffer. (a) control 1 mM Mg^{++}, sedimentation coefficients from right to left: 50s, 30s, 20s and 7s; (b) 5 mM putrescine added, sedimentation coefficients from right to left: 70s, 60s, 50s, 30s, 20s and 7s; (c) 10 mM putrescine concentration, sedimentation coefficients from right to left: 100s, 70s, 60s, 50s, 20s and 7s.

Figure 2

Analytical ultracentrifuge patterns of Pseudomonas ribosomes. (a) control, 1 mM Mg^{++}, sedimentation coefficients from right to left: 50s, 30s, 20s and 7s; (b) 5 mM Mg^{++}, sedimentation coefficients from right to left: 70s, 60s, 50s, 20s and 7s; (c) 10 mM Mg^{++}, sedimentation coefficients from right to left: 70s, 60s, 50s, 30s, 20s and 7s.

presence of 10 mM putrescine, while the aggregation pattern in the presence of 10 mM magnesium is shown in Figure 2c. Clearly, different patterns result. Magnesium leads to the association of 50s and 30s particles into 60s and 70s particles as well as some random aggregates. Putrescine, however, not only induces the formation of 60s and 70s particles, but also a distinct 100 s species.

When a ribosomal preparation which is in standard buffer is extensively dialyzed against the same buffer containing 10 mM putrescine and no magnesium, the profile shown in Figure 3b is obtained. Instead of the 50s, 30s, 20s and 7s components, 36s, 24s

Figure 3

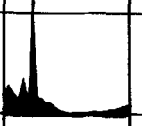
Analytical ultracentrifuge patterns of Pseudomonas ribosomes. (a) control, 1 mM Mg^{++}, sedimentation coefficients from right to left: 50s, 30s, 20s and 7s; (b) No Mg^{++}, 10 mM putrescine, sedimentation coefficients from right to left: 36s, 24s and 7s.

and 7s components are obtained. Thus it is clear that putrescine is not capable of replacing the minimal amount of magnesium (1 mM) which is required for maintainance of the basic 50s, 30s and 20s units.

In order to gain further information on the properties of the normal ribonucleoprotein-polyamine complex, the ability of this complex to catalyze poly U primed protein synthesis was investigated. The results are shown in Figure 4. It appears that 10 mM putrescine

Figure 4

Effects of Putrescine on Ribosomal Aggregation and Protein Synthesis

Addition	schlieren diagram	phenyl-alanine (cpm)	leucine (cpm)
1 mM Mg^{++}		512	681
1 mM Mg^{++} + 10 mM Put.		1620	107
10 mM Mg^{++}		3857	823
10 mM Mg^{++} + 10 mM Put.		3763	643

Reaction mixtures contained in 1.25 ml volume the following components (in μmoles unless otherwise specified): Tris-HCl buffer, pH 7.8, 125; potassium chloride, 63; β-mercaptoethanol, 7; ATP, 0.125; GTP, 0.0375; phosphoenolpyruvate, 9.4; phosphoenol-pyruvate kinase, 25 μg; poly U, 100 μg; C^{14}-L-amino acid 38 - 56; and the ribosomal and soluble fractions of the Pseudomonas (approximately 8 mg protein and 1.2 mg RNA). Magnesium acetate and putrescine were added at the final concentrations indicated. The mixtures were incubated at 37° for 15 minutes. Reactions were stopped with TCA, heated at 95° for 20 minutes, washed by membrane filtration and radioactivity determined using a Packard Tri-Carb Liquid Scintillation Counter. The specific activities of the C^{14}-L-amino acids were in the range of 200 - 300 mC/mmole. Values represent net polymer synthesis.

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and 1 mM magnesium together are capable of some stimulation of protein synthesis; however, 10 mM magnesium is about two and a half times more effective. Further addition of 10 mM putrescine to 10 mM magnesium results in no additional stimulation. The observation that polyamines act by increasing the fraction of 100s ribosomes is in agreement with the report of Martin and Ames[6].

The high sedimenting particles in our system, including 100s, do not appear to function too efficiently in protein synthesis as in E. coli. It thus appears that the mechanism of putrescine and magnesium are different in inducing ribosomal aggregation. The reasons for this anomalous situation are currently under investigation and will be reported elsewhere.

Recently there have been reports that external agents, such as streptomycin, putrescine and other cations, can cause misreading of the polynucleotide messenger. In order to test the possibility of miscoding[7], the incorporation of six other amino acids was examined in our system. Isoleucine, valine, serine, tyrosine and glycine were not incorporated. Only leucine was incorporated to any significant extent. Approximately 600 cpm is incorporated in the presence of 1 mM magnesium and this is reduced upon the addition of 10 mM putrescine. Increasing the magnesium concentration had little effect on the incorporation, but the putrescine inhibition was prevented. The incorporation of leucine in poly U catalyzed reactions had been observed earlier.[7] The mechanism by which putrescine inhibits leucine incorporation at lower magnesium concentration is not being further investigated.

Summary

In our Pseudomonas system, putrescine and magnesium both induce ribosomal aggregation. The high sedimenting particles formed in the presence of 10 mM putrescine appear to be less effective in stimulating protein synthesis than those formed in the presence of 10 mM magnesium.

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