POLYAMINE AND MAGNESIUM CONTENTS AND POLYPEPTIDE SYNTHESIS AS A FUNCTION OF CELL GROWTH

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SUMMARY: The polyamine and ribosome contents during the logarithmic phase of growth were much higher than those in the late logarithmic or stationary phase of growth. On the other hand, the Mg^{2+} content did not vary markedly throughout the different growth phases. These data, together with the results of a previous communication (1), suggest that increased polyamines during the logarithmic phase of growth may play significant roles not only in the neutralization of the negative charges of increased ribosomes but also in the increase of the velocity of polypeptide chain elongation by binding to the ribosomes. The polyphenylalanine synthetic activity of ribosomes from the logarithmic phase of growth was higher than that of ribosomes obtained from the stationary phase of growth in the presence of optimal concentrations of spermidine.

It has become evident that polyamines play an important role in the growth of various cells (2). We have proposed previously that a partial replacement of Mg^{2+} by polyamines *in vivo* usually can occur in protein synthesis (3-7). In addition, we have recently reported that polyamines not only shifted the optimal concentration of Mg^{2+} for polypeptide synthesis but also stimulated the ribosomal function (1). It has been reported also that spermidine is a necessary cofactor of polyphenylalanine synthesis in *B. thuringiensis* (8). Therefore, we have studied the relationship between polyamine and magnesium contents and polypeptide synthesis as a function of cell growth in *E. coli* B and *B. thuringiensis*.

MATERIALS AND METHODS

Materials - E. coli B and Bacillus thuringiensis Berliner were grown at 37° C in 2 liters of minimal salt medium (9) supplemented with 0.2% glucose and 0.2% casamino acids. The growth of the cells was followed with a Klett-Summerson colorimeter (No. 60 filter). The cells were harvested by centrifugation at 15,000 x g for 15 min at appropriate time intervals. When the polyamine and Mg^{2+} contents of the cells were measured, the cells were washed by centrifugation through a water-immiscible layer of silicones according to the method of Hurwits et al. (10). For the preparation of ribosomes and S-100, the cells were washed with Buffer I (10 mM Tris-HCl, pH 7.5, 10 mM magnesium acetate, 60 mM NH_4Cl, and 6 mM 2-mercaptoethanol) and centrifuged as indicated above.

Unwashed ribosomes and S-100 were prepared as described previously (11). A method for further purification of S-100 by Sephadex G-50 was described previously (1). Washed ribosomes were prepared by washing the ribosomes with a buffer containing 100 mM Tris-HCl, pH 7.5, 10 mM magnesium acetate, 60 mM KCl, 6 mM 2-mercaptoethanol and 500 mM NH_4Cl. Dialyzed ribosomes were obtained by dialyzing 10 mg of washed ribosomes (2 mg/ml) against 3 liters of Buffer I for 48 hr at 4° C.

Measurements of magnesium ions and of polyamines - Magnesium ions were analyzed in the presence of strontium chloride (1 mg/ml) by means of atomic absorption spectrometry using a Hitachi 208 instrument and a hollow-cathode lamp. Polyamines were measured with a slight modification (12) of the method of Dion and Herbst (13).

Procedures for polyphenylalanine synthesis - The reaction mixture (0.1 ml), which contained 80 mM Tris-HCl (pH 7.5), 1 mM dithiothreitol, 100 mM NH_4Cl, 1 mM ATP, 0.4 mM GTP, 4 mM phosphoenol-

pyruvate, 4 μg pyruvate kinase (Boehringer Mannheim GmbH), 20 μg E. coli B tRNA (General Biochemicals), 10 μg poly (U) (Boehringer Mannheim GmbH), 0.5 A_{260} unit of E. coli or B. thuringiensis dialyzed ribosomes, E. coli S-S100 (80 μg protein), 0.1 μCi of [^{14}C] phenylalanine (382 μCi/μmole), and magnesium acetate and polyamines at the specified concentrations, was incubated at 30° C for 15 min. A 0.08-ml aliquot of each reaction mixture was placed on a paper disc (25 mm diameter) and the hot trichloroacetic acid insoluble radioactivity was assayed with a liquid scintillation spectrometer. The counting efficiency was 1.2 x 10^6 cpm/μCi.

RESULTS

Polyamine, Mg^{2+} and ribosome contents in E. coli B at various phases of growth - The putrescine, spermidine, and Mg^{2+} contents per g of wet cells of E. coli are presented in Fig. 1 and Table 1. As shown in Fig. 1, the spermidine and putrescine contents during the logarithmic phase of growth were much higher than those in the late logarithmic or stationary phase of growth. Spermine was not detected in any phase of growth. On the other hand, the Mg^{2+} conten did not vary markedly throughout the different growth phases, although some increase (about 10% to 20%) occurred during the middle logarithmic phase. The values are tabulated in Table 1. The increase of total charge of cations per g wet cells during the logarithmic phase of growth was due primarily to the increase of charge of polyamines. At this stage, the percentage of polyamine-charge in total charge reached almost 50%, while it was about 30% during the late phase of growth. The ribosome contents of E. coli at the early logarithmic phase (Klett units: 100) was about 1.7 times as high as those of E. coli at the stationary phase (Klett units: 380) (Table 2).

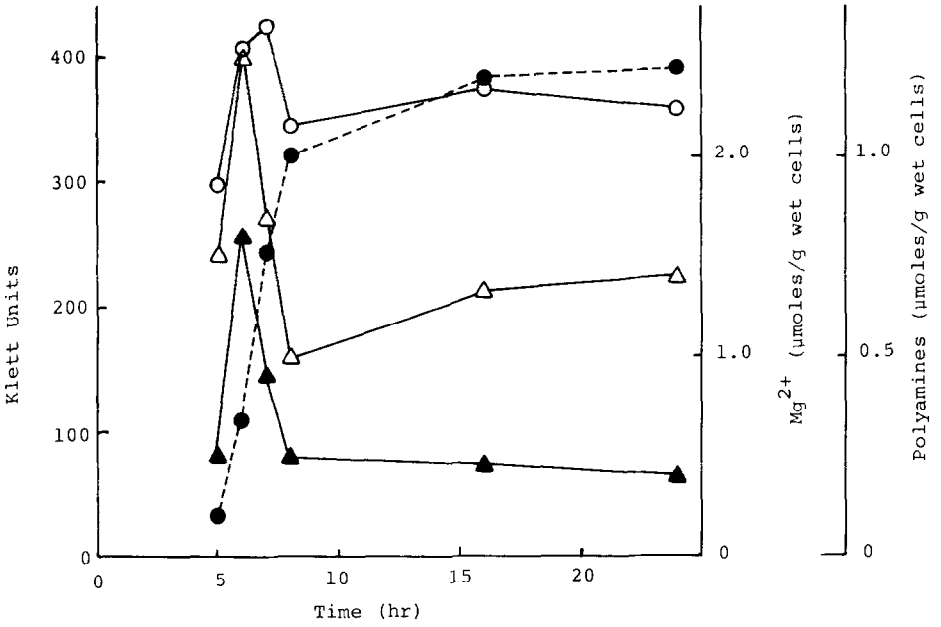

Fig. 1. Mg^{2+} and polyamines contents in E. coli B at various phases of growth. E. coli B cells were harvested at various time intervals and the Mg^{2+}, spermidine and putrescine contents were measured as described in the text.
●----●, Klett units; o——o, Mg^{2+}; Δ——Δ, putrescine; ▲——▲, spermidine.

Effect of spermidine on polyphenylalanine synthesis by various ribosome preparations - Since it has been reported that the components of ribosomes obtained from the cells at various growth phases may differ in their protein (14,15) and RNA (16) contents, it is of interest to study whether or not ribosomes from the logarithmic phase of growth respond better to spermidine than those from the stationary phase of growth. As shown in Table 2 and Table 3, the polyphenylalanine synthetic activity of ribosomes obtained from the logarithmic phase of growth was higher than that of ribosomes obtained from the stationary phase of growth either in the presence or absence of suitable amounts of spermidine. However, the percentage of stimulation by spermidine did not vary markedly among

Table 1. Charge concentrations of polyamines and Mg^{2+} in E. coli B harvested at various growth phases.

Time of harvest (hr)	Klett units	Charge concentration[a]				Percentage of charges	
		Mg^{2+}	Spermidine	Putrescine	Total	Mg^{2+}	Polyamines
5	36	3.70	0.87	1.50	6.07	61.0	39.0
6	111	5.16	2.52	2.56	10.24	50.4	49.6
7	246	5.30	1.38	1.72	8.40	63.1	36.9
8	320	4.54	0.84	1.00	6.38	71.2	28.8
16	385	4.68	0.72	1.30	6.70	69.9	30.1
24	390	4.58	0.63	1.38	6.59	69.5	30.5

[a]These values were calculated from the data of Fig. 1. Mg^{2+}, 2 x {Mg^{2+} (μmoles/g wet cells)}; Spermidine, 3 x {spermidine (μmoles/g wet cells)}; Putrescine, 2 x {putrescine (μmoles/g wet cells)}.

Table 2. Effect of spermidine on polyphenylalanine synthesis with ribosomes obtained from various growth phases of E. coli.

Klett units at time of harvest	Unwashed ribosome content ($\frac{A_{260}\text{ units}}{g\text{ wet cells}}$)	Ions (mM) Mg^{2+}	Ions (mM) Spermidine	(^{14}C)Phe* incorporated (CPM)	% Stimulation by spermidine
100	488	15 7	- 3.5	8933 18391	205
200	484	15 7	- 3.5	8838 18070	204
300	373	14 7	- 3.5	8106 16085	198
380	288	14 7	- 3.5	7549 14497	192

Polyphenylalanine synthesis was carried out under standard conditions. Ions specified in the table were the optimal concentrations for polyphenylalanine synthesis.
*Phe; phenylalanine.

the ribosomes from different growth phases. These phenomena were clearer in the ribosomes from B. thuringiensis.

DISCUSSION

It is well known that Mg^{2+} are essential for maintenance of ribosome function. The function of Mg^{2+} is partially substituted for by polyamines (17-20). Although the ribosome content of E. coli harvested at the logarithmic phase of growth was about 1.7 times greater than that of E. coli harvested at the stationary phase of growth, the Mg^{2+} content did not vary markedly throughout the different growth phases. On the other hand, the total charge concentration of E. coli harvested at the logarithmic phase of growth was about 1.6 times that of E. coli harvested at the station-

Table 3. Effect of spermidine on polyphenylalanine synthesis with ribosomes obtained from various growth phases of B. thuringiensis.

Klett units at time of harvest	Unwashed ribosome content ($\frac{A_{260}\text{ units}}{g\text{ wet cells}}$)	Ions (mM) Mg^{2+}	Ions (mM) Spermidine	[^{14}C]Phe incorporated (CPM)	% Stimulation by spermidine
100	336	18	-	1910	553
		10	7.5	10560	
200	168	18	-	1274	533
		10	7.5	6788	
300	157	20	-	927	522
		12	7.5	4838	
380	143	20	-	873	526
		12	7.5	4562	

The conditions of the experiment were as indicated in Table 2.

ary phase of growth. If the ratio of polyamines to Mg^{2+} in ribosomes is equal to that in whole E. coli cells, these values are compatible with our hypothesis that Mg^{2+} are replaced by polyamines, especially by spermidine, during protein synthesis in vivo.

In the E. coli cell-free system, the percentage of spermidine-charge in total polycationic charge found in the ribosomes incubated under conditions which yield the greatest polyphenylalanine synthetic activity was 32% (1). On the other hand, those in whole E. coli cells harvested at the logarithmic phase (Klett units: 111) and the stationary phase (Klett units: 390) of growth (Table 1) were 25% and 9.6%, respectively. These results suggest that spermidine may increase the velocity of polypeptide chain elongation at the logarithmic phase. Recently it has been reported that polyamine starvation in a conditional polyamine auxotroph of E. coli decreases

the rate of protein synthesis by limiting the velocity of polypeptide chain elongation (21). This observation supports the hypothesis that polyamines are important during *in vivo* protein synthesis.

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REFERENCES

1. Igarashi, K., Sugawara, K., Izumi, I., Nagayama, C. and Hirose, S. (1974) Eur. J. Biochem., 48, 495-502.
2. Cohen, S. S. (1971) Introduction to polyamines, Prentice Hall, Englewood Cliffs, N. J.
3. Takeda, Y. (1969) J. Biochem. Tokyo, 66, 345-349.
4. Takeda, Y. (1969) Biochem. Biophys. Acta, 179, 232-234.
5. Takeda, Y. and Igarashi, K. (1970) J. Biochem. Tokyo, 68, 937-940.
6. Takeda, Y., Matsuzaki, K. and Igarashi, K. (1972) J. Bacteriol., 111, 1-6.
7. Igarashi, K., Hikami, K., Sugawara, K. and Hirose, S. (1973) Biochim. Biophys. Acta, 299, 325-330.
8. Changchien, L. M. and Aronson, J. N. (1970) J. Bacteriol., 103, 734-740.
9. Davis, B. D. and Mingioli, E. S. (1950) J. Bacteriol., 60, 17-28.
10. Hurwits, C., Braun, C. B. and Peabody, R. A. (1965) J. Bacteriol., 90, 1692-1695.
11. Nirenberg, M. W. and Matthaei, J. H. (1961) Proc. Natl. Acad. Sci. U. S., 47, 1588-1602.
12. Igarashi, K., Izumi, I., Hara, K. and Hirose, S. (1974) Chem. Pharm. Bull. Tokyo, 22, 451-454.
13. Dion, A. S. and Herbst, E. J. (1970) Ann. N. Y. Acad. Sci., 171, 723-734.
14. DeWitt, W. and Price, R. P. (1974) Biochem. Biophys. Res. Commun., 56, 593-598.
15. Ramagapal, S. and Subramanian, A. R. (1974) Proc. Natl. Acad. Sci. U. S., 71, 2136-2140.
16. Mangiarotti, G., Turco, E., Ponzetto, A. and Altrada, F. (1974) Nature New Biol., 247, 147-148.
17. Weiss, R. L. and Morris, D. R. (1970) Biochim. Biophys. Acta, 204, 502-511.
18. Weiss, R. L. and Morris, D. R. (1973) Biochemistry, 12, 436-441.
19. Kimes, B. W. and Morris, D. R. (1973) Biochemistry, 12, 442-449.
20. Igarashi, K., Sugawara, K. and Hirose, S. (1975) J. Biochem. Tokyo, in press.
21. Jorstad, C. M. and Morris, D. R. (1974) J. Bacteriol., 119, 857-860.