DEPENDENCY OF SPERMIDINE STIMULATION OF POLYPEPTIDE SYNTHESIS ON THE URACIL CONTENT OF MESSENGER RIBONUCLEIC ACID

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SUMMARY: From a study of the translation of synthetic polynucleotides in the E. coli and B. thuringiensis cell-free systems, it is shown that the stimulation of polypeptide synthesis by spermidine depends on the uracil content of messenger ribonucleic acid. This stimulation can not be fulfilled by any amount of Mg^{2+} in the absence of polyamines.

In various cell-free systems, it has been reported that polyamines have not only a sparing effect on the Mg^{2+} requirement for polyphenylalanine synthesis but also a stimulating effect, which can not be fulfilled by any amount of Mg^{2+} (1-5). In attempting to elucidate the characteristics of the increase of polypeptide synthesis in the presence of polyamines by determining whether or not polyamines are necessary for maximum polypeptide synthesis with various synthetic polynucleotides, we have found that stimulation of polypeptide synthesis by spermidine depends on the uracil content of messenger RNA.

MATERIALS AND METHODS

Materials - E. coli Q 13 and Bacillus thuringiensis Berliner were grown as described previously (5), and the cells were harvested at the middle logarithmic phase (Klett units : 200). Dialyzed ribosomes from E. coli Q 13 and B. thuringiensis and Sephadex G-50 treated S100 from E. coli Q 13 (S-S100) were prepared as described previously (4,5). E. coli polysomes were isolated by the method of Flessel et. al. (6) and dialyzed by the same method

used for ribosomes. Synthetic polynucleotides (poly (U), poly (C), poly (A), poly (G,U), poly (A,C), poly (G,U_2), and poly (C,U_2)) were obtained from Boehringer Mannheim GmbH. Poly (C,U) was purchased from Miles Laboratories. Spermidine trihydrochloride was from Sigma Chemical Co.

Procedures for polypeptide synthesis - The reaction mixture (0.1 ml) contained 100 mM Tris-HCl (pH 7.5), 1 mM dithiothreitol, 100 mM NH_4Cl, 1 mM ATP, 0.4 mM GTP, 4 mM phosphoenolpyruvate, 4 μg pyruvate kinase (Boehringer Mannheim GmbH), 10 μg of E. coli B tRNA (General Biochemicals), and E. coli S-S100 (80 μg protein). In addition, it contained various kinds of mRNA, dialyzed ribosomes, ^{14}C-labeled and non-labeled amino acids, and magnesium acetate and polyamines at the specified concentrations. Incubation was carried out at 30° for 30 min. A 0.08-ml aliquot of each reaction mixture was placed on a paper disc (25 mm diameter) and the hot trichloroacetic acid (5%, w/v) insoluble radioactivity was measured with a liquid scintillation spectrometer. The counting efficiency was 1.2×10^6 cpm/μCi.

The mRNA, amino acids, and ribosomes used in each system were as follows : (A) poly (U) system, 10 μg of poly (U), 0.1 μCi of [^{14}C]phenylalanine (382 μCi/μmole), and 1 A_{260} unit of E. coli or B. thuringiensis ribosomes; (B) poly (A) system, 20 μg of poly (A), 0.1 μCi of [^{14}C]lysine (216 μCi/μmole), and 2 A_{260} units of ribosomes; (C) poly (C) system, 30 μg of poly (C), 0.1 μCi of [^{14}C]proline (163 μCi/μmole), and 3 A_{260} units of ribosomes; (D) poly (C,U) system, 15 μg of poly (C,U) or poly (C,U_2), 0.1 μCi of [^{14}C]phenylalanine, 0.1 μCi of [^{14}C]proline, 0.1 μCi of [^{14}C] leucine (280 μCi/μmole), 50 μM serine, and 2 A_{260} units of ribosomes; (E) poly (G,U) system, 15 μg of poly (G,U) or poly (G,U_2), 0.1 μCi of [^{14}C]phenylalanine, 0.1 μCi of [^{14}C]leucine, 0.1 μCi of [^{14}C]valine (225 μCi/μmole), 0.1 μCi of [^{14}C]glycine (99.7 μCi/μmole), 50 μM cysteine, 50 μM tryptophan, and 2 A_{260} units of ribosomes; (F) poly (A,C) system, 15 μg of poly (A,C), 0.1 μCi of [^{14}C]lysine, 0.1 μCi of [^{14}C]proline, 0.1 μCi of [^{14}C]histidine (50 μCi/μmole), 0.1 μCi of [^{14}C]threonine (50 μCi/μmole), 50 μM glutamic acid, 50 μM aspartic acid, and 2 A_{260} units of ribosomes. The reason why only coded amino acids were added to the reaction mixture is that non-coded aminoacyl-tRNA inhibits polypeptide synthesis at the step of the aminoacyl-tRNA binding to ribosomes (7,8). The precipitating reagents employed in (C) and (B and F)

Table 1. Effect of spermidine on polypeptide synthesis with *E. coli* ribosomes.

mRNA	Ions (mM)		^{14}C-amino acids incorporated (CPM)	% Stimulation by spermidine
	Mg^{2+}	Spermidine		
	13	-	24,362	
Poly(U)	8.5	2	47,623	195
	7	4	50,742	208
	13	-	4,955	
Poly(A)	10	2	5,124	103
	8	4	5,276	106
	16	-	2,396	
Poly(C)	11	2	2,340	98
	8	4	1,958	82
	13	-	7,165	
Poly (C,U)	11	2	9,604	134
	7	3.5	9,902	138
	13	-	17,807	
Poly (C,U_2)	8	2	27,168	153
	7	3	29,027	163
	16	-	23,480	
Poly (G,U)	10	2.5	32,992	141
	8	4	30,468	130
	16	-	12,994	
Poly (G,U_2)	10	2	19,750	152
	8	4	21,990	169
	13	-	20,335	
Poly (A,G)	10	2	18,982	93
	8	3.5	17,390	86

Polypeptide synthesis was carried out under standard conditions. Mg^{2+} specified in the table was the optimal concentrations for polypeptide synthesis in the presence or absence of the specified concentrations of spermidine.

were 10% trichloroacetic acid and 0.25% sodium tungstate in 5% trichloroacetic acid (9), respectively.

RESULTS

Effect of spermidine on synthetic polynucleotide dependent polypeptide syntheses - Table 1 clearly shows that the stimulation of polypeptide synthesis by spermidine depended on the uracil content of mRNA in an *E. coli* cell-free system. In poly (U) dependent polyphenylalanine synthesis, spermidine increased the polypeptide synthesis up to twice that synthesized at an optimal

Mg^{2+} concentration (13 mM) without spermidine. On the contrary, poly (C) dependent polyproline synthesis was inhibited gradually with an increased concentration of spermidine. At 4 mM spermidine, the concentration necessary for maximum polyphenylalanine synthesis, 18% inhibition of polyproline synthesis was observed although the optimal Mg^{2+} concentration was shifted from 16 mM to 8 mM. In the poly (A) dependent polylysine synthesis, only a shift of the optimal Mg^{2+} concentration from 18 mM to 8 mM was observed in the presence of 4 mM spermidine. When a copolymer was used as mRNA, the polypeptide synthesis with mRNA containing uracil was stimulated by spermidine, but the percentage of stimulation by spermidine was lowered gradually with a decrease in the uracil content of mRNA.

A similar dependency of the stimulation of polypeptide synthesis by spermidime on the uracil content of mRNA was observed with B. thuringiensis ribosomes (Table 2). However, in the poly (A) dependent polylysine synthesis, spermidine had not only a sparing effect on the Mg^{2+} requirement but also a stimulating effect on the polypeptide synthesis. The same result was obtained with poly (I) dependent polyvaline synthesis (19 mM Mg^{2+}, 107 cpm; 12 mM Mg^{2+} plus 5 mM spermidine, 157 cpm). These results suggest that the activity of B. thuringiensis ribosomes may be influenced more by polyamines than that of E. coli ribosomes.

Effect of spermidine on polypeptide synthesis with E. coli polysomes - If 25% of the bases of mRNA are uracil, it would be expected from the results of the preceeding section that spermidine would yield a 15 to 25% stimulation of polypeptide synthesis in an E. coli polysome dependent polypeptide synthetic system. As shown in Fig. 1, a 10 to 20% stimulation of polypeptide synthesis by E. coli polysomes occurred in the presence of spermidine.

Table 2. Effect of spermidine on polypeptide synthesis with *B. thuringiensis* ribosomes.

mRNA	Ions (mM)		^{14}C-amino acids incorporated (CPM)	% Stimulation by spermidine
	Mg^{2+}	Spermidine		
	18	-	4,220	
Poly(U)	10	4	17,424	413
	8	8	21,636	512
	18	-	7,056	
Poly(A)	10	4	9,824	139
	8	6	10,399	147
	16	-	2,388	
Poly(C)	11	2	2,318	97
	8	4	1,901	80
	19	-	5,529	
Poly (C,U)	12	3	8,439	153
	10	4	9,023	163
	19	-	13,410	
Poly (C,U_2)	10	4	24,442	182
	8	6	24,918	186
	16	-	28,246	
Poly (G,U)	12	2	43,202	153
	10	3,5	46,925	166
	18	-	15,207	
Poly (G,U_2)	10	4	28,293	186
	8	6	29,861	196
	16	-	38,165	
Poly (A,C)	10	2	40,104	105
	8	4	39,210	103

Polypeptide synthesis was carried out as described in the legend of Table 1.

DISCUSSION

The data presented show that the stimulation of polypeptide synthesis by spermidine is due to the existence of uracil in mRNA. The results suggest that polyamines not only change the configuration of ribosomes (4) but also interact with mRNA. Actually, the binding of spermidine to poly (U) has been observed by gel filtration (unpublished results), although it has been reported that spermidine or spermine binds preferentially to double stranded RNA (10,11).

In a previous communication (5), we have suggested that spermi-

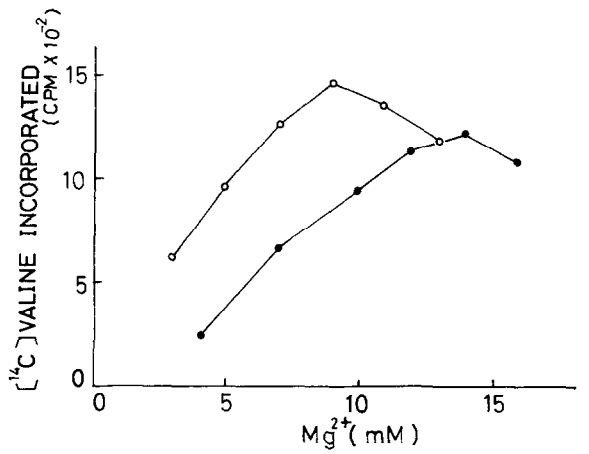

Fig. 1. Effect of spermidine on polypeptide synthesis with E. coli polysomes. Polypeptide synthesis was carried out under standard conditions except that the reaction mixture contained 2 A_{260} units of E. coli polysomes, 0.1 μCi of [^{14}C] valine (225 μCi/μmole) and 40 μM (each) of 19 nonradioactive amino acids instead of ribosomes, mRNA, and coded amino acids. •——•, no spermidine; o——o, 2 mM spermidine.

dine may increase the velocity of polypeptide chain elongation at the logarithmic phase. The increase of the velocity of polypeptide chain elongation by spermidine may be 15 to 20% in E. coli if uracil occupies 25% of the bases of mRNA. However, this value may increase because the substrate specificity of RNases is changed by polyamines. E. coli RNases stimulate the production of cytosine nucleotides in the presence of polyamines, but the production of uracil nucleotides is inhibited slightly by polyamines when this homopolymer is used as substrate (manuscript in preparation). Therefore, there is the possibility that the bases of mRNA in E. coli at the logarithmic phase may contain more than 25% uracil.

It would be of interest to learn how much stimulation of polypeptide synthesis by spermidine can be obtained in the B. thuringiensis polysome dependent polypeptide synthetic system bacause the activity of B. thuringiensis ribosomes may be influenced more by polyamines than that of E. coli ribosomes. However, attempts to

obtain B. thuringiensis polysomes thus far have been unsuccessful.

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