RESPONSIBILITY OF 16S RNA FOR THE STIMULATION OF POLYPEPTIDE SYNTHESIS BY SPERMIDINE

Kazuei Igarashi, Yasuhiro Watanabe, Etsuko Matsumoto, Atsuko Kogo, Masumi Yabuki, and Seiyu Hirose

Faculty of Pharmaceutical Sciences, Chiba University, Yayoi-cho, Chiba, Japan

Received March 10,1977

SUMMARY: From the studies on the spermidine stimulation of polyphenylalanine synthesis catalyzed by E. coli 50S and reconstituted 30S particles containing 16S RNA and 30S ribosomal proteins from E. coli and B. thuringiensis in different kinds of combinations, it is concluded that 16S RNA is mainly responsible for the stimulation of polypeptide synthesis by spermidine.

In various cell-free systems, it has been reported that polyamines have not only a sparing effect on the Mg^{2+} requirement for polypeptide synthesis but also a stimulating effect, which can not be fulfilled by any amount of Mg^{2+} (1-8). Polyphenylalanine synthesis with E. coli ribosomes was stimulated about 2-fold by SPD, while that with B. thur. ribosomes was stimulated about 5-fold by SPD (9). From the results of experiments determining polyphenylalanine synthesis catalyzed by reconstituted systems containing different combinations of 30S and 50S ribosomal subunits from E. coli and B. thur., it was concluded that 30S ribosomal subunits are responsible for the stimulation of polypeptide synthesis by SPD (10). In this communication, we present data showing that 16S RNA is mainly responsible for the stimulation of polypeptide synthesis by SPD.

MATERIALS AND METHODS

Materials - Dialyzed ribosomes from E. coli Q13 and B. thur.

Abbreviations: SPD, spermidine; B. thur., Bacillus thuringiensis.

ISSN 0006-291X

and Sephadex G-50 treated S100 from E. coli Q13 (S-S100) were prepared as described previously (3,9). The preparation of ribosomal subunits (30S and 50S) was carried out according to the procedure of Igarashi and Kaji (11) using Hitachi RPZ48T zonal rotor. The subunits were pelleted by centrifugation at 150,000g for 20 hr. The pellets were resuspended in Buffer I (10 mM Tris-HCl, pH 7.5, 30 mM NH_4Cl, 10 mM magnesium acetate, and 6 mM 2-mercaptoethanol) and stored at -70° until used. E. coli and B. thur. 16S RNA was prepared from 70S ribosomes by the method of Nomura et al. (12), except that macaloid was used instead of bentonite. The total 30S proteins of E. coli and B. thur. were prepared from 30S ribosomal subunits by the method of Nomura et al. (12). E. coli S1 protein and S1-depleted ribosomes were prepared according to the procedure of Tal et al. (13). One major and two minor bands were observed by polyacrylamide disc gel electrophoresis in our S1 preparation. Immobilized enzymes (Sepharose 4B-RNase A and Sepharose 4B-chymotrypsin) were prepared as described previously (14). About 1.0 mg of each enzyme was immobilized by 1.0 ml of Sepharose 4B.

Reconstitution of "30S" particles and procedures for polyphenylalanine synthesis - The reconstitution of 30S particles was performed according to the procedure of Traub et al. (15) with the following modifications: 1) the RNA solution (30 A_{260} units) was preheated at 42° for 7 min; 2) the mixture was incubated at 42° for 20 min; and 3) the mixture contained 14 mM magnesium acetate and 6 mM SPD instead of 20 mM magnesium acetate when 16S RNA of B. thur. was used for the reconstitution of 30S particles. Polyphenylalanine synthesis was carried out as described previously (3) except that ribosomes or ribosomal subunits were used as specified in the tables.

RESULTS

Determination of the component of 30S ribosomal subunits responsible for the SPD stimulation of polyphenylalanine synthesis - The effect of SPD on polyphenylalanine synthesis was studied by using E. coli 50S and 30S particles reconstituted in various combinations from 16S RNA and total 30S proteins prepared from E. coli and B. thur. ribosomes. As shown in Table 1, polyphenylalanine synthesis was stimulated about 2-fold by SPD with 30S particles reconstituted from E. coli 16S RNA and either E. coli or B. thur. proteins. In contrast, if the 16S RNA of the reconstituted particles were of B. thur. orgin, the polyphenylalanine synthesis was stimulated about 3.4-fold and 3.9-fold, when the proteins were of E. coli and B. thur. orgin, respectively. In all of these combinations, the 16S RNA appears to be the controlling factor for the stimulation. Thus, these results suggest that 16S RNA is mainly responsi-

Table 1. Activity of reconstituted 30S particles containing different combinations of 16S RNA and 30S proteins from *E. coli* and *B. thuringiensis*

Expt. No.	Source of 30S particles		Ions (mM)		[^{14}C]Phenyl-alanine incorporated (cpm)	Stimulation by SPD (-fold)
	16S RNA	Proteins	Mg^{2+}	SPD		
1	E[a]	E	13	-	2992	2.10
			6	4	6270	
	E	B[b]	15	-	1329	2.16
			8	4	2866	
	B	E	18	-	2009	3.41
			8	6	6843	
	B	B	18	-	2050	4.05
			9	7	8309	
2	E	E	12	-	2915	1.77
			8	3	5159	
	E	B	12	-	681	1.84
			8	3	1253	
	B	E	14	-	1515	3.35
			8	6	5076	
	B	B	16	-	1063	3.86
			8	6	4104	

a: *E. coli*, b: *B. thuringiensis*.

The reaction mixture (0.1 ml) for polyphenylalanine synthesis contained 0.25 A_{260} unit of reconstituted 30S particles and 0.4 A_{260} unit of *E. coli* 50S subunits. Ions specified in the table were at the optimal concentrations for polyphenylalanine synthesis.

ble for the stimulation of polyphenylalanine synthesis by SPD. In addition, SPD increased the yield of reconstituted 30S particles containing *B. thur.* 16S RNA (Table 2). The 30S particles reconstituted in the presence or absence of SPD showed almost equal specific activity for polyphenylalanine synthesis (Table 2) and each preparation sedimented at about 30S (data not shown). SPD did not have any effect on the yield of reconstituted 30S particles containing *E. coli* 16S RNA.

Table 2. Effect of spermidine on the reconstitution of 30S particles containing B. thuringiensis 16S RNA

Ionic conditions during reconstitution (mM)			Yield of 30S particles (A_{260} unit)		Activity of 30S particles reconstituted from B. thur. 16S RNA and 30S proteins of B. thur.		
			Source of 30S proteins		Ions (mM)		[^{14}C]Phenylalanine incorporated (cpm)
K^+	Mg^{2+}	SPD	E. coli	B. thur.	Mg^{2+}	SPD	
330	20	-	12.8	14.4	18	-	1207
					9	6	5590
330	17	3	15.6	16.7	18	-	1342
					9	6	5896
330	14	6	19.0	20.4	18	-	1282
					9	6	5729

The reaction mixture (8.5 ml), containing 30 A_{260} units of preheated B. thur. 16S RNA and 40 equivalents of E. coli or B. thur. total 30S ribosomal proteins, was incubated for 20 min at 42° under different ionic conditions as specified in the table. The reaction mixture was cooled to 4° and concentrated by centrifugation at 50,000 rpm for 3.5 hr in a Hitachi fixed-angle rotor 65. The pellet was dissolved in 0.25 ml of Buffer II (10 mM Tris-HCl, pH 7.5, 30 mM NH_4Cl, 0.3 mM magnesium acetate, and 6 mM 2-mercaptoethanol), and the solution was centrifuged for 10 min at 30,000 g. The Mg^{2+} concentration of the 30S particles solution was adjusted to 10 mM, and absorbance at 260 nm was measured. Polyphenylalanine synthesis was carried out as described in the legend of Table 1.

Effect of RNase A and chymotrypsin on the SPD stimulation of polyphenylalanine synthesis - In Experiment 1 in Table 3, RNase A or chymotrypsin was added to the reaction mixture. Although the content of poly(U) in the reaction mixture was decreased by the addition of RNase A, the content of poly(U) in the presence or absence of SPD did not change significantly in these experiments (data not shown). In Experiment 2, 30S subunits were pretreated with immobilized enzymes and then polyphenylalanine synthesis was performed in the presence of 50S subunits. In both experiments, the stimulation of polyphenylalanine synthesis by SPD was decreased

Table 3. Effect of RNase A and α-chymotrypsin on the stimulation of polyphenylalanine synthesis by spermidine

Expt. No.	RNase A or α-chymotrypsin		Ions (mM) Mg^{2+}	Ions (mM) SPD	[^{14}C]Phenylalanine incorporated (cpm)	Stimulation by SPD (-fold)
1	-		14	-	9516	2.15
			7	4	20488	
	RNase[a]	0.01 ng	14	-	4768	1.86
			7	4	7552	
		0.02 ng	14	-	1648	1.69
			7	4	2784	
	Chymo.[a]	0.5 ng	14	-	4112	2.07
			7	4	8510	
		1 μg	14	-	2820	2.06
			7	4	5796	
2	-		14	-	7507	2.06
			7	3	15429	
	RNase[b]	10 μl	14	-	3824	1.81
			7	3	6908	
		30 μl	14	-	1912	1.42
			7	3	2716	
	Chymo.[b]	20 μl	14	-	3186	1.97
			7	3	6266	
		60 μl	14	-	1775	1.91
			7	3	3397	

a: Free enzyme, b: Immobilized enzyme.

In Experiment 1, the reaction mixture (0.1 ml) for polyphenylalanine synthesis contained 0.75 A_{260} unit of E. coli ribosomes and RNase A (Sigma, Type 1-A) or α-chymotrypsin (Sigma, Type II) as specified in the table. In Experiment 2, E. coli 30S subunits (12 A_{260} units in 0.45 ml of Buffer I) were incubated at 30° for 10 min with immobilized enzymes as specified in the table. The immobilized enzymes were removed by centrifugation for 10 min at 20,000 g, and the enzyme-treated 30S subunits were used for the assay of polyphenylalanine synthesis after the dialysis against Buffer I for 2 hr. The reaction mixture (0.1 ml) contained 0.3 A_{260} unit of the enzyme treated 30S subunits and 0.5 A_{260} unit of E. coli 50S subunits. Ions specified in the table were at the optimal concentrations for polyphenylalanine synthesis.

by RNase A but not by chymotrypsin. These results again indicate that 16S RNA is mainly responsible for the stimulation of polyphe-

Table 4. Effect of *E. coli* S1 protein on the stimulation of polyphenylalanine synthesis by spermidine

Ribosomes	Ions (mM)		[^{14}C]Phenyl-alanine incorporated (cpm)	Stimulation by SPD (-fold)
	Mg^{2+}	SPD		
E. coli S1-depleted ribosomes	13	-	3211	1.87
	7	-	6005	
E. coli S1-depleted ribo. +S1	13	-	10076	1.93
	7	4	19470	
E. coli ribosomes	13	-	9218	1.95
	7	4	17942	
E. coli ribosomes +S1	13	-	12575	1.93
	7	4	24232	
B. thur. ribosomes	19	-	1330	6.02
	7	8	8004	
B. thur. ribosomes +S1	20	-	1966	5.83
	7	8	11466	

The reaction mixture (0.1 ml) for polyphenylalanine synthesis contained 0.75 A_{260} unit of the kind of ribosomes specified in the table. Where indicated, 3.2 μg of S1 protein were added to the reaction mixture. Ions specified in the table were at the optimal concentrations for polyphenylalanine synthesis.

nylalanine synthesis by SPD.

Effect of E. coli S1 protein on the SPD stimulation of polyphenylalanine synthesis - It has been reported that 30S subunits from *Bacillus* genus lacks a protein equivalent to *E. coli* S1 protein (16,17). As stated before, the degree of SPD stimulation of polyphenylalanine synthesis with 30S subunits reconstituted from *B. thur.* 16S RNA and *B. thur.* proteins was greater than the stimulation of 30S particles reconstituted from *B. thur.* 16S RNA and *E. coli* proteins. Therefore, we examined the possibility that S1 protein decreases the stimulatory effect of polyphenylalanine synthesis by SPD. As shown in Table 4, S1 proein did not alter the SPD stimulation of polyphenylalanine synthesis with *E. coli* S1-depleted

ribosomes or B. thur. ribosomes. However, the S1 protein did increase the polyphenylalanine synthetic activity of each kind of ribosomal preparation. These results suggest that S1 protein is not concerned with the SPD stimulation of polypeptide synthesis.

DISCUSSION

The data presented show that 16S RNA is mainly responsible for the stimulation of polyphenylalanine synthesis by SPD. However, we can not completely deny the possible involvement of 30S proteins in the SPD stimulation of polyphenylalanine synthesis, since the degree of SPD stimulation of polyphenylalanine synthesis with 30S subunits reconstituted from B. thur. 16S RNA and B. thur. proteins was greater than that with 30S particles reconstituted from B. thur. 16S RNA and E. coli proteins. The low activity of 30S particles reconstituted from E. coli 16S RNA and B. thur. proteins compares with the very low activity reported earlier for 30S particles reconstituted from E. coli 16S RNA and B. stearothemophilus proteins (12). If one could obtain more active 30S particles reconstituted from E. coli 16S RNA and B. thur. proteins, it might be elucidated as to whether or not 30S proteins are also responsible for the stimulation of polyphenylalanine synthesis by SPD. In this respect, it is of interest to mention that the inability to translate the coat cistron of RNA phage R17 protein with B. stearothermophilus 30S subunits has been attributed to both 16S RNA and S12 protein (18). However, contradictory results have been reported (19,20).

It is also of interest that the yield of reconstituted 30S particles containing B. thur. 16S RNA was increased if the reconstitution process was conducted in the presence of SPD. The effect of SPD became greater when the temperature of the incubation for reconstitution was decreased from 42° to 40°. There is a report that SPD may facilitate the recontitution of E. coli 50S ribosomal

subunits (21). Therefore, polyamines may play a role not only in protein synthesis but also in the biosynthesis of ribosomes in some kinds of bacteria.

ACKNOWLEDGEMENT

The authors would like to express their thanks to Dr. B. K. Joyce of Colorado State University for her help in preparing this manuscript. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

REFERENCES

1. Bretthauer, R. K., Marcus, L., Chaloupka, J., Halvorson, H. O. and Bock, R. M. (1963) Biochemistry, 2, 1074-1084.
2. Deutscher, M. P., Chambon, P. and Kornberg, A. (1968) J. Biol. Chem. 243, 5117-5125.
3. Igarashi, K., Sugawara, K., Izumi, I., Nagayama, C. and Hirose, S. (1974) Eur, J. Biochem. 48, 495-502.
4. Igarashi, K., Watanabe, Y. and Hirose, S. (1975) Biochem. Biophys. Res. Commun. 67, 407-413.
5. Konecki, D., Kramer, G., Pinphanichakarn, P. and Hardesty, B. (1975) Arch. Biochem. Biophys. 169, 192-198.
6. Atkins, J. F., Lewis, J. B., Amderson, C. W. and Gesteland, R. F. (1975) J. Biol. Chem. 250, 5688-5695.
7. Fleischer-Lambropoulos, H., Sarkander, H. I. and Brode, W. P. (1975) Biochem. Biophys. Res. Commun. 63, 792-800.
8. Salden, M. and Bloemendal, H. (1976) Biochem. Biophys. Res. Commun. 68, 157-161.
9. Igarashi, K., Hara, K., Watanabe, Y., Hirose, S., and Takeda, Y. (1975) Biochem. Biophys. Res. Commun. 64, 897-904.
10. Igarashi, K., Yabuki, M., Yoshioka, Y., Eguchi, K. and Hirose, S. (1977) Biochem. Biophys. Res. Commun. in press.
11. Igarashi, K. and Kaji, A. (1969) Proc. Natl. Acad. Sci. U. S. 62, 498-505.
12. Nomura, M., Traub, P. and Bechmann, M. (1968) Nature, 219, 793-799.
13. Tal, M., Aviram, M., Kanarek, A. and Weiss, A. (1972) Biochim. Biophys. Acta, 281, 381-392.
14. Igarashi, K., Terada, K., Tango, Y., Katakura, K. and Hirose, S. (1975) J. Biochem. (Tokyo) 77, 383-390.
15. Traub, P., Mizushima, S., Lowry, C. V. and Nomura, M. (1971) Method in Enzymology, 20, 391-407.
16. Sun, T., Bickle, T. A. and Traut, R. R. (1972) J. Bacteriol. 111, 474-480.
17. Isono, K. and Isono, S. (1976) Proc. Natl. Acad. Sci. U. S. 73, 767-770.
18. Held, W. A., Gette, W. R. and Nomura, M. (1974) Biochemistry, 13, 2115-2122.
19. Goldberg, M. L. and Steitz, J. A. (1974) Biochemistry, 13, 2123-2129.
20. Isono, S. and Isono, K. (1975) Eur. J. Biochem. 56, 15-22.
21. Hosokawa, K., Kiho, Y., Migita, L. K. (1973) J. Biol. Chem. 248, 4135-4143.