POLYAMINES AND PROTEIN SYNTHESIS. IV. STIMULATION OF AMINOACYL TRANSFER RNA FORMATION BY POLYAMINES

Yoshifumi Takeda and Kazuei Igarashi

Department of Bacteriology and Serology, Research Institute for Microbial Diseases, Osaka University, Yamada-kami, Suita, Osaka, Japan

Received October 7, 1969

SUMMARY

From a study of the effect of polyamines on aminoacyl-tRNA formation it is shown that spermine, spermidine and putrescine stimulate aminoacyl-tRNA formation. The stimulation occurs without adding magnesium ions in the reaction mixture. Spermine is the most effective polyamine among those tested for this stimulation.

The stimulation of amino acid incorporation into polypeptides by polyamines has been studied in bacteria (1,2), yeast (3), animal tissue (4) and plant tissue (5). We have reported the stimulatory effect of polyamines on polypeptide synthesis in E. coli and have proposed that magnesium ions are usually replaced by polyamines, especially by spermine, during protein synthesis in vivo (6,7).

To elucidate further the role of polyamines in protein synthesis, it is necessary to investigate in detail the effect of polyamines on various steps of protein synthesis. Cohen and Lichtenstein (8) have reported the aggregation of ribosomal subunits by polyamines and this was confirmed by other workers both in vivo (9) and in vitro (10). Stimulation of the specific binding of aminoacyl-tRNA to ribosomes by polyamines also has been reported (11,12).

In this communication, the effect of polyamines on aminoacyl-tRNA formation, the first step of protein synthesis, in E. coli is studied and evi-

dence is presented that polyamines stimulate aminoacyl-tRNA formation both in the absence and presence of magnesium ions.

MATERIALS AND METHODS

Preparation of aminoacyl-tRNA synthetase --- Aminoacyl-tRNA synthetase was prepared according to the method described by Kaji et al. (13) with a slight modification. Cell extracts of E. coli B were prepared as described previously (6) and the supernatant fluid obtained after centrifugation at 105,000 x g for 2 hours was used as the starting material. To this supernatant fluid, dihydrostreptomycin (100 mg/ml) and protamine sulfate (10 mg/ml) were added at final concentrations of 0.5% and 0.05%, respectively. This preparation was centrifuged for 20 min at 10,000 x g and to the supernatant fluid sufficient saturated ammonium sulfate solution (pH 7.0) was added to obtain 46% saturation with ammonium sulfate. The precipitate was collected by centrifugation and dissolved in a buffer containing 0.01 M Tris-HCl pH 7.8, 0.06 M NH_4Cl, 0.01 M magnesium acetate and 0.006 M β-mercaptoethanol. The protein concentration was adjusted to 8 mg per ml and the preparation was stored in small portions at -20°C. Just before use, dialysis against about 1,000 volumes of a buffer containing 0.01 M Tris-HCl pH 7.8, 0.06 M NH_4Cl and 0.006 M β-mercaptoethanol was carried out to obtain aminoacyl-tRNA synthetase free of magnesium ions.

Standard assay procedure for aminoacyl-tRNA formation --- The standard reaction mixture (0.1 ml) for the aminoacylation of tRNA contained the following: 0.01 M Tris-HCl pH 7.8, 0.05 M NH_4Cl, 0.006 M β-mercaptoethanol, 0.0025 M ATP, 200 μg of E. coli B tRNA (General Biochemicals) and 5 μl of enzyme solution. Radioactive amino acids (New England Nuclear Co. and Daiichi Kagaku Co.), magnesium acetate, spermine tetrahydrochloride, spermidine trihydrochloride, and putrescine dihydrochloride were added at the concentration as indicated in the figures and tables. Although, in some experiments, 0.01 M

phosphoenol pyruvate, and 10 µg of pyruvate kinase were added, little stimulation of aminoacyl-tRNA formation was observed. Specific activities of ^{14}C-amino acids used in the experiments were as follows: ^{14}C-phenylalanine, 42.8 µc per µmole; ^{14}C-lysine, 41.4 µc per µmole; ^{14}C-leucine, 41.7 µc per µmole; and ^{14}C-isoleucine, 40.5 µc per µmole.

After the reaction mixture was incubated at 22°C for 20 minutes, 0.07 ml aliquot of each reaction mixture was placed on a paper disc (25 mm diameter) and cold trichloroacetic acid insoluble radioactivity was counted by a Beckman liquid scintillation spectrometer. The counting efficiency was 1.5×10^6 cpm per µc.

RESULTS

Effect of spermine and magnesium acetate on phenylalanyl-tRNA formation --- The fact that magnesium ions are essential for the aminoacyl tRNA formation is now well established (14,15). This was confirmed in the phenylalanyl-tRNA formation we studied as shown in Fig. 1. Under our experimental condition, 4 mM magnesium ions are sufficient to obtain maximum aminoacylation of tRNA with ^{14}C-phenylalanine, while in the presence of 1 mM magnesium ions, ^{14}C-phenylalanyl-tRNA formation was about 27% of that of maximum value.

The experimental results in Fig. 2 indicate that spermine stimulates the phenylalanyl-tRNA formation both in the absence and presence of magnesium ions. Under the condition where no magnesium acetate was added in the reaction mixture, spermine stimulated the phenylalanyl-tRNA formation, 46.1 µµmoles of ^{14}C-phenylalanyl-tRNA being formed with the addition of 2 mM spermine. The amount of ^{14}C-phenylalanyl-tRNA formed in the presence of spermine varied with the different enzyme preparations and in some experiments it was more than 70.2 µµmoles. In the presence of 1 mM magnesium ions, 83.2 µµmoles of ^{14}C-phenylalanyl-tRNA were formed with the addition of 2 mM spermine. When the concentration of magnesium ions in the reaction mixture was 2 mM, 81.5 µµmoles of ^{14}C-phenylalanyl-tRNA were formed in the presence of 0.4 mM spermine.

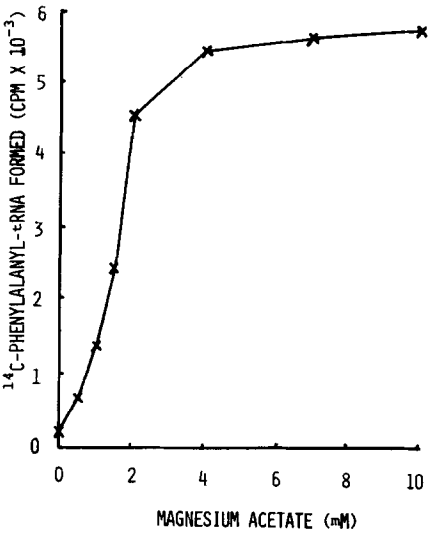

Fig. 1. Effect of magnesium ions on phenylalanyl-tRNA formation. The components of the reaction mixture and the assay procedure were described in the text. ^{14}C-phenylalanine added to each reaction mixture was 0.25 μc (5.85 mμmoles). Each value was the average of duplicate determinations. Radioactivity (647 cpm) adsorbed to a paper disc of a sample without enzyme was subtracted from each point.

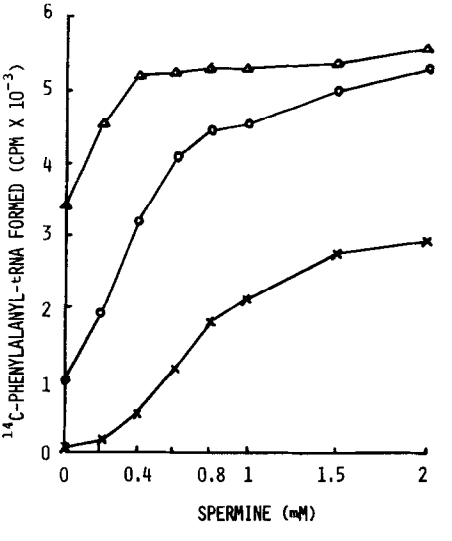

Fig. 2. Effect of spermine and magnesium acetate on phenylalanyl-tRNA formation. The conditions of the experiment are as described in the legend of Fig. 1. Control radioactivity subtracted from each point was 774 cpm.
x — x, without magnesium acetate; o — o, with 1 mM magnesium acetate;
Δ — Δ, with 2 mM magnesium acetate.

The amount of ^{14}C-phenylalanyl-tRNA formed in the presence of 10 mM magnesium acetate was 86.8 μμmoles, indicating that either 1 mM magnesium acetate plus 2 mM spermine or 2 mM magnesium acetate plus 0.4 mM spermine has the same effect on phenylalanyl-tRNA formation as 10 mM magnesium acetate. In the absence of magnesium ions, a maximum aminoacylation of tRNA with ^{14}C-phenylalanine was not observed even with the addition of 2 mM spermine. An experiment with a higher concentration of spermine was not possible because tRNA was sedimented with the addition of a higher concentration of spermine.

Effect of spermidine and putrescine on phenylalanyl-tRNA formation in the absence and presence of magnesium ions --- The stimulation of phenylalanyl-tRNA formation by other polyamines, spermidine and putrescine, was studied and the results were shown in Fig. 3 and Table 1. As was shown in Fig. 3, spermidine stimulated phenylalanyl-tRNA formation both in the absence and presence of magnesium ions. The degree of stimulation by spermidine in the absence of magnesium ions was not as striking as that by spermine, yet, in the presence of either 1 mM or 2 mM magnesium ions, more than 80% of a maximum aminoacylation of tRNA with ^{14}C-phenylalanine was observed. In the presence

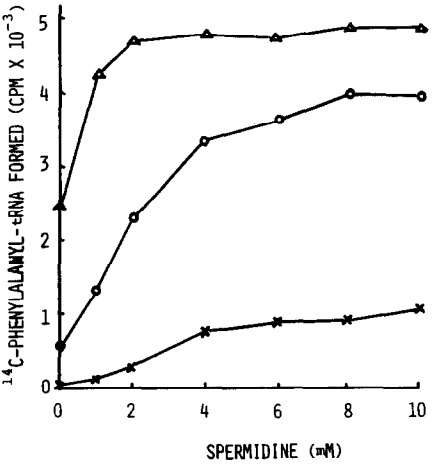

Fig. 3. Effect of spermidine and magnesium acetate on phenylalanyl-tRNA formation. The conditions of the experiment are as described in the legend of Fig. 1. Control radioactivity subtracted from each point was 845 cpm. x — x, without magnesium acetate; o — o, with 1 mM magnesium acetate; Δ — Δ, with 2 mM magnesium acetate.

Table 1. Effect of putrescine and magnesium acetate on phenylalanyl-tRNA formation

Putrescine (mM)	Magnesium acetate (mM)			
	0	1	2	10
0	109	1097	3724	4736
10	1464	3745	4655	-
20	1086	3556	4864	-

The conditions of the experiment are as described in the legend of Fig. 1. Control radioactivity subtracted from each point was 203 cpm. The symbol (-) indicates the experiment was not done in this case.

of 10 mM magnesium acetate the amount of ^{14}C-phenylalanyl-tRNA formed was 78.0 µµmoles, and almost the same aminoacylation was observed with the addition of both 2 mM magnesium ions and 2 mM spermidine. The concentration of spermidine necessary to obtain a maximum ^{14}C-phenylalanyl-tRNA formation in the presence of 2 mM magnesium acetate was about 5 times as much as that of spermine.

Putrecine also stimulated phenylalanyl-tRNA formation as shown in Table 1. In the presence of 10 mM putrescine and 2 mM magnesium acetate an almost maximum ^{14}C-phenylalanyl-tRNA formation was observed.

Effect of spermine and magnesium acetate on the aminoacylation of tRNA with various amino acids —— It was found that spermine stimulated phenylalanyl-tRNA formation both in the absence and presence of magnesium acetate (Fig. 2). Experimental data shown in Table 2 indicate that the stimulation by spermine was also observed in the aminoacylation of tRNA with several other amino acids studied. We studied lysyl-, leucyl-, and isoleucyl-tRNA formation and all of them were stimulated by spermine in the absence and presence of magnesium acetate. However, the degree of stimulation of the aminoacylation was different for each amino acid.

Table 2. Effect of spermine and magnesium acetate on the aminoacylation of tRNA with various amino acids

Concentration of ions (mM)		amino acids used for aminoacylation			
Mg^{2+}	spermine	phe	lys	leu	isoleu
0	0	71	268	3118	402
0	2	2957	450	5734	1536
1	0	966	783	3411	726
1	2	5340	2289	5954	1801
2	0	3467	2603	3029	1209
2	2	5621	4357	5824	1745
10	0	5562	8249	3094	1732
10	2	5041	6807	4535	1612

The components of the reaction mixture and the assay procedure were described in the text. The amounts of radioactive amino acids added to each reaction mixture were as follows; ^{14}C-phenylalanine 0.25 µc (5.85 mµmoles), ^{14}C-lysine 0.25 µc (6.05 mµmoles), ^{14}C-leucine 0.25 µc (6.0 mµmoles) and ^{14}C-isoleucine 0.25 µc (6.70 mµmoles). Each value was the average of duplicate determinations. Radioactivities adsorbed to paper disc of samples without enzyme were subtracted from each value; they were 774 cpm for ^{14}C-phenylalanine, 557 cpm for ^{14}C-lysine, 235 cpm for ^{14}C-leucine and 366 cpm for ^{14}C-isoleucine.

DISCUSSION

It has been reported that the aminoacylation of tRNA, the first step of protein synthesis, requires magnesium ions (14,15). Experimental results presented in this communication show that polyamines such as spermine, spermidine and putrescine can replace magnesium ions in this reaction.

Previously we have studied the effect of polyamines on amino acid incorporation into polypeptides by cell free extracts of E. coli and have proposed the hypothesis that replacement of magnesium ions by polyamines in vivo, especially by spermine, usually can occur in protein synthesis (6). This hypothesis is supported further by experimental data reported from this laboratory earlier (7,11) and by the present data.

The mechanism of the stimulatory effect of polyamines on aminoacylation of tRNA is not studied in this communication. Requirement of magnesium ions for aminoacylation has been explained that magnesium ions interact with aminoacyl-tRNA synthetase (16,17). It is possible that polyamines affect aminoacyl-tRNA synthetase itself by activating the enzyme. Recent findings from our laboratory (Igarashi and Takeda, manuscript in preparation) indicate another possibility. We have found that spermine can bind to tRNA in the absence of magnesium ions and that the bound spermine is effective on aminoacyl-tRNA formation. This suggests that the primary function of the polyamines is to bind to tRNA and through this binding polyamines stimulate aminoacyl-tRNA formation. The detailed results will be published elsewhere.

ACKNOWLEDGEMENT

The authors would like to express their thanks to Drs. T. Fujino and T. Miwatani for their interest and encouragement during the course of this study. Thanks are also due to Dr. B. K. Joyce for her help in preparing this manuscript.

REFERENCES

1. D. Nathans and F. Lipmann, Proc. Natl. Acad. Sci. U. S., 47, 497 (1961)
2. R. G. Martin and B. N. Ames, Proc. Natl. Acad. Sci. U. S., 48, 2171 (1962)
3. R. K. Bretthauer, L. Marcus, J. Chaloupka, H. O. Halvorson and R. M. Bock, Biochemistry, 2, 1079 (1963)
4. A. Hershko, S. Amoz and J. Mager, Biochem. Biophys. Res. Commun., 5, 46 (1961)
5. S. Coucci and N. Bagni, Life Sciences, 7, 113 (1968)
6. Y. Takeda, J. Biochem. Tokyo, 66, 345 (1969)
7. Y. Takeda, Biochim. Biophys. Acta, 179, 232 (1969)
8. S. S. Cohen and J. Lichtenstein, J. Biol. Chem., 235, 2112 (1960)
9. J. L. Colbourn, B. H. Witherspoon and E. J. Herbst, Biochim. Biophys. Acta, 49, 422 (1961)
10. N. Silman, M. Artman and H. Engelberg, Biochim. Biophys. Acta, 103, 231 (1965)
11. Y. Takeda, Biochim. Biophys. Acta, 182, 258 (1969)
12. M. J. A. Tanner, Biochemistry, 6, 2686 (1967)
13. A. Kaji, H. Kaji and G. D. Novelli, J. Biol. Chem., 240, 1185 (1965)
14. M. P. Deutscher, J. Biol. Chem., 242, 1123 (1967)
15. D. I. Hirsh, J. Biol. Chem., 243, 5731 (1968)
16. R. B. Loftfield and E. A. Eigner, J. Biol. Chem., 242, 5355 (1967)
17. R. B. Loftfield and E. A. Eigner, J. Biol. Chem., 244, 1746 (1969)