Vol. 91, No. 2, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 28, 1979 Pages 440-448

COMPARATIVE STUDIES ON THE INCREASE BY POLYAMINES OF FIDELITY OF PROTEIN SYNTHESIS IN ESCHERICHIA COLI AND WHEAT GERM CELL-FREE SYSTEMS

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Received October 2, 1979

SUMMARY: In the presence of polyamines, the fidelity of protein synthesis in a wheat germ cell-free system was increased significantly, while it was increased slightly in an E. coli cell-free system. The effective concentration of polyamines for the increase in fidelity of protein synthesis was nearly equal to that for the stimulation of protein synthesis in a wheat germ cell-free system.

A number of studies have led to the conclusion that polyamines participate in a wide variety of growth processes (1,2). As for the influence of polyamines on protein synthesis, there is now considerable evidence that polyamines can enhance protein synthesis in prokaryotic and eukaryotic cell-free systems (3-9). In addition, we have recently reported that the defect in the amount of some kinds of 30S subunit split proteins was responsible for the decrease of polypeptide synthesis in a polyamine-requiring mutant of Escherichia coli grown in the absence of polyamines (10).

One of the main molecular mechanisms that might contribute to aging is a progressive loss of fidelity in information transfer between macromolecules, especially in protein synthesis (11,12). Since the polyamine concentration decreases gradually with aging (1), we have investigated whether polyamines increase the fidelity of protein synthesis and have found that the fidelity of protein synthesis is increased in the presence of polyamines in a wheat

0006-291X/79/220440-09$01.00/0

germ cell-free system, and to a lesser degree in an E. coli cell-free system. Recently, Abraham et al. (13) have also reported that the fidelity of protein synthesis is increased in the presence of spermidine.

MATERIALS AND METHODS

Materials - The incubated 30,000 x g supernatant (IS-30) of E. coli Q13 was prepared according to the method of Nirenberg and Matthaei (14), and dialyzed for 20 hr at 4° C against a buffer containing 10 mM Tris-HCl (pH 7.5), 10 mM magnesium acetate, 60 mM NH_4Cl and 6 mM 2-mercaptoethanol. The incubated 30,000 x g supernatant (IS-30) of wheat germ was prepared by the method of Roberts and Paterson (15) except that powdered glass was used instead of sand (16) and 0.1 mM phenylmethylsulfonyl fluoride, an inhibitor of proteases (17), was added to the buffer. The tRNA of E. coli and of wheat germ was prepared with a slight modification (18) of the method of Zubay (19).

In vitro assays for poly(U)-dependent polyphenylalanine synthesis and poly(U) dependent incorporation of leucine into polypeptides - (1) E. coli cell-free system. - The reaction mixture (0.05 ml), which contained 20 mM K-phosphate buffer (pH 7.5), 50 mM KCl, 1 mM dithiothreitol, 1 mM ATP, 0.4 mM GTP, 4 mM phosphoenolpyruvate, 5 µg of pyruvate kinase (Boehringer Mannheim GmbH), 25 µg of tRNA, 10 µg of poly(U), 2.2 A_{260} units of IS-30, 0.05 µCi of [^{14}C]phenylalanine (85.5 µCi/µmol), and magnesium acetate and polyamine concentrations as indicated, was incubated at 30° C for 15 min. A 0.04 ml aliquot of each reaction mixture was placed on a paper disc (25 mm diameter) and the hot trichloroacetic acid (TCA) insoluble radioactivity was assayed with a liquid-scintillation spectrometer.

For measurement of the incorporation of leucine, the same reaction mixture was used except that 0.1 µCi of [^{14}C]leucine (354 µCi/µmol) and 6 µM phenylalanine were used instead of [^{14}C]-phenylalanine. Radioactivity without poly(U) was subtracted from each value.

The content of phenylalanine and leucine in IS-30 was calculated from the hot TCA insoluble radioactivities obtained from the constant amount of ^{14}C-amino acid and varying amounts of ^{12}C-amino acid. It was calculated by postulating that the amount of polypeptides synthesized in the presence of the different amounts of amino acid was the same.
(2) Wheat germ cell-free system. - The reaction mixture (0.05 ml), which contained 20 mM K-phosphate buffer (pH 7.5), 6 mM Hepes-KOH (pH 7.5), 30 mM KCl, 50 mM potassium acetate, 2 mM dithiothreitol, 1.5 mM ATP, 0.3 mM GTP, 8 mM creatine phosphate, 7.5 µg of creatine kinase (Boehringer Mannheim GmbH), 50 µg of tRNA, 10 µg of poly(U), 1.4 A_{260} units of IS-30, 0.05 µCi of [^{14}C]phenylalanine (85.5 µCi/µmol), 0.03 mM phenylmethylsulfonyl fluoride, and magnesium acetate and polyamine concentrations as indicated, was incubated at 30° C for 15 min. The other procedures were the same as those described in the E. coli cell-free system.

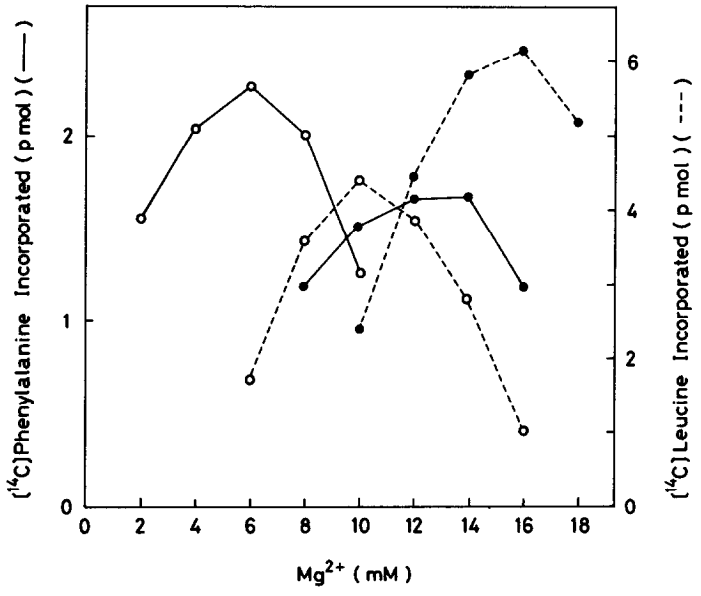

Fig. 1. Effect of spermidine on poly(U) dependent polyphenylalanine synthesis and incorporation of leucine into polypeptides in a wheat germ cell-free system. Polypeptide synthesis was carried out under standard conditions. (——), polyphenylalanine synthesis; (----), incorporation of leucine into polypeptides, (●), without spermidine; (o), 2 mM spermidine.

RESULTS

Effect of polyamines on the fidelity of polypeptide synthesis in a wheat germ cell-free system - The fidelity of ribosomes was examined by measuring the mis-incorporation of leucine (genetic code: GUX, UUA and UUG) in place of phenylalanine (UUU and UUC) into a polypeptide using poly(U) as a template. The incorporation of both correct and incorrect amino acids was dependent on the addition of poly(U). As shown in Fig. 1, the addition of spermidine to the reaction mixture not only reduced the optimal Mg^{2+} concentration of mis-incorporation of leucine, but also decreased the level of mis-incorporation. Stimulation of polyphenylalanine synthesis by spermidine was also observed under these conditions. The optimal Mg^{2+} concentration of mis-incorporation of leucine was higher than that of polyphenylalanine synthesis.

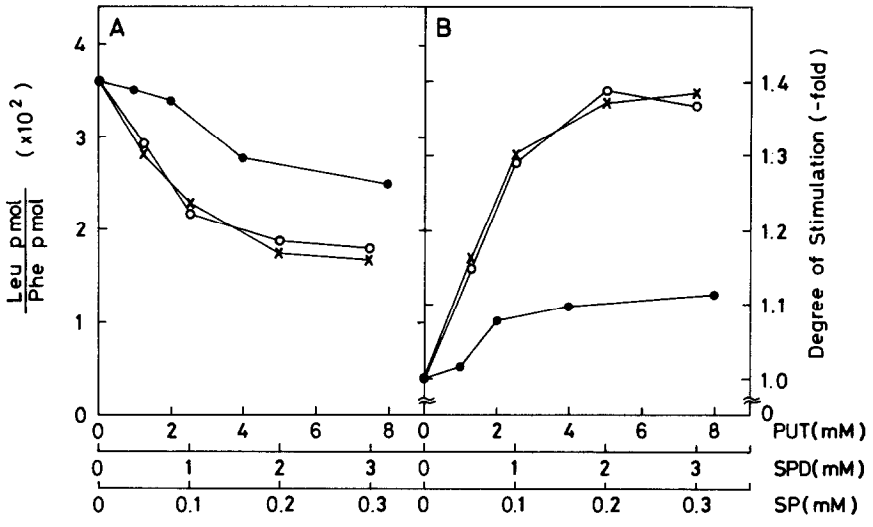

Fig. 2. Effect of polyamines on polyphenylalanine synthesis and the fidelity of polypeptide synthesis in a wheat germ cell-free system.
Polypeptide synthesis was carried out under standard conditions. The frequency of misreading (Leu pmol/Phe pmol) (A) was calculated from the value at the Mg^{2+} concentrations in which the highest incorporation of phenylalanine or leucine was obtained. The degree of stimulation of polyphenylalanine synthesis by polyamines (B) was calculated from the value at the optimal Mg^{2+} concentration in the presence or absence of the polyamine specified in the figure. (●——●), putrescine; (o——o), spermidine; (x——x), spermine.

The effects of various concentrations of polyamines on the fidelity of polypeptide synthesis were then examined (Fig. 2). The frequence of misreading was calculated by measuring the molar ratio of leucine to phenylalanine incorporated into hot TCA insoluble materials at the Mg^{2+} concentrations in which the highest incorporation of phenylalanine or leucine was obtained. The fidelity level gradually increased by the addition of spermidine, the optimal concentration being 2 mM (Fig. 2A). Spermine gave an effect similar to that of spermidine, although the effective concentration was lower. Putrescine was found to be less effective for the increase of fidelity of polypeptide synthesis. The effective concentration of polyamines for the increase of

Table 1. Increase of fidelity of polypeptide synthesis by spermidine at the optimal magnesium concentration in a wheat germ cell-free system.

Ions (mM)		[^{14}C]Phenyl-alanine incorporated (pmol)	Stimulation by SPD (-fold)	[^{14}C]Leucine incorporated (pmol)	Leu pmol / Phe pmol
Mg^{2+}	SPD				
12	-	136.0	-	3.98	0.029
8	0.5	171.1	1.26	1.24	0.0072
8	1.0	201.2	1.48	1.34	0.0067
6	2.0	201.8	1.48	1.25	0.0062
6	3.0	192.1	1.41	1.23	0.0064

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

fidelity of polypeptide synthesis was nearly equal to that for the stimulation of polypeptide synthesis (Fig. 2A and 2B).

The increase of fidelity of polypeptide synthesis by spermidine was then calculated at the optimal magnesium concentration for polyphenylalanine synthesis. As shown in Table 1, the effect of spermidine was very pronounced. The fidelity of polypeptide synthesis was increased up to 4.7-fold by 2 mM spermidine.

Effect of polyamines on the fidelity of polypeptide synthesis in an E. coli cell-free system - As shown in Fig. 3, the addition of spermidine did not influence significantly the fidelity of polypeptide synthesis, although it did decrease the optimal Mg^{2+} concentration in the mis-incorporation of leucine. This was further confirmed by measuring the molar ratio of leucine to phenylalanine incorporated hot TCA insoluble materials at the Mg^{2+} concentrations in which the highest incorporation of phenylalanine or leucine was obtained. The molar ratio was slightly decreased at low concentrarions of polyamines, concentrations

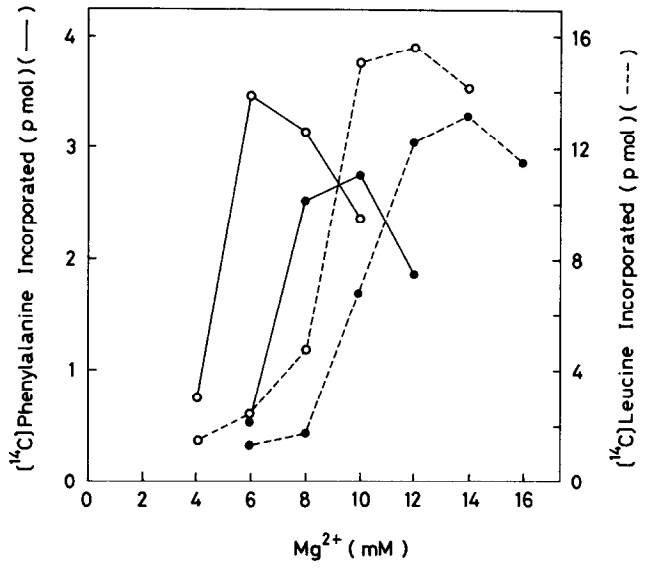

Fig. 3. Effect of spermidine on poly(U) dependent polyphenylalanine synthesis and incorporation of leucine into polypeptides in an E. coli cell-free system. Polypeptide synthesis was carried out under standard conditions. (——), polyphenylalanine synthesis; (----), incorporation of leucine into polypeptides. (●), without spermidine; (○), 0.5 mM spermidine.

not optimal for polyamine stimulation of polyphenylalanine synthesis (Fig. 4). However, when the fidelity of polypeptide synthesis was calculated at the magnesium concentration optimal for polyphenylalanine synthesis, the fidelity was increased up to 1.9-fold by 1 mM spermidine (Table 2).

DISCUSSION

The data presented show that the fidelity of protein synthesis *in vitro* is increased in the presence of polyamines. Although the loss of fidelity in protein synthesis has been thought to be a basic deteriorative reaction in the cellular mechanisms of the aging process (11,12), *in vitro* experiments of the error frequency in translation during aging have not given consistent results (20-23): namely the fidelity of ribosomes has been shown to be higher in old animals (20), higher in young ones (21) or did not

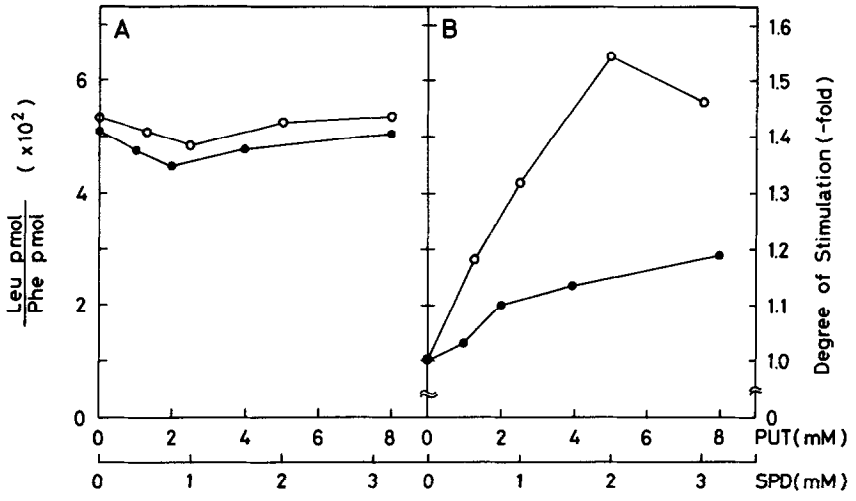

Fig. 4. Effect of polyamines on polyphenylalanine synthesis and the fidelity of polypeptide synthesis in an E. coli cell-free system.
Polypeptide synthesis was carried out under standard conditions. The frequency of misreading (Leu pmol/Phe pmol) and the degree of stimulation of polyphenylalanine synthesis by polyamines were calculated as described in the legend of Fig. 2. (●——●), putrescine; (o——o), spermidine.

Table 2. Increase of fidelity of polypeptide synthesis by spermidine at the optimal magnesium concentration in an E. coli cell-free system.

Ions(mM)		[^{14}C]Phenyl-alanine incorporated (pmol)	Stimulation by SPD (-fold)	[^{14}C]Leucine incorporated (pmol)	Leu pmol / Phe pmol
Mg^{2+}	SPD				
10	-	115.9	-	4.47	0.039
6	0.5	142.6	1.23	3.32	0.023
4	1.0	164.1	1.42	3.40	0.021
4	2.0	200.7	1.73	5.30	0.026
2	3.0	192.8	1.66	5.28	0.027

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

change during aging (22,23). Since it is clear that polyamines play an important role in the increase of fidelity of protein synthesis, the experiments of the error frequency in translation during aging should be performed carefully in the presence and absence of polyamines.

It was of interest that the fidelity of protein synthesis in an E. coli cell-free system was not influenced significantly by polyamines. In addition, the fidelity level without polyamines was lower in an E. coli cell-free system, when the data of Figs. 2 and 4 or Tables 1 and 2 were compared. These results suggest that eukaryotic ribosomes may have evolved to be more resistant to mistranslation. Experiments are now in progress to elucidate the mechanisms by which the fidelity level of the wheat germ cell-free system is high and increased by polyamines. In addition, we are studying the effect of polyamines on the fidelity of protein synthesis in a cell-free system of mammalian tissues.

ACKNOWLEDGEMENT

The authors whould like to express their thanks to Nisshin Seifun Co. for the gift of wheat germ. Thanks are also due to Dr. B. K. Joyce for her help in preparing this manuscript. This work was supported by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture (301053).

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