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Effects of Polyamines on Activities of Elongation Factors, Phenylalanyl-tRNA Synthetase and tRNA in Protein Biosynthesis

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The effects of polyamines on the activities of elongation factors EF-1 and EF-2, phenylalanyl-tRNA synthetase, and tRNA were investigated. The activities of EF-1 and EF-2 were mostly stimulated by spermidine among three kinds of polyamines. The activities of EF-1 and EF-2 were increased in the presence of spermidine by 230 and 181%, respectively. The activity of phenylalanyl-tRNA synthetase was slightly increased in the presence of polyamines. The effect of spermine on the synthetase was higher than that of the other polyamines. The tRNA activity in the presence of polyamines was increased by 206% with spermidine, by 144% with spermine, and by 114% with putrescine. According to these results, it is concluded that polyamines in higher plants stimulate the protein biosynthesis by promoting the activities of elongation factors EF-1 and EF-2, aminoacyl-tRNA synthetases, and tRNAs, but the effects of polyamines on the various components for protein biosynthesis are different in according to the kind of polyamines.

Keywords : polyamine, elongation factor, phenylalanyl-tRNA synthetase, tRNA

The polyamines are normal components of prokaryotic and eukaryotic cells in both animals and plants. Natural polyamines are polyvalent cations which appear to have a diversity of roles in DNA replication, transcription and translation (Tabor and Tabor, 1976; Algranati and Goldemberg, 1977; Tabor and Tabor, 1984). Studies using cell-free protein synthesis system demonstrated that polyamines have a sparing effect on the Mg^{2+} requirements for polypeptide synthesis (Igarashi *et al.*, 1974). Evidence is presented that polyamines maintain not only the folding, but also the active structure of tRNAs (Pochon and Cohen, 1972; Quigley *et al.*, 1978). There are several reports on the relationship between aminoacylation and polyamines. Polyamines increase the formation of some aminoacyl-tRNAs without added Mg^{2+} in an *Escherichia coli* cell-free system (Takeda and Igarashi, 1969). In a rat-liver cell-free system, Ile-tRNA formation by crude Ile-tRNA synthetase is much greater in the presence of polyamines than in the presence of Mg^{2+} (Igarashi *et al.*, 1974). Also, polyamines appears to stimulate the correct binding of aminoacyl-tRNA to ribosome (Thompson *et al.*, 1981; Igarashi *et al.*, 1982; Thompson and Kalim, 1982).

There are evidences for polyamine involvement in various growth and developmental phases of higher plants: cell division, embryogenesis, rooting, flowering, pollen tube growth and ethylene biosynthesis (Kuehn and Atmar, 1982; Feirer *et al.*, 1984; McConlouge *et al.*, 1984; Jarvis *et al.*, 1985; Altman *et al.*, 1988; Walker *et al.*, 1988; Prakash *et al.*, 1988; Bagni, 1989; Evans and Malmberg, 1989). Application of polyamines to potato tuber slices resulted in a considerable inhibition of protein synthesis (Isola and Franzoni, 1989), although polyamines have also been shown to stimulate many enzymes. Callose synthase involved in wound response was activated by spermine application (Fredrikson and Larsson, 1989). Moreover, spermidine and spermine at very low concentrations caused increase in the

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activity of β-1, 4-endoglucanase (Cho *et al.*, 1988). The activity of elongation binding factor, a protein crucial for protein biosynthesis during stratification, was stimulated by spermine (Twardowski and Szczotka, 1989).

However, the effect of polyamines on the protein biosynthesis in higher plant cells was not investigated thoroughly. In this study, we have studied on the effects of polyamines on the activities of several components necessary for protein biosynthesis, EF-1, EF-2, phenylalanyl-tRNA synthetase and tRNA.

MATERIALS AND METHODS

Seed germination

Maize seeds (*Zea mays* L. cv. Golden Cross Bantam) were sterilized in 2% sodium hypochlorite solution and washed 3 times with sterilized distilled water. The washed seeds were germinated in distilled water at 30℃. If necessary, 8 mM putrescine, 0.8 mM spermidine, or 80 μM spermine was added to distilled water, respectively. The germination was carried out at 30℃ with the same solution. The shoots of 2-d-old maize seedlings (5 to 10 mm in length) were used.

Preparation of S-30

Ten grams of maize shoot were homogenized with 30 mL of buffer I (100 mM Tris-Cl, pH 7.8; 50 mM KCl; 5 mM $MgCl_2 \cdot 6H_2O$; 5 mM β-mercaptoethanol; 450 mM saccharose) in a Buhler homogenizer. After filtration through cheesecloth, the suspension was centrifuged at 30,000*g* for 30 min. The supernatant was prereacted as previously described (Sim and Rho, 1985) and dialyzed for 3 h against buffer II (100 mM Tris-Cl, pH 7.8; 5 mM $MgCl_2 \cdot 6H_2O$; 50 mM β-mercaptoethanol; 250 mM saccharose) and the dialyzed solution was designated as S-30.

Preparation of S-100 for elongation factor purification

Ten grams of maize shoot were homogenized with 20 mL of buffer III (100 mM Tris-Cl, pH 7.0; 100 mM KCl; 2 mM $MgAC_2$; 4 mM β-mercaptoethanol) in a Buhler homogenizer. After filtration through cheesecloth, the suspension was centrifuged at 30,000 *g* for 30 min. The supernatant was again centrifuged at 150,000*g* for 2 h, and the resulting supernatant was dialyzed for 3 h against buffer IV (1 mM Tris-acetate, pH 7.0; 2 mM $MgAC_2$; 4 mM β-mercaptoethanol) containing 0.1 M KCl and the dialyzed solution was designated as S-100.

Purification of elongation factor EF-1

Maize S-100 supernatant was applied to 5×1 cm column of DEAE-cellulose (Whatman DE 23) that had been prewashed with the buffer IV containing 0.1 M KCl. 4.08 mL was discarded, 14.28 mL were collected as fraction C. The column was washed with an additional 10.2 mL of the buffer IV containing 0.1 M KCl and eluted with the same buffer containing 0.3 M KCl. 2.4 mL was again discarded and 7.8 mL were collected as fraction D.

After the dialysis of the fraction C for 45 min against buffer V (1 mM Tris-acetate, pH 7.6; 1 mM $MgAC_2$; 4 mM β-mercaptoethanol; 0.1 mM EDTA), fraction C was applied to 9×1 cm column of DEAE cellulose (Whatman DE 23) that had been prewashed with buffer V. The first 14.81 mL was eluted with buffer V containing 30 mM KCl. 3.8 g of $(NH_4)_2SO_4$ were added to 12 mL of the 14.81 mL and the solution was incubated at 0℃ for 20 min and centrifuged at 10,000*g* for 20 min. To the resulting supernatant, 0.85 g of $(NH_4)SO_4$ was added, and the solution was again incubated at 4℃ for 12 h and centrifuged at 10,000*g* for 25 min. The pellet was suspended in 1 mL of buffer VI (1 mM Tris-Acetate, pH 7.3; 50 mM KCl; 5 mM β-mercaptoethanol) containing 250 mM saccharose and 5 mM $MgCl_2$ and the solution was incubated at −18℃ for 12 h, dialyzed 2 h against the same solution, and centrifuged at 10,000*g* for 2 min. The supernatant was designated as purified EF-1.

Purification of elongation factor EF-2

After the dialysis of the fraction D from the S-100 for 1 h against buffer VII (1 mM Tris-Acetate, pH 7.6; 1 mM $MgAC_2$; 4 mM β-mercaptoethanol) containing 0.15 M KCl, the fraction D was applied to 5×1 cm column of DEAE-cellulose (Whatman DE-23) that had been prewashed with buffer VII containing 0.15 M KCl, eluted with the same solution, and the first 38.7 mL was collected. 7.4 g of $(NH_4)_2SO_4$

were added to 30 mL of the 38.7 mL. The solution was incubated at 0℃ for 20 min and centrifuged at 10,000*g* for 20 min. To the resulting supernatant, 3.18 g of $(NH_4)_2SO_4$ were added, and the solution was again incubated at 4℃ for 12 h and centrifuged at 10,000*g* for 25 min. The pellet was suspended in 1 mL of buffer VI containing 250 mM saccharose and 5 mM $MgCl_2$ and the solution was incubated at −18℃ for 12 h, dialyzed for 2 h against the same solution, and centrifuged at 10,000*g* for 2 min. The supernatant was designated as purified EF-2.

Purification of phenylalanyl-tRNA synthetase

S-100 was prepared in the same way as previous process except using buffer A (0.2 M Tris-base; 0.3 M NH_4Cl; 20 mM $MgSO_4$; 1 mM EDTA; 0.15 M D-(+)-Glucose). Purification of phenylalanyl-tRNA synthetase was performed as previously described (Haar, 1979). 16.5 g of $(NH_4)_2SO_4$ were added to 35 mL of the S-100 supernatant. The solution was adjusted to pH 7.0 with 1 N NH_4OH, slowly stirred for 30 min at 0℃ and centrifuged at 24,000*g* for 15 min. The pellet was suspended in 20 mL of buffer B (30 mM potassium phosphate, pH 7.2) and 4.1 g of $(NH_4)_2SO_4$ were added. The solution was stirred for 30 min at 0℃ and centrifuged at 24,000*g* for 15 min. Twenty-two mL of the supernatant were precipitated by adding 5.3 g of $(NH_4)_2SO_4$, stirred for 30 min at 0℃ and then centrifuged at 24,000*g* for 15 min. The pellet was suspended in 2 mL of buffer C (30 mM potassium phosphate, pH 7.2; 1 mM EDTA; 1 mM DTE; 0.01 mM PMSF) and dialyzed for 12 h against buffer C. The dialyzed solution was applied to 5×1 cm column of Sephadex CM-50 (Pharmacia) that had been washed with the buffer C. The column was then washed with the buffer D (30 mM potassium phosphate, pH 7.2; 1 mM EDTA; 1 mM DTE; 0.01 mM PMSF; 10% Glycerol) containing 50 mM KCl. The first 30 mL were eluted with the buffer D containing 0.15 M KCl. 14.2 g of $(NH_4)_2SO_4$ were added to the fraction and the solution was stirred for 30 min at 0℃ and centrifuged at 24,000*g* for 15 min. The pellet was suspended in 0.5 mL of the buffer D, dialyzed for 2 h against 1 L of buffer E (30 mM potassium phosphate, pH 7.2; 1 mM EDTA; 1 mM DTE; 50% Glycerol), and stored at −20℃.

Preparation of polyamine treated tRNA

Treatment of polyamine to tRNA was performed according to the procedure described by Quivy and Chroboczek (1988). The reaction was done in a volume of 100 μL of reaction mixture containing 50 mM Tris-actate (pH 7.6), 8 mM $Mg(OAc)_2$, 50 mM potassium acetate, and 150 μg wheat germ tRNA (Sigma). If necessary, 8 mM putrescine, 0.8 mM spermidine, or 80 μM spermine was added to the reaction mixture, respectively. The mixture was incubated at 25℃ for 30 min. The tRNA was precipitated with 0.1 vol. of 3 M sodium acetate (pH 5.2) and 2 vol. of 100% ethanol, incubated at −20℃ for 2 h, centrifuged at 10,000*g* for 10 min, washed with 70% ethanol, again centrifuged at 10,000*g* for 10 min, dried, and suspended in 50 μL 10 mM Tris-Cl (pH 7.5) containing 5 mM $Mg(OAc)_2$.

In vitro translation mixture

In vitro translation was performed according to Sim and Klambt (1976). 0.6 mL of reaction mixture contained 10 mM $MgCl_2 \cdot 6H_2O$, 70 mM KCl, 0.6 μM ATP, 0.3 μM GTP, 0.01 μM CTP, 0.01 μM UTP, 1.25 μM PEP, 10 μg phosphoenolpyruvate kinase, 150 μg tRNA, 200 μg poly U, 10 A260 S-30, and 0.0125 μM each of amino acid without ^{12}C-phenylalanine. If necessary, 40 μg EF-1 and/or 20 μg EF-2 was added to the reaction mixture, and then 0.35 μCi ^{14}C-phenylalanine was added. The reaction mixture was incubated at 35℃ for 1 h.

Measurements of synthesized protein

The incubation was terminated by cooling the mixture to 0℃ and adding 0.6 mL of 0.1 M ^{12}C-phenylalanine, 0.1 mL of 0.5% bovine serum albumine and 1.3 mL of 10% TCA. After incubation for 15 min at 0℃, the reaction mixture was centrifuged at 10,000 *g* for 20 min. The pellet was suspended in 5 mL of 5% TCA solution containing 0.05% of ^{12}C-phenylalanine, boiled at 90℃ for 15 min to hydrolyze aminoacyl-tRNA, again cooled at 0℃ for 15 min, and centrifuged at 10,000*g* for 10 min. The pellet was resuspended in 5 mL of 5% TCA solution, filtered through Satorius membrane filter SM 11306 (pore

size 0.45 μm), washed with 5% TCA solution 4 times, dried, and the radioactivity was determined with Beckman LS 6500 liquid scintillation counter.

Aminoacylation

Aminoacylation was performed using slightly modified method of Stepanov (1992). The reaction was conducted in 100 μL of reaction mixture containing 50 mM Tris-Cl (pH 8.5), 9 mM $MgCl_2 \cdot 6H_2O$, 5 mM ATP, 150 μg tRNA, 0.35 μL ^{14}C-phenylalanine, and 10 μg phenylalanyl-tRNA synthetase for 20 min at 37℃. Fifty μL of aminoacylated reaction mixture was dropped on filter paper disk (Whatman 3MM, 25 mm diameter), and the filter paper soaked in ice cold 5% TCA solution for 10 min, washed with ice cold 5% TCA solution two times and 95% ethanol once, dried, and the radioactivity was determined with Beckman LS 6500 liquid scintillation counter.

Protein determination

The amounts of S-30, EF-1 and EF-2 were determined according to the Warburg and Christian (1942).

RESULTS AND DISCUSSION

Effects of polyamines on the activities of EF-1 and EF-2

The polyamines are active substance which was known to be important in many *in vivo* metabolism (Tabor and Tabor, 1984; Smith, 1985). To study the effect of polyamines on the activities of elongation factor EF-1 and EF-2 of higher plant, in vitro polyU-dependent protein synthesis system by S-30 containing all components, ribosome, mRNA and protein factors for protein synthesis was used. In the youngest leaves of *Zea mays*, the content of polyamines, putrescine, spermidine and spermine amounted to 383, 237 and 50 ng/g fr wt, respectively (Birecka *et al.*, 1985). However, the optimal concentration of polyamines for *in vitro* protein synthesis was 8 mM putrescine, 0.8 mM spermidine and 80 μM spermine (Kim and Sim, 1993), and these concentrations were used to characterize the role of exogenous polyamines in this study. It is not certain whether the optimal concentration of polyamines used in this study is applicable to *in vivo* system or not.

It was found that the stimulatory rates of protein synthesis in the presence of both EF-1 and EF-2 extracted from polyamine-treated maize shoots were increased 208.9, 276.8 and 227.6%, respectively, by putrescine, spermidine and spermine (Table 1). The activity of EF-2 showed a rise of 129.2, 181.6 and 142.8%, respectively, by 8 mM putrescine, 0.8 mM spermidine and 80 μM spermine (Table 1). The activities of EF-1 isolated from the maize shoots treated

Table 1. Effects of polyamines on the activities of EF-1 and EF-2. EF-1 and EF-2 were prepared from the shoots which were not treated with polyamines and treated with 8 mM putrescine, 0.8 mM spermidine, or 80 μM spermine, respectively. The reaction mixtures contained 40 μg EF-1, 20 μg EF-2, 150 μg tRNA, 0.35 μCi ^{14}C-Phenylalanine and 200 μg polyU and were incubated at 35℃ for 60 min

cpm and increase rate	Incorporation of ^{14}C-Phe (cpm)			Rate of increase (%)		
Components of *in vitro* system	PUT (8 mM)	SPD (0.8 mM)	SPM (80 μM)	PUT (8 mM)	SPD (0.8 mM)	SPM (80 μM)
EF-1[a] EF-2[a] (Control)		641			100.0	
EF-1[b] EF-2[a]	1,192	1,474	1,203	186.0	230.0	187.7
EF-1[a] EF-2[b]	828	1,164	915	129.2	181.6	142.8
EF-1[b] EF-2[b]	1,339	1,774	1,459	208.9	276.8	227.6

[a]Factors were isolated from the shoots not treated with polyamines; [b]Factors were isolated from the shoots treated with 8 mM putrescine, 0.8 mM spermidine, or 80 μM spermine, respectively; The data indicate the mean value of three experiments.

with putrescine, spermidine and spermine were, respectively, about 1.9, 2.3 and 1.9 times greater than control (Table 1). It was shown that the activities of EF-1 and EF-2 were stimulated by three kinds of polyamine significantly. Especially, the effect of spermidine, divalent polyamine, on the activity of EF-1 and EF-2 was greater than the other two polyamines (Mitsui *et al.*, 1984; Kashiwagi *et al.*, 1989; Ito and Igarashi, 1990; Table 1). As shown in Table 1, the effect of polyamines on the activity of EF-1 was greater than that of EF-2. The activation of EF-1 by spermine is coincident with the previous observation (Twardowski and Szczotka, 1989).

According to these results, it is concluded that polyvalent polyamines concerned with DNA or RNA stimulate the protein synthesis by promoting the activities of elongation factors (Tabor and Tabor, 1984; Smith, 1985). However, it is difficult to discriminate whether polyamines act on elongation factor directly or several metabolism such as phosphorylation regulating the activity of protein. Further study should be conducted to make it clear.

Effect of polyamines on the activity of phenylalanyl-tRNA synthetase

Aminoacyl-tRNA synthetases catalyze the binding between amino acid and tRNA to form aminoacyl-tRNAs necessary for protein synthesis. In order to investigate the effect of polyamines on the activity of aminoacyl-tRNA synthetases, aminoacyl-tRNA synthetases were prepared from the maize shoots treated with three kinds of polyamines, 8 mM putrescine, 0.8 mM spermidine and 80 μM spermine, according to the procedure of Haar (1979). The activity of phenylalanyl-tRNA synthetase was measured by the amount of *in vitro* aminoacylation.

As results, the activities of phenylalanyl-tRNA synthetase prepared from polyamine-treated maize shoots were greater than that of the control. The increasing rates of phenylalanyl-tRNA synthetase activity were directly proportional to reaction time (Fig. 1). This result corresponds with the effect of polyamines on the activity of rat-liver isoleucyl-tRNA synthetase (Igarashi *et al.*, 1974; Igarashi *et al.*, 1978). However, the effect of polyamines on the activity of phenylalanyl-tRNA synthetase was most stimulated by spermine unlike the effects of polyamines on the elongation factors.

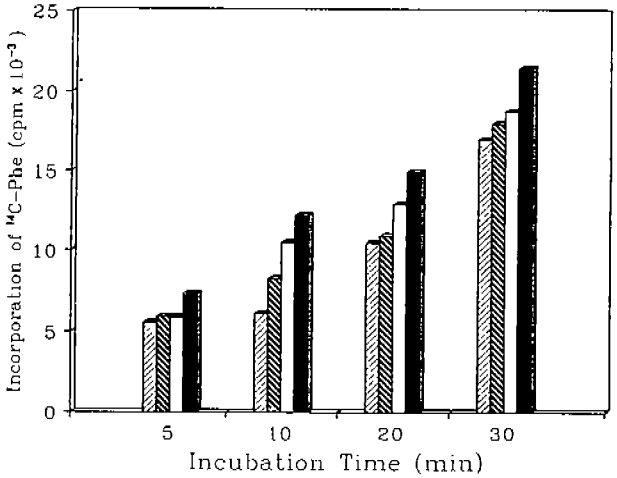

Fig. 1. Effect of polyamines on the activity of phenylalanyl-tRNA synthetase. Phenylalanyl-tRNA synthetase was prepared from the shoots which was not treated with polyamines and treated with 8 mM putrescinc, 0.8 mM spermidine, or 80 μM spermine, respectively. The reaction mixtures containing 10 μg of phenylalanyl-tRNA synthetase and 150 μg wheat germ tRNAs were incubated at 37℃ for 20 min. The data indicate the mean value of two experiments. ▨, Control; ▧, 8 mM Putrescine; □, 0.8 mM Spermidine; ■, 80 μM Spermine.

Effect of polyamines on the activity of tRNA

Polyvalent polyamines have been known to act on several polyanions, especially phosphate in nucleic acids. In order to study the effect of polyamines on the activity of tRNA, wheat germ tRNA (Sigma) were treated with 8 mM putrescine, 0.8 mM spermidine, and 80 μM spermine, respectively, according to the procedure of Quivy and Chroboczek (1988). The activity of tRNA was measured by the amount of aminoacylated tRNA.

The activity of tRNA was stimulated by 3 kinds of polyamine in the same way as the effect of polyamines on the activity of elongation factor and phenylalanyl-tRNA synthetase. tRNA activity was increased 206.7, 144.2 and 114.5%, respectively, by spermidine, spermine and putrescine (Table 2). This activation of tRNA by polyamines in higher plant seems to correlate with the reports that spermidine or Mg^{2+} stabilize secondary or tertiary structure of tRNA (Pochon and Cohen, 1972; Lövgren *et al.*, 1978), and that spermine maintains the stable shape of anticodon loop of yeast $tRNA^{Tm}$ (Peebles *et al.*, 1983). In prokaryote and animal cells, polyamines stimulate the activity of tRNA (Robison and Zim-

Table 2. Effect of polyamines on the activity of phenylalanyl-tRNA. Wheat germ tRNAs were treated with 8 mM putrescine, 0.8 mM spermidine, or 80 μM spermine, respectively. The reaction mixtures containing 150 μg of polyamine-treated wheat germ tRNAs and 10 μg phenylalanyl-tRNA synthetase isolated from the shoots were incubated at 37℃ for 20 min. The data indicate the mean value of two experiments

cpm and increase rate / Polyamines	Incorporation of ^{14}C-Phenylalanine (cpm)	Rate of increase (%)
Control	17,863	100.0
8 mM Putrescine	20,460	114.5
0.8 mM Spermidine	36,919	206.7
80 μM Spermine	25,752	144.2

merman, 1971; Berther *et al.*, 1974). In this study, it is demonstrated that tRNAs are more activated by divalent cation than by monovalent and trivalent cation. Further study will be carried out in order to elucidate the reasons.

In conclusion, it is apparent that polyamines stimulate the protein synthesis by activating the elongation factors EF-1, EF-2, phenylalanyl-tRNA synthetase and tRNA in higher plant cells. These results in higher plant are same as the effects of polyamines on the protein biosynthesis in prokaryote and animal cells (Mitsui *et al.*, 1984; Ito and Igarashi, 1990).

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蛋白質 生合成에 關與하는 伸張要因과 Phenylalanyl-tRNA Synthetase 및 tRNA의 活性에 미치는 Polyamine의 效果

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적 요

단백질 생합성에 관여하는 elongation factor EF-1, EF-2와 phenylalanyl-tRNA synthetase 및 tRNA의 활성에 미치는 polyamines의 효과를 조사하였다. EF-1과 EF-2의 활성은 polyamine 중 spermidine에 의하여 가장 많이 증가하였는데, EF-1은 대조구에 비하여 230%, EF-2는 181% 증가하였다. Phenylalanyl-tRNA synthetase의 활성은 polyamine에 의하여 약간의 증가양상을 보였을 뿐이나, 사용한 polyamine 중에서는 spermine의 효과가 가장 높게 나타났다. 반면 tRNA의 활성에 미치는 polyamine의 효과는 spermidine을 처리한 경우에 가장 높은 것으로서 206%의 증가율을 보였으며, putrescine을 처리한 경우에 가장 낮은 114%의 증가율을 보였다. 이러한 결과들로 보아 polyamine은 고등 식물의 단백질 생합성과정에 관여하는 EF-1, EF-2와 phenylalanyl-tRNA synthetase 및 tRNA의 활성을 증가시키나, 단백질 생합성에 관여하는 각 구성요소의 활성에 미치는 polyamine의 효과는 polyamine의 종류에 따라 다름을 알 수 있었다.

주요어 : polyamine, elongation factor, phenylalanyl-tRNA synthetase, tRNA

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