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EFFECT OF SPERMIDINE ON N-FORMYLMETHIONYL-tRNA BINDING TO 30S RIBOSOMAL SUBUNITS AND ON N-FORMYLMETHIONYL-tRNA DEPENDENT POLYPEPTIDE SYNTHESIS

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SUMMARY: The spermidine stimulation of AUG dependent F-met-tRNA binding to 30S ribosomal subunits and polypeptide synthesis was greater than that of GUG dependent F-met-tRNA binding and polypeptide synthesis. Spermidine stimulation of polypeptide synthesis was greatest when $AUG(U)_n$ was used as a template.

Polyamines have been implicated in numerous growth processes (1,2). As for the influence of polyamines on protein synthesis, it has been shown in various cell-free systems 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+} (3-9). In addition, it has been reported that the stimulation of polyphenylalanine synthesis by SPD is due mainly to a stimulation of the initiation of polypeptide synthesis (5,9).

In this communication, we have studied the effect of SPD on the F-met-tRNA binding to *E. coli* 30S ribosomal subunits and on F-met-tRNA dependent polypeptide synthesis using various synthetic polynucleotides or MS2 RNA.

MATERIALS AND METHODS

Materials - *E. coli* Q13 washed ribosomes and Sephadex G-50 treated S100(S-S100) were prepared as described previously (3). The preparation of ribosomal subunits (30S and 50S) was carried out according

Abbreviation: SPD, spermidine.

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to the procedure of Igarashi and Kaji (10) using a Hitachi RPZ 48T zonal rotor. Crude initiation factors were prepared from the ribosomal wash according to the procedure of Traub et al. (11). MS2 RNA was prepared from the phage MS2 according to the method described by Gierer and Schramm (12). AUG, GUG, poly(U), poly(A), poly(GU), poly(GU_2), and poly(AUG) were purchased from Boehringer Mannheim GmbH. $AUG(U)_n$, $AUG(A)_n$, $GUG(U)_n$, and $GUG(A)_n$ were prepared according to the method of Stanley Jr. et al. (13). F-[^{3}H]met-tRNA was prepared by the method of Nakamoto and Kolakofsky (14) using 2 μM [^{3}H]methionine (specific activity 3.3 Ci/mmol).

Assay of F-met-tRNA binding to 30S ribosomal subunits - The reaction mixture (0.1 ml), which contained 50 mM Tris-HCl (pH 7.5), 100 mM NH_4Cl, 1 mM dithiothreitol, 1 mM GTP, 20,000 cpm of F-[^{3}H]met-tRNA (20 μg of tRNA), 1 A_{260} unit of 30S subunits, 60 μg of crude initiation factors, template as described below, and magnesium acetate and SPD at the specified concentrations, was incubated at 30° C for 10 min. One of the following templates was used in this experiment: 20 μg of poly(AUG), poly(GU) or poly(GU_2); 30 μg of MS2 RNA; 5 μg of AUG or GUG; or $AUG(U)_n$, $AUG(A)_n$, $GUG(U)_n$ or $GUG(A)_n$ containing 5 μg of AUG or GUG. The amount of F-[^{3}H]met-tRNA bound to 30S subunits was measured by the procedure of Nirenberg and Leder (15).

Procedure for polypeptide synthesis - The reaction mixture (0.05 ml), which contained 50 mM Tris-HCl (pH 7.5), 100 mM NH_4Cl, 1 mM dithiothreitol, 1 mM ATP, 0.4 mM GTP, 2 mM phosphoenolpyruvate, 2.5 μg of pyruvate kinase (Boehringer Mannheim GmbH), 30 μg of crude initiation factors, 0.5 A_{260} unit of 30S subunits, 40 μg of S-S100 protein, a template as described above, 30 μM (each) of coded amino acids, 20,000 cpm of F-[^{3}H]met-tRNA (20 μg of tRNA), and magnesium acetate and SPD at specified concentrations, was incubated at 30° C for 3 min. Then, 1 A_{260} unit of 50S subunits (2 μl) was added to the reaction mixture and incubation was carried out at 30° C for 30 min. A 0.04 ml aliquot of each reaction mixture was placed on a paper disc (25 mm diameter) and the hot trichloroacetic acid insoluble radioactivity was assayed with a liquid scintillation spectrometer.

RESULTS

Effect of SPD on F-met-tRNA binding to 30S ribosomal subunits - The addition of SPD to a reaction mixture containing various templates increased the binding of F-met-tRNA to 30S subunits in comparison to a control system containing no SPD but optimal Mg^{2+} concentration (Table 1). The stimulation by SPD of AUG dependent F-met-tRNA binding to 30S subunits was greater than that of GUG dependent F-met-tRNA binding. When poly(AUG) was used as the template, both AUG and GUG dependent F-met-tRNA binding should occur. In this respect, it is of interest that the stimulation of poly(AUG) dependent F-met-tRNA binding by SPD was between the stimulation of AUG and GUG dependent F-met-tRNA binding by SPD. MS2 RNA dependent F-met-tRNA binding to

Table 1. Effect of spermidine on F-met-tRNA binding to 30S ribosomal subunits.

Template	Ions (mM)		F-[^{3}H]met-tRNA bound (cmp)	Stimulation by SPD (-fold)
	Mg^{2+}	SPD		
AUG	10	-	3175	1.56
	8	3	4968	
$AUG(U)_n$	14	-	897	1.67
	12	3	1494	
$AUG(A)_n$	14	-	2075	1.63
	12	3	3382	
Poly(AUG)	10	-	4395	1.33
	8	3	5844	
Poly(GU)	10	-	3710	1.26
	8	3	4665	
Poly(GU_2)	10	-	4629	1.23
	8	3	5691	
GUG	10	-	2809	1.23
	8	4	3466	
$GUG(U)_n$	14	-	1063	1.21
	12	3	1291	
$GUG(A)_n$	14	-	1942	1.31
	12	4	2538	
MS2 RNA	10	-	1751	1.30
	8	3	2279	

The assay was carried out under standard conditions. Ions specified in the table were at the optimal concentration for F-met-tRNA binding.

30S subunits was also stimulated by SPD.

Since SPD stimulation of polypeptide synthesis in a system with a template lacking an initiation codon depends on the uracil content of messenger RNA (4), it was of interest to know whether or not the base composition of nucleotides neighboring an initiation codon influenced the SPD stimulation of F-met-tRNA binding to 30S subunits. No significant difference was observed in the SPD stimulation of F-met-tRNA binding between $AUG(U)_n$ and $AUG(A)_n$, and among $GUG(U)_n$, $GUG(A)_n$, poly(GU) and poly(GU_2) (Table 1).

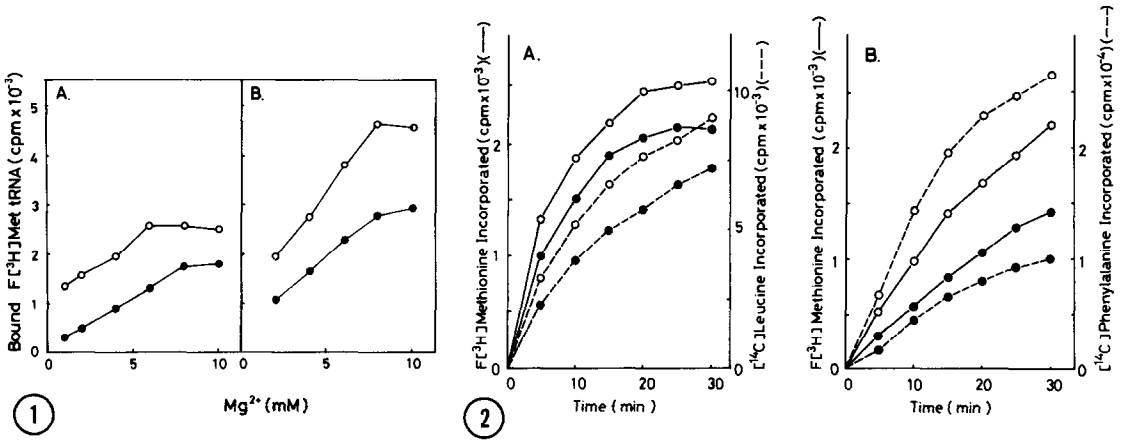

Fig. 1. Effect of spermidine on F-met-tRNA binding to 30S ribosomal subunits, and 30S and 50S ribosomal subunits using AUG as a template.
Assay of F-met-tRNA binding to ribosomes was carried out under standard conditions. (A) 0.5 A_{260} unit of 30S subunits and 1.0 A_{260} unit of 50S subunits were used with 50S subunits being the final component added to the reaction mixture; (B) 1.0 A_{260} unit of 30S subunits were used. (●——●), No SPD; (o——o), 2 mM SPD.

Fig. 2. Kinetics of poly(GU) and AUG$(U)_n$ dependent polypeptide synthesis.
Polypeptide synthesis was carried out under standard conditions except that the reaction mixture (0.05 ml) contained 1 A_{260} unit of 30S subunits, 2 A_{260} units of 50S subunits, and 60 µg of crude initiation factors. (---); [^{14}C]Leucine (A) or [^{14}C]phenylalanine (B) was used as labeled amino acid. Non-labeled F-met-tRNA (20 µg of tRNA) was added. (——); F-[^{3}H]-met-tRNA (A and B) was used as labeled material. (A) Poly(GU) system; (●) 12 mM Mg^{2+}, (o) 2 mM SPD and 7 mM Mg^{2+}. (B) AUG$(U)_n$ system; (●) 16 mM Mg^{2+}, (o) 3 mM SPD and 10 mM Mg^{2+}.

Fig. 1 shows the profiles of F-met-tRNA binding to 30S subunits, and 30S and 50S subunits using AUG as a template in the presence of various Mg^{2+} concentrations with and without the addition of SPD. The SPD stimulation of F-met-tRNA binding was also observed in a system containing 30S and 50S subunits. Similar profiles were observed when templates other than AUG were used (data not shown).

Effect of SPD on F-met-tRNA dependent polypeptide synthesis - The stimulatory effect of SPD on F-met-tRNA dependent polypeptide synthesis was studied using various synthetic polynucleotides or

Table 2. Effect of spermidine on polypeptide synthesis in the presence of F-met-tRNA.

Template	Ions (mM)		Amino acids incorporated (cpm)	Stimulation by SPD (-fold)
	Mg^{2+}	SPD		
$AUG(U)_n$	16	-	3950	3.63
	10	3	14353	
$GUG(U)_n$	16	-	4449	3.11
	10	3	14002	
$AUG(A)_n$	16	-	3748	2.48
	10	4	9293	
$GUG(A)_n$	16	-	4880	2.14
	10	3	10424	
Poly(GU)	12	-	2782	1.31
	7	2	3634	
MS2 RNA	9	-	1277	1.36
	5	2	1738	

Polypeptide synthesis was carried out under standard conditions except that non-labeled F-met-tRNA (20 µg of tRNA) was used instead of F-[^{3}H]met-tRNA. Amino acids used were as follows: $AUG(U)_n$ and $GUG(U)_n$ system, 0.05 µCi of [^{14}C]phenylalanine (448 mCi/mmol); $AUG(A)_n$ and $GUG(A)_n$ system, 0.05 µCi of [^{14}C]lysine (292 mCi/mmol); poly(GU) system, 0.05 µCi [^{14}C]leucine (300 mCi/mmol), and 30 µM (each) of phenylalanine, valine, cysteine, glycine, and tryprophan; MS2 RNA system, 0.05 µCi of [^{14}C]valine (225 mCi/mmol) and 30 mM (each) of 19 other amino acids. The precipitating reagent employed in $AUG(A)_n$ and $GUG(A)_n$ system was 0.25% sodium tungustate in 5% trichloroacetic acid (19). Ions specified in the table were at the optimal concentration for polypeptide synthesis.

MS2 RNA as a template (Table 2). The SPD stimulation of AUG dependent polypeptide synthesis was greater than that of GUG dependent polypeptide synthesis. In addition, the SPD stimulation of $AUG(U)_n$ dependent polypeptide synthesis was greater than the SPD stimulation of $AUG(A)_n$ dependent polypeptide synthesis. MS2 RNA dependent polypeptide synthesis was also stimulated by SPD. It should also be noted that polypeptide synthesis was stimulated more by SPD even in the absence of F-met-tRNA if the template contains AUG (Table 3). This is concluded from a comparison of the degree of stimulation by

Table 3. Effect of spermidine on polypeptide synthesis in the absence of F-met-tRNA.

Template	Ions (mM)		Amino acids incorporated (cpm)	Stimulation by SPD (-fold)
	Mg^{2+}	SPD		
$AUG(U)_n$	18	-	3461	3.11
	12	3	10771	
Poly(U)	14	-	7324	2.14
	7	3	15650	
$AUG(A)_n$	16	-	3512	2.15
	10	3	7551	
Poly(A)	14	-	11487	1.03
	7	3	11798	
Poly(GU)	12	-	2712	1.20
	7	2	3249	

Polypeptide synthesis was carried out as described in the legend of Table 2. Since F-met-tRNA was omitted from the reaction mixture, commercially available tRNA (20 μg) was added after the treatment of the tRNA with 0.2 M Tris-HCl (pH 8.8) at 37° C for 1 hr to hydrolyze aminoacyl bonds. Ions specified in the table were at the optimal concentration for polypeptide synthesis.

SPD of $AUG(U)_n$ and poly(U) dependent polypeptide synthesis or of $AUG(A)_n$ and poly(A) dependent polypeptide synthesis in the absence of F-met-tRNA. These results suggest that the complex of $AUG(U)_n$ or $AUG(A)_n$, $tRNA_F^{met}$, and 30S subunits may also function as an initiation complex as reported by Mosteller et al. (16) and the formation of this complex may be stimulated by SPD.

A kinetic study of poly(GU) and $AUG(U)_n$ dependent polypeptide synthesis was performed using F-[^{3}H]met-tRNA or [^{14}C]phenylalanine and [^{14}C]leucine as labeled materials (Fig. 2). When incubation was carried out for 20 min using poly(GU) as a template, the stimulation by SPD of the incorporation of F-[^{3}H]met-tRNA and [^{14}C]-leucine was about 1.2- and 1.3-fold, respectively. In case $AUG(U)_n$ was used as a template, the stimulation by SPD of F-[^{3}H]met-tRNA

and [^{14}C]phenylalanine incorporation into polypeptides was about 1.7- and 2.7-fold, respectively. The amount of F-[^{3}H]met-tRNA incorporated into polypeptides with AUG(A)$_n$ system could not be measured because of the release of formylmethionine from the polypeptides (unpublished results).

DISCUSSION

The data presented in the RESULTS section show that SPD is necessary for the maximum F-met-tRNA binding to 30S subunits and that the SPD stimulation of overall polypeptide synthesis is based at least partially on the stimulation of the formation of an initiation complex. Although the SPD stimulation of AUG(U)$_n$ dependent polypeptide synthesis was greater than the SPD stimulation of AUG(A)$_n$ dependent polypeptide synthesis, no significant difference was observed in the SPD stimulation of F-met-tRNA binding to 30S subunits when either AUG(U)$_n$ or AUG(A)$_n$ was used as a template. This suggests that the SPD stimulation of uracil dependent aminoacyl-tRNA binding to the aminoacyl site (A site) also influences the stimulation by SPD of overall polypeptide synthesis (4, 9).

It is of interest that the SPD stimulation of AUG dependent F-met-tRNA binding to 30S subunits and AUG dependent polypeptide synthesis was greater than that of GUG dependent F-met-tRNA binding to 30S subunits and GUG dependent polypeptide synthesis. There are reports (17,18) that some kinds of mRNA contain GUG as an initiation codon.

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