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STIMULATORY EFFECT OF UTP ON PEPTIDE CHAIN INITIATION IN STREPTOMYCES AUREOFACIENS

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The formation of the 30S and 70S initiation complex in Streptomyces aureofaciens differs from that in E.coli and B.stearothermophilus with respect to the requirement for nucleotide triphosphates for the maximum activity. In the presence of GTP and initiation factors from S.aureofaciens the codon specific binding of fMet-tRNA to ribosomes of S.aureofaciens was stimulated by ATP or UTP. UTP exhibited the most significant effect increasing the binding about 3.5 times, whereas CTP had no effect on the reaction. The stimulatory effect of UTP is GTP-dependent and was not observed in experiments with E.coli ribosomes.

1. INTRODUCTION

Initiation of polypeptide chain in Escherichia coli requires three initiation factors IF1, IF2 and IF3 and GTP for the codon specific binding of initiator tRNA to ribosomes (1-3). Guanosine triphosphate acts as a steric effector in this reaction permitting the association of IF2 with ribosomes at a low concentration of the factor. The energy of GTP hydrolysis is used to release the IF2 and bound GTP from the 70S complex.(4,5).

It has been shown that initiation complex formation in Bacillus stearothermophilus is stimulated by two initiation factors that are analogous to IF2 and IF3 of E.coli, and that the codon specific binding of fMet-tRNA to ribosomes is stimulated by ATP, as well as by GTP. The maximum stimulatory effect was obtained when both nucleotide triphospha-

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tes were present in the reaction mixture (6). The role of ATP is not trivial since artifacts such as Mg^{2+} complex or phosphorylation of GDP were eliminated.

In the present paper we estimated conditions for ApUpG or MS2 RNA dependent binding of fMet-tRNA to 30S subunits or 70S ribosomes of Streptomyces aureofaciens. Our results show that the formation of 30S and 70S initiation complex depends on the presence of initiation factors from S.aureofaciens and that both GTP and UTP are required for the maximum binding of fMet-tRNA to ribosomes.

2. MATERIALS AND METHODS

2.1. Material

ApUpG and MS2 RNA were Miles products. GTP, UTP, CTP, ATP, Guanosine-5'-[β,γ-imido] triphosphate, (GDPNP) and Puromycin were from Serva, Heidelberg, FRG. [^{3}H]fMet-tRNA was obtained from Prof. L.Bosch, University of Leiden, Holland.

2.2. Ribosomes and ribosomal subunits were isolated and purified as described previously (7). Ribosomal subunits were activated as recommended in (8).

2.3. Crude initiation factors were isolated as in (9).

2.4. fMet-tRNA binding

The binding of [^{3}H]fMet-tRNA to 30S subunits or 70S ribosomes was assayed in a reaction mixture (100 µl) containing: 50 mM Tris-HCl pH 7.6; 40 mM NH_4Cl; 6 mM $Mg(OAc)_2$, 10 mM 2-mercaptoethanol, 30 pmol 30S subunits or 25 pmol 70S ribosomes (as indicated), 0.3 O.D. MS2 RNA, or 0.1 O.D. ApUpG, [^{3}H]fMet-tRNA (42 pmol), nucleotide triphosphates as indicated in the figure legends. The reaction was preincubated for 5 min at 37 °C and started by adding [^{3}H]fMet-tRNA. The mixture was incubated for 20 min at 37 °C and than diluted with 3 ml of cold wash buffer (20 mM Tris-HCl, pH 7.6, 40 mM NH Cl, 6 mM $Mg(OAc)_2$) and filtered through nitrocellulose filters (Sartorius 0.45 µ), the filters were washed three times with 2 ml of wash buffer and dried. The radioactivity was determined in a liquid scintillation counter.

2.5. Puromycin reaction

The binding incubation mixtures were as above and 5 µl of an aqueous solution (5 µg/µl) were added to the reaction and the incubation continued for 20 min at 37 °C. The react-

ion was stopped with 1 ml of cold 0.1 M sodium phosphate pH 5.0 and fMet-puromycin was extracted with 1.5 ml of ethyl-acetate (10).

3. RESULTS AND DISCUSSION

At physiological Mg^{2+} concentrations (5-6 mM) the 30S initiation complex formation depends on the presence of initiation factors, GTP and mRNA or ApUpG. The effect of increasing concentrations of initiation factors on the fMet--tRNA binding to 30S subunits is shown in Fig. 1. The stimulatory effect of the crude fraction of initiation factors is strictly dependent on the presence of GTP or MS2 RNA. At the constant GTP level (0.2 mM) in the reaction mixtures the stimulatory effect of increasing concentrations of initiation factors was further increased in the presence of ATP.

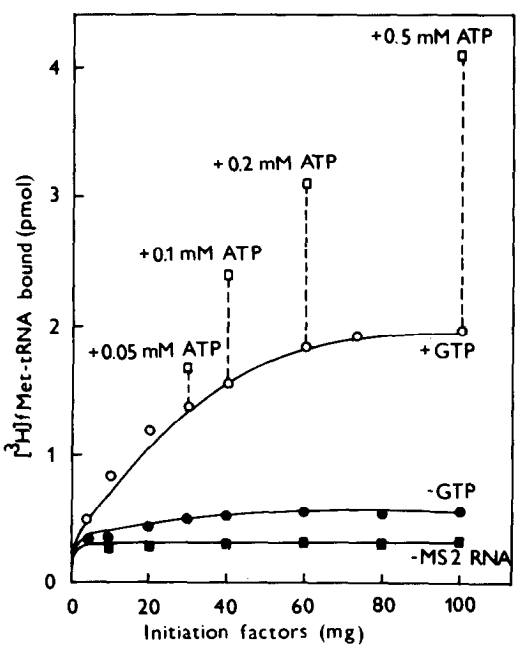

Fig. 1 Binding of fMet-tRNA to 30S subunits of S.aureofaciens in the presence of response to crude initiation factors, MS2 RNA and GTP.

The fMet-tRNA binding to 30S subunits was estimated as described in Materials and Methods. Stimulatory effect of initiation factors was estimated in the absence: of GTP (●), MS2 RNA (■), in the presence of 0.2 mM GTP only (○), or in the presence of increasing concentrations of ATP (□).

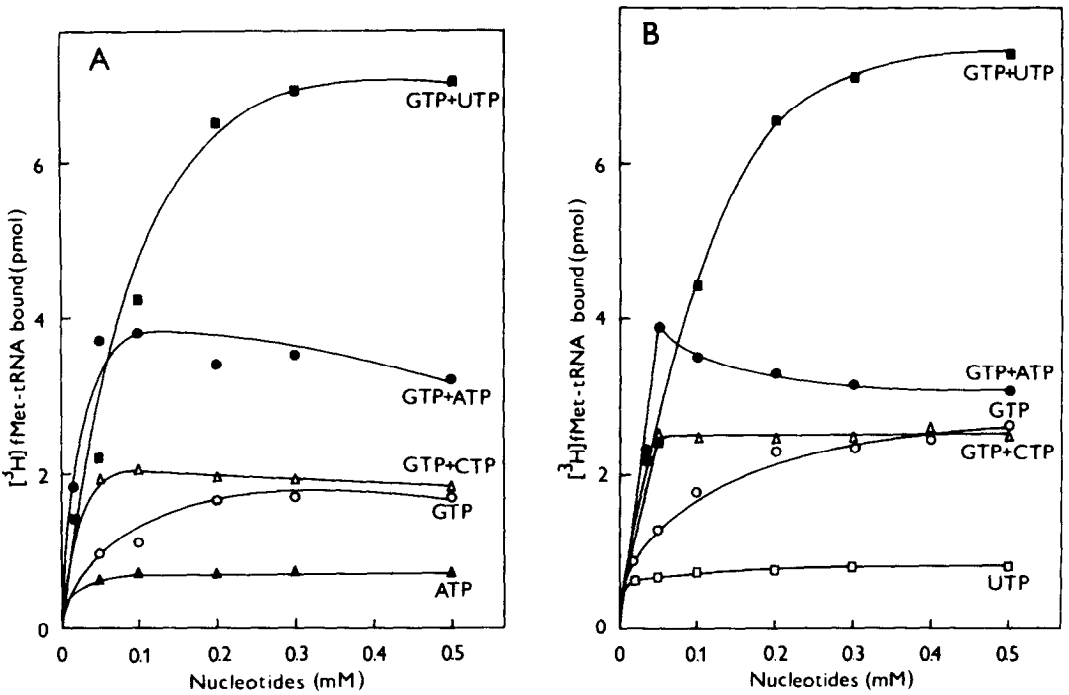

Fig. 2 The effect of nucleotide triphosphates on the binding of fMet-tRNA to 30S subunits of S.aureofaciens.

(A) MS2 RNA dependent binding: in the presence of ATP (▲), GTP (○), the following experiments were performed at a constant concentration of GTP (0.2 mM) and increasing concentrations of CTP (△), ATP (●) or UTP (□).

(B) ApUpG dependent binding: in the presence of UTP (□), GTP (○), the following experiments were performed at a constant concentration of GTP (0.2 mM) and increasing concentration of CTP (△), ATP (●), or UTP (□).

In the next experiments we studied, whether ATP alone, or a mixture of GTP and ATP or another nucleotide triphosphate, e.g. CTP or UTP, would stimulate the MS2 RNA dependent binding of fMet-tRNA to 30S subunits. These experiments were performed at optimum concentrations of initiation factors. As shown in figure 2A the increasing concentrations of ATP in the absence of GTP had a very small stimulatory effect on the fMet-tRNA binding. When only GTP was used in the reaction, the maximum binding was obtained at about 0.2 mM and increasing concentrations of GTP had no additional stimulatory

effect. Further experiments were performed in the presence of 0.2 mM of GTP and increasing concentrations of ATP, CTP or UTP. The addition of CTP to the reaction mixture had no pronounced effect on the fMet-tRNA binding. In the presence of 0.05 mM - 0.1 mM ATP the binding of fMet-tRNA was stimulated roughly twice. The increase of ATP concentration from 0.1 mM to 0.5 mM had a slight inhibitory effect on the reaction. UTP had the most significant effect on the reaction. The binding of the initiator tRNA was stimulated already by 0.05 mM UTP and reached the maximum at 0.3 mM. The fMet-tRNA binding was by about 3.5 times higher than in the presence of GTP alone. Essentially the same results were obtained when MS2 RNA was substituted by ApUpG (Fig. 2B). These experiments were performed to eliminate the possibility that UTP might be involved in the activation of noninitiator codons present in MS2 RNA (11). The data also demonstrate that UTP alone cannot fulfil the requirement for GTP in the binding reaction.

In order to investigate whether the stimulatory effect of ATP or UTP possibly influences the correct position of fMet-tRNA on the ribosome, the transfer of fMet-tRNA from 70S ribosomes to puromycin was assayed (Fig. 3). The initiation factor dependent binding of fMet-tRNA to 70S ribosomes at 0.2 mM GTP is remarkably stimulated by the presence of UTP and initiator tRNA is reactive with puromycin. These results indicate that the initiator tRNA is correctly positioned at the donor site. Since only the attachment of formylated tRNA to 70S ribosomes was stimulated by the initiation factors (12), we obtained an additional evidence that UTP really stimulates the fMet-tRNA binding.

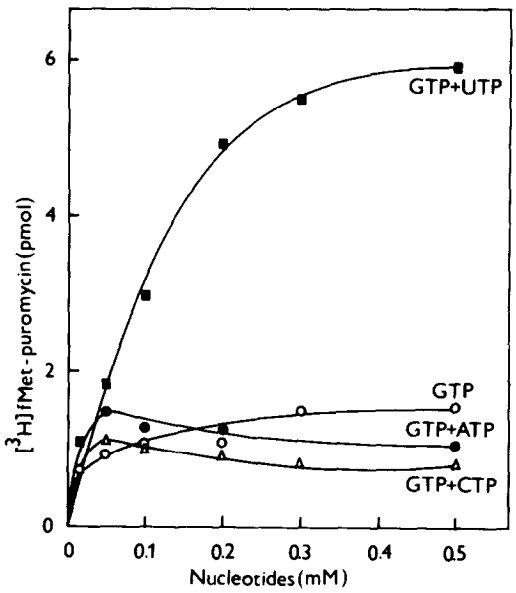

Fig. 3 The effect of nucleotide triphosphates on the binding of fMet-tRNA to 70S ribosomes of S.aureofaciens and the puromycin reaction.

The ApUpG dependent binding of fMet-tRNA in the presence of initiation factors and the puromycin reaction were performed as described in Materials and Methods.
The reaction proceeded in the presence of GTP only (O), the following experiments were carried out at 0.2 mM GTP and increasing concentrations of CTP (Δ), ATP (●) and UTP (■).

In additional experiments we wanted to find out whether the stimulatory effect of UTP is GTP dependent or whether GTP can be replaced with the nonhydrolyzable analogue guanylylimidodiphosphate (GDPNP). These experiments were performed with 30S subunits of S.aureofaciens (Fig. 4A) and with 70S ribosomes of S.aureofaciens and E.coli (Fig. 4B). The stimulatory effect of UTP on fMet-tRNA binding was determined in the presence of 0.2 mM GTP or GDPNP. As shown in Fig. 4A, in the presence of 0.2 mM GDPNP the increasing concentrations of UTP have only a small stimulatory effect on the reaction. The course of the reaction was almost the same as in the control experiments performed in the presence of GTP alone. The data suggest that UTP stimulates the GTP

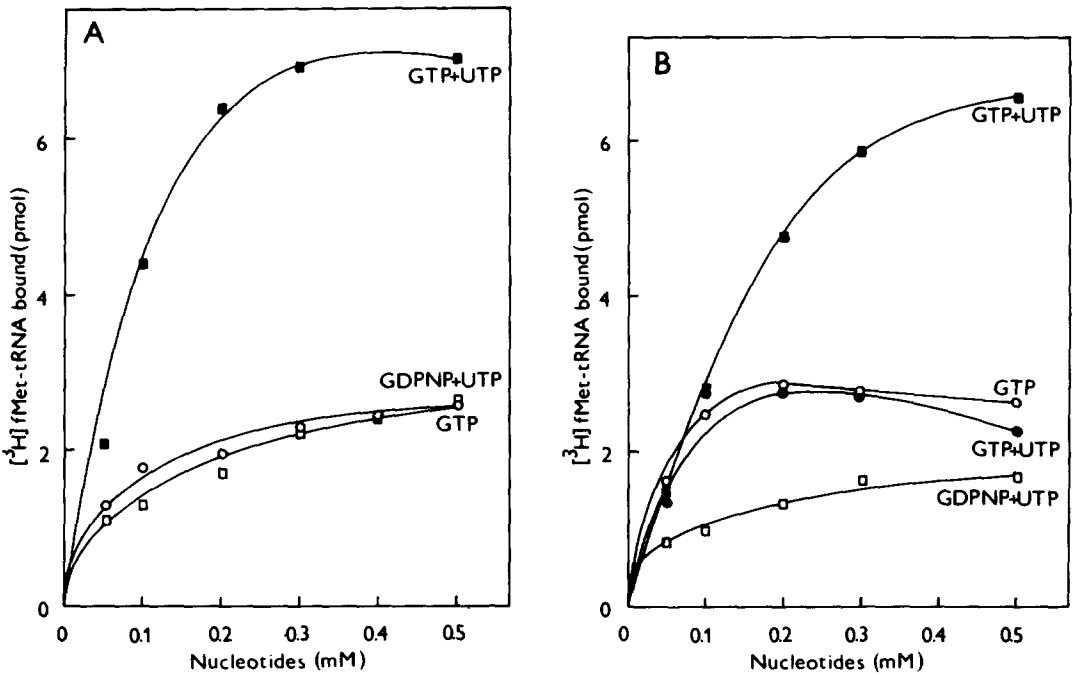

Fig. 4 The effect of the nonhydrolyzable analogue guanylyl-imidodiphosphate on the stimulation of the binding of fMet-tRNA by UTP.

(A) ApUpG dependent binding of the initiator tRNA to 30S subunits of S.aureofaciens. Stimulatory effect of GTP only (⊙), in the presence of 0.2 mM of GTP and increasing concentrations of UTP (■) and with 0.2 mM GDPNP (□) instead of GTP.

(B) ApUpG dependent binding of fMet-tRNA to 70S ribosomes. Experiments with ribosomes of E.coli: in the presence of increasing amounts of GTP alone (○) and at a constant concentration of GTP (0.2 mM) and increasing concentrations of UTP (●). Experiments with 70S ribosomes of S.aureofaciens were performed at 0.2 mM GTP and increasing concentrations of UTP (■) or with 0.2 mM GDPNP instead of GTP (□).

mediated binding of fMet-tRNA to ribosomes of S.aureofaciens. It follows from the results summarized in Fig. 4B that ribosomes can recognize the stimulatory effect of UTP. In the presence of initiation factors from S.aureofaciens and 0.2 mM GTP the binding of fMet-tRNA to ribosomes of E.coli is not stimulated by UTP. On the contrary, the UTP concentration higher than 0.2 mM has an inhibitory effect. In experiments with ribosomes of S.aureofaciens under similar

experimental conditions, the binding of initiator tRNA to 70S ribosomes was remarkably stimulated by UTP. The results also show that the substitution of GTP by GDPNP inhibits the fMet-tRNA binding to 70S ribosomes and that increasing concentrations of UTP have only a low stimulatory effect. At present, we can only hypothesize on the mode of UTP action. One of possible explanations for the stimulatory effect of UTP might be that UTP increases the activity by phosphorylation of initiation factor(s) or ribosomes. Additional experiments with $[^{32}P]$UTP can clarify, whether the phosphorylation of factor(s) or/and ribosomes is involved in the stimulatory effect of the nucleotide, or whether UTP stabilizes the 30S initiation complex of S.aureofaciens.

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