EFFECT OF GTP ON THE DISSOCIATION OF 70 S RIBOSOMES

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1. Introduction

Zamecnik's discovery of the GTP requirement for protein synthesis [1] was followed by a series of investigations on the role of this nucleotide both in microbial and mammalian systems. In bacterial extracts GTP is needed in several steps of the protein synthesis process: (a) The binding of *N*-formylmethionyl-tRNA to the ribosomes [2,3]. (b) An interaction with the soluble transfer factors T [4,5] and subsequent binding of the aminoacyl-tRNA to the ribosomes [6–10]. Apparently GTP is not hydrolyzed in these reactions, although there are some conflicting results on this point [6,7,9,10]. (c) The formation of a complex containing ribosomes and transfer factor G [11], and the translocation of peptidyl-tRNA [12] or *N*-formylmethionyl-tRNA [13] from the aminoacyl (A) to the peptidyl (P) site on the ribosome with concomitant hydrolysis of the nucleotide into GDP and orthophosphate.

This communication reports a new effect of GTP: an enhancement of the *in vitro* dissociation of 70 S ribosomes. The properties and physiological significance of dissociating factors from *Bacillus stearothermophilus* and *Escherichia coli* were recently investigated [14].

The ribosomal dissociation by extracts from *E. coli* was previously studied by Subramanian et al. [15] who did not mention any effect of nucleotides on the reaction.

2. Materials and methods

B. stearothermophilus 1503-4R was grown as described before [16].

E. coli D10, kindly supplied by R.F.Gesteland, was cultivated in a Casamino acids glucose medium [17].

The harvesting of bacteria and the preparation of extracts and ribosomes will be described elsewhere [14]. Dissociating factor was obtained by extracting ribosomes with solutions of high salt concentration [14]; this preparation should also contain initiation factors [18].

5'Guanylyl-methylene diphosphonate (GMP-PCP) was a generous gift of Drs. C. and J.E.Allende.

Standard assay. Each 0.2 ml reaction mixture contained 10 mM Tris-HCl buffer pH 7.8, 5 mM magnesium acetate, 50 mM KCl, 2.5 mM dithiothreitol (DTT), 0.5 to 1.0 A_{260} units of ribosomes, dissociating factor (5–80 μg of protein) and where indicated, 1 mM GTP; in this case magnesium acetate was increased to 6 mM. Incubations were carried out for 5 min at 55° in thermophilic systems or at 37° in *E. coli* systems, and the tubes were then chilled to 0°. Samples of 0.15 ml were immediately layered on 4.6 ml of 5–20% linear sucrose density gradients made up in 20 mM Tris-HCl buffer pH 7.8, 10 mM magnesium acetate and 50 mM KCl. After centrifugation for 100 min at 40,000 rpm in a Spinco SW 50 L rotor at 4°, gradients were analyzed with a Gilford recording spectrophotometer. Dissociation was calculated from the area under the absorbancy tracing of the 70 S peak or by using an empirical formula [14].

3. Results and discussion

In a recent study on the *in vitro* dissociation of 70 S ribosomes [14], we obtained some preparations from *B. stearothermophilus* with very low ribosomal

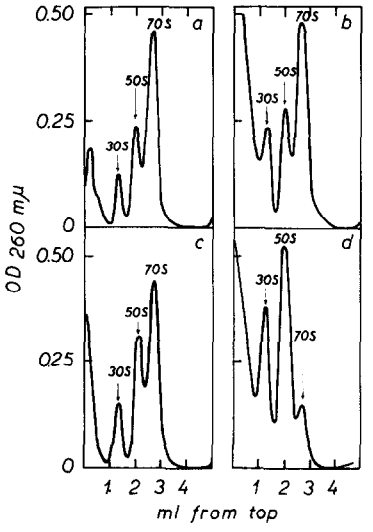

Fig. 1. Effect of GTP on ribosomal dissociation. Sucrose gradient profiles of *B. stearothermophilus* ribosomes after incubation: (a) without additions; (b) with GTP; (c) with dissociating factor; (d) with dissociating factor and GTP. All other components of mixtures are described under methods.

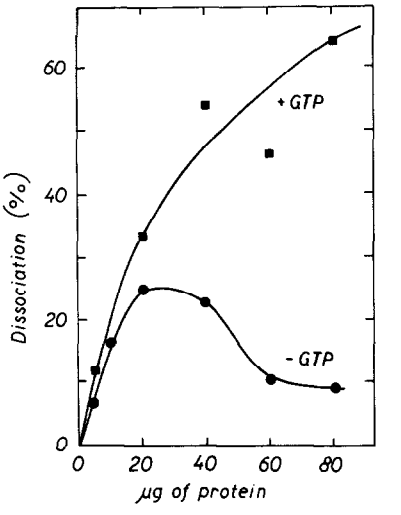

Fig. 2. GTP effect at different concentrations of dissociating factor. Assays and calculations were as indicated in the text.

dissociating activity (fig. 1a and c). When GTP was added to the incubation mixtures the dissociation notably increased as shown in fig. 1d. In the absence of dissociating factor, GTP has almost no effect on ribosomes (fig. 1b).

The nucleotide stimulation is not due to complexing of Mg^{++} with concomitant decrease of free metal ion, since the addition of more magnesium acetate to 7 mM total concentration did not alter the activation.

Different stimulations were obtained when one batch of ribosomes was tested with various dissociating factor preparations or when a particular factor acted on different 70 S particles. These results may indicate that GTP interacts with both the dissociating factor and the ribosomes, although it cannot be ruled out that a variable amount of the nucleotide remains in the preparations, even after extensive dialysis.

Fig. 2 illustrates the GTP effect in the presence of increasing amounts of dissociating factor. The decrease of dissociation at high concentrations of dissociating factor, in the absence of GTP, was not observed in other preparations, but in all cases GTP stimulation was much more pronounced at high levels of dissociating factor.

The specificity of GTP action was tested by incubating mixtures with different nucleotides (table 1). Neither ATP, CTP nor UTP could substitute for GTP. Guanosine 5'-diphosphate or GMP-PCP were about 60% as active as GTP, and GMP was inhibitory. GDP or GMP-PCP added together with GTP, both at 1 mM concentration, gave an activation somewhat similar to that obtained with 2 mM GTP alone. These results indicate that GTP and GDP or GMP-PCP effects were additive.

The GTP activation was abolished in the presence of GMP, pyrophosphate or orthophosphate.

The increase of ribosomal dissociation by GDP or GMP-PCP indicates that this effect does not require the hydrolysis of GTP at the linkage between β and γ phosphates. On the other hand GMP and PP_i counteract the activation of GTP, suggesting that the bond between α and β phosphates might be broken.

The ribosomal dissociation at increasing GTP concentrations is shown in fig. 3. The apparent $K\hat{m}$ calculated for GTP was 6.2×10^{-4} M. This high value could be attributed to some non-specific nucleotide triphosphate phosphohydrolases. When the experiment was repeated in the presence of 1 mM ATP (this nucleotide alone had little or no effect on dissociation), the apparent $K\hat{m}$ for GTP decreased to 3.5×10^{-5} M. This value is still high, but it is nearer the $K\hat{m}$ for oligopeptide synthesis reaction [19].

Table 1

Nucleotide specificity of the activation effect. When nucleotides or phosphate were used, equimolar amounts of magnesium acetate were also added in order to maintain the free Mg^{++} concentration. With PP_i, 1.5 μmoles/ml of magnesium acetate were added.

Additions	Per cent dissociation	Per cent increase
–	29.8	
1 mM GTP	60.0	101.5
2 mM GTP	67.2	125.9
1 mM ATP	36.6	22.8
1 mM CTP	37.3	25.2
1 mM UTP	30.8	3.4
1 mM GDP	46.6	56.4
1 mM GMP-PCP	49.0	64.4
1 mM GMP	20.0	-33.0
1 mM GTP + 1 mM GDP	64.5	116.8
1 mM GTP + 1 mM GMP-PCP	65.6	120.5
1 mM GTP + 1 mM GMP	38.2	28.3
1 mM GTP + 1 mM PP_i	34.6	16.2
1 mM GTP + 2 mM P_i	30.7	3.1

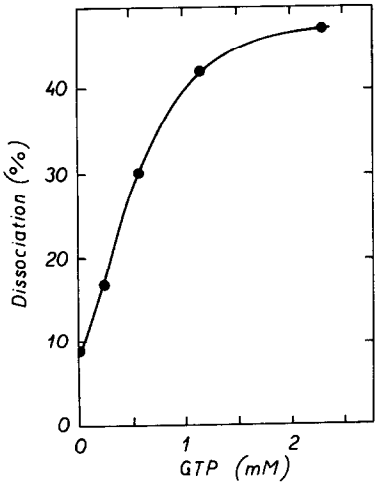

Fig. 3. Influence of GTP concentration on dissociation. Reaction mixtures were as already described, except that different levels of GTP and magnesium acetate (1:1) were added.

In an attempt to determine whether the activating effect of GTP is specific for thermophilic bacteria, *E. coli* systems or hybrid mixtures obtained from *B. stearothermophilus* and *E. coli*, were also assayed (table 2). A marked increase of dissociation was observed in all cases where at least one component came from *B. stearothermophilus*. In *E. coli* systems the GTP stimulation was smaller and variable.

Table 2

Effect of GTP on thermophilic, *E. coli* and hybrid systems. 0.8 A_{260} units of ribosomes and 40 μg of dissociating factor were used in all cases. Assays with hybrid systems were incubated for 5 min at 45°C. B.s., *Bacillus stearothermophilus*; E.c., *Escherichia coli*.

Ribosomes	Dissociating factor	GTP	Dissociation (%)
B.s..	B.s..	–	16.2
B.s.	B.s.	+	48.4
B.s..	E.c.	–	16.9
B.s.	E.c.	+	47.7
E.c.	B.s.	–	10.7
E.c.	B.s.	+	46.5
E.c.	E.c.	–	19.3
E.c.	E.c.	+	28.1

The 70 S particles used in the experiments described in this paper were obtained from bacteria harvested after slow cooling of the culture. Under these conditions the polypeptide chains have been completed and released from ribosomes, as shown previously [20]. This fact ruled out the possibility that the GTP activation occurred by translocation of peptidyl-tRNA with a hypothetical labilization of ribosomes [21]. Besides, the enhancing effect also appeared in the presence of GMP-PCP, a well known inhibitor of protein synthesis.

The mechanism of GTP effect on ribosomal dissociation and its physiological significance are under study.

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