RECONSTITUTION OF THE 50 S RIBOSOME SUBUNIT. LOCALIZATION OF G-DEPENDENT GTPase ACTIVITY

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1. Introduction

Recent work from our laboratory [1] has confirmed and supplemented the evidence from early reports [2] showing the existance of an active centre in the 50 S ribosome subunits responsible for the G-dependent hydrolysis of GTP. The use of protein deficient nucleoprotein core particles derived from the 50 S ribosome subunit [3] is a very valuable tool to study the minimal ribosomal structural components required for some ribosomal activities [4–6]. We have studied the G-dependent GTPase activity in 50 S derived cores and reconstituted particles and the results obtained are reported in this work.

2. Materials and methods

2.1. *Ribosomes, ribosome subunits and derived particles*

Ribosomes and ribosome subunits were prepared from *E. coli* D-10 as previously described [6]. α-, β- and γ-cores and two types of split proteins $SP_{50-\beta}$ and $SP_{\beta-\gamma}$ (released in the conversion of 50 S to β-cores and β- to γ-cores respectively) were obtained by isopycnic centrifugation in CsCl gradients [3]. Reconstitution of active particles from the nucleoprotein cores and split proteins was carried out by activation of the reaction mixtures at 50° for 90 min [3].

2.2. *G-dependent GTPase activity assay*

The G-dependent GTPase activity of the particles was tested under conditions described elsewhere [7] using 1 mg/ml of 50 S subunits or equimolecular amounts of derived cores in 50 μl volumes of incubation.

2.3. *Materials*

^{32}P-γ-labelled GTP was prepared by a modification of a method previously described [7]. The method used to obtain purified G factor was as described elsewhere [8].

3. Results

3.1. *Conditions for optimal G-dependent GTPase activity*

Our initial studies were aimed to optimize the G-dependent GTPase activity of the protein deficient cores. 30 S ribosome particles and alcohols known to be stimulatory agents for different activities in certain systems with 50 S subunits [5, 9–11] show a similar effect in this case as indicated in table 1. However, it is interesting to note that methanol has no stimulatory effect on intact 50 S subunits but is strongly stimulatory of the activity of the cores. We have found, in experiments not presented here, that ethanol is less effective than methanol in this system and isopropanol has no effect.

3.2. *G-dependent GTPase activity of 50 S derived particles*

Using optimal conditions for GTPase activity, namely, in the presence of 30 S subunits and 20% ($^{v}/_{v}$) methanol, the activity of the 50 S derived particles was tested. Different preparations of cores

Table 1
Effect of methanol and 30 S subunits in G-dependent GTPase of 50 S subunits and derived particles.

Additions	GTP hydrolyzed (molecules/particle)		
	β-cores	α-cores	50 S subunits
None	14.6	14.5	39.7
30 S	37.5	34.1	51.2
Methanol	48.3	44.1	42.3
30 S and methanol	51.4	53.2	55.5

G-dependent GTPase activity was assayed as described elsewhere [8]. Incubation were carried out at 30° for 30 min. Either 1 mg/ml 50 S subunits or equimolar amounts of derived cores were used; equimolar amounts of 30 S subunits were also present when indicated. Methanol was added when required at a final concentration of 20% ($^{v}/_{v}$).

Table 2
G factor-dependent GTPase activity of protein deficient 50 S derived particles.

Particle	GTP hydrolyzed (molecules/particle)		
	Exp 1	Exp 2	Exp 3
γ	3.4	2.4	0
$\gamma + SP_{\beta-\gamma}$	27.9	22.4	22.6
β	34.3	28.5	26.4
α	–	29.9	37.3
50 S	60.7	35.1	52.8

Each experiment was carried out with different sets of preparations. Incubations were at 30° for 15 min. In all cases G-dependent GTPase activity of 50 S and derived particles was tested in the presence of an equimolar concentration of 30 S subunits and 20% ($^{v}/_{v}$) methanol. (–) assay not carried out.

show variations in their activity due, at least in part, to differences in the activity of the 50 S subunits from which they were derived.

As shown in table 2, GTPase activity of β-cores differs with the preparations but is always smaller than in 50 S subunits. γ-cores are depleted of most of the G-dependent GTPase activity but this is recovered upon the addition of the split proteins released on the β- to γ-core conversion. These results are in agreement with those obtained by other workers on GDP binding in a slightly different system [12].

When G-dependent GTPase and the peptidyl transferase activities of a given β-core preparation were compared with the activities of the 50 S subunit preparation, it was found that more GTPase than peptidyl transferase activity was lost in the preparation of the cores (results not presented here). Differences in the activity of the different cores is in the rate rather than in the extent of the reaction, as shown in fig. 1 which presents the kinetics of the reaction carried out by the various particles. Whereas in studies on the peptidyl transferase activity, α-cores show a higher activity than the 50 S subunits from which they were derived [4, 6], this is not the case when the G-dependent GTPase is studied.

γ-cores, upon prolonged incubation, display a low but reproducible GTPase activity. However, we were able to show that this residual GTPase activity of γ-cores was resistant to thiostrepton (table 3), an antibiotic known to inhibit the G-dependent GTPase

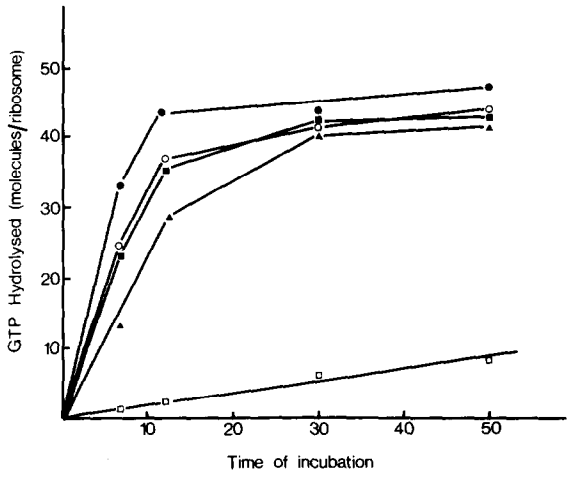

Fig. 1. Kinetics of the G-dependent GTP-hydrolysis by 50 S subunits derived particles. The assays were carried out as described in (2.2), in the presence of an equimolar concentration of 30 S subunits and 20% ($^{v}/_{v}$) methanol. (●——●) 50 S particles; (○——○) α-cores; (■——■) β-cores; (▲——▲) γ-cores + $SP_{\beta-\gamma}$; (□——□) γ-cores.

Table 3
G-dependent GTPase activity. Effect of Thiostrepton.

Particle	Additions	Experiment 1		Experiment 2	
		GTP hydrolyzed (molecules/particle)	Inhibition (%)	GTP hydrolyzed (molecules/particle)	Inhibition (%)
γ	–	12.3	–	7.8	–
γ	Thiostrepton	11.1	10	6.7	13
$\gamma + SP_{\beta-\gamma}$	–	42.0	–	28.5	–
$\gamma + SP_{\beta-\gamma}$	Thiostrepton	11.8	72	7.7	73
70 S	–	81.2	–	–	–
70 S	Thiostrepton	13.1	83	–	–

GTPase assay was carried out at 30° for 60 min in the presence of 30 S subunits and 20% ($^{v}/_{v}$) methanol. Thiostrepton was added when required at a final concentration of 2×10^{-5} M. Experiments 1 and 2 were carried out with different core and split protein preparations.

activity of intact ribosomes or 50 S subunits [2, 13, 14].

4. Discussion

The G-dependent GTPase activity of the ribosomes requires the proteins involved in the β- to γ-core step. A similar requirement has been shown for a number of activities of the peptidyl transferase centre [6]. However, the GTPase centre of the 50 S subunit is unique among those so far studied [4–6], requiring at least partially the proteins released in the conversion of 50 S subunits to α-cores for its normal function. This finding confirms other pieces of experimental evidence that show the different location of the peptidyl transferase and GTPase centre [1].

The residual GTPase activity of the γ-cores might be due to either non-specific GTPase of the particles (although in all our experiments is also a G-dependent activity) or the active centre being altered in its characteristics by structural changes that make it hardly sensitive to thiostrepton due to a lower affinity of the γ-particles for this antibiotic.

The stimulatory effect of G-dependent GTPase by the alcohols is not well understood. Since the action seems especially strong on the nucleoprotein cores, our results are compatible with an action at the level of the whole structure of the particle rather than directly on the G-dependent GTPase centre.

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Note added in proof: When this work was in press it was reported that two specific proteins of the 50 S subunit known as L7 and L12 are implicated in G-dependent GTPase activity (Kischa, Möller and Stöffler, Nature New Biology 233 (1971) 62). Recent analyses of our split protein fractions carried out in collaboration with Dr. H. G. Wittmann have shown that neither L7 nor L12 are present in the split protein fraction released in the preparation of β-cores, whereas protein L7 (but not L12) was present in the $SP_{\beta-\gamma}$ fraction. These results suggest that the GTPase activity of the γ-cores is due to the L12 protein being still integrated in the ribonucleoprotein

structure. Thiostrepton is shown above to inhibit the GTPase activity of β-cores but not in the case of γ-cores, suggesting that the binding centre of the antibiotic requires the L7 protein.

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