ROLE OF SPLIT PROTEINS FROM 30 S SUBUNITS IN THE RIBOSOME–EF-T GTPase REACTION

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1. Introduction

Both 30 S and 50 S ribosomal subunits have been shown to participate in the GTPase activities of elongation factor G (EF-G) and elongation factor T (EF-T = EF-Tu + EF-Ts) [1–5]. The 50 S subunit appears to contain a region which is shared by both elongation factors and at or near which the hydrolysis of GTP occurs [4–14]. In the case of EF-G, the turnover level of GTP hydrolysis is regulated by the 30 S subunit [3]. The GTPase of EF-T requires aminoacyl-tRNA in addition to both ribosomal subunits and is stimulated by poly(U) [4, 5]. Recently, 30 S proteins S5 and S9 have been shown to cooperatively reactivate CsCl-prepared 30 S cores that are themselves inactive in the ribosome–EF-G GTPase reaction. The reassembly of such functional 30 S particles is enhanced by S2 [3]. S5 and S9 appear to function by enabling the 30 S subunit to associate with the 50 S subunit forming particles active in the EF-G GTPase reaction. We now show in this communication that the CsCl 30 S core is also inactive in the ribosome–EF-T GTPase reaction and can be restored to original activity by the combined action of proteins S2, S5 and S9. In this case, S2 and S9 are the major components needed.

2. Materials and methods

EF-T and 70 S ribosomes were isolated from *E. coli* B/2 as previously described [15]. The NH_4Cl-washed ribosomes were separated into 30 S and 50 S subunits by sucrose density gradient centrifugation at 0.5 mM $MgCl_2$ using a Spinco 15 Ti rotor. The 30 S subunits were 98% pure and the 50 S at least 95% pure as measured by analytical sucrose gradient centrifugation. 30 S cores were prepared by CsCl isopycnic centrifugation of 30 S subunits in 20 mM Tris-HCl (pH 7.8)–20 mM $MgCl_2$–7 mM β-mercaptoethanol. Under these conditions, 8 split proteins are extracted: S1, S2, S3, S5, S9 S10, S14 and S20. The individual split proteins were purified as described. Their molecular weights were taken to be those used [3]. For reconstitution, 30 S cores were incubated for 5 or 10 min with the respective split proteins at 42° in 20 mM Tris-HCl (pH 7.8)–30 mM $MgCl_2$–250 mM NH_4Cl. The 75 μl GTPase reaction mixtures contained, in 20 mM Tris-HCl (pH 7.8)–30 mM $MgCl_2$–60 mM NH_4Cl, 10 pmoles of the different 30 S particles, 10 pmoles of 50 S subunits, 2 μg of poly(U), 120–150 pmoles of [^{14}C]Phe-tRNA^{Phe} (prepared from approx. 40% pure tRNA^{Phe}), 4–7 μg of EF-T and 150–200 pmoles of [^{32}P]GTP (specific activity, 2,000–4,000 Ci/mole).

1 A_{260} unit was taken to represent 67 pmoles of 30 S particles or 39 pmoles of 50 S subunits [16, 17]. GTPase activity was measured as the amount of $^{32}P_i$ liberated during a 5 min incubation at 30° [18]. Radioactivity was measured with a Tri-Carb liquid scintillation spectrometer.

3. Results and discussion

As in poly(U)-directed polyphenylalanine synthesis [19, 20] and the ribosome–EF-T GTPase reaction [3], 30 S cores produced from 30 S subunits by dissociation of a specific fraction of proteins in CsCl density gradients at 20 mM Mg^{2+} were found to be inactive in the ribosome–EF-T GTPase reaction (table 1). Full activity could be restored to the 30 S cores upon incubation

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Table 1
Omission of individual split proteins from 30 S subunits: effect on the ribosome–EF-T GTPase reaction.

System	Relative GTPase activity (complete system = 100%)
Core alone	0
Native 30 S control	105
Complete	100
–S1	68
–S2	64
–S3	135
–S5	51
–S9	72
–S10	95
–S14	82

The complete system contained 10 pmoles each of all the seven 30 S split proteins. For the GTPase assays, 10 pmoles of native 50 S subunits were added. Blanks of native 50 S + 30 S core have been subtracted. Residual 50 S activity, which was around 10% of that observed upon addition of native 30 S, was slightly reduced (to approx. 7%) when 30 S core was present.

Table 2
Ability of individual split proteins to restore activity to 30 S cores in the ribosome–EF-T GTPase reaction.

Split protein	(pmoles of protein added)	(pmoles of GTP cleaved per 10 pmoles of 30 S particles + 10 pmoles of 50 S subunits)
(Native 30 S control)		55
S1	10	5
	20	12
S2	10	20
	20	29
S3	10	12
	20	8
S5	10	17
	20	23
S9	10	20
	20	33
S10	10	5
	20	2
S14	10	0
	20	8

Blanks of native 50 S + 30 S core have been subtracted (see table 1).

Table 3
Effect of various combinations of split proteins with 30 S core on reconstitution of the ribosome–EF-T GTPase activity.

Split protein(s)	(pmoles of GTP cleaved per 10 pmoles of 30 S particles + 10 pmoles of 50 S subunits)
(Native 30 S control)	38
S2 + S5 + S9	34
S2 + S9	30
S5 + S9	21
S2 + S5	20
S2	15
S5	7
S9	11
S1 + S2 + S5	31
S1 + S2	12
S1 + S5	13
S3 + S9 + S10	11
S3 + S9	15
S3 + S10	9
S9 + S10	9

For reconstitution, 10 pmoles of 30 S cores were incubated with 10 pmoles of each split protein as indicated. Blanks of 50 S + 30 S cores have been subtracted (see table 1).

with the seven isolated split proteins S1, S2, S3, S5, S9, S10 and S14. The effect of split protein S20 was not tested. As can be further seen in table 1, the individual omission of S1, S2, S5 or S9 from otherwise complete reconstitution mixtures produced 30 S particles with much reduced activity. The effect of omitting S10 or S14 was relatively minor, while omission of S3 led to an increase in activity suggesting an inhibitory role for S3 under these conditions.

The action of the *individual* split proteins can be seen in table 2. Each split protein showed some ability to restore activity to the 30 S core. However, proteins S2, S9 and to a lesser extent S5 were clearly the most active species at higher protein concentrations. The stimulatory activity of S3 and S10 observed when they were present at an equimolar ratio to 30 S core was reduced when larger amounts were added.

To extend these findings, various combinations of split proteins with 30 S cores were tried (table 3). Together, S2, S5 and S9 could restore 90% of the 30 S activity, with S2 + S9 alone being able to restore 80% of the activity. In all cases tested, the restoration of 30 S activity was additive. The increase in activity seen when S1 was combined with S2 and S5 correlates

well with the loss in activity seen when it was omitted from an otherwise complete reconstitution mixture (table 1). This stimulatory action of S1 is most likely due to its contribution to the binding of poly(U) by 30 S particles [21]. The combinations containing S3 and S10 produced low stimulations, once again demonstrating that their participation in this reaction is of minor importance.

It is interesting to observe that in both the EF-G and EF-T GTPase activities S2, S5 and S9 are the split proteins involved in the reconstitution of active 30 S particles. Since we have earlier shown that S5 and S9 and, in an auxiliary fashion, S2 are required for 30 S–50 S association [3], stimulation of this association is the most likely explanation for their ability to restore 30 S activity. Association of the 30 S and 50 S ribosomal subunits seems to represent a basic requirement in both EF-T and EF-G GTPase activities [22]. In fact, we have recently found that in the presence of methanol the association of CsCl 30 S cores and 50 S subunits can take place in the absence of the 30 S split proteins, giving rise to a complex active in both the EF-T- and EF-G-dependent GTPase reactions (unpublished results).

The major contributors to the EF-T GTPase seem to be S2 and S9, with S5 playing a less prominent role; whereas, in the EF-G GTPase reaction S5 and S9 are the only proteins required for full reconstitution. With EF-T the action of the proteins assayed under our conditions is additive, while in the case of EF-G S5 and S9 showed a cooperative mechanism. These differences may be explained by the presence in the EF-T–ribosomal GTPase system of Phe-tRNA and poly(U). These additional components are probably also responsible for the more diffuse involvement of the 30 S split proteins in the EF-T GTPase. Indeed, Kurland and co-workers have observed that S2, S3 and S14 are necessary constituents for the interaction of aminoacyl-tRNA with ribosomes, while S1 plays an important role in mRNA binding to the 30 S [21, 23]. Investigation of the EF-G and EF-T GTPase reactions in the complete system for polypeptide synthesis will be essential to clarify the exact role of the 30 S split proteins in these two reactions.

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