EFFECT OF RIBOSOMAL PROTEIN L12 UPON INITIATION FACTOR IF-2 ACTIVITIES

A. Kay, G. Sander*, and M. Grunberg-Manago
Institut de Biologie Physico-chimique, rue P. et M. Curie, 75005 Paris
*Gesellschaft für Molekularbiologische Forschung mbH,
3301 Stöckheim, Germany

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Summary

The effect of removal of the 50S subunit proteins L7 and L12 upon initiation factor IF-2 activities is investigated. Both "coupled" and "non-coupled" GTPase activities are greatly reduced as is fMet-tRNA ribosomal binding. These activities can be restored by re-addition of L12. IF-2 activities are less affected by lack of L12 than EF-G dependent GTP hydrolysis. It is proposed that ribosomal sites for initiation factor and elongation factor -dependent GTP hydrolysis are closely associated.

Introduction

The initiation factor IF-2 exhibits a ribosomal-dependent GTPase activity [1-3]. This activity can be subdivided into two classes : an activity "non-coupled" to protein synthesis, in which GTP is hydrolyzed by IF-2 and ribosomes in the absence of mRNA and fMet-tRNA, and a "coupled" activity in which the amount of GTP hydrolyzed is increased by the addition of poly (A, U, G) and fMet-tRNA[1-3]. It is thought that the hydrolysis of GTP is necessary for the release of IF-2 after formation of the 70S ribosomes, the exit probably taking place from a site on the 50S subunit[4-7]. It is conceivable that both the coupled and non-coupled GTP hydrolysis occur at the same site on the ribosome, but there are no data yet.

The elongation factors EF-G and EF-T also exhibit ribosome dependent GTPase activities [8] and the action of IF-2 in initiation is sometimes compared to that of EF-T in elongation[2,5,9]. There is evidence that the ribosomal sites for EF-G and EF-T GTPase activities are very close, and possibly identical[10-13]. There is

also some evidence, obtained from using antibiotics such as thiostrepton and from competition with EF-G, which suggests that the site of IF-2-dependent GTP hydrolysis is also close to the elongation factor sites [7]. Recently, it has been shown that treatment of ribosomes with 50% ethanol and M NH_4Cl results in the loss of several ribosomal proteins, especially the acidic proteins L7 and L12 of the 50S subunit [13-15]. The loss of these proteins is accompanied by a sharp reduction in the ribosomal dependent GTPase activities of EF-G and EF-T which is reversed by adding back either protein [13-16]. In this communication, we report the influence that the removal and re-addition of L12 has upon the ribosomal dependent GTPase and fMet-tRNA binding activities of IF-2.

Materials and Methods

The preparation of E.coli ribosomal subunits, the treatment of the 50S subunit with NH_4Cl-ethanol, the isolation of L12, and the purification of EF-G have been described previously by Parmeggiani and collaborators [15].

E.coli IF-2 and IF-1, and f(^{3}H)Met-tRNA were prepared as in ref.(2). The f(^{3}H)Met-tRNA was prepared using (^{3}H)methionine (specific activity : 4 Ci/mmole) obtained from C.E.A., Saclay, France.

GTPase activity was assayed by measuring the liberation of $^{32}P_i$, using the method of Kolakofsky et al. [1]. The GTP-γ-(^{32}P) used was a commercial preparation obtained from C.E.A., Saclay, further purified by chromatography on Norite.

f(^{3}H)Met-tRNA binding to ribosomes was measured by a nitrocellulose (Millipore) filtration assay [2].

Results and Discussion

Ethanol-treated 50S particles were compared for EF-G and

IF-2 ribosomal dependent GTPase activity in the presence and absence of L12. The dependence of GTP hydrolysis upon EF-G and L12 is shown in Fig. 1. The addition of L12 greatly stimulates the GTPase activity of the treated ribosomes, although they still retain a significant activity in the absence of L12. This is in agreement with previous work [13-15].

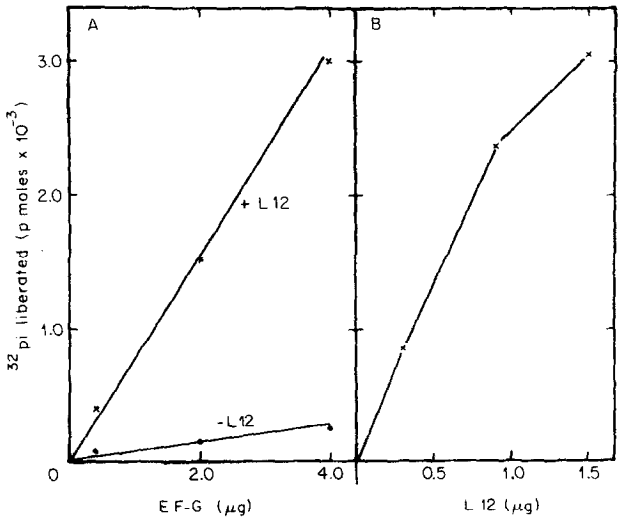

Figure 1

Dependence of GTP hydrolysis by NH_4Cl - ethanol treated 50S subunits upon : (a) EF-G, and (b) L12. The treated 50S (10.8 p-moles) were pre-incubated 10 min at 30°C with L12 or L12 buffer in final conditions (25 μl) of Tris-HCl, pH 7.8, 100 mM; $MgCl_2$, 13.8 mM; NH_4Cl, 100 mM; β-mercaptoethanol, 7 mM; glycerol (from the ribosomes), 4%. To this were then added untreated 30S subunits (19.5 pmoles); GTP-γ-^{32}P, 7500 pmoles (1 pmole = 33 cpm); Tris-HCl, NH_4Cl, and $MgCl_2$ to maintain conditions as in the pre-incubation to give a final volume of 50 μl. Incubation was for 10 min at 30°C. Activity was measured as liberation of $^{32}P_i$ (1). In figure (a), 1.5 μg L12/assay were added where indicated, and in figure (b), 4 μg EF-G/assay were used.

We then looked at the effect of L12 upon the ribosome-dependent GTPase activities of IF-2 (Table I). In these experiments, both the 30S and 50S subunits were pre-incubated at 37°C before addition of

TABLE I

Effect of L12 on IF-2 and EF-G-dependent GTPase activities

Additions	GTP-γ-^{32}P hydrolyzed (pmoles)		
	30S + 50S + IF-2	30S + 50S + IF-2 + poly (A, U, G) + fMet-tRNA	30S + 50S + EF-G
Untreated 50S particles			
Expt 1	287.8	697.3	-
Expt 2	-	-	-
NH_4Cl-ethanol treated 50S particles + L12			
Expt 1	336.1(100)	680.0(100)	-
Expt 2	78.2(100)	284.9(100)	134.5(100)
NH_4Cl-ethanol treated 50S particles			
Expt 1	111.1(33.0)	233.0(34.3)	-
Expt 2	32.7(41.8)	95.5(33.7)	24.1(17.9)

50S and 30S subunits (Expt 1, 23.5 pmoles each; Expt 2, 11.7 pmoles each) were pre-incubated with or without L12 (Expt 1, 5 μg; Expt 2, 1.5 μg) for 15 min at 37°C in final conditions (in 50 μl) of Tris-HCl, pH 7.5, 60 mM; $MgCl_2$, 10.5 mM; NH_4Cl, 100 mM; β-mercaptoethanol, 7 mM. To this were added : IF-2, 1.5 μg (in both Expts) or EF-G, 0.18 μg; GTP-γ-^{32}P, 2000 pmoles (1 pmole gives about 100 cpm in both Expts); poly (A, U, G), 0.15 A_{260} units; and fMet-tRNA, 0.5 A_{260} units (where indicated); together with Tris-HCl, NH_4Cl, and $MgCl_2$ to maintain the pre-incubation salt conditions (final volume 100 μl). Incubation was for 20 min at 37°. Activity was measured as the liberation of $^{32}P_i$ (1). The control values obtained with factors alone and/or 30S + 50S alone (10-20 pmoles) have been subtracted. Figures in bracket are percentage of activity (reconstituted 50S being taken as 100%).

IF-2 and GTP, since it was found that pre-incubation of the 30S subunits greatly stimulates IF-2-dependent GTPase activities. It has previously been reported that pre-incubation of the ribosomes also stimulates IF-2-dependent fMet-tRNA ribosomal binding (17). The

TABLE II

Influence of L12 upon IF-2-dependent fMet-tRNA ribosomal binding

Additions	f(^{3}H)Met-tRNA bound (pmoles)		
	30S	30S + ethanol treated 50S	30S + ethanol treated 50S + L12
IF-2(1.5 μg)	0.183	0.293(33.6)	0.51
IF-2(3.0 μg)	0.415	0.645(49.0)	0.885
IF-2(1.5 μg)+IF-1	0.415	0.725(31.5)	1.40
IF-2(3.0 μg)+IF-1	1.02	1.35 (37.5)	1.93

Pre-incubation (50 μl) of 30S (29.5 pmoles) and 50S (29.0 pmoles) with or without L12 (5 μg) was for 15 min at 37°C in Tris-HCl, pH 7.5, 60 mM; NH_4Cl, 100 mM; $MgCl_2$, 5.7 mM; β-mercapto-ethanol, 7 mM. To this were then added : IF-2, as indicated; IF-1, 1.3 μg (where indicated); poly (A, U, G), 0.3 A_{260} units; f(^{3}H)Met-tRNA, 1.0 A_{260} unit; GTP, 1mM; Tris-HCl, NH_4Cl, and $MgCl_2$ to maintain concentrations as in the pre-incubation mixture; and incubation was continued for 20 min at 37°C. f(^{3}H)Met-tRNA binding was measured by nitrocellulose (Millipore) filtration assay [2]. The values of f(^{3}H)Met-tRNA bound to the ribosomes in the absence of any factors (<0.1 pmole in every case) have been subtracted. The figures in brackets are percentage of stimulation of 30S by 50S depleted of L12 as compared to re-constituted 50S (taken as 100%).

addition of L12 to ethanol-treated 50S particles enhances the ribosomal dependent GTPase activities of IF-2, restoring the levels of GTP hydrolysis found when untreated 50S subunits are used. Both the "coupled" and "non-coupled" GTPase activities of IF-2 are augmented in approximately the same proportions. However, the residual activity remaining in the ethanol-treated ribosomes is greater for IF-2 (30-40%) than for EF-G (18%).

The effect of L12 upon IF-2-dependent attachment of fMet-tRNA to ethanol-treated ribosomes was also investigated (Table II).

Addition of 50S subunits augments the factor-dependent fMet-tRNA binding that occurs with the 30S subunits alone. The stimulation obtained by the addition of ethanol-treated 50S particles is only 30-40% that obtained when 50S subunits reconstituted with L12 are added. The lack of L12 in ethanol-treated 50S particles does not appear to have an effect upon the stimulatory activity of IF-1.

Our results show that the lack of L12 impairs all the IF-2 activities associated with the 50S subunits, but that these activities can be restored by addition of L12. However, the GTPase activity of IF-2 is less affected by loss of L12 than the GTPase activity of the elongation factor EF-G.

The effects of L12 can be explained in several ways. On the 50S subunit the protein may not be close to the sites of GTP hydrolysis, but its loss induces a global change of conformation of the subunit, resulting in a reduction of GTPase activity. A large change is unlikely since treated 50S particles are still able to form 70S ribosomes [15]. Another possibility is that L7 or L12 form an essential part of the GTPase site. However, it has been shown recently that lack of L12 can be partly compensated by the addition of 20% methanol, which restores part of the elongation factor activities affected[18, 19]. That L7 and L12, in the absence of methanol, are required for most of the reactions involved in translocation indicates, as Hamel and Nakamoto have suggested [18], that although the sites involved are basically intact, L7 and L12 play an auxiliary role. It is probable that they are closely associated with these sites.

Our results showing that the "non-coupled" and "coupled" GTPase activities of IF-2 are both affected to the same extent by loss of L12 from the 50S subunit indicate that both of these activities may be mediated by the same ribosomal site, as was suggested in the Introduction. That L12 influences both IF-2 and elongation factors

ribosomal dependent GTPase activities indicates that the sites involved may also be closely associated upon the 50S subunits, as was suggested previously from work with thiostrepton and competition experiments [7]. However, the lack of L12 affects IF-2-dependent GTPase activities less than it affects EF-G-dependent GTP hydrolysis. This could mean that the sites, while close, are not identical and that local perturbations caused by the loss of L12 affect the IF-2 site less than the EF-G site. There is, however, the possibility that the two sites are identical or partly shared, but that the spatial requirements for IF-2-dependent GTP hydrolysis are less strict than those for EF-G; the loss of an auxiliary protein, L12, may therefore have less influence upon IF-2 GTPase activities.

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