AN INITIATION FACTOR CAUSING DISSOCIATION OF *E. COLI* RIBOSOMES

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Purification of crude initiation factors, essential for polypeptide synthesis in cell-free systems of *E. coli*, yielded a fraction DF which causes dissociation of 70 S ribosomes. Its stoichiometric action on 70 S ribosomes is antagonized by increasing Mg^{2+} concentrations but not by the addition of 30 S and 50 S subunits washed with high salt concentration. GTP did not stimulate this dissociating action. 2 μg of our most purified preparation caused 100% dissociation of 100 μg of 70 S ribosomes without added GTP. DF-induced dissociation is a very rapid process at 37°C and is temperature-dependent in the range of 0°–37°C. DF, which is thermolabile factor, is much less or not effective with complexed 70 S ribosomes bearing peptidyl-tRNA and mRNA.

1. Introduction

Initiation of polypeptide synthesis in *E. coli* requires a number of so-called initiation factors, which can be washed off the ribosomes by high salt [1]. Fractionation [2–4] and purification [5–8] of these factors revealed the existence of at least three protein fractions, designated F_1, F_2 and F_3 by Iwasaki et al. [2], each with a different function. Recently a factor (DF) was described by Subramanian et al. [9] which caused dissociation of 70 S ribosomes into subunits (compare also Gonzalez et al. [10]). In the present paper we report the isolation and partial purification of a ribosomal protein fraction with both F_3 and DF activity. Some of its properties will be described.

2. Materials and methods

The preparation of ^{3}H-MS_2-RNS, *N*-formyl-^{35}S-methionyl-tRNA, purified ribosomes free of initiation factors, and the isolation and separation of the initiation factors F_1, F_2 and F_3 has been described previously [11, 12].

DF activity was assayed by incubating 100 μg of purified ribosomes with DF at 37°C for 10 min (unless stated otherwise) in 0.3 ml reaction mixture containing 50 mM tris-HCl, pH 7.8, 50 mM KCl, 12 mM NH_4Cl, 5 mM Mg acetate (or 6 mM when 1.0 mM GTP was included) and 6 mM β-mercaptoethanol. The mixture was analysed by centrifugation in a 15–30% sucrose gradient of the same ionic composition for 15.5 hr at 20,000 rpm in a SW 25.3 rotor. Absorbance at 260 mμ was monitored continuously with a Gilford spectrometer and the areas under the ribosomal peaks were determined planimetrically.

Prelabelled ribosomes were prepared from *E. coli* cells pulse-labelled for 30 sec with ^{3}H-uridine and for 5 sec with ^{14}C-amino acids. The cells were ground with alumina and ribosomes were sedimented from an iS_{30}-extract [13].

3. Results and discussion

Crude factors were fractionated on a DEAE-column (cf. fig. and legend 1) [2, 4, 6]. DF activity emerged from the column in the fractions 21–40. The latter fractions were pooled and fractionated on Sephadex G-75 (fig. 2). DF activity was separated from the bulk of protein as illustrated. After either of the purification steps, a linear relationship was found between the percentage of ribosome dissociation and the amount of DF added (fig. 3), enabling the extent of

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Table 1
Purification DF activity.

Number	Fraction of DF	μg of DF causing 100% dissociation	Purification factor
I	Crude fraction [a]	67	1
II	After DEAE fractionation [b]	19	3.5
III	After Sephadex G-75 fractionation [c]	9	34

[a] Ribosomal wash precipitated with 75% $(NH_4)_8SO_4$ saturation
[b] Pool of fractions 21–40 (Fig. 1).
[c] Fraction 38 of fig. 2.

Table 2
Effect of Mg^{2+} on the dissociation.

Factor preparation	Percentage dissociation		
	5 mM Mg^{2+}	7 mM Mg^{2+}	10 mM Mg^{2+}
50 μg of crude factors	27	8.7	0
40 μg of fraction II (table 1)	53	20	0

For experimental details see Materials and methods.

Table 3
Effect of GTP on the dissociation.

Exp. number	Factor preparation	Percentage dissociation	
		+GTP (1 mM)	-GTP
1	25 μg of fraction II (table 1)	17	17
	55 μg of fraction II (table 1)	51	50
2	25 μg of fraction II (table 1)	16	16
	50 μg of fraction II (table 1)	30	32

For experimental details see Materials and methods.

purification to be calculated (table 1).

Subramanian et al. [9] found that the ability of crude factors to dissociate 70 S ribosomes decreased with increasing Mg^{2+} concentrations. A similar effect was seen with the more purified DF preparation (table 2). No antagonistic effect of added 30 S and 50 S subunits also washed with high salt concentration were observed. The straight lines relating concentration and activity of DF (fig. 3) coincided when separated 70 S ribosomes or a mixture of the latter with subunits were studied.

Recently Gonzalez et al. [10] reported that GTP stimulated the dissociating activity of their (crude) DF preparation, particularly at high levels of the latter. No effect was detectable in our case with DF after DEAE-fractionation (table 3). Even after purification on Se-

Table 4
Dissociation with DF [a] of prelabelled ribosomes.

Amount of DF	Percentage dissociation	Specific radioactivity 70 S ribosomes [b]	
		^{3}H	^{14}C
none	0	0.62	0.67
16 μg	14	0.92	0.93
55 μg	64	1.2	1.3

[a] Fraction II table 1.
[b] After sucrose gradient centrifugation 25 fractions per gradient were collected, precipitated with cold TCA, filtered through glass fibre filters and counted in a liquid scintillation counter. Counts in the 70 S region were enumerated and divided by the area under the 70 S peak. For further experimental details see text.

Table 5
MS_2-RNA-directed binding of F-^{35}S-Met-tRNA to 70 S ribosomes in the presence of initiation factors.

Exp. number	Factors added	μμmoles of F-^{35}S-Met-tRNA bound [c]
1	Fraction A [a]	0.05
2	Fraction A + DF [b]	<0.01
3	Fraction A + DF + F_2 + F_3	1.96
4	DF + F_2 + F_3	2.09
5	F_2 + F_3	0.01

[a] Fractions 1–20 of fig. 1.
[b] Fractions 21–40 of fig. 1.
[c] μμmoles of F-^{35}S-Met-tRNA bound in the absence of MS_2-RNA were substracted. 100 μg of 70 S ribosomes, 40 mμmoles GTP, 40 μμmoles of F-^{35}S-Met-tRNA, 10 μg of MS_2-RNA and 10 μg of each factor indicated in the table were incubated in the presence of 1.2 μmoles Mg acetate, 10 μmoles KCl, 4 μmoles NH_4Cl in 50 mM tris acetate buffer pH 7.2 for 12 min at 37°C (total volume 0.2 ml). After incubation the reaction was diluted with 5 ml of the same buffer, filtered through Millipore filters and the radioactivity assayed.

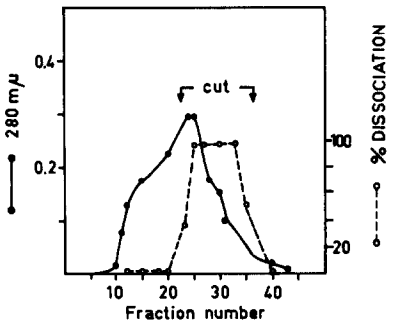

Fig. 1. Separation of DF on DEAE-cellulose. 150 mg of crude factors (fraction I, table 1, concentration 5 mg/ml) were applied to a DEAE-cellulose (1.1 X 35, Serva, 0.80 meq/g) in 0.01 M tris-HCl buffer pH 7.8, containing 6 mM β-mercaptoethanol and 0.03 M NH_4Cl (buffer A) and washed with 2 column volumes of the same buffer. Fractions of 48 drops were collected and 0.1 ml samples were taken for the dissociation tests (cf. Materials and methods).

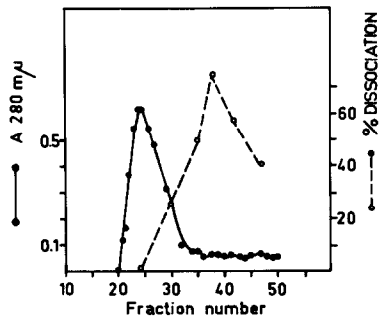

Fig. 2. Separation of DF on Sephadex G-75. Fractions 21–40 from DEAE-cellulose were pooled, precipitated with 80% $(NH_4)_2SO_4$ saturation, dissolved in about 3 ml of buffer A, and passed through the Sephadex G-75 column (1 X 80 cm). Fractions of 18 drops were collected. Absorbancy at 280 mμ was measured and dissociating activity tested as decribed in the legend to the fig. 1.

phadex G-75, 100% dissociation could be obtained in the absence of added GTP. The extent to which the various fractionation procedures are able to eliminate added GTP from DF has not been studied.

At 37°C the dissociation reaction proceeded at a high rate. As soon as the incubation mixture reached 37°C, no further dissociation was observed (results not presented). At this time DF was still active indicating that DF affects ribosomes stoichiometrically rather than catalytically, a conclusion already reached by Subramanian et al. [9]. The low dissociation at 0°C, although noticeable upon prolonged incubation, is also in agreement with Subramanian et al. [9]. DF is thermolabile. Preheating at 70°C for 5 min abolished the activity by about 90%. After 3 min incubation at in-

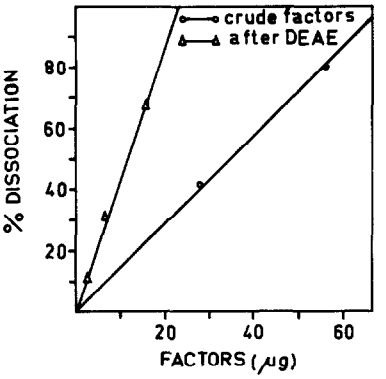

Fig. 3. Dependence of the dissociation on factor concentration. For experimental details see: Materials and methods.

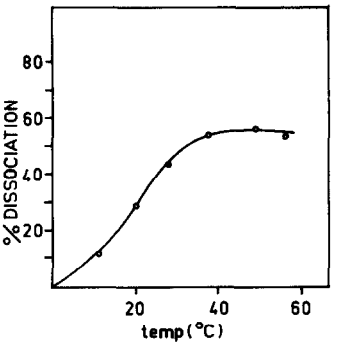

Fig. 4. Temperature-dependence of the dissociation. Conditions as described in Materials and methods. Incubation time: 3 min.

termediate temperature in the range 0–37° no full inactivation of DF occurred indicating that the dissociation reaction is temperature-dependent in this range (fig. 4). Above 37°C the stoichiometric reaction may have reached completion or may have stopped due to inactivation of the system.

Table 4 indicates that the effect of DF is restricted to so-called free 70 S ribosomes and that DF is much less or not effective with 70 S particles complexed with peptidyl-tRNA and a messenger (fragment). The specific activity of a mixture of free and complexed 70 S ribosomes, isolated from cells pulse-labelled *in vivo* with ^{3}H-uridine and ^{14}C-amino acids, increased upon DF-induced dissociation, suggesting a preferential dissociation of the most labile 70 S particles (presumably free 70 S, compare also Ron et al. [14], Subramanian et al. [9] and Algranati et al. [15]).

Closer examination of fractions 21–40 of fig. 1 revealed that they contained both F_1 and F_3 activities. A better separation of the latter two activities can be obtained by loading the DEAE column with crude factors at a lower concentration (3 mg/ml). Identification of F_1, F_3 and F_2 was achieved by measuring the MS_2-RNA-directed binding of F-^{35}S-Met-tRNA to the ribosomes in the presence of different factors and combinations (table 5). DF activity was predominantly found in fractions containing F_3. Whether more extensive purification of DF and F_3 will separate the two activities has to await further experimentation.

DF is essential for initiation of polypeptide synthesis in cell-free systems of *E. coli* lacking free 30 S ribosomes. This could be shown by similar F-Met-tRNA binding studies using purified ribosomes freed completely of 30 S ribosomes and particles by sucrose gradient centrifugation. As binding of F-Met-tRNA to such ribosomes is assumed to take place through the intermediate formation of the so-called initiation complex: 30 S-mRNA-F-Met-tRNA (compare [1]), dissociation of the 70 S ribosomes is a requisite for binding Accordingly DF(F_3) in addition to F_1, F_2 and GTP was found to be essential for binding of F-Met-tRNA using the 70 S ribosomes.

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