STUDIES ON THE RIBOSOMAL INITIATION FACTOR F_3. MULTIPLICITY OF F_3 AND DF ACTIVITIES

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1. Introduction

Among the ribosomal factors implicated in polypeptide chain initiation in *E. coli* F_3 plays a crucial role. It is required for specific translation of natural messengers and is assumed to mediate the binding of the 30 S ribosomal subunit to the messenger [1–3]. Another ribosomal factor has been described by Subramanian et al. [4–7] which causes dissociation of 70 S ribosomes into subunits and which is designated DF. The relationship of DF to initiation factor F_3 has been studied by purifying F_3 [5] and DF [8, 9]. A preparation of F_3 has been obtained by Sabol et al. [10] and Dubnoff and Maitra [11] which behaves as a homogeneous protein and exhibits DF activity suggesting that F_3 and DF represent one and the same protein (compare also [7]).

Recently, evidence for specific recognition of different initiator sites on messengers has been obtained [12–16, 25] suggesting either the existence of multiple F_3 factors or modification of F_3 specificity by other proteins. Accordingly several species of F_3 have been isolated which were characterized by their differences in activity for the translation of T_4 mRNA and MS_2-RNA [17]. After T_4 infection of *E. coli* cells, the modification in template specificity has been accounted for by a change in F_3 factor activity [18].

In the present paper we report on our fractionation of F_3. Multiple F_3 fractions have been obtained with differential activities towards different messengers. Some of them display significant F_3 activity, but are virtually inactive in causing dissociation of 70 S ribosomes.

2. Materials and methods

2.1. *Preparation of purified ribosomes*

Crude ribosomes (see [23]) were washed with 1 M NH_4Cl in 0.01 M Tris-HCl, pH 7.8, 0.01 M Mg acetate and 0.006 M β-mercaptoethanol by stirring overnight at 4°. They were spun down, resuspended in the same buffer containing 0.06 M, instead of 1 M, NH_4Cl and dialysed against the latter buffer.

When used as substrate for DF assay, the ribosomes were dialysed a second time at 0.005 M Mg and fractionated in a B XIV zonal rotor using a 750 ml convex exponential sucrose gradient (10–40%). The 70 S particles were collected; they were virtually free of 50 S and 30 S subunits (cf. fig. 1). When used for the F-met-tRNA binding reaction (cf. legend of fig. 2) the ribosomes were washed twice with 1 M NH_4Cl and dialysed against 0.01 M Mg acetate.

2.2. *Preparation of F_1, F_2, F_3, MS_2-RNA and F-^{3}H-met-tRNA*

Crude factors were isolated as described previously [9]. To the preparation $(NH_4)_2SO_4$ was added to 55% saturation. The precipitate was used to prepare F_2 according to Hershey et al. [19]. The supernatant was supplemented with $(NH_4)_2SO_4$ to 80% saturation. The precipitate was dissolved in buffer A (20 mM NH_4Cl, 6 mM β-mercapto-

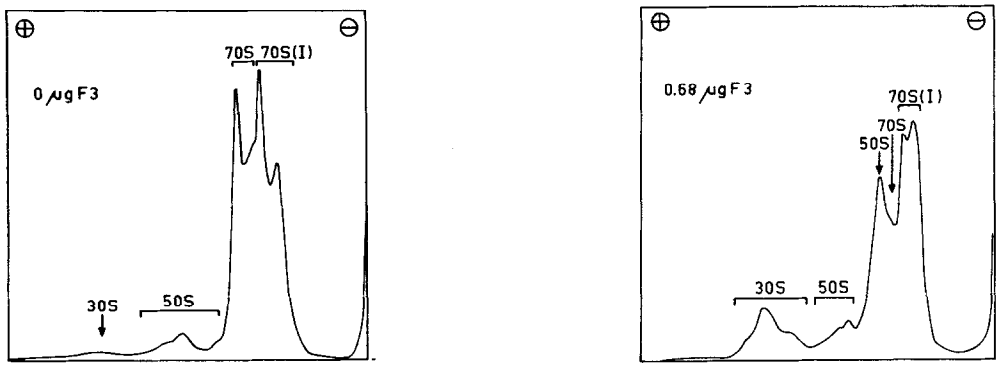

Fig. 1. Electrophoretic analysis on polyacrylamide gels of separated 70 S ribosomes incubated in the presence and absence of DF. For experimental details compare sect. 2.3 and the legend to fig. 2.

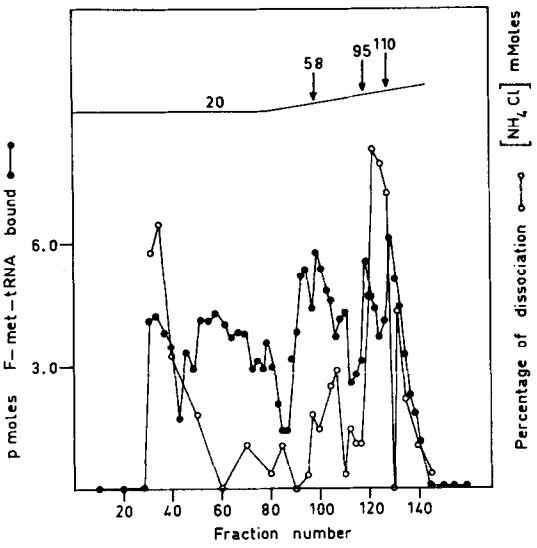

Fig. 2. Fractionation of F_3 (DF) on DEAE cellulose. A dialyzed solution containing F_3 (compare sect. 2.2) was put on a column (2.5 × 50 cm) of Serva-DEAE-SH (1.0 meq/g) and eluted with buffer A. After about 50 fractions (each of 48 drops) were collected, a gradient of 20–250 mM NH_4Cl in buffer A was started. F_3 activity was assayed in 0.01 ml of each fraction by adding an excess of purified F_1 and F_2, 120 μg of purified ribosomes, 30 pmoles of F-^{3}H-met-tRNA, 10 μg of MS_2-RNA and 0.02 μmole of GTP. The final solution (0.1 ml) contained 7 mM Mg acetate, 50 mM K acetate, 10–30 mM NH_4Cl, 6 mM β-mercaptoethanol and 50 mM Tris-HCl, pH 7.2. DF activity was assayed in 0.05 ml of each fraction by adding 15 μg of purified 70 S ribosomes and incubating them for 10 min at 37° in a reaction mixture (total volume 0.1 ml) containing 0.01 M Tris-HCl, pH 7.8, 0.05 M KCl, 0.012 M NH_4Cl, 0.004 M Mg acetate and 0.006 M β-mercaptoethanol (buffer B). The reaction mixtures were submitted to electrophoresis on polyacrylamide gels (compare sect. 2.3).

ethanol, 20 mM Tris-HCl, pH 7.7) and dialysed against buffer A. The dialysed solution containing F_3 was put on a DEAE-cellulose column and fractionated according to the legend of fig. 1. Initiation factor F_1 was prepared as described by Hershey et al. [19]. MS_2 phage was grown and isolated according to Strauss and Sinsheimer [20] and the RNA was extracted as described by Boedtker and Stumpp [21].

For the preparation of F-^{3}H-met-tRNA, see Voorma et al. [22].

2.3. *Polyacrylamide gel electrophoresis of ribosomes*

The preparation of polyacrylamide gel has been described previously [23]. During electrophoresis the buffer from both cathode and anode compartments was pumped around and cooled simultaneously in a cryostate. For further details compare [23] except that buffer C of the previous paper was modified as follows: 0.01 M Tris-acetate, pH 7.8, 0.05 M K acetate, 0.012 M NH_4 acetate and 0.004 M Mg acetate. In general about 30 gels were run simultaneously. The electrophoresis procedure thus slightly modified revealed even more heterogeneity than previously [23] observed. A representative experiment showing the effect of DF on separated 70 S ribosomes is illustrated in fig. 1.

3. Results

A crude factor preparation containing F_1, F_3 and DF but no F_2 activity was obtained by $(NH_4)_2SO_4$ precipitation of the ribosomal wash (compare sect.

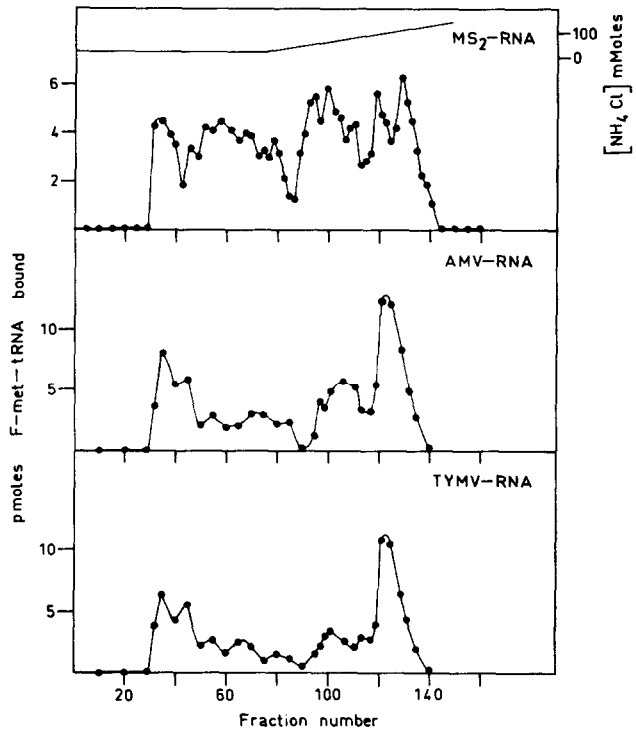

Fig. 3. Multiplicity of F_3 assayed in the presence of different viral messengers. F_3 assays were identical to those of fig. 2. In the experiments with the plant viral messengers 10 μg of each messenger was used.

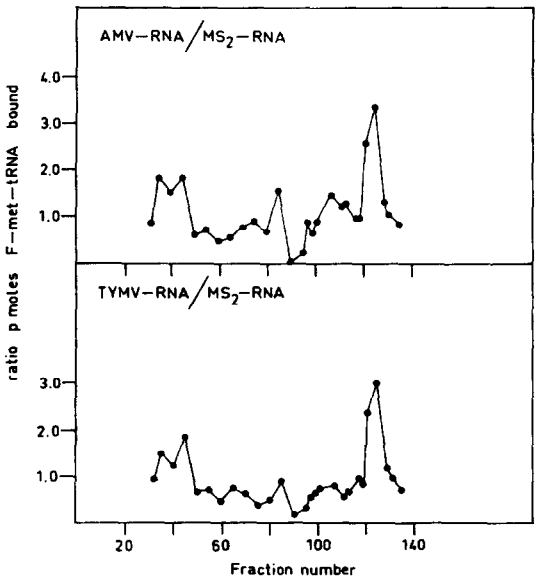

Fig. 4. Ratios of F_3 activities assayed in the presence of different viral messengers. The ratios were computed from the data of fig. 3.

2.2). This preparation was fractionated on a column of DEAE cellulose (fig. 2). Elution with 20 mM NH_4Cl was followed by applying a gradient of 20–250 mM NH_4Cl. F_3 activities were assayed by supplementing each fraction with purified F_1 and F_2 and measuring the binding of F-^{3}H-met-tRNA to complexes of ribosomes and MS_2-RNA in the presence of GTP. As is evident from fig. 2 a great number of fractions with F_3 activity can thus be separated. The various fractions display differential activity towards different messengers. This is illustrated in fig. 3, in which the fractions were similarly assayed for F_3 activity but using 2 plant viral messengers: RNA derived from alfalfa mosaic virus (AMV) and RNA from turnip yellow mosaic virus (TYMV). The differences are brought out more clearly by computing the ratios of the binding activities for the various fractions (fig. 4). Fraction 125, for instance, preferentially stimulates ribosome binding to plant viral messengers.

Although this fractionation separates fractions with different messenger selection abilities, no cistron selection on one and the same messenger could be detected with the same procedure (see footnote*). The relative accessibility of the 3 cistrons on MS_2-RNA was not found to be altered by any F_3 fraction. (This obviously does not necessarily imply that further fractionation cannot yield F_3 fractions with different cistron specificities.)

The elution of F_3 from DEAE cellulose is altered profoundly after gel filtration in the presence of urea. This follows from experiments in which fractions 31–40, 96–105, 117–123 and 125–135 were combined, respectively. The 4 combinations were each supplemented with 6 M urea and submitted to gel filtration on Sephadex G-75 equilibrated with 6 M urea. The Sephadex fractions were dialysed to

* Cistron selection was determined by assaying the fractions of fig. 2 for their ability to recognize the initiator regions on MS_2-RNA after disruption of the secondary structure of the latter according to Voorma et al. [22]. The binding of labelled alanyl-tRNA, seryl-tRNA and arginyl-tRNA to the ribosomes was determined in the presence of cold F-met-tRNA, GTP and each fraction complemented with purified F_1 and F_2 (not illustrated).

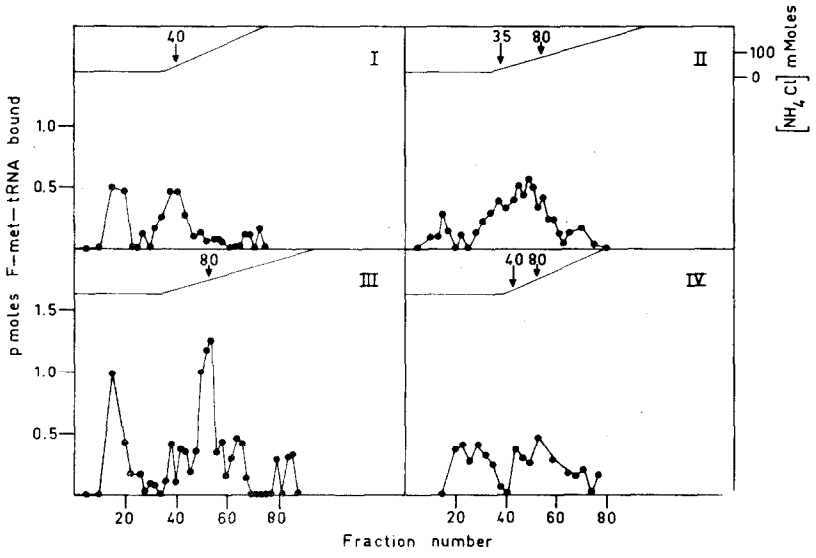

Fig. 5. Refractionation of F_3 on DEAE cellulose after gel filtration in the presence of urea. Diagram I illustrates the refractionation of the fractions 31–40 of fig. 2. The latter fractions were combined, concentrated, supplemented with urea to 6 M and fractionated on Sephadex G-75 in 6 M urea. After dialysis (see text) the active fractions were combined and refractionated on DEAE cellulose. Diagrams II, III and IV show the results obtained with the following combinations of fractions: 96–105, 117–123 and 125–135, respectively.

eliminate urea, assayed for F_3 activity and the combined active fractions in each of the 4 cases were rechromatographed on DEAE cellulose (fig. 5). The procedure separates F_3 activity from inactive proteins. The second fractionation on DEAE cellulose still reveals, however, a multiplicity of F_3 peaks.

What is the relationship of the various F_3 fractions of fig. 2 to DF? Do they all display DF activity? From fig. 2 it can be concluded that like F_3, DF also varies from fraction to fraction, although not to the same extent. DF activities were assayed by incubating the fractions with separated 70 S ribosomes and analyzing the reaction mixtures by electrophoresis on polyacrylamide gels (compare sect. 2.3 and [23]). The latter procedure not only permits a rapid assay of a great number of fractions simultaneously but also reveals changes in the ribosomes which cannot be observed with sucrose gradient centrifugation (compare discussion).

Fig. 2 shows a great variation in DF/F3 ratios which even reach zero values in a number of fractions (fractions 60, 90 and 130). In a previous paper [23] it has been shown that 70 S ribosomes incubated with DF are converted to 70 S particles which migrate slower than the original 70 S ribosomes during gel electrophoresis. In fig. 6 the effect of the DEAE fractions 60, 90 and 130 of fig. 2 on 70 S ribosomes is illustrated. These fractions do not cause dissociation of 70 S ribosomes but their effect on the migration profile during polyacrylamide gel electrophoresis is profound.

4. Discussion

This paper shows a multiplicity of F_3 and DF activities when a rather crude factor preparation is fractionated on DEAE cellulose. It should be emphasized that both the F_3 and DF activity profile can be drastically altered by changing the elution scheme. For instance, lowering of the NH_4Cl concentration from 20 to 0 mM before starting the gradient yields one large F_3 zone containing DF, but without separation of subfractions. This finding and the altered behaviour of F_3 on DEAE cellulose after gel filtration in the presence of urea (fig. 5)

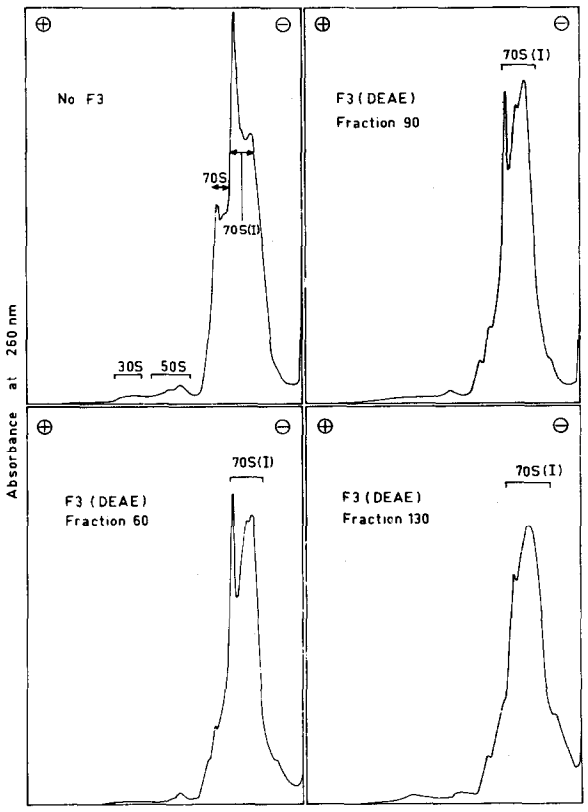

Fig. 6. Electrophoresis on polyacrylamide gel of 70 S ribosomes treated with various F_3 fractions obtained after fractionation on DEAE cellulose.

suggest that F_3 molecules readily complex with other protein molecules or with themselves. It is not excluded that such a complex formation is responsible for the alteration in specificity of F_3 towards different messengers (compare fig. 3) and/or for the variation in DF/F_3 ratios in the various fractions (fig. 2). In particular the fractions 60, 90 and 130 of fig. 2 which do not cause dissociation of 70 S ribosomes under the experimental conditions of the assay are noteworthy. Unless one assumes that protein molecules exist, endowed with F_3 but devoid of DF activity, one is led to postulate that the factor activity can be modified by other proteins in each fraction or by complexing of two or more factor molecules themselves. Modulating proteins may exert a differential effect on F_3 and DF activity.

The cardinal question is, of course, how F_3 is able to mediate a specific interaction between the ribosome and mRNA. It is conceivable that specific spatial conformations of the initiator sites on the messenger compel the ribosome to accommodate to these structural requirements. If F_3 is to mediate such an interaction it may well itself induce conformational alterations in the ribosome structure. Alternatively, F_3 interacts directly with structural elements of the messenger and thus "recognizes" initiator regions. *A priori,* both possibilities need not be mutually exclusive. In favour of an indirect function of F_3 is the finding that F_3 is able to alter the behaviour of the ribosomes when analyzed by polyacrylamide gel electrophoresis. Even when dissociation into subunits does not occur, for instance at low F_3 (DF) concentrations [23] or in the case of fig. 6 (with fractions 60, 90 and 130) such alterations are clearly detectable. Free electrophoresis of the ribosomes under comparable conditions does not reveal such alterations in electrophoretic mobility. (In fact it does not even separate 70 S and ribosomal subunits; [24].) These data suggest that F_3 (DF) induces conformational changes in the 70 S ribosome, which may result in dissociation when the F_3 (DF) concentration is raised beyond a certain value [23]. Although it is hypothetical that F_3 fulfils its specific function of messenger and cistron selection by altering the ribosomal conformation, this concept relates operationally the 2 functions of F_3: translational control and ribosome dissociation. A multiplicity of F_3 with differential activities towards a variety of messengers has also been demonstrated by Grünberg-Manago, Gros and their colleagues [25]. They also found F_3 fractions devoid of DF activity.

Acknowledgements

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