DISSOCIATION OF 70 S *E. COLI* RIBOSOMES INDUCED BY A RIBOSOMAL FACTOR (DF). ELECTROPHORETIC STUDIES OF THE RIBOSOMAL PARTICLES

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The dissociation of purified 70 S *E. coli* ribosomes, induced by the dissociation factor DF, has been studied by submitting the reaction mixtures to electrophoresis on polyacrylamide gels. The electrophoretic analysis of the ribosome mixtures revealed a heterogeneity which escaped detection by conventional sucrose gradient centrifugation. Increasing amounts of DF in the reaction mixtures converted 70 S ribosomes to particles (designated 70 S (I)) which migrate slower in the electric field than the original 70 S ribosomes. These 70 S (I) ribosomes still consist of both subunits. They dissociate upon further raising the DF concentration.

1. Introduction

Recently Subramanian et al. [1, 2] described a ribosomal protein factor which causes dissociation of 70 S ribosomes of *E. coli*. This so-called dissociation factor (DF) has attracted the attention of various investigators [3–7] as it is assumed to be essential for run-off 70 S ribosomes to enter a new round of protein synthesis [1, 2, 8]. In a previous paper we reported on the partial purification of DF and on some of its properties [6]. We have now studied the dissociation process in more detail using polyacrylamide gel electrophoresis. A new class of ribosomal particles can be detected after interaction of DF with 70 S ribosomes, which dissociate at higher DF concentrations.

2. Materials and methods

2.1. *Preparation of purified 70 S ribosomes*

Crude ribosomes were isolated from an iS30 extract prepared according to Nirenberg and Matthaei [9] from *E. coli* Q 13 (RNase I⁻). The 70 S particles were separated in a B XIV zonal rotor using a 750 ml convex exponential sucrose gradient (10–40%). Fractions of the 70 S peak were collected, diluted with buffer A (0.01 M tris-HCl, pH 7.8, 0.06 M NH_4Cl, 0.01 M Mg-acetate) and centrifuged overnight in the Spinco rotor 30 at 30,000 rpm. The particles were washed twice with buffer A containing 1 M instead of 0.06 M NH_4Cl. The final pellet was suspended in buffer A with a Mg concentration of 0.005 M instead of 0.01 M and the 70 S particles were isolated by zonal centrifugation.

2.2. *Preparation of DF*

Crude factors were fractionated on DEAE-cellulose as described previously [6]. Similar results were obtained with DF which was purified further on Sephadex G-75.

2.3. *Dissociation of 70 S ribosomes by DF*

Purified 70 S ribosomes (15 μg) were incubated with DF at 37° for 10 min in 0.1 ml reaction mixture containing 0.05 M tris-HCl, pH 7.8, 0.05 M KCl, 0.012 M NH_4Cl, 0.005 M Mg acetate and 0.006 M β-mercaptoethanol (buffer B).

2.4. *Preparation of polyacrylamide gels*

Gels (4% w/v) were prepared essentially according

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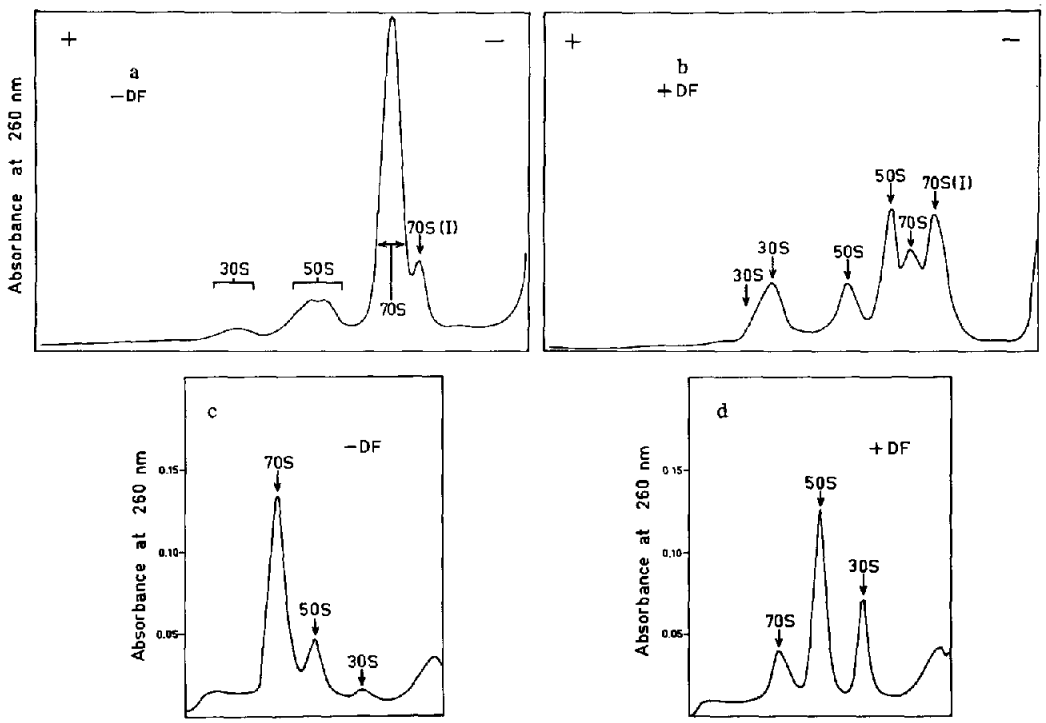

Fig. 1. DF-induced dissociation of 70 S ribosomes. In the experiments (b) and (d) 4.8 μg of DF per 15 μg of ribosomes was used. Sucrose gradient experiments were performed with 90 μg of ribosomes.

to Loening [10] by diluting stock acrylamide solution (19% acrylamide and 1% of methylenebisacrylamide in H_2O) five fold with buffer and H_2O to reach the ionic composition of buffer C (0.05 M tris-acetate pH 7.8, 0.074 M ammonium acetate and 0.005 M Mg acetate). After adding *N, N, N', N'*-tetramethylethylenediamine (0.033 ml) and 10% (w/v) ammonium persulphate (0.33 ml) per gram of acrylamide–bisacrylamide, the mixture was pipetted into perspex tubes (0.8 cm diameter). The polymerized gels were blown out of the tubes and stored in buffer C for at least 72 hr at 4° (during which the gels swell). Pieces of 4 cm lengths were inserted into electrophoresis tubes.

2.5. *Electrophoretic analysis of ribosomal mixtures*

Pre-electrophoresis was performed for 30 min at 5 V/cm and 15 mA/gel. Ribosome mixtures (0.1 ml) supplemented with 0.03 ml of 20% sucrose in buffer C were then applied and electrophoresis was continued for 10 min at 2.5 V/cm and 7.5 mA/gel and subsequently for 3 hr at 5 V/cm and 15 mA/gel. After electrophoresis the gels were scanned continuously at 260 nm in a Zeiss spectrophotometer. Areas under the ribosomal peaks were determined with an electronic curve resolver (Dupont 310).

3. Results and discussion

A two-dimensional electrophoretic separation of bacterial ribosomes and subunits in polyacrylamide–agarose composite gels has been described by Dahlberg et al. [11]. We have modified their procedure by performing the electrophoresis in cylinders of 4% polyacrylamide gel (omitting agarose; for further details see Materials and methods). As is evident from fig. 1a and b these gels can readily be scanned directly at 260 nm. For comparison aliquots of the same mixtures have also been analyzed by the conventional sucrose gradient centrifugation technique (fig. 1c and d). Electrophoresis apparently reveals a heterogeneity which escapes detection by sucrose gradient centrifugation. The individual zones of ribosomal particles were identified by cutting 0.5 mm slices out of the gel and in-

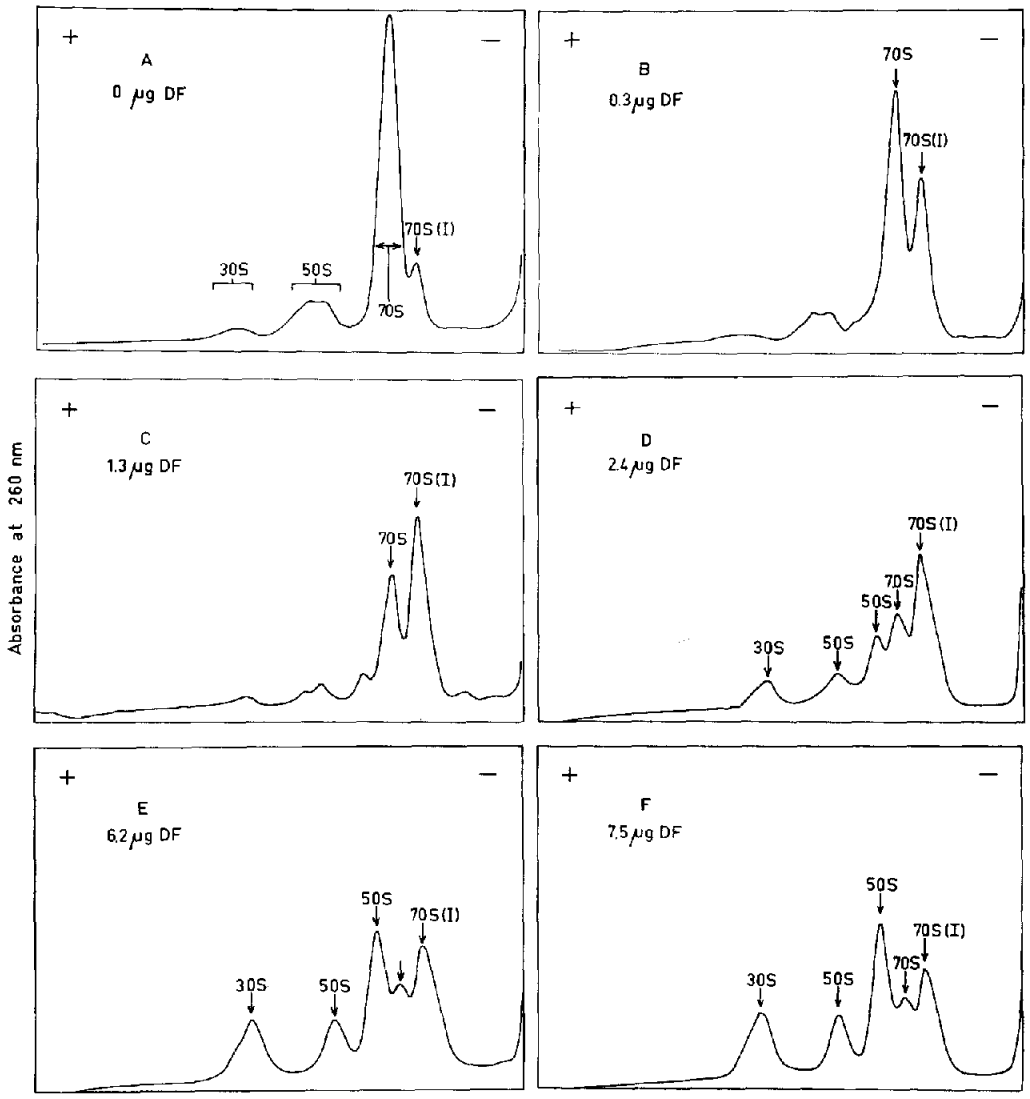

Fig. 2. Dissociation of 70 S ribosomes with increasing amounts of DF (compare Materials and methods).

troducing them into a new gel containing 0.002 M EDTA, 0.04 M tris-acetate, 0.02 M Na acetate pH 7.8 and 0.2% SDS. In this new gel RNA was released from the ribosomal particles and could be analysed electrophoretically. Identification of 16 S and/or 23 S RNA on these gels enabled us to assign sedimentation coefficients to the ribosomal particles in the electropherograms of fig. 1.

The interaction of DF with 70 S ribosomes results in the formation of ribosomes (designated 70 S (I)) which migrate slower during electrophoresis than the original 70 S particles (fig. 1b). The dissociation also results in the appearance of at least two classes of 50 S ribosomes and two classes of 30 S ribosomes. In this paper we will pay special attention to the 70 S (I) particles.

In a second series of experiments (fig. 2) an increase in the concentration of DF caused the original 70 S particle to dissociate more, proportionally raising the amount of 70 S (I) particles until a maximum was

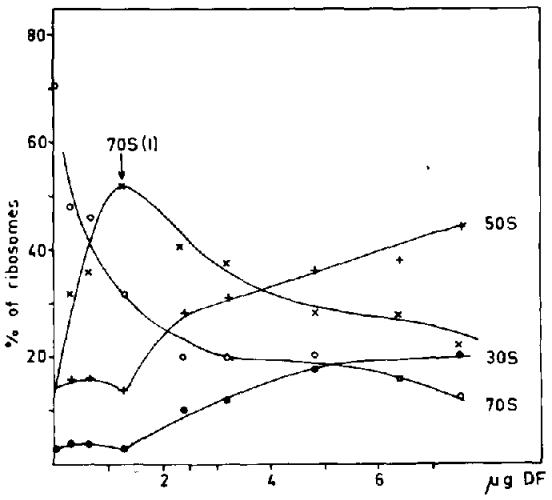

Fig. 3. The relation between the amount of DF and the proportion of each ribosomal species (see text and fig. 2).

reached. No increase in the amount of 30 S and 50 S ribosomes was observed with DF in concentrations required for sub-maximal conversion of 70 S to 70 S (I) ribosomes (fig. 2A, B and C). Dissociation into subunits did occur, however, when the DF concentration was raised further (fig. 2D, E and F). At concentrations of DF which did not cause significant increases in the total quantity of 50 S ribosomes (fig. 2A, B and C) an alteration of the 50 S profile was always detected. Other electrophoresis experiments [12] with larger amounts of separated 50 S ribosomes also revealed an interaction of DF with these particles. Experiments are in progress to study the consequences of this interaction.

To estimate the proportions of the various particles in the reaction mixtures more quantitatively, an electronic curve resolver (see Materials and methods) was used. The quantity of each ribosomal species was expressed as a percentage of the total amount of ribosomes and plotted against the DF concentration (fig. 3). These data suggest that under the present conditions 70 S (I) ribosomes function as intermediates in the DF-induced dissociation of purified 70 S ribosomes (washed with 1 M NH_4Cl and fractionated twice on sucrose gradients, see Materials and methods).

Comparison of the results of electrophoretic and sedimentation analysis shows that part of the 70 S (I) ribosomes dissociates spontaneously during sucrose gradient centrifugation but remains intact during migration in the gel (data not illustrated here). This could explain our previous observation [6] and those of others [7, 13] that a linear relationship exists between the percentage dissociation (as measured by sedimentation) and the quantity of DF. Such a relationship was not apparent in fig. 3.

What is the nature of the 70 S (I) ribosomes? Electrophoretic analysis of the RNA released from these particles, revealed the presence of both 16 S and 23 S RNA. The sedimentation coefficient of these ribosomal particles is slightly lower than 70 S as may be concluded from an experiment in which a reaction mixture with DF was analysed by sucrose gradient centrifugation (fig. 4, upper left diagram). Individual sucrose fractions A, B and C, subsequently analysed by electrophoresis (diagrams A, B and C) revealed a preponderance of 70 S (I) particles at the slower sedimenting side of the 70 S peak in the sucrose gradient. The difference in sedimentation rate between 70 S and 70 S (I) ribosomes apparently is quite small and has recently [12] been estimated to range between 2 and 4 S. Presumably it reflects a DF-induced conformational alteration. Although the suggestion (see above) that such an alteration represents an intermediate stage in the dissociation process is an attractive one, no decisive evidence on this point is available as yet. It remains possible that under the present conditions part of the 70 S particles dissociate directly to the free subunits and another part via the 70 S (I) ribosomes. Furthermore it may be envisaged that conversion to the latter particles is brought about by an agent other than DF, present in the DF preparation employed. From figs. 2 and 3 it is clear, however, that the fall in 70 S (I) ribosomes caused by increasing amounts of DF, is accompanied by a rise in subunit content.

The specific activity of DF cannot be defined unless the methods to estimate the extent of dissociation and to prepare the ribosomal substrate, are rigidly standardized. This conclusion is based on our finding [12] that the amount of DF required for full dissociation is dependent upon the history of the 70 S ribosomes. Preincubation at 5 mM Mg^{2+} in the absence of DF makes the 70 S ribosomes more liable to dissociation by DF, whereas preincubation at 15 mM Mg^{2+} or in the presence of tRNA makes them more resistant to DF. The DF-in-

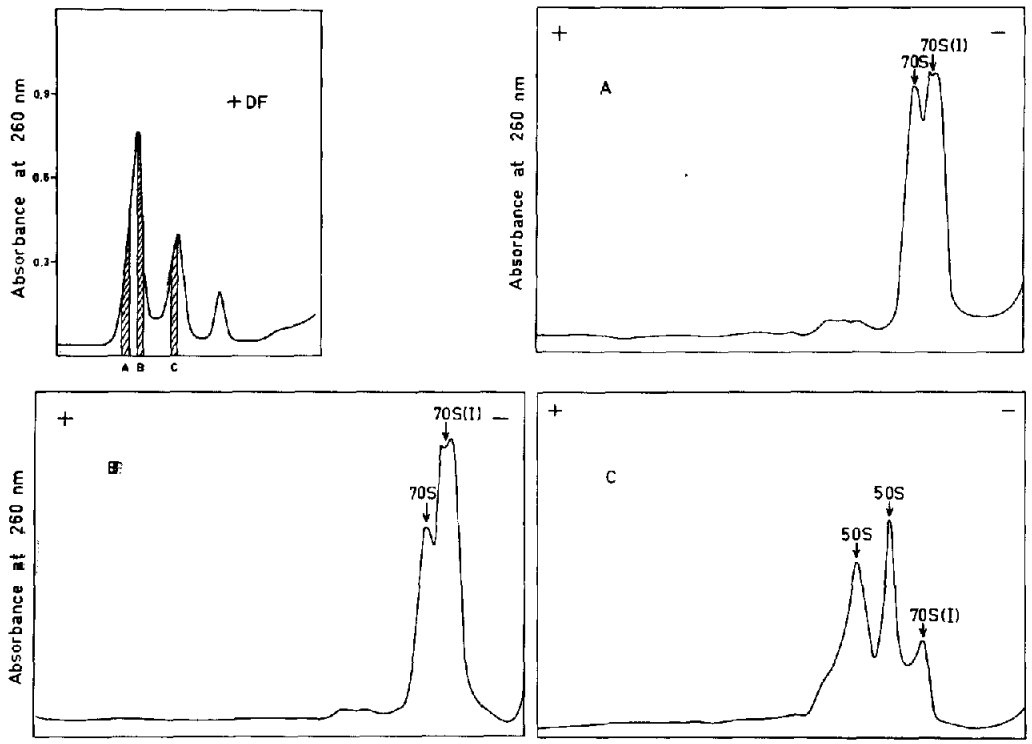

Fig. 4. Electrophoretic analysis of individual fractions obtained after sucrose gradient centrifugation of a reaction mixture containing DF. Purified 70 S ribosomes (300 μg) were incubated with 38 μg of DF and submitted to sucrose gradient centrifugation. Sucrose fractions A, B and C varied from 0.2 to 0.3 ml.

duced conversion of 70 S ribosomes to 70 S (I) particles reported here, was observed with highly purified ribosomes at 5 mM Mg^{2+}. Additional experiments are necessary to see to which extent this is a general phenomenon. Polyacrylamide gel electrophoresis of intact ribosomal particles seems to be the analytical procedure of choice for studying such ribosomal changes.

Acknowledgments

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Note added in proof

Recently Dr. M.M. Mirault, ISREC, Lausanne, Switserland informed us that he has also worked out an electrophoretic separation of ribosomal particles.

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