DISSOCIATION OF MITOCHONDRIAL RIBOSOMES OF NEUROSPORA CRASSA BY A BACTERIAL DISSOCIATION FACTOR.

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Summary

From ribosomes of *Escherichia coli* a protein factor can be obtained that promotes dissociation of bacterial ribosomes into subunits. Incubation of mitochondrial ribosomes from *Neurospora crassa* with the bacterial dissociation factor also leads to the formation of subunits. Under the same conditions no dissociation of cytoplasmic ribosomes from *Neurospora crassa* was observed.

Introduction

It has been shown previously (1-3) that ribosomes from *E.coli* contain a protein factor that is capable of dissociating *E.coli* ribosomes into subunits. This dissociation factor (DF) is present in the 1 M NH_4Cl wash from the ribosomes.

We have investigated the effect of *E.coli* DF on mitochondrial and cytoplasmic ribosomes from *N.crassa*. We know that mitochondrial and bacterial protein synthesis have many aspects in common. As far as the ribosomes are concerned the resemblance is more clearly demonstrated by functional behaviour than by physical characteristics (4). Both types of ribosomes have in common e.g. the need of fMet-tRNA for the initiation of protein synthesis (5) and the sensitivity of their peptidyl transferase to chloramphenicol (6). On the other hand physical characteristics such as the molecular weights of ribosomes and ribosomal RNAs show a much greater range for mitochondrial than for bacterial ribosomes. Since dissociation of ribosomes is a functional step in protein synthesis, it was tempting to investigate whether bacterial DF could also induce dissociation of mitochondrial ribosomes,

or that mitochondrial and bacterial ribosomes are different in this respect.

Materials and Methods

Ribosomes and DF were isolated from *E.coli* strain Q13 as described by ALBRECHT et al.(3). The DF used in our experiments was the crude preparation obtained as the precipitate between 30 % and 80 % saturation with ammonium sulphate from the 1 M NH_4Cl wash from *E.coli* ribosomes.

Mitochondria were obtained from *N.crassa*, strain 5256, using a grindmill as described previously (6). Mitochondrial ribosomes from *N.crassa* were isolated following a procedure of GRIVELL et al. (7) for mitochondrial ribosomes from yeast. This includes the incubation of mitochondria with puromycin. Cytoplasmic ribosomes from *N.crassa* were isolated as described by KÜNTZEL (8).

For the dissociation experiments *E.coli* ribosomes and cytoplasmic ribosomes were washed with 1 M NH_4Cl. Mitochondrial ribosomes were used unwashed, since treatment with 1 M NH_4Cl caused already complete dissociation. Dissociation was studied on ribosomes isolated from isokinetic sucrose gradients. Incubations with DF were carried out in a total volume of 0.5 ml for 10 min at 30° C in a medium containing 50 mM tris/HCl pH 7.8, 5 mM $MgCl_2$, 60 mM NH_4Cl, 6 mM β-mercaptoethanol, about 100 µg ribosomes and 0.05 to 0.15 mg DF. Control experiments were done under the same conditions without adding DF. Dissociation was followed on isokinetic sucrosegradients.

Results

Ribosomes from *E.coli* and from mitochondria of *N.crassa* showed sedimentation values of 70 S and 71 S - 73 S respectively as compared to 77 S cytoplasmic ribosomes from *N.crassa*. In previous experiments it has been demonstrated that the peptidyltransferase of mitochondrial ribosomes is inhibited by chloramphenicol and not by anisomycin, this in contrast to the peptidyltransferase of cytoplasmic ribosomes (6). This means that mitochondrial ribosomes are similar in this functional

Table 1. Poly(U)- and MS_2-directed phenylalanine incorporation by *E.coli* and *N.crassa* mitochondrial ribosomes

Type of ribosomes	[^{14}C] Phe incorporation (pmoles/mg RNA)			
	Experiment 1		Experiment 2	
	+poly(U)	-poly(U)	+MS_2	-MS_2
E.coli	4990	235	460	170
N.crassa mitochondrial	1970	230	324	187

Phenylalanine incorporation was measured according the procedure as described by NATHANS (8). In all systems an *E.coli* S100 was used, isolated according NIRENBERG (9). Incubation took place during 30 min at 30° C. All values are corrected for blanks without ribosomes.

respect to bacterial ribosomes. We thought it useful however to test the mitochondrial ribosomes for functional activity also under less artificial conditions then used in the assay for peptidyl transferase activity. As the test for functional activity we choose the poly(U)- and MS_2-directed phenylalanine incorporation measured in a cell free system. In table 1 the results of two typical experiments are shown. In the different experiments incorporation activities ranged from 10 to 40 % of the values obtained with *E.coli* ribosomes. Notwithstanding this variability in results we may conclude that intact and functionally active ribosomes were isolated.

Upon incubation with DF mitochondrial ribosomes showed an increase in the amount of subunits, accompanied by a concommitant decrease of ribosomes present (Fig.1). The dissociation followed a pattern that resembled that of *E.coli* ribosomes quite closely; the formation of subunits increased with higher concentrations of DF. The dissociation process differed on the following minor points. Firstly, the degree of dissociation was somewhat less then with *E.coli* ribosomes at the concentrations of DF used. Secondly, when the incubation was carried on for longer periods of time or at higher temperatures the 2 : 1 ratio for the large and small subunit peaks was lost; a relatively higher peak was formed at the

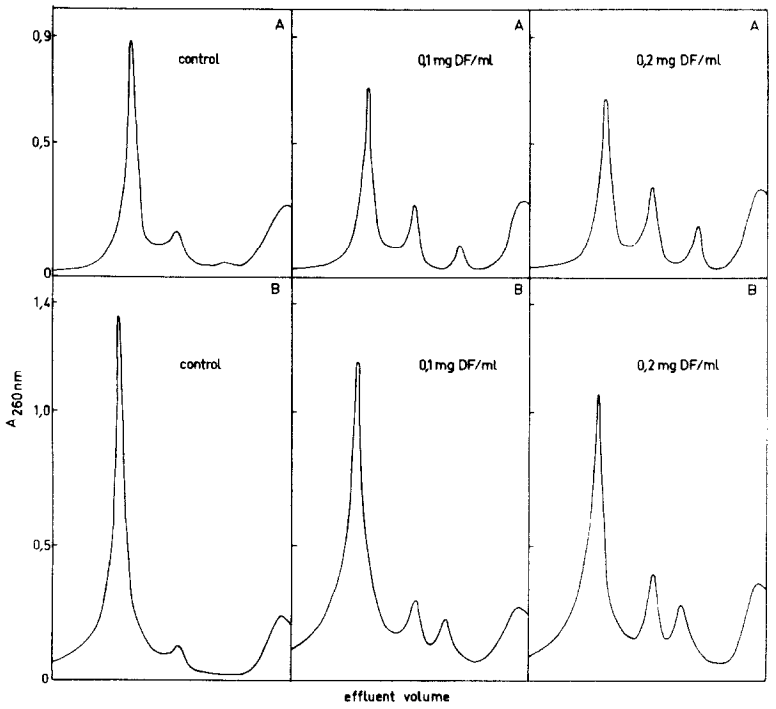

Fig. 1. Sedimentation profiles of *E.coli* ribosomes (A) and *N.crassa* mitochondrial ribosomes (B) after incubation with *E.coli* DF. After incubation the ribosomes were spun through isokinetic sucrose gradients in SW27-1 tubes during 15 h at 22,000 rpm at 4° C in a Spinco ultracentrifuge L2-65B. The sucrose concentration at the top of the gradient was 15 % (w/v). Salt concentrations in the gradient were 100 mM NH_4Cl, 10 mM $MgCl_2$ and 10 mM Tris/HCl (pH 7.8).

position of the small subunit peak. This effect is not likely to be caused by ribonucleases in the DF preparation, as could be concluded from the fact that 28 S liver ribosomal RNA appeared not at all to be degraded after incubation with excess DF. RNA analysis was performed by polyacrylamidegel electrophoresis. Most probably the relative greater instability of the mitochondrial ribosome as compared to bacterial and cytoplasmic ribosomes is responsible for this phenomenon.

The effect of *E.coli* DF on cytoplasmic ribosomes from *N.crassa* was investigated too. As can be seen in fig. 2 no formation of subunits took place under conditions where *E.coli* ribosomes readily formed subunits.

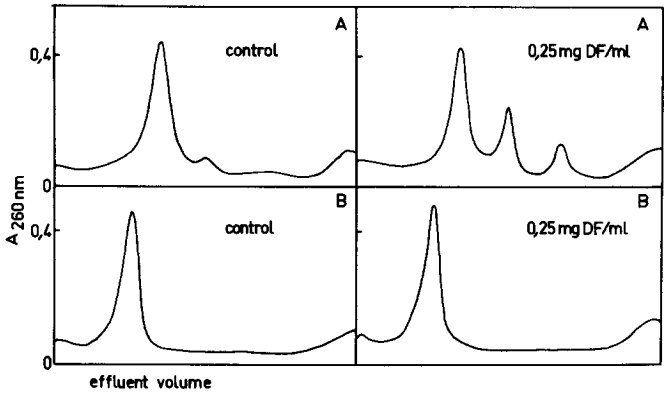

Fig. 2. Sedimentation profiles of *E.coli* ribosomes (A) and *N.crassa* cytoplasmic ribosomes (B) after incubation with *E.coli* DF. For technical data see legend to fig. 1.

From these experiments it follows that ribosomes from *N.crassa* mitochondra and *E.coli* are both susceptible to dissociation by a *bacterial* protein factor. This property is not shared by the cytoplasmic ribosomes from *N.crassa*. The role generally alloted to DF is to supply subunits for the formation of subsequent initiation complexes. The fact that *E.coli* DF also dissociates mitochondrial ribosomes indicates that in both systems factor mediated dissociation of ribosomes occurs. The absence of any effect of *E.coli* DF on cytoplasmic ribosomes once again stresses the functional similarities of mitochondrial and bacterial protein synthesis.

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