RUN OFF RIBOSOMES OF *ESCHERICHIA COLI*: THEIR ACCUMULATION IN RESPONSE TO COLD TREATMENT AND THEIR DISSOCIATION DURING CENTRIFUGATION

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1. Introduction

Incubation of *Escherichia coli* at low temperature is one of the means which may be used to obtain polysomal run off [1–3]. In this context, Friedman et al. [2] reported that the products of polysomal run off induced at low temperature ($\leqslant 8°$) are ribosomal subunits. In the experiments reported here, we show that under our conditions a low (5–6°) temperature treatment results in the accumulation of 70 S ribosomes while the proportion of subunits increases only slightly; these run off monosomes are subject to dissociation by ribonuclease. There have been several reports of bacterial ribosomes with apparently reduced sedimentation coefficients [4–6]. However, studies of the sedimentation properties of free ribosomes from the sea urchin egg have led Infante and Baierlein [7] to conclude that dissociation during centrifugation provoked by high hydrostatic pressure can produce sucrose gradient profiles in which ribosomes appear to sediment with a lower sedimentation constant. In this report we show that during prolonged centrifugation the 70 S ribosomes produced by low temperature-induced run off in *E. coli* appear to sediment more slowly than complexed 70 S ribosomes, and that this effect is due in part at least to dissociation during centrifugation.

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2. Materials and methods

E. coli strain MRE-600 was grown in rich medium [8] at 37°. When the A_{550} reached 0.7 (Zeiss PMQII spectrophotometer), the cultures were cooled in $\leqslant 15$ sec to 2°, harvested, and lysed [9]. Polyacrylamide gel electrophoresis was carried out at a gel concentration of 2.6% in the presence of sodium dodecyl sulfate [10, 11].

3. Results

Fig. 1a shows a typical polysomal profile of cells which were lysed immediately (T_0) after chilling. Fig. 1b and c show the effects of an incubation at 5–6° for 10 min (T_{10}) and 210 min (T_{210}), respectively. A progressive disappearance of polysomes accompanied by an accumulation of run off monosomes are the consequences of incubation at low temperature. The effect of RNase on these lysates is shown in fig. 1d–f. RNase provokes a total degradation of polysomes to 70 S particles (fig. 1d and e), and it has a dissociative effect on the run off monosomes accumulated at low temperature (fig. 1f).

The gradients of fig. 1 were centrifuged for a short time (2 hr) to display polysomes, 70 S particles and subunits. When the extracts used in fig. 1 are subjected to more prolonged centrifugation (4 3/4 hr) a reduction is evident in the proportion of 70 S material in comparison to subunits; furthermore, a new peak appears with an apparent sedimentation coefficient of 63 S, the relative importance of which increases with

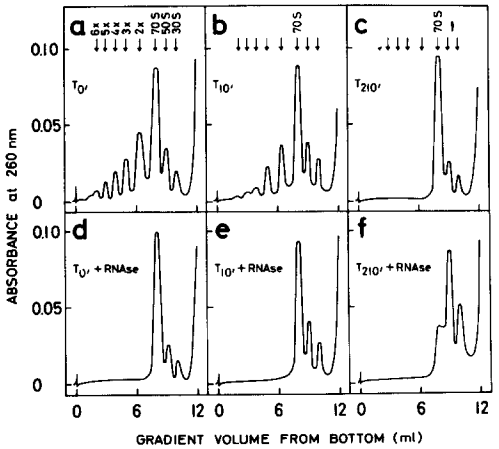

Fig. 1. The effects of cold incubation prior to lysis on the polysome profiles. After cooling 40 ml of culture was centrifuged for 3 min at 5000 rpm (3000 *g*), and the pellet was resuspended in 0.2 ml of a solution of 25% sucrose in 10 mM Tris-Cl, pH 8.1. A freshly-prepared solution of Tris–EDTA–lysozyme was added so that the final conc. in a vol of 0.3 ml were 1 mM EDTA, 0.1 mg/ml lysozyme, and 0.05 M Tris-Cl, pH 8.1. After 2 min this mixture was transferred to a tube containing 0.33 ml of the lytic mixture to give a final conc. of 0.48% Brij 58, 9.5 mM $MgSO_4$, 48 μg/ml DNase, 37 mM Tris-Cl, pH 7.7. Following 4 min of incubation the lysate was clarified by centrifugation at 7000 rpm (6000 *g*) for 5 min, and the supernatant was analyzed immediately or stored at −70° in small aliquots. Linear sucrose gradients (12 ml, 15–30%, w/v, in 10 mM Tris-Cl, pH 7.5., 10 mM $MgSO_4$, 30 mM KCl) were centrifuged at 2° in a Beckman SW41 rotor at 40,000 rpm for 2 hr. The absorption profiles were read at 260 nm with a Gilford recording spectrophotometer equipped with a linear flow cell. The control culture T_0, was lysed immediately after chilling, load 6 A_{260} units (a); culture T_{10} was incubation at 5–6° for 10 min before lysis, load 3.5 A_{260} units (b); culture T_{210} was incubated at 5–6° for 210 min before lysis, load 3 A_{260} units (c). Panels (d), (e) and (f) show the sedimentation profiles of the same extracts as panels (a), (b) and (c), respectively, but RNase at 3 μg/ml was added to the lytic mixture and was allowed to act at 2° for approx. 20 min before centrifugation, load 2–3 A_{260} units per gradient.

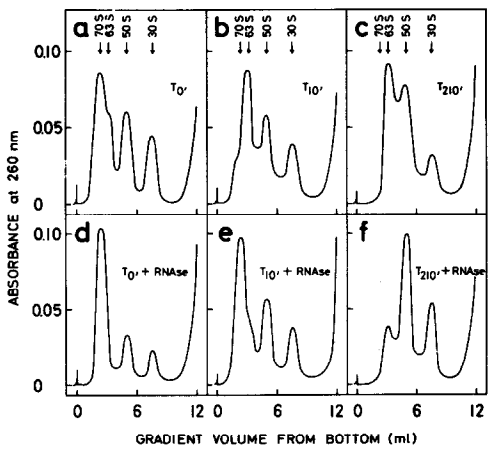

Fig. 2. The effects of prolonged centrifugation on the sedimentation profiles of the extracts of fig. 1. Conditions are as described in the legend to fig. 1, except that the time of centrifugation was 4 3/4 hr.

increasing periods of cold treatment (fig. 2 a–c). Simultaneously, the relative amount of 70 S material diminishes, such that in T_{210} a separate peak of 70 S material is no longer resolvable (fig. 2c). The profiles obtained following prolonged centrifugation of these same extracts treated with RNase are shown in fig. 2d–f. In T_0 + RNase (fig. 2d) and in T_{10} + RNase (fig. 2e), a substantial peak of complexed 70 S ribosomes is evident; this peak, which is derived from breakdown of polysomes by RNase, is absent from T_{210} + RNase (fig. 2f) because, as shown in fig. 1c, no polysomes are present in that extract. A further effect of RNase is to convert a large portion of the material which sediments in the 63 S region into subunits; this is particularly evident from a comparison of fig. 2c and 2f. There remains, however, a residual peak of material sedimenting in the 63 S region in T_{10} + RNase and T_{210} + RNase.

Stimulated by the results of Hardy and Turnock [12, 13], we examined the effect of the presence in the gradient buffer of mercaptoethanol or spermidine on the sedimentation profiles obtained after prolonged centrifugation of the ribosomes contained in an extract of cells subjected to 210 min of cold treatment. We found that mercaptoethanol (6 mM) is without effect, but that in the presence of spermidine (1–2 mM) the peak of material with an apparent sedimentation constant of 63 S disappears and is replaced by material sedimenting at 70 S.

Recentrifugation of fractions collected from a sucrose gradient of a lysate of cells treated 210 min at 5–6° and centrifuged 4 3/4 hr permits an estimation of the relative percentages of the various ribosomal particles in selected fractions. After such recentrifugations we found a clear separation of 30 S and 50 S subunits in fractions collected in the 50–70 S

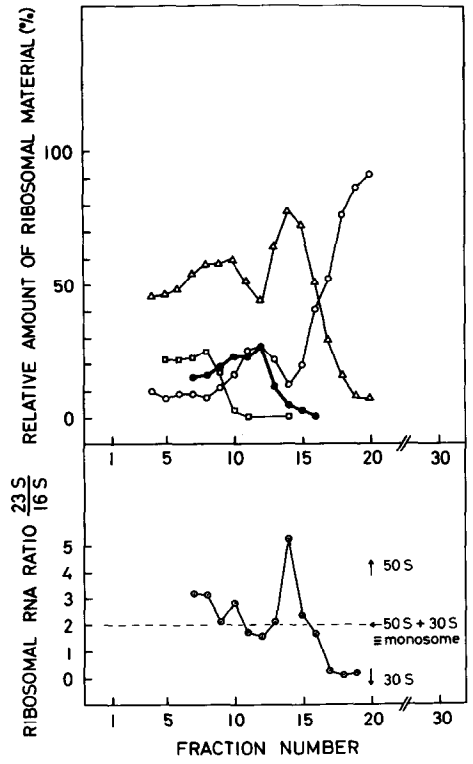

Fig. 3. Relative amounts of ribosomal components in fractions collected after preparative sucrose gradient centrifugation of an extract containing run off ribosomes. A lysate of cells incubated for 210 min at 5–6° was prepared and centrifuged as described in the legend to fig. 2, except that the gradient buffer contained in addition mercaptoethanol (6 mM) and the load was 40 A_{260} units. Thirty fractions of 400 μl each were collected. Recentrifugations of portions corresponding to 300 μl of selected fractions were carried out under the same conditions as was the preparative centrifugation; the relative proportions of the various ribosomal particles in each fraction analyzed were then determined planimetrically (a). Portions of selected fractions were also subjected to polyacrylamide gel electrophoresis; the gels were scanned in a Gilford recording spectrophotometer equipped with a gel scanner, and the ratio of 23 S to 16 S RNA in each fraction analyzed was determined planimetrically (b) (△–△–△) = 50 S; (○–○–○) = 30 S; (●–●–●) = 63 S; (□–□–□) = 70 S.

region (fig. 3a) indicating that dissociation had occurred during centrifugation. Although we cannot exclude the possibility that some stable 63 S particles exist, most of the material which resediments as a peak in the 63 S region (fig. 3a) presumably represents a reassociation of subunits which were dissociated but not separated by the first centrifugation [7]. Parallel to the recentrifugations, the content of selected fractions in 16 S and 23 S ribosomal RNA was determined by polyacrylamide gel electrophoresis. The results of these analyses (fig. 3b), as anticipated from the recentrifugations, show an excess of 23 S RNA in fractions collected in the 60–70 S region, and an excess of 16 S RNA in the fractions collected in the 50–60 S region.

4. Discussion

Our results show that low temperature incubation of *E. coli* leads to the accumulation of 70 S ribosomes with only a slight increase in the relative proportion of subunits. These free ribosomes are subject to dissociation during high speed centrifugation. Whether the free 70 S ribosomes are the primary products of polysomal run off, or whether they are formed secondarily from subunits which promptly recombine after their release from the messenger, is a question which has not yet been satisfactorily resolved [14]. Under our conditions RNase, in addition to its anticipated effect of degradation of polysomes, provokes the dissociation of the 70 S ribosomes accumulated during cold-induced run off. Others [15–17] have also noted that free 70 S ribosomes are sensitive to dissociation by RNase under certain conditions. Our results are in conflict with those of Friedman et al. [2] who reported that ribosomal subunits are the products of cold-induced run off in the presence and absence of RNase in the lysis buffer. At present we have no satisfactory explanation for this conflict, although it is conceivable that differences [18] between the strain we used (MRE-600) and the strain used routinely by Friedman et al. (Q_{13}, [2]) could, in part at least, account for it.

The significance of the persistence of material sedimenting at approx. 63 S in our extracts subjected to RNase treatment (see fig. 2e, 2f) as well as in those of Friedman et al. (see fig. 2c [2]) is still somewhat unclear. This material could represent stable pre-existing 63 S particles, but we feel it is rather more likely that it represents some artefactual product of ribonuclease degradation.

Free ribosomes are stable to sucrose gradient centrifugation of short duration in buffers containing potassium as the monovalent cation, whereas they are

dissociated immediately in buffers where the potassium has been replaced by sodium [19]. Hardy and Turnock [12, 13] have recently reported that contamination of sucrose gradient buffers by trace quantities of silver can lead to dissociation of free ribosomes during centrifugation even when the monovalent cation is potassium. The dissocation which they observed could be prevented by the addition of spermidine or mercaptoethanol to the gradient buffers. We believe that the dissociation of free ribosomes during centrifugation which we describe here is not due to contamination of our buffers by silver ions. For one thing we only observed such dissociation following prolonged centrifugation at higher rotor speeds (40,000 rpm) than those (27,000 rpm) used by Hardy and Turnock [12], and even then the newly dissociated subunits are not resolved in one centrifugation but sediment as a single peak in the 63 S region. Secondly, and more important, the dissociation we observe is inhibited by spermidine and not by mercaptoethanol. Following a suggestion of Hardy and Turnock [13], we explain the effect of spermidine as being one of stabilization of free 70 S ribosomes against any dissociative influence (in this case, high hydrostatic pressure), whereas the effect of mercaptoethanol would be to neutralize any contaminating silver ions. Since, in our experiments, mercaptoethanol did not prevent dissociation during centrifugation, we think it unlikely that there was any significant contamination of our gradients by silver ions. On the contrary, it seems that the dissociation provoked by high hydrostatic pressure which we observed is due simply to the inherent properties of free 70 S ribosomes.

Recent studies of the product of association *in vitro* of native *E. coli* 30 S and 50 S subunits have shown that it too is subject to dissociation during centrifugation [20].

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