SENSITIVITY AND RESISTANCE TO STREPTOMYCIN IN RELATION WITH FACTOR-MEDIATED DISSOCIATION OF RIBOSOMES

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1. Introduction

Much progress in understanding the complex role of ribosomes during protein synthesis has been made possible by the use of antibiotics. Streptomycin inhibition has been well documented [1–3]. We wish to report some data on the interference of this antibiotic with the factor-mediated dissociation of the ribosome, with special emphasis on the specificity of this effect in streptomycin sensitive (*str*^{s}) bacteria. Moreover, we distinguish a particular class of ribosomes using high speed gravitational field centrifugations. This class of ribosomes is clearly affected by the *str*A mutations.

It is well known that ribosomal preparations derived from exponential cultures of bacteria contain three classes of particles: the 30 S and 50 S subunits and the 70 S ribosome. Mild preparation techniques allow the observation of various additional polysomal peaks. The 70 S peak is usually considered to consist of breakdown products from the polysomes called monosomes.

In high centrifugal fields, we have resolved the 70 S peak into two clearly distinct components.

2. High *g* centrifugations reveal a 'slow' sedimenting ribosome

Whereas centrifugation at 170,000 *g* displays the usual 70 S, 50 S and 30 S peaks, as shown in fig. 1a, centrifugation at 300,000 *g* reveals a distinct split of the associated ribosomes into two subclasses, one sedimenting at 70 S and the other around 63 S (fig. 1b). It is striking to note that, in at least two instances where particular classes of slow ribosomes were characterised, high gravitational field centrifugation techniques were applied. Schreier and Noll [4], centrifuging at 400,000 *g*, showed the existence of a 60 S

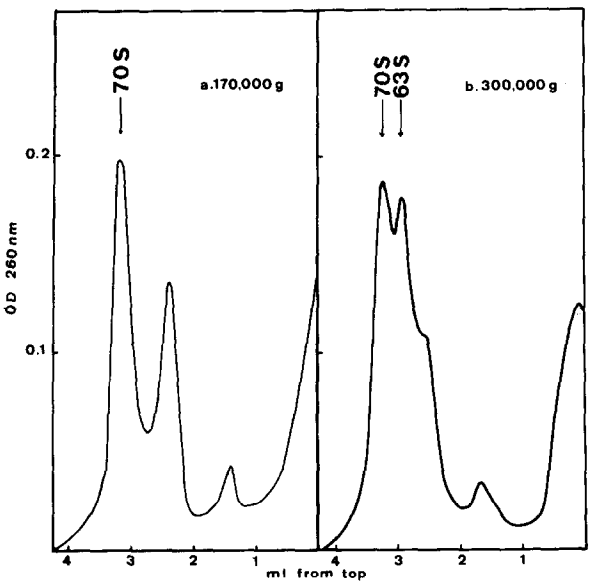

Fig. 1. Ribosomal samples run in different centrifugal fields. Ribosomes were obtained from *Escherichia coli* in exponential growth using standard techniques. Identical samples containing 15 μg of material were layered on 5 ml 10 to 30% sucrose density gradients prepared in tris HCl 10 mM pH 7.4, KCl 60 mM, Mg acetate 5 mM. One gradient (a) was spun for 150 min at 39,000 rpm in a SW 39 rotor (170,000 *g*). The other one (b) was spun for 105 min at 49,000 rpm in a SW 50.1 rotor (300,000 *g*). Analysis was performed using the continuous flow method described by Edwards and Mathias [12].

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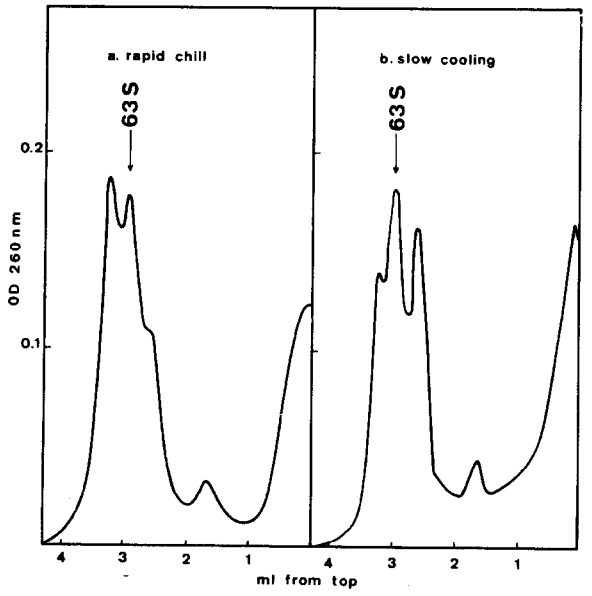

Fig. 2. Comparison of exponential phase ribosomes and ribosomes obtained after slow cooling. The slow cooling process followed the conditions described by Algranati et al. [7]. The exponential phase ribosomes were obtained as in fig. 1. Both samples were spun for 105 min at 300,000 *g* and analysed as described above.

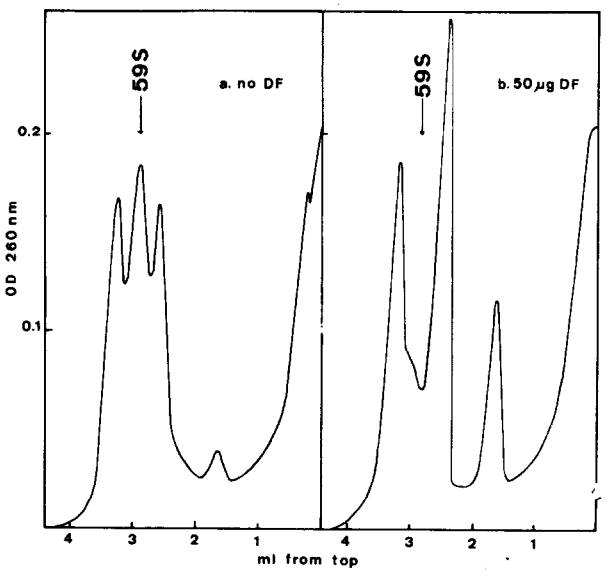

Fig. 3. Differential sensitivity of 70 S and 'slow' particles to the dissociation factor. The reaction mixture prepared as described by Algranati et al. [7] contained (a) 15 µg of ribosomes, and (b) 15 µg of ribosomes plus crude dissociation factor (50 µg protein). After 15 min incubation at 30°, samples were analyzed as in fig. 1b.

ribosomal intermediate which appears during protein initiation, while Kaempfer [5] produced so-called 'single' ribosomes which sedimented around 65 S in gradients spun at 285,000 *g*.

3. The slow sedimenting ribosome is the substrate of the dissociation factor

When the ribosomal preparation under study is derived from a slowly cooled culture, the 63 S peak markedly increases compared to an extract from an exponential culture, as shown in fig. 2. This process of slow cooling converts the monosomes into so-called 'runoff' ribosomes which are particularly sensitive to the dissociation factor recently described [6–9]. The following results demonstrate that the slow ribosome is the preferential substrate for the dissociation factor. Fig. 3 compares the sedimentation profiles of a ribosomal preparation either untreated (a) or treated with a small amount of crude dissociation factor (b). The slow peak has almost completely disappeared in (b), whereas the 70 S remains intact. (We chose a ribosomal preparation from a *str*^{r} strain to illustrate this phenomenon because the slow ribosome is better separated in this case (see below)). However, the same high sensitivity to DF is found for the slow component from a *str*^{s} preparation). The 70 S component can be partially split into subunits by the dissociation factor, but this requires much higher amounts of the factor and is probably a side effect.

It appears that the increased sensitivity of ribosomes towards DF after a slow cooling process which has been reported is merely due to the enrichment of the population in 'slow' ribosomes. Furthermore, it is clear from published results that DF action is never complete, even at very high factor concentrations. The residual 70 S ribosomes which always remain are probably 'true' monosomes.

4. Slow ribosomes from *str*^{s} and *str*^{r} strains have different sedimentation coefficients

We compared high *g* sedimentation profiles of ribosomal populations from streptomycin sensitive (*str*^{s}) and streptomycin resistant (*str*^{r}) strains (fig. 4). The sedimentation coefficients of the slow ribosomes were

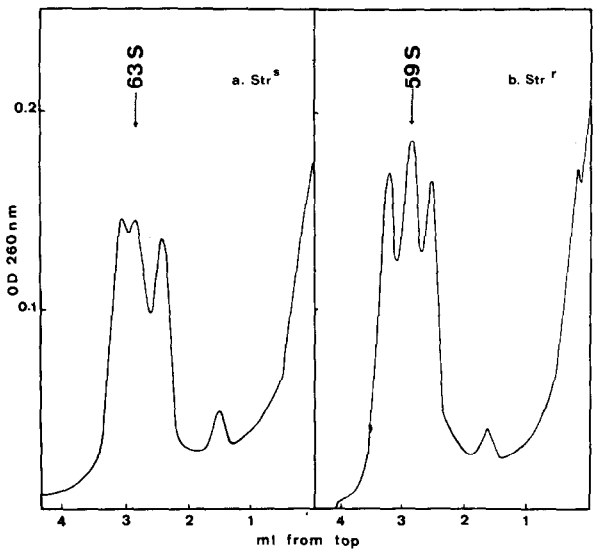

Fig. 4. Comparison of ribosomal populations in *str*^{r} strains. Samples obtained either from *str*^{S} strains C600 and 112–12 (a) or from *str*^{r} strains C600 #R_4 and 112–12 #R_4 (b) were analysed at 300,000 *g* as in fig. 1b. (112–12 #R_4 is the transduced strain for the *str*^{r} R_4 character). The sedimentation values were obtained by interpolation and are means calculated for *str*^{S} from five measurements on two independent preparations of strain C600 and for *str*^{r} from four measurements on extracts from C600 #R_4 and 112–12 #R_4.

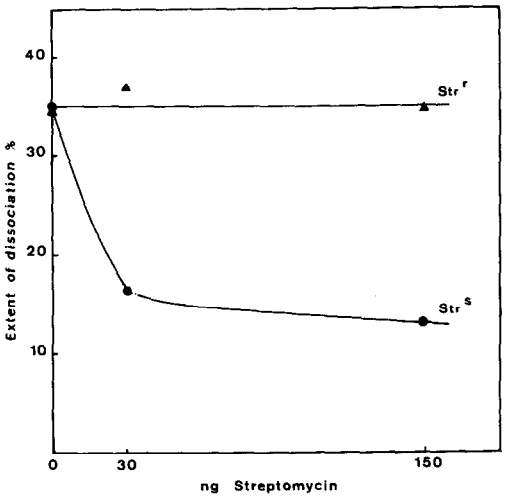

Fig. 5. Inhibition by streptomycin of factor-mediated dissociation. Dissociation assays were performed as described in fig. 3b in the absence or in the presence of 30 ng or 150 ng of streptomycin. Areas under the various peaks were computed using a planimeter. The remaining amount of 70 S + 63 S (for *str*^{S} strain) or 70 S + 59 S (for *str*^{r} strain) was taken as measure of the extent of dissociation. • ribosomes from strain C600 *str*^{S}, ▲ ribosomes from strain C600 #R_4 *str*^{r}.

estimated by linear interpopulation, taking as references the 70 S and 50 S peaks. The values differ markedly according to whether the ribosomes originate from the normal strain (62.9 ± 0.4 S) or the *str*^{r} derivative (59.3 ± 0.4 S). The shift in sedimentation value from 63 S to 59 S is clearly associated with the *str*^{r} mutation, since the 59 S peak is observed in a strain which received the *str*^{r} character by phage P_1 transduction.

5. Streptomycin inhibits DF-mediated dissociation of *str*^{S} but not of *str*^{r} ribosomes

Fig. 5 illustrates the factor-mediated dissociation of ribosomes from *str*^{S} and *str*^{r} strains at various drug-to-ribosome ratios. The graph shows that streptomycin inhibits the dissociation of *str*^{S} ribosomes whereas the dissociation of the *str*^{r} particles appears completely indifferent to the presence of the drug. (Inhibition of DF activity by streptomycin has already been mentioned by Algranati [10]).

A simple explanation of this result would be that the drug acts by blocking the ribosomal receptor for the dissociation factor. This inhibition of DF activity by streptomycin might be responsible for the accumulation of 'stuck' ribosomes in streptomycin treated cultures of *str*^{S} strains, an effect we pointed out a few years ago [11].

Acknowledgements

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