BINDING OF DIHYDROSTREPTOMYCIN TO RIBOSOMES AND RIBOSOMAL SUBUNITS FROM STREPTOMYCIN-RESISTANT MUTANTS OF *CHLAMYDOMONAS REINHARDI*

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1. Introduction

Today mitochondria and chloroplasts are thoroughly investigated with respect to a possible regulatory function in the synthesis of macromolecules and cell structures in eukaryotes. There is evidence of a mutual exchange of informatory molecules, such as RNA and enzymes, between the organelles and the cytoplasm and of a mutual influence of their respective protein synthesizing systems [1,2]. In this connection a comparative study of the ribosomes from organelles and cytoplasm is of great importance. For the investigation of chloroplast ribosomes and chloroplast – cytoplasmic interactions the unicellular green alga *Chlamydomonas reinhardi* is widely used [3], and mutants with altered 70 S ribosomes have been decribed [4,5].

In three mutant strains of this alga [6], we found 70 S ribosomes which show a different dissociation behaviour into subunits and a different effect of streptomycin on this dissociation [7]. Depending whether the mutant was streptomycin-sensitive (*ss*), streptomycin-resistant with a Mendelian (sr_3) or with a miparental pattern of inheritance (sr_{35}), streptomycin induced in vivo the formation of 'stuck' 70 S ribosomes to a different, gradually diminishing degree. In accordance with these findings, streptomycin inhibits in vivo the protein synthesis on the 70 S chloroplast ribosomes in sr_3 more severely than in sr_{35} [8]. In this paper we now report on the binding of [^{3}H]dihydrostreptomycin to the ribosomes and their subunits.

The antibiotic is bound differently by the 70 S ribosomes of the three mutants. The site of the antibiotic resistance seems to lie in the 30 S subunits in both sr_3 and sr_{35}, in spite of the different pattern of inheritance of the streptomycin-resistance, indicating, that nuclear and extranuclear genes must participate in the synthesis of the 70 S ribosomes.

2. Materials and methods

The three mutants *ss*, sr_3 and sr_{35}, their cultivation, as well as the isolation of ribosomes have been described [6,7].

The separation of ribosomes and subunits was essentially carried out according to Chua et al. [9].

For the antibiotic binding assay we used two methods. The adsorption of the ribosome – dihydrostreptomycin complex to Millipore-filters (0.45 μ) [10] was used in order to compare the binding capacity of 70 S and 80 S ribosomes in our three mutants. Since for the subsequent experiments the standard deviation was too large, we used the more sensitive binding assay of Yamada and Davis [11] in a modified form: The ribosomal pellets were suspended in 200 μl of a 25 mM Tris–MCl buffer, containing 5 mM $MgCl_2$ Tris–MCl buffer, containing 5 mM $MgCl_2$ and 25 mM KCl, so that the sample contained 10–15 A_{260}-units of ribosomes. Various amounts (see Results) of [^{3}H] dihydrostreptomycin in 200 μl of the above buffer were added (spec. activity 3 Ci/mmole Amersham). The mixtures were incubated for 30 min at 27°C. After addition of 2.5 ml of ice-cold buffer, the suspensions were layered on 8 ml sucrose

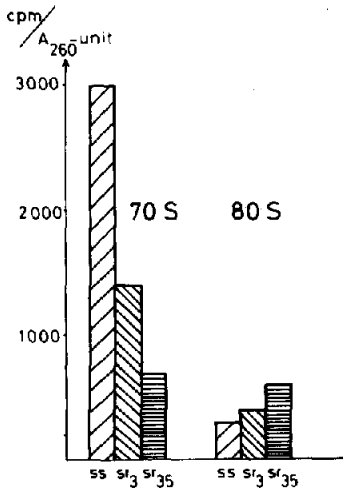

Fig. 1 Binding mutants *ss*, sr_3 and sr_{35} of [^{3}H]dihydrostreptomycin to 70 S and 80 S ribosomes from the mutants *ss*, sr_3 and sr_{35} measured with the Millipore filter method and a dihydrostreptomycin concentration in the assay medium of 1 nmol/l. Each value is the mean of 10 measurements from 2 separate experiments. The standard deviation is 250–300 cpm/A_{260}-unit.

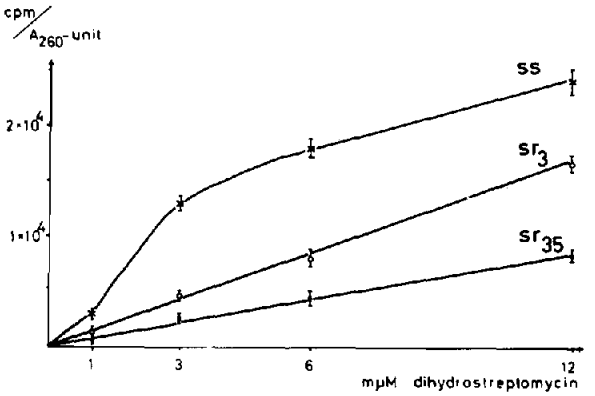

Fig. 2. Binding of [^{3}H]dihydrostreptomycin to the 70 S ribosomes of the strains *ss*, sr_3 and sr_{35} as a function of the drug concentration in the assay medium. Each curve represents the mean of two independent experiments. The bars indicate the difference between the two corresponding values.

cushions in the same buffer.

Centrifugation was carried out in the Ti-50 rotor of a Spinco model L 2 preparative ultracentrifuge for 14 hr at 40 000 rpm over 30% sucrose in the case of 70 S ribosomes, whereas in the case of subunits, 16 hr at 48 000 rpm and 20% sucrose was used.

3. Results

The binding of dihydrostreptomycin to 70 S and 80 S ribosomes from our three mutant strains is given in fig. 1. The binding to the 70 S ribosomes is greatest in the strain *ss* and smallest in sr_{35}. The 80 S ribosomes bind only an insignificant amount of the antibiotic. The Millipore filter assay used in these experiments, however, was not sensitive enough to prove quantitative differences between the binding abilities of the 70 S ribosomes from the mutants sr_3 and sr_{35}. Therefore we used a modified method of Jamada and Davis for the binding experiments with 70 S ribosomes and with subunits.

Fig. 2 shows the binding of dihydrostreptomycin to the 70 S ribosomes of the three mutant strains as a function of the drug concentration in the assay medium. The binding curve for 70 S ribosomes of the *ss*-strain seems to reach a plateau at higher drug concentrations. In the resistant strains, however, the binding of dihydrostreptomycin to the 70 S ribosomes is lower and the curves are almost linear in the range of antibiotic concentrations we used. Here, a significant difference is observed between the binding capacities of the 70 S ribosomes of strain sr_3 and strain sr_{35}. At a concentration of 12 nmol/l dihydrostreptomycin in the binding assay, roughly 0.6, 0.3 and 0.15 molecules of the drug were bound by one 70 S ribosome of the strains *ss*, sr_3 and sr_{35} respectively. (The assumption was made that 1 A_{260}-mit = 66 μg = 24 pmoles ribosomes [12].)

Having found differences in the binding of dihydrostreptomycin to the 70 S organelle ribosomes of the three strains, we measured the binding to their subunits in order to determine the site of the antibiotic attachment (fig. 3).

To our astonishment the 30 S subunit of the *ss*-strain bound twice as much dihydrostreptomycin than the 70 S ribosomes at the same conditions (figs. 2 and 3). Here a difference to the bacterial ribosomes is visible, since the 70 S ribosomes of *E. coli* bind more antibiotic than the 30 S subunits [11].

On the other hand, the 30 S subunits of both resistant strains did not bind any significant amount of dihydrostreptomycin and no difference in their

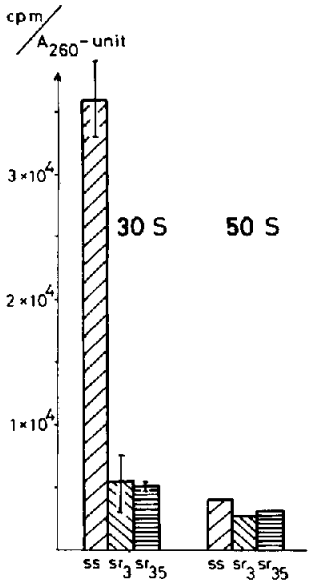

Fig. 3. Binding of [^{3}H] dihydrostreptomycin to the subunits of 70 S ribosomes. The drug concentration in the assay medium was 6 nmol/l. The values for the 30 S subunits are the mean of two independent measurements.

binding capacity could be observed, in contrast to the behaviour of the 70 S ribosomes.

As expected, The 50 S subunits of all three mutants failed in binding dihydrostreptomycin.

4. Discussion

The different binding of dihydrostreptomycin by the 70 S ribosomes of the three mutant strains corresponds very well to the different formation of 'stuck' 70 S ribosomes under the influence of streptomycin in vivo [7]. Our previous finding that streptomycin induces to a small extent the formation of di- and oligomers from 80 S ribosomes in vivo [13] is not reflected by the present binding experiments.

This in vitro method doesn't allow to detect a probably weak and reversible action of the drug on the 80 S ribosomes, as it has been made plausible also by the recent discovery of streptomycin-dependent 80 S ribosomes [14].

The fact that the 30 S subunits of both resistant strains do not bind dihydrostreptomycin at all, shows that in both strains the streptomycin resistance is caused by a mutation in the 30 S subunits. Assuming that our 70 S and 30 S ribosomal preparations are mainly of chloroplast origin, the present results cannot confirm the hypothesis that in sr_3 the mitochondrial ribosomes are resistant to streptomycin. Considering the different patterns of inheritance of our two mutants, it seems that two different proteins of the 30 S subunit are altered, so that nuclear and cytoplasmic gene products are present in the 30 S subunits. Similarly Mets and Bogorad [4] found in the 50 S subunit of a Mendelian and of an uniparental erythromycin-resistant mutant of *Chlamydomonas reinhardi* different altered ribosomal proteins. Both mutated ribosomal proteins in sr_3 and sr_{35} cause the same loss of binding capacity for dihydrostreptomycin to the 30 S subunit. However, in the 70 S ribosomes the binding capacity is different in the two mutants. One could suggest that by the addition of the 50 S subunit a conformational change in the 30 S ribosome is induced, which is different in both strains and causes their different response to streptomycin.

Acknowledgement

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