Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

Clara Calvori, Laura Frontali, Luisa Leoni, Giorgio Tecce

Effect of rifamycin on ribosomes from B. subtilis

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **42** (1967), n.4, p. 563–566.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1967_8_42_4_563_0>

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Accademia Nazionale dei Lincei, 1967.

Biologia molecolare. — *Effect of rifamycin on ribosomes from* B. subtilis. Nota di CLARA CALVORI, LAURA FRONTALI, LUISA LEONI e GIORGIO TECCE, presentata (*) dal Corrisp. M. AGENO.

RIASSUNTO. — La dietilamide della rifamicina B provoca un aumento significativo della percentuale di subunità ribosomiali in estratti di cellule di *B. subtilis* incapaci di riassociarsi in alte concentrazioni di Mg. Tale effetto si nota solo quando lo antibiotico agisce *in vivo*.

Questi risultati, e le loro relazioni con l'inibizione da parte della rifamicina sulla sintesi proteica *in vitro*, vengono brevemente discussi in relazione al problema della sintesi e dissociazione *in vivo* dei ribosomi.

Several antibiotics active on protein and nucleic acids synthesis are known to alter the sedimentation patterns of bacterial ribosomes.

Besides the well known effect of chloramphenicol which produces the accumulation of protein deficient particles [1], a significant effect on the ribosomes was demonstrated for streptomycin [2], mitomycin [3], edeine [4].

The relationship between this effect and the primary mechanism of the antibiotic is not always well understood but the study of the characteristics of the altered particles might give useful information on the biosynthesis of ribosomes.

The diethylamide of rifamycin B has been previously shown to inhibit amino-acid incorporation into protein by cell-free extracts of *B. subtilis* [5]. Alterations in the sedimentation patterns of ribosomes prepared from *B. subtilis* cells treated whit this antibiotic are now reported.

The effect of rifamycin SV on ribosomes prepared from rat liver was studied by Gualerzi et al. [6].

EXPERIMENTAL.

B. subtilis strain ATCC 6633 was cultivated in a medium previously described [7]. Rifamycin B diethylamide, at concentration of 20 μg/ml or 1 μg/ml, was added to exponentially growing cultures. The addition of 20 μg/ml of the antibiotic stopped the growth completely whereas the addition of 1 μg/ml slowed it down. In control cultures growth was stopped or slowed by adding penicyllin G or by chilling, centrifuging and resuspending the cells in fresh medium, or by anaerobiosis. The same results were to be obtained with these different methods. After 60′ or 90′ from the addition of the antibiotic, cells were chilled, centrifuged, washed with 0.01 M pH 7.7 tris-HCl

(*) Nella seduta dell'8 aprile 1967.

buffer and disrupted by grinding with alumina Norton. Extracts were suspended in the same tris–HCl buffer containing 10^{-2} or 10^{-4} M Mg^{++} and centrifuged twice at 30,000 g for 30'; particles were sedimented at 105,000 g for two hours and resuspended in the same buffer. Sedimentation velocity experiments were performend in Spinco model E analytical ultracentrifuge at a speed of 31,410 rpm.

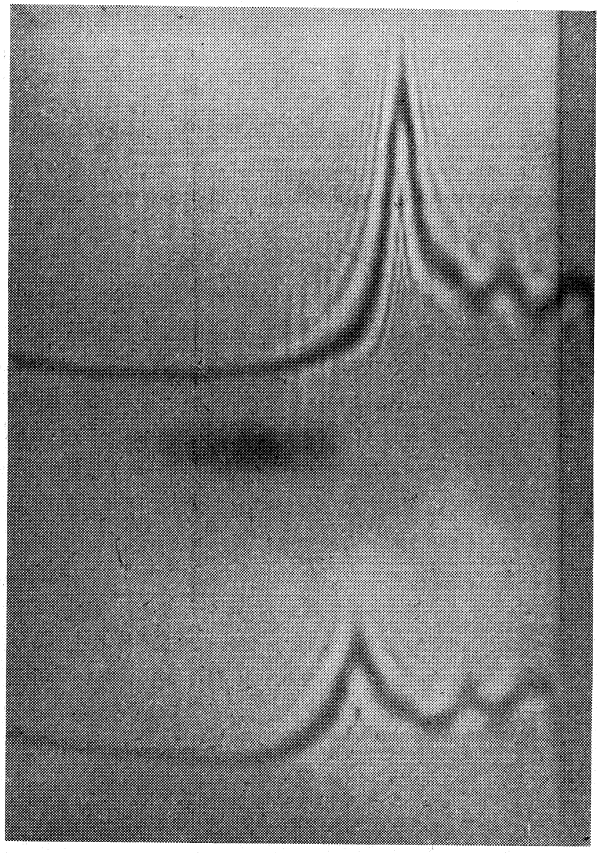

Fig. 1. – Sedimentation patterns of ribosomes extracted with tris–HCl buffer containing 10^{-2} M Mg^{++} from *B. subtilis* cells treated with penicyllin (upper diagram) and with rifamycin (lower diagramm).

Fig. 1 shows the sedimentation patterns of ribosomes from untreated *B. subtilis* cells (upper curve) and from cells treated with 20 μg/ml rifamycin (lower curve), extracted with tris–HCl buffer containing 10^{-2} M Mg^{++}. Sedimentation coefficients are the same in both cases, but the ratio of 70–S ribosomes to 50–S and 30–S subunits is quite different: in fact the percentage of subunits is 18 % for the upper curve and 38 % for the lower curve. This means that a large proportion of particles from rifamycin treated cells are unable to associate in 10^{-2} M Mg^{++}.

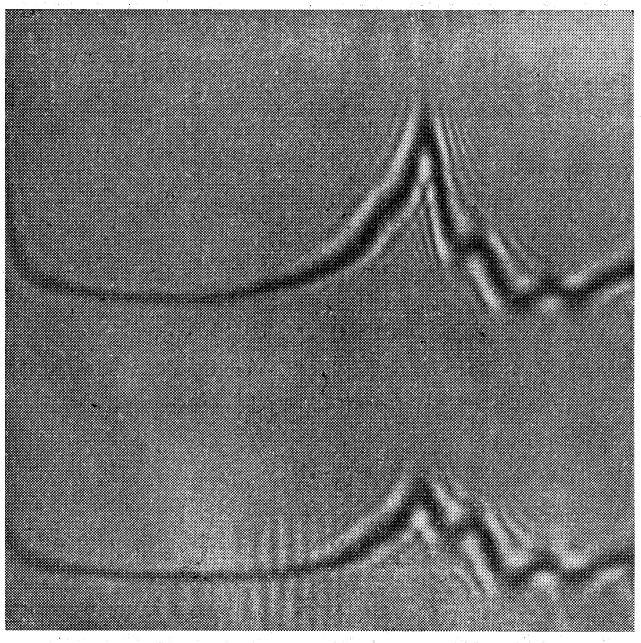

Fig. 2. – Sedimentation patterns of ribosomes extracted with tris–HCl buffer containing 10^{-4} M Mg^{++}, after riassociation in 10^{-2} M Mg^{++}. The upper diagram refers to ribosomes from cells treated with penicyllin; the lower diagram refers to ribosomes from cells treated with rifamycin.

The sedimentation patterns in 10^{-4} M Mg^{++} was the same for ribosomal subunits from treated and untreated cells; but when the association of subunits was studied by incubating them in an ice bath the two hours [8], after having increased the Mg^{++} concentration to 0.01 M, the percentage of subunits was 42 % for the sample from rifamycin treated cells and 23 % for the sample from untreated cells (fig. 2). Figures are summarized in Table I.

TABLE I.

RIBOSOMES	30–S + 50–S %	70–S %
Extracted in 10^{-2} M Mg^{++} from rifamycin treated cells	38.7 ± 4.6	61.3 ± 4.6
Extracted in 10^{-2} M Mg^{++} from penicyllin treated or untreated cells	18.3 ± 3.2	81.7 ± 3.2
Extracted in 10^{-4} M Mg^{++} from rifamycin treated cells after association in 10^{-2} M Mg^{++}	42.0 ± 6.4	58.0 ± 6.4
Extracted in 10^{-4} M Mg^{++} from penicyllin treated or untreated cells after association in 10^{-2} M Mg^{++}	23.1 ± 2.6	76.9 ± 2.6

When treatment with rifamycin is performed on extracts no effect of the antibiotic is to be observed on the sedimentation properties of ribosomes. This is true even when rifamycin is added to subunits before raising Mg^{++} concentration to associate them.

Discussion and conclusion.

The above results indicate that rifamycin B diethylamide increases the proportion of ribosomal subunits which are unable to associate in high Mg^{++} concentration. The fact that rifamycin does not affect *in vitro* the association of ribosomal subunits from untreated cells rules out the possibility that the drug acts by complexing Mg^{++} or binding to ribosomal sites responsible for interaction of subparticles.

The fact that rifamycin inhibits protein synthesis *in vitro* [5], while only its addition to living cells increases the percentage of subunits, suggests the hypothesis that in the presence of the antibiotic, ribosomal precursors are accumulated which are incapable of interacting to form 70–S ribosomes; alternatively the antibiotic might increase the dissociation of active ribosomes. Both effects might be consequent to the inhibition by the drug of protein synthesis.

This work was supported by a grant from the C.N.R.

References.

[1] M. Nomura and J. D. Watson, « J. Mol. Biol. », *1*, 204 (1959).
[2] A. Herzog, « Bioch., Bioph. Res. Comm. », *15*, 172 (1964).
[3] H. Suzuki, and W. W. Kilgore, « Science », *146*, 1585 (1964).
[4] Z. Kurylo–Borowska and M. Hierowsky, « Bioch. Bioph. Acta », *95*, 590 (1965).
[5] C. Calvori, L. Frontali, L. Leoni and G. Tecce, « Nature », *207*, 417 (1965).
[6] C. Gualerzi, M. T. Mangiantini and A. Trentalance, « Boll. Soc. It. Biol. Sper. », in press.
[7] L. Frontali, L. Leoni and G. Tecce, « Nature », *203*, 84 (1964).
[8] F. N. Chang, C. J. Sih and B. Weisblum, « Proc. Natl. Acad. Sci. US », *55*, 431 (1966).