KIRROMYCIN, AN INHIBITOR OF THE 30 S RIBOSOMAL SUBUNITS FUNCTION*

H. WOLF and H. ZÄHNER**
Institut für Biologie, Lehrbereich Mikrobiologie, Universität Tübingen, D-74 Tübingen, Im Schönblick 47, Germany

and

K. NIERHAUS
Max-Planck-Institut für Molekulare Genetik, D-1 Berlin 33, Ihnestr. 63–73, Germany

Received 14 February 1972

1. Introduction

This paper describes the effects of kirromycin on the protein and nucleic acid synthesis in *B. brevis* cells, as well as on the polypeptide synthesis in the cell-free systems of *B. brevis* and *E. coli*.

Kirromycin, a new antibiotic produced by *Streptomyces collinus*, inhibits the growth of some bacteria, e.g. *B. brevis*, however, no activity against *E. coli* (*in vivo*) was observed. Isolation, characterization, and biological properties of kirromycin have been reported in a previous paper [1].

2. Materials and methods

2.1. Materials

Ribosomes, 30 S and 50 S ribosomal subunits, and supernatant enzymes (S-100 fraction) from *E. coli* A19 [2], a RNAase I-less mutant, and *B. brevis* ATCC 9999 were prepared as described [3,4]. After high-speed centrifugation, a fraction of supernatant fluid was dialyzed with 10 mM Tris-HCl buffer pH 7.6 containing 10 mM magnesium acetate and 6 mM 2-mercaptoethanol. The ribosomes were suspended in 10 mM magnesium acetate, the ribosomal subunits, however, in 1 mM magnesium acetate. All colected enzymes and ribosomal particles were kept until use at -80° (see sect. 2.3).

U-^{14}C-L-isoleucine (10 mCi/mM), U-^{14}C-L-phenylalanine (10 mCi/mM), 2-^{14}C-thymidine (56 mCi/mM), and 2-^{14}C-uracil (52.5 mCi/mM) were purchased from the Radiochemical Centre, Amersham and chloramphenicol from Parke Davis and Co., Detroit, Mich., USA. Kirromycin was isolated according to methods described in a previous paper [1].

2.2 Synthesis of cellular macromolecules

Isotopic incorporation studies were performed by the addition of 14 μg ^{14}C-isoleucine, 7 μg ^{14}C-uracil, or 7 μg ^{14}C-thymidine to growth cuvettes containing 7 ml synthetic medium [5], which were inoculated with enough log phase *B. brevis* cells to give a turbidometrically-determined concentration of 10^7 cells per ml. The growth cuvettes were then agitated at 37° in a biophotometer (Jouan, Paris). At various times (see fig. 1) during the growth period, a 0.2 ml sample of the cell suspension was withdrawn and then given to 1 ml of cold 7% trichloracetic acid. The precipitate formed was collected on a membrane filter (0.45 μ pore size), washed with 5% trichloracetic acid, dried, and the radioactivity measured.

* Metabolic products of microorganisms, 100; for preceding publication see [1].

** To whom to address correspondence.

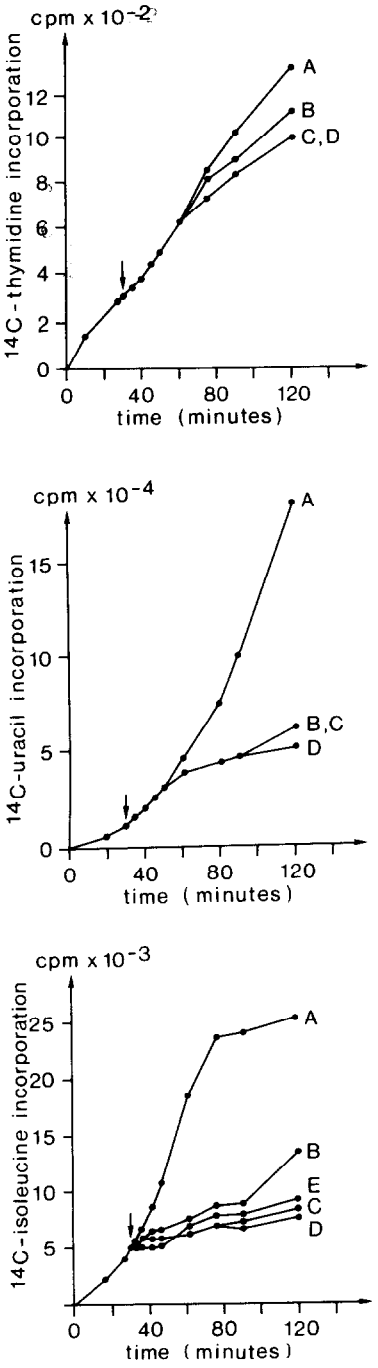

Fig. 1. Effect of kirromycin on the incorporation of ^{14}C-thymidine, ^{14}C-uracil, and ^{14}C-isoleucine by intact cells of *B. brevis* (time of addition of the antibotic is indicated by the arrow). A: control; B: 1.25 μg/ml kirromycin; C: 5 μg/ml kirromycin; D: 10 μg/ml kirromycin; E: 20 μg/ml chloramphenicol.

2.3. Polyphenylalanine synthesis

Experiments for polyphenylalanine synthesis were carried out by a method similar to that of Traub et al. [6]. Each incubation mixture (150 μl) contained: 10 mM Tris-HCl pH 7.8, 30 mM NH_4Cl, 10 mM magnesium acetate, 1 mM ATP, 0.03 mM GTP, 6 mM 2-mercaptoethanol, 5 mM phosphoenolpyruvic acid, 3 μg pyruvate kinase, 0.075 μCi ^{14}C-phenylalanine, 40 μg poly(U), 12 μl enzyme fraction (S-100), 1 A_{260} unit of 70 S ribosomes or 50 S and 30 S ribosomal subunits, and 5 μl antibiotic solution (kirromycin was dissolved in ethanol).

The reaction mixture was incubated for 45 min at 30°, stopped by the addition of 2 ml of 5% trichloracetic acid and heated for 15 min at 90°. The resulting precipitate was collected on a glassfibre filter, and washed with 5% trichloracetic acid. The filter was dried and the radioactivity measured.

3. Results and discussion

The effect of kirromycin on the incorporation of ^{14}C-thymidine, ^{14}C-uracil, and ^{14}C-isoleucine by intact cells of *B. brevis* was determined. Kirromycin,

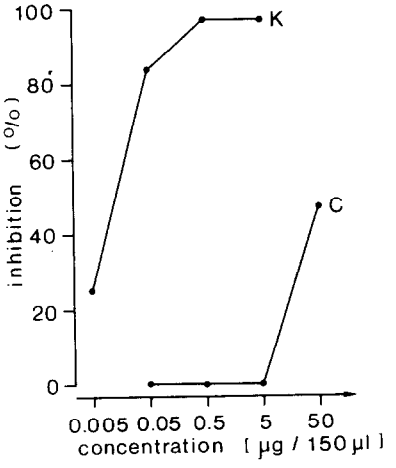

Fig. 2. Effect of concentrations of kirromycin and chloramphenicol on polyphenylalanine synthesis in the cell-free system of *B. brevis*. K: kirromycin (M.W. 296). C: chloramphenicol (M.W. 323)

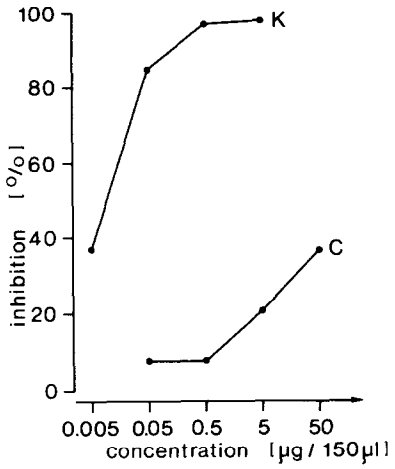

Fig. 3. Effect of concentrations of kirromycin and chloramphenicol on polyphenylalanine synthesis in the cell-free system of *E. coli*. K: kirromycin; C: chloramphenicol.

like chloramphenicol, inhibited isoleucine incorporation within less than 5 min after addition, whereas the rate of thymidine and uracil incorporation remained unchanged for 20–30 min after addition of the antibiotic (fig. 1). We inferred from these data that kirromycin acts as a specific inhibitor of protein synthesis in *B brevis* cells. This conclusion was further substantiated by *in vitro* experiments.

As shown in fig. 2, kirromycin, compared to chloramphenicol, more strongly inhibited the poly U-directed polyphenylalanine synthesis in the *in vitro* system of *B. brevis*.

Peptide synthesis in the *in vitro* system of *E. coli* was also inhibited, whereas the inhibition of growing cells was not observed (fig. 3). Thus the difference in susceptibility of *B. brevis* and *E. coli* may be due to a difference in factors other than those contained in the protein-synthesizing system.

Studies were continued to localize the binding site of the antibiotic with respect to ribosomal subunits. The binding experiments were carried out with separated subunits of *E. coli* ribosomes. As shown in table 1, the combination of kirromycin-treated 30 S subunits and control 50 S subunits showed in much lower peptide synthesizing activity than that of treated 50 S subunits and control 30 S subunits.

Further, the effect of the antibiotic on the polyphenylalanine synthesis was examined for different ratios of 30 S to 50 S subunits of *E. coli* ribosomes. As shown in fig. 4, the inhibitory effect of kirromycin on polypeptide synthesis was strongly decreased by the addition of excess 30 S ribosomal subunits, however not by the addition of 50 S ribosomal subunits.

These data clearly indicate that kirromycin binds with 30 S subunits of *E. coli* ribosomes and interferes with the polypeptide-synthesizing function.

Acknowledgements

We would like to thank Professor H.-G. Wittmann for allowing us the use of laboratory facilities. We wish to thank F.W. Ebensperger for reviewing the manuscript.

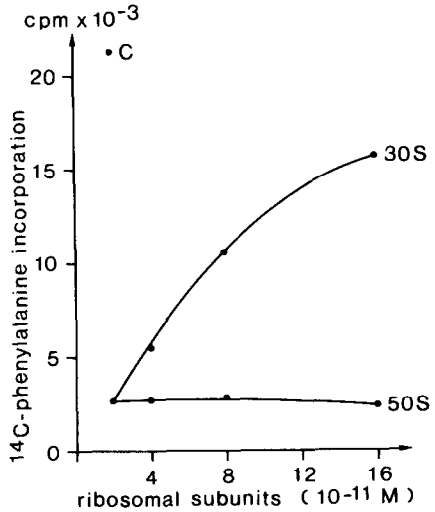

Fig. 4. Reversal of inhibition, in a kirromycin-inhibited polypeptide synthesizing system, by addition of 30 S ribosomal subunits (*E. coli*). 30 S: Reaction mixture (without ribosomes) + 0.03 µg kirromycin + 2×10^{-11} M 50 S particles + 2, 4, 8 or 16×10^{-11} M 30 S particles; 87–22% depression. 50 S: Reaction mixture (without ribosomes) + 0.03 µg kirromycin + 2×10^{-11} M 30 S particles + 2, 4, 8 or 16×10^{-11} M 50 S particles; 87–88% depression. C: Without antibiotic.

Table 1
Effect of kirromycin-treated ribosomal subunits on the polyphenylalanine synthesis in the cell-free system of *E. coli.*

Ribosomal subunits added (2×10^{-11} M)		Concentration of kirromycin (M)	Polyphenylalanine synthesized (cpm/150 μl)	Inhibition (%)
30 S	–	–	3,011	85
30 S*	–	–	2,235	89
–	50 S	–	2,770	86
–	50 S*	–	1,478	93
30 S	50 S	–	20,110	0
30 S*	50 S*	–	5,515	73
30 S	50 S	7×10^{-7}	2,684	87
30 S*	50 S	–	6,816	66
30 S	50 S*	–	11,334	44

The samples contained the standard reaction mixture, however the 30 S and 50 S subunits were added according to the data in the table

* 2×10^{-11} M ribosomal subunits preincubated with 0.03 μg kirromycin (5 min at 30°).

References

[1] H. Wolf und H. Zähner, Arch. Mikrobiol., in press.
[2] R.F. Gesteland, J. Mol. Biol. 16 (1966) 67.
[3] T. Staehelin and D.R. Maglott, in: Methods in Enzymology, Vol. 20, eds. K. Moldave and L. Grossman (Academic Press, New York and London, 1971) p. 449.
[4] I. Hindennach, G. Stöffler, and H.-G. Wittmann, European J. Biochem. 23 (1971) 7.
[5] T.S. Eikhom, J. Jonsen, S. Laland, and T. Refsvik, Biochim. Biophys. Acta 76 (1963) 465.
[6] P. Traub, S. Mizushima, C.V. Lowry, and M. Nomura, in: Methods in Enzymology, Vol. 20, eds. K. Moldave and L. Grossman (Academic Press, New York and London, 1971) p. 391.