Notes

STUDIES ON THE MECHANISM OF ACTION OF RAVIDOMYCIN (AY-25,545)

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Isolation and characterization of ravidomycin (AY-25,545), an antibiotic produced by *Streptomyces ravidus*, has been previously reported[1]. The antibiotic is mainly active against Gram-positive organisms and exhibits strong antitumor activity. In the present communication, we are reporting the effect of ravidomycin on macromolecular biosynthesis in *Bacillus subtilis*.

A stock solution of ravidomycin was prepared in methanol (1 mg/ml). Further dilutions were made with 50% (v/v) aqueous methanol. The final concentration of methanol in assay mixture was 1% or less.

The effect of ravidomycin on the growth of *B. subtilis* is shown in Fig. 1. Growth was significantly inhibited at 0.5 μg/ml and at 1 μg/ml complete cessation of growth was observed within 2 hours; ravidomycin treated cells (1~2 μg/ml) suffered an almost complete loss in viability. At 1.0 μg/ml lysis of cells was also observed (Fig. 1) indicating that ravidomycin acts as a bactericidal antibiotic.

The effect of ravidomycin on protein, RNA and DNA syntheses was monitored by determining the incorporation of L-[*U*-^{14}C]leucine, [2-^{14}C]-

Fig. 1. Effect of ravidomycin on the growth of *B. subtilis*.

Medium contained 2% glucose and 1% yeast extract; incubation time 60 minutes on a rotary shaker at 37°C. The numbers on the curves indicate amounts of ravidomycin (μg/ml).

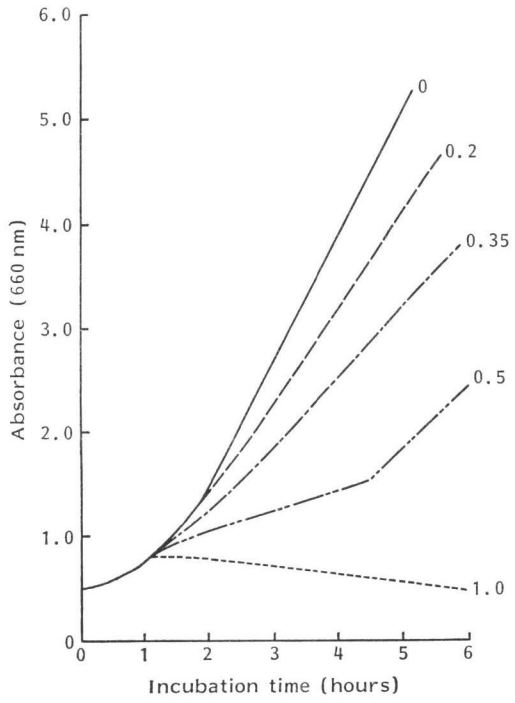
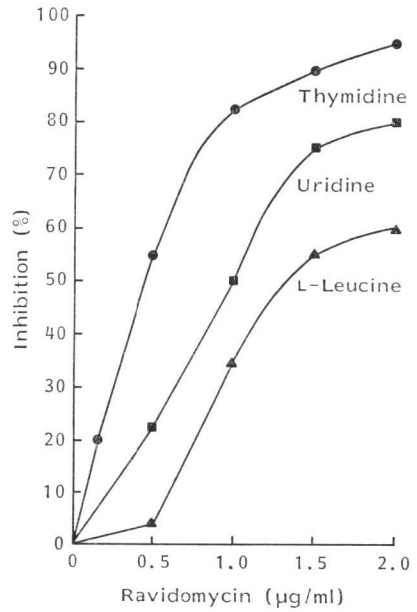


Fig. 2. Effect of ravidomycin on macromolecular syntheses in *B. subtilis*.

Each 1 ml reaction mixture contained 0.50 ml of cells, 0.46 ml GYE dil., 20 μl antibiotic and 20 μl of labeled precursor (1 mM, 10 mCi/mmol) incubation 60 minutes at 37°C. Results are expressed as % inhibition of incorporation of labeled precursor into the acid-insoluble fraction.



uridine and [2-^{14}C]thymidine, respectively, into cold trichloroacetic acid-insoluble precipitates.

Bacillus subtilis cells grown to exponential phase (two to three hours) in a medium containing 2% glucose and 1% yeast extract (GYE) in shake flasks were collected by centrifugation. The cells were suspended in a medium containing 0.4% glucose and 0.2% yeast extract (GYE dil.) to give an absorbancy of 3.0 at 660 nm.

The 1-ml assay mixture contained 0.5 ml of the cell suspension, 0.46 ml GYE dil., and 20 μl of ravidomycin solution of the required concentration. After 10 minutes at 25°C, 20 μl of the labeled precursor (1 mM, 10 mCi/mmol); was added. The reaction tubes were incubated at 37°C, on a rotary shaker. The reaction was terminated by the addition of 1 ml of 10% trichloroacetic acid. The insolubles were collected on a Whatman GF filter mounted on top of a Millipore filter (0.8 μm). The dried filters were placed in vials containing 15 ml of a toluene-based scintillation counting fluid, and radioactivity was counted in a Packard Model 2375 liquid scintillation counter.

Ravidomycin inhibited syntheses of DNA and RAN (Fig. 2). At a concentration of 0.5 μg/ml, ravidomycin inhibited DNA synthesis more (50~60%) than RNA synthesis (20~30%), while protein synthesis was only slightly inhibited (<5%).

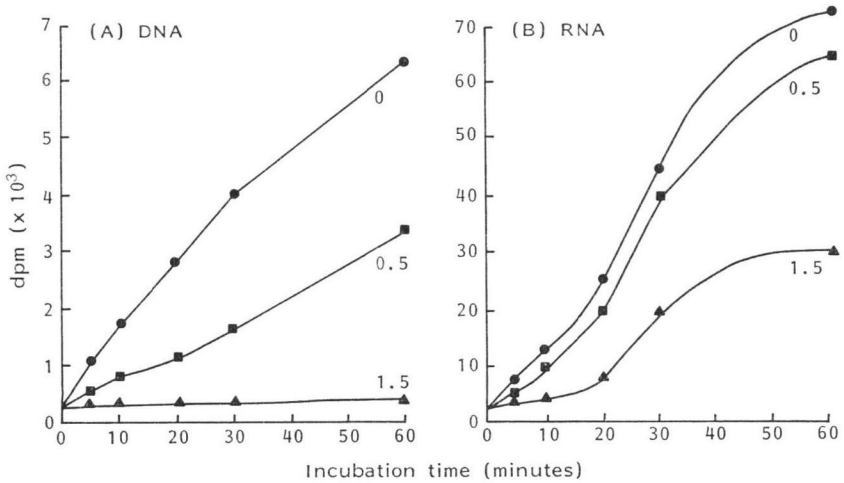
The time courses of DNA and RNA syntheses by *B. subtilis* are shown in Fig. 3. Significant inhibition of RNA and DNA syntheses took place within the first five minutes. However, ravidomycin at 1.5 μg/ml almost instantaneously inhibited synthesis of DNA, while synthesis of RNA proceeded at a much diminished rate.

Although low concentrations of ravidomycin inhibit RNA and DNA syntheses, the results suggest that ravidomycin primarily inhibits DNA synthesis followed by inhibition of RNA synthesis. Similar results were recently reported with new antitumor antibiotics, gilvocarcins and chrysomycin[2,3].

The action of ravidomycin on intracellular macromolecules was assessed. *B. subtilis* was grown for five hours with shaking at 37°C in GYE (50 ml) containing 10 μCi of [2-^{14}C]thymidine. Labeled cells were washed with GYE and resuspended in fresh ravidomycin containing medium without isotope. After a two-hour incubation period, the samples were removed, added to 1 volume of 10% cold trichloroacetic acid, and the radioactivity in the acid-insolubles was determined. Ravidomycin (4 μg/ml) had no detectable effect upon cellular DNA. Similarly ravidomycin did not cause any loss in radioactivity from cells labeled with [2-^{14}C]uridine or [*U*-^{14}C]leucine. In its action on intracellular DNA, ravidomycin resembles chrysomycin A[2].

Fig. 3. Time-course of inhibition of RNA and DNA syntheses.

The reaction mixture contained per ml: 0.50 ml of cell suspension, 0.46 ml GYE dil., 20 μl of the labeled precursor (1 mM, 10 mCi/mmol). Samples were removed at indicated time intervals and radioactivity in cold trichloroacetic acid-insolubles determined. The numbers on the curve represent μg/ml of ravidomycin.



Acknowledgment

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References

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