Inhibition of Protein Synthesis in *Mycobacterium smegmatis* by a New Antibiotic Lividomycin

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Summary

Lividomycin inhibited incorporation of acetate-1-^{14}C, glutamic acid-1-^{14}C, leucine-1-^{14}C, and glycine-1-^{14}C into protein of *Mycobacterium smegmatis* (strain Jucho). Incorporation of acetate-1-^{14}C and glycine-1-^{14}C into nucleic acids was also inhibited, but incorporation of these compounds into lipids was not inhibited in the presence of lividomycin. Incorporation of uridine-2-^{14}C into nucleic acids was significantly inhibited, but incorporation of thymidine-2-^{14}C into the nucleic acid fraction was not inhibited in the presence of the antibiotic. It is suggested that lividomycin inhibits the synthesis of protein, probably as a result of inhibition of RNA synthesis.

INTRODUCTION

Lividomycin is a new aminoglycoside antibiotic produced by Kowa Company, Tokyo (ODA, MORI, ITO & KUNIEDA, 1971 ; MORI, ICHIYANAGI, KONDO, TOKUNAGA & ODA, 1971 ; ODA, MORI & KYOTANI, 1971 ; ODA, MORI, KYOTANI & NAKAYAMA, 1971). Its *in vitro* and *in vivo* antituberculous activity was reported by TSUKAMURA, MIZUNO & YAMAMOTO (1970). Clinical investigations on lividomycin are being done in several clinics. The present study concerns with the mode of action of lividomycin.

METHODS

Mycobacterium smegmatis, strain Jucho, was used. The organism was cultivated in SAUTON medium at 37°C for 5 days, washed three times in saline, and used for experiments. The organism was incubated in a reaction mixture (4.0 ml in centrifugation tube) containing various radioactive compounds in the presence or absence of lividomycin. After incubation at 37°C for 0, 3, 6, and 24 hours, the mixture was cooled in ice water bath, and the organism was collected by centrifugation at 500 G for 15 minutes. The organism was washed three times with 4.0 ml of cold distilled water and fractionated according to the procedure of SCHNEIDER (1945). The fractionation was carried out as follows : (TCA-soluble fraction) extracted twice with 2.0 ml of cold 10% (w/v) trichloroacetic acid (TCA) solution ; (Lipid fraction) extracted twice with 2.0 ml of ethanol and twice with 3.0 ml of boiling ethyl ether-ethanol (1.5 ml ethyl ether+1.5 ml ethanol) ; (Nucleic acid fraction) extracted twice with 2.0 ml of 5% (w/v) TCA solution in water bath (100°C) ; (Protein fraction) the residue dissolved in 2.0 ml of 1% (w/v) NaOH solution by heating at 100°C.

A 0.02 ml sample of each fraction was placed in a stainless planchet, dried by heating, and measured for radioactivity by a gas flow counter (Kobe Electric Company, Osaka). A total of the radioactivity in each fraction was calculated from the radioactivity (counts/minute) measured in the sample and the volume of the fraction.

The compositions of the reaction mixtures are shown in the legends of figures. Final concentration of lividomycin was 100 μg/ml.

The following radioactive compounds were used: Sodium acetate-1-^{14}C (specific activity, 0.630 mc/mg) ; DL-glutamic acid-1-^{14}C (specific activity, 0.103 me/mg) ; L-leucine-1-^{14}C (specific activity, 0.374 mc/mg) ; glycine-1-^{14}C (specific activity, 0.552 mc/mg) ; thymidine-2-^{14}C (specific activity, 0.237 mc/mg) ; uridine-2-^{14}C (specific activity, 0.227 mc/mg). These were the products of the Radiochemical Centre, Amersham, Buckinghamshire, England.

TCA-soluble and protein fractions were subjected to the paper chromatography. The TCA-soluble fraction was extracted five times, to remove TCA, with ethyl ether and concentrated until a 1/10 volume under reduced pressure (2 mmHg). The protein fraction was put into an ampoule containing 4.0 ml of 6 N HCl and heated at 100°C for 24 hours. After this hydrolyzation, the fraction was concentrated until a 0.2 ml amount by heating at 100°C. The

concentrate was placed on a Toyo filter paper strip (no. 50) and subjected to paper chromatography. The solvents used were (1) tertiary butanol+formic acid+water (75+15+15) and (2) n-butanol+acetic acid (4+1). The radioactivity in paper chromatograms was measured by automatic paper chromatogram scanner (slit, 3 mm ; recording size, 1 : 2 ; recording, 300 mm/hour ; range, 300 or 1,000 counts per 5 seconds) (Nihon Musen Company, Tokyo). After measurement of the radioactivity, the spots of paper were coloured by ninhydrin reagent, and the Rf value was measured and compared with the Rf value of authentic amino acids. The number of viable organisms (the number of colony-forming units) in the reaction mixture was measured after washing the organisms three times with saline.

RESULTS

The results obtained are shown in Figures 1 to 6.

Radioactive acetate was incorporated at a large amount into the lipid fraction, and radioactive glycine also at a little amount. These incorporations were not affected by the presence of lividomycin.

Radioactivities from acetate, glutamic acid, leucine and glycine were found at a large amount in the protein fraction. The incorporations of these radioactive compounds into the protein fraction were significantly inhibited in the presence of lividomycin.

Radioactive acetate and glycine were incorporated at a considerable amount into the nucleic acid fraction. The incorporations of these compounds were inhibited significantly by lividomycin.

Radioactive thymidine and uridine were incorporated almost exclusively into the nucleic acid fraction, the rdioactivity in the lipid and protein fractions remaining very scanty. A considerable amount of the radioactivity was found in the TCA-soluble fraction. The incorporation of the radioactivity from thymidine, which labels DNA exclusively, was not inhibited, whereas that of radioactive uridine was significantly inhibited in the presence of lividomycin.

Under the conditions tested, the antibiotic appeared bacteriostatic. No significant loss of the viable count was observed.

A few suggestions were obtained from the paper chromatographic studies. In experiment of acetate incorporation, TCA-soluble fraction of both samples, control and lividomycin, showed single radioactive spot that corresponded to glutamic acid as shown previously (TSUKAMURA & TSUKAMURA, 1964). Radioactive spot of glutamic acid was observed also in the protein fraction, but the dimension of the radioactivity in lividomycin sample was approximately 1/10 of that of control sample. The result suggests that the process of formation of glutamic acid from acetate is not inhibited by lividomycin, but the incorporation of glutamic acid into protein molecules is inhibited.

In experiment of glutamic acid incorporation, both control and lividomycin samples of the TCA-soluble and protein fractions showed two radioactive spots, one large and another small. These corresponded to glutamic acid and leucine, respectively.

Radioactive spot of leucine was found in the protein fraction of both control and lividomycin samples. No other spot was found.

In experiment of glycine incorporation, radioactive spots corresponding to serine and aspartic acid were found in the protein fraction of control sample, whereas only spot corresponding to serine was found in the protein fraction of lividomycin sample.

The above observations on paper chromatograms were made on the samples of 24 hours-incubation.

In view of the above observations, it is considered that lividomycin inhibits protein synthesis and this inhibition of protein synthesis is probably due to inhibition of RNA synthesis. The inhibition of RNA synthesis is suggested from the finding that the incorporation of radioactive thymidine, which labels DNA exclusively, has not been inhibited but that of radioactive uridine has significantly been inhibited in the presence of lividomycin.

Referenccs

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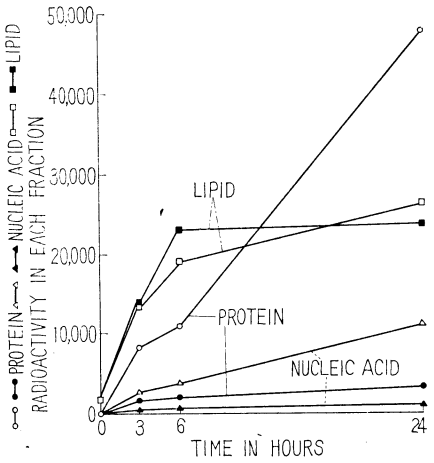
Fig. 1. Effect of lividomycin on incorporation of acetate-1-^{14}C into lipid, nucleic acid and protein fractions of *Mycobacterium smegmatis* Jucho.

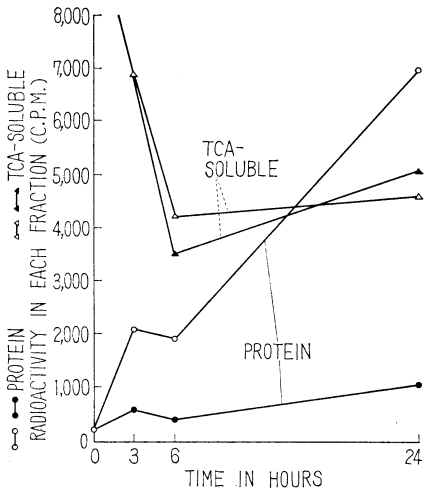


Open: Control. Closed: lividomycin.

The radioactivity is expressed as counts per minute in each fraction obtained from 16 mg dry weight of bacterial cells.

Reaction mixture consisted of the following: Bacterial suspension (40 mg wet weight/ml) in M/15 phosphate buffer, pH 7.1, 2.0 ml ; acetate-1-^{14}C (sodium salt) solution (10 μc/ml), 1.0 ml; sodium acetate solution (5 μg/ml), 0.5 ml; distilled water (control) or lividomycin solution (800 μg/ml), 0.5 ml. After incubation the cells were collected by centrifugation, washed once with 0.1% (w/v) sodium acetate solution, and thereafter washed three times with distilled water. The cells were fractionated according to the procedure of SCHNEIDER.

Fig. 2. Effect of lividomycin on incorporation of glutamic acid-1-^{14}C into TCA-soluble and protein fractions of *Mycobacterium smegmatis* strain Jucho.

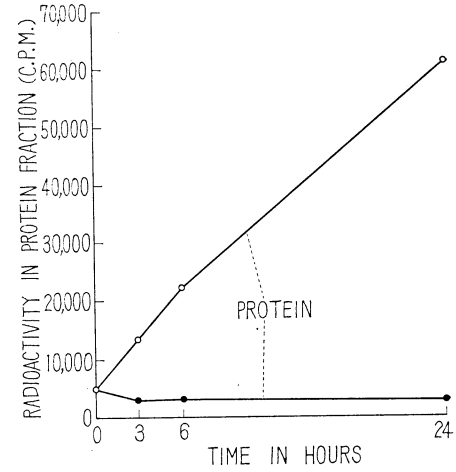


Open: Control. Closed: Lividomycin.

The radioactivity is expressed as counts per minute in each fraction obtained from 16 mg dry weight of bacterial cells.

The reaction mixture consisted of the following: Bacterial suspension (40 mg wet weight/ml) in M/15 phosphate buffer, pH 7.1, 2.0 ml ; glutamic acid-1-^{14}C solution (10 μc/ml), 1.0 ml ; sodium glutamate solution (5 μg/ml), 0.5 ml; distilled water (control) of lividomycin solution (800 μg/ml), 0.5 ml.

Fig. 3. Effect of lividomycin on incorporation of leucine-1-^{14}C into protein fraction of *Mycobacterium smegmatis* strain Jucho.



After incubation, the cells were washed once with 0.1% (w/v) sodium glutamate, and then three times with distilled water. The cells were fractionated according to the procedure of SCHNEIDER.

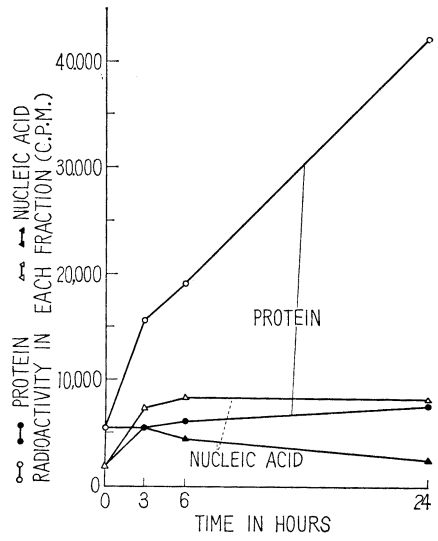
Open: Control. Closed: Lividomycin.

The radioactivity is expressed as counts per minute in protein fraction obtained from 48 mg dry weight of bacterial cells.

The reaction mixture consisted of the following: Bacterial suspension (120 mg wet weight/ml) in M/15 phosphate buffer, pH 7.1, 2.0 ml; L-leucine-1-^{14}C solution (10 μc/ml), 1.0 ml; L-leucine solution (5 μg/ml), 0.5 ml ; distilled water (control) or lividomycin solution (800 μg/ml), 0.1 ml.

After incubation, the cells were washed once with 0.1% (w/v) L-leucine solution and thereafter three times with distilled water. The cells were fractionated according to the procedure of SCHNEIDER.

Fig. 4. Effect of lividomycin on incorporation of glycine-1-^{14}C into nucleic acid and protein fractions of *Mycobacterium smegmatis* strain Jucho.



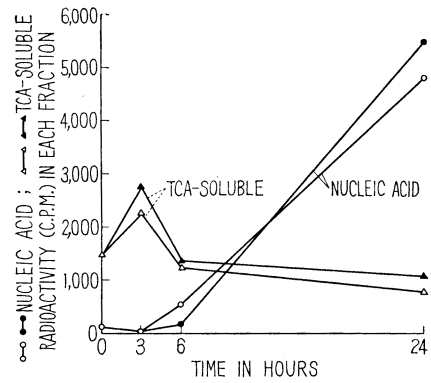
Open: Control. Closed: Lividomycin.

The radioactivity is expressed as counts per minute in each fraction obtained from 48 mg dry weight of bacterial cells.

The reaction mixture consisted of the following: Bacterial suspension (120 mg wet weight/ml) in M/15 phosphate buffer, pH 7.1, 2.0 ml; glycine-1-^{14}C solution (10 μc/ml), 1.0 ml; glycine solution (5 μg/ml), 0.5 ml ; distilled water (control) or lividomycin solution (800 μg/ml), 0.5 ml.

After incubation, the cells were washed once with 0.1% (w/v) glycine solution and thereafter three times with distilled water. The cells were fractionated according to the procedure of SCHNEIDER.

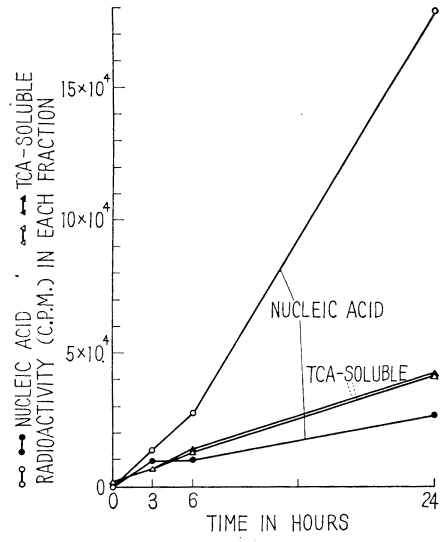
Fig. 5. Effect of lividomycin on incorporation of thymidine-2-^{14}C into nucleic acid and TCA-soluble fractions of *Mycobacterium smegmatis* strain Jucho.



Open: Control. Closed: Lividomycin.

The radioactivity is expressed as counts per minute in each fraction obtained from 60 mg dry weight of bacterial cells. The reaction mixture consisted of the following : Bacterial suspension (150

Fig. 6. Effect of lividomycin on incorporation of uridine-2-^{14}C into nucleic acid and TCA-soluble fractions of *Mycobacterium smegmatis* strain Jucho.



mg wet weight/ml) in M/15 phosphate buffer, pH 7.1, 2.0 ml; thymidine-2-^{14}C solution (10 μc/ml), 1.0 ml; distilled water (control) or lividomycin solution (400 μg/ml), 1.0 ml.

After incubation, the cells were washed three times with distilled water and then fractionated according to the procedure of SCHNEIDER.

Open: Control. Closed: Lividomycin.

The radioactivity is expressed as counts per minute in each fraction obtained from 20 mg dry weight of bacterial cells.

The reaction mixture consisted of the following: Bacterial suspension (50 mg wet weight/ml) in M/15 phosphate buffer, pH 7.1, 2.0 ml; uridine-2-^{14}C solution (10 μc/ml), 1.0 ml; distilled water (control) or lividomycin solution (400 μg/ml), 1.0 ml.

After incubation, the cells were washed three times with distilled water and then fractionated according to the procedure of SCHNEIDER.