

ON THE EFFECT OF N-METHYL-BIS (3-MESYLOXYPROPYL) AMINE HYDROXYCHLORIDE ON *BACILLUS SUBTILIS* CELLS

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N-Methyl-bis (3-mesyloxypropyl)amine hydrochloride is now in use as an anti-tumor drug. In view of its activity against some bacteria the present work was conducted to study its mode of action on *Bacillus subtilis*. The compound was found to induce irreversible damage to bacterial DNA whereas its effect on RNA was temporary and depending on maintenance of effective concentrations of the compound.

In 1965 EL-MERZABANI and SAKURAI¹⁾, studied the antitumor effect of some new derivatives of sulphonic acid esters of aminoglycols, most of which proved to be effective against YOSHIDA sarcoma. However, N-methyl-bis (3-mesyloxypropyl)amine hydrochloride (substance 838) was the only compound which proved effective in the prolongation of the life span of tumor-bearing animals. This compound is now in use as a chemotherapeutic drug for tumor treatment. The effect of this compound on *Bacillus subtilis* is described in the present paper.

Materials and Methods

Organisms and culture conditions: *Bacillus subtilis*icc and *Escherichia coli* Juhl were grown in shake cultures (220 rpm) at 35°C in nutrient broth having the following composition (g/100 ml): meat extract 0.15; yeast extract 0.15; peptone 0.5 and NaCl 0.5 and harvested at the end of their log. phase of growth (O.D. 1.4 at 660 nm). Cells were collected by centrifugation at 15,000×g for 10 minutes and washed twice with sterile saline solution. Release of cellular materials absorbing at 260 nm was conducted by the method of ITO and KOYAMA²⁾.

Extraction and sucrose density-gradient centrifugation analysis of *B. subtilis* RNA: Cells were collected by centrifuging (15,000×g) 3 ml portions of cultures supplemented with 0, 1 and 5 µg/ml of substance 838. These were separately transformed to their protoplasts by the method of WEIBULL³⁾, the RNA was extracted by the SDS-phenol method and this was then precipitated with ethanol. The precipitates were centrifuged and dissolved in 1.2-ml portions of phosphate buffer of pH 6.7. Aliquots of supernatant (0.6 ml) were applied to sucrose gradients (5~15 w/v %, 13-ml tube) and were then centrifuged at 45,000 rpm (115,461×g) for 7 hours at 4°C. Immediately after centrifugation the tubes were fractionated by puncturing the bottoms of the centrifuge tubes to collect 0.38-ml portions for which the absorbance at 260 mµ was measured. Results are shown in Figs. 4a, b and c.

Analysis of *B. subtilis* DNA by alkaline sucrose gradients centrifugation: The method of MCGRATH and WILLIAMS⁴⁾ was applied. *B. subtilis* cells were converted to protoplasts and 10⁷ protoplasts were added to the tops of (5~20 % linear) alkaline sucrose gradients containing 0.7 M NaCl; 0.3 M NaOH and 1 mM EDTA and prepared after NOLL⁵⁾. The tubes were centrifuged at 40,000 rpm for 1 hour and then punctured from their bottoms to collect 0.53-ml fractions. The O.D. for each fraction was determined at 260 mµ. Results are given in Figs. 5a, b and c.

Fig. 1. Effect of substance 838 on the release of 260 nm absorbing materials

Bacterial cells (10^8 /ml) obtained as described in text were suspended in phosphate saline buffer of pH 7.0. Different concentrations of the drug were added. Control cultures lacking the drug were run alongside. At the predetermined incubation period 3 ml samples were withdrawn, centrifuged at $15,000 \times g$ and the O.D. at 260 nm of the supernatant was measured.

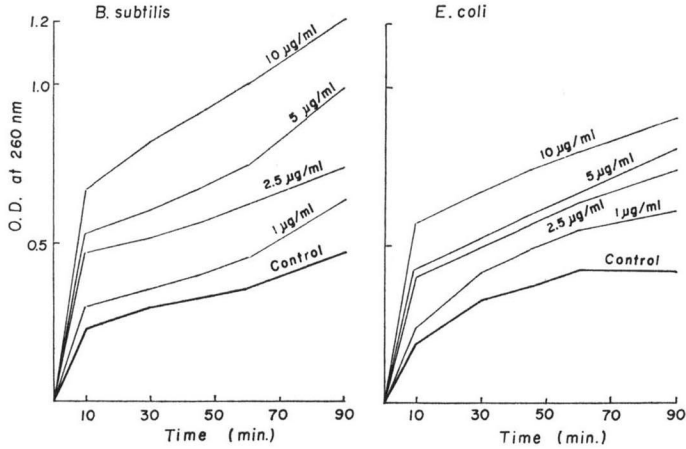


Fig. 2. The UV spectra of the released substances

The UV spectra of samples collected after 60 minutes and obtained from the experiment of Fig. 1

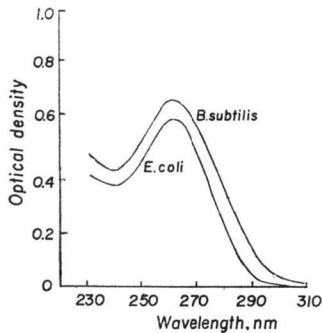
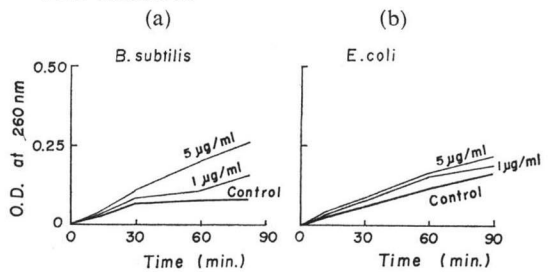


Fig. 3. OD measurements of ice-cold 5% TCA soluble fraction

Bacterial cells (10^8 /ml) were suspended in fresh nutrient broth medium; 1 and 5 µg/ml of substance 838 were added and cultures lacking the drug served as controls. Three ml samples were withdrawn after different incubation periods, the cells were collected by centrifugation at $15,000 \times g$, washed with sterile saline solution and treated with ice-cold 5% TCA for 30 minutes. The O.D. at 260 nm of the supernatants were measured



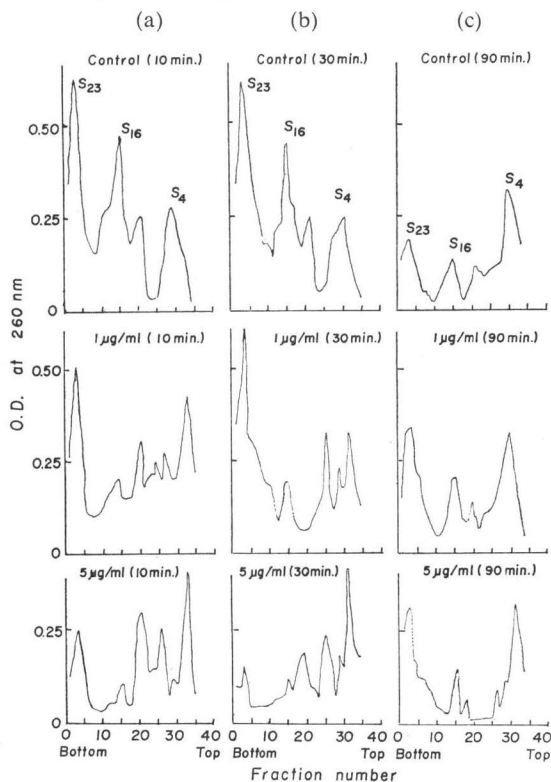
Results

Incubating *B. subtilis* and *E. coli* cells resting in minimal phosphate medium with 1~10 µg/ml of substance 838 caused the release of 260 nm absorbing material (Figs. 1a and b). The UV spectrum of these released materials (Fig. 2) corresponded to that of nucleic acids. The effect on gram-positive bacteria was more pronounced than that on gram-negative ones. Concentrations of 1 and 5 µg/ml of the substance were chosen for subsequent investigations since they represented concentrations causing mild and severe effects on the resting cells.

The effect of the tested substance on the DNA of *B. subtilis* and *E. coli* cells growing normally in nutrient medium was assessed by measuring the O.D. at 260 nm of ice-cold 5% TCA extracts of cells. The effect on *B. subtilis* was quite pronounced whereas these was little

Fig. 4. Sucrose-density gradient profile of *B. subtilis* RNA

B. subtilis cells were resuspended in fresh nutrient broth containing 0.0, 1.0 and 5.0 $\mu\text{g}/\text{ml}$ of substance 838; and 3 ml samples were withdrawn after 10, 30 and 90 minutes of treatment. The cells were transferred to protoplasts, then RNA was extracted and loaded on sucrose gradients (5~15 w/v %) prepared in Tris-HCl buffer of pH 6.7 containing 0.01 M EDTA. The rest of the experimental steps were as given in text.



effect on *E. coli* (Figs. 3a and b).

The ultracentrifugal patterns of the RNA of *B. subtilis* cells are illustrated in Figs. 4a, b and c. Substance 838 caused abnormalities in the sedimentation profile of the RNA between the region of S16 and S4. Such an effect was observed after 10 minutes of the treatment (Fig. 4a) and remained for the next 20 minutes (Fig. 4b). After 90 minutes there appeared to be a repair in the RNA damage (Fig. 4c).

Substance 838 caused the scission of the DNA into smaller fragments. The sedimentation profile of the DNA obtained from normal and treated microbial cells at various incubation periods is shown in Figs. 5a, b and c. With longer incubation periods the effect on the DNA was increased.

Substance 838 rapidly lost activity when its aqueous solutions were incubated at 37°C as illustrated in Fig. 6. Similar results were recorded by EL-MERZABANI¹³ when the substance was injected into the peritoneal cavity of rats.

Discussion

Several drugs with antitumor activities induced their effects through the inhibition of purine and pyrimidine biosynthesis⁹⁻⁸⁾, and others break the DNA strands⁹⁻¹¹⁾.

Fig. 5. Alkaline sucrose-gradient profile of *B. subtilis* DNA

The experimental steps were those of legened to Fig. 4

B. subtilis protoplasts were layered on the top of alkaline sucrose-gradients. The rest of the experimental steps were as given in the materials and methods.

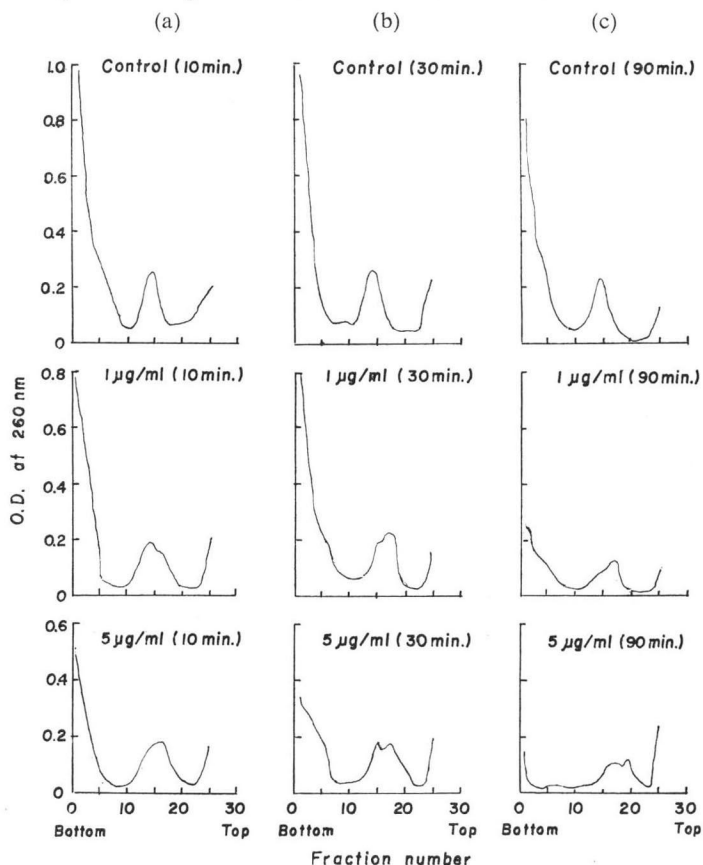
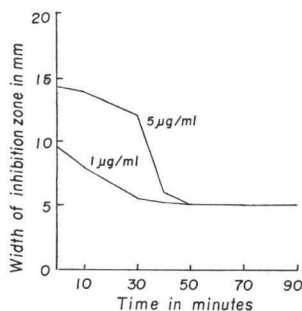


Fig. 6. Effect of lapse of time on the antimicrobial activity of substance 838

Antimicrobial activity against *B. subtilis* was determined by the agar disc technique. Diameters of discs used were 5 mm.



DNA strands producing two uneven fragments. This effect was persistent once it was induced by the drug even though the drug loses its activity after the first 45 minutes. On the other

Substance 838 caused the release of 260 nm absorbing materials from static cultures of *E. coli* and *B. subtilis*. When growing cells were subjected to the drug, the increase in the O.D. at 260 nm of the ice-cold 5% TCA soluble fraction could still be recorded with cultures of *B. subtilis* whereas such an effect was not observed in case of *E. coli*.

Figures (4a, b and c) of the sedimentation profile of RNA showed a tendency for the RNA to return to normal after 90 minutes of treatment with the drug whereas the disturbance in the sedimentation profile of the DNA persisted.

The effect of the drug on DNA seemed likely to induce irreversible fragmentation of

hand, the effect on RNA was temporary and seemed to be dependent on the existence of an effective concentration of the drug.

Acknowledgement

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