THE BIOCHEMICAL EFFECT OF AUROMOMYCIN ON BACTERIAL CELLS

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The mechanism of action of auromomycin was studied with intact cells of *Bacillus subtilis*. The antibiotic exhibited a preferential inhibition of DNA synthesis over RNA and protein syntheses. The strand scission of cellular DNA was induced by the drug, and DNA was degraded into acid-soluble fragments. The results suggested that DNA is the chemoreceptor of auromomycin.

Auromomycin, a new antitumor antibiotic, was isolated from the culture broth of *Streptomyces macromomyceticus*, a macromomycin-producing organism, by YAMASHITA *et al.*[1] The antibiotic is an acidic polypeptide with a molecular weight of 12,500 and an isoelectric point of pH 5.4, and consists of 16 different amino acids. The physicochemical and biological properties of auromomycin are different from those of macromomycin, but auromomycin is converted into macromomycin by adsorption chromatography on Amberlite XAD. Auromomycin inhibits growth of Gram-positive and Gram-negative bacteria, whereas the antimicrobial spectrum of macromomycin is limited to Gram-positive organisms.

We have studied the mechanism of action of auromomycin with cultured tumor cells and isolated viral DNA, and found that the antibiotic causes DNA strand breaks *in vivo* and *in vitro*[2]. The studies on the mode of action have been extended to bacterial cells, and the results are presented in this publication.

Materials and Methods

Auromomycin (Lot 11-1 and T-0504) was generously supplied by Kanegafuchi Chemical Industries, Osaka, and was dissolved in saline just prior to use. Pluramycin A was kindly donated by Dr. K. MAEDA, and bleomycin A_2 by Dr. T. TAKEUCHI, Institute of Microbial Chemistry, Tokyo. [^{3}H]Thymine (13.2 Ci/mmole), and [^{3}H]uridine (37.6 Ci/mmole) were purchased from New England Nuclear, Boston, Mass. [^{14}C]Valine (280 mCi/mmole) was obtained from Radiochemical Centre, Amersham, England. Nuclease-free pronase was generously provided by Dr. H. SAITO, Institute of Applied Microbiology, University of Tokyo, Tokyo. The other chemicals were commercial products.

Bacillus subtilis Marburg 168 (thy$^-$) was grown at 37°C with aeration in nutrient broth, containing (g/liter): glucose 5, peptone 10, NaCl 0.5, KCl 0.1, and thymine 0.02. The cells at an early logarithmic phase of growth were used in all the experiments.

Cell growth and viability

The cells were incubated with various concentrations of auromomycin in 5 ml medium at 37°C under gentle shaking, and the growth was measured every hour by turbidimetry at 660 nm. For determination of cell viability, 0.5 ml of the culture was withdrawn at 10 and 60 minutes, diluted with saline to stop further reaction with the drug, and plated on nutrient agar containing 20 μg/ml of thymine after appropriate dilutions. The colonies were counted after overnight incubation.

Syntheses of macromolecules

The cell suspension of 5 ml was incubated, as described above, with auromomycin and appropriate labelled precursor ([^{3}H]thymine 0.2 μCi/ml, [^{3}H]uridine 0.2 μCi/ml plus cold uridine 40 μg/ml, or [^{14}C]valine 0.2 μCi/ml). At the time indicated, 1 ml of the culture was withdrawn and mixed with an equal volume of 10% ice-cold trichloroacetic acid (TCA). The insoluble materials were collected on glass fiber filters (Whatman GF83), and washed with cold 5% TCA. In the case of [^{14}C]valine incorporation, the samples were heated at 90°C for 20 minutes before filtration. The radioactivity was determined in toluene-PPO-POPOP, using a liquid scintillation counter.

Labelling DNA with radioactivity

The cells were incubated with 1 μCi/ml of [^{3}H]thymine for 2~3 hours in the medium containing 2 μg/ml of thymine. The labelled cells were washed and resuspended in fresh medium with 20 μg/ml of thymine.

DNA degradation into the acid-soluble fraction

The [^{3}H]thymine-labelled cells were incubated at 37°C for 10 minutes with antibiotics without shaking in 0.2 ml of nutrient broth. The incubation was terminated by the addition of an equal volume of 10% chilled TCA. The samples were centrifuged, and the TCA-soluble radioactivity, after extracting TCA with ether, was determined in 10 ml of BRAY's solution. The TCA-insoluble radioactivity was measured by solubilizing the DNA fraction with 10% TCA at 90°C for 20 minutes. The degradation of DNA into acid-soluble fragments was calculated from the ratio of the TCA-soluble radioactivity to the total radioactivity of both TCA-soluble and TCA-insoluble fractions.

DNA strand scission

The cell suspension incubated with antibiotics, as described above, was chilled, diluted with cold saline-EDTA (0.15 M NaCl and 0.01 M EDTA), centrifuged and resuspended in 0.2 ml of saline-EDTA. It was then treated at 37°C with 40 μg of lysozyme for 4 minutes, 40 μg of pronase for 4 minutes, and 0.2% (final) SDS for 10 minutes; and then 1/10 volume of 1 N NaOH was added at room temperature. The whole cell lysate was gently layered on the top of 4.6 ml of 5~20% alkaline sucrose gradient, and centrifuged at 40,000 rpm for 2.5 hours at 20°C in a Beckman SW50L rotor. The medium for sucrose gradients contained 0.7 M NaCl, 0.01 M EDTA and 0.3 N NaOH. The fractionation and determination of radioactivity were carried out as described previously[8].

Results

Growth Inhibition by Auromomycin of *B. subtilis*

Turbidimetric and survival studies showed that auromomycin inhibited growth of *B. subtilis* Marburg 168 at antibiotic concentrations higher than 0.06 μg/ml. As illustrated in Fig. 1, the action was bactericidal.

Effects of Auromomycin on Macromolecular Syntheses of the Intact Cells

The incorporation of [^{3}H]thymine was more markedly blocked by auromomycin at concentrations of 1 and 10 μg/ml than those of [^{3}H]uridine and [^{14}C]valine (Fig. 2). The results suggested that the primary action of the antibiotic may be involved in DNA synthesis, and the inhibition of RNA and protein syntheses may be the secondary action.

Degradation of Cellular DNA into Acid-soluble Materials

The [^{3}H]thymine-labelled cells were incubated with antibiotics at 37°C and the degradation of DNA into the TCA-soluble fraction was examined. The results are summarized in Fig. 3. In control cells without antibiotics, the radioactivity appeared slightly in the TCA-soluble fraction during the incubation. Auromomycin induced degradation of cellular DNA into acid-soluble fraction even in 30 minutes; and approximately 80 and 70% of the total radioactivity changed into TCA-soluble frag-

Fig. 1. The effect of auromomycin on growth and viability of *B. subtilis*.

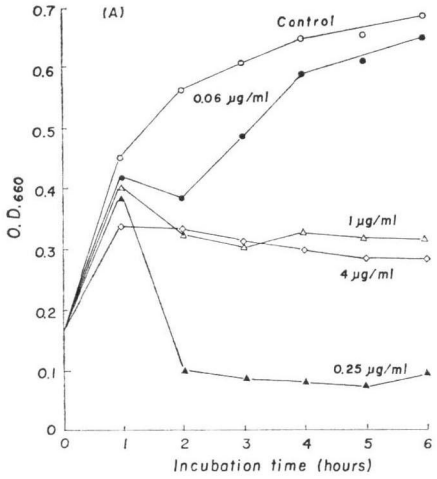


Fig. 2. The effect of auromomycin on macromolecular syntheses in *B. subtilis*.

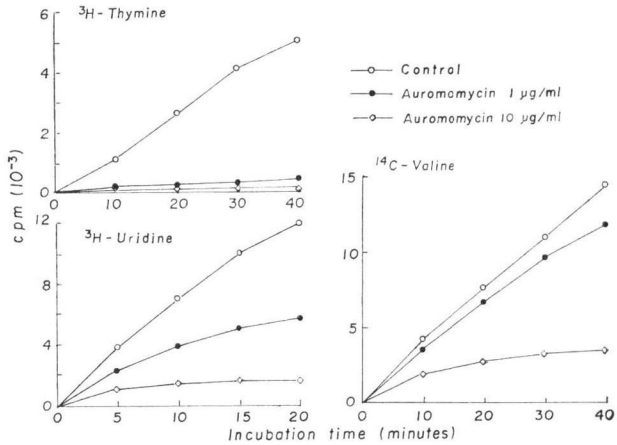
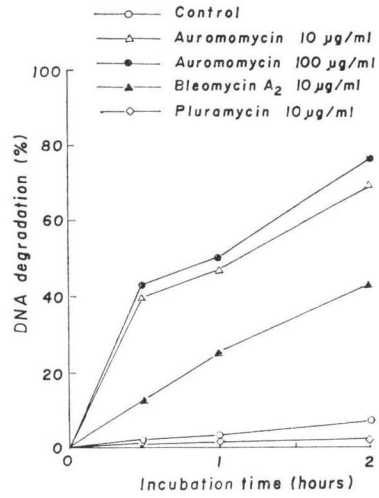


Fig. 3. DNA degradation by antibiotics in intact cells of *B. subtilis*.



ments at 10 and 100 μg/ml of the antibiotic, respectively, by the 2-hour incubation. In parallel experiments, bleomycin A_2, which was reported to cause degradation of cellular DNA of *E. coli*[4], was also observed to induce DNA degradation in *B. subtilis* up to 45% of the total DNA during 2 hours of incubation. Contrary to these antibiotics, pluramycin A showed a stabilizing effect on cellular DNA, which seemed to be in accord with the results that pluramycin A binds to and stabilizes the double strand structure of DNA[5].

No significant degradation of cellular RNA into acid-soluble fraction was found at concentration of 100 μg/ml of auromomycin (data are not shown).

The degradation of cellular DNA by auromomycin was examined over a wide range of drug concentrations, and the results are presented in Table 1. A marked degradation was observed at concentrations higher than 0.1 μg/ml of auromomycin, although the dose response was not clear at high antibiotic concentrations.

DNA Strand Scission Induced by Auromomycin

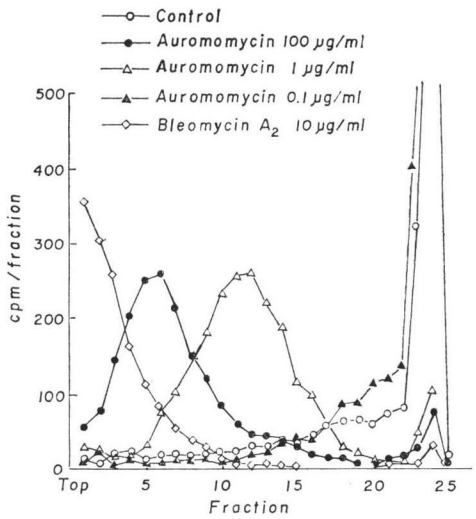
The single strand scission of cellular DNA of auromomycin-treated cells was observed by alka-

Table 1. Degradation of cellular DNA into acid-soluble fragments by auromomycin.

The reaction mixture was incubated at 37°C for 70 minutes.

Antibiotics		% Degradation
None		5.1
Auromomycin	0.01 μg/ml	8.7
	0.1 μg/ml	41.5
	1 μg/ml	64.1
	10 μg/ml	68.7
	100 μg/ml	68.7
Bleomycin A_2	10 μg/ml	46.6

Fig. 4. DNA strand scission induced by antibiotics in intact cells of *B. subtilis*.



line sucrose density gradient centrifugation. By the method employed, DNA of control cells sedimented at the bottom of the tube. When the cells were exposed to 100 or 1 μg/ml auromomycin at 37°C for 10 minutes, fragmentation of cellular DNA occurred and DNA peak appeared at different positions in the gradient depending upon the antibiotic concentrations (Fig. 4). At drug concentration of 0.1 μg/ml, no new peak was observed in the gradient, heavy fractions near the bottom slightly increased, indicating a slight scission of DNA strand. Bleomycin A_2, which was reported to interact with DNA and to induce DNA strand scission *in vitro* and *in vivo*[6,7], also significantly fragmented DNA of intact cells of *B. subtilis*.

Discussion

It has been demonstrated in the current experiments that auromomycin induces a single strand scission of DNA and exhibits a preferential inhibition of DNA synthesis over RNA and protein syntheses in *B. subtilis*. The results, obtained with bacterial cells, are well in accord with those observed in mammalian tumor cells[2]. Therefore, auromomycin seems to show the same mode of action in bacterial cells as in mammalian cells.

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