MECHANISM OF ACTION OF ANTITUMOR ANTIBIOTIC, STUBOMYCIN

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Stubomycin, a macrocyclic lactam involving β-phenylalanine, has been shown to have marked antitumor activity on several kinds of murine tumors[1~3]. The antibiotic also showed direct cytotoxic activity on mammalian cells and some microorganisms. It seemed that the primary action of the antibiotic is similar to that of polyene antibiotics[4]. The experiments reported herein were designed mainly to elucidate the action of stubomycin on *Saccharomyces cerevisiae*.

To determine the effect of stubomycin on the release of low molecular substances from *S. cerevisiae*, the microorganisms were suspended in saline at a final concentration of 20 μg/ml, and the amount of K^+, glucose and the UV-absorbing materials released into the medium were determined. As shown in Fig. 1, rapid release of these substances from *S. cerevisiae* was observed.

The morphological changes in the plasma membranes of *S. cerevisiae* were examined by freeze-fracture electron microscopy according to the procedure described previously[5]. Fig. 2 shows structural alterations in the plasma membrane of *S. cerevisiae* which was treated with 20 μg/ml of stubomycin for 30 minutes. There was no significant alteration in the distribution of membrane particles. However, many depressions were produced by treatment with stubomycin.

The effects of stubomycin (1 or 0.25 μg/ml) on the uptake of acetate into *S. cerevisiae* were investigated. One and three hours after addition of the drug and radioactive precursors, the uptake of [^{3}H]acetate and [^{3}H]uridine was determined. At the end of the incubation period, cells were collected on a glass fiber filter and washed with ice-cold saline, three times with

Fig. 1. Effect of stubomycin on the release of the low molecular substances from *Saccharomyces cerevisiae*.
Stubomycin was added to *S. cerevisiae* cells suspended in saline at time zero. At certain periods of incubation, part of the cell suspention was centrifuged, and the resulting supernatant was used for analysis. K^+ was measured using an atomic absorption flame spectrophotometer (Shimadzu AA 610 S). Glucose was determined by anthrone-H_2SO_4. The absorbance at 260 nm was measured using a Shimadzu UV 200S spectrophotometer.
○: OD, ●: glucose, △: K^+.

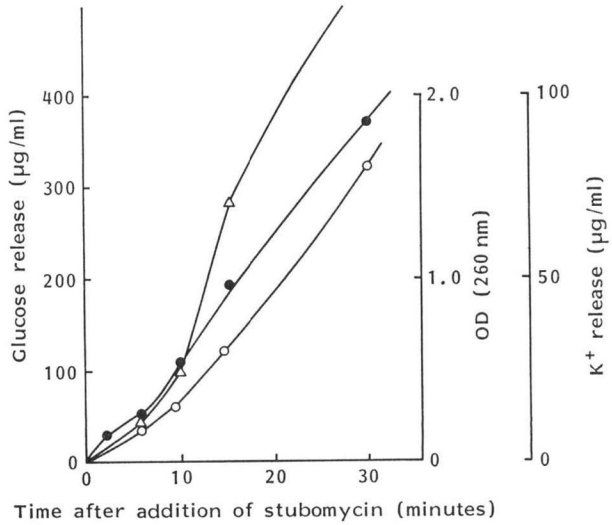

Fig. 2. Morphological change of membrane of *Saccharomyces cerevisiae* exposed to stubomycin.

S. cerevisiae cells suspended in saline were treated with 20 μg/ml of stubomycin for 30 minutes, and morphological changes in the plasma membrane were observed by freeze-fracture electron microscopy.

A: Control, B: treated with stubomycin.

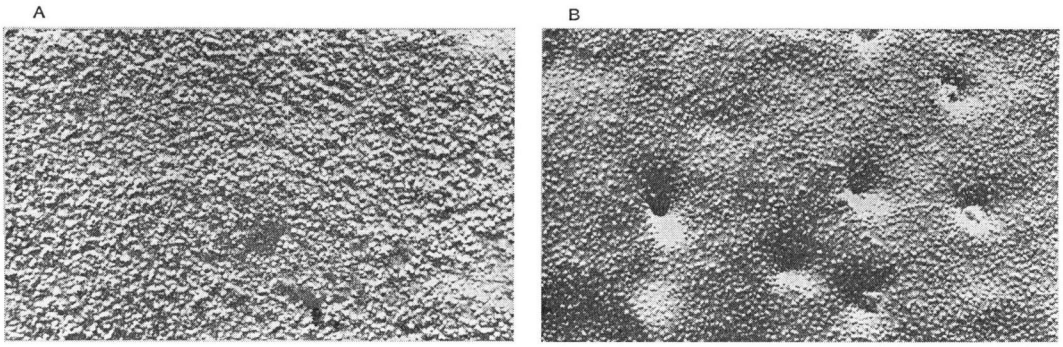

Table 1. Inhibitory action of stubomycin on the incorporation of acetate and uridine into acid precipitable fractions of *Saccharomyces cerevisiae*.

Drug	Concentration (μg/ml)	Percent of inhibition			
		1 hour		3 hours	
		[^{3}H]Acetate	[^{3}H]Uridine	[^{3}H]Acetate	[^{3}H]Uridine
Stubomycin	1.0	62	87	87	100
	0.25	13	53	57	64
Cerulenin	0.25	29	6	71	35

Medium: Glucose 3%, yeast extract 0.3%, peptone 0.3%, pH 7.3.

Precursors: [^{3}H]Acetate (1 μCi/ml), [^{3}H]uridine (0.5 μCi/ml).

S. cerevisiae were labeled for 1 hour with radioactive precursors before the termination of culture.

ice-cold 5% TCA, and with H_2O. The dried acid-precipitable material was dissolved in Protosol (New England Nuclear), and the radioactivity was counted by an Aloka liquid scintillation spectrometer. As shown in Table 1, stubomycin almost equally inhibited the uptake of [^{3}H]acetate and [^{3}H]uridine but cerulenin, known as an inhibitor of fatty acid synthesis, mainly inhibited the uptake of [^{3}H]acetate.

In our previous report, stubomycin showed DNA, RNA and protein synthesis inhibition at almost the same time and to the same degree[3], and some phospholipids reduced the anti-*S. cerevisiae* activity of the antibiotic[4]. Stubomycin inhibited growth of fungi as well as Gram-positive bacteria whereas polyene antibiotics inhibited mainly fungi because the presence of sterols in the membrane is a requirement for polyene antibiotic sensitivity[6~8]. Therefore, it appeared that stubomycin is incorporated into the membrane phospholipid bilayer, and increases the permeability of the plasma membrane, ultimately leading to lysis and death of the cells. At present, it is only clear that the difference between stubomycin and polyene antibiotics on the action of antibacterial activity was the target existing in the plasma membrane.

Acknowledgment

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