8006-I, AN ANTIBIOTIC FROM *AMBLYOSPORIUM SPONGIOSUM* (PERS.) HUGHES SENSU PIROZYNSKI

II. BIOLOGICAL PROPERTIES*

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8006-I is an antibacterial antibiotic with a rather broad spectrum of activity. The minimum inhibitory concentrations for the most sensitive bacteria are in the range of one to ten μg/ml. Yeasts are not affected by concentrations up to 100 μg/ml. Some filamentous fungi like *Fusarium oxysporum* and *Mucor miehei* are inhibited at 100 μg/ml. In Ehrlich carcinoma ascitic cells the incorporation of uridine and leucine and to a lesser extent that of thymidine is reduced.

In isolated nuclei of these cells the incorporation of UTP into RNA is inhibited. At low concentrations, the incorporation of uracil into trichloroacetic acid-precipitable material is almost completely inhibited in cells of *Bacillus subtilis*; at higher concentrations all macromolecular syntheses are affected. No reduction of respiration of the cells is observed. The antibiotic exhibits weak hemolytic activity and lytic activity towards bacteria.

In vitro an inhibition of both DNA- and RNA polymerase from *Escherichia coli* is observed. Poly(U)-directed poly(Phe) synthesis is not affected.

As described in the preceding paper[1], 8006-I, an antibiotic with a carotenoid structure, was isolated from submerged cultures of *Amblyosporium spongiosum* HA 8006. In the present paper, we wish to report the biological properties of antibiotic 8006-I.

Materials and Methods

Test Organisms and Growth Conditions

Bacteria (except *Actinomycetales*) were grown on nutrient broth (NB) or on meat extract, peptone medium (MP) composed of (per liter): lab lemco powder (Oxoid) 3 g, peptone (Merck) 5 g, and NaCl 2.5 g, the pH was adjusted to 7.2. *Lactobacillus casei* was grown on micro inoculum broth (Difco; MIB) and *Streptococcus faecalis* on a medium containing (per liter): tryptone (Difco) 10 g, yeast extract (Difco) 5 g, K_2HPO_4 2 g, glucose 11 g, the pH was adjusted to 7.5. The following synthetic media were used: MM1 according to EIKHOM *et al.*[2] and MM2 see preceding paper[1]. *Actinomycetales*, yeasts and filamentous fungi were grown on YMG medium[1], MA medium (20 g malt extract) or on a synthetic medium, MM3, composed of (per liter): glucose 10 g, $(NH_4)_2SO_4$ 1 g, $CaCl_2$ 0.1 g, NaCl 0.1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, K_2HPO_4 0.125 g, KH_2PO_4 0.875 g, HOAGLAND solution[3], 10 ml. For solid media agar, 20 g, was added to one liter of medium.

Assays

Incorporation experiments with bacteria, Ehrlich carcinoma ascitic (ECA) cells and nuclei from ECA cells were performed as described before[4]; protoplasts from *E. coli* were prepared according to the method of WEISS[5]. Tests for hemolytic activity were carried out as previously described[6]. DNA polymerase from *E. coli* was purchased from Boehringer, Mannheim; the assay is described in[7].

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DNA-dependent RNA polymerase from *E. coli* (Boehringer) was tested according to FUCHS *et al.*[8]. Chitinsynthase from *Coprinus cinereus* was prepared and tested as described by ADAMS and GOODAY[9]. Phosphodiesterase (Boehringer) was assayed according to FURUTANI *et al.*[10], and the assay of alcohol dehydrogenase from yeast (Boehringer) is described in[11]. All biochemicals were purchased from Boehringer, Mannheim; all radiochemicals from Amersham Buchler.

Results and Discussion

Antibiotic 8006-I exhibits a rather broad spectrum of antibacterial activity with minimum inhibitory concentrations (MIC) ranging from 1 μg to 100 μg/ml. Gram-positive as well as Gram-negative bacteria were sensitive. Yeasts were not affected by concentrations up to 100 μg/ml, and only a few filamentous fungi were sensitive at high concentrations, for example *Fusarium oxysporum*, *Humicola grisea*, and *Mucor miehei* at 100 μg/ml. The antimicrobial spectrum is given in Table 1.

In Fig. 1 the bactericidal effect of 8006-I on cells of *B. subtilis* grown in MM2 is shown. When 8006-I was added to the culture during the lag phase (a), 5 μg/ml (=MIC) prevented growth, when the same amount was applied during the growth phase (b) to a population of higher density, growth was only delayed for three hours; after the addition of 20 μg/ml, however, lysis of the cells was observed.

In exponentially growing cells of *B. subtilis* the effect of 8006-I on the macromolecular syntheses was measured. The incorporation of uracil into trichloroacetic acid-precipitable material was almost completely inhibited by 5 μg/ml (Fig. 2). At higher concentrations all syntheses came to a halt. Spheroplasts from *E. coli* K 12, an organism not sensitive to the antibiotic, were sensitive; the lytic effect of

Table 1. Spectrum of antimicrobial activity of antibiotic 8006-I.
Minimum inhibitory concentrations (MIC) in the serial dilution test; size of inoculum: 10^6 cells or spores per ml.

Organism	Temperature	Medium	MIC (μg/ml)	Organism	Temperature	Medium	MIC (μg/ml)
Bacteria				*Mycobacterium* sp.	37	YMG	100
Acinetobacter calcoaceticus	30°C	MP	10	*Nocardia brasiliensis*	27	YMG	100
Arthrobacter citreus	27	NB	10	*Proteus vulgaris*	37	NB	10
Bacillus brevis ATCC 9999	37	NB	5~10	*Proteus vulgaris*	37	MM2	5
Bacillus brevis ATCC 9999	37	MM1	1	*Pseudomonas fluorescens*	27	NB	>100
Bacillus subtilis ATCC 6633	37	NB	10	*Staphylococcus aureus*	37	NB	20
Bacillus subtilis ATCC 6633	37	MM2	5	*Streptococcus faecalis*	37	YT	50
Bacillus subtilis ATCC 6051	37	NB	10	*Streptomyces antibioticus*	37	YMG	100
Bacillus subtilis ATCC 6051	37	MM2	5	*Streptomyces bambergiensis*	37	YMG	50
Escherichia coli D 22	37	MP	>100	*Streptomyces viridochromogenes*	37	YMG	10
〃 〃 K12	37	MP	>100	Yeasts and filamentous fungi			
〃 〃 A 19	37	MP	100	*Alternaria kikuchiana*	27	YMG	>100
〃 〃 A 19-15	37	MP	100	*Botrytis cinerea*	27	YMG	>100
Lactobacillus casei	37	MIB	>100	*Fusarium oxysporum*	27	YMG	100
Micrococcus luteus	37	NB	50	*Humicola grisea*	27	YMG	100
				Mucor miehei	27	YMG	100
				Penicillium notatum	27	MA	>100
				Saccharomyces cerevisiae	27	MM3	>100

Fig. 1. Bactericidal effect of antibiotic 8006-I on cells of *B. subtilis* in MM2; (a) and (b) indicate the addition of the antibiotic.

(a) 1: control without antibiotic. 2: 1 μg/ml. 3: 5 or 10 μg/ml.

(b) 4: 1 μg/ml. 5: 5 μg/ml. 6: 20 or 50 μg/ml.

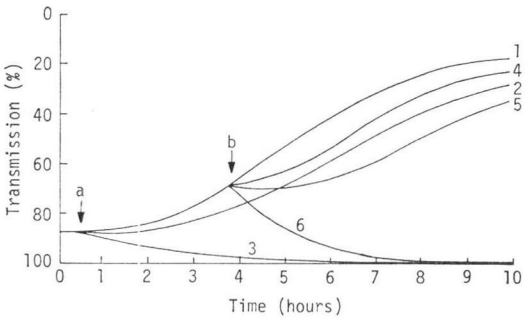


Table 2. Lytic effect of antibiotic 8006-I (100 μg/ml) on protoplasts from *E. coli* K12.

The optical density (E_{578}) is given as a percentage of the control containing no antibiotic.

Time (minutes)	E_{578} (%)
0	100
15	93
30	74
45	57
60	52
75	48
120	40

Table 3. Effect of antibiotic 8006-I on the incorporation of thymidine, uridine, and leucine into TCA-precipitable material in ECA cells and on the incorporation of UTP into RNA in isolated nuclei of ECA cells.

The incorporation was calculated as a percentage of the control containing no antibiotic.

8006-I (μg/ml)	Incorporation (%)			
	Thymidine	Uridine	Leucine	UTP
5	100	81	99	82
10	84	67	85	65
100	68	11	21	38

Fig. 2. Effect of 8006-I on the incorporation of thymidine, uracil, leucine, and *N*-acetylglucosamine into TCA-precipitable material of cells of *B. subtilis* in MM2.

The incorporation was calculated as a percentage of the control containing no antibiotic.

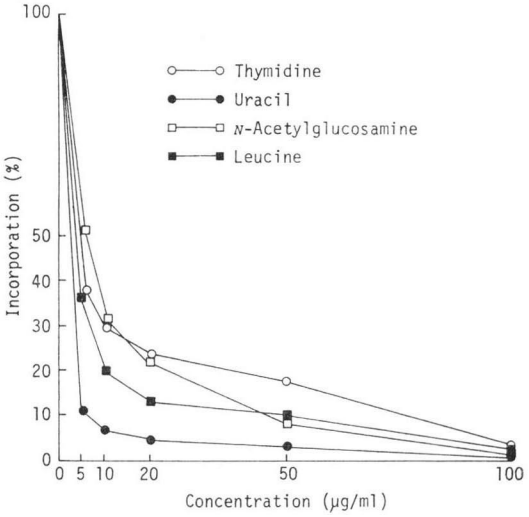
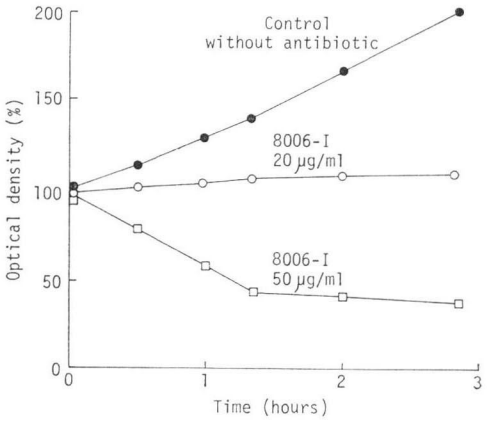


Fig. 3. Effect of 8006-I on EDTA-treated cells of *E. coli* K 12.

The cells were suspended in MM2 and different amounts of 8006-I were added.



8006-I on the spheroplasts is shown in Table 2. If the permeability of *E. coli* cells was altered by treatment with EDTA[12] the cells also became sensitive to 8006-I. As shown in Fig. 3, 20 μg/ml prevented growth and 50 μg/ml or higher concentrations caused lysis of EDTA-treated cells of *E. coli*. Therefore, the resistance of *E. coli* K 12 towards 8006-I is due to the barrier function of the outer membrane.

In cells of the ascitic form of Ehrlich carcinoma (ECA) the incorporation of uridine and leucine into

Table 4. Hemolytic activity of antibiotic 8006-I on bovine erythrocytes.

8006-I (μg/ml)	Hemolysis (%)
0	0
10	25
50	39
100	52
control	100

Table 5. Inhibition of DNA polymerase and DNA-dependent RNA polymerase from *E. coli* by 8006-I. The incorporation of TMP (DNA polymerase) and UMP (RNA polymerase) was calculated as a percentage of the control containing no antibiotic.

8006-I (μg/ml)	Incorporation (%)	
	TMP	UMP
5	92	91
10	84	82
100	39	35

TCA-precipitable material was greatly affected by 100 μg/ml of 8006-I. At lower concentrations the incorporation of UTP into RNA with isolated nuclei of ECA cells was inhibited to the same extent as was the incorporation of uridine (Table 3), indicating an inhibitory effect of 8006-I on RNA synthesis.

Antibiotic 8006-I exhibits weak hemolytic activity as shown in Table 4. At a concentration of 100 μg/ml, 50% of bovine erythrocytes were lysed.

The respiration rate of cells of *B. subtilis* was not reduced after the addition of 50 μg/ml of 8006-I for a time period of 5 minutes; the antibiotic had also no effect on the respiration of freshly germinated spores of *Penicillium notatum*. *In vitro* experiments with DNA polymerase and DNA-dependent RNA polymerase from *E. coli* revealed, that both enzymes are inhibited to the same degree by 8006-I, as shown in Table 5.

No effect was observed on the polypeptide elongation and $tRNA^{Phe}$-charging reactions in bacteria as measured by poly (U)-directed poly(Phe) synthesis (we are grateful to H. WOLF, Tübingen, for conducting this experiment). Therefore, the observed inhibition of the incorporation of leucine is most likely a secondary effect of the action of 8006-I.

Other enzyme systems tested were: alcohol dehydrogenase from yeast, phosphodiesterase from *E. coli* and chitinsynthase isolated from fruiting bodies of *Coprinus cinereus*, but none of these enzymes was impaired by 8006-I.

Acknowledgement

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