GLOBOPEPTIN, A NEW ANTIFUNGAL PEPTIDE ANTIBIOTIC

Sir:

A new antibiotic named globopeptin was found in a culture broth of *Streptomyces* sp. strain MA-23. The organism is a methylammonium chloride-resistant strain isolated from a soil sample collected in Kiyose-shi, Tokyo. Globopeptin is active against phytopathogenic fungi, inducing large pseudo-spheroplasts in sensitive fungi. The present communication describes the fermentation, isolation, and physico-chemical and biological properties of the antibiotic.

Fermentation was carried out in a 50-liter fermentor containing 33 litters of a medium (glycerol 3%, soybean powder 0.5%, glucose 0.2%, NaCl 0.2% and $Mg_3(PO_4)_2 \cdot 8H_2O$ 0.5%, pH 7.5 before autoclaving) at 27°C with agitation (250 rpm) and aeration (16 liters/minute). Antibiotic activity in the supernatant fraction of culture broth was monitored by the paper disc method with *Mucor racemosus* KF-223 as test organism (potato - glucose agar, pH 6, incubation at 27°C for 20 hours). Under these conditions the antibiotic titer reached its maximum at day 3. Addition of magnesium phosphate to production medium increased the production of the antibiotic by about 2.5-fold.

Globopeptin was isolated from the culture broth as outlined in Fig. 1. The supernatant fluid (30 liters) of 3-day culture of strain MA-23 was extracted with butanol (18 liters). After evaporation of the organic layer, the residue was applied on a column of the synthetic porous polymer Diaion HP-20 (Mitsubishi Chemicals Co., Tokyo), which was eluted with 10% and then 50% aqueous acetone. The active fractions were collected, concentrated *in vacuo*, and freeze-dried to give 1.32 g of crude powder. To the aqueous solution (20 ml) of the crude powder (1.3 g) 80 ml of acetone was added. The resulting precipitate (680 mg) was chromatographed on silica gel with chloroform - methanol solutions (10: 1, 5: 1, 2: 1 and 1: 1). The active fractions collected and concentrated were freeze-dried to give a white amorphous powder of pure globopeptin (237 mg). It gave a single spot on silica gel TLC with several solvent systems using DRAGENDORFF's reagent for detection. A single peak was observed on HPLC when monitored at 224 nm with only minor peaks amounting less than 1% of total peak area.

Globopeptin is soluble in water, methanol, butanol, but is insoluble in ethyl acetate, chloroform and benzene. It is readily soluble in chloroform - methanol (1: 1). *Anal* found: C 47.8, H 5.0, N 15.3, S 12.0 (Table 1). Electron impact (EI)-MS and field desorption (FD)-MS did not give a peak indicative enough of the molecular weight. UV spectrum in methanol shows a major peak at 224 nm and a minor peak at 270 nm, which did not shift in both acidic and alkaline conditions (Fig. 2). In an acid hydrolysate of a purified preparation, equimolar amounts of Asp, Thr, Pro, Gly and Cys (and no other amino acids) were detected with an amino acid analyzer (Jeol, model 200A). The ether extract of the acid hydrolysate contained an unidentified UV-absorbing material detectable by silica gel TLC. The antibiotic gives a positive reaction to

Fig. 1. Isolation procedures for globopeptin.

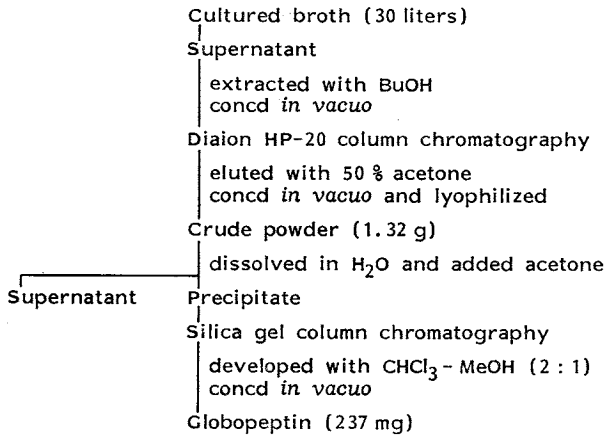
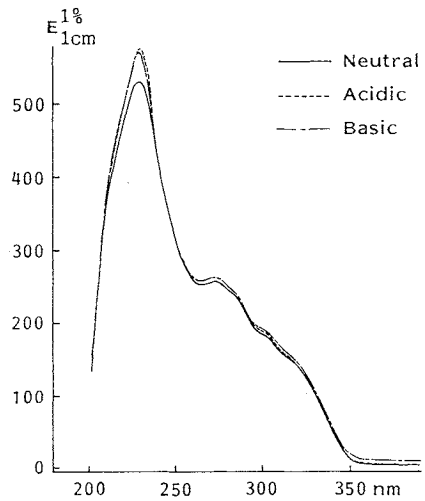


Table 1. Physico-chemical properties of globopeptin.

Appearance	White amorphous powder
MP	200°C
$[\alpha]_D^{25}$	+62° (*c* 1.0, $CHCl_3$ - MeOH, 1: 1)
Anal (%)	C 47.8, H 5.0, N 15.3, S 12.0.
UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$)	224 (536), 270 (262), 283 (sh, 244)
IR ν_{max} (KBr) cm^{-1}	3330, 2990, 1680, 1520, 1450, 1300, 1180, 1080, 1050
Amino acid anal (molar ratio)	Asp (0.94), Thr (0.92), Pro (1.19), Gly (1.00), Cys (1.13)
Color reaction	Positive: DRAGENDORFF, anisaldehyde - H_2SO_4 Negative: Ninhydrin, aniline phthalate

Fig. 2. UV spectra of globopeptin (MeOH).



DRAGENDORFF's reagent, and a negative reaction to H_2SO_4 and ninhydrin. It is suggested that the antibiotic molecule consists of a peptide moiety, to which a chromophore is bound by means of amino groups.

In comparison with known antibiotics with reference to the above physico-chemical properties, globopeptin bears some resemblance to SF-2049 substance.[1] However, globopeptin is different from SF-2049 substance in amino acid composition (Cys present *vs.* not present), in the number of the constituent amino acids (5 *vs.* more than 7), and in electrophoretic behavior (neutral *vs.* acidic). None of the known antibiotics with S content of around 12% contains all the five amino acids of globopeptin molecule. Therefore, it is concluded that globopeptin is a new antibiotic.

Globopeptin inhibits the growth of *Mucor racemosus*, and several phytopathogenic fungi such as *Piricularia oryzae*, *Botrytis cinerea*, and *Alternaria kikuchiana* (Table 2), but is only weakly active against yeasts and bacteria tested. Microscopical observation revealed that the antibiotic induces strongly round-shaped cell morphology in sensitive fungi. This suggests that globopeptin is a cell-wall active antibiotic. Preliminary results show that the antibiotic inhibits chitin synthesis in *P. oryzae*. Aculeacin,[2] lipopeptin[3] and neopeptin[4] are among antifungal peptide antibiotics with cell wall synthesis-inhibiting activity. Globopeptin is an additional example. Intraperitoneal injection of the antibiotic at 200 mg/kg had no toxic effect on mice.

Table 2. Antifungal spectrum of globopeptin.

Test organism	MIC* (μg/ml)
Candida albicans KF-1	>100
Mucor racemosus KF-223	0.2
Piricularia oryzae KF-180	0.2
Monilinia fructicola KF-242	>100
Botrytis cinerea KF-241	0.78
Alternaria kikuchiana KF-185	1.56
Rhizoctonia solani KF-240	100

* Potato - glucose agar, 27°C, 72 hours.

Detailed description of the producing organism and the mode of action of the antibiotic will be reported elsewhere.

Acknowledgment

The authors gratefully acknowledge Y. ARAI, Fuji Plant, Kyowa Hakko Kogyo Co., Ltd., for large scale fermentation.

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(Received September 9, 1986)

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