Short Communication

Neopeptins A and B, New
Antifungal Antibiotics

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Two new antifungal antibiotics, neopeptins A and B have been discovered in our screening for the inhibitors of microbial cell wall biosynthesis. The antibiotics were isolated from a fermentation broth of *Streptomyces* sp. K-710, which resembles *Actinomyces malachiticus*. The strain was isolated from a soil sample collected in Aizuwakamatsu-shi, Fukushima-ken.

Fermentation was carried out at 30°C for 72 hr in a jar fermenter containing 18 liters of a medium composed of glucose (2%), soluble starch (1%), meat extract (0.1%), dry yeast

(0.4%), soybean flour (2.5%), NaCl (0.2%) and K_2HPO_4 (0.005%). The fermentation broth (pH 7.6) was filtered with the aid of Celite and the mycelium was extracted with 70% methanol. The filtrate and the mycelium extract were combined and passed through a column of Diaion HP-10. The column was washed with 10% methanol and the antibiotics were eluted with 70% methanol. The eluate was concentrated *in vacuo* to a small volume and extracted with butanol. The extract was concentrated *in vacuo* to dryness and the residue was purified with silicic acid chromatography using the following stepwise solvent systems: chloroform $\rightarrow$ chloroform-methanol (9:1 $\rightarrow$ 4:1 $\rightarrow$ 1:1). The active fractions were combined and concentrated to give a crude powder. Further purification was done on a Sephadex LH-20 column using methanol which gave 950 mg of a white powder. The final purification was achieved by HPLC using a Nucleosil 5C₁₈ column with 65% methanol and producing two separated biologically active peaks. From the first fraction, neopeptin A was obtained as a purified white powder. Similarly neopeptin B was obtained from the second peak. The yield ratio of neopeptins A

FIG. 1. IR Spectra of Neopeptins A (Lower) and B (Upper) in KBr.

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and B was approximately 2:1.

Neopeptins A and B were obtained as an amorphous white powder which decomposed gradually above 200°C. They were soluble in water and lower alcohols but hardly soluble in acetone, chloroform, ether and hexane. They showed a positive reaction to Rydon-Smith and iodine vapor tests, but were negative to ninhydrin and anthrone tests. They were optically active: $[\alpha]_D^{20} -9.2^\circ$ (*c* 0.48, methanol) for neopeptin A and $[\alpha]_D^{20} -28.6^\circ$ (*c* 0.124, methanol) for neopeptin B. Paper-electrophoresis showed that they are amphoteric compounds having isoelectric points at an acidic pH (~4).

Elementary analysis and molecular weight determination by secondary ion mass spectroscopy

gave the following molecular formulae.

Neopeptin A. SIMS: $(M+1)^+$ 1176, $(M+Na)^+$ 1198, $(M+K)^+$ 1214. Calcd. for $C_{53}H_{81}N_{11}O_{19}$: C, 54.11; H, 6.94; N, 13.10. Found: C, 53.94; H, 7.19; N, 12.78.

Neopeptin B. SIMS $(M+1)^+$ 1190, $(M+Na)^+$ 1212, $(M+K)^+$ 1228. Calcd. for $C_{54}H_{83}N_{11}O_{19}$: C, 54.49; H, 7.03; N, 12.95. Found: C, 53.96; H, 7.12; N, 12.24.

They showed end absorption in the UV region and the IR spectra of neopeptins A and B were nearly identical (Fig. 1). The presence of amide (1650, 1530 cm^{-1}) and lactone (1740 cm^{-1}) was suggested. After an acid hydrolysis (6 N HCl) of neopeptin A, aspartic acid, serine, *N*-methylphenylalanine and β -hydroxyglu-

TABLE I. ANTIFUNGAL ACTIVITY OF NEOPEPTINS

The conventional agar dilution method was employed using a potato-sucrose medium.

Test organism	Minimal inhibitory concentration ($\mu g/ml$)	
	Neopeptin A	Neopeptin B
<i>Cochliobolus miyabeanus</i> IFO 5277	62.5	125
<i>Colletorichum lagenarium</i> IFO 7513	4	250
<i>Pyricularia oryzae</i> IFO 5994	500	250
<i>Glomerella cingulata</i> IFO 9767	250	250
<i>Botrytis cinerea</i> IFO 5365	250	> 500
<i>Alternaria mali</i> IFO 8984	250	> 500
<i>Rhizoctonia solani</i> IFO 6258	> 500	> 500
<i>Aspergillus oryzae</i>	> 500	> 500
<i>Penicillium chrysogenum</i>	> 500	> 500

TABLE II. INHIBITION OF CELL WALL GLYCAN SYNTHESIS BY NEOPEPTINS

The preparation of β -1,3-glucan synthetase from *Saccharomyces cerevisiae* GS-1-36 and the assay procedure were essentially similar to Cabib's method⁽⁴⁾ with slight modifications. The proteoheteroglycan synthetase assay has already been described.⁽³⁾ The enzyme preparations from *S. cerevisiae* for neopeptin A and *P. oryzae* for neopeptin B were used in the experiments. The peptidoglycan synthetase assay was the same as already described.⁽³⁾

Enzyme	ID ₅₀ ($\mu g/ml$)	
	Neopeptin A	Neopeptin B
β -1,3-Glucan synthetase (<i>S. cerevisiae</i>)	300	300
Proteoheteroglycan synthetase (<i>S. cerevisiae</i>)	350	350
Peptidoglycan synthetase (<i>E. coli</i>)	200	150

tamic acid were identified by amino acid analysis.

The antibiotics inhibited the growth of plant pathogenic fungi (Table I). This inhibition was accompanied by swelling of the mycelium. The neopeptins were effective to cucumber powdery mildew disease (*Sphaerotheca fuliginea*) in a pot test. Administration of 30 ppm of neopeptins showed a preventive value of over 90%. As shown in Table II, they inhibited β -1,3-glucan synthetase and proteoheteroglycan synthetase from *Saccharomyces cerevisiae* and peptidoglycan synthetase from *Escherichia coli*. However they were not inhibitory to bacteria and yeasts. Chitin synthetase from *Pyricularia oryzae* was not inhibited by 1 mg/ml of neopeptins. Inhibition of β -1,3-glucan and proteoheteroglycan synthesis as well as swelling of fungal mycelia may explain that the site of action of neopeptins is related to fungal cell wall biosynthesis. The antibiotics are of low toxicity to mice. Intravenous injection of 200 mg/kg of a mixture of neopeptins (A:B, approximately 2:1) was not lethal to mice.

In comparison with the known antifungal

peptide antibiotics, lipopeptins A and B¹⁻³⁾ are very similar to the present compounds. However they are different in amino acid composition and non-identity was proved by a direct comparison. Structural determination and details of the mechanism of action are currently under study in this laboratory.

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