Journal of Microbiology and Biotechnology
Vol. 7. No. 1, 49-51, 1997

Antifungal Activities of Magainin-2 Hybrid Peptides against *Trichosporon beigelii*

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In order to obtain a synthetic peptide with a more potent antifungal activity than magainin-2 but without hemolytic activity, four hybrid peptides were designed from the sequences of magainin 2 and cecropin A and their antifungal activities against *Trichosporon beigelii* were investigated. The result showed that analogue 2 and 4 exhibited better antifungal activity against *T. beigelii* than magainin-2 but no hemolytic activities. The peptides, therefore, could be used as models for the development of potent antifungal peptides.

Trichosporon beigelii is a pathogenic fungi which causes white piedra in immunologically normal patients and disseminated trichosporonosis in immunocompromised hosts (7). Disseminated infection caused by *Trichosporon beigelii* is frequently fatal in granulocytopenic patients, despite the administration of current available antifungal drugs. Thus, the development of new antifungal agents is needed for the effective therapy of the fungal infections.

Magainins and cecropins are antimicrobial peptides which have broad antibacterial spectrum against both Gram positive and Gram negative bacteria and have antitumor activity for several tumor cell lines without hemolytic activity (2, 5). The two peptides have a commom structural feature that form an amphipathic α-helix in a membrane environment. It is generally believed that the amphipathic α-helical structure of these peptides leads to the formation of ion channels across the cell membrane and cell death.

Although magainins have been known to have antifungal activity (9), there have been few additional studies on the antifungal activity of magainins and their derivatives. Previously it was reported that hybrid peptides of cecropin A (CA) and melittin (ME) have more potent antibacterial activities than parental peptides without hemolytic activity toward eucaryotic cells (1, 6). In this study, in order to design synthetic hybrid peptides with better antifungal properties than magainin 2 (MA), inverted peptides and CA hybrid peptides of MA were synthesized by the solid phase method. The antifungal activities of the synthetic peptides were evaluated by their growth inhibition of *Trichosporon beigelii* using turbidity measurement.

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Key words: antifungal activity, magainin-2, hybrid synthetic peptides, *Trichosporon beigelii*

MATERIALS AND METHODS

Fungal Strain and Growth Condition

T. beigelii KCTC 7251 was obtained from the Korean Collection for Type Cultures, Korea Research Institute of Bioscience & Biotechnology, Korea. *T. beigelii* was grown at 28°C in YM medium (1% glucose, 0.3% malt extract, 0.5% peptone, and 0.3% yeast extract).

Chemical Reagents

Chemicals for solid phase peptide synthesis were purchased from Sigma Chemical Co. (USA). Fmoc (Nα-fluorophenylmethoxycarbonyl)-amino acids and Rink Amide MBHA-Resin were obtained from Nova Biochem (San Diego, U.S.A.).

Peptide Synthesis

Peptide synthesis was carried out by the solid phase methods (4) using Fmoc as the Nα-amino protecting group. All peptides were purified by HPLC on a reverse phase C_{18} column.

Hemolytic Activity Assay

Hemolytic concentrations (HCs) of synthetic peptides were measured using the agarose hole method (1). The agar plates were prepared with 6 ml of a medium containing 1% agarose, 0.9% sodium chloride and 10% (v/v) human red blood cells (hRBCs) suspended in Alsever's solution (pH 6.1) (1). Three μl of the serially diluted peptides were applied in the 3 mm wells and then the plates

were incubated overnight at 37°C. HCs were calculated from the plot of the square of the inhibition zone diameter and peptide concentration.

Antifungal Activity Assays

T. beigelii was innoculated at the concentration of 1×10^5 cells per tube containing 1 ml YM medium and peptides diluted serially from an initial concentration, 40 μg/ml were then added. The tubes were incubated at 28°C for 30 h under constant shaking (140 rpm). The absorbance at 660 nm was measured. Minimal inhibitory concentrations (MICs) of the peptides were determined.

RESULTS AND DISCUSSIONS

Antimicrobial peptides, including CA, ME and MA have been known to play an important role in insect immunity and host defense. The amino acid sequences of these peptides are given in Table 1. Only magainins among several antimicrobial peptides have been known to have potent antifungal activity. Thus, the development of synthetic peptides with improved antifungal activity could be useful for effective therapy.

It has been reported that CA(1-13)ME(1-13), CA(1-8)ME(1-18) and CA(1-8)ME(1-12) hybrid peptides derived from amino terminal sequences of CA and ME are more active and bactericidal against Gram-positive and Gram-negative bacteria than parental peptides and have no hemolytic activity (1, 6). Thus, in order to design hybrid peptides having improved antifungal activity over MA, its two inverted peptides (analogues 1 and 2), CA-MA hybrid peptide (analogue 3) and its derivative (analogue 4) were synthesized. These synthetic peptides are designed to form the amphipathatically basic-flexible-hydrophobic structure found in the CA-ME hybrid peptides. The amino acid sequences of all analogues synthesized in this study are summerized in Table 2. Helical wheel diagrams of these analogues are shown in Fig. 1. The purity of synthetic peptides was considered up to 95% by analytical reverse phase(RP)-HPLC.

The turbidity measurement has generally been used as an assay for antifungal activity against fungi growing in a unicellular form such as *T. beigelii*, therefore the method was applied for measurement of the antifungal activities of our synthetic peptides against *T. beigelii*.

The MIC value of MA-2, analogue 1 and 3 was 10 μg/ml and that of analogue 2 and 4 was 5 μg/ml, respectively (Table 3). Analogue 2 in which the KKF sequence

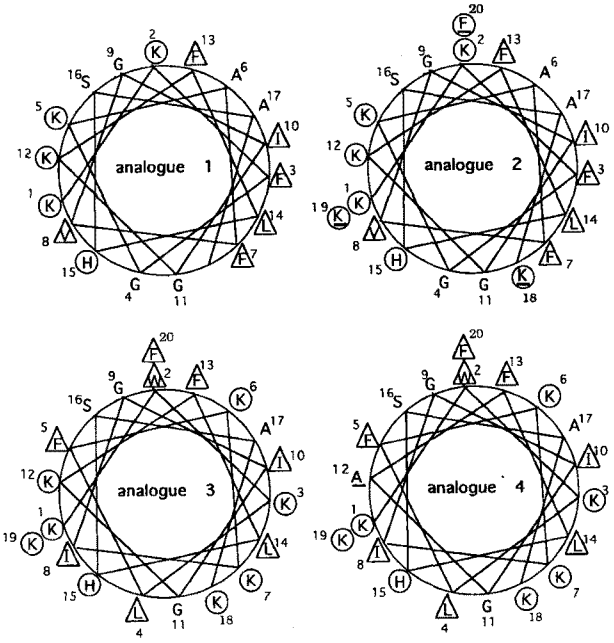

Fig. 1. Helical wheel diagrams of analogues. The numbers indicate the amino acid position, starting at the amino terminal end. Straight lines indicate bonds between amino acids.
Circle, positive charged amino acids; Triangle, hydrophobic amino acids.

Table 1. Amino acid sequences of CA, ME and MA.

Peptides	Amino acid sequences
	1 5 10 15 20 25 30 35
CA	K W K L F K K I E K V G Q N I R D G I I K A G P A V A V V G Q A T Q I A K - NH_2
ME	G I G A L K V L T T G L P A L I S I K R K R Q Q - NH_2
MA	G I K F L H S A K K F G K A F V G I N S

Table 2. Amino acid sequences of the synthetic peptides used in this study.

Peptides	Amino acid sequences
	1 5 10 15
analogue 1	K K F G K A F V G I G K F L H S A - NH_2
analogue 2	K K F G K A F V G I G K F L H S A K K F - NH_2
analogue 3	K W K L F K K I G I G K F L H S A K K F - NH_2
analogue 4	K W K L F K K I G I G A F L H S A K K F - NH_2

Table 3. Minimal inhibitory concentrations (MIC) of peptides against *T. beigelii*.

Peptides	MIC (μg/ml)
MA	10
analogue 1	10
analogue 2	5
analogue 3	10
analogue 4	5

was added at the C-terminus of analogue 1 showed more potent activity than analogue 1, which is an inverted peptide of MA-2 with 17 amino residues. Therefore, the increase in amphipathicity by the addition of $_{8}$KKF sequence seemed to result in a slight improvement in antifungal activity. Analogue 4 in which Lys at position 12 of analogue 3 was substituted with Ala showed more potent antifungal activity than analogue 3. Thus, an increase of hydrophobicity by replacing Lys which is contiguous to the hydrophobic region of analogue 3 with Ala resulted in more potent antifungal activity (Fig. 1 and Table 3). However, as recorded in our previous report, analogue 1 showed no antifungal activity against *Fusarium oxysporum* (3). The difference in antifungal activities of peptide between *F. oxysporum* and *T. beigelii* may be related to the difference of the plasma membrane or the cell surface composition of the two strains.

Although the bee venom, ME, has been known to have powerful antibacterial activity, this peptide shows an undesirable hemolytic activities. None of the synthetic peptides used in our study showed hemolytic activity against hRBCs at the concentration, 1000 μM (data not shown).

In conclusion, analogue 2 and 4 showed more potent antifungal activity against *T. beigelii* than the naturally occurring peptide, MA, and without hemolytic activities against hRBCs. The result also showed that the amphipathatically basic-flexible-hydrophobic structure of these peptides plays an important role in antifungal activity. Also, analogue 2 and 4 will be potentially useful models for the design of peptides with powerful antifungal activities and the study of structure-function activity relationships of antifungal peptides.

Acknowledgement

This work was supported by a grant (NB110M) from Korea Ministry of Science and Technology.

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(Received August 8, 1996)