J. Microbiol. Biotechnol. (1998), **8**(6), 595 – 600

JOURNAL OF
MICROBIOLOGY AND
BIOTECHNOLOGY

Structure-Antifungal Activity Relationships of Cecropin A-Magainin 2 and Cecropin A-Melittin Hybrid Peptides on Pathogenic Fungal Cells

LEE, DONG GUN, ZHE ZHU JIN[1], SONG YUB SHIN, JOO HYUN KANG, KYUNG-SOO HAHM, AND KIL LYONG KIM*

Peptide Engineering Research Unit, Korea Research Institute of Bioscience and Biotechnology, KIST, P.O. Box 115, Yusong, Taejon 305-600, Korea
[1]Medical college, Yanbian University, Yanji 133000, China

Received: June 11, 1998

Abstract In order to investigate a relationship of the structure-antifungal and hemolytic activities between cecropin A(1-8)-magainin 2(1-12) and cecropin A(1-8)-melittin(1-12) hybrid peptides, several analogues with amino acid substitution at positions 10 (Ile) and 16 (Ser) were designed and synthesized. The increase of the hydrophobicity by substituting with Leu, Phe, and Trp at position 16 in cecropin A(1-8)-magainin 2(1-12) did not have a significant effect on antifungal activity but caused a remarkable increase in hemolytic activity. These results indicate that the hydrophobic property at position 16 of cecropin A(1-8)-magainin 2(1-12) is more correlated to hemolytic activity than to antifungal activity. Replacement with Pro at position 10 of cecropin A(1-8)-magainin 2(1-12) and cecropin A(1-8)-melittin (1-12) caused a remarkable decrease in α-helical contents in the 50% TFE solution and induced a reduction in lytic activity against *Aspergillus flavus*, and *Aspergillus fumigatus*. These results demonstrate that flexibility at the central hinge region is essential for lytic activity against fungal cells and α-helicity of the peptides.

Key words: Cecropin A-magainin 2, cecropin A-melittin, structure, antifungal activity, hemolytic activity

Infections caused by fungi have become more frequent, in part because of improved diagnosis and the increasing number of immunocompromised patients [5]. Recently, the pathogenic fungi have been documented as etiologic agents of human disease. Because of the ubiquity of various fungi within our environment, human is constantly exposed to these fungi. The filamentous fungi, *Aspergillus fumigatus* and *Aspergillus flavus*, produce a thermogenic mycotoxin which causes a diverse spectrum of human diseases including allergic bronchopulmonary aspergillosis, asthma, aspergilloma, and invasive infection in immunocompromised hosts [2, 10]. *A. fumigatus* is the most common human pathogen which brings on both invasive and noninvasive aspergillosis. In particular, *A. fumigatus* is responsible for several types of respiratory mycosis. *A. flavus*, the second most common *Aspergillus* species, is isolated from invasive aspergillosis of immunosupressed patients as well as lesions originating in the nasal sinuses. Also, the yeast form, *Candida* species, is frequently encountered in certain clinical diseases. *Candida* species may cause various clinical forms of Candidiasis [4]. These infections can be acute or chronic, superficial or deep. In particular, *C. albicans* constitutes at least 60% of *Candida* species isolated from sites of infection. The *A. fumigatus*, *A. flavus*, and *C. albicans* used in this study are known to be pathogenic fungi.

A large number of natural and synthetic compounds of diverse types have been used for therapeutic treatments of several diseases caused by these pathogenic fungi. However, currently available antifungal agents are not entirely satisfactory because of low efficacy and severe toxicity. For example, amphotericin B produced by aerobic actinomycetes, *Streptomycetes nodosus*, has specific antifungal activity in candidiasis, aspergillosis, histoplasmosis, and blastomycosis. However, this chemical antibiotic does not often respond in most of the patients infected by pathogenic fungi because of an undesirable side effects [9]. Moreover, the emergence of multiple-chemical drug-resistant fungal strains requires constant new strategies for the treatment of several diseases by micropathogenic fungi.

During the last decade, many antibiotic compounds, including antimicrobial peptides, have been discovered from insects to mammalians containing cecropins, magainins, defencins, and melittin. These antimicrobial peptides are thought to protect the host from potential microbial or

*Corresponding author
Phone: 82-42-860-4163; Fax: 82-42-860-4593;
E-mail: kimkl@kribb4680.kribb.re.kr

fungal threats. Magainins 2 (MA) is known to show broad spectrum of antibiotic activity on both bacteria and fungi [3, 18]. Cecropin A (CA) can lyse bacterial cells but does not lyse fungal cells. MA and CA have been found to be unable to lyse mammalian cells such as human erythrocytes [15]. On the other hand, melittin (ME) has powerful antifungal and antibacterial activity while it shows a strong hemolytic activity against human erythrocytes [16].

The CA-ME hybrid peptides derived from N-terminal regions of CA and ME have high antimicrobial activity with a broader spectrum than the parental peptides but show no lytic effect on sheep erythrocytes [1, 6]. In our previous study, CA(1-8)-MA(1-12) hybrid peptide composed of CA(1-8) and MA(1-12) and its analogues were found to have powerful lytic activity against bacterial and tumor cells with little or no hemolytic activity [13, 14]. The hydrophobicities at position 12 of CA(1-8)-MA(1-12) and the hinge sequence (Gly-Ile-Gly) of CA(1-8)-MA (1-12) and CA(1-8)-ME(1-12) were reported to be important in antibacterial activity [13, 14]. However, the effect of position 12 in CA(1-8)-MA(1-12) and the hinge sequence in CA(1-8)-MA(1-12) and CA(1-8)-ME(1-12) in antifungal activity against pathogenic fungal cells was not yet known.

In this study, in order to investigate the effects of position 12 in CA(1-8)-MA(1-12) and the hinge sequence (Gly-Ile-Gly) in CA(1-8)-MA(1-12) and CA(1-8)-ME(1-12) in antifungal activity against pathogenic fungal cells, and obtain more improved antifungal peptides without hemolytic activity, several analogues based on CA(1-8)-ME(1-12) and CA(1-8)-MA(1-12) were synthesized by the solid phase method using Fmoc-chemistry [11]. The antifungal activity of the synthetic peptides were measured by growth inhibition against *C. albicans*, *A. flavus*, and *A. fumigatus* known as pathogenic fungi. Cytotoxicity activity of the peptides against human erythrocytes is displayed as percent hemolysis at the peptide concentration of 100 μM. The secondary structure of the peptides in various solutions was investigated by circular dichroism (CD) spectra.

MATERIALS AND METHODS

Fungal Strains

C. albicans (KCTC 7121), *A. flavus* (KCTC 7375), and *A. fumigatus* (KCTC 6145) were obtained from the Korean Collection for Type Cultures, Korea Research Institute of Bioscience & Biotechnology, Korea. These fungal strains were grown at 28°C in YM medium (1% glucose, 0.3% malt extract, 0.5% peptone, and 0.3% yeast extract).

Peptide Synthesis

All peptides were synthesized by the solid phase method using Fmoc-chemistry. Rink Amide p-methyl benzhydrylamine (MBHA) resin (0.55 mmol/g; Bova Biochem, U.S.A.) was used as support. Coupling of Fmoc-amino acids was performed by dicyclohexyl-carbodiimide (DCC)/1-hydroxybenzotriazole (HOBt). After completion of peptide chain elongation, the protected final peptide resins were treated with a deprotecting solution (88% trifluoroacetic acid, 2.5% phenol, 2.5% thioanisole, 2.5% H_2O, 2.5% 1,2-ethanedithiol, 2% triisopropylsilane, v/v). The crude peptides were purified by preparative HPLC on a C_{18} reverse-phase (RP) column. The purities of the purified peptides were determined by analytical RP-HPLC. The amino acid compositions of the purified peptides were determined by amino acid analysis (HITACHI 8500 A, Japan).

Antifungal Activity

The fungal cells were seeded on 96-well plates (NUNC, U.S.A.) at a density of 2×10^3 cells per well in a volume of 100 μl of YM media. One hundred μl of the serially diluted peptides were added to each well, and the plates were incubated for 24 h at 30°C. After incubation, 10 μl of a 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) solution (5 mg/ml MTT in phosphate-buffered saline, pH 7.4) was added to each well, and the plates were then incubated at 37°C for 4 h. Then, 30 μl of 20% SDS solution containing 0.02 N HCl was added and the reaction incubated at 37°C for 16 h to dissolve the formed formazan crystals. The optical density of each well was measured at 570 nm by a microtiter ELISA reader (Molecular Devices Emax, U.S.A.).

Hemolytic Activity

Prior to the assay, 1~2 week-old human red blood cells (hRBCs) were washed three times with PBS (35 mM sodium phosphate/150 mM NaCl, pH 7.0) prior to assay. The peptides to be tested were dissolved in 0.5 ml PBS and then 0.5 ml of a 4% (v/v) solution of the hRBC suspended in PBS was added (final peptide concentration: 100 μM). The cell suspension was incubated for 1 h at 37°C and centrifuged at $1,000\times g$ for 5 min. The absorbance of the supernatant was measured at 414 nm. Zero percent hemolysis and 100% hemolysis were determined by absorbance values from hRBC incubated in PBS and 1% Triton X-100, respectively. The percentage hemolysis was calculated by the following formula: % hemolysis=[($A_{414\,nm}$ in the peptide solution-$A_{414\,nm}$ in PBS)/($A_{414\,nm}$ in 1% Triton-X 100-$A_{414\,nm}$ in PBS)]$\times$100 [13, 14].

Circular Dichroism Analysis

CD spectra of peptides were recorded using a J720 spectropolarimeter (Jasco, Japan). All samples were maintained at 25°C during analysis. Four scans per sample were performed over the wavelength range 190~250 nm at 0.1 nm intervals. The spectra were measured in 10 mM

sodium phosphate buffer, pH 7.0, 50% (v/v) trifluoroethanol (TFE) in 10 mM sodium phosphate buffer, pH 7.0, and 30 mM sodium dodecyl sulfate (SDS) in 10 mM sodium phosphate buffer, pH 7.0, at 25°C using a 1 mm pathlength cell. The peptide concentrations were 100 μg/ml. The mean residue ellipticity, [θ], is given in deg·cm^2 (dmol^{-1}: $[\theta]=[\theta]_{obs}$ (MRW/10 l c), where $[\theta]_{obs}$ is the ellipticity measured in millidegrees, MRW is the mean residue molecular weight of the peptide, c is the concentration of the sample in mg/ml, and l is the optical path-length of the cell in cm. The percent helicity of the peptides were calculated with the following equation [17].

$$\% \text{ helicity} = 100\ ([\theta]_{222} - [\theta]^{0}_{222})/[\theta]^{100}_{222}$$

where $[\theta]_{222}$ is the experimentally observed mean residue ellipticity at 222 nm. Values for $[\theta]^{0}_{222}$ and $[\theta]^{100}_{222}$, corresponding to 0% and 100% helical contents at 222 nm, are estimated to be -2,000 and -30,000 deg·cm^2/dmol, respectively [17].

RESULTS AND DISCUSSION

Design and Synthesis of the Peptides

In our previous study, CA-MA hybrid peptide, CA(1-8)-MA(1-12), was observed to have nearly similar antibacterial and antitumor activity when compared to CA-ME hybrid peptide, CA(1-8)-ME(1-12) [12, 13]. CA(1-8)-ME(1-12) exhibited 14% hemolysis against human erythrocytes at the peptide concentration of 200 μg/ml, but CA(1-8)-MA(1-12) displayed no hemolytic activity [13, 14]. Also, the analogue peptide, L^{16}-CA(1-8)-MA(1-12), with Leu substitution of Ser at position 16 in CA(1-8)-MA(1-12) was observed to show more potent antibacterial and antitumor activity than CA(1-8)-MA(1-12) without a significant increase in hemolytic activity [13, 14]. These results suggested that the hydrophobicity at position 16 plays an important role in the antibacterial and antitumor activities of CA(1-8)-MA(1-12). However, the antifungal effect of the amino acid residue at position 16 in CA (1-8)-MA(1-12) has not yet been reported. In order to investigate the effects of the hydrophobicity of the residue at position 16 in the antifungal activity of CA(1-8)-MA (1-12), A^{16}-CA(1-8)-MA(1-12), F^{16}-CA(1-8)-MA(1-12) and W^{16}-CA(1-8)-MA(1-12) were synthesized (Table 1).

Table 1. Amino acid sequences of the analogues with amino acid substitution at position 16 in CA(1-8)-MA(1-12).

Peptides	Amino acid sequences
CA(1-8)-MA(1-12)	KWKLFKKIGIGKFLHSAKKF-NH_2
A^{16}-CA(1-8)-MA(1-12)	KWKLFKKIGIGKFLHAAKKF-NH_2
L^{16}-CA(1-8)-MA(1-12)	KWKLFKKIGIGKFLHLAKKF-NH_2
F^{16}-CA(1-8)-MA(1-12)	KWKLFKKIGIGKFLHFAKKF-NH_2
W^{16}-CA(1-8)-MA(1-12)	KWKLFKKIGIGKFLHWAKKF-NH_2

Table 2. Amino acid sequences of the analogues with Pro-substitution at position 10 in CA(1-8)-MA(1-12) and CA(1-8)-ME(1-12).

Peptides	Amino acid sequences
CA(1-8)-MA(1-12)	KWKLFKKIGIGKFLHSAKKF-NH_2
P^{10}-CA(1-8)-MA(1-12)	KWKLFKKIGPGKFLHSAKKF-NH_2
CA(1-8)-ME(1-12)	KWKLFKKIGIGAVLKVLTTG-NH_2
P^{10}-CA(1-8)-ME(1-12)	KWKLFKKIGPGAVLKVLTTG-NH_2

The *N*-terminal amphipathic basic sequence and *C*-terminal hydrophobic α-helical region in CA(1-8)-MA (1-12) and CA(1-8)-ME(1-12) is linked by the flexible hinge sequence (Gly-Ile-Gly). The flexible hinge sequence is important to the antibacterial activity of antimicrobial peptides [13]. Therefore, in order to investigate the effect of the central hinge sequence in antifungal activity of CA(1-8)-ME(1-12) and CA(1-8)-MA(1-12), the analogues with Pro substitution at position 10 were synthesized (Table 2).

The synthetic peptides were observed as one peak in the analytical reversed-phase HPLC on C_{18} column (data not shown). The correct amino acid compositions of the synthetic peptides was confirmed by amino acid analysis (data not shown).

Antifungal and Hemolytic Activity of the Peptides

The increase of the peptide hydrophobicities by substituting with Ala, Leu, Phe, or Trp at position 16 in CA(1-8)-MA(1-12) did not have significant effect on antifungal activity against *C. albicans*, *A. flavus*, or *A. fumigatus* (Table 3). Leu, Phe, and Trp-substituted analogues displayed a remarkable increase in hemolytic activity compared to

Table 3. Antifungal and hemolytic activities of the analogues with amino acid substitution at position 16 in CA(1-8)-MA(1-12).

Peptides	IC_{50} (μg/ml) (fungal cells)			% Hemolysis (100 μM)	Mean hydrophobicity[a]
	C. albicans	*A. flavus*	*A. fumigatus*		
CA(1-8)-MA(1-12)	1.5	1.2	1.3	0	-0.153
A^{16}-CA(1-8)-MA(1-12)	2.1	2.9	3.4	0	-0.128
L^{16}-CA(1-8)-MA(1-12)	1.0	1.2	1.1	6.2	-0.114
F^{16}-CA(1-8)-MA(1-12)	1.1	1.6	1.8	15.3	-0.084
W^{16}-CA(1-8)-MA(1-12)	3.5	3.7	3.0	20.4	-0.122

[a]Mean hydrophobicity was calculated using the consensus value of the hydrophobicity scale for each amino acid residue [8].

CA(1-8)-MA(1-12) at the peptide concentration of 100 μM but the Ala-replaced analogue showed no hemolytic activity (Table 3). In a previous study, the increase of the hydrophobicity at position 16 had a remarkable increase in antibacterial activity (data not shown). Therefore, the hydrophobic property at position 16 of CA(1-8)-MA(1-12) is expected to be important to antibacterial activity rather than antifungal activity. These results suggested that the difference in lytic activity against fungal and bacterial cells according to the increase of the hydrophobicity of the peptides may be due to the rigid fungal cell surface composed of β-1,3-glycan and chitin, unlike that of bacteria. The lipid composition of the outer leaflet of red blood cell membranes was reported to be composed of mainly neutral phospholipids, such as phosphatidylcholine (PC) and sphingomyelin [12]. Thus, the significant increase in hemolytic activity induced by substitution of hydrophobic residues (Phe or Trp) at position 16 in CA

Table 4. Antifungal and hemolytic activities of the analogues with Pro-substitution at position 10 in CA(1-8)-MA(1-12) and CA(1-8)-ME(1-12).

Peptides	IC_{50} (μg/ml) (fungal cells)			% Hemolysis
	C. albicans	*A. flavus*	*A. fumigatus*	(100 μM)
CA(1-8)-MA(1-12)	1.5	1.2	1.3	0
P^{10}-CA(1-8)-MA(1-12)	1.9	2.8	3.1	0
CA(1-8)-ME(1-12)	1.0	1.3	1.5	15.5
P^{10}-CA(1-8)-ME(1-12)	1.2	3.2	2.9	0

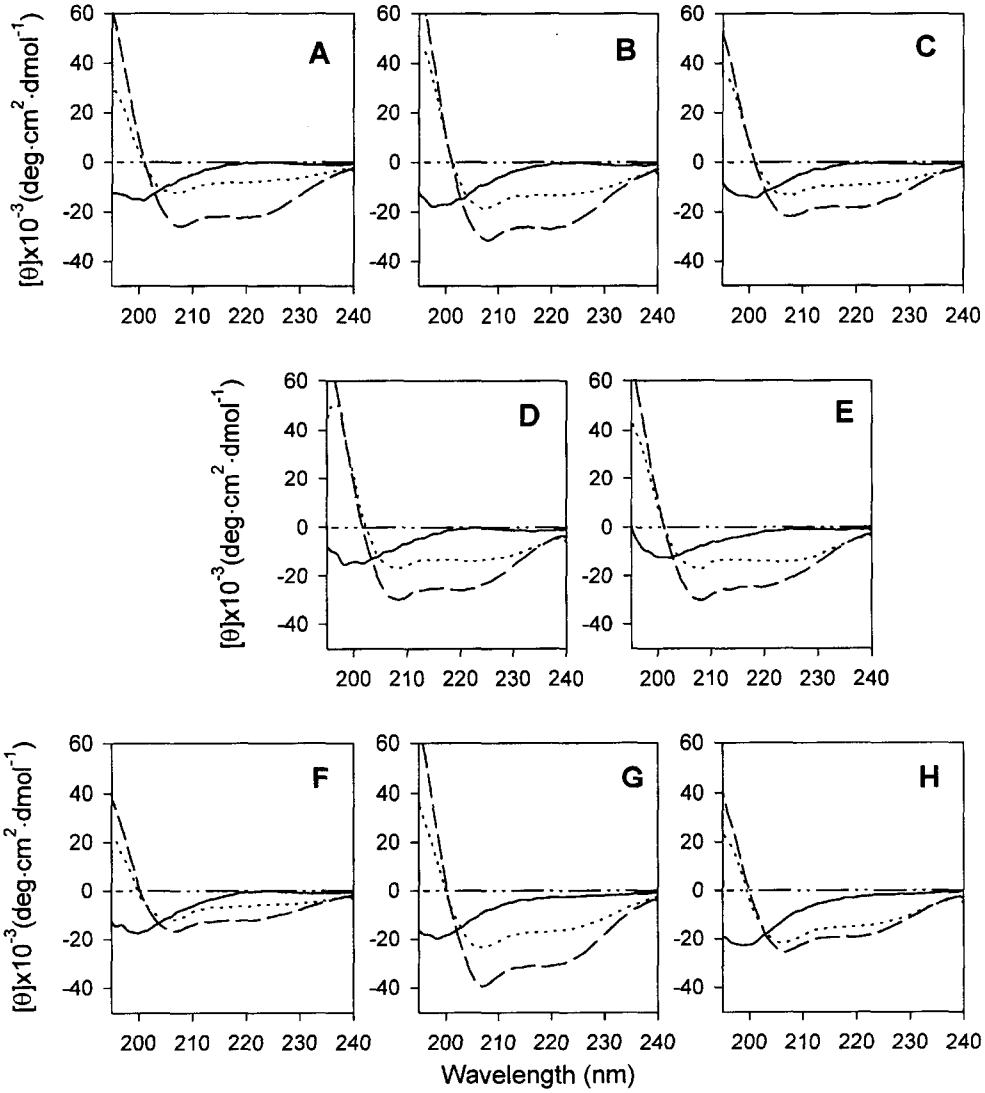

Fig. 1. CD spectra of the peptides in 0% TFE (——), 50% TFE (- - - - - -) and 30 mM SDS (·····) containing 10 mM sodium phosphate buffer, pH 7.0.

A: CA(1-8)-MA(1-12); B: A^{16}-CA(1-8)-MA(1-12); C: L^{16}-CA(1-8)-MA(1-12); D: F^{16}-CA(1-8)-MA(1-12); E: W^{16}-CA(1-8)-MA(1-12); F: P^{10}-CA(1-8)-MA(1-12); G: CA(1-8)-ME(1-12); H: P^{10}-CA(1-8)-ME(1-12).

(1-8)-MA(1-12) was due to strong hydrophobic interaction of the peptide with neutral lipids on the red blood cell surface.

As shown in Table 4, Pro-substituted analogues [P^{10}-CA(1-8)-MA(1-12) and P^{10}-CA(1-8)-ME(1-12)] at position 10 showed 2~3 times lower antifungal activity against *A. flavus* and *A. fumigatus* than CA(1-8)-MA(1-12) and CA(1-8)-ME(1-12). Also, CA(1-8)-ME(1-12) exhibited 15.5% hemolysis at the peptide concentration of 100 μM but P^{10}-CA(1-8)-MA(1-12) showed no hemolysis. However, substitution with Pro at position 10 in CA-MA and CA-ME hybrid peptides induced a dramatic decrease in both antibacterial and antitumor activities (data not shown). The reduction in antibiotic activity against bacteria, tumors, and red blood and fungal cells of Pro-substituted analogues seems to be due to the β-turn formation provided by Pro-introduction at the hinge region of CA-MA and CA-ME hybrid peptides. Therefore, the results suggested that the flexibility at the hinge region of CA-MA and CA-ME hybrid peptides is required in maintaining their antibiotic activities.

Since the analogue peptides designed in the present study showed powerful antifungal activity with little or no hemolytic activity, the development of an antibiotic agent based on these peptides may lead to improved therapeutics for the treatment of a variety of fungal infectious diseases.

Secondary Structure of the Peptides

The α-helical contents of antimicrobial peptides are responsible for antibacterial activity [6, 7, 14]. Therefore, in order to determine the content of α-helicity of the peptides, a CD analysis was performed in 10 mM sodium phosphate buffer, pH 7.0, and 50% (v/v) TFE and 30 mM SDS containing 10 mM sodium phosphate buffer, pH 7.0. The CD spectra of the peptides used in this study is shown in Fig. 1. The percent α-helicity of the peptides was calculated according to Wu *et al.* [17] as presented in Table 5. The peptides showed an unordered conformation in an aqueous sodium phosphate buffer. In 50% TFE or 30 mM SDS micelles, the peptides displayed the spectra characteristic of α-helical conformation (Fig. 1).

Increased α-helical content of the peptides in 50% TFE and SDS micelles was more involved in their hemolytic activity than in antifungal activity. Pro-substitution in the hinge region of CA(1-8)-MA(1-12) and CA(1-8)-ME (1-12) brought about a reduction in α-helix content under 50% TFE and 30 mM SDS conditions. These results suggest that the central hinge sequence, Gly-Ile-Gly, is important to the α-helical structure of the peptides.

Table 5. Percent α-helicity of the peptides deduced from CD spectra.

Peptides	α-helicity (%)		
	Phosphate buffer	50% TFE	30 mM SDS
CA(1-8)-MA(1-12)	6.3	65.7	18.5
A^{16}-CA(1-8)-MA(1-12)	5.3	79.7	37.0
L^{16}-CA(1-8)-MA(1-12)	5.4	53.5	23.4
F^{16}-CA(1-8)-MA(1-12)	6.0	77.6	39.1
W^{16}-CA(1-8)-MA(1-12)	1.4	72.0	40.9
P^{10}-CA(1-8)-MA(1-12)	5.3	33.4	13.0
CA(1-8)-ME(1-12)	1.9	93.9	47.8
P^{10}-CA(1-8)-ME(1-12)	1.4	55.6	41.6

Acknowledgments

This work was supported in part by a grant (AG370M) from the Ministry of Agriculture, Fishery and Forestry, Korea.

REFERENCES

1. Andreu, D., J. Ubach, A. Boman, B. Wahlin, D. Wade, R. B. Merrifield, and H. G. Boman. 1992. Shorted cecropin A-melittin hybrids: Significant size reduction retains potent antibiotic activity. *FEBS Lett.* **296:** 190–194.
2. Bardana, Jr. E. J. 1981. The clinical spectrum of aspergillosis-part2: Classification and description of saprophytic, allergic and invasive variants of human disease. *CRC Crit. Rev. Clin. Lab. Sci.* **13:** 85–159.
3. Berkowitz, B. A., C. L. Bevins, and M. Zasloff. 1990. Magainins: A new family of membrane-active host defense peptides. *Biochem. Pharmacol.* **39:** 625–629.
4. Bodey, G. P. 1984. Cadidiasis in cancer patients. *Am. J. Med.* **77:** 13–19.
5. Bodey, G. P. 1998. Fungal infections in cancer patients. *Ann. N.Y. Acad. Sci.* **544:** 431–442.
6. Boman, H. G., D. Wade, I. A. Boman, B. Wahlin, D. Wade, R. B. Merrifield, and H. G. Boman. 1989. Antibacterial and antimalarial properties of peptides that are cecropin-melittin hybrids. *FEBS Lett.* **259:** 103–106.
7. Chen, H. C., J. H. Brown, J. L. Morell, and C. M. Huang. 1988. Synthetic magainin analogues with improved antimicrobial activity. *FEBS Lett.* **236:** 462–466.
8. Eisenberg, D. 1984. Three-dimentional structure of membrane and surface proteins. *Annu. Rev. Biochem.* **53:** 595–623.
9. Keay, S., D. W. Denning, and D. A. Stevens. 1991. Endocarditis due to *Trichosporon beigelii*: *In vitro* susceptibility of isolates and review. *Rev. Infect. Dis.* **13:** 383–386.
10. Land, C. Y., K. Hult, R. Fuchs, S. Hagelberg, and H. Lundstr. 1987. Thermogenic mycotoxins from *Aspergillus fumigatus* as a possible occupational health problem in sawmills. *Appl. Enviro. Microbiol.* **53:** 787–790.
11. Merrifield, R. B. 1986. Solid phase synthesis. *Science* **232:** 341–347.
12. Op den Kamp, A.F. 1979. Lipid asymmetry in metabolite. *Annu. Rev. Biochem.* **48:** 47–71.

13. Shin, S. Y., J. H. Kang, and K. S. Hahm. 1998. Cecropin A-Magainin 2 hybrid peptides having potent antimicrobial activity with low hemolytic effect. *Biochem. Mol. Biol. Intl.* **44:** 1119–1126.
14. Shin, S. Y., M. K. Lee, K. L. Kim, and K. S. Hahm. 1997. Structure-antitumor and hemolytic activity relationships of synthetic peptides derived from cecropin A-magainin 2 and cecropin A-melittin hybrid peptides. *J. Pept. Res.* **50:** 279–285.
15. Steiner, H., D. Hulmark, A. Engstrom, H. Bennich, and H. G. Boman. 1981. Sequence and specificity of two antibacterial proteins involved in insect immunity. *Nature* **292:** 246–248
16. Tosteson, M. T., S. J. Holmes, M. Razin, and D. C. Tosteson. 1985. Melittin lysis of red cells. *J. Membr. Biol.* **87:** 35–44.
17. Wu, C.-S. C., K. Ikeda, and J. T. Yang. 1981. Ordered conformation of polypeptides and proteins in acidic dodecyl sulfate solution. *Biochemistry* **20:** 566–570.
18. Zasloff, M. 1987. Magainins, a class of antimicrobial peptides from *Xenopus* skin: Isolation, characterization of two active forms and partial cDNA sequence of precusor. *Proc. Natl. Acad. Sci. USA* **84:** 5449–5453.