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Effects of the Hinge Region of Cecropin A(1-8)-Melittin 2(1-12), a Synthetic Antimicrobial Peptide on Antibacterial, Antitumor, and Vesicle-Disrupting Activity

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CA(1-8)-ME(1-12) [CA-ME], composed of cecropin A(1-8) and melittin(1-12), is a synthetic antimicrobial peptide having potent antibacterial and antitumor activities with minimal hemolytic activity. In order to investigate the effects of the flexible hinge sequence, Gly-Ile-Gly, of CA-ME on antibiotic activity, CA-ME and three analogues, CA-ME1, CA-ME2, and CA-ME3, were synthesized. The Gly-Ile-Gly sequence of CA-ME was deleted in CA-ME1 and replaced with Pro and Gly-Pro-Gly in CA-ME2 and CA-ME3, respectively. CA-ME1 and CA-ME3 showed a significant decrease in antitumor activity and phospholipid vesicle-disrupting ability. However, CA-ME2 showed similar antitumor and vesicle-disrupting activities, as compared with CA-ME. These results suggest that the flexibility or β-turn induced by Gly-Ile-Gly or Pro in the central part of CA-ME may be important in the electrostatic interaction of the *N*-terminus cationic α-helical region with the cell membrane surface and the hydrophobic interaction of the *C*-terminus amphipathic α-helical region with the hydrophobic acyl chains in the cell membrane. CA-ME3 exhibited lower antitumor and vesicle-disrupting activities than CA-ME and CA-ME2. This result suggests that the excessive β-turn structure caused by the Gly-Pro-Gly sequence in CA-ME3 seems to interrupt ion channel/pore formation in the lipid bilayer. We concluded that the appropriate flexibility or β-turn structure provided by the central hinge is responsible for the effective antibiotic activity of the antimicrobial peptides with the helix-hinge-helix structure.

Keywords: Antitumor activity, CA(1-8)-ME(1-12), Helix-hinge-helix structure, Hinge region, Vesicle-disrupting activity.

Introduction

Antimicrobial peptides are an important component of the defense system and innate immunity of insects, amphibians, and mammals (Brevins and Zasloff, 1990; Boman, 1991; 1995; Hancock, 1997). Cecropin A from the *Hyalophora cecropia* pupae consists of 37 amino acid residues (Steiner *et al.*, 1982) and has potent antibacterial activity against Gram-positive and Gram-negative bacteria (Zasloff, 1992). Melittin, another antimicrobial peptide, is the main component of honey bee venom and consists of 26 amino acid residues (Tosteson *et al.*, 1985). Melittin exhibits potent antibacterial activity, but is very cytotoxic to normal eukaryotic cells such as erythrocytes (Tosteson *et al.*, 1985; Blondelle *et al.*, 1993). These two peptides are believed to cause cell lysis by the insertion of peptide monomers into the cell membrane, followed by oligomerization and ion channel/pore formation which permeabilizes the membrane and leads to cell death (Saberwal and Nagaraji, 1994; Matsuzaki *et al.*, 1997; 1998).

In the course of the structure-activity studies aimed at developing antibacterial peptides having improved antibiotic activity with no hemolytic effects, a series of cecropin A-melittin hybrid analogues consisting of different chain lengths of cecropin A (CA) at the *N*-terminus and melittin (ME) at the *C*-terminus were synthesized (Boman *et al.*, 1989; Andreu *et al.*, 1992;

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Wade *et al.*, 1992). These cecropin A-melittin hybrid peptides displayed more effective antibacterial activity than CA with less hemolytic activity. One of these hybrid peptides, CA(1-8)-ME(1-12) [CA-ME] composed of CA(1-8) and ME(1-12), was found to have effective antitumor activity against different human small cell lung cancer cells along with antibacterial activity with hemolytic activity (Shin *et al.*, 1996; 1997; 1999).

The secondary structure of CA was predicted to have a cationic amphipathic helix-hinge-hydrophobic helix motif from sequence analysis and circular dichroism (CD) measurements (Merrifield *et al.*, 1982; Steiner *et al.*, 1982). This prediction was later confirmed for CA by 2D-NMR analysis (Holak *et al.*, 1988). ME contains cationic and hydrophobic segments similar to CA, but these segments are arranged in opposite order. CA-ME peptides are also composed of an *N*-terminal cationic amphipathic α-helix and a *C*-terminal hydrophobic α-helix joined by a flexible hinge sequence of Gly^9-Ile^{10}-Gly^{11} like CA. However, the involvement of the hinge region of CA-ME in the antibacterial, antitumor, and vesicle-disrupting activities have not yet been investigated. Therefore, in this study, the antibacterial, antitumor, and vesicle-disrupting effects of the deletion (CA-ME1), substitution with Pro (CA-ME2), a helix breaker, or substitution with Gly-Pro-Gly (CA-ME3), a β-turn inducer, of the CA-ME flexible hinge sequence (Gly^9-Ile^{10}-Gly^{11}) were investigated. The vesicle-disrupting activities of the peptides were investigated by the measurement of released dye from dye-encapsulated vesicles. Human erythrocyte and mouse fibroblast cell lines were used to measure the cytotoxicity of the peptides against normal eukaryotic cells. The secondary structures of the peptides were investigated by circular dichroism (CD) spectra in hydrophobic environments mimicking cell membranes, such as trifluoroethanol (TFE) or sodium dodecyl sulfate (SDS).

Materials and Methods

Peptide synthesis Peptides were synthesized by the solid phase method (Merrified 1986; Shin *et al.*, 1995; 1996; 1998; Ha *et al.*, 1996) using Fmoc (9-fluorenylmethoxycarbonyl)-chemistry. Rink Amide 4-methylbenzhydryl-amine (MBHA) resin (0.55 mmol/g) was used as the support for *C*-terminal amidate peptide synthesis. The coupling of Fmoc-amino acids was performed with *N*-hydroxybenzotriazole (HOBt) and dicyclohexylcarbodiimide (DCC). Amino acid side chains were protected as follows: *t*-butyl (Ser and Thr), trityl (His), *t*-butyloxycarbonyl (Lys). Deprotection and cleavage from the resin were carried out for 2 h at room temperature using a mixture of trifluoroacetic acid, phenol, water, thioanisole, 1,2-ethanedithiol and triisopropylsilane (82.5:5:5:5:2.5:2, v/v). The crude peptide was then repeatedly washed with diethylether, dried in a vacuum, and purified by reversed-phase preparative HPLC on a Waters 15-μm Deltapak C_{18} column (19 × 30 cm). Purity of the peptides was checked by analytical reversed-phase HPLC on an Ultrasphere C_{18} column (Beckman, USA), 4.6 × 25 cm. Peptide concentration was determined by amino acid analysis. The molecular masses of the peptides were confirmed with MALDI (matrix-assisted laser desorption/ionization) mass spectrometry.

Hemolytic activity The hemolytic activity of the peptides was evaluated by determining the hemoglobin release of 0.4% suspensions of fresh human erythrocytes at 414 nm. Human red blood cells (hRBC) were centrifuged and washed three times with phosphate-buffered saline (PBS: 35 mM phosphate buffer/0.15 M NaCl, pH 7.0). One hundred μl of an 0.8% (v/v) suspension of in HRBC in PBS was plated into 96-well plates, and then 100 μl of the peptide solution was added to each well. The plates were incubated for 1 h at 37°C, and centrifuged at 150 × g for 5 min. One hundred μl aliquots of the supernatant were transferred to 96-well plates. Hemolysis was measured by absorbance at 414 nm with an ELISA plate reader (Molecular Devices Emax, Sunnyvale, California). Zero percent hemolysis and 100% hemolysis were determined in PBS and 0.1% Triton-X 100, respectively. The hemolysis percentage was calculated using the following equation: % hemolysis = [($Abs_{414\ nm}$ in the peptide solution − $Abs_{414\ nm}$ in PBS)/($Abs_{414\ nm}$ in 0.1% Triton-X 100 − $Abs_{414\ nm}$ in PBS)] × 100.

Antitumor activity Growth inhibitory activity of the peptides against cancer and normal fibroblast cells was determined as 50% inhibition concentration (IC_{50}) using a tetrazolium (MTT) colorimetric assay. Human chronic myelogenous leukemia (K-562: KCTC cat no. H013), human acute T-cell leukemia (Jurkat: KCTC cat no. H154), and human lung carcinoma cancer cells (A-549: KCTC cat no. H017 and MDA-MB-361: KCTC cat no. H258) were used for the growth inhibitory activity assay of the peptides against cancer cells. Mouse NIH-3T3 fibroblast cell line (KCTC cat no. M016) was used for the growth inhibitory activity assay of the peptides against normal cells. All cells were purchased from the Cell Bank, Korea Research Institute of Bioscience & Biotechnology (Taejon, Korea). Cells were grown in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 units/ml penicillin G sodium, and 100 μg/ml streptomycin sulfate. The cells were plated on 96-well plates at a density of 2.0×10^4 cells/well in 150 μl of the same medium. After incubating the plates overnight at 37°C in 5% CO_2 atmosphere, 20 μl of serially diluted peptides was added and the plates were further incubated for 3 d. Twenty μl of MTT solution (5 mg/ml MTT in phosphate-buffered saline) was added to each well and the plates were incubated at 37°C for 4 h. Forty μl of 20% SDS solution containing 0.02 M HCl was added to dissolve the dark blue crystals (MTT-formazan product) which were formed in each well, and plates were incubated overnight. Absorbance was measured at 570 nm on an ELISA plate reader (Molecular Devices Emax, Sunnyvale, California).

Antibacterial activity *Escherichia coli* (KCTC cat no. 1682), *Salmonella typhimurium* (KCTC cat no. 1926), *Pseudomonas aeruginosa* (KCTC cat no. 1637), *Bacillus subtilis* (KCTC cat no. 1918), *Streptococcus pyogenes* (KCTC cat no. 3096), and *Staphylococcus aureus* (KCTC cat no. 1621) were supplied from the Korean Collection for Type Cultures (KCTC), Korea Research Institute of Bioscience & Biotechnology (Taejon, Korea). The bacteria were grown to the mid-logarithmic growth

phase in pH 7.0 medium consisting of 10 g/l bactotryptone, 5 g/l yeast, and 10g/l NaCl. Peptides were filtrated with a 0.22 μm filter and stepwise-diluted in a medium of 1% bactopeptone. The tested organism [final bacterial suspension: 2×10^6 colony formation units (CFU)/ml] suspended in growth medium (100 μl) was mixed with 100 μl of the test peptide solution in a microtiter well plate, with three replicates for each test solution. Microbial growth was determined by the increase in optical density at 620 nm after 10 h incubation at 37°C. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of peptide at which there was no change in optical density.

Carboxyfluorescein leakage measurements Carboxyfluorescein (CF)-encapsulated large unilamellar vesicles (LUV) composed of PC/PS (4:1, w/w) were prepared by the reversed-phase ether evaporation method (Duzgunes *et al.*, 1983) using 100 mM CF. The initially formed vesicles were extruded through a 0.1 μm Nuleopore filter. To remove free CF dye, the vesicles were passed through a Bio-Gel A 0.5 m (Bio-Rad, Richmond, USA) column (1.5 × 30 cm) using phosphate buffered saline, pH 7.4, as the eluting buffer. The separated LUV fraction, after appropriate dilution to a final concentration of 6.36 μM, was mixed with the peptide solution in a 2 ml cuvette at 25°C. The leakage of CF from the LUV was monitored on a Shimazu RF-5000 spectrofluorometer (Tokyo, Japan) by measuring fluorescence intensity at 520 nm with excitation at 490 nm. The apparent percent leakage value at a fluorescence intensity, F, was calculated by the following equation: % leakage (apparent) = $100 \times (F - F_0)/(F_t - F_0)$, where F_t denotes the fluorescence intensity corresponding to 100% leakage after the addition of 20 μl of 10 % Triton X-100 and F_0 represents the fluorescence of the intact vesicle.

Circular dichroism (CD) spectroscopy CD spectra of peptides were recorded using a Jasco J720 spectropolarimeter with a 1 mm pathlength cell (Japan). The CD spectra of the peptides in 10 mM sodium phosphate buffer (pH 7.2), 50% (v/v) TFE, or 30 mM SDS were recorded at 25°C in the 190–240 nm wavelength range at 0.1 nm intervals with peptide concentrations of 100 μM. The mean residue ellipticity $[\theta]$ was calculated using the molecular weight of each peptide as determined from the amino acid composition. The percentage helicity of the peptides were calculated with the equation (Chen *et al.*, 1974): % helix = $100\ ([\theta]_{222} - [\theta]^0_{222})/[\theta]^{100}_{222}$, where $[\theta]_{222}$ is the observed mean residue ellipticity per residue at 222 nm in deg·cm^2·dmol^{-1}. $[\theta]^0_{222}$ and $[\theta]^{100}_{222}$, are the estimated ellipticities corresponding to a random coil ($-1{,}000$ deg·cm^2·dmol^{-1}) and 100% helical peptides ($-36{,}500$ deg·cm^2·dmol^{-1}), respectively.

Results and Discussion

The hybrid peptide, CA-ME, was found to have effective antibiotic activity against bacterial and tumor cells together with low hemolytic activity (Shin *et al.*, 1996; 1997; 1999). Unlike CA and cecropin B (CB) with an amphipathic helix-flexible-hydrophobic helix motif, CA-ME has the structural motif of a cationic short helix-flexible-amphipathic helix. In our previous study, the tertiary structure of CA-ME in an aqueous 50% TFE solution was determined by two-dimensional NMR study. Under these conditions, the Gly-Ile-Gly sequence was observed to be rather flexible (Oh *et al.*, 1999). This flexible sequence in the central region of CA-ME is thought to play an important role in its antibacterial, antitumor, and vesicle-disrupting activities. Therefore, in this study, the effect of the flexible Gly-Ile-Gly sequence was investigated on the antibacterial, antitumor, and vesicle-disrupting activities of CA-ME. All peptides (Table 1) were synthesized by the solid-phase method using Fmoc-chemistry. The amino acid composition and molecular weight of the synthetic peptides were confirmed by amino acid analysis and MALDI mass spectra (data not shown). Antitumor activity of the peptides against four different cancer cells were determined as the 50% growth inhibition concentration (IC_{50}) obtained using plots of cell survival (%) versus concentration of peptides (Fig. 1). The IC_{50} values of the peptides against the four different tumor cells are summarized in Table 2. The MIC values of the peptides against the six different bacterial cells are summarized in Table 3. The deletion (CA-ME1) and Gly-Pro-Gly substitution (CA-ME3) of Gly-Ile-Gly in CA-ME caused a significant reduction in the antitumor activity. In contrast, the Pro substitution (CA-ME2) in the hinge region did not affect antitumor activity, as compared with CA-ME.

CA-ME and its three analogues were found to have similar antibacterial activities against the six different

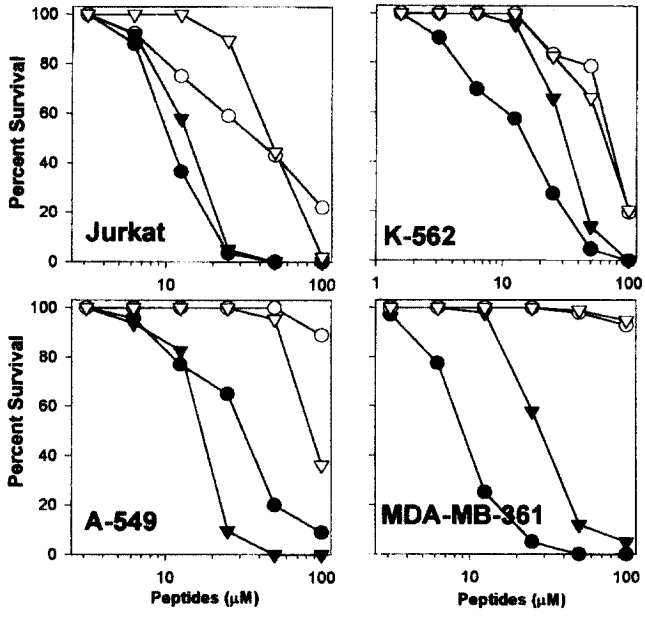

Fig. 1. Concentration-response curves of CA-ME (●), CA-ME1 (○), CA-ME2 (▼), and CA-ME3 (▽) in growth inhibition against four different transformed tumor cells.

Table 1. Amino acid sequences of CA-ME and its analogues.

Peptide	Amino acid sequence	Remarks
CA-ME	KWKLFKKIGIGAVLKVLTTG-NH_2	CA(1-8)-ME(1-12)
CA-ME1	KWKLFKKI------AVLKVLTTG-NH_2	CA(1-8)-ME(1-12):$G^9I^{10}G^{11}$ deletion
CA-ME2	KWKLFKKI---**P**--AVLKVLTTG-NH_2	CA(1-8)-ME(1-12):$G^9I^{10}G^{11} \rightarrow$ P
CA-ME3	KWKLFKKIG**P**GAVLKVLTTG-NH_2	CA(1-8)-ME(1-12): $I^{10} \rightarrow$ P

Table 2. Antitumor and hemolytic activities of CA-ME and its analogues.

Peptide	Tumor cell (IC_{50}: μM)				Fibroblast cell (IC_{50}: μM)	% Hemolysis (100 μM)
	K-562	A-549	Jurkat	MDA-MB-361	NIH-3T3	human erythrocytes
CA-ME	12.5	20.6	10.0	10.0	> 100	6.4
CA-ME1	80.2	> 100	40.2	> 100	> 100	0
CA-ME2	20.7	10.5	12.5	28.0	> 100	0
CA-ME3	70.4	90.0	50.0	> 100	> 100	0

Table 3. Antibacterial activities of CA-ME and its analogues.

Peptide	Gram-negative bacteria MIC (μM)			Gram-positive bacteria MIC (μM)		
	E. coli	*S. typhimurium*	*P. aeruginosa*	*B. subtilis*	*S. pyogenes*	*S. aureus*
CA-ME	6.25	0.78	1.56	1.56	1.56	6.25
CA-ME1	6.25	0.78	1.56	0.78	1.56	3.125
CA-ME2	6.25	1.56	1.56	1.56	3.125	6.25
CA-ME3	6.25	0.78	1.56	1.56	1.56	3.125

bacterial cells (Table 3). The deletion and Gly-Pro-Gly or Pro substitution of Gly-Ile-Gly in CA-ME did not cause a significant change in antibacterial activity. This result may be due to the presence of a cell wall on the outside of bacterial cells unlike eukaryotic cells. All the peptides displayed no lytic activity (IC_{50}: >100 μM) in the growth inhibition against normal NIH-3T3 fibroblast cells (Table 2). Hemolytic activity of the peptides was measured by monitoring the hemoglobin released from incubation with a 0.4% human red blood cell suspension and 100 μM peptide. No peptides exhibited hemolytic activity at concentrations below 100 μM except CA-ME (Table 2).

In order to investigate the membrane interaction with the peptides, vesicle perturbation activities induced by the peptides were measured using CF-entrapped LUV (Fig. 2). The phospholipid vesicle as an artificial model membrane was made of PC/PS (4:1, w/w) at a mixture of zwitterionic and negatively-charged phospholipids. The release of CF from the vesicle was displayed as a function of peptide to lipid ratio (P/L) and in a dose-dependent manner. CA-ME, the most effective peptide for antitumor activity, exhibited the highest activity in vesicle disruption. The relative

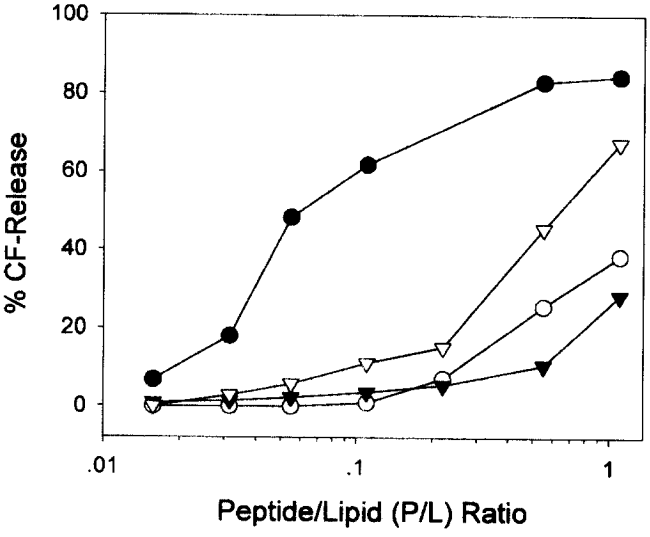

Fig. 2. Release of CF from LUVs composed of PC:PS (4:1). The extents of vesicle disruption induced by peptide are indicated as a function of peptide to lipid ratio (P/L). The released CF fluorescence was measured at $\lambda_{ex.}$ = 490 nm and $\lambda_{em.}$ = 520 nm. ●, CA-ME; ▼, CA-ME1; ▽, CA-ME2; ○, CA-ME3.

Table 4. Percent α-helicity of CA-ME and its analogues in various media.

Peptide	Buffer		50% TFE		30 mM SDS	
	$[\theta]_{222}$	α-helix (%)	$[\theta]_{222}$	α-helix (%)	$[\theta]_{222}$	α-helix (%)
CA-ME	−2,523.8	1.4	−26,475.2	71.1	−18,271.4	46.3
CA-ME1	−3,338.2	1.0	−30,983.5	84.8	−17,597.4	44.2
CA-ME2	−2,583.0	1.3	−12,717.3	29.4	−14,585.9	35.1
CA-ME3	−3,071.9	0.2	−24,590.8	65.4	−15,563.9	38.1

efficiencies of these peptides to perturb the lipid membranes correlated with their antitumor activities. This result suggests that the antitumor activities induced by these peptides are due to the interaction and perturbation of peptides with the cell membrane. The Gly-Ile-Gly fragment as the hinge region of the CA-ME hybrid peptide exhibited suitable flexibility to disturb a phospholipid vesicle by which the flexible region might assist the helical part of the peptide to penetrate the lipid bilayer. Therefore, the hinge region of the CA-ME hybrid peptide seemed to be important for the interaction and perturbation of phospholipid vesicle membranes.

The CD spectra of the peptides were measured in phosphate buffer with or without α-helix inducing solvents TFE or SDS, consisting of an aliphatic tail and a negatively-charged head group mimicking the lipid membrane. As shown in Fig. 3 and Table 4, the CD spectra

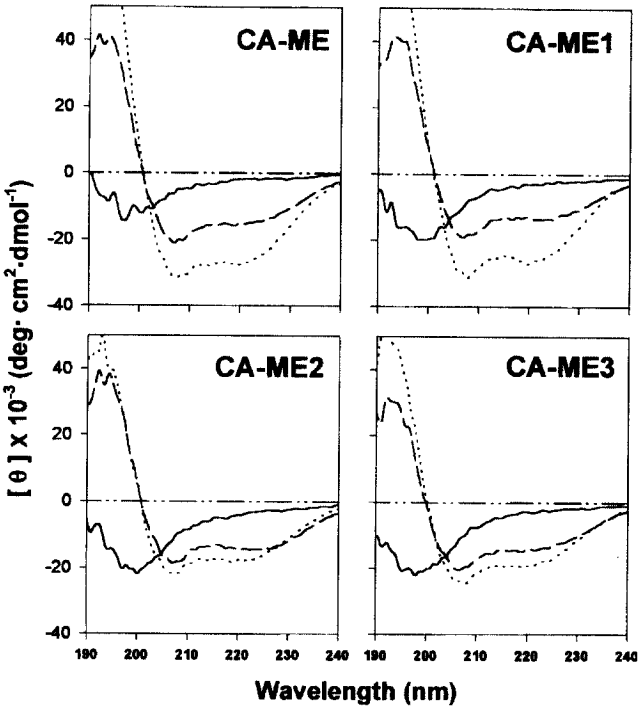

Fig. 3. CD spectra of CA-ME and its analogues in 10 mM sodium phosphate buffer, pH 7.2 (——), 50% (v/v) TFE (------), and 30 mM SDS(– – –) containing 10 mM sodium phosphate buffer, pH 7.2.

indicated that CA-ME and its analogues have a random coil structure in phosphate buffer, but a well-defined α-helical structure in 50% TFE and 30 mM SDS solutions. In 50% TFE and 30 mM SDS solutions, the Gly-Ile-Gly deletion (CA-ME1) and Gly-Pro-Gly substitution (CA-ME3) did not affect the a-helical content. In contrast, substitution of Gly-Ile-Gly with Pro (CA-ME2) resulted in a remarkable decrease in α-helical content in 50% TFE. Nevertheless, although CA-ME2 had a lower α-helical content than CA-ME and CA-ME1 in cell membrane-mimicking environments such as TFE or SDS micelles, it had a stronger antitumor activity than the other peptides. This result suggests that the α-helix characteristics of the peptide may be an important role in lytic action against tumor cells, but that α-helical content may not be a key factor.

In conclusion, the deletion of Gly-Ile-Gly (CA-ME1) and substitution with Gly-Pro-Gly (CA-ME3) in CA-ME resulted in a significant decrease of antitumor activity against four different transformed cancer cells and PC/PS vesicle-disrupting activity. However, CA-ME2 with a Pro residue instead of the Gly-Ile-Gly sequence showed a similar antitumor and PC/PS vesicle-disrupting activity when compared with CA-ME. Accordingly, these results suggest that the flexibility or bend potential induced by the Gly-Ile-Gly sequence or Pro residue in the central part of CA-ME may be important in the electrostatic interaction of the *N*-terminus cationic short α-helical region with the cell membrane surface and the hydrophobic interaction of the *C*-terminus amphipathic α-helical region with the hydrophobic acyl chains in the cell membrane. CA-ME3 exhibited lower antitumor and vesicle-disrupting activities than CA-ME and CA-ME2, suggesting that the extended turn structure provided by Gly-Pro-Gly in CA-ME3 seems to interrupt the ion channel/pore formation in the cell membrane.

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References

Andreu, D., Ubach, J., Boman, A., Wahlin, D., Wade, D., Merrifield, R. B. and Boman, H. G. (1992) Shortened

cecropin A-melittin hybrids: Significant size reduction retains potent antibiotic activity. *FEBS Lett.* **296**, 190–194.

Bevins, C. L. and Zasloff, M. (1990) Peptides from frog skin. *Annu. Rev. Biochem.* **59**, 395–414.

Blondelle, S. E., Simpkins, L. R., Perez-Paya, E. and Houghten, R. A. (1993) Influence of tryptophan on melittins hemolytic activity. *Biochim. Biophys. Acta* **1202**, 331–336.

Boman, H. G. (1991) Antibacterial peptides: Key components needed in immunity. *Cell* **65**, 205–207.

Boman, H. G. (1995) Peptide antibiotics and their role in innate immunity. *Annu. Rev. Immunol.* **13**, 61–92.

Boman, H. G., Wade, D., Boman, A., Wahlin, B. and Meriffield, R. B. (1989) Antibacterial and antimalarial properties of peptides that are cecropin-melittin hybrids. *FEBS Lett.* **259**, 103–106.

Chen, Y.-H., Yang, J. T. and Chau, K. H. (1974) Helix and β-form of proteins in aqueous solution by circular dichroism. *Biochemistry* **13**, 3350–3359.

Duzgunes, N., Wilschut, J., Hong, K., Fraley, R., Perry, C., Friend, D., James, T. L. and Paphadjopoulos, P. (1983) Physiochemical characterization of large unilamellar phospholipid vesicles prepared by reverse-phase evaporation. *Biochim. Biophys. Acta* **732**, 289–299.

Ha, J.-M., Shin, S. Y., Hong, H. N., Suh, D. J., Jang, T. S., Kang, S. W., Kuean, S. J. and Ha, B. J. (1996) Structure-antagonistic activity relationships of an NK-2 tachykinin receptor antagonist, L-659,877, and its analogues. *J. Biochem. Mol. Biol.* (formerly known *Korean Biochem. J.*) **29**, 429–435.

Hancock, R. E. (1997) Peptide antibiotics. *Lancet* **349**, 418–422.

Holak, T. A., Engstrom, A., Kraulis, P. J., Lindeberg, G., Bennich, H., Jones, T. A., Gronenborn, A. M. and Clore, G. M. (1988) The solution conformation of the antibacterial peptide cecropin A: a nuclear magnetic resonance and dynamic resonance and dynamical simulated annealing study. *Biochemistry* **27**, 7620–7629.

Matsuzaki, K., Nakamura, A., Murase, O., Sugishita, K., Fujii, N. and Miyajima, K. (1997) Modulation of magainin 2-lipid bilayer interactions by peptide charge. *Biochemistry* **36**, 2104–2111.

Matsuzaki, K., Sugishita, K., Ishibe, N., Ueha, M., Nakata, S., Miyajima, K. and Epand, R. M. (1998) Relationship of membrane curvature to the formation of pores by magainin 2. *Biochemistry* **37**, 11856–11863.

Merrifield, R. B. (1986) Solid phase synthesis. *Science* **232**, 341–347.

Merrifield, R. B., Vizioli, L. D. and Boman, H. G. (1982) Synthesis of the antibacterial peptide cecropin A (1-33). *Biochemistry* **21**, 5020–5031.

Oh, D., Shin, S. Y., Kang, J. H., Hahm, K.-S. and Kim, Y. (1999) NMR structural characterization of cecropin A(1-8)-magainin 2(1-12) and cecropin A(1-8)-melittin (1-12) hybrid peptides. *J. Peptide Res.* **53**, 578–589.

Saberwal, G. and Nagaraji, R. (1994) Cell-lytic and antibacterial peptides that act by perturbing the barrier function of membranes: facts of their conformational features, structure-function correlation and membrane-perturbing abilities. *Biochim. Biophys. Acta* **1197**, 109–131.

Shin, S. Y., Kang, S. W. and Ha, J.-Y. (1995) Chemical synthesis and determination of biological activity of the epidermal growth factor-like domain in mouse betacellulin. *J. Biochem. Mol. Biol.* (formerly *Korean Biochem. J.*) **28**, 87–93.

Shin, S. Y., Kang, J. H., Lee, M. K. and Hahm, K.-S. (1996) Antibacterial activities of peptides designed as hybrids of antimicrobial peptides. *J. Biochem. Mol. Biol.* (formerly *Korean Biochem. J.*) **29**, 545–548.

Shin, S. Y., Lee, M. K., Kim, K. L. and Hahm, K.-S. (1997) Structure-antitumor and hemolytic activity cecropin A-magainin 2 and cecropin A-melittin hybrid peptides. *J. Peptide Res.* **50**, 279–285.

Shin, S. Y., Park, J.-H., Jang, S. Y., Lee, M. K. and Hahm, K.-S. (1998) Characterization and epitope mapping of KI-41, a murine monoclonal antibody specific for the gp41 envelope protein of the immunodeficiency virus-1. *J. Biochem. Mol. Biol.* (formerly *Korean Biochem. J.*) **31**, 58–63.

Shin, S. Y., Kang, J. H. and Hahm, K.-S. (1999) Structure-antibacterial, antitumor and hemolytic activity relationships of cecropin A-magainin 2 and cecropin A-melittin hybrid peptides. *J. Peptide Res.* **53**, 82–90.

Steiner, H. (1982) Secondary structure of the cecropins: antibacterial peptides from the moth *Hyalophora cecropia*. *FEBS Lett.* **137**, 283–287.

Tosteson, M. T., Holmes, S. J, Razin, M. and Tosteson, D. C. (1985) Melittin of red cells. *J. Membr. Biol.* **87**, 35– 44.

Wade, D., Andreu, D., Mitchell, S. A., Silveira, A. M. V., Boman, A., Boman H. G. and Merrifield, R. B. (1992) Antibacterial peptides designed as analogs or hybrids of cecropins and melittin. *Int. J. Peptide Protein Res.* **40**, 429–436.

Zasloff, M. (1992) Antibiotic peptides as mediators of innate immunity. *Curr. Opin. Immunol.* **4**, 3–7.