ELSEVIER

Biochimica et Biophysica Acta 1336 (1997) 171–179

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Effects of the anti-bacterial peptide cecropin B and its analogs, cecropins B-1 and B-2, on liposomes, bacteria, and cancer cells

Hueih Min Chen *, Wei Wang, David Smith, Siu Chiu Chan

Department of Biochemistry, Hong Kong University of Science and Technology, Kowloon, Hong Kong

Received 19 November 1996; revised 24 January 1997; accepted 4 February 1997

Abstract

Custom designed analogs of the natural anti-bacterial peptide cecropin B (CB) have been synthesized; cecropin B-1 (CB-1) was constructed by replacing the C-terminal segment (residues 26 to 35) with the N-terminal sequence of CB (positions 1 to 10 which include five lysine residues). The second analog, CB-2, is identical to CB-1 except for the insertion of a Gly-Pro residue pair between Pro-24 and Ala-25. These peptides were used to investigate their anti-liposome, anti-bacterial and anti-cancer activities. The strength of anti-liposome activity is reduced two- to three-fold when the analogs are used instead of natural CB based on DL_{50} analysis. Similarly, the potency of these analogs on certain bacteria is about two- to four-fold lower than those of CB based on LC measurements. In contrast, on leukemia cancer cells, the potency of CB-1 and CB-2 is about two- to three-fold greater than that of natural CB based on IC_{50} measurements. All CB, CB-1 and CB-2 peptides have comparable helix contents according to CD measurements. These results indicate that the designed cationic lytic peptides, having extra cationic residues, are less effective in breaking liposomes and killing bacteria but more effective in lysing cancer cells. The possible interpretations for these observations are discussed. © 1997 Elsevier Science B.V.

Keywords: Anti-bacteria; Anti-cancer; Cecropin

1. Introduction

Cecropins, a family of anti-bacterial peptide, are found in the immune hemolymph of silkworm pupae [1], insects, and mammals [2,3] and contribute to the animal's innate immunity. This family consists of cecropins A, B, C, D, E and F which have highly homologous sequences of 35 to 39 amino acids in length [4]. These peptides were originally discovered to have a broad spectrum anti-bacterial activity on both Gram-positive and Gram-negative bacteria but were found to be unable to lyse eukaryotic cells [5]. Therefore, they may become valuable peptide antibiotics, especially when certain bacteria develop resistance to chemical antibiotics. Moreover, experimental results [6] have shown that cecropins can lyse cancer cells, giving them a further potential for medical applications [7].

Natural cecropin B (CB), consisting of 35 amino acids, possesses the highest anti-bacterial activity [4] in the family. Its tertiary structure has not yet been determined. However, based on the NMR structure of cecropin A [8], we predict that CB will consist of an N-terminal amphipathic α-helix and a C-terminal

* Corresponding author. Fax: +852 2358 1552; E-mail: bchmc@usthk.ust.hk.

PII S0304-4165(97)00024-X

hydrophobic α-helix joined by a flexible hinge at Gly-23 and Pro-24. The cell killing ability of the peptides is given credence by their helical conformation. However, CB has a random coil form in solution unless certain polar organic solvents such as hexafluoroisopropyl alcohol [9] or lipid bilayers such as liposomes [10] are involved. The detailed mechanism of cell lysis by the peptides is not clearly known yet but it is believed to involve the formation of an ion-channel or pore [11] prior to cell death. A channel-forming action by the amphipathic α-helix segment of the botulinum neurotoxin A HC (BoTxATM) on liposomes has recently been established [12] and supports this premise.

In this study, we synthesized cecropin B-1 (CB-1) by replacing the C-terminal segment of CB (positions 26 to 35, the hydrophobic α-helix) with the sequence of CB from positions 1 to 10 (part of the amphipathic α-helix) [13]. This rearrangement, which added five cationic residues to the peptide, was used to test the peptide's anti-liposome, anti-bacterial and anti-cancer activities. In addition, cecropin B-2 (CB-2) was generated with the same sequence as CB-1 but including an extra inserted residue pair of Gly-Pro immediately after Pro 24. We used CB-2, with its similar length, to examine the effect of an extended flexible linker and to compare the results to those obtained from CB-1. Also, synthetic cecropin A (CA) was used to compare its anti-bacterial activity to that of CB [4].

2. Materials and methods

2.1. Materials

Penicillin G, streptomycin, 1,1,1,3,3,3-hexafluoro-2-propanol, egg phosphatidyl choline, egg phosphatidic acid and Triton X-100 were purchased from Sigma, USA. The fluorescent dye, calcein, was produced by Behring Diagnostics, USA. Cell culture media, Dulbecco's modified eagle medium (D-MEM), F-12 nutrient mixture (Ham) medium and fetal bovine serum (FBS), were purchased from Gibco, USA. The RPMI-1640 medium, sodium bicarbonate, hydrochloric acid and sodium hydroxide used were products of Sigma. Water used in this experiment was deionized and distilled. The cancer cell lines, HL-60, K-562, Jurkat (E6-1), CCRF-CEM and fibroblast cells 3T6, 3T3 were purchased from the American Type Culture Collection (ATCC), USA.

2.2. Peptide synthesis

Peptides CA, CB, CB-1, and CB-2 were synthesized by an Applied Biosystems (ABI) 431 A Peptide Synthesizer from the Department of Medical Biochemistry, University of Calgary, Canada. Their sequences are shown in Fig. 1. Fmoc chemistry was applied with HBTU/HOBT coupling (ABI Fastmoc 0.25 chemistry cycles). The final products were deprotected and cleaved from the resin using a trifluoroacetic acid (TFA) solution containing water, phenol, thioanisole and ethanedithiol. Resin was removed by filtration and, the peptides were precipitated with diethyl ether after the organic solvents were evaporated. These crude peptide preparations were desalted on Sephadex G-10 (20% acetic acid), and purified using reverse phase HPLC (Vydac C-18 column, 0.1% TFA in H_2O–acetonitrile). The purity of the peptides was determined by analytical HPLC (Vydac C-18, TFA/H_2O/acetonitrile) to be about 95%. Their molecular weight and amino acid composition were determined by mass spectrometry. The results show that the total number of amino acids in the peptide are: CA: 37.143; CB: 35.257; CB-1: 34.327; CB-2: 36.307 and the molecular weights (m/z; $z = +1$) are: CA: 4003.86; CB: 3834.74; CB-1; 4113.15; CB-2: 4267.32. These data agree well with the sequences. After purification, the peptide solutions were lyophilized. The dried products were weighed using a microbalance (Sartorius Re-

Fig. 1. Amino acid sequences of the natural cecropins A (CA) and B (CB) and custom cecropin CB-1 and CB-2. In CA the first underlined segment is the amphipathic helix and the second underlined segment is the hydrophobic helix. The turn is located between the two helices. CB-1 was constructed by repeating the segment from positions 1 to 10 of CB (underlined in CB) in the region between position 25 and the C-terminus of CB-1 (underlined in CB-1). CB-2 is similar to CB-1 but with the addition of a Gly-Pro residue pair immediately after Pro-24.

search Model R200D with the resolution up to 0.01 mg). The net weight of each peptide was calculated by multiplying the raw weight by the purity (%) and peptide content (%) obtained by analytical HPLC. The results show that the purity/peptide content for CA, CB, CB-1 and CB-2 are 95%/78%, 95%/71%, 95%/73%, and 95%/73%, respectively. The concentrations of the peptide solutions are determined from the net weight of peptides and their molecular weights.

2.3. Circular dichroism of peptides

The secondary structure of the peptides was investigated by spectropolarimetry (Jasco Model J-720). The concentrations of CB, CB-1 and CB-2 used for circular dichroism (CD) measurements were 0.08 mg/ml with 2.5mM sodium phosphate buffer and the pH was adjusted to 6.4. The spectrum was scanned from 250 to 190 nm (far UV region) for the peptide backbone study. A standard chemical, d-10-camphor sulfonate, at a concentration of 0.06% (w/v in water) in a 1 cm pathlength cuvette was used for calibration and the CD was taken as +190.4 mdeg at 290.5 nm. After the calibration, experiments were conducted at a temperature of 20°C maintained by a circulating water bath. The conditions used for running the machine were: 1.0 nm band width, 0.25 s response time, 0.1 nm/data step resolution, 10 nm/min scan speed and 5 times accumulation.

2.4. Preparation of liposomes and membrane perturbation

The liposomes without fluorescent dye were constructed by mixing 15 mg of egg phosphatidyl choline (PC) with 5 mg of egg phosphatidic acid (PA), and a total volume of 1 ml chloroform was added into the mixture. After gentle shaking to dissolve the solid, the chloroform was removed by an argon stream. The lipid was then hydrated and dispersed into 2 ml of PBS at pH 7.4. The milky solution was sonicated (Laboratory Supplies Co., Model G112 SPIT) for at least four hours until the solution became clear. A similar process was applied to produce fluorescent dye-encapsulating liposomes. Calcein dye (100nM) was mixed with the dried mixture of PC and PA (3:1, 20 mg) in 2 ml PBS at pH 7.4. The solution was then sonicated for more than four hours. Those dyes that were not incorporated into the liposome were removed by passing the transparent solution through a Sephadex G-25 column. The liposome solutions (0.5 ml each) were added to various aliquots of peptide stock solutions to give varied lipid/peptide ratios. The samples with peptide concentrations of 0.5–5 μM were then incubated at 23°C for 2 h before the experiments. Peptide-induced calcein leakage, reflected by an increase in fluorescence, was investigated by a Perkin-Elmer luminescence spectrofluorimeter (Model LS 50B) at an excitation and an emission wavelengths of 496 and 517 nm, respectively. Instead of adding peptides, 100% leakage was achieved by adding 0.1% Triton X-100. Using this reference, the degree of leakage induced by various concentrations of peptides was calculated using the following equation:

$$\text{dye leakage}(\%) = \left[(F - F_0)/(F_r - F_0)\right] \times 100 \quad (1)$$

where F represents the fluorescence intensity induced by the peptides, F_0 is the intensity obtained from the solution without peptides, and F_r represents the fluorescence intensity obtained after adding Triton X-100.

2.5. Measurements of the lethal concentrations (LC) of CA, CB and analogs

The inhibition zone assay [4] was used to determine the LC of CA, CB, CB-1 and CB-2 on *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*. LB broth (6 ml) containing 1% agarose and 50 μl of bacterial suspension (O.D. = 0.5 at $A_{550\ nm}$) was poured into a flat-bottom petri dish (inner diameter: 8.5 cm), and a number of small holes (diameter: 0.2 cm) were made inside the agarose plate by a 0.2 cm gel puncher (Pharmacia). A constant volume (1.5 μl) of CA, CB, CB-1 and CB-2 at various concentrations was added to the holes of several dishes, and the zone diameters were recorded after 18 h. The LC (mol/l) was then obtained from the slope of the plot of η^2 versus according to the following equation [4]:

$$\eta^2 = 0.47(\lambda^{-1}\mathrm{LC}^{-1}) \quad (2)$$

where η is the zone diameter (unit: dm), indicates moles of peptide and λ represents the thickness of

the agarose layer in the plate (0.02 dm in this experiment).

2.6. Cell culture

Cell suspensions of leukemia cell lines HL-60, K-562, Jurkat (E6-1), and CCRF-CEM were maintained in a RMPI-1640 medium with 10% FBS, penicillin G (100 unit/ml) and streptomycin (100 μg/ml). Cells (1×10^5 cells/ml) were seeded into 25 cm^2 culture flasks (Falcon) and incubated at 37°C (5% CO_2, 95% air) for two days and then centrifuged at 1000 rpm for 5 min. The pellets were washed twice with culture medium before they were suspended in the RMPI-1640 medium containing 0.5% FBS. The cell suspension was incubated overnight and then diluted to a concentration of 1×10^5 cells/ml for the cytotoxicity assay (see cytotoxicity Section 2.7). Monolayer cells, 3T3 (contact-inhibited fibroblast cells) and 3T6 (collagen-secreting fibroblast cells) at 1×10^4 cells/ml were seeded into 25 cm^2 culture flasks containing D-MEM medium supplemented with 10% FBS, penicillin G (100 unit/ml) and streptomycin (100 μg/ml). Cells were grown to about 80% confluence before being removed by trypsinization. These monolayer cells were resuspended in the D-MEM medium with 0.5% FBS and then prepared for cytotoxicity assays.

2.7. Cytotoxicity assays

A 96-well plate (Costar) was used for this assay. Each well in the plate contained a 90 μl cell suspension (1×10^5 cells/ml). Wells in the first and second columns acted as blanks (medium only) and 100% survival controls (cells and medium only), respectively. Dulbecco's phosphate-buffered saline (10 μl) was added to the first and second columns and the various concentrations of peptides (10 μl each), freshly prepared from the stock solution (500 μM), were added to the remaining columns (three wells for each concentration of peptide solution) with final concentrations of 1, 5, 10, 15, 20, 25, 30, and 50 μM. The plate was then incubated for 24 h before a microtetrazolium (MTT)-based colorimetric assay was undertaken. MTT works by being metabolized by mitochondrial dehydrogenases in living metabolically active cells to form a formazan salt from which strong absorption at 570 nm is observed. During the assay, 20 μl of MTT (5 mg/ml; Boehringer Mannheim) was added to each well and the plate was incubated for 4 h. Subsequently, 100 μl of solubilization solution (10% SDS in 0.01 mol/ml HCl) was added to each well and the plate was then incubated at 37°C overnight. The absorbance at 570 nm was measured by a microtiter plate reader (Bio-Rad model 450). Cell survival (%) was then determined from the $\Delta A_{570\ nm}$ relative to the control and final results were recorded by averaging at least three repeated experiments. The IC_{50} for each peptide was obtained from the curve of *cell survival* versus *concentration of peptide* and taken from the concentration at which cell viability was 50%.

2.8. Time course of cytotoxicity on HL-60 cancer cells

A mixture of peptide (25 μM) and HL-60 cell suspension (1×10^6 cells/ml) was placed in a glass coverslip holder (30×10 mm). This sample holder was then placed in an inverted microscope (Zeiss:Axiovert 35) with a video tape recorder. The temperature of the holder was maintained at 37°C by an oil bath (Medical System: TC-202). The time course of cell lysis by the peptides was recorded over a period of about one hour.

3. Results and discussion

3.1. Circular dichroism spectroscopy

The CD curves of CB, CB-1, and CB-2 are shown in Fig. 2. The CD at 222 nm was negligible for all peptides when HFP (1,1,1,3,3,3-hexafluoro-2-propanol) was not involved in the solution (group I in Fig. 2). This indicates that the peptides have a random coil conformation. However, after adding HFP (20% v/v), normal CD curves for helix structure were obtained for CB, CB-1 and CB-2 (group II in Fig. 2). The constituents of the secondary structures were analyzed [14] and showed that the α-helix content of the peptides were approximately equal (CB: 52.9% ± 5.9; CB-1: 56.2% ± 5.6; CB-2: 53.4% ± 6.2). Similar experiments were done using acidic liposomes without fluorescent dye instead of HFP

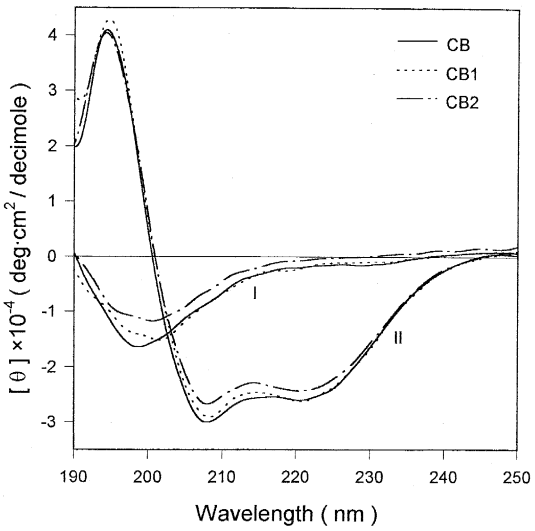

Fig. 2. CD spectra of peptides without (I)/with (II) HFP.

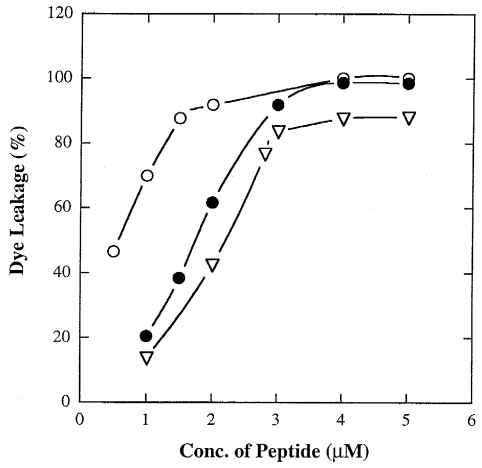

Fig. 4. Measurements of the DL_{50} of the peptides. CB, CB-1 and CB-2 are shown by open circles, filled circles and open triangles, respectively. The DL_{50} of each peptide is obtained from the concentration at which dye leakage is 50%.

[10]. The CD spectra of CB, CB-1 and CB-2 (0.08 mg/ml) with acidic liposomes (1 mg/ml) are shown in Fig. 3. The α-helix contents are $32.9\% \pm 0.3$, $31.7\% \pm 0.7$ and $29.4\% \pm 0.2$ for CB, CB-1 and CB-2, respectively. The α-helix content of CB-2 is lower than that of CB-1 in either HFP or acidic liposome. This implies that the insertion of Gly-Pro may diminish the helix conformation slightly. Another possible reason is that some aggregation of CB-2 may occur based on the results shown in Fig. 4 (see Section 3.2 for detailed discussion). Nevertheless, all the peptides have very similar α-helix contents but have different cell lysis abilities (see the following sections). This suggests that the content of α-helix in the peptide may not be a key factor in killing cells but that the characteristics of the helix itself may be important.

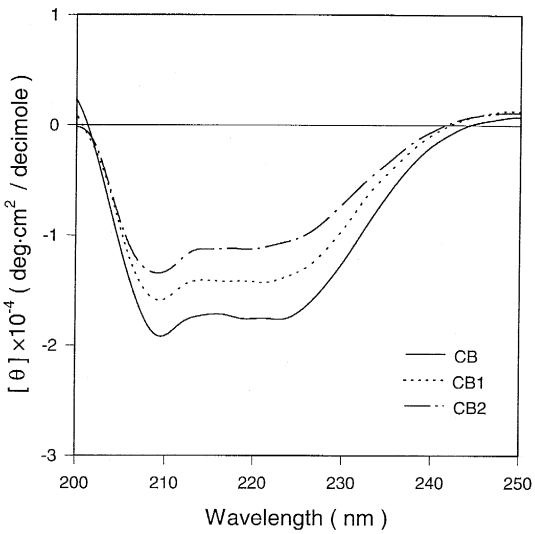

Fig. 3. CD spectra of peptides with lipid vesicles. CD signals below 200 nm were vigorously perturbed by the liposomes and therefore are not shown in the figure.

3.2. Anti-liposome activities of CB, CB-1 and CB-2

Time course experiments for fluorescent dye-encapsulating liposomes with various concentrations of peptides were conducted. In all cases, fluorescent saturation of the solution was reached within a two-hour incubation period. The measurement of dye leakage, therefore, was carried out following at least two hours incubation. Dye leakage (%) from liposomes as a function of the concentration of peptide is shown in Fig. 4. For CB-2 (open triangles), the plateau of the dye leakage curve is not consistent with those of CB (open circles) and CB-1 (filled circles) which reach 100% leakage. A possible explanation is that a precipitate in the liposome solution gradually forms as CB-2 concentration is increased. Beyond 5 μM of CB-2, the amount of precipitate causing a decrease in fluorescent intensity may have a greater effect than that of broken liposomes causing an increase in fluorescent intensity. The greater ease

of forming a precipitate by CB-2 than by the other peptides may be due to the extended linker and slightly lower helix content allowing greater movement of the helices leading to a concentration dependent aggregation. The DL_{50} (50% of dye leakage) of CB, CB-1 and CB-2 were obtained from the curves shown in Fig. 4. These results show that CB has the lowest DL_{50} (0.6 μM) as compared with those of CB-1 (1.7 μM) and CB-2 (2.2 μM). This indicates that the potency of CB in liposomes is higher than that of its analogs, CB-1 and CB-2. These results are consistent with the observations from bacteria (see the following section).

3.3. Anti-bacterial activities of CA, CB, CB-1 and CB-2 on several bacteria

The LC of the above peptides on *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa* were measured and calculated from the plot of Eq. (2) with the known zone diameters. Table 1 shows the summary of the *LCs* of CA, CB, CB-1 and CB-2. We used CA as a reference to identify whether the concentrations of peptides used in the experiments were accurate or not. The results show that the potencies of CA on the three bacterial strains are weaker (higher LC) than those of CB. These are the expected observations and confirm previous results that CB has higher anti-bacterial activity than CA [4]. The LC of CB-1 and CB-2 were shown to be higher than those of CB. In particular, for *Pseudomonas aeruginosa*, the LC of CB-1 and CB-2 are about four times higher than those of CB. A similar trend was obtained from the experiments for artificial liposomes above (see section on anti-liposome activities). This demonstrates that the cationic residues (five lysine residues between position 25 to the C-terminus) in CB-1 and CB-2 do not improve the potency of killing bacteria or of breaking the liposome, even though the liposomes are acidic. Our results showed that the peptide does not form a secondary structure in a neutral liposome solution (data not shown). A further implication is that the killing effect of the peptides on bacteria (or in breaking liposomes) may not only depend on the electrostatic interaction between the lipid and the peptide but that the hydrophobic amino acids in the C-terminal segment may play a significant role. For example, anti-bacterial activity may be enhanced by the involvement of the hydrophobic helix in the non-polar environment of the tail-to-tail lipid bilayer of the cell membrane. If this hydrophobic helix is replaced by an amphipathic one, the mechanism of lysis may be changed resulting in the lower observed activity. Further experiments to investigate this hypothesis will be carried out. As the LC of CB-1 and CB-2 are similar for the bacteria tested, the additional length in the flexible region between the N- and C-terminal α-helices may not influence anti-bacterial activity. The similar results obtained for both CB-1 and CB-2 confirm the consistency of the experiments.

3.4. Anti-cancer activities of CB, CB-1 and CB-2 on cancer cells: some observations / conclusions

Examples of the anti-cancer activities on HL-60 leukemia cells after treatment with CB, CB-1 and CB-2 for 30 min are shown in Fig. 5a, Fig. 5b, and Fig. 5c, respectively; Fig. 5d shows the result of the control (without peptides). As seen from the figures, the viability of the cells is about 68.5% for CB; 32.2% for CB-1; 40.6% for CB-2, and 100% for the control. These experiments illustrate that the potency of CB-1 and CB-2 in killing HL-60 cancer cells is greater than that of CB. Other examples of HL-60 cell lysis at equilibrium (incubation for 24 h) are

Table 1
Measurement of the lethal concentrations (LC) for various peptides on *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*; results are the average of three experiments (*P. aeruginosa* of 5 experiments) and the average deviations are shown

Species	Lethal concentration of peptides (μM)			
	CA	CB	CB-1	CB-2
Klebsiella pneumoniae	0.33 ± 0.021	0.26 ± 0.012	0.39 ± 0.015	0.36 ± 0.003
Escherichia coli	0.45 ± 0.022	0.36 ± 0.003	0.49 ± 0.002	0.49 ± 0.002
Pseudomonas aeruginosa	1.43 ± 0.004	0.46 ± 0.022	1.48 ± 0.021	1.51 ± 0.008

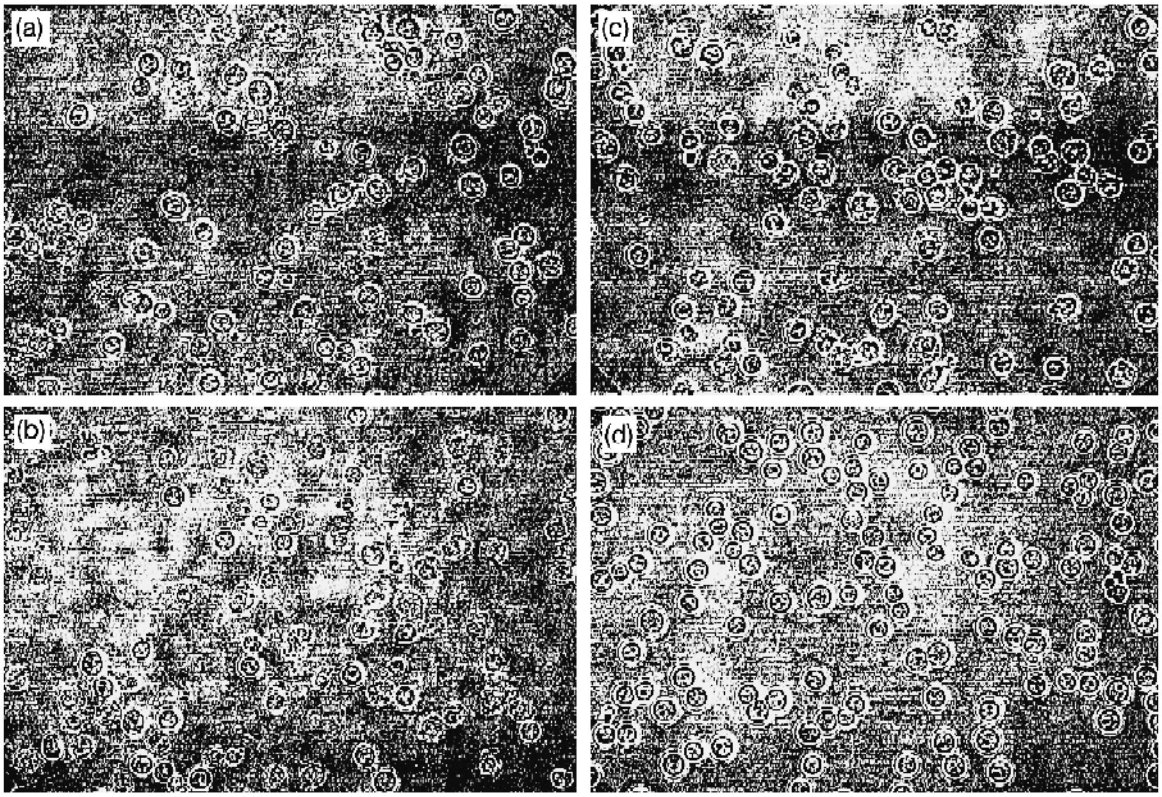

Fig. 5. Viability of HL-60 cancer cells after treatment with the peptides for 30 min. The populations of cells killed by CB, CB-1 and CB-2 after 30 min are shown in (a), (b) and (c), respectively. The control (without peptide) is shown in (d). Killed cells can be identified by their irregular or broken shapes.

shown in the plots of cell survival (%) versus the concentration of the peptides being investigated with the cytotoxicity assay (see Fig. 6). The IC_{50} of CB, CB-1 and CB-2 in killing HL-60 cancer cells obtained from the curves are 14.3 μM for CB, 7.5 μM for CB-1 and 9.6 μM for CB-2. This again indicates that the anti-cancer activities of the peptide anologs, CB-1 and CB-2, are greater than those of the natural cecropin, CB. Similar experiments using these peptides on other cell lines are summarized in Table 2. In general, the cancer cells were obtained from leukemia patients of different age, sex and race. These differences cause the cells to have different growth rates and life cycles (data not shown). Their characteristics vary with the cell line and consequently the resistance to a certain peptide is varied. For example, the IC_{50} of CB is 14.1 μM for HL-60 but is 12.3 μM for CCRF-CEM. If a particular cell line affected by the

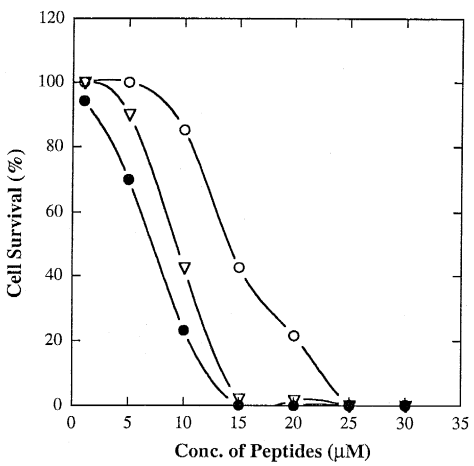

Fig. 6. Measurements of IC_{50} of the peptides on HL-60 cancer cells. CB, CB-1 and CB-2 are shown by open circles, filled circles and open triangles, respectively. The IC_{50} of these peptides were obtained at cell survivals of 50%.

Table 2
Measurement of the IC_{50} of the peptides, CB, CB-1 and CB-2, on various cancer cell lines; the data are the average of three experiments with the average deviation shown

Species	IC_{50} (μM)		
	CB	CB-1	CB-2
Leukemia cancer cells			
HL-60	14.1 ± 1.3	7.5 ± 0.5	9.2 ± 0.8
K-562	17.6 ± 1.7	10.2 ± 0.7	11.1 ± 1.5
Jurkat (E6-1)	8.0 ± 0.4	2.4 ± 0.3	3.1 ± 0.3
CCRF-CEM	12.3 ± 0.8	8.6 ± 0.5	7.0 ± 0.6
Fibroblast cells			
3T6	> 50	> 50	> 50
3T3	> 50	> 50	> 50

different peptides is focused on, we found that the anti-cancer activity of CB is the weakest among these peptides. Both CB-1 and CB-2 are about two times more potent than natural CB. For Jurkat (E6-1) leukemic cells, the killing ability of CB-1 and CB-2 is around three times greater than that of CB. This suggests that the cationic amino acids (five lysine residues) located in the C-terminus of the peptide enhance its ability to lyse cancer cells. Insertion of Gly-Pro into the flexible region of CB-1 (to create CB-2) decreases the potency of cell killing except for CCRF-CEM cancer cells. All the peptides have a much higher IC_{50} (over 50 μM) in lysing normal fibroblast cells such as 3T6 and 3T3 cells. This confirms previous results that cecropins have little ability to lyse eukaryotic cells [5]. However, current study has shown that the tumor cells are exceptional. A possible reason for the difference between the activity on eukaryotic cancer cells and other eukaryotic cells may be that tumor cell membranes have a greater susceptibility to cationic lytic peptides. Transformed cancer cells usually have more exposed anionic lipids (for example, leukemic GRSL cells, see Ref. [15]) or a greater accessible surface, in particular, that are caused by microvilli on the cell membrane [16]. However, attributing the greater potency on tumor cells of both CB-1 and CB-2 than CB to their higher cationic content affecting the greater anionic nature of tumor cell membranes [15] contradicts the effect of the peptides on anionic liposomes as reported above. Anionic membranes may only be a prerequisite for cell killing as the peptides did not have a defined structure in neutral liposomes. This similar hypothesis for melittin has been mentioned [17]. Once the helical structure is formed, however, the anionic lipid content of the membrane might no longer be a significant factor in cell lysis. The difference in potency of the peptides could reflect different cell lysis mechanisms. The extra cationic segment of the anologs may allow a more effective mechanism of cell lysis than that allowed by the hydrophobic segment of natural CB.

Acknowledgements

The authors thank Professor Donald Chang for the use of his inverted microscope and Ms. Camilla Erskine for help with the manuscript. We are also grateful to Mr. Denis McMaster for his help with peptide synthesis.

References

[1] H. Steiner, D. Hultmark, A. Engstrom, H. Bennich, H.G. Boman, Nature 292 (1981) 246–248.
[2] H.G. Boman, D. Hultmark, Ann. Rev. Microbiol. 41 (1987) 103–126.
[3] J.-Y. Lee, I.A. Boman, C. Sun, M. Andersson, H. Jornwall, V. Mutt, H.G. Boman, Proc. Natl. Acad. Sci. USA 86 (1989) 9159–9162.
[4] D. Hultmark, A. Engstrom, H. Bennich, R. Kapur, H.G. Boman, Eur. J. Biochem. 127 (1982) 207–217.
[5] H.G. Boman, D. Wade, I.A. Boman, B. Wahlin, R.B. Merrifield, FEBS Lett. 259 (1989) 103–106.
[6] A.J. Moore, D.A. Devine, M.C. Bibby, Peptide Research 7 (1994) 265–269.
[7] H.G. Boman, Annu. Rev. Immunol. 13 (1995) 61–92.
[8] T.A. Holak, A. Engstrom, P.J. Kraulis, G. Lindeberg, H. Bennich, T.A. Jones, A.M. Gronenborn, G.M. Clore, Biochemistry 27 (1988) 7620–7629.
[9] H. Steiner, FEBS Lett. 137 (1982) 283–287.
[10] L. Zhong, R.J. Putnam, W.C. Johnson Jr., A.G. Rao, Int. J. Peptide Protein Res. 45 (1995) 337–347.
[11] B. Christensen, J. Fink, R.B. Merrifield, D. Mauzerall, Proc. Natl. Acad. Sci. USA 85 (1988) 5072–5076.
[12] M. Oblatt-Montal, M. Yamazaki, R. Nelson, M. Montal, Protein Sci. 4 (1995) 1490–1497.
[13] D. Wade, D. Andreu, S.A. Mitchell, A.M.V. Silveira, I.A. Boman, R.B. Merrifield, Int. J. Peptide Protein Res. 40 (1992) 429–436.

[14] J.T. Yang, C.-S. Wu, H.M. Martinez, Methods Enzymol. 130 (1986) 208–269.
[15] W.J. Van Blitterswijk, G. De Veer, J.H. Krol, P. Emmelot, Biochim. Biophys. Acta 688 (1982) 495–504.
[16] M. Sekignchi, T. Suzuki, Gastric tumor cell lines, in: R.T. Hay, J.-G. Park, A. Gazdar (Eds.), Atlas of Human Tumor Cell Lines, Academic Press, Inc., San Diego, USA, 1994.
[17] C.R. Dawson, A.F. Drake, J. Helliwell, R.C. Hider, Biochim. Biophys. Acta 510 (1978) 75–86.