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Effect of Secondary Structure on Biological Activities of Antimicrobial Peptides

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Abstract: A 15-mer cationic α-helical antibacterial peptide was used as the framework to study the effect of peptide secondary structure on antimicrobial activities. We designed an α-helical peptide with higher helical propensity compared with the original peptide, a β-sheet peptide and a random coiled peptide without changing the original amino acid composition of the peptide sequence. Three truncated peptides were also designed. The secondary structures of the peptides were determined by circular dichroism spectra both in aqueous solution and in hydrophobic environment. The biological activities of the peptides were detected against three Gram-negative bacterial strains, three Gram-positive bacterial strains and human red blood cells. The results showed that the two helical peptides exhibited comparable antibacterial activities but their hemolytic potency (cytotoxicity) varied from extreme hemolysis to no hemolysis, which was positively correlated with their helical propensity. The β-sheet peptide partially lost both of the biological activities. The random coiled peptide with the lowest improvement in hemolytic activity showed comparable antibacterial activity against Gram-positive bacteria but weaker antibacterial activity against Gram-negative bacteria. Truncated peptides showed inevitable weaker antimicrobial activity compared to the parent peptide. Our results show that peptide secondary structure is strongly correlated with hemolytic activity and relatively less correlated with antimicrobial activity, which provides an insight into the mechanism of action of the antimicrobial peptide.
Keywords: Antimicrobial peptide, secondary structure, specificity, mechanism of action.

1. Introduction

In recent years, the microbial resistance to traditional antibiotics has resulted in the emergence of many antibiotic-resistant strains of bacteria, prompting an urgent requirement for new classes of antibiotics [1-4]. Alpha-helical and β-sheet cationic antimicrobial peptides have been proposed as potent candidates, having characteristics which includes the strong ability to kill target cells, a wide spectrum of activity against both gram-negative and gram-positive bacteria, activity against pathogens resistant to traditional antibiotics, and a relative difficulty in selecting resistant mutants *in vitro*[1, 5, 6]. Although the

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exact mode of action of antimicrobial peptides has not been established, all cationic amphipathic peptides interact with membranes and it has been proposed that the cytoplasmic membrane is the main target of some peptides, whereby peptide accumulation in the membrane causes increased permeability and a loss of barrier function[2, 7]. Recently, factors believed to be important for antimicrobial activity have been identified, including peptide hydrophobicity, the presence of positively charged residues, an amphipathic nature that segregates basic and hydrophobic residues, and secondary structure. And, Hodges and coworkers increased this list to include (i) the importance of a lack of structure in nondenaturing conditions but an inducible structure in the presence of the hydrophobic environment of the membrane, (ii) the presence of a positively charged residue in the center of the nonpolar face of amphipathic cyclic β-sheet and α-helical peptides as a determinant for locating the peptides at the interface region of prokaryotic membranes and decreasing transmembrane penetration into eukaryotic membranes and (iii) the importance of peptide self-association in an aqueous environment to the biological activities of these peptides[1, 2]. Many studies have previously shown that peptide self-association in the membrane-bound state is correlated with antimicrobial activity[8] while peptide self-association in an aqueous environment has no effect on antimicrobial activity. Hydrophobicity and amphipathicity are considered crucial parameters for peptides whose sole target is the cytoplasmic membrane. In the present study, in order to investigate the *Modification of Secondary Structure has effect on biological activities of antimicrobial peptides,* we designed an antimicrobial peptide HPRP-A1, HPRP-A2 (W12K/K15W), a β-sheet peptide and a random coiled peptide without changing the original amino acid composition of the peptide sequence and three truncated peptides. The secondary structures of peptides were determined by circular dichroism spectra both in aqueous solution and in hydrophobic environment. The template peptide HPRP-A1 showed a high level of activity against various gram-negative and gram-positive bacteria and, more importantly, negligible hemolytic activity.

2. Materials and methods

Peptide synthesis and purification

Synthesis of the peptides were carried out by standard solid-phase synthesis methodology using 9-fluorenylmethyloxycarbonyl (Fmoc) chemistry on rink amide 4-methylbenzhydrylamine (MBHA) resin (125mg, 0.1mmol) as previously described[9]. The Fmoc protecting group was removed at each cycle with 4mL of 20% piperidine in N,N'-dimethylformadine (DMF) for 30 min at 25^0C. Amino acid couplings were carried out by adding Fmoc amino acids with 0.8ml (0.45M) O-benzotriazole-1-yl-N,N,N',N'-tetramethyl-uroniumhexafluorophosphate (HBTU), 0.8 ml (0.35M) 1-hydroxybenzotriazole (HOBt) and 110µl (0.742g/ml) N,N'-diisopropylethylamine (DIEA) in DMF/DCM (dichloromethane) to resin by shaking for 3.5h. Finally, at the completion of the synthesis, the peptides were acetylated with TFA/H_2O/TIS (90:5:5) for 2h. The cleaved peptide–resin mixtures were washed with cold ether and the peptides extracted with neat acetonitrile. The resulting peptide solutions were then lyophilized prior to purification[10]. The crude peptides were purified by preparative reversed

phase high-performance liquid chromatography (RP-HPLC) on a Shimadzu LC-8A using a Zorbax 300 SB-C8 column (250x9.4 mm I.D, 6.5µm particle size, 300 Å pore size) with a linear AB gradient (0.2% acetonitrile/min) at a flow rate of 2mL/min, where eluent A was 0.1% aqueous trifluoroacetic acid (TFA) and eluent B was 0.1% TFA in acetonitrile.

Analytical RP-HPLC of Peptides

Peptides were analyzed on a Shimadzu LC-20A high-performance liquid chromatography. Runs were performed on a Zorbax 300 SB-C8 column (150 x 4.6 mm I.D, 5µm particle size, 300 A^o pore size) from Agilent Technologies using a linear AB gradient (1% acetonitrile/min) and a flow rate of 1 mL/min, where solvent A was 0.1% aqueous trifluoroacetic acid and solvent B was 0.1% trifluoroacetic acid in acetonitrile. Temperature profiling analyses were performed in 5^{o}C increments.

Characterization of peptide secondary structure

The mean residue molar ellipticities of the peptides were determined by circular dichroism (CD) spectroscopy with a J-810 spectropolarimeter (Jasco, Easton, MD, USA) at 25^{o}C under benign (nondenaturing) conditions (50 mM KH_2PO_4, Na_2HPO_4, 100 mM KCl, pH 7.4), here after referred as KP buffer, as well as in the presence of an a-helix inducing solvent, 2,2,2-trifluoroethanol (TFE), with the buffer and TFE present at 1:1 (vol/vol). The concentration of 75µM stock solution of the peptide analogs was loaded into a 1-mm fused silica cell and its ellipticity was scanned from 190 to 250 nm at a sensitivity of 100 millidegrees, response time of 1s, bandwidth of 1 nm, and a scan speed of 100nm/min. The values of molar ellipticities of the peptide analogs at the wavelength of 222 nm were used to estimate the relative α-helicity of the peptides.

Bacterial strains used in this study

Bacterial strains were activated by transferring bacterial cells from refrigerated agar slant to tryptic soy agar (TSA) (Difco, Sparks, MD) and cultured plates were incubated at 37^{o}C for 24 h. A representative colony was then inoculated into 50mL of brain-heart infusion broth and the strains was grown aerobically at 37^{o}C for 24 h to reach the concentration of approximate $1x10^9$ CFU per mL.

Escherichia coli ATCC25922

Pseudomonas aeruginosa ATCC27853

Klebsiella pneumoniae ATCC700603

Staphylococus aureus ATCC25923

Bacillus subtilis ATCC6633

Staphylococus epidermidis ATCC12228

Measurement of antimicrobial activity

Minimal inhibitory concentrations (MICs) were determined by a standard microtiter dilution method in a modified Luria-Bertani medium with no added salt (LB, composed exclusively 10 g of tryptone and 5 g of yeast extract/liter). Briefly, cells were grown overnight at 37^{o}C in LB and were diluted in the same medium. Serial dilutions of the peptides were added into 96-well microtiter plates in a volume of 90µl/well, followed by the addition of 10µl of bacteria to give final inoculums of 5 x 10^5colony-forming units(CFU)/ml. The plates were incubated at 37^{o}C for 24h and the MICs were determined as the lowest peptide concentration that inhibited growth[1].

Measurement of hemolytic activity (minimal hemolytic concentration [MHC])

Peptide samples were serially diluted by phosphate-buffered saline (PBS, 0.08 M NaCl, 0.043 M Na_2HPO_4, 0.011 MKH_2PO_4, pH 7.4) in 96-well plates to give a volume of 70µl sample solution in each well. Human erythrocytes anticoagulated by EDTA were collected by centrifugation for 5 min and washed twice by PBS, then diluted to a concentration of 2% in PBS. Peptide samples were added in each well with 70µl of 2% human erythrocytes and reactions were incubated at 37°C for 4h. The plates were then centrifuged for 10 min at 3000 rpm and the supernatant (80µl) was transferred to 96-well plates. Hemoglobin release was determined spectrophotometrically at 540 nm. The hemolytic activity was determined as the maximal peptide concentration that caused no hemolysis of erythrocytes after 1h. The control for no release of hemoglobin was a sample of 1% erythrocytes without any peptide added. Since erythorocytes were in an isotonic medium, no detectable release of hemoglobin was observed from this control during the course of the assay [2]. For the hemolysis time study, hemolytic activity of peptides at concentration of 4, 8, 16, 32, 64, 125, 250 and 500µg/ml was at 4h at 37°C.

Calculation of Therapeutic Index (MHC/MIC Ratio)

It should be noted that both the MHC and MIC values are carried out by serial twofold dilutions; thus, for individual bacteria and individual peptides, the therapeutic index (MHC/MIC) could vary as much as fourfold if the peptide is very active in both hemolytic and antimicrobial activities[11]. However, if there is no detectable hemolytic activity, then the maximum possible error in the therapeutic index would be only twofold from variations in the antimicrobial activity. When there was no detectable hemolytic activity at 500µg/ml, a minimal hemolytic concentration of 1000µg/ml was used to calculate the therapeutic index[2]. As for the antimicrobial activity, 500 µg/ml would be used because of the top limit of MIC was 250 µg/ml.

3. Results

Peptide design

The template peptide HPRP-A1 is derived from an α-helical amphipathic peptide, referred to as HPA3NT1[12], with double amino acid substitutions as R3→K and I11→L. The substitutions were intended to simplify the amino acid composition to reduce side effects. The idealized amphipathic helix of HPRP-A1 is shown in Fig. 1 to present the non-polar face and polar face with hydrophobic green colored residues and hydrophilic blue colored residues which are present along the opposite side of the helix.

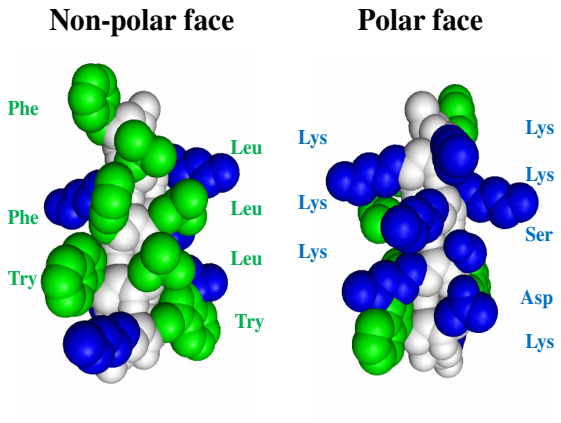

Fig. 1. Space-filling model of parent peptide HPRP-A1.

Hydrophobic amino acids on the nonpolar face of the helix are green colored; hydrophilic amino acids on the polar face of the helix are blue colored. The models were created with the PyMOL (version 0.98) program. The peptide sequences are shown in Table 1.

In an idealized helical conformation, a hydrophilic lysine residue is present at the edge of the non-polar face (position 15). To gain increased helical potential, we exchanged the original position of K15 with W13 to get peptide HPRP-A2. Thus, an hydrophobic face was formed and Tryptophan, possessing low helical potency, was moved to the C-terminus.

Table 1. Sequences of peptides used in this study

Peptides	Amino acid sequence[a]	t_R(min)[b]
HPRP-A1	Ac-FKKLKKLFSKLWNWK-amide	41.2
HPRP-A2	Ac-FKKLKKLFSKL***K***NW***W***-amide	41.5
HPRP-B	Ac-FKLKLKFSNKLKWKW-amide	34.3
HPRP-C	Ac-LFKKNKLWFKSKKWL-amide	33.3
HPRP-T10	Ac-FKKLKKLFSK-amide	30.8
HPRP-T12	Ac-FKKLKKLFSKLW-amide	40.5
HPRP-T14	Ac-FKKLKKLFSKLWNW-amide	43.2

[a] One-letter codes are used for the amino acid residues; Ac, N^{α}-acetyl; amide, C-terminal amide; the bold and italic letters denote the substituting amino acids of the parent peptide HPRP-A1. All amino acids are L-amino acids.

[b] Retention times of the peptide analogs were determined on a Shimadzu LC-20A high-performance liquid chromatograph at 25 °C. Runs were performed on a Zorbax 300 SB-C_8 column (150 × 4.6 mm inner diameter, 5 µm particle size, 300 Å pore size) from Agilent Technologies using a linear AB gradient (1% acetonitrile/min) and a flow rate of 1 ml/min, where solvent A was 0.1 % aqueous TFA and solvent B was 0.1 % TFA in acetonitrile.

In order to demonstrate the effect of the secondary structure of the helical amphipathic peptides on their cytotoxic activity and antimicrobial specificity, peptides with different secondary structures were designed which share the same amino acid composition. For all peptide analogs used in this study, the N-terminus is acetylated to enhance what and the C-terminus is amidated.

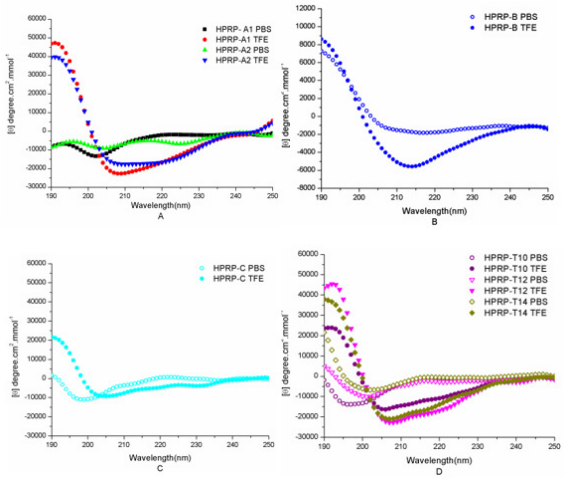

Fig. 2. Circular dichroism spectra of peptides
CD spectra of peptide HPRP at 25 °C in KP buffer
A. α-Helical peptides HPRP-A1, A2
B. β-Sheet peptide HPRP-B
C. Random coiled peptide HPRP- C
D. Truncated α-helix peptides HPRP- T10, 12, 14

Biophysical properties

The conformation of the peptide analogs was assessed by CD spectroscopy both in benign buffer and a hydrophobic environment. The CD spectra of each peptide are shown in Fig. 2. In benign buffer, all peptides were exhibited as a random coiled structure below 200 nm. As for in the attenuated polar condition (50% TFE), the secondary structures were differentiated. As a polarity moderating reagent, TFE is largely used as a mimic of membrane to decrease the polarity of the solvent. For most amphipathic membrane active peptides, the

secondary structure would undergo a transformation to present the polar face and non-polar face in this condition. The minimum at 222 nm the helical contents for peptide HPRP-A1 and HPRP-A2. For peptide HPRP-B, one minimum peak below 200 nm the β-sheet contents. For peptide HPRP-C, the hydrophobic environment induced very little secondary structure exchanges when compared with the buffer condition. The helical percents for helical peptides in the hydrophobic environment were also indicated in the reference to the peptide HPRP-A2, which presented the largest mean residue molar ellipticity. The CD spectra results confirmed the desired alteration as designed.

Table 2. Circular dichroism data of the peptide analogs

Peptides	t_R(min)[a]	Benign[b]		50% TFE[c]	
		$[è]_{222}$	% helix[d]	$[è]_{222}$	% helix[d]
HPRP-A1	41.2	-1700	10.8	-14650	93.3
HPRP-A2	41.5	-6350	40.4	-15350	97.8
HPRP-B	34.3	-	-	-	-
HPRP-C	33.3	-	-	-	-
HPRP-T10	30.8	-2150	13.7	-9950	63.4
HPRP-T12	40.5	-2800	17.8	-15700	100
HPRP-T14	43.2	-650	4.1	-12100	77.1

[a] Peptides are ordered by relative hydrophobicity during RP-HPLC at 25 °C (Table 1) .

[b] The mean residue molar ellipticities, $[\theta]_{222}$ (degree·cm^2·dmol^{-1}) at wavelength 222 nm were measured at 25 °C in KP buffer .

[c] The mean residue molar ellipticities, $[\theta]_{222}$ (degree·cm^2·dmol^{-1}) at wavelength 222 nm were measured at 25°C in KP buffer diluted at 1:1 (v/v) with TFE.

[d] The helical content (in percentage) of a peptide relative to the molar ellipticity value of peptide HPRP-T12 in 50% TFE.

As mentioned above, all peptides contain the same composition of amino acids, thus the intrinsic hydrophobicity of all analogs are identical. However, the retention times of these analogs on RP-HPLC vary widely as shown in Table 2. HPRP-A2 with a defined amphipathic structure possessed the longest eluting time. In contrast, the random coiled peptide HPRP-C exhibited the shortest retention time. The conformational transition resulted in the increased or decreased binding stability with the matrix of the HPLC column.

Cytotoxic activity and therapeutic index

The inhibitory activity of the peptide analogs against both Gram-negative and Gram-positive bacteria was assessed by series diluted method and the minimum inhibitory concentration (MIC) is compiled in Table 3 for Gram-negative bacteria and Table 4 for Gram-positive bacteria. The geometric mean MIC values from three microbial strains in these tables were calculated to provide an overall evaluation of antimicrobial activity against bacteria. For the α-helical conformation peptide, i.e. peptide HPRP-A1 and HPRP-A2, their geometric mean of MIC against Gram-negative and Gram-positive bacteria were roughly at the same level which was 1.6-6.4 μM. The β-sheet peptide HPRP-B lost the antibacterial activity with the MICs beyond 12.5-25 μM except for *B.subtilis*. Most interestingly, peptide HPRP-C with random coiled structure both in buffer and hydrophobic conditions exhibited the weakest anti-Gram-negative and anti-Gram-positive bacterial activity compared to template peptide HPRP-A1. This result indicates that the secondary structure more strongly affected the anti-Gram-negative bacterial activity than anti-Gram-positive bacterial activity.

Table 3. Antimicrobial (MIC) and hemolytic (MHC) activities of peptide analogs against Gram-negative bacteria and human red blood cells

Peptides[a]	MIC[b]				MHC[c] hRBC	Therapeutic Index[d]
	E. coli ATCC25922	*P. aeruginosa* ATCC27853	*K.pneumoniae ATCC700603*	*GM*[e]		
	μM				μM	
HPRP-A1	1.6	3.2	3.2	2.5	32	12.8
HPRP-A2	1.6	3.2	3.2	2.5	16	6.4
HPRP-B	12.5	12.5	25	15.7	64	4.1
HPRP-C	50	50	50	50.0	500	20.0
HPRP-T10	100	100	25	63.0	500	15.9
HPRP-T12	3.2	3.2	12.5	5.0	32	6.4
HPRP-T14	1.6	6.4	6.4	4.0	16	4.0

[a] Peptides are ordered by relative hydrophobicity during RP-HPLC at 25 °C.
[b] Antimicrobial activity (minimal inhibitory concentration) was determined as the minimal concentration of peptide to inhibit microbial growth.
[c] Hemolytic activity (minimal hemolytic concentration) was determined on human red blood cells (hRBC).
[d] Therapeutic index = MHC (μM)/geometric mean of MIC (μM). Larger values indicate greater antimicrobial specificity.
[e] *GM* denotes the geometric mean of MIC values from all three microbial strains in this table.

The cytotoxicity of the peptides was assessed against human red blood cells and defined as the minimal hemolytic concentration (MHC) (Table 3 and Table 4). Peptide HPRP-A2 with the greatest α-helical potency is the most hemolytic. The template peptide HPRP-A1, with the moderate α-helical potency, exhibited moderate hemolytic activity. The lowest cytotoxicity is ascribed to peptide HPRP-T10 and HPRP-C, which presented lowest α-helical potency and no defined secondary structure. For peptide HPRP-B, which exhibited β-sheet contents in 50% TFE aqueous, the cytotoxicity is also moderate but lower than that of template peptide HPRP-A1.

Table 4. Antimicrobial (MIC) and hemolytic (MHC) activities of peptide analogs against Gram-positive bacteria and human red blood cells

Peptides[a]	MIC[b]				MHC[c] hRBC	Therapeutic Index[d]
	S.aureus ATCC25923	*B.subtilis* ATCC6633	*S.epidermidis* ATCC12228	*GM*[e]		
	μM				μM	
HPRP-A1	6.4	1.6	1.6	2.5	32	12.8
HPRP-A2	3.2	1.6	1.6	2.0	16	8.0
HPRP-B	25	3.2	12.5	10.0	64	6.4
HPRP-C	50	6.4	25	20.0	500	25.0
HPRP-T10	200	50	25	63.0	500	7.9
HPRP-T12	6.4	3.2	6.4	5.1	32	6.3
HPRP-T14	6.4	3.2	6.4	5.1	16	3.1

[a] Peptides are ordered by relative hydrophobicity during RP-HPLC at 25 °C.
[b] Antimicrobial activity (minimal inhibitory concentration) was determined as the minimal concentration of peptide that inhibits microbial growth. When no antimicrobial activity was detected at 100 μM, a value of 200 μM was used for calculation of the therapeutic index.
[c] Hemolytic activity (minimal hemolytic concentration) was determined on human red blood cells (hRBC).
[d] Therapeutic index = MHC (μM)/geometric mean of MIC (μM). Larger values indicate greater antimicrobial specificity.
[e] *GM* denotes the geometric mean of MIC values from all four microbial strains in this table.

A therapeutic index is a widely employed parameter that is used to represent the specificity of antimicrobial reagents. It is calculated by the ratio of MHC (hemolytic activity) and MIC (antimicrobial activity); thus, larger values in therapeutic index indicate greater antimicrobial specificity. The Therapeutic indexdata in Table 3 and Table 4 show that peptide HPRP-B possesses the poorest specificity with regard to both Gram-negative bacteria and Gram-positive bacteria. For peptide HPRP-C, anti-Gram-positive bacteria activity and hemolytic activity of HPRP-C is the lowest, but this is not so with regards to anti-Gram-negative bacteria activity.

4. Discussion

Most antimicrobial peptides present amphipathic structures in either α-helical or β-sheet conformation, especially in the state of membrane-binding. Still, there exist antimicrobial peptides that present no specific secondary structure, i.e. random coiled structure[13, 14]. To elucidate the conformational effects, two α-helical peptides with the helical potency gradient of HPRP-A2 >HPRP-A1 (template), one β-sheet HPRP-B peptide and one coiled HPRP-C peptide were designed which share the same amino acid composition. Thus, the intrinsic hydrophobicity of the peptides is maintained. However, the retention times of the peptides in RP-HPLC are closely correlated with the conformational organization of the peptide, as determined in this study that the retention time follows the sequence of HPRP-A2 >HPRP-A1>HPRP-B >HPRP-C. Thus, it can be determined that the more amphipathic a peptide is, the stronger combination with the column it will exhibit.

The conformational style of a peptide is also strongly correlated with its cytotoxic activity towards eukaryocytes. Better organization corresponded to higher hemolytic activity[15, 16], as shown by the fact that HPRP-T10 and HPRP-C showed no hemolytic activity. The antimicrobial results demonstrated that it is imperative to maintain certain helical contents for the desirable antimicrobial activity. The β-sheet HPRP-B peptide showed the weaker antimicrobial activity.

It is largely accepted that most antimicrobial peptides(AMPs) will be induced into an amphipathic conformation with a polar moiety and a non-polar moiety upon interaction with membrane.

5. Conclusions

Peptide secondary structure plays an important role in the biological activity of AMPs. The helical potency of a present peptide is correlated more with hemolytic activity and less with antibacterial activity. Inconsistent with some previous concepts, it seems that amphipathic structure is not the critical property of AMPs for the specificity of activity in the present study, and the activity of AMPs depends on the maintenance of a proper ratio between hydrophobic amino acid and hydrophilic amino acid. There is a frontier for peptide hydrophobicity. If a peptide hydrophobicity crosses this frontier, the increase in hydrophobicity would not increase antimicrobial activity but it would increase undesired hemolytic activity. Through careful design that does not change the original amino acid composition, we can get antimicrobial peptides with strong activity specificity. The HPRP-C peptide, with its greater specificity

towards bacteria,is a good candidatefor further development. This work would provide another approach towards improvingthe specificity of AMPs.

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Ảnh hưởng của cấu trúc bậc hai đến hiệu quả hoạt động sinh học của peptit kháng khuẩn

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Tóm tắt: Một peptide xoắn alpha 15 amino acid được sử dụng làm khuôn mẫu để nghiên cứu ảnh hưởng của cấu trúc bậc hai đối với hoạt động kháng khuẩn. Chúng tôi thiết kế một peptide xoắn alpha với mức độ xoắn cao hơn bản mẫu, thiết kế một peptide gấp beta và một xoắn ngẫu nhiên mà không thay đổi thành phần và số lượng các amino acid trong các chuỗi peptide. Đồng thời, dựa trên khuôn mẫu ban đầu thiết kế 3 peptide cắt ngắn tương ứng 14, 12 và 10 amino acid. Cấu trúc bậc hai được xác định bởi máy đo lưỡng sắc vòng trong hai môi trường nước đẳng trương và môi trường kị nước. Hoạt tính sinh học của các peptide được thử nghiệm trên ba chủng vi khuẩn gram âm, ba chủng vi khuẩn gram dương và tế bào hồng cầu người. Kết quả cho thấy hai peptide xoắn alpha có hoạt động kháng khuẩn mạnh đồng thời cũng có hoạt tính tan huyết cao. Peptide phiến gấp beta có hoạt tính kháng khuẩn và tính tan huyết cũng thấp hơn. Peptide xoắn ngẫu nhiên có tính tan huyết rất thấp nhưng vẫn có khả năng kháng khuẩn, tuy nhiên khả năng kháng vi khuẩn gram dương cao hơn kháng vi khuẩn gram âm. Các peptide bị cắt ngắn không thể tránh khỏi bị ảnh hưởng so với peptide mẫu. Kết quả cho thấy cấu trúc bậc hai của peptide rất tương quan với hoạt tính tan huyết nhưng ít tương quan hơn so với hoạt tính kháng khuẩn, từ đó hiểu hơn về cơ chế hoạt động của peptide kháng khuẩn.

Từ khóa: Peptide kháng khuẩn, cấu trúc bậc hai, tính đặc hiệu, cơ chế hoạt động.