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De novo generation of antimicrobial LK peptides with a single tryptophan at the critical amphipathic interface‡

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***De novo* design of amphipathic model peptides has been successful for generating many antimicrobial peptides with various lengths and amino acid compositions. Here, we suggest a very simple strategy to design antimicrobial peptides with a short length and a simple amino acid composition. Amphipathic helical properties were conferred by using only leucines and lysines and a single tryptophan was positioned at the critical amphipathic interface between the hydrophilic ending side and the hydrophobic starting side, in the helical wheel projection. According to this rule, the model peptides with 7 to 13 residues exhibited antimicrobial activity. Among them, the most potent activity against both Gram-positive and Gram-negative bacteria, covering all of the nine bacterial strains tested in this study, was found for the 11-mer sequences having a 1 : 1 ($L_5K_5W^6$) or a 3 : 2 ($L_6K_4W^6$) ratio of leucines to lysines. In particular, the former peptide $L_5K_5W^6$ could be evaluated as the most useful agent, as it showed no significant hemolytic activity with a broad-spectrum of antimicrobial activity. Copyright © 2009 European Peptide Society and John Wiley & Sons, Ltd.**

Keywords: *de novo* design; antimicrobial peptides; amphipathic helix; LK peptides; tryptophan; critical amphipathic interface

Introduction

The membrane-lytic, antimicrobial peptides (AMPs) from all species of life have emerged as potential therapeutic agents [1–6]. Comprehensive investigations on their structure-activity relationships have generated many potent analogs of the AMPs [1–8]. Commercial approaches are also being widely attempted to develop clinical therapeutics for future use [1–6]. However, some obstacles in their commercial and clinical applications are still indicated, such as the rather large molecular sizes, high manufacturing costs, and poor pharmaceutical and pharmacokinetic properties [1,7]. Thus, it would be a valuable effort to generate shorter AMPs with a simple amino acid composition, which can be a more favorable lead molecule to reduce production costs and to facilitate pharmaceutical optimization. In this respect, it is worth to note the minimalistic *de novo* approaches to design model amphipathic helical peptides having antimicrobial activities (partially reviewed in [8,9]).

The cationic, amphipathic α-helical peptides represent a particularly abundant and widespread, and the most well characterized class of naturally occurring AMPs [1–11]. Accordingly, extensive researches have been promoted to generate amphipathic helical model AMPs with a simple composition of amino acids, such as leucines and lysines, which have strong helix-forming potential in proteins [12]. For example, Blondelle and Houghten [13] designed a series of model peptides composed of leucyl and lysyl residues (namely LK peptides), to be completely amphipathic when adopted a helical structure. The peptides varied in length from 8 to 22 residues and the highest antimicrobial activity was found for the 14-mer sequence: Ac-KKLLKKLKKLLKKL-NH_2. Similar approach by Béven *et al*. [14] also proposed a 15-residue LK peptide with a strong antimicrobial activity: KLLKLLLKLLLKLLK. Those kinds of systematic screenings of model peptides are also aiming to generalize a specific rule to design a potent AMP. For this, in the present work, we introduced our recent concept of AMP engineering using tryptophan, into the LK model peptide designing.

Trp residues in proteins and peptides often play a crucial role in the membrane interaction [15–20] and stabilize the helical structure of the membrane-interacting peptides [12,15–18]. In addition, some AMPs are particularly rich in Trp [21,22]. As part of an effort to develop new, low molecular mass peptide antibiotics, we have sought to find a method to restore the antimicrobial activity of the short, inactive fragment of natural AMPs. We have found that a single tryptophanyl substitution at certain positions of inactive fragments of the amphipathic helical AMPs could confer the antimicrobial activity, without increasing any significant hemolytic activity [7,15,16]. For example, the D16W substitution in the inactive N-terminal 23-residue fragment (named $GGN4^{N23}$) of a 37-residue AMP esculentin-2EM (formerly known as gaegurin 4 [23]) promoted a full recovery of the antimicrobial activity [7,15].

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Similarly, the N-terminal 11-residue fragment (named GGN5^{N11}) of a 24-residue AMP brevinin-1EMa (formerly known as gaegurin 5 [23]) is inactive, but its A4W analog (named A4W-GGN5^{N11}) is as active as the intact peptide [7,16]. The insertion of a Trp residue not only stabilized the amphipathic helical structure but also had an important role in the membrane interaction, by anchoring the peptide to the membrane [7,15,16]. To play this specific role, Trp was the most efficient amino acid, since its amphipathic architecture could contribute to both the hydrophobic and hydrophilic interactions. The most important point was the position of the Trp. To maximize the activity-conferring effect, Trp had to be located at the critical amphipathic interface between the hydrophilic ending side and the hydrophobic starting side, in the helical wheel projection. The position for Trp that could meet this criterion in the GGN4^{N23} and GGN5^{N11} was the position 16 and 4, respectively [7]. It has been further surprising that those tryptophanyl substitutions did not confer a significant hemolytic activity, whereas they promoted a full recovery of the antimicrobial activity.

On the basis of the previous findings, we hypothesized that an amphipathic helical peptide having a Trp at the critical amphipathic interface would possess an antimicrobial activity. As a preliminary test to ascertain this concept, we designed 12 kinds of $L_lK_mW^n$ model peptides (LK peptides with a single tryptophan at the designated *n*th position) and their activities are reported in this paper.

Materials and Methods

Peptide Preparation

Peptides were synthesized automatically on a peptide synthesizer (Model 90 manufactured by Advanced Chemtech, Inc.) by solid-phase methods using standard Fmoc chemistry. Fmoc-protected amino acids and Rink Resins were obtained from Advanced Chemtech, Inc. and HPLC solvents were from Fisher Scientific. All other chemicals were either analytical or biotechnological grade, obtained from various manufacturers. To obtain the peptide amide, 4-(2′,4′-dimethoxyphenyl-Fmoc-amino-methyl) phenoxy resin was used. Side-chain protection groups included Fmoc-O-t-butyl-L-Serine and N-Fmoc-N-Boc-L-Lysine. Double coupling procedures were performed with diisopropylcarbodiimide/1-hydroxybenzotriazole activation. Fmoc group removal from the peptide chain was performed with 25% piperidine in DMF. Cleavage of the resin and the protecting group was performed with 10% TFA in DCM. Purification and analysis of the products were performed by analytical reversed-phase HPLC on C-18 columns from Merck–Hitachi. Acetonitrile/water mixed with 0.1% TFA was used as eluent and a gradient of 20–80% acetonitrile was applied at a flow rate of 1 ml/min. The correct mass of the product peptides was identified by MS. The same peptides were also purchased from the peptide manufacturer company ANYZEN (Kwangju, Korea; URL, http://www.anygen.com), to check reproducibility of the activity test. Both the synthesized and purchased peptides were applied to the antimicrobial activity test.

Activity Test

Antimicrobial activity was assessed by the standard broth microdilution method measuring the MIC values. The activity of each peptide was determined against four strains of Gram-positive (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538p, *Staphylococcus epidermis* ATCC 12 228, *Micrococcus luteus* ATCC 10 240) and five strains of Gram-negative bacteria (*Escherichia coli* ATCC 25 922, *Shigella dysentariae* ATCC 9752, *Salmonella typhomurium* ATCC 14 028, *Klebsiella pneumoniae* ATCC 10 031, *Pseudomonas aeruginosa* ATCC 27 853). In brief, from cell cultures (10^6–10^8 colony-forming units/ml in LB broth media) incubated in the presence of various concentrations (1.6–200 μg/ml in 2-fold dilution) of peptides, the MIC was defined as the lowest peptide concentration that completely inhibits the cell growth.

In order to measure hemolytic activities, suspensions of human red blood cells (10% v/v) were incubated for 30 min at 37 °C, in the presence of various concentrations (6.3–200 μg/ml in 2-fold dilution) of peptides. After centrifugation, the absorbance at 550 nm of the supernatant was measured. The relative attenuation, as compared with that of the blood suspension treated with 0.2% Triton X-100, was defined as the percentage of hemolysis.

CD Spectroscopy

CD measurement of 50-μM peptide dissolved in 10-mM SDS micelles, which is above its critical micellar concentration [7,23], was performed on a Jasco J-720 spectropolarimeter at 20 °C, using a 0.2 cm path-length cell. The correct concentration of the peptide was ensured spectrophotometrically at 280 nm using the known value of molar absorptivity for Trp. CD scans were taken from 250 to 190 nm, with a 1-nm bandwidth, a 4-s response time, a scan speed of 50 nm/min, and a 0.5-nm step resolution. Three scans were added and averaged, followed by subtraction of the CD signal of the solvent. Finally, the CD intensity was normalized as the mean residue molar ellipticity (MRME) [15].

Results

Amino acid sequences of the present $L_lK_mW^n$ model peptides are summarized in Figure 1. To design each sequence, we used helical wheel diagram shown in Figure 2. All of the peptides were designed to be perfectly amphipathic when folded into α-helical structures, by converging the hydrophobic leucines into one side and the cationic lysines into the other side of the helical axis. The

no.	*Amino acid sequence* (1 3 5 7 9 11 13)	*Name (Formula)*
1.0	L K K ***W*** L K -NH_2	$L_2K_3W^4$
1.1	L K K ***W*** L K K -NH_2	$L_2K_4W^4$
1.2	L L K ***W*** L K K -NH_2	$L_3K_3W^4$
1.31	L L K ***W*** L L K -NH_2	$L_4K_2W^4$
1.32	L L K ***W*** L K K L -NH_2	$L_4K_3W^4$
2.0	K L L K ***W*** L L K -NH_2	$L_4K_3W^5$
2.1	K L L K ***W*** L L K L -NH_2	$L_5K_3W^5$
3.0	K K L L K ***W*** L K K L L -NH_2	$L_5K_5W^6$
3.1	K K L L K ***W*** L L K L L -NH_2	$L_6K_4W^6$
3.2	L K L L K ***W*** L L K L L -NH_2	$L_7K_3W^6$
4.0	L K K L L K ***W*** L L K L L K -NH_2	$L_7K_5W^7$
4.1	L L K L L K ***W*** L L K L L K -NH_2	$L_8K_4W^7$

Figure 1. Amino acid sequences of the $L_lK_mW^n$ model peptides. Each peptide sequence is labeled with the serial number on the left and the peptide name as a formula on the right. Tryptophan (***W***) residues are indicated in bold, italicized letters. Amino acid variation from the preceding number of peptide is underlined.

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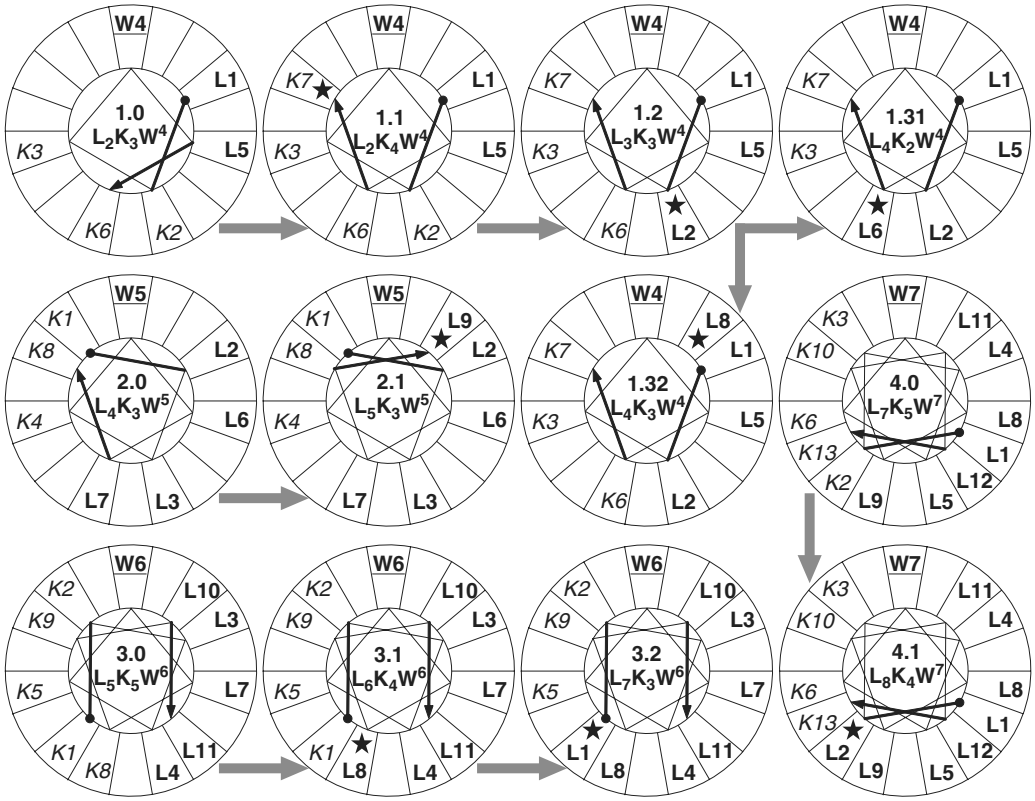

Figure 2. Helical wheel diagrams for the $L_lK_mW^n$ model peptides. The model number is presented with the peptide name (formula), in the middle of each diagram. The order of design is depicted by the flow of the gray arrows. Then, the amino acid variation from the preceding number of peptide is indicated by an asterisk. The leucines and lysines are indicated in bold and in italicized letters, respectively. Tryptophans are in bold and underlined.

single Trp was always located at the critical amphipathic interface between the hydrophilic (lysyl) ending side and the hydrophobic (leucyl) starting side, in the helical wheel projection. Under this rule, four template sequences (model No.0) were first generated with different position of Trp in the primary structure (Figure 1): Trp position 4 for model 1.0, position 5 for 2.0, position 6 for 3.0, and position 7 for 4.0. Peptide length of the templates was chosen to approximately center the Trp in the sequence: 6-residue length for model 1.0, 8-residue for 2.0, 11-residue for 3.0, and 13-residue for 4.0. The L : K ratios of the templates were distributed from 0.7 : 1 for model 1.0 to 1.4 : 1 for model 4.0. From each template, one to four analogs were designed step-by-step to alter the peptide length and/or L : K ratio, either by a single (L to K or K to L) substitution or by a C-terminal addition of an amino acid (L or K). For example, the K6L substitution of the model 1.2 generated the model 1.31, while the model 1.32 was designed by adding the C-terminal leucine to the model 1.2. The model 1.32 has an isomeric configuration to the model 2.0. Finally, 12 model peptides with the $L_lK_mW^n$ formula were generated and they could be varied in the length from 6 to 13 residues and in the L : K ratio from 0.5 : 1 to 2.3 : 1.

Antimicrobial activities of the 12 $L_lK_mW^n$ model peptides are summarized in Table 1. Most peptides, except the shortest one (model 1.0), exhibited detectable activities against certain bacterial species and were broadly more active against Gram-positive bacteria than against Gram-negative bacteria. However, strong and broad spectrum of antimicrobial activity was observed for the models 2.1, 3.0, and 3.1, against all of the nine bacterial strains tested; MIC values ranged from 1.6 to 50 μg/ml. Among them, the most potent antimicrobial activity was found for the 11-residue $L_6K_4W^6$ peptide (model 3.1). In particular, its activity against Gram-positive bacteria was very strong with the smallest MIC value of 1.6 μg/ml. Even for the Gram-negative bacteria, MIC value of the $L_6K_4W^6$ was less than 6.3 μg/ml against three species and was 25 μg/ml against the other two species. The peptide length and the tryptophanyl position of the $L_6K_4W^6$ is the same as those of the other two model 3 series peptides ($L_5K_5W^6$ and $L_7K_3W^6$), while its L : K ratio (1.5 : 1) is higher than that of $L_5K_5W^6$ (model 3.0; L : K is 1 : 1) and lower that that of $L_7K_3W^6$ (model 3.1; L : K is 2.3 : 1). Thus, the highest activity of the $L_6K_4W^6$ indicates that the 1.5 : 1 (3 : 2) ratio of L : K would be an optimal value to maximize antimicrobial activity, in the designing strategy suggested in this work. However, the 1 : 1 ratio of L : K seems also favorable, since the antimicrobial activity of $L_5K_5W^6$ (model 3.0) is quite comparable to that of $L_6K_4W^6$ (model 3.1). Compared to the $L_6K_4W^6$ and $L_5K_5W^6$, other peptides with similar L : K ratios but with different lengths, including models 1.2, 1.32, 2.0, 2.1, and 4.0, showed significantly decreased activity. Particularly in the longer peptides (model 4 series, $L_7K_5W^7$, and $L_8K_4W^7$), the activity against Gram-negative bacteria was totally abolished (Table 1). Thus, restricted to our screening, the optimal peptide length for high antimicrobial activity is 11 residues. An additional point to note is the isomeric relationship between models 1.32 ($L_4K_3W^4$) and 2.0 ($L_4K_3W^5$), which showed nearly identical activities (Table 1). Thus, translocation of Trp within the middle of the primary structure seems to be tolerable, once it is located at the critical amphipathic interface with the same L : K ratio.

To evaluate the model peptides in regard of therapeutic candidates, their hemolytic activities were examined. As shown in Table 2, the hemolytic activities of the peptides with less than 11 residues (models 1.0–2.1) were negligible even at

Table 1. Antimicrobial activities of the $L_lK_mW^n$ model peptides

	$L_2K_3W^4$ **1.0**[a]	$L_2K_4W^4$ **1.1**	$L_3K_3W^4$ **1.2**	$L_4K_2W^4$ **1.31**	$L_4K_3W^4$ **1.32**	$L_4K_3W^5$ **2.0**	$L_5K_3W^5$ **2.1**	$L_5K_5W^6$ **3.0**	$L_6K_4W^6$ **3.1**	$L_7K_3W^6$ **3.2**	$L_7K_5W^7$ **4.0**	$L_8K_4W^7$ **4.1**
Minimal inhibitory concentration (μg/ml)[b]												
Gram-positive bacteria												
B. subtilis	>200	>200	100	25	50	50	12.5	6.3	1.6	1.6	3.1	6.3
S. aureus	>200	>200	200	25	25	25	3.1	6.3	1.6	3.1	12.5	25
S. epidermis	>200	n.a.[c]	>200	100	100	200	12.5	6.3	1.6	3.1	12.5	50
M. luteus	n.a.	100	25	n.a.	100	100	25	6.3	1.6	3.1	3.1	6.3
Gram-negative bacteria												
E. coli	>200	>200	>200	100	100	100	12.5	12.5	6.3	50	>200	>200
S. dysentariae	>200	>200	100	100	200	200	12.5	6.3	3.1	12.5	>200	>200
S. typhimorium	>200	>200	>200	200	>200	>200	25	25	25	>200	>200	>200
K. pneumoniae	>200	>200	>200	200	200	100	6.3	6.3	3.1	50	>200	>200
P. aeruginosa	>200	>200	>200	100	50	100	50	25	25	>200	>200	>200

[a] Amino acid sequences are represented by the corresponding model numbers (refer to Figure 1).
[b] Minimal inhibitory concentrations are the consistent values obtained with the synthesized and the purchased peptide samples, each measured in duplicate.
[c] Not available; the MIC values were not reproducible in repetitive experiments.

Table 2. Hemolytic activities of the $L_lK_mW^n$ model peptides

	$L_2K_3W^4$ **1.0**[a]	$L_2K_4W^4$ **1.1**	$L_3K_3W^4$ **1.2**	$L_4K_2W^4$ **1.31**	$L_4K_3W^4$ **1.32**	$L_4K_3W^5$ **2.0**	$L_5K_3W^5$ **2.1**	$L_5K_5W^6$ **3.0**	$L_6K_4W^6$ **3.1**	$L_7K_3W^6$ **3.2**	$L_7K_5W^7$ **4.0**	$L_8K_4W^7$ **4.1**
200 μg/ml	4.84[b]	4.90	5.19	4.61	5.01	5.42	7.09	7.26	57.58	64.43	47.72	36.37
100 μg/ml	4.90	5.13	4.96	6.05	4.84	5.99	5.36	5.76	14.92	34.29	20.86	10.90
50 μg/ml	4.73	4.90	4.73	4.73	4.84	5.13	4.73	4.72	7.38	17.58	12.80	7.61
25 μg/ml	5.07	5.19	4.84	4.44	5.01	5.13	4.84	5.07	5.01	14.99	10.78	5.24
12.5 μg/ml	5.13	5.01	4.96	4.78	4.44	4.73	5.19	4.78	4.96	6.51	5.31	5.24
6.3 μg/ml	5.07	4.90	4.78	4.61	4.78	5.47	4.84	5.30	4.73	5.13	5.07	4.44

[a] Amino acid sequences are represented by the corresponding model numbers (refer to Figure 1).
[b] Percent hemolysis relative to that by 0.2% Triton X-100.

200 μg/ml concentration. The other peptides with relatively higher antimicrobial activities (models 3.0–4.1) also showed no significant hemolytic activity at less than 50 μg/ml concentrations. The most notable peptide was $L_5K_5W^6$ (model 3.0) that exhibited little hemolytic activity, even at 200 μg/ml concentration, despite its potent antimicrobial activity. Even for the most potent antimicrobial peptide $L_6K_4W^6$ (model 3.1), the hemolytic activity was not significant at less than 100 μg/ml concentrations. These results are consistent with the previous finding of D16W-$GGN4^{N23}$ and A4W-$GGN5^{N11}$, where the single tryptophanyl substitution of the inactive parent molecule ($GGN4^{N23}$ and $GGN5^{N11}$) conferred antimicrobial activity with depressed hemolytic activity [7,15,16]. Thus, it can be suggested that our strategy was favorable to generate AMPs with little hemolytic activity.

Finally, we examined whether the model peptides indeed adopted a helical structure as expected at designing stage. In SDS micelles, which are generally employed as one of simple membrane-mimetic environments, their conformational preferences were examined by CD spectroscopy. As shown in Figure 3, helical adoption of many model peptides could be evidenced by the negative band near 222 nm. Only the two short peptides, $L_2K_3W^4$ and $L_2K_4W^4$ (models 1.0 and 1.1), appeared to be totally disordered, as evidenced by single minimum at 199 nm and a positive band at 228 nm, which is well correlated with their inertness. For the other peptides, the helicity or helical stabilization, deduced from the signal intensification at 222 nm [7,24,25], could be correlated with antimicrobial activity; i.e., more potent group showed more stabilized helical conformation. However, the helicity was not linearly proportional to the activity and the detailed variation of activity between models 3.0, 3.1, 3.2, 4.0, and 4.1 could not be clearly explained solely by the helicity. This observation is consistent with our previous investigation of tryptophanyl substitution for natural AMP sequences [7,15,16], which indicated that, in addition to the helicity, many other parameters, such as amphipathicity, hydrophobic/hydrophilic ratio, peptide length, and the position and membrane insertion of Trp, would cooperate for the activity.

Discussion

This study constitutes the first demonstration of a new concept for AMP engineering using a specific amino acid, Trp. The present results on a series of $L_lK_mW^n$ model peptides testify that an amphipathic helical model peptide with a single Trp at the critical amphipathic interface between hydrophilic ending side and hydrophobic starting side in the helical wheel projection could possess a strong and broad spectrum of antimicrobial

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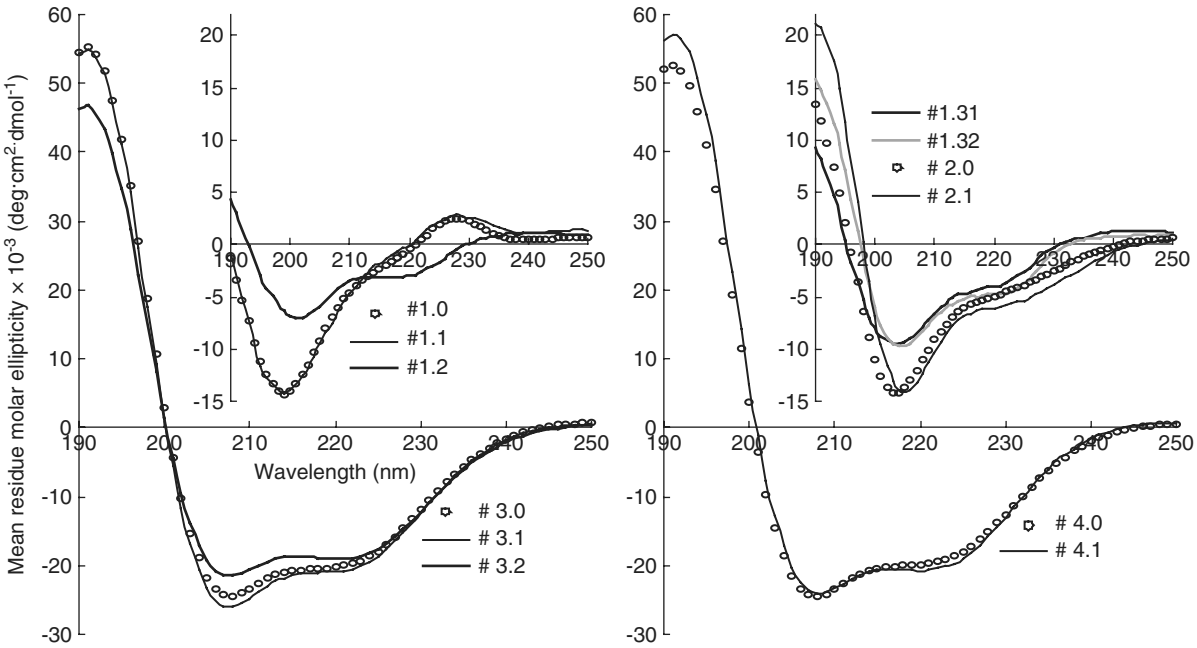

Figure 3. Far-UV CD spectra of the $L_IK_mW^n$ model peptides in 10 mM SDS micelles. Corresponding model numbers are indicated for each spectrum in every panel.

activity. According to this rule of Trp, the peptides with rather short length of 7–11 residues showed antimicrobial activity, and CD spectroscopy proved their helical propensity at a membrane-mimetic environment. In our designing strategy, the Trp can play the designed role as the critical amphipathic interface only when the peptide adopts a helical conformation. It means that the antimicrobial activity of the designed peptide would be regulated by its helical stability; i.e., as the helical structure is more stabilized, the position of Trp should be more strictly arranged to constitute the critical amphipathic helical interface, thereby conferring more potent antimicrobial activity. In this respect, the present CD results showed a good correlation between helicity and potency of the peptides.

Trp residues have often appeared in *de novo* engineering of AMPs [9,25–33], where they were just employed as hydrophobic amino acids or spectroscopic probes. Accordingly, the tryptophans were randomly distributed without considering any specific rule and the potent peptides possess longer sequences than those suggested in this work. The $L_IK_mW^n$ sequences are also found in the literature; the $K_6L_6W^2$ (KWKKLLKKLLKLL-NH_2), $K_4L_7W^6$ (KLLLKWLLKLLK-NH_2), and their analogs have been evaluated as potent antimicrobial agents [25–27]. However, they are longer than the present peptides and their tryptophanyl location, for which no explanation was given, is in the middle of the hydrophobic face.

On the basis of the present rule for Trp, the other detailed factors to optimize the activity remain to be further investigated by a comprehensive and more systematic approach, which would be desirable to examine in relation to 3-dimensional structures. To be more usefully generalized, the factors determining selectivity between bacterial and human cells would be also worthy of investigation. Restricted to the present preliminary investigation, the 3:2 or alternatively 1:1 ratio of L:K and the 11-residue length of peptide were optimal to generate the most potent model peptide (models 3.1 and 3.0). This finding is also supported by the amino acid composition of the A4W-GGN5^{N11} (sequence: FLGWLFKVASK [14,15]), which has been previously derived from a systematic sequence modification of a 24-residue natural AMP, brevinin-1EMa (formerly known as gaegurin 5 [23]), to search for the shortest bioactive analog of the peptide (refer to the Introduction Section). The A4W-GGN5^{N11} that has also 11 residues in total was the shortest and the most potent analog of the parent molecule. In the 3-dimensional structure of the A4W-GGN5^{N11}, the Trp constitutes the critical amphipathic α-helical interface [14,15]. The A4W substitution was the most effective one to confer activity to the inactive GGN5^{N11} [14].

In the present study, the peptides $L_6K_4W^6$ and $L_5K_5W^6$ could be derived as potent antibacterial agents, which are even stronger than the A4W-GGN5^{N11}. Since they are relatively short, strong, and simple in composition, compared with other *de novo* designed or natural AMPs, the peptides could be good templates to generate a useful antimicrobial agent. They can be also regarded as therapeutic candidates, since the peptides, like A4W-GGN5^{N11}, showed no significant hemolytic activity at the minimal concentrations exerting the antimicrobial activity. Among the two peptides, the antimicrobial activity of the $L_6K_4W^6$ appears slightly stronger than that of the $L_5K_5W^6$. However, considering significant hemolytic activity of $L_6K_4W^6$ at more than 100 μg/ml concentrations, the peptide $L_5K_5W^6$ can be alternatively chosen as the most favorable candidate to be developed as a therapeutic agent; its antimicrobial potency is comparable to that of $L_6K_4W^6$, while hemolytic activity is not significant even at 200 μg/ml.

It has been generally accepted that the membrane permeation of amphipathic α-helical AMPs is accomplished via either the 'pore-forming' mechanism or the 'carpet-like' mechanism [9–11]. In the present results, the longest peptides $L_7K_5W^7$ and $L_8K_4W^7$ (models 4.0 and 4.1) were not active against Gram-negative bacteria while they inhibited Gram-positive bacteria. Many natural AMPs are also selectively active against Gram-positive bacteria [34,35]. It has been suggested that they do not readily cross the outer membrane of Gram-negative bacteria, since they function via the 'pore-forming' mechanism, where they form oligomers upon

membrane binding [34,35]. In contrast, the peptides acting via the 'carpet-like' mechanism are expected to possess activity against both Gram-positive and Gram-negative bacteria. Thus, the present 13-residue peptides $L_7K_5W^7$ and $L_8K_4W^7$ might act via the pore-forming mechanism, while the others act via the carpet-like or via both the mechanisms. However, the exact mode of action remains to be investigated in detail.

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