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Improving the antibacterial activity and selectivity of an ultra short peptide by hydrophobic and hydrophilic amino acid stretches

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ABSTRACT

The principle of amino acid stretches tagged at the C terminal of Luecrocin I, which is an ultra-short antibacterial peptide, by tryptophan and arginine or lysine has been reported. The choice of amino acid type at each stretch position depends on the hydrophobic and hydrophilic regions visualized in the helical wheel pattern of Luecrocin I. Oligopeptide tagging should also consider the properties such as positive charge, hydrophobicity, the content of hydrophobic amino acids, polar angle, the properly hydrophilic and hydrophobic facets. Amidation at C terminal and lysine substitute for arginine can increase selectivity between mammalian cells (hemolytic and MTT assay) and bacterial cells tested. KT2 and RT2 which have 53% hydrophobic residues, 7 positive charges, 160° polar angle, −0.02 (KT2) and −0.04 (RT2) hydrophobicity were effective against *S. typhi* DMST 22842, *S. epidermidis* ATCC 12228, *E. coli* ATCC 25922 and *V. cholerae* non-O1, non-O139. The SEM images implied that the antibacterial mechanism of RT2 and KT2 may depend on concentration rather than time. Finally, RT2 and KT2 can be new antibacterial agents or may be further developed for alternative antibiotics.

In antibacterial peptide synthesis, some ultra short antimicrobial peptides found in or derived from living things and chemical synthesis such as GKH17, HKH17, K4X7W (where *X* designates Gly, Ala, Val, or Leu), K5L7, KNK10, GRR10-OH, RRP9-OH and Luecrocin I had poor antibacterial activities which may be due to their low proteolytic stabilities.[1–6] Palmitoic acid (PA), Undecanoic acid (UA) and hydrophobic oligopeptide (tryptophan and phenylalanine) tagging have been reported for improving antibacterial activities.[1–6] In order to expand the new systematic approach of antibacterial peptide design by amino acid stretches, the helical wheel pattern was introduced to depict the hydrophobic and hydrophilic areas of a short peptide template (Luecrocin I), which is a naturally occurring antibacterial peptide, extracted from white blood cells of *Crocodylus siamensis*, and has a NGVQPKY amino acid sequence.[7] Its amino acid sequence was divided into two areas (hydrophobic and hydrophilic areas) which were almost equal sizes. Tryptophan (W), which is a hydrophobic amino acid, was used to increase the hydrophobic area, whereas arginine (R) and lysine (K) were selected to extend the hydrophilic area and to increase charge. So, at the positions 8–17 of these peptides are the same (ABBABBAABB: A designates hydrophilic amino acids (R and K) and B designates hydrophobic amino acid (W)). Generally, antibacterial peptides have net positive charges at around 2–9,[8] we therefore engineered the peptides to have the charges around the mean of previous reports at the range of 6–7 (include the positive charge at N terminal). Polar angles of these peptides were the equal values (160°). The other parameters were calculated in order to study their relations in antibacterial and hemolytic activities (Table 1).

Designed peptides were synthesized by using standard Fmoc solid phase of GL biochem (Shianghai, PR China). Each peptide was purified to ⩾95% purity by using (RP)-HPLC (stationary phase: C-18, mobile phase: varying from 5% to 20% acetonitrile in water, 0–20 min). The molecular weight of each peptide was confirmed via ESI-MS (Table 1).

Minimum Inhibitory Concentrations (MICs) and bactericidal activities were determined as a previously described method.[9] Briefly, 6 bacterial strains (Department of Medical Science, Thailand) were inoculated to enter their log growth-phases. The microorganism concentration was adjusted to an optical density of 0.001 at 600 nm. 100 μL of each microorganism in nutrient broth solution (Himedia, India) was added to 96 well plates. 10 μL of various peptide concentrations in 20 mM Tris–HCl buffer (pH 7.4) were added. 20 mM Tris–HCl buffer (pH 7.4) was used as control. % inhibition = $100 - (O.D._{600\ \text{at}\ 24h} - O.D._{600\ \text{at immediate treatment}} \times 100 / O.D._{600\ \text{at 24h of control}} - O.D._{600\ \text{at immediate treatment of control}})$. MIC was taken as the concentration where the growth inhibition was

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Table 1
Amino acid sequences, measured molecular weights by ESI-TOF MS, theoretical molecular weights, positive charges and hydrophobicities of the designed peptides

Peptide	Sequence	Theoretical Mw (Da)	Measured Mw (Da)	Positive Charge	Hydrophobic moment (M_H)[a]	Hydrophobicity (H)[b]	Hydrophobic amino acid content (%)	Polar angle, θ (°)[c]
Luecrocin I	NGVQPKY	804.90	804.91	+2	1.47	−2.71	43	140
KT2	NGVQPKYKWWKWWKKWW-NH_2	2433.88	2433.92	+7	3.41d	−0.02[d]	53	160
RT2	NGVQPKYRWWRWWRRWW-NH_2	2545.93	2545.97	+7	3.42[d]	−0.04[d]	53	160
KT3	NGVQPKYKWWKWWKKWWW-NH_2	2620.09	2620.13	+7	2.81[d]	0.51[d]	56	160
CRT2	NGVQPKYRWWRWWRRWW	2546.93	2546.95	+6	3.42	−0.04	53	160
CRT3	NGVQPKYRWWRWWRRWWW	2732.14	2732.18	+6	2.82	0.49	56	160
KWKT2	NGVQPWYKWWKWWKKWW-NH_2	2491.92	2491.96	+6	4.49[d]	1.12[d]	59	160
RT4	NGVQPKYRWWRWWRRWWWW-NH_2	2918.36	2918.40	+7	2.43[d]	0.97[d]	58	160
RW2	RWWRWWRRWW-NH_2	1759.04	1759.2	+6	6.21[d]	1.81[d]	60	140
RW3	RWWRWWRRWWW-NH_2	1945.2	1945.29	+6	4.88[d]	2.53[d]	64	140

[a,b] Mean hydrophobic moment (M_H) and hydrophobicity of all peptides listed were calculated in the CSS.[19]
[c] Polar angle or θ (°) indicates the amphipathicity obtained from the angle measurement of the area of hydrophilic amino acid
[d] The estimated hydrophobicity and amphathicity values because of the amidated peptides.

Table 2
MICs defined as the concentration (μg/ml) that inhibited bacterial growth at least 90% and 100% bactericidal activities of the peptides against bacterial strains

Peptide	Sequence	*S. epidermidis* ATCC 12228	*S. typhi* DMST 22842	*E. coli* ATCC 25922	*S. aureus* ATCC 25923	*P. aeruginosa* ATCC 27853	*V.cholerae* non-O1, non-O139
Luecrocin I	NGVQPKY	>200	>200	>200	>200	>200	>200
KT2	NGVQPKYKWWKWWKKWW-NH_2	23[a]	23	11[a]	46	91	11
RT2	NGVQPKYRWWRWWRRWW-NH_2	23	23	46[a]	91	×	46
KT3	NGVQPKYKWWKWWKKWWW-NH_2	23[a]	23[a]	11[a]	>91	×	×
CRT2	NGVQPKYRWWRWWRRWW	23	>91	46[a]	>91	×	×
CRT3	NGVQPKYRWWRWWRRWWW	91[a]	>91	>91	>91	×	×
KWKT2	NGVQPWYKWWKWWKKWW-NH_2	91[a]	>91	>91	>91	×	91
RT4	NGVQPKYRWWRWWRRWWWW-NH_2	91	>91	>91	>91	>91	×
RW2	RWWRWWRRWW-NH_2	>91	>91	>91	>91	46	23
RW3	RWWRWWRRWWW-NH_2	>91	>91	×	>91	×	>91

[a] The peptide concentrations that could kill bacteria 100%. × represents there is no significant inhibition. > means there is a bacterial inhibition lower than 90%.

observed at greater than 90%. After incubation at 37 °C for 24 h, 5 μL was removed from each well and plated on agar (Himedia, India) and incubated at 37 °C for 48 h. 100% bactericidal activity was obtained as the concentration at which a 99.9% reduction in CFU of the starting inoculums was observed.

The MICs of all designed peptides and the 100% bactericidal activities are shown in Table 2. Peptides which had good antibacterial activity against 3 bacterial strains (*S. epidermidis* ATCC 12228, *S. typhi* DMST 22842 and *E. coli* ATCC 25922) are KT3, KT2, RT2 and CRT2. For *S. epidermidis* ATCC 12228, the MICs of KT3, KT2, RT2 and CRT2 were 23 μg/mL. These MICs of KT3, KT2, RT2 and CRT2 against this strain were able to kill in practice, but only KT2 and KT3 had 100% bactericidal values at their MICs. Similarly, KT2, RT2 and KT3 had the equal MICs against *S. typhi* DMST 22842. However, only KT3 had 100% bactericidal value at its MIC. In *E. coli* ATCC 25922, KT3 and KT2 had the best MICs, which also were 100% bactericidal values at 11 μg/ml, followed by CRT2 and RT2, which were bactericidal values at 46 μg/ml. Other bacterial strains were quit more resistant these designed peptides than 3 strains above. For *S. aureus* ATCC 25923, KT2 had the lowest MIC (46 μg/mL) and other peptides had poor antibacterial activities. Similarly, KT2 had the lowest MIC value, which was 11 μM, against *V. cholerae* non-O1, non-O139, followed by RW2 and RT2 (23 and 46 μg/mL respectively). *P. aeruginosa* ATCC 27853 could resist almost all these designed peptides. It is probably because of extra cellular protease, composition of bacterial membrane and mechanism of drug resistance.

The antibacterial activity results indicated that the increasing in amphipathicity, hydrophobic amino acid amount and hydrophobicity (Table 1) could not improve antibacterial activity, which is in agreement with a previous report.[10] However, the increasing in positive charge, polar angle, hydrophilic facet could support this activity.

The arginine replacement with lysine (KT2) resulted in slightly increasing antibacterial activity against some bacterial strains, which may cause even though the properties such as charge (7), polar angle (160°), facets (Fig. 1) and hydrophobic amino acid content (53%) of RT2 and KT2 are equal, the hydrophobicity and PI value of arginine and lysine are different (−0.04 and −0.02 hydrophobicity respectively; 10.76 and 9.74 PI respectively). CRT2 (the peptide has an amino acid sequence the same as RT2, but it is not amidated at C terminal.) was found that it had lower antibacterial activity than that of RT2 which has amide at both side chains. This corresponded to previous reports that amidation at the C terminal can increase the antibacterial activity of aurein 2.2, 2.3 and other antibacterial peptides.[11,12] The effect of adding one tryptophan of CRT2 (called CRT3) results in antibacterial activity decrease. Also, it showed much lower antibacterial activity than RT2, which is shorter. This indicates that amidation at the C terminal of CRT2 is more effective in improving the antibacterial activity of RT2 than tryptophan addition (CRT3). This may be due to the more positive charge by NH_2 and the more appropriate hydrophilic facet of RT2 as can be seen in Figure. 1. In addition, the hydrophobic facet of RT2 tend to be more interrupted by lysine at position 6 and amide at position 17 resulting in the size of hydrophobic facet smaller. KWKT2 is the peptide which was designed to replace lysine at position 6 with tryptophan for studying its effect. Its helical wheel pattern is visualized in Figure 1 that it has a bigger hydrophobic area when compared with KT2. The lower antibacterial activity of KWKT2 than that of KT2 indicated that lysine at position 6 of KT2 was important in the antibacterial mechanism. The size of the hydrophobic facet and the amount of hydrophobic amino acids

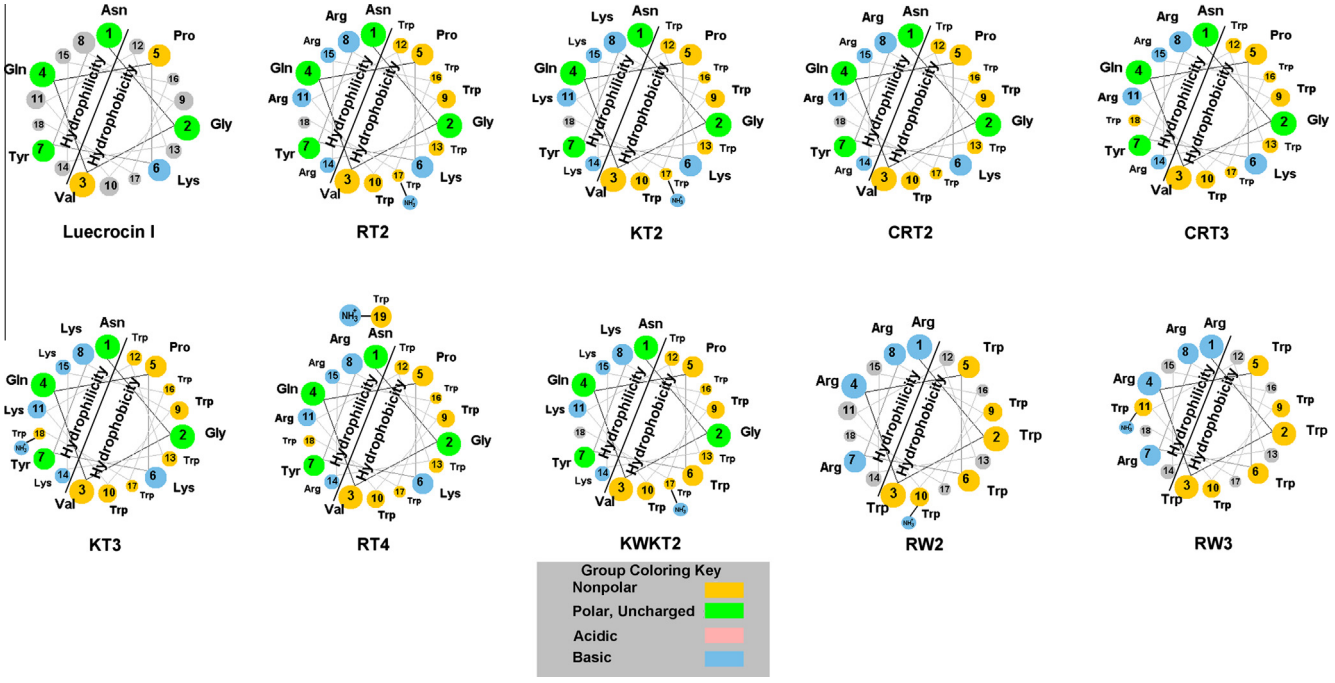

Figure 1. Helical wheel patterns[20] of the designed peptides. The template (Luecrocin I) was divided into hydrophobic and hydrophilic regions in the almost equal sizes.

(59%) of KWKT2 were less appropriate, since they are greater than that of KT2 (53% hydrophobic residue content). Also, its charge is lesser. The attachment of one tryptophan at the C terminal of KT2 (called KT3) results in increase in hydrophobicity (0.51), hydrophobic amino acid content (56%), but the MICs against 3 bacterial strains were the same in Table 2. The reason for this is probably because its hydrophobic facet slightly bigger than that of KT2 (Fig. 1), but its hydrophilic facet isalmost the same because its amide at position 18 stabilizes the hydrophilic facet, even though it has tryptophan at position 18 to perturb the hydrophilic facet as well as that of CRT3. The effect of two tryptophan stretches of RT2 (called RT4) on antibacterial activity was measured, and found that it was clearly lower antibacterial potency, even though it has a longer amino acid sequence (18 residues). This contradicted previous reports that four tryptophan residue tagging of ultra short peptides can increase the antibacterial potency and selectivity[1–3,6] indicating that this cannot be applied to increase antibacterial activity of all peptides and considering of the properties of peptide templates is important as well. For this case, maybe the tryptophan at position 18 of RT4 interrupts its hydrophilic region without any amide to preserve like that of KT3, resulting in the smaller hydrophilic facet, high hydrophobicity (0.97) and hydrophobic amino acid content (58%). The amino acid sequence of RW2 is just the part that extends from the template (Luecrocin I), whereas RT2 retains the template. The antibacterial activities of RW2 and Luecrocin I were much lower than those of RT2 (except in *P. aeruginosa* ATCC 27853 and *V. cholerae* non-O1, non-O139 for RW2), which indicated that the extension of Luecrocin I with the amino acid sequence of RW2, which modifies the sizes of hydrophobic and hydrophilic facets can increase antibacterial activity. The reason why RW2 and RW3 had poor antibacterial activity may be the smaller polar angle (140°) which corresponded to a previous report that smaller polar angle results in poorer antibacterial activity against gram negative bacteria.[13] Moreover, the high

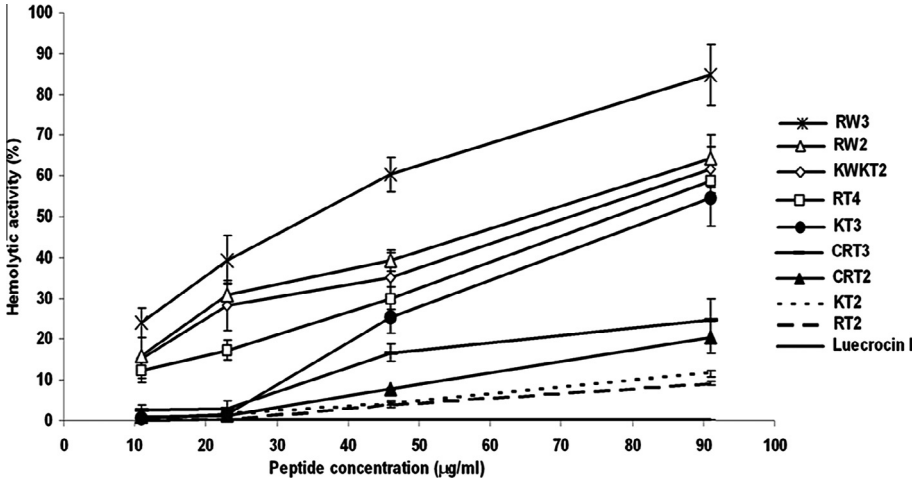

Figure 2. Comparison of hemolytic activity of all designed antibacterial peptides at the same concentration in the range of 1–91 μg/ml.

Table 3
Therapeutic indexes of each peptide against the bacterial strains tested

Peptide	Sequence	Therapeutic index[a]					
		S. epidermidis ATCC 12228	*S. typhi* DMST22842	*E. coli* ATCC 25922	*S. aureus* ATCC 25923	*P. aeruginosa* ATCC 27853	*V. cholerae* non-O1, non-O139
KT2	NGVQPKYKWWKWWKKWW-NH_2	2	2	4	1	0.5	4
RT2	NGVQPKYRWWRWWRRWW-NH_2	2	2	1	0.5	n.a.[b]	1
CRT2	NGVQPKYRWWRWWRRWW	1	<0.25	0.5	<0.25	n.a.	n.a.
CRT3	NGVQPKYRWWRWWRRWWW	0.25	<0.25	<0.25	<0.25	n.a.	n.a.
KT3	NGVQPKYKWWKWWKKWWW-NH_2	1	1	2	<0.5	n.a.	n.a.
RT4	NGVQPKYRWWRWWRRWWWW-NH_2	0.06	<0.06	<0.06	<0.06	<0.06	n.a.
KWKT2	NGVQPWYKWWKWWKKWW-NH_2	0.06	<0.06	<0.06	<0.06	n.a.	0.06
RW2	RWWRWWRRWW-NH_2	<0.02	<0.02	<0.02	<0.02	0.04	0.08
RW3	RWWRWWRRWWW-NH_2	<0.02	<0.02	n.a.	<0.02	n.a.	<0.02

[a] Greater values in therapeutic index (MHC/MIC) indicate better antimicrobial specificity.
[b] Not available due to very poor antibacterial activity.

hydrophobicity (1.81 and 2.53) and hydrophobic amino acid content (60% and 64%) may be also the reasons for this.

Each designed peptide was tested their toxicity against human red blood cells (hRBCs) as previously described.[14] The minimal hemolytic concentration (MHC) was measured as the lowest peptide concentration that produces 5% hemolysis.[14] The therapeutic index is defined as the ratio of MHC and MIC (MHC/MIC).[15]

The hemolytic activities of all designed peptides are shown in Figure 2. The peptide that had the lowest hemolytic activity is RT2, followed by KT2. However, their hemolytic activities were almost equal. CRT2 was clearly more toxicity than both RT2 and KT2 at the same concentration, followed by CRT3, KT3, RT4, RWKT2, RW2 and RW3 respectively. For therapeutic index data (Table 3), KT2 showed the highest value, which were 4 for *E. coli* ATCC 25922 and *V. cholerae* non-O1, non-O139, followed by those for *S. epidermidis* ATCC 12228 and *S. typhi* DMST 22842 (therapeutic index: 2), then for *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853 (therapeutic index: 1 and 0.5 respectively). In RT2, its therapeutic indexes against *S. epidermidis* ATCC 12228 and *S. typhi* DMST 22842 were highest (therapeutic index: 2), followed by those against *E. coli* ATCC 25922 and *V. cholerae* non-O1, non-O139 (therapeutic index: 1). The therapeutic index of KT3 against *E. coli* ATCC 25922 exhibited the highest at 2, followed by those against *S. epidermidis* ATCC 12228 and *S. typhi* DMST 22842 at 1. CRT2 had the hightest therapeutic index which was 1 against *S. epidermidis* ATCC 12228, and had therapeutic indexes less than 1 against other strains. For other peptides, their therapeutic indexes were very low, which were less than 0.5.

RT2 showed % hemolytic activity slightly lower than KT2 at the same concentration (Fig. 2). It is probably that arginine has a PI value (10.76) which is higher than that of lysine (9.74), resulting in arginine having more positive charge in 20 mM Tris–HCl, pH 7.4 than that of lysine. Also, the hydrophobicity of RT2 (−0.04) is lower than that of KT2 (−0.02). Therefore, RT2 had slightly lower hemolytic activity than that of KT2. But, KT2 had better therapeutic indexes due to its lower MICs against some bacterial strains. Comparing between RT2 and CRT2, amidation (NH_2) at the C terminal of RT2 had lower toxicity against mammalian cells than that of the native peptide (CRT2) because amide increases the positive charge, which also decreases toxicity against hRBCs. Thus, the increase in positive charge by replacing arginine with lysine and amidation at C terminal can support selectivity against these bacterial strains. However, KT3, RT4, RWKT2, RW2 and RW3 increased hemolytic activity respectively (Fig. 2) as the increasing in hydrophobicity (Table 1) resulting in lower therapeutic index shown in Table 3. RW2 and RW3 were the most toxicity toward hRBCs, which may be due to the large hydrophobicity (1.81 and 2.53 respectively), hydrophobic amino acid content (60 and 64 respectively), low positive charge (5 and 6 respectively) and smaller polar angle (140°), which corresponded to a previous report that smaller polar angle results in higher hemolytic activity.[10] In addition, the hydrophobic area of RW3 is clearly bigger as can be seen in Figure 1. Therefore, the increasing in hydrophobicity and hydrophobic amino acid content (Table 1) were the reasons for this, which corresponded with previous reports.[8,10,13] Moreover, the lower charge, smaller polar angle and bigger hydrophobic facet were also the parameters that can affect the hemolytic activity. However, the amphipathicity (Table 1) seemed not to correlate with this activity in this study, which contradicted the previous study.[10] Perhaps, other parameters above also affect this.

Designed peptides (KT2, RT2, KT3 and CRT2), which had good selectivity, were tested for MTT assay.[16] Vero cells are a kidney epithelial cell line extracted from an African green monkey, representing normal mammalian cells. RAW 264.7 cells are a mouse leukaemic monocyte macrophage cell line, representing mammalian cells in the immune system. The lower toxicity of RT2 in the MTT assay when compared with KT2 (Fig. 3) indicated that argi-

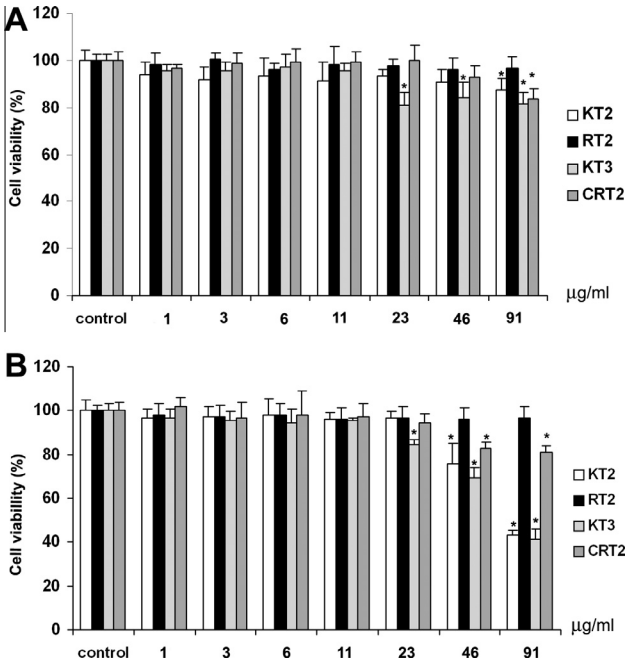

Figure 3. Effects of four designed peptides (KT2, RT2, KT3 and CRT2) on Vero and RAW 264.7 cell viability (Fig. A and B respectively). Bars represent mean and SEM ($n = 8$). * indicates $p < 0.05$ significant difference Data are expressed as mean ± - S.E.M. Statistical evaluation was considered by one-way analysis of variance (ANOVA followed by Scheffe's multiple range test).

nine replacing lysine showed lower toxicity, which corresponded to the results in the hemolytic activity assay. Comparing between RT2 and CRT2, CRT2 had slightly more toxicity than that of RT2 indicating that amidation at the C terminal can increase selectivity as well. Moreover, an increased fraction of tryptophan, resulting in higher hydrophobicity, supports the effectiveness of peptides against mammalian cells, as can be seen in KT3 and other peptides as reported previously.[2]

The bacterial strains that were the most susceptible against designed antibacterial peptides (KT2, RT2, KT3 and CRT2) were selected for visualizing the bacterial morphology when treated with them by using scanning electron microscope.[7] The abnormal morphologies of bacterial cells were visualized since 1 min (Figs. 4 and 5), which indicated that they could kill or inhibit bacteria rapidly, and their antibacterial mechanisms may depend on concentration rather than time. In RT2 and CRT2 against *S. epidermidis* ATCC 12228 and *S. typhi* DMST 22842 respectively, it was not clear that the bacterial surfaces had no blebs. But, the rough and wavy morphologies indicated that RT2 and CRT2 may have the different antibacterial mechanism against these bacterial strains, compared with those of KT3 and KT2 against *E. coli* ATCC 25922, which many blebs were visualized. However, only these results could not reply what their main antibacterial mechanisms are. The further study is needed to consider and confirm their main antibacterial mechanisms.

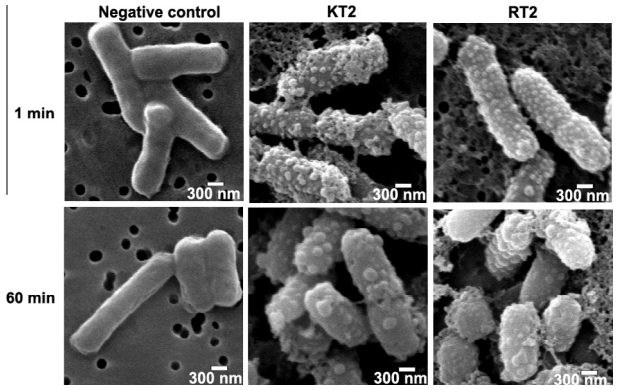

Figure 4. SEM images of *E. coli* ATCC 25922 after incubation with KT2 and KT3 for 1 min and 60 min (10XMIC, bacterial suspension O.D.600 = 0.1). The abnormal shapes such as blebs of bacteria can be seen as soon as 1 min of incubation in comparison with those of negative controls (20 mM Tris–HCl buffer).

The secondary structures of RT2 and KT2 were determined in various environments (1 mM liposome, 50 mM SDS, 20 mM Tris–HCl and 50% TFE) by dissolving in each environment above to a final concentration of 0.1 mg/mL. A Jasco J-715 spectropolarimeter was used to measure the average spectra over five scans in the range 190–260 nm, using a 1 mm optical path length quartz cell at room temperature (25 °C). The parameters were set to 20 nm/min scanning speed at an interval of 0.1 nm, 1 s response time and 1.0 nm bandwidth. The spectra obtained were subtracted from the spectra of the environments before calculating the mean residue ellipticity as reported previously.[17] Percentage of the α-helix contents of the peptides were calculated as a previous study.[18]

Comparing the α-helical contents (Table 4) of RT2 and KT2, KT2 had more the α-helical content than that of RT2 in all environments tested. This indicated that the higher hydrophobicity of KT2 (−0.02) can maintain the α-helical structure better than that of RT2 (−0.04), which corresponded with a previous report that hydrophobicity is important in maintaining the α-helical structure.[10] Their secondary structures tend to be more ordered in the α-helical contents as can be seen in Figure 6 and Table 4 when they interacted with bacterial monolayer mimics (50 mM SDS) and bacterial bilayer mimics (1 mM liposome). However, they did not have their maximum α-helical content (in 50% TFE) when interacted with bacterial membrane mimics.

In conclusion, individual parameters of peptide templates such as positive charge, hydrophobicity, polar angle, content of hydrophobic amino acids and facets should be also considered in amino acid tagging. In addition, hydrophobic and hydrophilic regions in the helical wheel pattern are helpful in choosing amino acids to create proper facets of short antibacterial peptides which also have

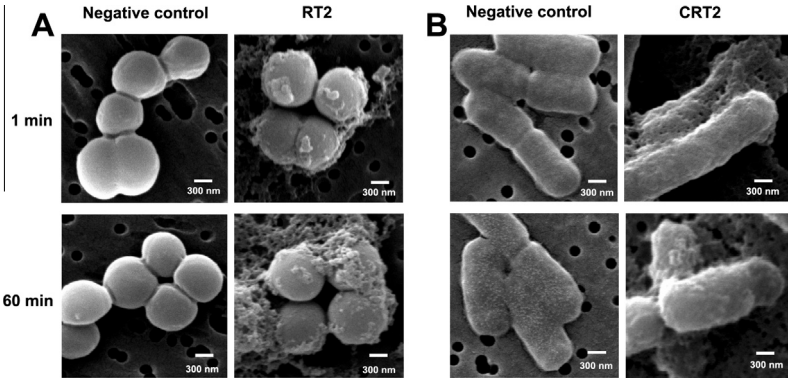

Figure 5. SEM images of *S. epidermidis* ATCC 12228 and *S. typhi* DMST 22842 (A and B respectively) after incubation with RT2 and CRT2 (10XMIC, bacterial suspension $O.D._{600}$ = 0.1) for 1 min and 60 min. The abnormal morphologies of bacteria can be seen as soon as 1 min of incubation in comparison with those of negative control (20 mM Tris–HCl buffer) at 1 min.

Table 4
Percent α-helical contents of the antibacterial peptides in each environment

Environments	Buffer[a]		50% TFE		25 mM SDS		1 mM Liposome	
	$[\theta]_{222}$	% α-Helix	$[\theta]_{222}$	% α-Helix	$[\theta]_{222}$	% α-Helix	$[\theta]_{222}$	% α-Helix
KT2	−3398.6	12.6	−17,772	65.9	−6652.3	24.7	−11,044.7	41
RT2	−1454	4.1	−13,957.9	39.6	−2854.3	8.1	−12,984.9	36.8

[a] 20 mM Tris–HCl buffer (pH 7.4).

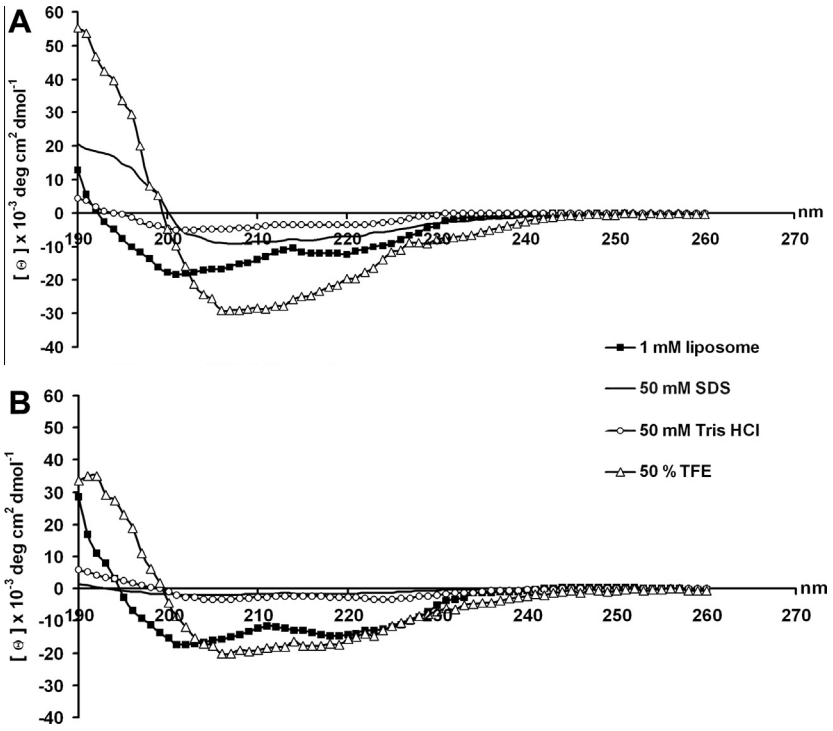

Figure 6. Secondary structures of KT2 and RT2 (A and B respectively) in various environments (1 mM liposome, 50 mM SDS, 20 mM Tris–HCl and 50% TFE). 20 mM Tris–HCl represents the environment that supports native conformation which does not interact with bacterial cells of peptides. 50 mM SDS and 1 mM liposome represent the environments of lipid monolayer and lipid bilayer of bacterial membrane respectively. 50% TFE is the environment that supports maximum α-helical contents of peptides.

selectivity. Overlarge tryptophan stretches can decrease antibacterial activity and increase toxicity because of hydrophobicity. However, not only amidation at C terminal but also lysine substitute for arginine can increase selectivity against these bacterial strains. The expected mechanisms of RT2 and KT2 may depend on concentration rather than time. Finally, RT2 and KT2 are presented in this study as new alternative antibacterial agents, and they may be further applied as alternative antibiotics against infectious diseases.

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