Activity Improvement of Antimicrobial Peptides by a Chemical Modification Approach: Toward the Creation of Novel Types of Antimicrobial Agents

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Abstract: There is currently a threat posed by the rapid emergence of antibiotic-resistant pathogens due to the abuse of conventional antibiotics. To overcome such a resistance problem, it is necessary to develop peptide agents with potent antimicrobial activities and high selectivity for target bacterial strains. Conventional approaches, such as the deletion, addition, and replacement of amino acid residues in the template peptide have been employed to alter the properties of antimicrobial peptides (AMPs). In this review, we summarize a recently developed approach, chemical modification, for the improvement of various types of naturally-occurring AMPs. By applying this design strategy to these peptide sequences, new AMPs with enhanced antimicrobial activities and improved selectivity for microorganisms have been successfully generated. This potential strategy should facilitate the creation of novel class of peptide antibiotics with properties suitable for pharmaceutical application.

Keywords: Antimicrobial activity, activity spectrum, cell membrane, amino acid, peptide, disulfide bond.

INTRODUCTION

As a result of the extensive use and overuse of conventional antibiotics, the rapid emergence of multi-drug resistant bacterial strains has become a threat to human health in many parts of the globe. Therefore, the development of novel potent and safer antibiotics able to effectively combat such strains is urgently needed. Recently, antimicrobial peptides (AMPs) have attracted considerable attention as next-generation therapeutics [1-6]. These peptides are widely distributed in nature, ranging from insects to mammals, including humans. They play an important role in the innate immune system, forming a first line of defense against invading pathogens.

To date, more than 880 AMPs have been isolated from a variety of organisms. AMPs have certain common features of small size (generally 12-50 amino acids), and cationic nature that facilitate interaction with the negatively-charged bacterial surface, and an overall amphipathic structure with both polar and hydrophobic regions. Although their sequences and structures are highly diverse, they can be classified into four structural classes: (i) linear α-helix peptides (magainin and cecropin) [7-9], (ii) β-sheet peptides with multiple intramolecular disulfide bonds (defensin and protegrin) [10-12], (iii) extended peptides with predominant amino acids (tryptophan-rich indolicidin and proline-rich apidaecin) [13,14], (iv) loop peptides with just one disulfide bond (bactnecin and thanatin) [15,16]. The primary target of the majority of AMPs is the bacterial cellular membrane. They can usually form transient pores on the membrane, or disrupt the local membrane structure, although the mechanism is not well elucidated [2,5,6]. Such activities eventually lead to bacterial cell lysis and are thought to be responsible for their bactericidal effect. Thus, a structural analysis of the peptide-membrane complex is crucial for the understanding of the structure-activity relationship of AMPs. Such studies are expected to contribute to the development of novel AMP as well as a deeper understanding of their mechanisms. Based on the information provided by the structural analysis, the attempt to alter the antimicrobial activity and target bacterium specificity of natural AMP has been made in an effort toward the generation of peptide agents with new biological properties.

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Numerous studies using synthetic peptide analogues with specific amino acids replaced by others have been performed to investigate the functional role of the residues within the AMP sequence [17-21]. Additionally, peptide analogues with an oligopeptide inserted into the AMP backbone or deleted from the backbone were also designed to investigate the effect of sequence alteration on antimicrobial activity [22-25]. Fortunately, significant activity enhancement has been achieved for several AMPs by these peptide mutation approaches. Aside from this established approach for activity improvement, particular attention is now being given to the generation of new AMPs by chemical modification of the original peptide. Furthermore, an *in vivo* monitoring assay system has been utilized to increase the activity of proline-rich AMPs [26-29].

In the present report, we provide an overview of the development of new AMPs by a promising approach, chemical modification, as a strategy for the improvement of the antimicrobial properties. The emphasis will be on the alteration achieved by incorporating chemically modified unnatural amino acids into the AMP backbone. It should be noted that we will not mention very small AMPs prepared by combinatorial chemistry in this review.

LIPID ATTACHMENT AND INCORPORATION OF AMINO ACIDS

There are two different types of design approaches for the improvement of AMP activity by chemical modification (Fig. **1**). One is the conjugation of a functional moiety to the peptide backbone and the other is an incorporation of artificial amino acids with a featured chemical group into the peptide sequence. These approaches have been shown to be effective for altering the physicochemical properties of native AMPs, by which modified AMPs can sometimes exhibit higher antimicrobial activities and expanded activity spectra against microorganisms, including Gram-positive and Gram-negative bacteria, yeast, filamentous fungi.

Lipid attachment-Acylation of AMPs with fatty acids has been explored as a way to increase the overall hydrophobicity of the peptides and their binding affinity for the bacterial cell membrane. As a consequence of modification of lipid chains, significant enhancement of antimicrobial activities has been reported for certain cationic peptides [30-33]. For instance, naturally derived helix-forming peptide SC4 was conjugated with dodecyl and

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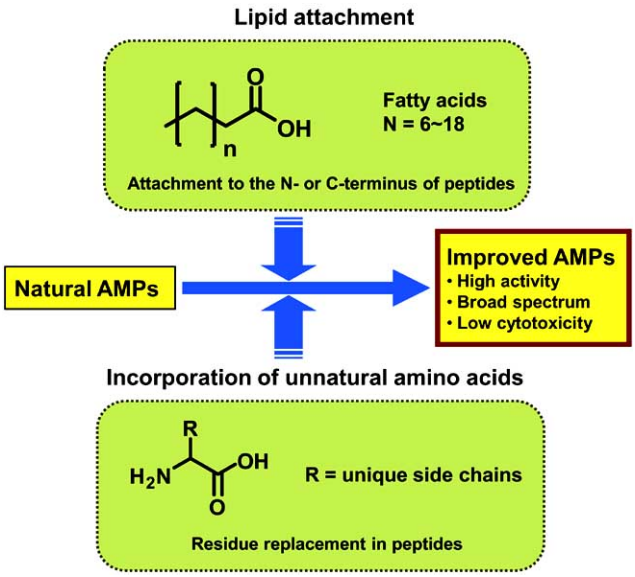

Fig. (1). Schematic drawing of the chemical modification approach for the improvement of AMPs.

octadecyl fatty acids (C12-SC4 and C18-SC4, respectively, Table **1**) to increase the membrane affinity and stabilize the secondary structure in the membrane environment [34,35]. Bactericidal activities of peptide conjugates were investigated against Gram-positive (*Staphlococcus aureus*, *Streptococcus pyogenes*, and *Bacillus anthracis*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria, and two drug-resistant strains of *S. aureus*. Both conjugates exhibited significant activity increase against Gram-positive bacteria, but little or no activity increase against Gram-negative bacteria. The conjugate C12-SC4 was particularly active against Gram-positive *S. pyogenes*, displaying a dramatic increase of antimicrobial activity (30-fold compared with SC4). It is noteworthy that drug-resistant strains, which are susceptible only to the conventional antibiotic vancomycin, were effectively killed at sub-micromolar concentrations of C18-SC4. The hemolytic activities of both conjugates against red blood cells (RBC) were also tested to determine the cytotoxicity against mammalian cells. Although both conjugates exhibited some degree of lytic activity against human erythrocytes, hemolysis occurred at concentrations 10- to 100-fold higher than those required for bacterial cell lysis. Biophysical studies demonstrated that fatty acid conjugation enhances membrane affinity and helix formation in membrane-bound SC4-peptide conjugates. Thus, fatty acid conjugation was shown to be effective for the potentiation of cationic AMP.

Successful enhancement of the antimicrobial activity was also observed for the *N*-acylated peptide fragment which was obtained from proteolytic digestion of bovine iron-transporting protein lactoferrin (LFcinB, Table **1**). A peptide based on the cationic core sequence of LFcinB was *N*-terminally modified by the covalent attachment of alkyl chains of six to eighteen carbons, and their antimicrobial activities were tested for five microorganisms (*E. coli*, *P. aeruginosa*, *S. aureus*, *Candida. albicans*, and *Trichophyton mentagrophytes*) [36]. Among the peptide conjugates, the one with the 11-carbon-chain acyl group was found to be most active for all of the bacteria and fungi tested, displaying a two to eight times lower minimum inhibitory concentaration (MIC) than LFcinB. The influence of lipophilic modification of the lactoferrin peptide on the antimicrobial activity was also investigated using a human lactoferrin peptide fragment (LF12, Table **1**) [37]. This fragment contains a region that forms an amphipathic α-helix of human lactoferrin and has been shown to be more potent against microorganisms than the intact protein. LF12 analogues modified with a series of acyl chains (C_6~C_{18}) at the C-terminus were prepared and their activities were evaluated against Gram-positive *S. aureus* and Gram-negative *E. coli*. The result of the assay indicated that the optimal acyl chain length was 12 carbon units, with the highest enhancement of antibacterial activity being 50-fold against *E. coli*, and 75-fold against *S. aureus* when compared with the parent peptide. The chain length of the C_{12} peptide was suggested to be related to the capacity to bind bacterial surface lipopolysaccaride (LPS).

Carbon chain length-dependent antimicrobial activities were also studied for the antibacterial domain of human lysosomal protease cathepsin G (CG 117-136, Table **1**). A series of acylated CG117-136 analogues having C-2~C-18 hydrocarbon chains at the

Table 1. Peptide-Lipid Conjugates Used in the Chemical Modification Studies

SC4	KLFKRHLKWKII-$CONH_2$
C12-SC4	$CH_3(CH_2)_{11}CO$-KLFKRHLKWKII-$CONH_2$
C18-SC4	$CH_3(CH_2)_{17}CO$-KLFKRHLKWKII-$CONH_2$
LFcin B	FKCRRWQWRMKKLGAPSITCVRRAF
LFcin B core	RRWQWRMKK-$CONH_2$
Acyl-11-core	$CH_3(CH_2)_9CO$-RRWQWRMKK-$CONH_2$
LF12	FQWQRNIRKVR-homoserine lactone
LF12-C12	FQWQRNIRKVRS- $CONH(CH_2)_{11}CH_3$
CG 117-136	RPGTLCTVAGWGRVSMRRGT
C-12-CG 117-136	$CH_3(CH_2)_{11}CO$-RPGTLCTVAGWGRVSMRRGT
K_4S4(1-13)	ALWKTLLKKVLKAA-$CONH_2$
Lauroyl- K_4S4(1-13)	$CH_3(CH_2)_{10}CO$-ALWKTLLKKVLKAA-$CONH_2$
Aminolauroyl-K_4S4(1-13)	$NH_2(CH_2)_{11}CO$-ALWKTLLKKVLKAA-$CONH_2$

The peptide sequences are shown in letter code. The hydrophobic amino acids are shown in blue. The –$CONH_2$ at the C-terminus of each sequence indicates the amidation of a carboxylate group.

N-terminus were synthesized and their bactericidal activities were evaluated using three unrelated strains of *Neisseria gonorrhoeae*, *P. aeruginosa*, and *S. aureus* [38]. The Gram-positive bacteria include methicillin-resistant and vancomycin-intermediate (VISA) isolates of *S. aureus*. It was found that elongation of the acyl chain in such analogues resulted in increased activity, reaching a maximum with the C-12-modified analogue. This analogue was substantially more active against all three Gram-positive bacteria (4- to 64-fold activity increase), including vancomycin-resistant isolates. The analogues having longer acyl moieties, on the other hand, displayed decreased activities. Thus, the favorable acyl chain is assumed to more readily anchor the peptide in a membrane and that this facilitates the peptide to fold into an α-helical structure. Interestingly, the C-12-modified analogue exhibited the highest propensity for the formation of an α-helical structure and the ability to damage the target membrane, which led to improved bactericidal action of the acylated peptide.

The effect of acylation and aminoacylation on antimicrobial activities has been systematically investigated for tree frog-derived dermaseptin S4, a 28-residue polycationic peptide [39-41]. Acyl derivatives, having various chain lengths ranging from acetyl to palmitoyl, were prepared by conjugating the chain moieties to the shortest active fragment K_4S4(1-13) (Table **1**), and subjected to MIC assay using *S. aureus* and *E. coli* as test strains. The bacteria displayed quite different sensitivities to those acyl conjugates. The conjugates with intermediate acyl chains exhibited significantly increased activities against *S. aureus* (up to 6-fold relative to acyl-free peptide), while the ones with the longer acyls gradually resulted in a total loss of activity. In contrast, no increased activities against *E. coli* were observed for any of the acyl conjugates. To further explore the effect of *N*-acylation of K_4S4(1-13) on antimicrobial activity, certain acyl derivatives were converted to the aminoacyl form to compare the biological properties. Interestingly, significantly increased antimicrobial activities were observed for the conjugates with long aminoacyls. For instance, the conjugate with the aminolauryl moiety exhibited 12-fold and 3-fold enhanced activities against *S. aureus* and *E. coli*, respectively. Furthermore, this conjugate was significantly less hemolytic than the corresponding acyl conjugate due to the acylation-enhanced hemolytic activity against human RBC compared with the acyl-free peptide. Structural analysis revealed that the addition of the amino group did not alter the secondary structure of the corresponding acyl conjugate. Thus, it is proposed that less hydrophobic aminoacyl derivatives can avoid peptide aggregation in solution and gain access to the bacterial plasma membrane. Taken together, these data demonstrate that both potency and target selectivity can be modulated by the nature of the acyl moiety.

Incorporation of artificial amino acids-The incorporation of nongenetically coded amino acids into the peptide sequence is also a useful approach to alter the structural and physicochemical properties of AMPs [42, 43]. A wide variety of unusual amino acids having structurally and functionally featured side-chain groups have been made for biochemical studies [44, 45]. By replacement of the key amino acid residue with such amino acids in the template sequence, improvement of the antimicrobial activity has been achieved in several types of AMPs.

A successful case study was reported for the alteration of a membrane active form of bovine lactoferrin peptide (LFB, Table **2**). This peptide has two tryptophan (Trp) residues, which are known to insert into the membrane bilayer and serve as a membrane anchor in Trp-rich AMPs [46-53]. Preliminary structure-activity relationship study revealed that both Trp residues in position 6 and 8 are essential for the exertion of antimicrobial activity [54]. In order to investigate the effect of bulky aromatic side chain on the activity, a series of peptide analogues, in which Trp 6 and Trp 8 were replaced by natural and aromatic amino acids having different structural features, were synthesized (Fig. **2**) [55, 56]. The antimicrobial activities of these analogues were tested using two representative bacterial strains, *S. aureus* and *E. coli*. It was found that all of the analogues having the artificial aromatic group were more active against the test strains than native LFB. This indicates that the Trp residues in position 6 and 8 can be favorably replaced with larger, aromatic amino acids. There is a clear correlation between the antimicrobial activities of the analogues and the size of the residues employed as substitutes for Trp. The larger residues impart higher antimicrobial activity to the LFB analogues. However, the difference in the topology of side-chain also seems to influence the activities. For instance, the two isomeric artificial amino acids Bip (biphenylalanine) and Dip (diphenylalanine) shown in Fig. (**2**) resulted in analogues of different bacterial selectivity when incorporated as replacements for the Trp residues in both positions. The Dip peptide was clearly most active against *E. coli* (a 6.7-fold activity increment), while the Bip peptide most active against *S. aureus* (a 33-fold activity increment). Since these two residues are of essentially identical size, the topology of biphenyl and diphenyl side chains seems to determine the biological activity of the peptide analogues in which they are incorporated. It is interesting to note that the bacterial specificity can be altered by introducing aromatic amino acid with different structural properties. The impact of large aromatic side chains on antimicrobial activity was more evident against *S. aureus* than *E. coli*. It is speculated that the introduction of large aromatic amino acids renders the peptide analogue less selective against bacterial membranes.

The incorporation of artificial aromatic amino acid into the dermaseptin S1 fragment (DS1, Table **2**), derived from the skin secretions of *Phyllomedusinae* frogs, also led to augmentation of the antimicrobial activity [57]. The DS1 peptide is comprised of a characteristic polypeptide chain of 34 amino acids, with four lysine residues and a highly conserved tryptophan residue in the third position from the *N*-terminus. The antimicrobial action of this peptide is reported due to membrane permeabilization and subsequent bacterial lysis [58]. In an attempt to improve the potency of the core peptide DS(1-15)-NH_2, the analogue with a Trp indole ring in the third residue which was replaced with an naphthyl group was designed. This DS1 analogue had the same antimicrobial activity as the template peptide against *S. aureus* and *E. coli*, indicating the tolerance of the third position to artificial aromatic amino acids. Interestingly, the addition of a Lys residue to the *N*-terminus of this analogue generated a more potent DS1 analogue. The combination of the two different peptide modifications had a synergistic effect on the antimicrobial activity of DS1. All of the DS1 analogues in Table **2** showed mild hemolysis against human RBC at a concentration of 100 μM. This cytotoxicity suggests that these analogues selectively act on the bacterial membrane.

Marked improvement of antimicrobial activity was achieved by incorporating fluorinated amino acids into the magainin analogue. Fluorocarbons are extremely hydrophobic, chemically inert, and their sizes are relatively small [59]. These useful properties were exploited to develop novel proteins which incorporate fluorinated hydrophobic amino acids into their hydrophobic cores. These proteins exhibited increased stability toward heat and chemical denaturants [60-63]. Although the effect of fluorination on antimicrobial activity has also been studied using short AMP [64], a fluorinated amino acid of leucine was incorporated into MSI-78 (Table **2**), a synthetic analogue of the well-characterized AMP, magainin 2 [63, 65, 66]. This peptide itself is unstructured in solution, but dimerizes to form an antiparallel α-helical coiled-coil structure in a membrane environment. Replacement of two leucine and isoleucine residues in MSI-78 with the fluorous amino acid hexafluoro leucine (hFLeu) produced the peptide analogue fluorogainin-1. The antimicrobial activity of this analogue was evaluated against eleven bacterial strains, including both Gram-posi-

Table 2. Peptide Analogues having Artificial Amino Acid Residues

LFB	FKCRRWQWRMKKLGA
LFB analogue	FKCRR**X**Q**X**RMKKLGA
	X=Bal, 1-Nal, 2-Nal, Dip, Bip, Ath, Tbt
DS1(1-15)-NH_2	ALWKTMLKKLGTMAL-$CONH_2$
[1-Nal^3]DS1(1-15)-NH_2	AL**X**KTMLKKLGTMAL-$CONH_2$
[2-Nal^3]DS1(1-15)-NH_2	AL**X**KTMLKKLGTMAL-$CONH_2$
[Lys^{0}1-Nal^3]DS1(1-15)-NH_2	KAL**X**KTMLKKLGTMAL-$CONH_2$
	X=1-Nal or 2-Nal
MSI-78	GIGKFLKKAKKFGKAFVKILKK
Fluorogainin-1	G**X**GKF**X**KKAKKFGKAFVK**XX**KK
	X=hFLeu
LeuA	KYYGNGVH**C**TKSG**C**SVNWGEAFSAGVHRLANGGNGFW (S—S)
LeuA 1	KYYGNGVH**X**TKSG**X**SVNWGEAFSAGVHRLANGGNGFW
LeuA 2	KYYGNGVH**X**TKSG**X**SVNWGEAFSAGVHRLANGGNGFW
LeuA 3	KYYGNGVH**F**TKSG**F**SVNWGEAFSAGVHRLANGGNGFW
	X=AllylGly for LeuA 1, **X**=norvaline for LeuA 2

The peptide sequences are shown in letter code. The hydrophobic amino acids are shown in blue. X denotes artificial amino acid residues incorporated into the peptide sequence. The –$CONH_2$ at the C-terminus of each sequence indicates the amidation of the carboxylate group. Artificial amino acid abbreviations: Bal, benzothienylalanine; 1-Nal, (naphth-1-yl)alanine; 2-Nal, (naphth-2-yl)alanine; Dip, diphenylalanine; Bip, biphenylalanine; Ath, anthracenylalanine; Tbt, (tri-*tert*-butyl-indolyl)alanine; hFLeu, hexafluoroleucine.

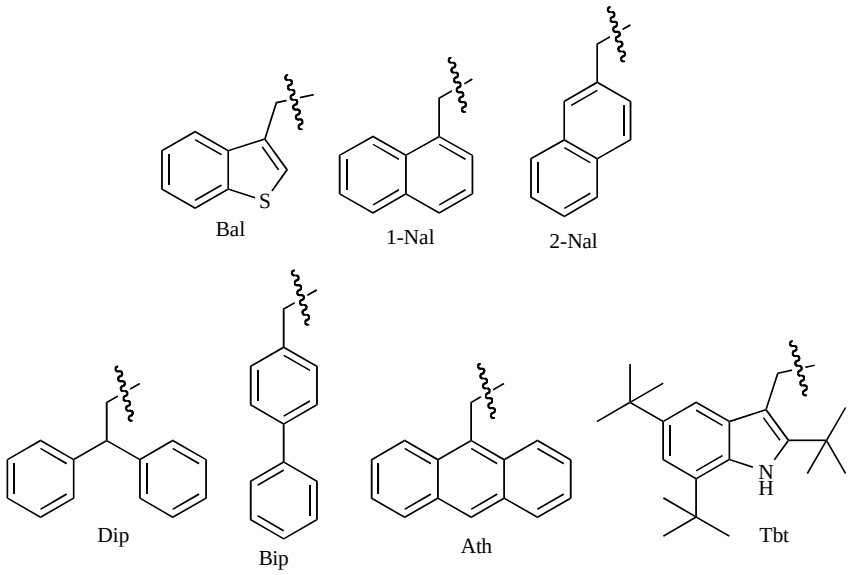

Fig. (2). Chemical structure of side chains and abbreviations for the corresponding aromatic amino acid residues employed in chemical modification of the LFB peptide.

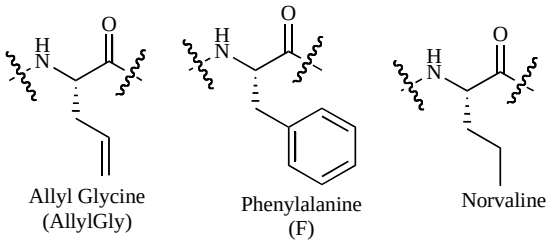

Fig. (3). Chemical structure of the key amino acid residues used in the disulfide bond mimics.

itive and Gram-negative bacteria. It was shown that fluorogainin-1 retained the broad-spectrum antimicrobial activity of MSI-78 despite the incorporation of eight trifluoromethyl groups into the peptide chain. Importantly, fluorogainin-1 was much more potent against the two target pathogenic bacteria, *Klebsiella pneamoniae* (MSI-78 insensitive) and *S. aureus* (4-fold increment in MIC relative to MSI-78). The only bacterium tested for which fluorogainin-1 was significantly less effective than MSI-78 was *S. pyogenes*. The hemolytic activities of both peptides were tested against sheep erythrocytes. No lytic activities were detected up to 250 μg/mL, while many AMPs activity at concentrations well below 100 μg/mL. Furthermore, the resistance of fluorogainin-1 to proteolysis was observed in the presence of liposomes under conditions in which MSI-78 is rapidly degraded. One explanation for this result is that the incorporation of the more hydrophobic hFLeu side chain strengthens the hydrophobic interaction between the dimmers, which would, in turn, promote the formation of structural dimers that are resistant to proteolysis.

There is considerable interest in engineering a disulfide bond in AMP which forms parallel and/or antiparallel β-sheet structure [67, 68]. It is reported that the antimicrobial activities of certain β-sheet peptides are retained by double substitution of cysteine residues with aromatic amino acids [69]. This finding implied that the disulfide bridge in such peptides is dispensable for antimicrobial activity. Hence, there is a possibility of designing a β-sheet peptide analogue with two strands connected by a non-natural linkage. Replacement of the disulfide bridge with a hydrophobic interaction was performed by chemical modification of leucocin A (LeuA, Table **2**), known to be the member of the bacteriocins produced by lactic acid bacteria to destroy competing microorganisms [70, 71]. The C-terminal region of this AMP forms an amphipathic α-helix which is probably involved in the interaction with chiral receptors in the bacterial cell membrane [72-74]. On the other hand, the *N*-terminal region consists of an antiparallel β-sheet which is constrained by one intramolecular disulfide bridge. Previous studies indicated that the substitution for both cysteines (residues 9 and 14) with serine led to a complete loss of antimicrobial activity, indicating the necessity of these residues for the antimicrobial activity. As an exploration of a linkage that can be substituted for the disulfide bond, two cysteine residues in LeuA were replaced with allylglycines to produce the peptide analogue LeuA 1, and the antimicrobial activity of this analogue was tested against the three bacterial strains *Carnobacterium maltaromaticum*, *Carnobacterium divergens* and *Listeria monocytogenes* (Fig. **3**). Interestingly, the analogue LeuA 1 was almost as active as the native peptide LeuA for all of the test strains. It is evident that the hydrophobic interactions of the allyl side chains in LeuA 1 maintained the correct peptide conformation for full antimicrobial activity. In order to obtain insight into the nature of the interaction between the two modified residues in LeuA 1, its saturated counterpart (LeuA 2) and L-phenylalanine analogue (LeuA 3), in which the π-stacking interactions are potentially enhanced, were synthesized. Unexpectedly, all of the peptide analogues showed the same activity as the parent LeuA against the three test strains. This result

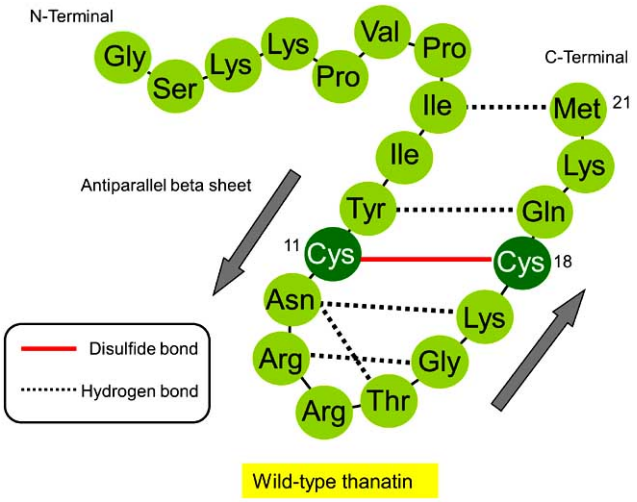

Fig. (4). Schematic illustration of the thanatin structure.

demonstrates that hydrophobic interactions can replace the disulfide bond in LeuA and the π-stacking interaction does not seem to contribute significantly to the stabilization of the required secondary structure. Therefore, hydrophobic interaction facilitates the peptide adopting an active conformation for membrane-receptor binding. It is possible that the hydrophobic interaction provided by the diallyl moieties is mimicked by other lipophilic side chains.

To investigate the utility of chemical modification of loop peptides, we focused on thanatin and performed engineering of the disulfide bond included in this AMP [75]. Thanatin is a 21-residue non-hemolytic AMP isolated from the hemipteran insect *Podisus maculiventris* (Fig. **4**) [16]. This peptide has a broad spectrum of antimicrobial activity against Gram-positive and Gram-negative bacteria as well as fungi. The peptide contains two cysteine residues (residues 11 and 18) linked by a single disulfide bond to form an antiparallel β-sheet structure consisting of 14 amino acid residues [76]. These cysteine residues are necessary for the exertion of antimicrobial activity against gram-negative bacteria, because the substitution for both residues with alanine resulted in a significant reduction of the activity [77,78]. In an effort to explore the possibility of replacing the disulfide bridge with a non-covalent interaction, we designed a thanatin analogue in which two hydrogens of the sulfhydryl groups were modified with bulky *tert*-butyl groups (*t*-Bu, Fig. **5**). To our surprise, the antimicrobial activity of this cysteine-free analogue was increased 4-fold against the gram-positive bacteria *Micrococcus luteus* compared with native thanatin. In contrast, the *t*-Bu thanatin exhibited greatly reduced activity against *E. coli*. Although the activity enhancement as a result of the substitution for cysteine residues with aromatic amino acids has been reported for the mutation of tachyplesin [69], it has not been achieved by the incorporation of artificial amino acids into the β-sheet of AMP. Structural analysis using two-dimensional NMR revealed that the analogue exhibited a β-sheet structure similar to that of native thanatin, implying the stabilization of the former structure by hydrophobic interaction between the two modified residues. In order to investigate the relationship between side-chain hydrophobicity and antimicrobial activity, a series of thanatin analogues modified with methyl, ethyl, *t*-Bu, and octyl groups were designed and their antimicrobial activities were evaluated against two bacterial strains [79]. A good correlation was found between side-chain hydrophobicity and antimicrobial activity against *M. luteus*, whereas all of the analogues were almost inactive against *E. coli*. This result suggests that thanatin has different modes of action depending on the target microorganisms. Hence, the target bacterial specificity is thought to

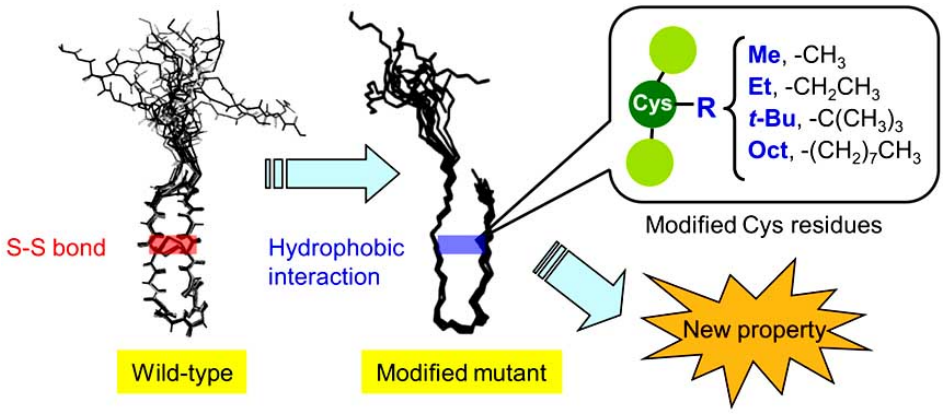

Fig. (5). Chemical modification of the cysteine residues involved in loop formation of thanatin.

be altered by the extent of hydrophobic interaction between the lipophilic side chains in the modified cysteine residues. This new type of β-sheet peptide may have the capacity to maintain the active hairpin conformation under reducing conditions, under which the usual β-sheet peptide would probably be inactive due to the cleavage of intramolecular disulfide bridge.

CONCLUSION

In conclusion, this review demonstrates the utility of chemical modification as an approach to improving the antimicrobial properties of natural AMPs. The conjugation of fatty acid chains to a peptide sequence, or replacement of the appropriate residue in the peptide with non-natural amino acids containing unique side chain, yielded new peptide agents with enhanced antimicrobial activities, improved target selectivity, and low cytotoxicity. However, there are still a limited number of chemically improved AMPs. It is also noted that the biological properties obtained by the chemical modification compete favorably with those by conventional means. This is partly due to the lack of a general design principle for improving the antimicrobial activity. Further studies aimed at exploring useful functional units which can be incorporated into peptides should be conducted to establish just such a guiding design principle.

The structure-activity relationship investigation of AMPs is crucial for the rational design of chemically-modified peptide agents. Additionally, understanding the interaction of the AMP with the target bacterial cell membrane would provide valuable information for molecular design. Based on these studies, it might be possible to design tailor-made peptide agents which have potent antimicrobial activity for particular bacterial strains. We hope that chemical modification will pave the way for the creation of practically useful AMP analogues.

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