

## Dimerization of Antimicrobial Peptides: A Promising Strategy to Enhance Antimicrobial Peptide Activity



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**Abstract:** Antimicrobial resistance is a global health problem with strong social and economic impacts. The development of new antimicrobial agents is considered an urgent challenge. In this regard, Antimicrobial Peptides (AMPs) appear to be novel candidates to overcome this problem. The mechanism of action of AMPs involves intracellular targets and membrane disruption. Although the exact mechanism of action of AMPs remains controversial, most AMPs act through membrane disruption of the target cell. Several strategies have been used to improve AMP activity, such as peptide dimerization. In this review, we focus on AMP dimerization, showing many examples of dimerized peptides and their effects on biological activity. Although more studies are necessary to elucidate the relationship between peptide properties and the dimerization effect on antimicrobial activity, dimerization constitutes a promising strategy to improve the effectiveness of AMPs.

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### 1. INTRODUCTION: ANTIMICROBIAL PEPTIDES (AMPs)

The occurrence of pathogenic microorganisms resistant to antibiotics has been increasing, while few new antibiotics have been discovered and approved for commercialization [1]. This situation has led to a global health problem, with strong social and economic impacts [2]. Therefore, there is an urgent need for new molecules to control diseases caused by these resistant microorganisms. Among these molecules, Antimicrobial Peptides (AMPs) appear to be an interesting alternative since they act through mechanisms in which the pathogens rarely develop resistance [3]. In contrast to conventional antibiotics, which exert their toxic activity by binding to specific targets, most AMPs have been considered membrane-destabilizing molecules.

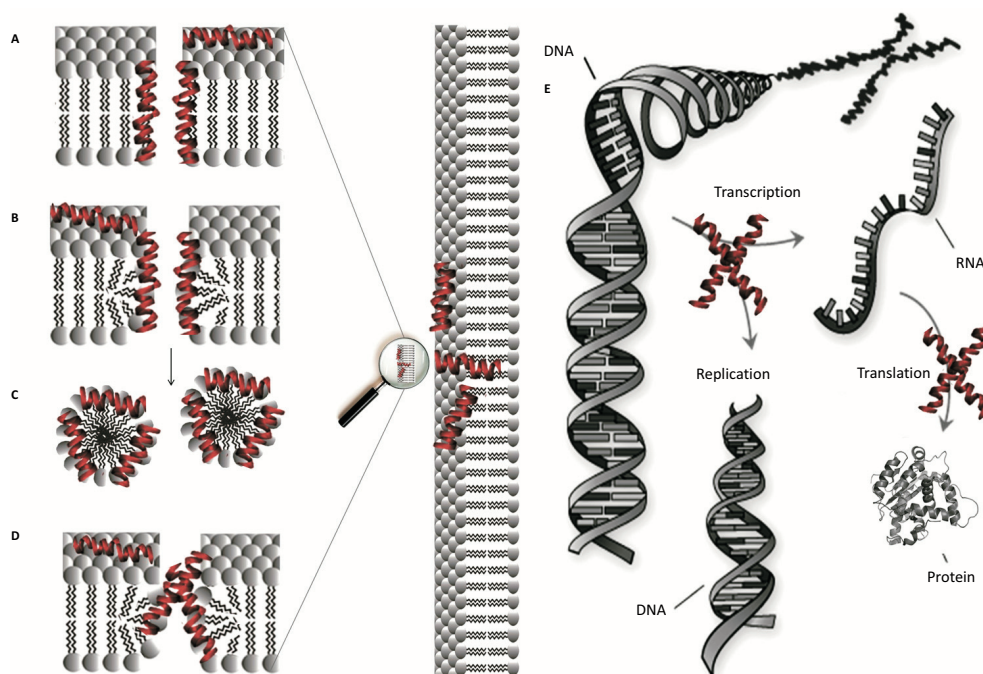
Antimicrobial peptides usually show a broad spectrum of action against gram-negative and gram-positive bacteria [4-8], fungi [9], viruses [10] and tumor cells [11, 12]. Although the main mechanism of action is membrane disruption [13], other mechanisms include inhibiting intracellular targets, such as DNA and RNA, and inhibiting protein synthesis and microbial enzymes (Figure 1). The exact mechanism of action of AMPs in the membrane of the target cell remains controversial and is dependent on the peptide concentration and the lipid composition [14, 15].

The broad spectrum of action, the rapid microbicide activity and the ability to be used in combination with other antibiotics make studies with AMPs an increasing line of research, showing the high potential of these molecules [16, 17]. The number of publications related to AMPs is growing exponentially, indicating that research on this topic is a current and important subject (Figure 2).

#### 1.1. AMPs Properties

Despite the remarkable biological diversity from which the AMPs were discovered, these molecules have certain characteristics in common. Membrane-active AMPs exhibit common physicochemical properties that characterize them as a special group of biomolecules: 1) normally consist of 12 to 50 amino acid residues, 2) have a net positive charge, 3) have approximately 50% hydrophobic residues, and 4) form an amphipathic  $\alpha$ -helix in contact with the membrane. Cationicity is due to the presence of basic amino acid residues such as arginine and lysine. Many models show that this character is crucial for the initial attraction of AMPs on the membrane surface. Amphipathicity is characterized by the separation of the  $\alpha$ -helix in a hydrophobic and a hydrophilic face. The amphipathic character is formed by a periodic sequence of polar and apolar residues in a range of three to four amino acid residues. Thus, the polar and apolar side chains of amino acid residues are positioned appropriately for their segregation between opposite faces [18-21].

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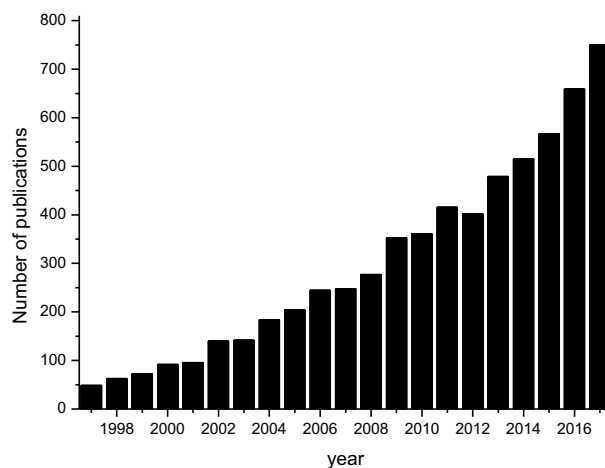


**Figure 1.** Mechanism of action proposed for AMPs: “barrel-stave” pore (a); “toroidal pore” (b); “detergent-like” (c); “disordered toroidal pore” (d) and intracellular targets (e).

## 1.2. Mechanism of Action of AMPs

The molecular understanding of the mechanism of action of AMPs is still not entirely clear [22, 23]. The model known as “barrel-stave” pores (Figure 1A) describes the formation of barrel-shaped pores, where each stave can be represented by a peptide chain. In this model, the hydrophobic face of the peptide interacts with the hydrophobic chain of the phospholipids, while the hydrophilic surface remains oriented inside the pore. According to the “toroidal pore” (Figure 1B), the peptides remain associated with the head groups of the phospholipids, inducing a curvature in the lipid bilayer. This model differs from the “barrel-stave” model because in addition to the peptides, the polar head groups of the lipids also form part of the pore. An alternative to the classic “toroidal pore” is the so-called “disordered toroidal pore”, which predicts that the inside of the pore would not be well structured, showing higher entropy (Figure 1D). Finally, the mechanism called “carpet-like” or “detergent-like” model (Figure 1C) proposes membrane permeabilization by the means of the detergent action of the peptides without the formation of pores. Thus, when a “threshold concentration” of the peptide molecules on the surface of the membrane is reached, micellar aggregates occur, starting the process of solubilization. It has been suggested that this mechanism can be considered an extreme form of the “toroidal pore” mechanism [24-27].

Even though widely studied for the last three decades, the mechanism of action of AMPs remains elusive [28, 29]. The knowledge of the mechanism of action of a particular bioactive molecule is always an essential issue to support its activity and is important in the design of new molecules. In this regard, various techniques have been used to evaluate the mechanism of action, especially nuclear magnetic resonance,



**Figure 2.** Evolution of the number of publications about AMPs (Source: PubMed, 2018. Search criteria: “antimicrobial peptides” in title/abstract).

molecular dynamics, isothermal titration calorimetry, optical microscopy, leakage of carboxyfluorescein, fluorescence spectroscopy and circular dichroism, and others [30-33].

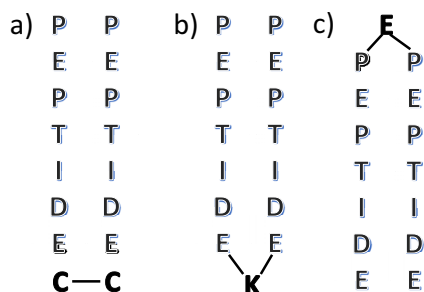
## 2. RATIONAL DESIGN OF AMPs: DIMERIZATION

Innumerable synthetic variants of AMPs have been produced, but few are able to reach clinical application. The main reasons are the low stability in physiological conditions and the lack of selectivity to prokaryotic cells [34, 35]. To increase the stability of AMPs against proteases, different strategies have been addressed, highlighting peptide dimerization. Many studies have shown that regardless of the mechanism of action, the aggregation/oligomerization of

AMP molecules is a prerequisite for its action. Therefore, in addition to the factors described above, it is currently suggested that peptide oligomerization also contributes to its activity and selectivity [25, 36-40]. Considering these studies, several AMPs were dimerized to increase their antimicrobial activity and selectivity [41-47]. In addition, dimeric versions have the potential to be more resistant to proteases compared to monomers. The peptide A3-APO is an example; it retains full antibacterial activity in the presence of mouse serum [48].

### 2.1. Strategies for AMPs Dimerization

Different strategies have been applied for the synthesis of dimeric peptides. Dimers have been synthesized mainly by disulfide bonds, by incorporating cysteine residues in any position of peptide, and by amide bonds, by incorporating lysine or glutamate residues in the C- or N-terminal position, respectively (Figure 3) [37, 41, 43, 49-56].



**Figure 3.** Common dimerization strategies. Peptides linked by a disulfide bond. The figure shows cysteine residues at the C-terminal position but residues could be at any position (a). C-terminal lysine-linked peptides (b). N-terminal glutamate linked peptides (c).

In the case of disulfide bond-linked peptides, cysteine residue can be incorporated as an extra residue (if not present as a constituent residue of the peptide) or by substituting a particular residue (generally a serine residue, if present, due to the similar side chain structure). The substitution/incorporation can be in any position of the peptide sequence, given the possibility of testing several dimer analogs. However, it is important to note that disulfide dimer synthesis involves an additional step after peptide chain elongation. Cysteine residues must be oxidized to produce the dimeric molecule. This procedure is generally achieved by air oxidation and significantly reduces the yield of peptide synthesis [55, 57, 58]. On the other hand, amide bond linked peptides can be produced by the incorporation of a lysine residue for C-terminal dimerization or glutamate residue for N-terminal dimerization. The first approach uses the alpha and epsilon amino groups of a lysine residue to produce amide bonds with the alpha amine groups of the first residue of the two-peptide chains. Fmoc-Lys(Fmoc)-OH can be used for this purpose by attaching it to the resin, and after alpha and epsilon-Fmoc group deprotection, the two peptide chains are simultaneously elongated. The second approach requires, before the cleavage from resin, the use of Fmoc-Glu-OH with its alpha and delta carboxylic group without the protection group, to link two elongated peptide chains [37, 52, 59]. It must be considered that C-terminal dimerization produces a dimeric molecule with two free N-terminal amines (posi-

tively charged at biological pH) from the first residue. On the other hand, N-terminal dimerization produces only one free N-terminal amine when an amino acid like glutamate is used as a linker. This difference could be responsible for the different antimicrobial activities of these analogs. It is well established that N-terminal modifications could affect the activity of AMPs [60-62]. We have showed that the charge of the N-terminus plays an important role in driving the selectivity of the AMP peptides. By modification of N-terminus, the peptide was active only against Gram-positive bacteria [61]. In this manner, dimerization on N-terminus is not a good strategy to improve biological activity.

### 2.2. Linker and Spacers

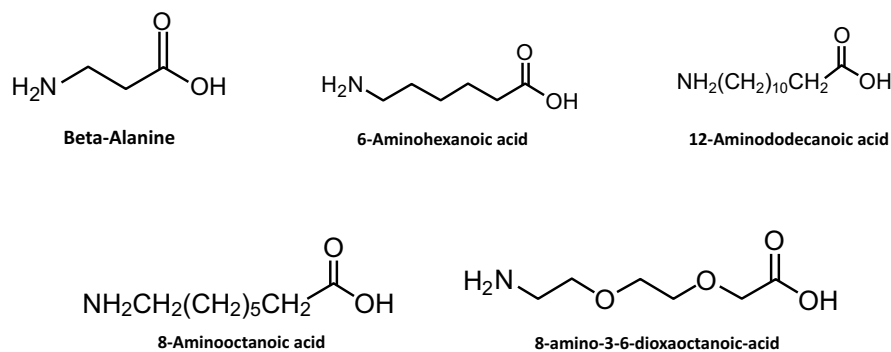
Linkers and spacers are molecules used to link and separate other molecules to avoid interactions among them, once biological activity can be affected by the distance between the molecules. In nature, they are used to connect and separate many protein domains without interfering in their functions [63]. They are also employed to link peptides to form multimeric molecules such as peptide dimers, trimers, tetramers and oligomers [64, 65]. For the dimerization of peptides, linkers are used to attach two molecules, which can be identical or different, resulting in homodimers and heterodimers, respectively. As an example, in Figure 3, lysine and glutamic acid are used as linkers for the formation of a homodimer peptide. The linkers possess functional groups as carboxyl or amine groups, which are responsible for attaching to the C or N terminus of peptides. In addition, spacers can be used, but they are not essential in the dimerization of peptides; nevertheless, they are very useful, especially when the molecules require space between them to conserve or improve its biological activity (Figure 4).

Lorenzón and coworkers reported the effects of different spacers used for Ctx-Ha peptide dimerization (sequence: Gly-Trp-Leu-Asp-Val-Ala-Lys-Lys-Ile-Gly-Lys-Ala-Ala-Phe-Asn-Val-Ala-Lys-Asn-Phe-Leu-CONH<sub>2</sub>). The dimeric forms were obtained by the addition of a Lys residue at the C-terminus of the peptide Ctx-Ha. The Fmoc-8-amino-3,6-dioxaoctanoic acid contains an ethoxy group and was used as a polar spacer, while the Fmoc-8-amino-octanoic acid contains methyl groups and was used as an apolar spacer; however, both spacers are the same size. The antimicrobial activity was better for the peptide containing the polar spacer, which was due to the additional interaction with the head groups of the membrane phospholipids or cell wall. The apolar group decreased the initial interaction and the biological activity [49]. Thus far, the results demonstrate that linker flexibility and polarity play key roles in hemolytic and biological activities in antimicrobial peptide dimers [49, 56, 64]. Linkers and spacers can be used to synthesize dimeric forms of peptides efficiently; however, they need to be carefully designed, with consideration of the polarity, length and position, to assure that they do not decrease the biological activity of the peptides.

## 3. DIMER vs MONOMER

### 3.1. Magainin 2 Dimers

The AMP Magainin 2 (MG2) was one of the first AMPs discovered and might be one of the most studied since its



**Figure 4.** Examples of spacers with different lengths and polarities that can affect the biological activity of a peptide.

**Table 1.** Antimicrobial activity of MG2 dimers.

Peptide Name	Sequence	Increase in Antibacterial Activity <sup>a</sup>		References
		<i>E. coli</i>	<i>S. aureus</i>	
(MG2) <sub>2</sub> K	(GIGKFLHSAKKFGKAFVGEIMNS) <sub>2</sub> K	16X	8X	[59]
MG2-CC	(GIGKFLHSAKKWGKAFVGEIMNSβAC) <sub>2</sub>	2X	2X	[50]
(MG2N22C) <sub>2</sub>	(GIGKFLHSAKKWGKAFVGEIMCS) <sub>2</sub>	2-4X	ND	[51]

discovery by Michael Zasloff in 1987. MG2 belongs to the magainin family, which are AMPs isolated from the skin of *Xenopus laevis* [14, 66, 67]. In addition to most AMPs, MG2 lacks a defined secondary structure in water but adopts an amphipathic helical structure in the presence of membrane mimetics or secondary structure-inducing solvents. In terms of biological activity, the peptide has a wide spectrum of action against gram-positive and gram-negative bacteria, fungi, protozoa, and even cancer cells [68-70]. This multifunctional activity makes MG2 a very interesting molecule to study, with great potential as a new drug. Since its discovery, a large number of MG2 analogs have been synthesized in an attempt to increase its biological activity and improve its pharmacotechnical properties [71-74]. MG2 dimerization has led to an increase in its antimicrobial activity. Table 1 shows the effects of dimerization on the antimicrobial activity of MG2 dimers. The molecules are slightly different, although the three versions correspond to molecules dimerized by the extreme C-terminus

Lysine-linked MG2 dimers showed an increased antibacterial activity of 8-16 times when compared to the monomeric MG2 peptide. This C-terminal lysine-linked increased the proximity and orientation of peptide chains. On the other hand, both cysteine-linked dimers showed an increased antibacterial activity of 2-4 times. It is important to note that the linkage of chains by a lysine comprises four aliphatic carbons, while a disulfide bond comprises just one. This difference in length could affect the interactions and flexibility of the chains. Specific modifications of the sequence (F12W, N22C, and the addition of βA) might also be responsible for the differential increase in antimicrobial activity of the dimeric versions. N-terminal dimerization using glutamic acid-linked did not promote the increase of antimicrobial activities of MG2 [52]. This result showed that the best linkage

position to produce a dimeric molecule is the C-terminus position. The C-terminal dimerization preserve two free N-terminal amines charged positively while N-terminal dimerization could affect the charge and the initial interaction with the membrane. The dimerization of (MG2)<sub>2</sub>K did not change the peptide structure and initial interaction and/or mechanism of action, but promote the proximity of the peptide chains and decrease the number of molecules required to pore formation and increase the biological activity.

### 3.2. Aurein 1.2 Dimers

Several AMPs from the aurein family, which were originally isolated from the Australian frogs *Litoria aurea* and *Litoria raniformis*, have been extensively studied. One of the most active peptides of the aurein family is aurein 1.2 (AU), a short 13-residue peptide with a molecular mass of 1480 g mol<sup>-1</sup>. Aurein 1.2 is active against microorganisms and tumor cells and possesses low toxicity against red blood cells [52, 75]. Lorenzón and coworkers synthesized two AU dimers: (AU)<sub>2</sub>K and E(AU)<sub>2</sub>, with lysine and glutamic acid residues used as linkers, respectively. Circular dichroism spectra indicated that these AU dimers have a “coiled coil” structure in water, while AU displayed a typical spectrum for disordered structures. Hemolytic and vesicle permeabilization assays showed that AU has a concentration-dependent activity, while this effect was less evident for the dimeric versions. In addition, carboxyfluorescein release experiments with LUVs showed that both dimer and monomeric peptides were able to permeabilize vesicles, although the ratio of leakage response to increases in peptide concentration were different. Optical microscopy experiments showed that both versions induced pore opening and promoted the burst of the vesicles. In addition, isothermal titration calorimetry on the LUVs also showed significant differences in peptide membrane

interactions. Together, these data clearly demonstrated that dimerization changes the mechanism of action of AU [52].

As shown in Table 2, dimerization of aurein 1.2 decreases the ability of the peptide to inhibit the growth of bacteria and fungi. (AU)<sub>2</sub>K was unable to inhibit the growth of *C. albicans* but promoted the aggregation of cells, which was elucidated as an interaction of the peptides with yeast cell wall carbohydrates called mannans [76]. In addition, its ability to aggregate yeast cells makes the dimeric versions of AU a promising future drug candidate for preventing *C. albicans* adhesion to biological targets and medical devices, such as prostheses and catheters, preventing diseases caused by this fungus [75, 76].

**Table 2. Antimicrobial activity of AU dimers.**

Peptide	MIC (μmol/L)		
	<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>
AU	16	8	32
(AU) <sub>2</sub> K	128	>128	>128
E(AU) <sub>2</sub>	128	>128	>128

These results indicated that the effect of the dimerization on biological activity of peptides could change the AMP mechanism of action and their biological activity. The change of the structure in solution support different initial interactions with the cell wall and change the mechanism of action of aurein 1.2. Melittin peptide also exhibited the same behavior (as mentioned below).

### 3.3. Other Peptides

Several research groups have studied the effects of dimerization on the biological activity of AMPs. In addition to the increase in antimicrobial activity and its velocity achieved, researchers were also attracted by the potential increase in other properties promoted by dimerization. Table 3 and 4 shows various AMPs that have been used as a template for studying the effects of dimerization. Specifically, we showed some peptides properties and the effects of dimerization on antimicrobial and hemolytic activities. The effects of dimerization on the biological activity of AMPs showed that the increase in biological activity is not a general rule. It is clear that dimerization affects the biological activity of the peptides, sometimes by increasing the antimicrobial activity, sometimes decreasing. In addition, some dimeric versions are hemolytic. According to some authors, the improvement in antimicrobial activity is correlated with an increase in hydrophobicity and net positive charge of its surface area, which enhances LPS binding and neutralization [77-79]. Furthermore, the ability to aggregate and to adopt a well-defined structure could be important to enhance the antimicrobial activity of dimers [54], since dimerized peptides have a lower concentration dependence for reaching the permeabilization threshold compared with the monomers. The "preassembling state" of peptide dimers leads to a reduced number of molecules necessary to form effective pore structures in membrane bilayers. Additionally, the peptide

chain proximity imposed by dimerization could also reduce the time required to form those pore structures. However, some studies have shown that dimeric peptides could lose their microbial properties. An acceptable explanation is that the dimers could be inhibited from passing through the cell walls of microbial cells [49, 80]. Moreover, the interaction with cell wall components, the conformational changes or the peptide aggregation prior to membrane binding could explain the lower capacity of some dimeric AMPs reaching the membrane of microorganisms [75]. These controversial studies show that the effects of dimerization of AMPs need to be better studied. Then, further work is needed to determine the parameters that must be taken into account when choosing an AMP to be dimerized.

The data presented in Tables 3 and 4 showed that most of the peptides that have an increased antimicrobial activity have been dimerized at the C-terminus. As discussed for Magainin 2, C-terminal dimerization preserve two free N-terminal amines charged positively, while N-terminal dimerization decrease the positive charge, affecting the initial interaction with the membrane. Dimeric peptides derived from 1037 and anapolin(J-AA)/RW are exceptions because those peptides were dimerized by N-terminus and have an increased activity. In the case of peptide 1037, N-terminus dimerization were achieve by the use of a cysteine (not glutamate) preserving two amines charged positively [81]. J-AA/RW dimers have lost N-terminus positive charges, but the overall charge/length ratio is high enough to minimize the reduced charge due to dimerization [82]. In our understanding, there is not a clear pattern of the effect of the charge/length relationship. However, the higher charge/size ratio may favor the activity of dimeric versions by the electrostatic repulsion of the peptide chains, preventing aggregation. The peptide secondary structure changes imposed by dimerization also affect biological activity. Dimeric peptide with increased antimicrobial activity has in common the same structure in solution and in membrane mimetics than monomer. The proximity of the peptides chains and decrease the number of molecules required to pore formation may explain the increase in activity. In this case, we believe that the mechanism of action is the same for both peptides.

It is interesting to note that dimerization of Ctx-Ha, aurein 1.2 and melittin peptides decreased the antimicrobial activity but increased the hemolytic activity and the percentage of membrane permeabilization. The red blood cells and vesicle have only lipids on the surface, lacking the components of bacterial cell walls, as peptidoglycan. The change of the structure in solution promote different initial interactions with the cell wall, as polysaccharide, and change the mechanism of action [76]. Peptide di-K18Hc is the only one with reduced toxicity, plus retained antimicrobial activity in elevated concentrations of NaCl or MgCl<sub>2</sub> [53].

### CONCLUSION

Several AMPs were linked together as dimers to improve the antimicrobial activity, although, for some AMPs, dimerization results in a decrease of activity. It appears that the assembled-state of dimers contributes to the proximity and orientation of peptide chains, enhancing pore formation and antimicrobial

Table 3. Dimeric antimicrobial peptides with increased activity.

Peptide	Linker Unit/Position	Sequence	Length	Charge	Charge/Length	MON and DIM structure (Aqueous/Membrane Mimetic)	Antimicrobial Activity	Hemolytic Activity	Other Effects	References
DH (histatin)	K/C-term	KRKFHEKHSHRGY	14	+8	0.67	No information	Increased against <i>S. aureus</i>	Not determined	Same "in vivo" activity	[41]
Ctx-Ha	K-AEEAc/C-term	GWLDVAKKIGKAAFVAKNFL	21	+4	0.19	MON: random coil/helicoidal DIM: random coil/helicoidal	Increased against gram-	Increased	Higher velocity and percentage of membrane permeabilization	[49]
Magainin 2	$\beta$ AC/C-term	GIGKFLHSAKKFGKAFVGEIMNSAC	25	+4	0.16	No information	Increased against gram- and gram+	Increased	Higher membrane permeabilization	[50]
Magainin 2	C/C-term	GIGKFLHSAKKFGKAFVGEIMCS	23	+4	0.17	MON: random coil/helicoidal DIM: random coil/helicoidal	Increased against gram-	Increased	Higher membrane permeabilization	[51]
Magainin 2	K/C-term	GIGKFLHSAKKFGKAFVGEIMNS	23	+4	0.17	MON: random coil/helicoidal DIM: random coil/helicoidal	Increased against gram- and gram+	Increased	Higher velocity and percentage of membrane permeabilization	[59]
di-K18Hc	C/C-term	WLNALLKKGLNCAKGVLA	18	+4	0.22	MON: random coil/helicoidal DIM: random coil/helicoidal	Increased against antibiotic-resistant bacteria	Decreased	Active in elevated concentrations of NaCl or MgCl <sub>2</sub>	[53]
V2-dimer	K/C-term	RGRKVRRKK	10	+7	0.7	No information	Increased against gram-	Not determined	Broader spectrum of antimicrobial activity	[54]
p-BthTX-I	C/C-term	KKYRYHLKPFCKK	13	6+	0.46	MON: random coil/random coil DIM: random coil/random coil	Increased against gram- and gram+	Similar	None	[55]
J-AA/ J-RR	Htrz (triazole) /N-term	GLLKRIKTL / RRWWR	10/6	4+/4+	0.4/0.67	MON: random coil/helicoidal DIM: random coil/helicoidal	Increased	Similar	None	[82]
cys-pep1037	C/N-term	KRFRIRVRV	9	6+	0.67	No information	Increased	Not determined	None	[81]



**Table 4. Dimeric antimicrobial peptides with decreased activity.**

Peptide	Linker Unit/Position	Sequence	Length	Charge	Charge/Length	MON and DIM structure (Aqueous/Membrane Mimetic)	Antimicrobial Activity	Hemolytic Activity	Other Effects	References
Ctx-Ha	K/C-term	GWLDVAKKIGKAAFNVAKNFL	21	+4	0.19	MON: random coil/helicoidal DIM: random coil/helicoidal	Decreased against gram+	Increased	Higher velocity and percentage of membrane permeabilization	[49]
Ctx-Ha	K-Aoc/C-term	GWLDVAKKIGKAAFNVAKNFL	21	+4	0.19	MON: random coil/helicoidal DIM: random coil/helicoidal	Decreased against gram+, gram- and yeast	Increased	Higher velocity and percentage of membrane permeabilization	[49]
Aurein 1.2	K/C-term	GLFDIHKIAESF	13	+1	0.08	MON: random coil/helicoidal DIM: coiled-coil/helicoidal	Decreased against gram+, gram- and yeast	Increased	Dimerization changes the mechanism of action	[83]
Aurein 1.2	E/N-term	GLFDIHKIAESF	13	+1	0.08	MON: random coil/helicoidal DIM: coiled-coil/helicoidal	Decreased against gram+, gram- and yeast	Similar	Dimerization changes the mechanism of action	[83]
PST13-RK	C/C-term	KKKFPWWPFKKK	13	+7	0.54	MON: ND/ $\beta$ -turn DIM: ND/ $\beta$ -turn	Decreased against gram+ and gram-	Not determined	Mammalian cell toxicity	[37]
PST13-RK	K/C-term	KKKFPWWPFKKK	13	+7	0.54	MON: ND/ $\beta$ -turn DIM: ND/ $\beta$ -turn	Decreased against gram+ and gram-	Not determined	Mammalian cell toxicity	[37]
Melittin	C/middle of polypeptide chain	GIGAVLKVLTGLCALISWIKRKRQQ	26	+5	0.19	MON: random coil/helicoidal DIM: helicoidal/helicoidal	Decreased against gram+ and gram-	Increased	-	[56]

ND: Not Determined

activity. The factors that lead a monomeric AMP to become a more active dimeric molecule are not well established. However, several factors must be considered for the design of a new dimeric antimicrobial peptide. The examples analyzed in this work showed that peptide N-terminus charge, linker position, structure, and interaction with cell wall components could affect the biological activity of dimeric peptides. In summary, dimerization constitutes a promising strategy to improve the effectiveness of some AMPs, although more studies are necessary to elucidate the relationship between peptide properties and the dimerization effect on antimicrobial and hemolytic activity. To date, hundreds of synthetic AMPs are in clinical development, and even more are in advanced stages of preclinical development. These AMPs, and new AMPs isolated from natural organisms or designed by computational methods, could have their antimicrobial activity optimized by dimerization.

**CONSENT FOR PUBLICATION**

Not applicable.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest, financial or otherwise.

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