A Review on Structure - Activity Relationship of Antimicrobial Peptide Magainin 2

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ABSTRACT: The rapid development of antibacterial resistance has increased the need for the discovery of novel antimicrobial agents. In this context, an emerging class of molecules called antimicrobial peptides (AMPs) has been considered an excellent material for introducing novel antibiotics. Among the wide variety of AMPs, magainin 2 is a 23 residue (GIGKFLHSAKKFGKAFVGEIMNS) peptide obtained from the African clawed frog *Xenopus laevis*. Different mutants of magainin 2 have been studied to investigate the structure-function relationship. In the present study, we recapitulate the effects of modifying several structural parameters of magainin 2 such as peptide length, amino acid composition, net charge, alpha helicity, hydrophobicity, hydrophobic moment and amphiphilicity on its antibacterial activity. Moreover, we summarized the hemolytic activity regarding these modifications. An optimum change of these parameters would provide desired antibacterial activity with minimum hemolysis. This structure-function relationship study could be used as a template in designing novel functional antibacterial agents.

Key words: Antimicrobial peptide, Magainin 2, Hydrophobicity, Net charge, Chain length, Amphiphilicity

INTRODUCTION

Antibiotic resistance is a serious threat to world's public health, resulting in increased hospital stays, increased medical expenses and a higher mortality rate.[1] Globally, more than half-million individuals die annually due to several antibiotic-resistant infections.[2] To get rid of this situation, scientists are trying to synthesize new antibacterial agents that can fight against notorious bacterial species efficiently. Over the last few decades, an emerging class of molecules called antimicrobial peptides (AMPs) has been studied as potent antibiotic compounds.[3]

AMPs are peptide molecules produced by virtually every living organism- mammals, animals, insects or plants, and protect their host from the attack of a wide range of pathogenic microbes such as bacteria, yeasts, fungi and viruses.[4-7] Despite having varieties of structural features, the AMPs possess several common characteristics. They are usually short peptides having 10-50 amino acid residues. Most AMPs are cationic, showing a net positive charge ranging from +3 to +11, therefore, they can selectively bind with negatively charged surface of the pathogen through electrostatic interaction.[8,9] Moreover, AMPs exhibit amphipathic characteristics[10], which means that the arrangement of amino acid sequences has the hydrophilic and the hydrophobic residues located in opposite directions of the molecule. This arrangement enables them to bind with both the hydrophilic and hydrophobic surfaces of the target membranes. The hydrophobic amino acid residues of peptides are crucial for antimicrobial activity as it controls the binding and insertion behavior into the lipid membrane of microbes.[11-13] Furthermore, many AMPs remain unstructured in aqueous solvent. However, they are transformed into a well-defined secondary structure

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(e.g., α-helix or β-sheet) in the presence of lipid membrane, which plays a vital role in antimicrobial activities.[14-16] These peptides have been protecting their hosts for millions of years and provide a low chance for the development of microbial resistance. Therefore, researchers are highly interested in discovering the structure-function relationships of these molecules.

Magainins are a class of AMPs found in the African clawed frog *Xenopus laevis*. They have been assigned the name "magainins" (Hebrew word "magain" meaning "shield"), indicating their ability to make a shield against the microbes. Magainin 1 and 2, both 23-residue peptides, were discovered by Dr. Michael Zasloff in 1987.[4] These peptides are differed by two substitutions at positions 10 and 22 as shown in Table 1. Magainin 2 exhibits broad-spectrum antibacterial activity compared to magainin 1[17] and therefore, researchers are found to be more interested in the structure of magainin 2.

Table 1. Sequence of two isoforms of magainin.

Magainin 1: GIGKFLHSAGKFGKAFVGEIMKS
Magainin 2: GIGKFLHSAKKFGKAFVGEIMNS

Several analogues of magainin 2 have been synthesized in the last few decades by changing some structural parameters to investigate its antibacterial action for discovering therapeutically potential antibiotics. In this review, we summarize the effects of changing these structural parameters on the antibacterial activity of magainin 2.

Structural features and antibacterial action of magainin 2

Magainin 2 is highly basic with a net charge of +4 at physiological pH (Figure 1A). Therefore, it can selectively bind with the negatively charged surface of bacteria by electrostatic interaction. The cytoplasmic membrane of Gram-negative bacteria is enclosed by an additional outer membrane which is enriched with anionic lipopolysaccharide, while that of Gram-positive bacteria is surrounded by a thick peptidoglycan layer enriched with anionic teichoic acids. Moreover, the cytoplasmic membrane of both Gram-positive and Gram-negative bacteria contain anionic phospholipids phosphatidylglycerol (PG) and cardiolipin (CL). Thus, magainin 2 bind with both the Gram-positive and Gram-negative bacteria by electrostatic interaction. In contrast to bacteria, the cell membrane of human RBC is exclusively enriched with neutral phospholipids such as phosphatidylcholine, sphingomyelin and phosphatidylethanolamine.[7,18,19] So, this peptide attaches to this neutral membrane only through weak hydrophobic interaction. Due to these fundamental structural differences, magainin 2 exhibit less toxicity to mammalian cell (*i.e.*, lower hemolytic activity) but show higher toxicity against both Gram-positive and Gram-negative bacteria. Thus, magainin 2 is considered a promising antibiotic and could provide a solution to the current antibiotic resistance problem.

Magainin 2 molecule is amphiphilic in nature, *i.e.,* the hydrophobic and hydrophilic amino acids are distributed in two distinct regions. Figure 1B demonstrates the helical wheel projection of magainin 2 molecule where the dotted line separates two distinct regions. Another important feature of magainin 2 is that it is unstructured (*i.e.*, no definite conformation) in an aqueous solvent. However, this peptide adopts an α-helical structure (Figure 1C) in acidic phospholipid bilayers, and the helix arranges itself parallel to the surface of the membrane.[16,20] This relatively rigid secondary structure forms the "backbone" of the α-helical structure. The hydrophobic residues (e.g., phenylalanine, leucine) are located on the helix's concave side and facing the membrane interface, while the amino acids with cationic hydrophilic groups (e.g., lysine residues) are faced opposite to hydrophobic residues, thus, forming an overall facially amphiphilic architecture. This arrangement sometimes plays a critical role in the rupture/lysis of the bacterial cytoplasmic membrane.[5] Cationic residues of magainin 2 are responsible for electrostatic interactions with negatively charged bacterial surfaces, while hydrophobic residues drive the molecule to insert deeply into the hydrophobic region of lipid membranes.[19] Finally, in the subsequent step, membrane permeabilization or pore

formation occurs, which causes bacterial death. An elaborate *in vitro* study has been performed to explore the size and dynamics of magainin 2-induced pore in bacteria mimetic model membrane.[21-23] Experimental evidence suggested that magainin 2 forms toroidal pores in the lipid membrane where the perpendicularly inserted α-helix peptide induces rearrangement of the lipid packing where both the peptides and phospholipids line the pore wall.[24-26] After membrane permeation through the toroidal pore, magainin 2 induces programmed cell death through apoptosis.[27]

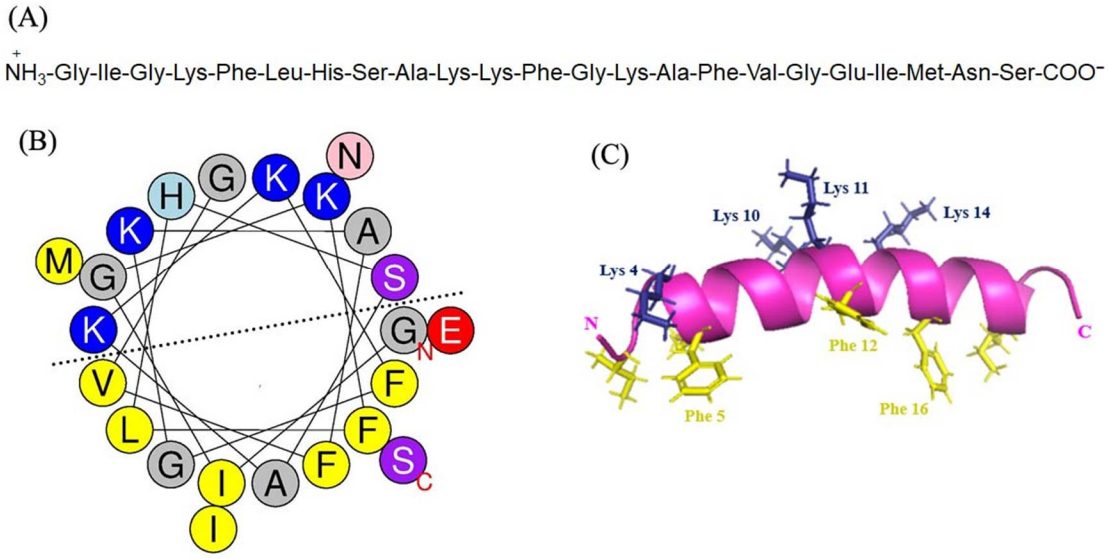

Figure 1. Structure of Magainin 2. (A) Linear structure of magainin 2 (at neutral pH both α-amino and His groups are considered completely protonated) (B) Helical wheel representation of magainin 2 obtained from HeliQuest server (https://www.heliquest.ipmc.cnrs.fr/) (C) The structure of magainin 2 in presence of DPC micelles (PDB ID: 2MAG)[16,20,28] visualized in PyMOL (version 2.4.1.). In both B & C hydrophobic and cationic residues are represented in blue and yellow color, respectively. The letters 'N' and 'C' stand for the N-terminal and C-terminal, respectively.

Several structural parameters of this alpha-helical peptide have been modified to enhance its activity. The extensively studied parameters are peptide length, amino acid composition, net charge, alpha helicity, hydrophobicity, hydrophobic moment and amphiphilicity which are discussed in the following section.

Modification in peptide chain

Magainin 2 is a 23-residue peptide with a molecular mass of 2466.9 g/mol.[29] A definite length of the chain (12 to17 amino acids) is required for antibacterial activity.[30] Removal of 1st two amino acids from N terminal[31] and five amino acids from C terminal[30] do not affect the antimicrobial activity. Addition of a group of alanine at the N-terminal causes complete loss of activity.[31] Substitution or modification at N-terminus will decrease the activity as a free amino terminus is necessary for full activity.[32] Substitution of amino acids with alanine at the position of 1, 8, 13, 18, and 19 yielded analogue with more significant activity than wild magainin 2.[17] The effects of chain length are summarized in Table 2.

Net Charge

Magainin 2 is a cationic AMP having a +4 charge at physiological pH.[4] Therefore, it can selectively bind and accumulate on the surface of Gram-positive and Gram-negative bacteria by electrostatic interaction.[33] It has been reported that the antimicrobial and hemolytic activity can be increased by increasing the value of peptide charge. Bessalle *et al.*, has reported that the addition of extra four positive charges on the N-terminal resulted in

decreased antimicrobial activity whereas adding that of ten increased the activity.[31] However, at peptide charge +5, the hemolytic activity was considerably low, having significant antimicrobial activity. In another experiment, the maximum antimicrobial effect was observed at charge +13 with considerable hemolytic activity. Analogues having charges more than 13, showed decreased antimicrobial and increased hemolytic activity.[31]

In addition to peptide charge modification, the angle subtended by the alpha helix face (charged residues) significantly affects the antimicrobial activity of magainin 2. The positively charged lysine residues provide an angle of 120° in the helical wheel projection of magainin 2 analogue. Wieprecht *et al*. synthesized several analogues of magainin 2 having various angles without changing overall charge and hydrophobicity.[34] It was observed that analogues with large angles show greater antibacterial action, for example, MIC value against *E. coli* decreased to eight times when the angle is increased from 120° (Figure 2A) to 180° (Figure 2B). However, these analogues showed larger hemolytic activity than magainin 2.[34]

Table 2. Effects of amino acid residues in the peptide chain.

Changes in Chain length	Effects	Reference
_ _ GKFLHSAKKFGKAFVGEI MNS	No effect on antibacterial activity	31
GIGKFLHSAKKFGKAFV_ _ _ _ _		30
GIGKFLHSAKKF_ _ _ _ _ _ _ _ _ _ _	Loss of activity	30
$(A)_{10}$ GIGKFLHSAKKFGKAFVGEI MNS		31
_ _ _ KFLHSAKKFGKAFVGEIMNS	Decreased activity	30
Ac-GIGKFLHSAKKFGKAFVGEIMNS		32
AIGKFLHAAKKFAKAFVAAIMNS	Increased activity	17

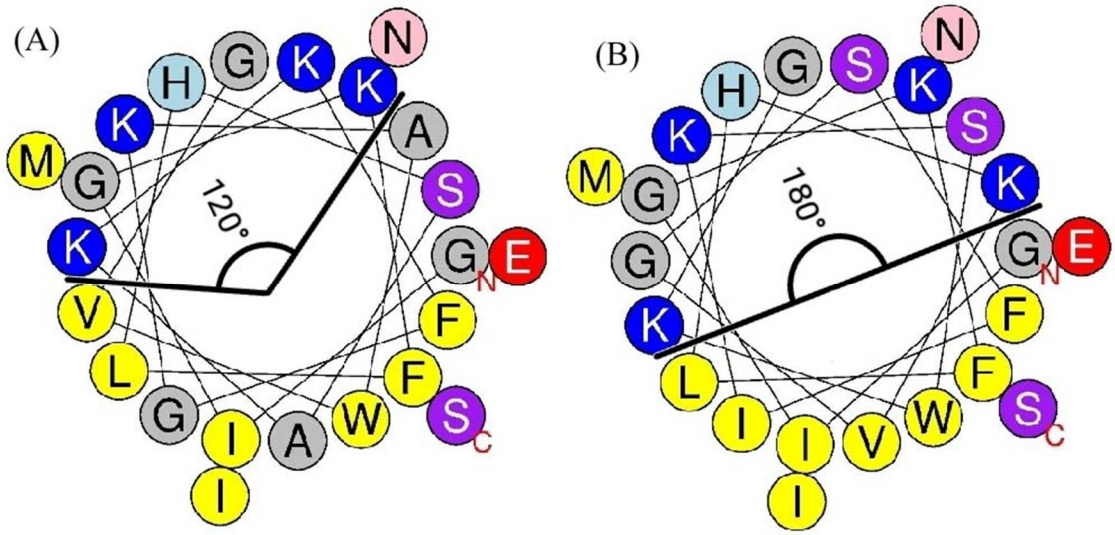

Figure 2. Angle comprised by the positively charged residues (four lysine represented by blue color and angle measured in degree) in the helical wheel projection of two magainin 2 analogues.

Alpha helicity

Alpha helicity is a prerequisite for AMPs[35,36] and higher helicity causes better activity.[37] Replacement of Gly at positions 1, 13 and 18 by Ala improved helicity and antibacterial (*E. coli, K. pneumoniae, P. aeruginosa, S. aureus* and *S. fecalis*) activity.[32] The

addition of Arg or Lys residues at either the C- or N-terminus and addition of α, α-disubstituted amino acids stabilize the helix conformation as well as improves antibacterial activity.[32]

Hydrophobicity

Magainin 2 molecule contains several hydrophobic residues (phenylalanine, leucine, and isoleucine) and has a hydrophobicity of 0.373.[38] Increasing the hydrophobicity increases both antibacterial (*E. coli, P. aeruginosa,* and *B. subtilis*) and hemolytic activities.[33]

The effect of hydrophobicity on antimicrobial and hemolytic activity can be explained using the index.

$$\text{Index} = \frac{\text{Concentration of 50\% hemolysis}}{\text{Minimmum inhibitory concentration}}$$

Figure 3 represents the indices of different analogues of magainin 2 having constant net charge and variable hydrophobicity.[29] No direct relationship has been found between indices of magainin 2 and hydrophobicity. However, the optimum activity against *E. coli* and *S. aureus* is observed at hydrophobicity of 0.460 and 0.438, respectively.

Amphiphilicity

Most of the alpha helices are amphiphilic where the hydrophilic and hydrophobic residues are located in opposite direction. The hydrophobic moment is a quantitative measure of amphiphilicity which is expressed as follows:[39]

$$< \mu H > = \sum_{i=1}^{N} Hi/_{N}$$

Here, μH = Mean helical hydrophobic moment

Hi = Hydrophobicity of the side chains

N = Number of residues

The amphiphilic helical structure is crucial for antibacterial activity.[36] Substitution of 3rd-Gly, 7th-His, 17th-Val with L-Lys, and 13th-Gly with L-Val, respectively, causes enhanced amphiphilicity as well as antibacterial activity.[30]

The relationship between hydrophobic moment and MIC value of different strains of *E. coli* is described in Figure 4, where the antibacterial activity of magainin 2 is directly proportional to the hydrophobic moment.[33,40,41]

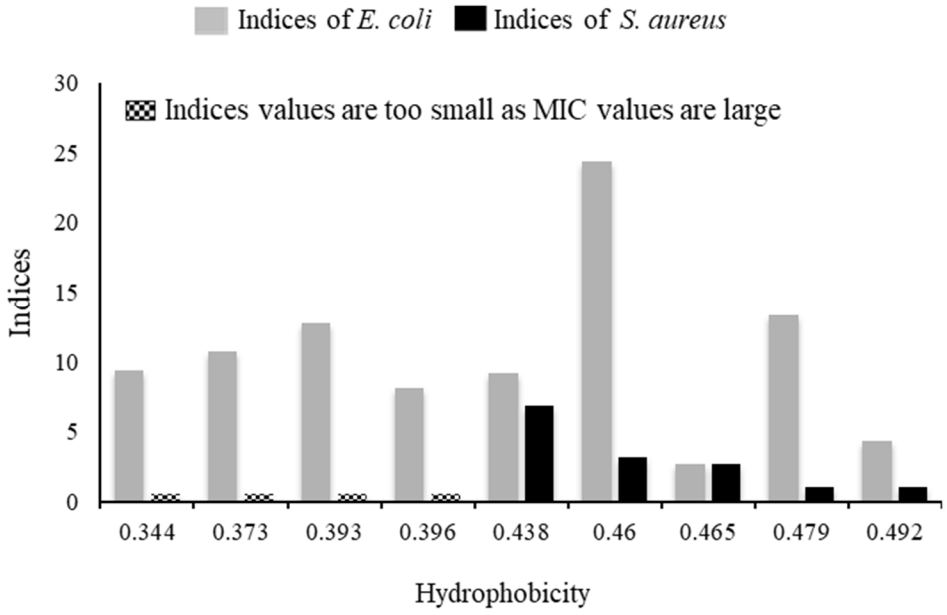

Figure 3. Effect of hydrophobicity on antimicrobial and hemolytic activity of Magainin 2.[29]

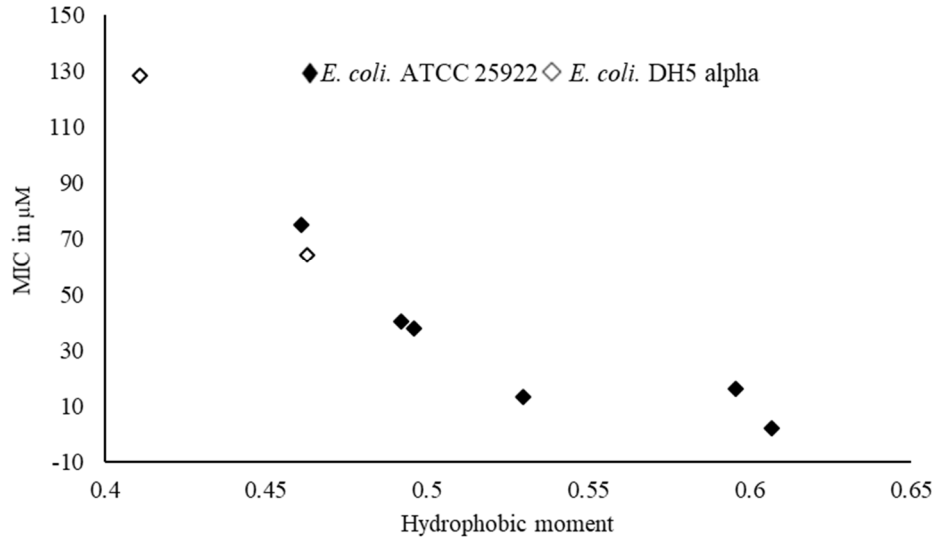

Figure 4. Relationship between hydrophobic moment and MIC value of two strains of *E. coli.*

CONCLUSION

The antibacterial activity of magainin 2 is greatly affected by several factors like peptide length, amino acid composition, alpha helicity, amphiphilicity, net charge, hydrophobicity and hydrophobic moment. Magainin 2 contains 23 amino acid residues in which a minimum length of ~ 12 amino acid residues is required for antimicrobial activity. The addition or removal of several amino acids from the peptide chain significantly changes the activity. It is a cationic peptide, and increasing net charge results in significantly increased antibacterial activity. The cationic property of magainin 2 can be enhanced by adding or substituting the native residues with positively charged amino acids. Amphiphilicity and alpha helicity are crucial factors of magainin 2 that have a directly proportional relationship with antibacterial activity. Increasing hydrophobicity can increase the antibacterial effect. These factors also affect the hemolysis of human RBC. Therefore, researchers should be more concerned while modifying these structural parameters. Moreover, the researchers also should be concerned during the measurement of MIC, which depends on several factors, such as type of microbial strains, the density of microbes in culture and conditions of the culture media. To compare the value of MIC with the current marketed antibiotics, a validated standard operating procedure is necessary. To avoid strain-related activity variation, we reviewed the antibacterial activity of magainin 2 as a function of its different structural parameters using similar bacterial strains from published reports. These structure-activity relationship studies can be used as a template for designing novel functional antibacterial agents or antibiotics.

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