© 2013 Journal of Pharmacy & Pharmacognosy Research, 1 (2), 39-53
ISSN 0719-4250
http://jppres.com/jppres

Review | Revisión

Antimicrobial peptides: The role of hydrophobicity in the alpha helical structure

[Los péptidos antimicrobianos: El papel de la hidrofobicidad en la estructura helicoidal alfa]

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Abstract

The antimicrobial peptides (AMPs) are a class of molecule obtained from plants, insects, animals, and humans. These peptides have been classified into five categories: 1. Anionic peptide, 2. Linear alpha helical cationic peptide, 3. Cationic peptide, 4. Anionic and cationic peptides with disulphide bonds, and 5. Anionic and cationic peptide fragments of larger proteins. Factors affecting AMPs are sequence, size, charge, hydrophobicity, amphipathicity, structure and conformation. Synthesis of these peptides is convenient by using solid phase peptide synthesis by using FMOC chemistry protocol. The secondary structures of three synthetic peptides were determined by circular dichroism. Also, it was compared the stability of the α-helical structure and confirmed the percentage of helix of these peptides by using circular dichroism. Some of these AMPs show therapeutic properties like antimicrobial, antiviral, contraceptive, and anticancer. The formulations of some peptides have been entered into the phase I, II, or III of clinical trials. This article to review briefly the sources, classification, factors affecting AMPs activity, synthesis, characterization, mechanism of action and therapeutic concern of AMPs and mainly focussed on percentage of α-helical structure in various medium.

Keywords: Anticancer; antimicrobial peptide; antiviral; circular dichroism; contraceptive.

Resumen

Los péptidos antimicrobianos (AMP) son una clase de molécula obtenida a partir de plantas, insectos, animales y seres humanos. Estos péptidos han sido clasificados en cinco categorías: 1. Péptido aniónico, 2. Péptido alfa lineal catiónico helicoidal, 3. Péptido catiónico, 4. Péptidos aniónicos y catiónicos con enlaces de disulfuro, y 5. Fragmentos de péptidos aniónicos y catiónicos de proteínas más grandes. Los factores que afectan a los AMP son secuencia, tamaño, carga, hidrofobicidad, anfipaticidad, estructura y conformación. La síntesis de estos péptidos es conveniente mediante el uso de síntesis de péptidos en fase sólida, mediante el protocolo de química FMOC. Las estructuras secundarias de tres péptidos sintéticos se determinaron por dicroísmo circular. También ha sido comparada la estabilidad de la estructura α-helicoidal y confirmado el porcentaje de hélice de estos péptidos mediante el uso de dicroísmo circular. Algunos de estos AMP muestran propiedades terapéuticas como antibióticas, antivirales, anticonceptivas y anticáncer. Las formulaciones de algunos péptidos se encuentran en fases I, II o III de ensayos clínicos. Este artículo revisa brevemente las fuentes, clasificación, factores que afectan a la actividad de los AMP, la síntesis, caracterización, mecanismo de acción y la acción terapéutica de los AMP y se centra principalmente en el porcentaje de la estructura de α-helicoidal en diversos medios.

Palabras Clave: Anticáncer; anticonceptivo; antiviral; dicroismo circular; péptido antimicrobiano.

List of Abbreviations: AMPs - antimicrobial peptides; BOC - acid labile tertiary butyl oxycarbonyl group; CA-P1,2 or 3 - cecropin-A peptide 1,2 or 3; CD - circular dichroism; DCM – dichloromethane; DIPEA - diiso-propyl ethylamine; DMA - Dimethyl acetamide; DMF - N-N-dimethyl formamide; EL - absorption coefficient of left circularly polarized light; ER - absorption coefficient of right circularly polarized light; FMOC - base labile fluorenyl methyl oxycarbonyl group; HBTU - tetramethyl uranium hexafluorophosphate; HF - hydrogen fluoride; HOBT – Hydroxy benzotriazole; LPPS – Liquid phase peptide synthesis; NPS – 2-nitro phenyl sulfenyl group; PAA – Poly acryl amide; PEG – Poly ethylene glicol; PS – Polystyrene; SDS - sodium dodecylsulphate; SPPS – Solid phase peptide synthesis; TFA - trifluoro acetic acid; TFE – trifluoroethanol; TFMSA - trifluoro methane sulphonic acid.

ARTICLE INFO
Received | Recibido: November 16, 2013.
Received in revised form | Recibido en forma corregida: December 8, 2013.
Accepted | Aceptado: December 15, 2013.
Available Online | Publicado en Línea: December 31, 2013
Declaration of Interests | Declaración de Intereses: The authors declare that they have no conflict of interests.
Funding | Financiación: none stated.

INTRODUCTION

Peptides are a class of compounds of low molecular weight, which yield amino acids on hydrolysis. The living organisms are constantly exposed to the potentially harmful pathogens through contact, ingestion and inhalation (Hultmark, 2003). While during pathogenic invasion the first line of defence involves the innate immunity followed by acquired immunity (Fearon and Locksley, 1996). In contrast of acquired immune mechanism, endogenous peptides (which are in the gastrointestinal, respiratory, and genitourinary tracts), which are induced a fast and effective defence against pathogens. This group of molecules is termed as 'antimicrobial peptides' (AMPs). These peptides were found in plants, insects, animals, and humans (Maróti et al., 2011). The AMPs are short peptides, generally between 12 to 50 amino acids present in the sequence of the peptides and these peptides are potent, broad spectrum antibiotics, which exhibited potential as novel therapeutic agents (Zanetti et al., 2002; McPhee and Hancock, 2005; Koczulla and Bals., 2003). These peptides are also called as host defence peptides (Tossi et al., 2005).

AMPs demonstrated to be effective against Gram positive and Gram negative bacteria, mycobacteria (including *Mycobacterium tuberculosis*), fungus, viruses and cancer cells (Toke, 2005; Mader and Hoskin, 2006; Suttmann et al., 2008; Da Silva et al., 2008; Wang et al., 2008; Thennarasu and Nagaraj, 1999) and contains more than 50% of hydrophobic residues and positively charged residues (Devine and Hancock, 2002). AMPs are believed to have a mechanism of action entirely distinct from those of current clinically-used antibiotics, and there is a great interest in their development for treatment of drug-resistant infections (Ge et al., 1999; Zhang and Falla, 2009; Liu et al., 2007; Glukhov et al., 2005).

SECONDARY STRUCTURE

AMPs can be broadly classified based on secondary structure and composition. The secondary structures of these peptides consist of four parts: i) alpha-helical, ii) beta-stranded, iii) beta-hairpin or loop, and iv) extended. These peptides contain a variety of antimicrobial activities from membrane permeabilization to cytoplasm. Ribosomally-synthesized AMPs, containing only natural amino acids can be grouped into linear, alpha-helical peptides (such as cecropins, magainins, and mellitin), peptides characterized by enrichment in one or two amino acids (proline arginine-rich PR39, indolicidin), and peptides containing disulfide bonds (e.g., defensins, protegrins). Large number of peptides with potent antimicrobial activity that were synthesized extra-ribosomally or contain substantial post-translational modifications, for example lipopeptides (polymyxin, dermaseptin) and the lantibiotics, which contain non-native amino acids or non-peptide backbone structures. In addition, a wide variety of synthetic AMPs have been developed utilizing either a combinatorial synthesis approach (Lu et al., 2006; Eckert et al., 2006; Gottler and Ramamoorthy, 2009). The aim of this article to review briefly the sources, classification, factors affecting AMPs activity, synthesis, characterization, mechanism of action and therapeutic concern of AMPs. The second version of the antimicrobial peptide database (ADP2) contains detailed structural information (Wang and Wang, 2004).

Fig. 1 shows the secondary structure of antimicrobial peptides such as α-helix and antiparallel β-sheets.

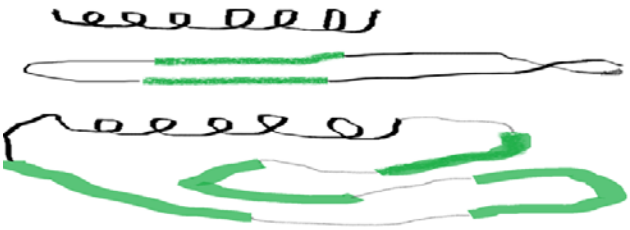

Figure 1. Secondary structure of the antimicrobial peptides.

SOURCES

More than 900 AMPs have been identified in various organisms from plants, insects, animals, and humans (Iwanaga et al., 1998; Selsted et al., 1993; Schnapp et al., 1998). These peptides have been grouped based on their primary structure, amino acid composition and their size. Tables 1-4 show the detailed information of AMPs obtained from plants, insects, animals, and humans.

Antimicrobial peptides from plants

The AMPs were obtained from various plants (Castro et al., 2005) and its examples with details are given in Table 1.

Antimicrobial peptides from insects

The AMPs were obtained from various insects (Bulet et al., 1999) and its examples with details are given in Table 2.

Antimicrobial peptides from animals

The AMPs were obtained from various animals (Iwanaga et al., 1998; Nakamura et al., 1998; Rosa and Barracco, 2010) and its examples with details are given in Table 3.

Table 1. Details of the antimicrobial peptides from plants.

Peptide name	Source	Amino acid number	Antimicrobial activity
Hevein	Latex of rubber trees	43	F
Purothionins	Wheat endosperm	45	G^+, G^-

F - Fungus; G^+ - Gram positive; G^- - Gran negavive.

Table 2. Details of the antimicrobial peptides from insects.

Peptide name	Source	Amino acid number	Antimicrobial activity
Acaloleptin	*Acalolepta luxuriosa*	71	G^+, G^-
Andropin	*Drosophila melanogaster*	34	G^+
Apidaecin IA	*Apis mellifera*	18	G^-
Cecropin	*Hyalophora cecropia*	37	G^-
Defensin- α	*Aedes aegypti*	40	G^+, G^-
Drosomycin	*Drosophila melanogaster*	44	F
Holotricin	*Holotrichia diomphalia*	43	G^+, G^-
Sapecin-α	*Sarcophaga peregrina*	40	G^+, G^-
Tenicin 1	*Tenebrio molitor*	43	G^+, G^-
Thanatin	*Podisus maculiventris*	21	G^+, G^-

F- Fungus; G^+- Gram positive; G^- - Gran negavive.

Table 3. Details of the antimicrobial peptides from animals.

Peptide name	Source	Amino acid number	Antimicrobial activity
Androctonin	*Androctonus australis*	25	F, G^-, G^+
Bactenecin	Bovine neutrophils	12	G^+, G^-
Brevinin	*Rana brevipora porsa*	24	G^-, G^+
Cupiennin	*Cupiennius salei*	35	G^+, G^-
Dermaseptin S1	*Phyllomedusa sauvagii*	34	G^-, G^+
Lycotoxin	*Lycosa carolinensis*	27	G^+, G^-
Tachyplesins	*Tachypleus tridentatus* (horseshoe crab)	17	G^-

F- Fungus; G^+- Gram positive; G^- - Gran negavive.

Antimicrobial peptides from humans

The AMPs were obtained from humans (Jenssen et al., 2006; Schroder and Harder, 1999; Zanetti et al., 1997) and its examples with details are given in Table 4.

CLASSIFICATION

AMPs are classified into five categories and its examples with details are given in Table 5.

FACTORS AFFECTING ANTIMICROBIAL PEPTIDES ACTIVITY

The factors affecting the antimicrobial activity (Chen et al., 2007) such as sequence, charge, conformation and structure, size, hydrophobicity, amphipathicity and its details as follows:

Sequence

Peptides contain the basic amino acid residues like lysine or arginine, the hydrophobic residues like tryptophan, alanine, phenylalanine, leucine, isoleucine, tyrosine and valine. Ratios of hydrophobic residues to charged (cationic or anionic) residues can vary from 1:1 to 2:1.

Charge

Anionic peptides contain more amount of aspartic acid and glutamic acids. The cationic peptides contain more amounts of arginine, histidine and lysine. Anionic peptides were complexed with zinc. Highly cationic peptides have more chance for complexation with zinc.

Table 4. Details of the antimicrobial peptides from humans.

Peptide name	Source	Amino acid number	Antimicrobial activity
Cathelicidins	Human neutrophils	30	F, G^-, G^+
α Defensins	Human neutrophils	12-80	F, G^-, G^+
Human Histatin 8	*Homo sapiens*	12	F, G^-, G^+
LL37	Neutrophils (*Homo sapiens*)	37	F, G^-, G^+

F- Fungus; G^+- Gram positive; G^- - Gran negavive.

Table 5. Antimicrobial peptides classification.

Classes	Characteristics	Examples
Anionic peptides	Rich in glutamic and aspartic acids	Maximin H5 from amphibians
Linear alpha helical cationic peptides	Lack in cysteine	Cecropins from insects, magainin and dermaceptin from amphibians, LL37 from humans
Cationic peptides	Rich in proline, arginine, phenylalanine, glycine, tryptophan	Indolocidin fromcattle, prophenin from pigs
Anionic and cationic peptides that contain disulphide	Cysteine	Peptides with 1 disulphide bond – brevinins, 2 disulphide bonds – protegrin and 3 disulphide bonds – drosomycin and defencins
Anionic and cationic peptide fragments of larger proteins	Tryptophan, lysine, leucine, histidine, proline, arginine, valine	Haemoglobin from humans, lysozyme, ovalbumin and lactoferricin from lactoferrin

Conformation and structure

Antimicrobial peptides can assume a variety of secondary structures including alpha-helices, relaxed coils and anti-parallel beta-sheet structures. Amphipathic alpha-helical peptides are more active than peptides with less-defined secondary structures. Peptides with a gamma-core motif (two anti-parallel beta-sheets with an inter-posed short turn) are very active.

Size

The size of antimicrobial peptides differs from six amino acid residues for anionic peptides to more than 60 amino acid residues. Even di- and tri-peptides with antimicrobial activity were reported.

Hydrophobicity

Water-soluble (hydrophilic) antimicrobial peptides create the partition into the membrane lipid bi-layer.

Amphipathicity

Peptides contain hydrophilic amino acid residues in one side and hydrophobic amino acid residues in the opposite side of a helical molecule. Quantification of hydrophobic residues is less easy in the non helical peptides.

ANTIMICROBIAL PEPTIDE SYNTHESIS

To synthesize AMPs can be used two important methodologies: Solid phase peptide synthesis and liquid phase peptide synthesis.

Solid phase peptide synthesis

Solid phase peptide synthesis (SPPS) was introduced by Bruce Merrifield in 1963 (Stewart and Young, 1984; Fields, 1994; Fields and Fields, 1994; Date et al., 1998). The most of the peptide synthesized by SPPS usually contain less than 20 amino acids. Synthesis of such peptides is routine and straightforward without significant complications.

SPPS is based on addition of the N terminal amino group (depends upon the sequence of the peptide) with C terminal of the side chain protecting amino acid residues to an insoluble polymeric support. The acid labile tertiary butyl oxycarbonyl (BOC) group or base labile fluorenyl methyl oxycarbonyl (FMOC) group is used for protect the functional group of amino acid (n-alpha protection). The second protected amino acid is added after deprotection, using either a coupling reagent. The resulting peptide is attached to the resin through C terminals and cleaved to yield a amide, depending on the coupling agent used in the side chain protecting groups are selected so as to be cleaved both at the same time with detachment of the peptide from the resin.

Deprotection of the BOC protecting group is achieved by 20% trifluoro acetic acid (TFA) in dichloromethane (DCM) and the FMOC protecting group by 20% piperidine in N-N-dimethyl formamide (DMF). Cleavage of the BOC amino acid containing peptide is achieved by liquid hydrogen fluoride (HF) and trifluoro methane sulphonic acid (TFMSA). DCM and DMF are the primary solvents used for resin deprotection, coupling and washing of peptide. In the continuous flow method the resin is contained in a column through, which reagents and solvents are pumped continuously again under manual or automatic control. FMOC strategy is 100% compatible with the continuous flow method, which depending on the instrument used for real time spectrophotometric monitoring of the progress of coupling and deprotection.

Cleavage of the FMOC amino acid containing peptide and side chain deprotection requires TFA.

Advantages

- Simple filtration.
- Synthesis can be carried out in one container
- In SPPS, synthesize a large peptide (more amino acid present in the sequence of the peptide).
- All the reactions involved in the synthesis should be carried 100% to completion.
- All the laborious purification at intermediate steps in the synthesis is eliminated.

Solid support

The solid support is more appropriated in describing the insolubility of the polymer, which

allows filtration or centrifugation and separation of reactants from the peptides. Variety of solid support has been developed for SPPS.

The characteristics of solid support is as follows:

- It should be physically stable.
- It must be inert.
- It must be swell extensively in the solvents using synthesis.
- It should be attaching the first entity either amino acid or organic molecules by the formation of covalent bond.

Types of solid support

- Brush polymers.
- Composites.
- Gel type supports.
- Supported gels.
- Surface-type supports.

Gel type supports

It contains four types of resins:

- Polystyrene (PS) resins.
- Poly acryl amide (PAA) resins.
- Poly ethylene glycol (PEG) grafted resins.
- PEG- based resins.

Protective groups in peptide synthesis

The reagents which are used to protect the functional group (amino group) present in the amino acid are termed as protective groups.

Types of protective groups

- Acid labile.
- Base labile.
- Other protecting groups.

Acid labile

It contain BOC group. The BOC group itself is sufficiently stable that the amino acid derivatives can be stored at room temperature. The deprotection of BOC amino acid can be obtained by 30 min treatment with 0.2% TFA in DCM.

Base labile

It contains FMOC amino acid. Base labile alpha protecting is a main role in the solid phase peptide synthesis. This derivative removed from amino acid and peptide by treating with the solution of secondary amine (mostly piperidine) in DMF.

FMOC amino acids provide a desirable orthogonal system for solid phase peptide synthesis and it is used for the synthesis of several peptides.

The FMOC group loss may produce undesirable short N terminal sequences of the peptide being synthesized due to attachment of amino acid to the exposed hydroxyl groups thus starting new peptide chains. This problem is usually overcome by the use of an acid-labile resin, such as ether resin. When FMOC amino acids are used in coupling reaction the reaction is slow.

Since the acid-labile ether resin should be used for FMOC solid phase peptide synthesis final cleavage of the peptide can be carried out by treatment with 25% TFA in DCM.

Other protecting group

Among the other types of alpha-protecting groups available, the 2-nitro phenyl sulfenyl (NPS) group has been used to some extent in solid phase peptide synthesis. This group can be removed from amino acid by very dilute anhydrous acid or nucleophiles.

Treatment of NPS peptide or NPS amino acid with HCL causes formation of NPS chloride as by-product this is essentially a reversal of the reaction used for the synthesis of NPS derivatives.

Peptide synthesis using BOC-chemistry protocol

The mostly used solvent is DCM. It can be purified by refluxing over phosphorous pentoxide (30 min) and normal distillation. TFA is the deprotection reagent. It can be bought in two purifies reagent grade and biograde, and should be stored in glass. Its boiling point is 71-73°C and a simple distillation is sufficient. BOC chemistry can be easily found in SPPS.

The purity of the best peptide and made by BOC chemistry was comparable with that of the best made by FMOC chemistry.

Another interesting alpha-protecting group removable by nucleophillic attack is the di-thio-succinyl group proposed. This may be very useful protecting group for solid phase peptide synthesis.

Some protecting group for amino acids, acetyl, benzoyl, benzyl, butyl, ter-butyl, oxycarbonyl, 2,6,-dichlorobenzyl.

Peptide synthesis using FMOC-chemistry protocol

In the FMOC chemistry (Abatino and Papini, 2008) the most popular solvent is dimethyl foramide (DMF). DMF can be purified by refluxing over ninhydrin and distillation under reduced pressure it should be stored over for molecular sieves.

Dimethyl acetamide (DMA) is used in solid phase peptide chemistry. It is slower decomposition than DMF and stock solution of tetramethyl uranium hexafluorophosphate (HBTU), diisopropyl ethylamine (DIPEA) are more stable in this solvent; DMA has a boiling point of 165-167°C.

N-methyl pyrrolidine-one has excellent solvating properties and can improve coupling rates on the resin by reducing folding and aggregation of the glowing peptide chain. It can be used in combination with DMF on its own. It has a boiling point of 202-204°C. It can distill under high vacuum. Hydroxy benzotriazole (HOBT) is added during the coupling steps. Piperidine is used for the deprotection of the FMOC group. It can be distilled over KOH. It has boiling point of 104-106°C.

A major problem in SPPS is the peptide chain aggregation due to either hydrophobic interactions or interchain hydrogen bonding. This occurs between 5-15 residues from the C-terminus and can lead to incomplete coupling and deprotection.

Liquid phase peptide synthesis

Liquid-phase peptide synthesis (LPPS) (Chan and White, 2000) is an old method still used for large-scale synthesis. This method is slow, because the product has to be manually removed from the reaction solution after each step and requires another chemical group to protect the C-terminus of the first amino acid.

Advantage

- The product is purified after each step.
- Side reactions are easily detected.
- Separate peptides are synthesized and then coupled together to create the larger peptides.

Disadvantages

- Synthesis cannot be carried out in one container, hence the product wastage is high.
- In LPPS, synthesis of a large peptide is not possible.
- Laborious purification at intermediate steps is complicated.

CHARACTERIZATION

The secondary structure of the AMPs can be characterized (Merrifield, 1963; Corsini et al., 2010) by circular dichroism (CD) and its detailed discussion is as follows:

Circular dichroism

The secondary structures (helical structure) of the antimicrobial peptides were characterized by circular dichroism. Here we discussed the following criteria regarding the basics of the circular dichroism in detailed way. Circularly polarized light is produced by passing a plane polarized light through a bi-refringent plate (it is a z-direction plate) which splits the light into two plane-polarized beams oscillating along different axis (x axis and y axis). When one of the beams is retarded by 90°, then the two beams which are 90° out of phase are added together, final result is circularly polarized light of one direction. The two axes are inverted to produce circularly polarized light of the other direction. Finally, adding the right and left circularly polarized that passes through the optically active sample is elliptically polarized light, this is termed as circular dichroism. It is equivalent to ellipticity I. When the plane polarized light passed through the optically active medium, it changes the left and right circularly polarized right refractive index with diverese the rotation speed.

The medium is called circularly bi-refringent. In circular birefringence effect in addition to the speeds of the absorption coefficient of left circularly polarized light (EL) and the absorption coefficient of right circularly polarized light (ER) is also possible that these two components get absorbed to different extents. The absorption coefficient of the left polarized light is not equivalent to the absorption coefficient of the right polarized light and the absorption coefficient will not oscillate alone for extended. The medium is said to exhibit CD and the transmitted light would become elliptically polarized.

The percentage of peptide helix can be calculated by the following formula:

Molar ellipticity = 2000/-33000 • 100

Where,
2000 – Base line of CD spectrum.
-33300 – Standard value of 100% helix.

CD spectroscopy has been extensively used for the determination of secondary structures of proteins and peptides. We have used three media of different polarity to assess the conformational flexibility of the peptides. The peptide CA-P1 was largely unordered in water, the polar solvent. The peptide failed to adopt any regular structure because of hydrogen bonding between water and peptide back-bone. However, in the presence of sodium dodecyl sulphate (SDS) micelles, a condition that mimics bio-membrane, the peptide folds into α-helical conformation, which was stabilized by intra-molecular hydrogen bonding between peptide back-bone amide bonds. In the presence of trifluroethanol (TFE), a solvent known to promote and stabilize α-helix, CA-P1 displayed more α-helical content as seen in Fig. 2.

CD spectroscopy was used for the determination of secondary CA-P2 peptide. Were used three media of different polarity to assess the conformational flexibility of the peptides. The peptide CA-P2 was largely unordered in water, the polar solvent. The peptide fails to adopt any regular structure because of hydrogen bonding between water and peptide back-bone. However, in the presence of SDS micelles, the peptide folds into α-helical conformation, which was stabilized by intra-molecular hydrogen bonding between peptide back-bone amide bonds. In the presence of TFE, a solvent known to promote and stabilize α-helix, CA-P2 displayed more α-helical content as seen in Fig. 3.

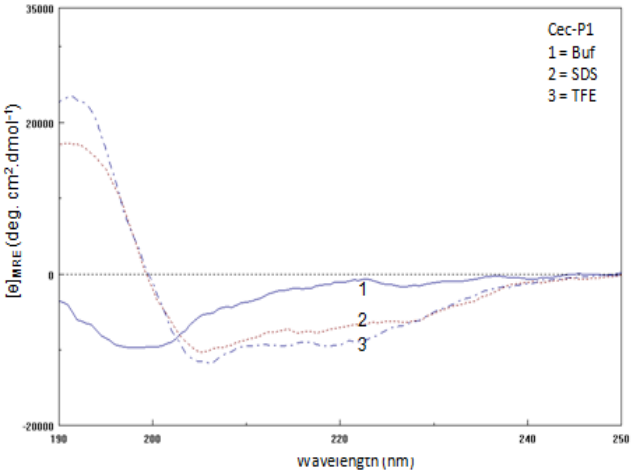

Figure 2. Circular dichroism spectrum of cecropin-A peptide 1 (CA-P1) derivative in the medium of buffer, sodium dodecylsulphate (SDS) and trifluoroethanol (TFE).

CD spectroscopy was used for the determination of secondary CA-P3 peptide. Were used three media of different polarity to assess the conformational flexibility of the peptides. The peptide CA-P3 was largely unordered in water, the polar solvent. The peptide failed to adopt any regular structure because of hydrogen bonding between water and peptide back-bone. However, in the presence of SDS micelles, the peptide folded into α-helical conformation, which was stabilized by intra-molecular hydrogen bonding between peptide back-bone amide bonds. In the presence of trifluroethanol, a solvent known to promote and stabilize α-helix, CA-P3 displayed less α-helical content as compared to CA-P2 (see Figs. 4 and 5).

Fig. 5 shows an overlay of CD profiles of all three peptides viz., CA-P1, CA-P2 and CA-P3 in TFE-water (70:30). As it is clear from the traces, the peptide CA-P2 in more ordered than CA-P1. The peptide containing the modified tryptophan CA-P3 displays the lowest α-helical content, and is more likely to be non-toxic to human cells.

The helix percentage of the CA-P3 was very less as compared with remaining two peptides due to more hydrophobicity. The parameters are shown in the Table 6.

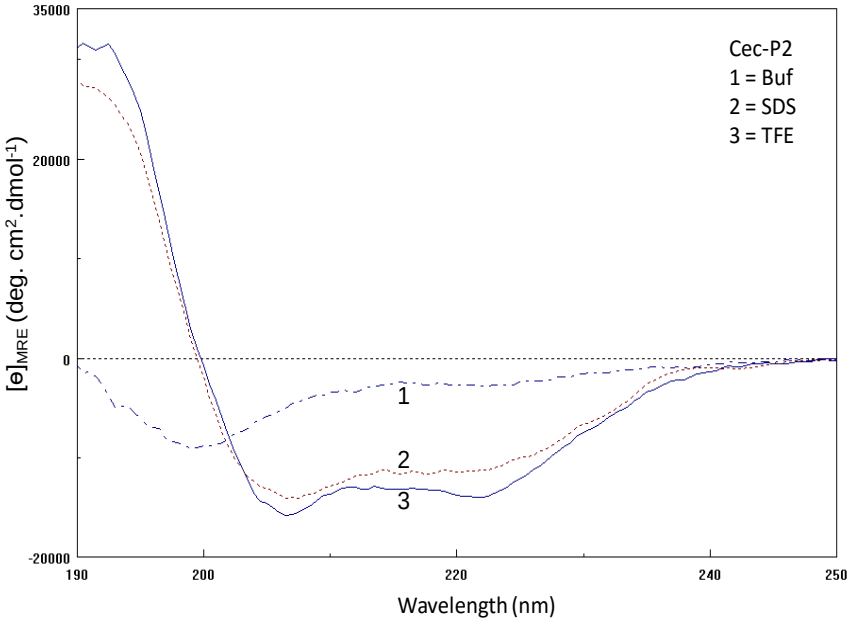

Figure 3. Circular dichroism spectrum of octyl cecropin-A peptide 2 (CA-P2) derivative in the medium of buffer, sodium dodecylsulphate (SDS) and trifluoroethanol (TFE).

Table 6. The helix percentage of the peptides.

Peptide name	Solvent name	Ranges	Wavelength (nm)	Helix (%)
CA-P1	Sodium dodecyl suplhate	-6808	222	14.43
	Trifluoroethanol	-9040	222	21.14
CA-P2	Sodium dodecyl suplhate	-11420	222	28.28
	Trifluoroethanol	-14022	222	36.10
CA-P3	Sodium dodecyl suplhate	-7730	222	17.20
	Trifluoroethanol	-6273	222	12.83

CA-P1 - cecropin-A peptide 1; CA-P2 - cecropin-A peptide 2; CA-P3 - cecropin-A peptide 3.

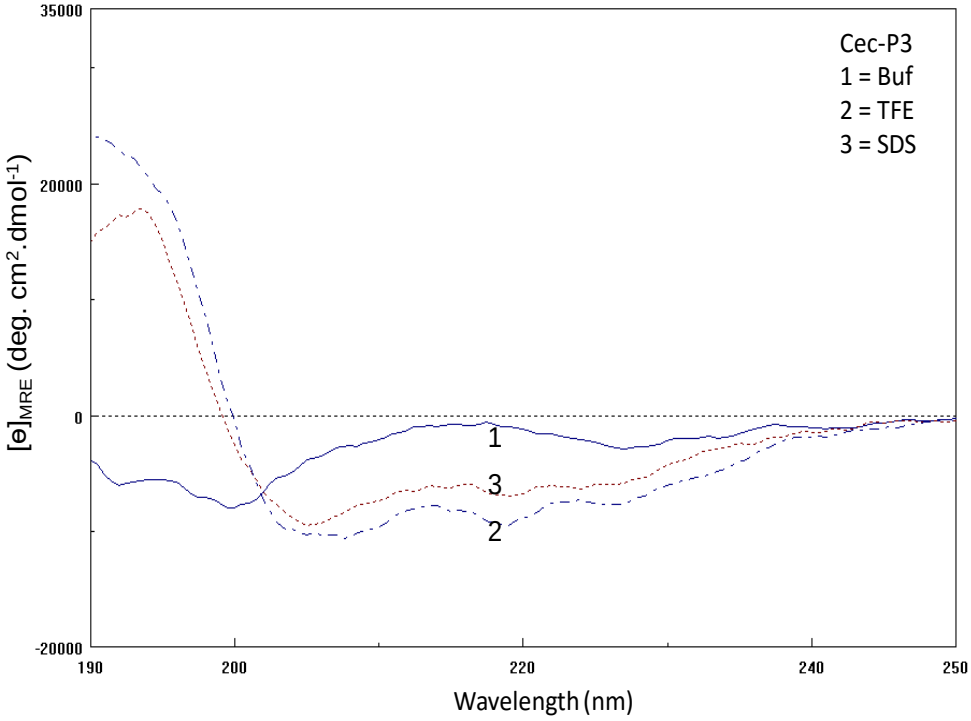

Figure 4. Circular dichroism spectrum of modified cecropin-A peptide 3 (CA-P3) derivative in the medium of buffer, sodium dodecylsulphate (SDS) and trifluoroethanol (TFE).

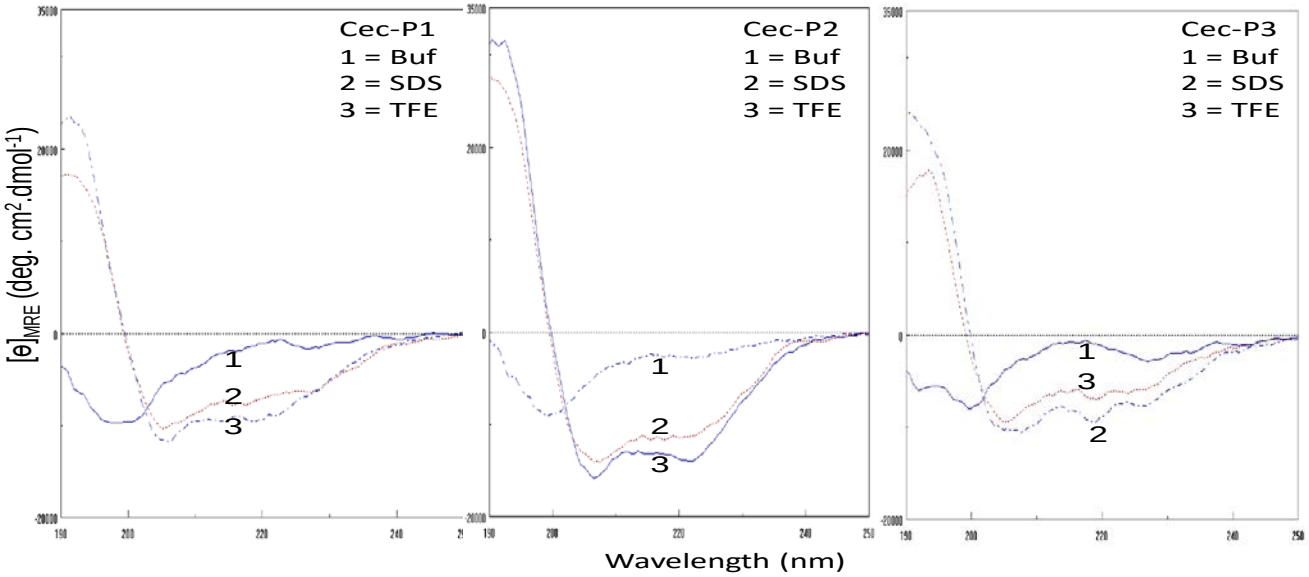

Figure 5. Overlay of circular dichroism profiles of all three peptides viz., cecropin A-peptide 1 (Cec-P1), cecropin A-peptide 2 (Cec-P2) and cecropin A-peptide 3 (Cec-P3).

MECHANISM OF ACTION

The AMPs are divided into two types of mechanisms (Lee et al., 2011; Brogden, 2005; Mangoni and Shai, 2011).

- Transmembrane pore–forming mechanisms.
- Intracellular killing mechanisms.

Transmembrane pore–forming mechanisms

It contains three types of model and their examples are shown in Table 7.

Table 7. Transmembrane pore–forming mechanisms.

Model name	Peptides examples	References
Barrel stave	Alamethicin	Bechinger, 1999
Carpet	Cecropin	Gazit et al., 1995
Toroidal pore	LL-37	Henzler Wildman et al., 2003

The barrel-stave model of antimicrobial peptide induced killing

In this model, the attached peptides aggregate and insert into the membrane bilayer so that the hydrophobic peptide regions align with the lipid core region (Bechinger, 1999; Yang et al., 2001) and the hydrophilic peptide regions form the interior region of the pore.

The carpet model of antimicrobial peptide induced killing

In this model, the peptides disrupt the membrane by orienting parallel to the surface of the lipid bilayer (Hallock et al., 2003) and forming an extensive layer or carpet.

Toroidal model of antimicrobial peptide induced killing

The attached peptides aggregate and induce the lipid monolayers to bend continuously through the pore so that the water core was lined by both the inserted peptides and the lipid head groups (Nissen-Meyer et al., 2010; Matsuzaki et al., 1993).

Intracellular killing mechanisms

It contains seven types of mechanisms and their examples are shown in Table 8.

Table 8. Intracellular killing mechanisms of some peptides.

Type of mechanism	Peptide	Reference
Flocculation of intracellular contents	Human histatin	Andreu & Rivas, 1998
Alters cytoplasmic membrane septum formation	Indolicidin	Subbalakshmi & Sitaram, 1998
Inhibits cell-wall synthesis	Mersacidin	Brotz et al., 1998
Binds nucleic acids	Tachyplesin	Yonezawa et al., 1992
Inhibits nucleic-acid synthesis	Dermaseptin	Patrzykat et al., 2002
Inhibits protein synthesis	Indolicidin	Subbalakshmi & Sitaram, 1998
Inhibits enzymatic activity	Apidaecin	Otvos, 2000

THERAPEUTIC CONCERN

The therapeutic concern of the AMPs is clearly explained about the broad spectrum activity of AMPs against bacteria, fungus, virus and active against respiratory infections, sexually transmitted infections. AMPs were used for contraceptive potentials. It is also explained about AMPs in clinical trials with recent updates. The AMPs were particularly much active against resistance multi-drug pathogens including bacteria, fungus, virus and protozoa.

Antimicrobial peptides in anti-microbials

The membrane active peptides were active against gram positive and gram negative organisms (Giacomeitti et al., 1998). Most of the peptides were lytic types. The antimicrobial activity of the peptides and their examples with details are given in Table 9 (Miyakawa et al., 1996).

Table 9. Antimicrobial activity of AMPs inducing lysis.

Peptide	Source	Target
Defensin NP-1	Rabbit granulocyte	*Cryptococcus neoformans*
Defensin NP-2	Rabbit granulocyte	*Aspergillus fumigatus*
Human defensin	Human neutrophil	*Mycobacterium tuberculosis*
Magainin-2	*Xenopus laevis*	*Candida albicans*
Tripticin	Human	*Aspergillus flavus*

Antimicrobial peptides against sexually transmitted infections causing pathogens

The AMPs were active against the various sexually transmitted infection causing pathogens and their examples are given in Table 10 (Zhang et al., 2002).

Table 10. Antimicrobial peptides against sexually transmitted infections causing pathogens.

Peptide	Mode of action	Target
Cecropin	Cytotoxic to the pathogen	*Chlamydia trachomatis*
Human α defensin 1, 2 and 3	CD8 antiviral factor secreted by CD8 T cells	Human immunodeficiency virus
Mellitin	Suppression of viral transcription	Human immunodeficiency virus
Protegrin	Membrane disruption of bacteria	*Neisseria gonorrhoeae*
Protegrin	Prevents uptake of elementary bodies by target cells	*Chlamydia trachomatis*

Antimicrobial peptides for contraceptive potency

Magainin-A caused 100% sperm immobilization in rat (50 µg/ml), rabbit (400 µg/ml) and monkey and human (800 µg/ml) (Reddy et al., 2004).

Antimicrobial peptides in clinical trials

P-113 a derivative of histatin, a human salivary peptide is undergoing phase I/II trials to treat oral candidiasis (Paquette et al., 2002). Indolicidin analogue, MBI-549 is in Phase II trials for treatment of acne infections (Fella and Hancock, 1997).

Pexiganan (Gaenera, USA), a 22-aminoacid analogue of magainin 2, was the first antimicrobial peptide to undergo commercial development as an antibiotic cream for the topical treatment of diabetic foot ulcers named Locilex™. In 1999 FDA approval was denied because Pexiganan showed insufficient evidence of efficacy despite unanimous agreement about the drug's performance in phase II trials at which stage most drugs (60-70%) fail due to low efficacy (Lamb and Wiseman, 1998).

P113 (developed by Periodontix, USA, then acquired by Demegen, USA) is a 12-amino-acid cationic peptide based on histatins, naturally occurring AMPs in the saliva (Gordon et al., 2005) that demonstrated excellent *in vitro* activity against *Candida albicans* and common Gram-positive and Gram-negative pathogens.

Demegen licensed P113 to Pacgen (Canada) for treatment as a mouth rinse for oral candidiasis in HIV patients (approval for Phase I/II clinical study received on March 2006).

AM-Pharma (The Netherlands) was focused on the development of lactoferricin-based peptides (11-mer peptide from the N-terminus of human lactoferricin, hLF-11) for the prevention of infections in patients undergoing hematopoietic stem cells transplantation (Phase I completed).

Protegrin-1 is undergoing phase II/III trials to treat ventilator associated pneumonia.

rBPI-21 derived from a human neutrophil peptide is undergoing phase II/III trials for treatment of severe paediatric meningococcaemia and Crohn's disease. Role of antimicrobial peptides in host defence against vaccinia virus, trial estimated enrollment: 311, study started on June 2005, study completion on February 2010, clinical trials gov identifier is NCT00407069.

CONCLUSIONS

Antimicrobial peptides are a class of molecule of innate host defence obtained from plants, insects, animals, and humans. Synthesis of the peptides is more easy and convenient by using the methodology of solid phase peptide synthesis as compared with liquid phase peptide synthesis.

The secondary structure of these peptides can be confirmed by using circular dichroism. Peptide CA-P1 spliced from cecropin-A adopts α-helical structure. Lipopeptide CA-P2 is more α-helical, more hydrophobic. Peptide CA-P3 is less α-helical, more hydrophobic. The percentage of helix is very less in modified tryptophan containing Peptide CA-P3 than other two peptides and non-toxic to human cells. Some of these peptides show therapeutic properties like antimicrobial, antiviral, contraceptive, and anticancer with strong efficacy. Formulations of some peptides have been entered into phase I/II/III trials. AMPs are active against topical infections in combination with usual antibiotics.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

ACKNOWLEDGEMENTS

The authors thank to Dr. S. Thennarasu, Head of the Department of Pharmacy, Central Leather Research Institute (CLRI), and Chennai for his valuable support.

REFERENCES

Abatino G, Papini AM (2008) Advances in automatic, manual, and microwave-assisted solid-phase peptide synthesis, Current Op Drug Disc Dev 11: 762-770.

Andreu D, Rivas L (1998) Animal antimicrobial peptides: an overview. Biopolymers 47: 415–433.

Bechinger B (1999) The structure, dynamics and orientation of antimicrobial peptides in membranes by multi-dimensional solid-state NMR spectroscopy. Biochim Biophys Acta 1462: 157–183.

Brogden KA (2005) Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? Nat Rev Microbiol 3: 238-250.

Brotz H, Bierbaum G, Leopold K, Reynolds PE, Sahl HG (1998) The lantibiotic mersacidin inhibits peptidoglycan synthesis by targeting lipid II. Antimicrob Agents Chemother 42: 154–160.

Bulet P, Hetru C, Dimarcq J-L, Hoffmann D (1999) Antimicrobial peptides in insects; structure and function. Dev Comp Immunol 23: 329–344.

Castro MS, Fontes W (2005) Plant defense and antimicrobial peptides. Protein Pept Lett 12: 13–18.

Chan WC, White PD (2000) FMOC-solid phase peptide synthesis - a practical approach. Oxford University Press. p. 365.

Chen Y, Guarnieri MT, Vasil AI, Vasil ML, Mant CT, Hodges RS (2007) Role of peptide hydrophobicity in the mechanism of action of α-helical antimicrobial peptides. Antimicrob Agents Chemother 51: 1398-1406.

Corsini G, Karahanian E, Tello M, Fernandez K, Rivero D, Saavedra JM, Ferrer A (2010) Purification and characterization of the antimicrobial peptide microcin N. FEMS Microbiol Lett 312: 119-125.

Da Silva AP, Unks D, Lyu SC, Ma J, Zbozien-Pacamaj R, Chen X, Krensky AM, Clayberger C (2008) In vitro and in vivo antimicrobial activity of granulysin-derived peptides against *Vibrio cholera*. J Antimicrob Chemother 61: 1103–1109

Date A, Satta Y, Takahata N, Chigusa SI (1998) Evolutionary history and mechanism of the *Drosophila* cecropin gene family, Immunogenetics 47: 417-429.

Devine DA, Hancock RE (2002) Cationic peptides: distribution and mechanisms of resistance. Curr Pharm Des 8: 703-714.

Eckert R, Qi F, Yarbrough DK, He J, Anderson MH, Wenyuan S (2006) Adding selectivity to antimicrobial peptides: rational design of a multidomain peptide against *Pseudomonas* spp. Antimicrob Agents Chemother 50: 1480–1488.

Fearon D, Locksley R (1996) The instructive role of innate immunity in the acquired immune response. Science 272: 50-53.

Fella TJ, Hancock REW (1997) Improved activity of a synthetic indolicidin analog. Antimicrob Agents Chemother 41: 771–775.

Fields CG, Fields GB (1994) Solvents for solid-phase peptide synthesis. Methods in Molecular Biology. In Peptide Syntheses Protocols. Pennington MW and Dunn BM (Eds.). Humana Press Inc. Totowa NJ. 35: 29–40

Fields GB (1994) Methods for removing the FMOC group. Methods in Molecular Biology. In Peptide Synthesis Protocols. Pennington MW and Dunn BM (Eds.). Humana Press Inc. Totowa NJ. 35: 17–27

Gazit E, Boman A, Boman HG, Shai Y (1995) Interaction of the mammalian antibacterial peptide cecropin P1 with phospholipid vesicles. Biochemistry 34: 11479–11488.

Ge G, MacDonald DL, Holroyd KJ, Thornsberry C, Wexler H, Zasloff M (1999) In vitro antibacterial properties of pexiganan, an analog of magainin**.** Antimicrob Agents Chemother 43: 782–788.

Giacomeitti A, Cirioni O, Greganti G, Quarta M, Scalise G (1998) In vitro activities of membrane active peptide against Gram-positive and Gram-negative aerobic bacteria. Antimicrob Agents Chemother 42: 3320–3324.

Glukhov E, Stark M, Burrows LL, Deber CM (2005) Basis for selectivity of cationic antimicrobial peptides for bacterial versus mammalian membranes. J Biol Chem 280: 33960–33967.

Gordon YJ, Romanowski EG, McDermott AM (2005) A review of antimicrobial peptides and their therapeutic potential as anti-infective drugs. Curr Eye Res 30: 505–515.

Gottler L, Ramamoorthy A (2009) Structure, membrane orientation, mechanism, and function of pexiganan - A

highly potent antimicrobial peptide designed from magainin. Biochim Biophys Acta 1788: 1680–1686.
Hallock KJ, Lee DK, Ramamoorthy A (2003) MSI-78, an analogue of the magainin antimicrobial peptides, disrupts lipid bilayer structure via positive curvature strain. Biophys J 84: 3052–3060.
Henzler Wildman KA, Lee DK, Ramamoorthy A (2003) Mechanism of lipid bilayer disruption by the human antimicrobial peptide, LL-37. Biochemistry 42: 6545–6558.
Hultmark D (2003) Drosophila immunity: paths and patterns. Curr Opin Immunol 15: 12–19.
Iwanaga S, Kawabata S, Muta T (1998) New types of clotting factors and defense molecules found in horseshoe crab hemolymph: their structures and functions. J Biochem 123: 1–15.
Jenssen H, Hamill P, Hancock REW (2006) Peptide antimicrobial agents. Clin Microbiol Rev 19: 491–511.
Koczulla AR, Bals R (2003) Antimicrobial peptides: current status and therapeutic potential. Drugs 63: 389-406.
Lamb HM, Wiseman LR (1998) Pexiganan acetate. Drugs 56: 1047–1052.
Lee SB, Li B, Jin S, Daniell H (2011) Expression and characterization of antimicrobial peptides Retrocyclin-101 and Protegrin-1 in chloroplasts to control viral and bacterial infections. Plant Biotechnol J 9: 100-115.
Liu Z, Young AW, Hu P, Rice AJ, Zhou C, Zhang Y, Kallenbach NR (2007) Tuning the membrane selectivity of antimicrobial peptides by using multivalent design. ChemBioChem 8: 2063–2065.
Lu J-X, Blazyk J, Lorigan GA (2006) Exploring membrane selectivity of the antimicrobial peptide KIGAKI using solid-state NMR spectroscopy. Biochim Biophys Acta 1758: 1303–1313.
Mader JS, Hoskin DW (2006) Cationic antimicrobial peptides as novel cytotoxic agents for cancer treatment. Expert Opin Investig Drugs 15: 933-946.
Mangoni ML, Shai Y (2011) Short native antimicrobial peptides and engineered ultrashort lipopeptides: similarities and differences in cell specificities and modes of action. Cell Mol Life Sci 13: 2267-2280.
Maróti G, Kereszt A, Kondorosi E, Mergaert P (2011) Natural roles of antimicrobial peptides in microbes, plants and animals. Res Microbiol 162: 363-374.
Matsuzaki K, Murase O, Fujii N, Miyajima K (1993) An antimicrobial peptide, magainin 2, induced rapid flip-flop of phospholipids coupled with pore formation and peptide translocation. Biochemistry 35: 11361–11368.
McPhee JB, Hancock RE (2005) Function and therapeutic potential of host defence peptides. J Pept Sci 11: 677-687.
Merrifield RB (1963) Solid phase peptide synthesis. I. The synthesis of a tetrapeptide. J Amer Chem Soc 85: 2149-2154.
Miyakawa Y, Ratnakar P, Rao AG (1996) In-vitro activity of the antimicrobial peptides human and rabbit defensins and porcine leukocyte protegrin against *Mycobacterium tuberculosis*. Infect Immunol 64: 926–932.
Nakamura T, Furunaka H, Miyata T, Tokunaga F, Muta T, Iwanaga S. Tachyplesin,(1998) A class of antimicrobial peptide from the hemocytes of the horseshoe crab (*Tachypleus tridentatus*). J Biol Chem 263: 16709–16713.
Nissen-Meyer J, Oppegård C, Rogne P, Haugen HS, Kristiansen PE (2010) Structure and mode-of-action of the two-peptide (class-IIb) bacteriocins. Probiotics and Antimicrob Proteins 2: 52-60.
Otvos L Jr. (2000) Interaction between heat shock proteins and antimicrobial peptides. Biochemistry 39: 14150–14159.
Paquette DW, Simpson DM, Friden P, Braman V, Williams RC (2002) Safety and clinical effects of topical histatin gels in humans with experimental gingivitis. J Clin Periodontol 29: 1051–1058.
Patrzykat A, Friedrich CL, Zhang L, Mendoza V, Hancock RE (2002) Sublethal concentrations of pleurocidinderived antimicrobial peptides inhibit macromolecular synthesis in *Escherichia coli*. Antimicrob Agents Chemother 46: 605–614.
Reddy KVR, Yedery RD, Aranha C (2004) Antimicrobial peptides: premises and promises. Intern J Antimicrob Agents 24: 536–547.
Rosa RD, Barracco MA (2010) Antimicrobial peptides in crustaceans. Invertebrate Surviv J 7: 262–284.
Schnapp D, Reid CJ, Harris A (1998) Localization and expression of human-defensin-1 in the pancreas and kidney. J Pathol 186: 99–103.
Schroder JM, Harder J (1999) Human beta-defensins-2. Int J Biochem Cell Biol 31: 645–51.
Selsted ME, Tang YQ, Morris WL, McGuire PA, Novotney MJ, Smith W, Henschen AH, Cullor JS (1993) Purification, primary structures, and antibacterial activeties of beta-defensins, a new family of antimicrobial peptides from bovine neutrophils. J Biol Chem 268: 6641–6648.
Stewart JM, Young JD (1984) Laboratory techniques in solid phase peptide synthesis. In Solid Phase Peptide Synthesis. Second Edition. Pierce Chemical Company. Rockford. IL. pp. 53-124.
Subbalakshmi C, Sitaram N (1998) Mechanism of antimicrobial action of indolicidin. FEMS Microbiol. Lett. 160: 91–96.
Suttmann H, Retz M, Paulsen F, Harder J, Zwergel U, Kamradt J, Wullich B, Unteregger G, Stöckle M, Lehmann J (2008) Antimicrobial peptides of the Cecropin-family show potent antitumor activity against bladder cancer cells. BMC Urol 8:5-7.
Thennarasu S, Nagaraj R (1999) Synthetic peptides corresponding to the beta-hairpin loop of rabbit defensin NP-2 show antimicrobial activity. Biochem Biophys Res Comm 254: 281–283.
Toke O (2005) Antimicrobial peptides: new candidates in the fight against bacterial infections. Biopolymers 80: 717-735.
Tossi A (2005) Host defense peptides: roles and applications. Curr Protein Pept Sci 6: 1-3.
Wang G, Watson KM, Buckheit RW Jr (2008) Anti-HIV-1 activity of antimicrobial peptides derived from human and bovine cathelicidins. Antimicrob Agents Chemother 52: 3438-3440.

Wang Z, Wang G (2004) APD: The antimicrobial peptide database. Nucl Acids Res 32: D590-D592.

Yang L, Harroun TA, Weiss TM, Ding L, Huang HW (2001) Barrel-stave model or toroidal model? A case study on melittin pores. Biophys J 81: 1475–1485.

Yonezawa A, Kuwahara J, Fujii N, Sugiura Y (1992) Binding of tachyplesin I to DNA revealed by footprinting analysis: significant contribution of secondary structure to DNA binding and implication for biological action. Biochemistry 31: 2998–3004.

Zanetti M, Gennaro R, Romeo D (1997) The cathelicidins family of antimicrobial peptide precursors: a component of the oxygen independent defense mechanisms neutrophils. Ann N Y Acad Sci 832: 147–162.

Zanetti M, Gennaro R, Skerlavaj B, Tomasinsig L, Circo R (2002) Cathelicidin peptides as candidates for a novel class of antimicrobials. Curr Pharm Des 8: 779-793.

Zhang L, Falla TJ (2009) Cosmeceuticals and peptides. Clin Dermatol 27: 485–494.

Zhang L, Tu W, He T (2002) Contribution of human alpha-defensin 1, 2 and 3 to the anti-HIV-1 activity of CD8 antiviral factor. Science 298: 995–1000.