We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Cationic Peptide Interactions with Biological Macromolecules

Monde Ntwasa

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/48492>

1. Introduction

Cationic amphiphilic peptides (CAPs) are widely studied as effectors that are activated by microbial pathogens in immune signaling pathways of invertebrates and vertebrates. These peptides are non-specific effectors that can kill bacteria, fungi, viruses and protozoan parasites [1, 2]. They are a universal feature in all forms of life and are often found in all the major barriers such as the skin and epithelia that are naturally designed for protection against invading microorganisms. In the case of invertebrates, they play a pivotal role in innate immunity upon which these animals depend for defense against infection. The two immune response strategies are interdependent and innate immunity has significant impact on the development of adaptive immunity [3-6]. In addition to innate immunity, vertebrates also rely on acquired immunity which is mediated by antibodies and cytotoxic T lymphocytes [7]. Identification of these antimicrobial peptides and the study of their structural features have led to the development of peptide drugs, sometimes through the design of synthetic peptides based on the known structures of the natural ones. A subset of cationic peptides has been found to have anti-tumour as well as wound-healing properties extending the prospects of these peptides as templates for drug design strategies against cancer and wound treatment [8]. The mechanisms by which these latter properties are manifested are not fully understood. Indeed, the mechanisms by which cationic peptides exert their wide biological activities are still under investigation and many theories have been proposed.

The mode of action of cationic peptides appears to be reliant heavily but not entirely, on their structural and biophysical features. As their name suggests, they are characterized by a net positive charge which contrasts conveniently with the negative charge that is characteristic of microbial membranes and cancer cells.

Studies on the antibacterial peptide mode of action produced several models that suggest that the phospholipid bilayer forming membranes is the main target of peptide action. There

is, however, evidence that shows that some cationic peptides can cross the plasma membrane and interact with intracellular macromolecules.

The mechanism by which cationic peptides inhibit viral infections is also not fully understood. They are understood to act primarily against enveloped RNA and DNA viruses but there are exceptions such as the non-enveloped adenovirus and a few others. Cationic peptides appear to target viral adsorption or the entry process, replication and gene expression [9]. It remains to be seen if the mode of action against viruses can be correlated to secondary structure features of the cationic peptides. Current knowledge points to interactions with the extracellular matrix and with membrane or viral envelope proteins. Intracellular targets whereby the host is stimulated to act against the virus are also suggested.

Antifungal peptides tend to be rich in polar and neutral amino acids suggesting a functional significance that is important for interfering with a unique fungal property. Furthermore, it has been shown that in peptides with activities against both fungi and bacteria different substitutions were required for optimizing the different types of activities. Overall, it seems that these peptides interact mainly with the phospholipid bilayer to effect lysis of certain microbes. However, mounting evidence that shows existence of intracellular targets that could be polypeptide or nucleic acid in nature, suggests a wider scope for investigation to establish how these peptides execute their biological functions.

CAPs are attractive candidates for therapeutic use but their development for commercialization is hampered by certain crucial obstacles. In this chapter, biochemical interactions of CAPs together with prospects for commercialization are discussed.

2. Classes of cationic peptides

Broadly, there are two major classes of cationic peptides with antimicrobial activities (Table 1). One group is produced by bacteria and fungi and consists of non-ribosomally synthesized peptides. These peptides are assembled by multifunctional peptide synthases in large and ordered multi-enzyme and co-factor systems following the “multiple carrier model” for peptide biosynthesis [10, 11]. Examples include Gramicidin, bacitracin, polymyxin B, streptogramins, vancomycin and others. This biosynthetic process results in an extensive chemical variety that includes peptides containing hydroxyl-, L-, D- and unusual amino acids which can be further modified by methylation, acylation, glycosylation or cyclic ring formation [11]. The major disadvantage of these peptides is that bacteria develop resistance to them e.g. vancomycin-resistant *Staphylococcus aureus* and enterococci [12, 13].

The second major class includes gene-encoded ribosomally synthesized peptides which are further subdivided into bacteriocins (produced by bacteria) and antimicrobial peptides (produced by eukaryotes). The latter are the main object of this chapter. A prominent group of bacteriocins, composed of rare and modified amino acids is also called lantibiotics. A good example is nisin, a peptide produced by *Lactococcus lactis* with rare amino acids such

Non-ribosomally synthesized	Gene-encoded
Contain a chemical variety of amino acid	Mainly D-amino acids
	Generally amphipathic with (12 – 20 amino acids)
	Have a high net positive charge and hydrophobic residues
Highly active at low concentrations	Active at higher concentrations
May be modified	Carry no unusual posttranslational modifications
Narrow spectrum	Broad spectrum

Table 1. Comparison between gene-encoded and non-ribosomally synthesized antimicrobial peptides

as lanthionine, 3-methylanthionine, dehydroalanine and dehydrobutyrine [14]. Lantibiotics act by either pore formation leading to disruption of the bacterial cell wall or by interfering with biosynthesis of molecules such as the peptidoglycan component of the bacterial cell wall. This results in a thinner cell wall and eventual lysis of the bacterium [15].

Antimicrobial peptides are divided into four major structural groups namely; (a) peptides that form α -helical structures, (b) cysteine-rich peptides with intramolecular disulfide bonds, (c) peptides that form β -sheets connected by a single or two disulfide bridges, and (d) peptides rich in particular amino acids such as histidine, glycine, arginine and proline or tryptophan [16-20] (Table 2 and Figure 1). They have considerable sequence diversity but share important physicochemical properties. They are 12 – 50 amino acids long, carry a positive (+2 to +9) charge and are composed of 40 – 50% hydrophobic residues. In their folded state, residues segregate into hydrophilic and hydrophobic clusters producing an amphipathic structure thus allowing them to be soluble in phospholipid membranes. The combination of these electrostatic and hydrophobic interactions results in membrane disruption and key structural features that contribute to their mode of action as described later in this chapter.

It is worth noting that the antimicrobial activity of cationic peptides is dependent upon physiological conditions [1]. They are regarded as antimicrobial peptides if they can kill pathogens at physiological concentrations of divalent cations such as Mg^{2+} and Ca^{2+} (1-2 mM), monovalent cations such as Na^+ and K^+ (100 mM) and polyanions and mucins.

Peptide type	Example	References
α -helical structures,	Cecropin A, magainins, dermaseptin, bombinin, mellitin, cathelicidin	[16, 115, 116]
Rich in cysteine residues,	HNP-1, 2 and 3 (human defensins)	[117]
β -sheets	Tachyplesins polyphemusin II (T22), lactoferricin	[18-20]
Rich in certain amino acids such as histidine, glycine, arginine and proline or tryptophan	Histatin (histidine), indolicidin (tryptophan), tritrypticin, holotricin (glycine & histidine), coleopteracin (glycine), pyrrhocoricin (proline)	[69, 118-120]

Table 2. Classes of AMPs

Figure 1. Structures and examples of cationic peptides representing different classes.. **A.** α -helical peptide - BMA-27 (PDB ID: 2KET). **B.** β -sheet peptide with disulfide bridges - human defensin (PDB ID: 3GNY) **C.** anti-parallel β -sheet - tachyplesin 1 (PDB ID: 1WO1), **D.** peptides with amino acid bias - Indolicidin (PDB ID: 1G89).

2. Modes of action of cationic peptides

The activity of antimicrobial peptides (AMPs) and their potential use as therapeutic agents rely on differences between mammalian and bacterial subcellular structures as well as between normal and abnormal (apoptotic and tumour) mammalian cells. The current dogma for microbial killing by cationic peptides is that they target the phospholipid bilayer and kill microorganisms by pore formation or membrane disruption leading to cell lysis. There is, however, growing evidence showing that some peptides act on intracellular macromolecular targets. In some cases it is debatable whether killing is due to intracellular targeting or a combination of this and membrane disruption. Nevertheless, good understanding of the mechanisms of action of AMPs should provide promising opportunities for drug design. Before reviewing their mode of action it is therefore necessary to consider the landscape of human and pathogen cell membranes. Generally, the cationic nature of antimicrobial peptides facilitates electrostatic attraction to the negatively charged microbial membrane phospholipids and their hydrophobicity facilitates cell membrane penetration. However, there are subtle differences in the mechanism of action of the various peptides.

Structural features of animal and bacterial cells

The distribution of phospholipids on the outer and inner leaflets of the plasma membrane in eukaryotic cells is asymmetric. Typically, the outer surface of normal mammalian cells is composed of neutral zwitterionic phospholipids and cholesterol [8]. It is largely composed of choline-containing phospholipids such as sphingolimyelin and phosphatidylcholine,

while aminophospholipids such as phosphatidylserine and phosphatidylethanolamine dominate the inner leaflet [21]. In addition to the heterogeneity of headgroups and acyl chains, the presence of cholesterol in animal cells introduces more complexity to the membrane landscape by promoting the formation of lipid microdomains [22].

On the other hand, bacterial membranes are predominantly composed of acidic phospholipids (such as phosphatidylglycerol and cardiolipin) that confer a net negative charge to the surface of the membrane while phosphatidylethanolamine and phosphatidylserine are not detectable [23-25]. Since AMPs have to cross the negatively charged lipopolysaccharide layer before reaching the membrane, the possible impact of this barrier has been investigated. The negative charge on the lipopolysaccharide (LPS) rather than the size of the saccharide moiety is important for susceptibility of the bacterial cell to antimicrobial activity of the cationic peptides. This was demonstrated by experiments using bacterial LPS mutants with varying lengths of the polysaccharide moiety but an equal number of phosphate groups. In these mutants the phosphate groups would, however, be heterogeneous due to further modifications resulting in diverse phosphorylation patterns amongst mutants. It was found that the LPS mutants display differential susceptibility to cationic peptides in a manner that seems to be related to charge location and magnitude and to absence or presence of the O-antigen side chain [23, 26]. It is proposed that because of their greater affinity for LPS than divalent cations and their bulkiness, cationic peptides competitively displace these ions and create a passage through the outer bacterial membrane thus propelling themselves to the cytoplasmic membrane by a “self-promoted uptake” [27].

Loss of asymmetry with distinct bias in phospholipid types is observed in tumorigenic cells when compared to animal cells. Cancer cells are known to carry a predominantly negative charge due to high levels of the anionic phosphatidylserine, O-glycosylated mucins, sialylated gangliosides and heparin sulphates [28, 29]. The membranes of tumorigenic cells also contain a significantly higher number of microvilli compared to normal cells effectively increasing the surface area of cancer cells [21].

Cationic peptides interactions with the phospholipid bilayer of membranes

Cationic peptides are attracted to the negatively charged prokaryotic membranes and kill microbial pathogens by causing disintegration of their membranes and subsequent collapse of electrochemical gradients [23, 30, 31]. Various models of membranolytic activities of AMPs have been proposed. These include the (i) barrel stave (ii) Carpet (iii) toroidal model, and (iii) channel-forming models reviewed in [2, 32, 33].

- i. *The barrel stave model* – this model is based on the amphipathic α -helical peptides forming contacts with headgroups on the inner and outer surfaces of the membrane bilayer using their hydrophilic ends while their hydrophobic regions make contact with the acyl chains of the phospholipids. This results in transmembrane pore channels whose inner surface or lumen consists of the hydrophilic regions of the peptides. Binding to the membrane is probably driven by hydrophobic interactions with the

membrane hydrophobic core and requires aggregation of several peptide monomers or oligomers in an α -helical form. This model proposes a stepwise sequence beginning with the peptides reaching the membrane and assembling at the surface. After recruitment of more monomers, they insert into the core of the membrane. Only a few pores are required to dissipate the transmembrane potential in cells [34]. The "barrel stave" model applies to certain peptides such as the non-ribosomally synthesized antibiotic, alamethicin [35] and the gene-encoded pardaxin, a polypeptide *Purdachirus marmoratus* toxin with a helix-hinge-helix structure [36, 37].

- ii. *The carpet model* – was first described for the action of dermaseptin and later for cecropin, the human cathelicidin LL-37 and others [33]. Binding of these peptides to the membrane is initially electrostatically driven and the peptides are not required to adopt a particular structural form. It is proposed that binding to bacterial membranes takes place in four defined steps [33, 34]. Initially, the peptides make contact with the LPS on Gram-negative bacteria or the teichoic acids on Gram-positive bacteria and traverse the membrane in a carpet-like fashion. The peptides then align themselves such that their hydrophobic regions face the lipids and their hydrophilic regions face the phospholipid headgroups. This is followed by the accumulation of peptides until a threshold concentration is reached. Finally, the peptides permeate the membrane and disrupt it causing the collapse of the bilayer. This model is sometimes referred as the *detergent model* and is characterized by the accumulation of the peptide which drives the eventual catastrophic collapse of the membrane.
- iii. *The toroidal model* – was first proposed by [38, 39] to describe the action of the *Xenopus laevis* AMP, magainin 2. Later it was found that peptides such as mellitin and protegrins also induce transmembrane pores in the toroidal fashion [35]. In this model, the peptides aggregate such that both the phospholipid headgroups of the monolayers and the peptides line the lumen of the pore. This results in the formation of a dynamic core consisting of the lipid monolayers and peptides with a characteristic lipid flip-flop.
- iv. *The aggregate or channel-forming model* – appears to be a subtle variation of the toroidal mechanism. It was first suggested after a study using short (10 – 14 amino acids) peptides and a membrane potential-sensitive cyanide dye. This model portrayed concentration- and voltage dependent peptide aggregation within the membrane without any fixed stoichiometry [40]. It was also described for sapecin, an antibacterial insect defensin isolated from the flesh fly, *Sarcophaga peregrina* [41]. In this study, the initial attraction to the membrane was found to be electrostatic with cardiolipin playing an important role. It had been shown previously that sapecin has a remarkable affinity for cardiolipin which is abundant in *Staphylococcus aureus*. Furthermore, *E. coli* mutants defective in cardiolipin synthesis were resistant to sapecin compared to wild type *E. coli* [42]. Using glucose leakage experiments it was shown that membrane permeabilization is dose-dependent and follows a sigmoidal curve. This cooperativity suggests that oligomerization is an important factor during permeabilization [41]. A similar mechanism was noted in a previous study involving the wasp venom mastoparan which was found to exhibit pore formation dynamics that are concurrent with mellitin but with some differences [43, 44].

A recent review of these peptide modes of action introduces new models that have been proposed. Some of these are variations of the older ones described above [45]. They include the *detergent model*, the *sinking raft model*, the *lipid clustering mode*, the *interfacial activity models* and *molecular shape model*. These models have the common premise of non-pore formation.

Cationic peptide interaction with nucleic acids

There is considerable evidence that shows that some antimicrobial cationic peptides can pass the membrane with minimum disruption, suggesting that they may have intracellular targets. Furthermore several peptides have been shown to bind DNA *in vitro*. Others inhibit important cytosolic proteins thereby interfering with key cellular processes.

When, tachyplesin, a 17 residue arginine-rich peptide, was isolated it was shown to kill bacteria at low concentrations and to form complexes with bacterial lipopolysaccharide [46]. While evidence indicates that tachyplesin interacts with lipid membranes and kills bacteria by leakage, the exact mechanism of leakage and killing remains poorly defined [47]. Tachyplesin I is a cyclic broad-spectrum antimicrobial peptide with a rigid, antiparallel β -sheet and two intramolecular S-S linkages [46]. This structural motif is known to contribute to DNA binding [48]. Indeed, using DNase1 protection and other DNA footprinting-like techniques [49] showed that tachyplesin binds DNA. Furthermore, they showed that it probably binds to the minor groove as methylation of a guanine in the major groove was not affected by the presence of the peptide. However, the antiparallel β -sheet motif has been shown, by 3D solution structures of DNA complexes with proteins, to be involved in DNA binding by making contacts with the major groove [50, 51]. The chemical configuration in the major and minor groove is important as it indicates specificity and non-specificity or interactions respectively.

Another member of the tachyplesin family, polyphemusin I also accumulates in the cytoplasm fairly rapidly without causing membrane damage and shows subtle signs that it may interact with DNA [52]. In crossing the plasma membrane these peptides induce transient pore formation and membrane permeability [53-55]. Using unmodified and PEGylated versions tachyplesin I was shown to induce lipid flip-flops characteristic of the toroidal mode of pore formation. In these experiments, PEGylation did not alter the mode of interaction between the peptide and lipid membranes but lowered both DNA binding ability and antimicrobial activity. It may be reasonable therefore to assume that tachyplesin targets both the membrane and DNA but the main method of bacterial killing is still elusive.

Buforins represent another group of AMPs that translocate across the membrane via transient pores. The 21 amino acid peptide buforin 2 is a more potent derivative of buforin 1 and has broad spectrum antimicrobial activity [56]. It is translocated across the lipid bilayer in a manner similar to maganin2 but without inducing severe membrane permeabilization due to a proline (Pro¹¹) that distorts the helical form of the peptide, concentrating basic amino acids in a limited amphipathic region and thereby enhancing electrostatic repulsion within and efficient translocation through the pore. The rapid and transient nature of the translocation limits membrane permeabilization by buforin 2. DNA-binding studies show that buforin 2 binds DNA and RNA and that buforin influences cellular processes to do with

nucleic acid metabolism [56, 57]. Buforin IIb, an anticancer synthetic analogue of buforin II, crosses the membrane without causing damage and accumulates in the nucleus. Furthermore, buforin IIb accumulates primarily in nuclei of Jurkat cells and induces mitochondria-dependent apoptosis in a mechanism that is not clearly understood [58]. Buforin I and II share complete sequence identity with the N-terminal region of histone H2A (H2A tail) that interacts directly with nucleic acids [59]. The H2A tails play a crucial role in maintaining the stability of the nucleosome by making specific interactions with DNA. In the nucleosome particle, they adopt a disordered conformation with many residues not making contact with DNA. The arginines, however, interact with the minor groove [60]. It is not clear whether the H2A tail interaction with chromatin can be taken as a model for buforin interactions. The helix-hinge-helix structure of buforin has been evaluated using phospholipid interactions but interactions with DNA have so far been demonstrated using techniques such as electrophoretic mobility shift assays. A 3D solution structure of a buforin- DNA complex may elucidate the exact nature of their interaction.

The actual contact between an AMP and DNA was demonstrated with indolicidin, a potent cationic peptide that enters bacterial cells without causing lysis and inhibits DNA replication [61]. These experiments showed that indolicidin assumes different environment-dependent conformations and prefers to bind certain sequences of double stranded DNA and that it binds poorly to single stranded DNA. This provides evidence that peptide-DNA interactions are not simple electrostatic attractions. Specific DNA-peptide interactions are often facilitated by the major groove environment which has richer chemical information than the minor groove [62, 63]. It may be expected then that the peptide makes specific contacts such as hydrogen bonds and hydrophobic interactions in the major groove and electrostatic contacts with the phosphate backbone. Other peptides may interact with nucleic acids. .

The DNA-binding property of cationic peptides together with subcellular localization into the nucleus may provide opportunities for development of delivery systems. Indeed a cationic amphipathic peptide called KALA was designed for delivery of DNA into cells [64]. Similarly the histidine-rich synthetic peptide known as LAH4 was also developed as a DNA carrier that can be used in a wide variety of applications including basic research, therapy and vaccination [65]. These prospects underline the importance of investigating the precise nature of the interaction between cationic peptides and nucleic acids.

Cationic peptide interaction with other subcellular targets

As stated earlier, some antimicrobial peptides have the ability to transiently permeabilize and translocate across the plasma membrane and cause death of the target pathogen without causing cell lysis. This indicates that these peptides may have intracellular targets. It is recorded that such cellular targets could include macromolecules in protein and lipid biosynthetic pathways and in nucleic acid metabolism (Table 3 and 4). It has not been established whether there are unique characteristics possessed by this class of peptides enabling them to target intracellular molecules.

Peptides	Mode of action	Reference
Buforin II and buforin IIb	Binds DNA	[56, 58, 70]
Tachyplesin	Binds DNA	[48, 121]
Mersadin	Inhibits cell wall synthesis	[122]
PR-39	Inhibits replication and protein synthesis	[123]
PR-26	Alters cytoplasmic membrane	[124]
Indolicidin	Replication, Alters cytoplasmic membrane	[61, 125]
Microcin 25	Alters cytoplasmic membrane	[126]
Pleurocidin	Inhibits nucleic acid metabolism	[127]
HNP-1	Inhibits nucleic acid metabolism	[128]
HNP-2	Inhibits nucleic acid metabolism	[128]
Dermaseptin	Inhibits nucleic acid metabolism	[127]
Histatins	Inhibits enzyme activity	[129]
Pyrrhocoricin	Inhibits enzyme activity	[129]
Drosocin	Inhibits enzyme activity	[129]
apidaecin	Inhibits enzyme activity	[129]
Pre-elafin/trappin-2	Binds DNA	[130, 131]
Lactoferricin	Regulation of transcription	[54] and references therein
Cecropin A	Gene expression	[132]

Table 3. Cationic peptides with intracellular killing activities

AMP	Interacting molecule	Reference
PR-39	Membrane receptor, multiple, SH3 domain-containing intracellular proteins and p85a (regulatory subunit of phosphatidylinositol 3-kinase, (nucleic acids unconfirmed)	[123, 133]
Buforin II	Nucleic acids (both RNA and DNA), inhibits transcription or translation	[56]
Mellitin	Hyperactivation of phospholipase A ₂	
Tachyplesin	C1q activating the class complement pathway	[91]
Lactoferricin B	Heparin-like molecules preventing angiogenesis	[96]
Histatin 5	67 kDa fungal protein	[74]
Histatin 5	<i>B. gingivalis</i> trypsin-like protease	[78]
Pyrrhocoricin, drosocin, apidaecin	DnaK preventing chaperone-assisted protein folding	[66, 67]
Apidaecins	Probably a permease transporter and protein involved in protein synthesis	[85]
Cathelicidin LL-37/ hCAP-18	binds to formyl peptide receptor-like 1 (FPRL1), a G protein-coupled, seven-transmembrane cell receptor found on various cell types including macrophages, neutrophils and subsets of lymphocytes	[100]
Mouse Cathelin-related antimicrobial peptide (CRAMP)	binds to formyl peptide receptor-like 1 (FPRL1)	[101]

Table 4. Putative non-lipid molecular targets of CAPs

Peptides that belong to the proline-rich family, pyrrocoricin, apidaecin and drosocin enter bacterial cells and macrophages and are distributed in all cellular compartments. These peptides bind specifically to the *E. coli* 70 kDa heat shock protein, DnaK preventing chaperone-assisted protein folding and death of the bacterium [66]. They appear to enter the cell in a LPS-mediated manner. Importantly, they do not bind to the equivalent human Hsp70 protein, pointing to a potential pharmaceutical benefit [67].

Proline is known to be a unique amino acid in facilitating macromolecular binding. Due to some unique biophysical reasons proline was found to facilitate macromolecular interactions by means of proline-rich motifs or even as a single proline residue [68]. Indeed it has been suggested that proline-rich modules may be a natural occurrence that facilitates membrane penetration [69]. Several examples have been recorded indicating that proline is important in AMP activity. The DNA-binding buforin II has a proline hinge which is crucial for membrane penetration [59, 70]. Cathelicidins have a α -helical N-terminus with antibacterial activity and a proline-containing C-terminus that is required for membrane penetration [69]. The endogenous proline-arginine (PR)-rich peptide, PR-39 inhibits NADPH oxidase by docking to the Src homology 3 (SH3) domain of this enzyme [71]. PR-39 is also implicated in blocking DNA replication [72]. This is consistent with established observations that proline-rich motifs are crucial for bind to signaling molecules with domains such as SH3 [68]. Detailed Structure-based analysis of the proline-rich motif and SH3 domain interaction shows how a crucial RXL motif in proline-rich ligands binds to the SH3 domain [73]. Systematic mutations of residues in the SH3 domain and the proline-rich ligand revealed that two crucial prolines interact directly with the domain while others form a molecular scaffold. Furthermore, arginine and lysine residues are involved in extensive interactions conferring specificity.

Some AMPs that are likely to have intracellular targets use unconventional mechanisms to enter the cell. These include the histatin family and apidaecins. Histatins, a family of histidine-rich AMPs found in human saliva enter the cell in a receptor-mediated manner and target the mitochondria [74]. The histatin family consists of AMPs that have potent activity against fungi and constitutes an important aspect of antifungal and wound healing activity in the oral cavity [74, 75]. It was found that histatin 5 kills intact *Candida albicans* without causing lysis and that spheroplasts (fragile with fragments of the cell wall) were 14-fold less susceptible compared to the intact cells. Binding studies showed that histatin 5 targets at least one specific protein that was detected in whole cell extracts and crude membrane fractions but not in the cell wall fraction and in spheroplasts [76]. Surprisingly, the human neutrophil defensin 1 (HNP-1) which differs structurally to histatins appeared to act in the same manner as histatin 5, probably sharing the same molecular target in *Candida albicans* [77]. Besides, histatin 5 was found to be an inhibitor of *B. gingivalis* trypsin-like protease probably accounting for natural protection against periodontitis [78]. They reduce the activity of a *Bacteroides gingivalis* trypsin-like protease by competitive inhibition [78]. This protease may be responsible for the periodontitis caused by *B. gingivalis* [79, 80], implying that histatins play an important role in combating oral pathogens. This was initially observed with lantibiotics such as nisin Z which uses Lipid II as a receptor [81] and

mesentericin Y, a 37 amino acid peptide isolated from *Leuconostoc mesenteroides*. This peptide targets a specific receptor found only on the food-borne *listeria*. Generally, these peptides have a characteristic structure with two domains; a recognition domain for binding to a receptor and an α -helical domain responsible for pore formation. Removal of the recognition domain results in loss of pathogen selectivity.

Some peptides exhibit anti-viral activity by mechanisms that albeit poorly understood, appear to be non-membrane dependent. The synthetic [Tyr5,12,Lys7]-polyphemusin II peptide (T22) inhibits HIV replication apparently by competition with cellular proteins required for viral attachment e.g. CD4 [82]. Mellitin and its inactive analogue can competitively inhibit the infectivity of the tobacco mosaic virus due to structural similarities with the virus capsid region required for RNA interaction [83].

Apidaecins are short proline-arginine-rich and highly antibacterial peptides that kill Gram-negative bacteria without forming pores [84, 85]. Their activity is limited to Gram-negative bacteria. Interestingly, they are distant relatives of the mammalian peptide PR-39. Apidaecin uptake was found to be actively driven by an energy-dependent mechanism, stereospecific and irreversible. The transporter-mediated model was demonstrated by the fact that pretreatment of cells with an oxidative phosphorylation uncoupler reduced uptake of apidaecin but did not prevent the uptake of a known pore-former, D-Mag (all D-magainin isomer). Furthermore, uptake of the apidaecin peptide was reduced by the presence of a proline-rich peptide (L-Pro) but not its enantiomer (D-Pro) indicating receptor dependence. Apidaecin may also act downstream on at least one indispensable cellular target as some peptide analogs entered the cell without killing it. Inhibition of protein synthesis by apidaecin suggests that it interferes with the translation machinery of the bacterium. The probable target is the 30S ribosomal subunit as cooperative inhibition by tetracycline (a known inhibitor of this subunit) and apidaecin was demonstrated [85]. Apidaecin is also implicated in interfering with protein folding by inhibiting the activity of DnaK [67, 86]. Since apidaecins are non-toxic to human cells better understanding of their mode of action is necessary. It seems probable that the intracellular targets of apidaecin are unique to Gram-negative bacteria. Nevertheless, the identification of intracellular targets of AMPs in general is important for the design of species- or strain-specific drugs.

Role of cationic peptides in anticancer therapy and wound healing

Current anticancer agents have limited success due to non-selective killing of cancer and normal cells and often result in the development of resistance. The discovery of new anticancer strategies is therefore urgent. Many studies have shown that cationic AMPs have anticancer properties. These peptides are divided into two classes; one that consists of peptides that are toxic to bacteria and cancer cells but not to human cells and another class with peptides that are toxic bacteria and to both cancer and normal human cells [87]. It is believed that they have membranolytic and non-membranolytic modes of action [8, 28]. The membranolytic activity is presumed to be based on the different compositions of cancer and normal membranes and includes the disruption of mitochondrial membrane. The disruption of the membrane probably occurs by some of the modes describes earlier; such as

the “carpet” model. These peptides can also cause permeation of the mitochondrial membrane releasing cytochrome c followed by apoptosis. Such apoptosis would also cause caspase 9 activation and conversion of pro-caspase3 to caspase 3. Buforin IIb which displayed selective cytotoxicity against 62 cell lines provides a good example in this instance. It crosses cell membranes without causing damage and causes mitochondria-dependent apoptosis characterized by caspase 9 activation [58]. The exact mechanism of apoptotic killing is not clear as it is for many other cationic peptides. Mitochondria-dependent apoptosis can also occur by the death receptor associated pathway [88].

It seems that different Amps induce different apoptotic pathways and membranolytic mechanisms. A COOH-terminal fragment of the cathelicidin LL-37 pre-protein, hCAP-18 was found to selectively kill oral squamous carcinoma cells and not healthy human fibroblast or HaCaT cells by apoptosis that is characterized by mitochondrial depolarisation with no detectable caspase 3 or in a caspase-independent mechanism [89]. Tachyplesin that was conjugated to an integrin homing peptide killed both tumour and endothelial cells by a mitochondrial and death receptor -dependent pathways [90]. On the other hand, tachyplesin was shown to kill tumour cells by interacting with hyaluronan and C1q a key component of the complement pathway thus activating the classic complement pathway leading to loss of membrane integrity and cell lysis [91].

The non-membranolytic mechanism is probably facilitated by interaction with specific proteins or through processes that activate specific intracellular molecules. Mellitin is reported to selectively promote the destruction of ras oncogene-transformed cells by preferentially activating phospholipase A2 and causing calcium influx [92, 93]. Lactoferricin B (LfcinB), a cationic AMP that is cytotoxic to human and rodent cancer cells, kills human leukaemia and breast carcinoma cells by a sequential process involving generation of reactive oxygen species, mitochondrial membrane depolarization and activation of the caspase cascade leading to death by apoptosis [94]. However, LfcinB kills human B-lymphoma cells in a caspase-independent mechanism [95]. Furthermore, LfcinB was found to prevent angiogenesis by interacting with a heparin-like molecule on the surface of human umbilical vein endothelial cells (HUVECs) [96].

There is a growing number of AMPs that appear to promote wound healing. It is generally noticeable that wounds in the oral cavity heal faster than skin lesions for example but it has emerged recently that this may be attributable to the histatin family, at least in part. At least two histatins have been identified as the major wound healing factors in human saliva [75]. Moreover this property was associated with active uptake of histatin by epithelial cells and the activation of an extracellular signal-regulated kinases $\frac{1}{2}$ signalling pathway suggesting a mechanism by which these peptides effect their non-AMP role. Wound healing is a localized process which involves inflammation, wound cell migration and mitosis, neovascularization, and regeneration of the extracellular matrix and is known to be mediated by peptide growth factors such as the epidermal growth factor (EGF) and transforming growth factor alpha (TGF- α) [97]. At least TGF- α has been shown to act by activating the expression of AMPs hCAP-18/LL-37 and human β -defensin 3 in addition to the larger proteins often found during

injury, the neutrophil gelatinase-associated lipocalin, and secretory leukocyte protease inhibitor in human keratinocytes [98]. And cathelicidins are known to regulate cellular responses including cell proliferation, cell migration of inflammatory cells, release of cytokines and angiogenesis [99]. The cathelicidin hCAP-18 interacts with formyl peptide receptor-like 1 (FPRL1), a G protein-coupled, seven transmembrane cell receptor [100]. And the only known mouse cathelicidin-like protein the cathelin-related antimicrobial peptide (CRAMP) known to be angiogenic was further shown to be chemotactic for human monocytes, neutrophils, macrophages, and mouse peripheral blood leukocytes [101]. Clearly, as the multi-functional role of antimicrobial peptides unravels, the number of peptides involved in non-infection related processes and new molecular targets are set to increase.

3. Drug design strategies

Cationic antimicrobial peptides have key characteristics that make them attractive candidates for pharmaceutical development: (i) they are active against a broad spectrum of Gram-negative and Gram-positive bacteria (including the multiple drug resistant strains), fungi, viruses and protozoa – a single peptide can act against all these pathogens (ii) generally, they do not target specific pathogen molecules reducing development of resistance, and (iii) they are potent and kill pathogens rapidly [1, 102]. There are, however, obstacles that hinder the commercialization of AMPs. Commercialization of antimicrobial peptides is hindered by various pharmacokinetic obstacles that may require some engineering to resolve and are indeed the object of intensive research worldwide. Absorption, distribution, metabolism and excretion (ADME) are vital pharmacokinetic parameters that must be satisfied by successful drug candidates, and major challenges have emerged with respect to peptide drugs. Some of the key shortcomings that should be addressed to improve rational peptide-based drug design are:

- i. Low bioavailability
- ii. Toxicity
- iii. High cost of production

There are several ideas about to overcome some of them (Table 5) and many researchers are investigating ways to remove these obstacles and move to commercialization.

Bioavailability and biodistribution

Peptide drugs have to overcome barriers that affect absorption, transport (systemic distribution) and translocation through membranes. These barriers are associated with the physicochemical properties of peptides such as aqueous solubility, lipophilicity, hydrogen bond formation and metabolic stability. Rapid degradation by proteolytic enzymes of the digestive tract, blood plasma and tissues is one of the major limitations attributed to peptide drugs as it limits oral availability and injection. These scenarios are further complicated by the fact that peptides are also subjected to rapid clearance by the liver and kidneys. Their physicochemical properties such as hydrophilicity and high conformation flexibility (no selectivity by specific receptors) also affect biodistribution.

Limitation	Cause	Solution	Reference
High cost	Regulatory and technological factors	Development of efficient and robust process of chemical synthesis Design short and compositionally simple peptides	[114, 134]
Low (especially oral) bioavailability	Peptides being substrates of digestive enzymes, blood plasma and tissues Rapid hepatic clearance Rapid renal clearance Poor biodistribution	Use of D-amino acids Peptide backbone alterations Protective delivery systems Chemical modification of protease cleavage sites	[1, 135]
Poor biodistribution	Hydrophilicity High conformational flexibility – non selective		
Toxicity	Immunogenicity Non-specific targets Some act on growth factors (wound healing) – may promote tumourigenesis	Pro-drug use e.g mellitin-biotin conjugates	[111]

Table 5. Challenges in drug design

There are several approaches to optimize lead peptides to circumvent bioavailability and biodistribution obstacles. These include (i) replacement of natural with unnatural or D- (rather than L-) amino acids, (ii) use of peptidometrics introducing non-peptidic backbones, (iii) adopting alternative formulations such as liposomes and (iv) modification to create protease resistant prodrugs [103]. The routes of drugs given systemically and orally are shown schematically in Figure 2 and Box 1 to indicate pharmacokinetic obstacles.

Figure 2. Distribution of drugs given intravenously and orally and obstacles that affect bioavailability. To be read together with Box 1.

BOX 1

When a drug is given intravenously it enters the systemic circulation via the right ventricle of the heart, flows past the lungs, into the left ventricle and finally into the rest of the circulatory system (Figure 2). Oral administration introduces the peptide into the strongly acidic environment of the stomach and later to high levels of proteolytic enzymes in the intestines. The main limitations to bioavailability of antimicrobial peptides are pre-systemic and systemic enzymatic degradation. When the peptide is given orally it could also undergo “first-pass” metabolism in the liver and the gastrointestinal tract. The major threat to the peptides lies in the small intestine where there are large quantities of peptidases [136]. Oral and intravenous delivery of peptides is therefore a major challenge for pharmaceutical science and demands innovative strategies. Biological barriers such as the Blood Brain Barrier (BBB) and placenta are additional obstacles to delivery of AMPs.

Strategies that can be considered to circumvent these problems are:

- i. Alternative routes of administration
 - Subcutaneous injection
 - Intramuscular
 - Mucosal (nasal sprays)
 - Sublingual delivery
 - Transdermal routes (patches)
- ii. Penetration enhancers
- iii. Protease inhibitors

Toxicity

Broadly, antimicrobial peptides are able to disrupt prokaryotic but not eukaryotic membranes because the latter are composed of zwitterionic phospholipids and contain cholesterol. Consequently, they appear to be non-toxic to animals. However, some peptides have been shown to translocate into cells and even carry other molecules with them. Indeed some cationic peptides are proposed as carriers for macromolecules such as DNA in certain instances [104, 105]. Due to incomplete knowledge about the action of AMPs on the eukaryotic cell, toxic effects of their application cannot be ignored or taken lightly. Indeed, all the commercially available AMPs are for topical applications and there is lack of confidence in other forms of administration.

About 38% of drug candidates are abandoned in Phase I clinical trials because of toxicity [106]. However, many cationic antimicrobial peptides appear to have no cytotoxicity against mammalian and are therefore considered good candidates for treating infections. One example is plecostasin, a defensin with a derivative known as NZ2114 and shown to have additional physicochemical benefits that allow it to cross the blood brain barrier making it attractive for treating meningitis [107]. Furthermore plecostasin can be used at high doses without toxicity to animal cells [108]. Apidaecins constitute another group of apparently non-toxic candidates and have been discussed earlier in the chapter. Toxicity, of antimicrobial peptides is still a matter not rigorously investigated to date. Often their hemolytic activity is tested.

There are many unresolved issues about the mechanism of killing of AMPs compounded by the probable existence of intracellular targets. Fears are caused by the possibility that toxicity could emerge *in vivo* based on interaction between the AMPs and unknown subcellular targets. Currently, there is accumulating evidence showing that AMPs can kill eukaryotic cells by apoptosis. Two cathelicidins, BMAP-27 and BMAP-28 were shown to be toxic to transformed cell lines, fresh tumor cells and proliferating lymphocytes at microbicide concentrations. This cytotoxicity is associated with membrane disruption, calcium influx and subsequent apoptosis [109]. AMPs are apparently attracted to these cells because of an increase in negative charge introduced by sialylation of glycoproteins on transformed cells and activated lymphocytes as treatment of U937 cell by neuraminidase abrogated the toxic effect. Furthermore, the human cathelicidin LL-37 was shown to induce apoptosis *in vitro* in a human airway epithelial cell line and *in vivo* in a murine airway [110]. LL-37 induced dose-dependent and caspase 3 dependent apoptosis in human lung epithelial cell line A549 [110]. This cell death was inhibited by caspase 3 inhibitor and by human but not by bovine serum. Clearly, there is need to investigate the physiological impact of AMPs. Toxicity can be addressed by several means including the use of prodrug format whereby a drug conjugate is designed to be activated at specific tissues. For instance, the anticancer peptide mellitin was conjugated to a biotin moiety which could be selectively cleaved in ovarian carcinoma cells by matrix metalloproteinase-2 which are highly expressed in these cells [111].

Cost of production

Cost of peptide production tended to increase at an alarming rate in the past decade due to regulatory and technological factors [112]. Technically, the cost of producing a peptide is dependent on size. Size also determines the method of production. Peptides may be produced by chemical synthesis, recombinant DNA technology, cell free expression systems, enzymatic synthesis and by the use of transgenic animals and plants. Since these peptides can sometimes involve unnatural amino acids, chemical synthesis may provide a wide range of peptide derivatives. To this end the discovery of the solid phase peptide synthesis method was a major step boosting peptide drug production [113]. It is now possible to produce peptides as long as 50 amino acids by chemical synthesis and produce therapeutic peptides on a large scale [114]. Indeed, now the chemical synthesis of peptides provides cheaper manufacturing costs compared to recombinant production [103]. This obstacle is likely to be overcome in the near future.

4. Conclusion

Cationic peptides are attractive molecules for clinical use. They have multifunctional properties as anti-infective agents that are able to kill bacteria, fungi, viruses and parasites as well as cancer cells. Their activity depends largely on their structural features and unique features in the landscape of prokaryotic and eukaryotic cell membranes. Some of these peptides have gone through clinical trials and reached commercialization but only for topical application. There are many obstacles that hinder development of cationic peptides for administration by the oral route or by injection. These are bioavailability, biodistribution and potential toxicity. These obstacles can be overcome by better understanding of the mechanism of action and killing of these peptides. More research is required in this area. The high cost of production has been a major obstacle for a long time. New advances in the chemical synthesis technology have greatly reduced the cost of production and now large scale chemical synthesis is possible. This method is preferable because of the opportunities it provides for the inclusion of unnatural amino acids during production. Recombinant DNA synthesis is another method that can be perfected to manufacture peptides at low cost.

Author details

Monde Ntwasa

School of Molecular & Cell Biology, University of the Witwatersrand

5. References

- [1] Hancock REW, Sahl H-G. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nat Biotech.* [10.1038/nbt1267]. 2006;24(12):1551-7.
- [2] Wiesner J, Vilcinskas A. Antimicrobial peptides: The ancient arm of the human immune system. *Virulence.* 2010;1(5):440-64.

- [3] Cederlund A, Gudmundsson GH, Agerberth B. Antimicrobial peptides important in innate immunity. *FEBS Journal*. 2011;278(20):3942-51.
- [4] Medzhitov R, Janeway JCA. Innate immunity: The virtues of a nonclonal system of recognition. *Cell*. 1997;91:295-8.
- [5] Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell*. 2006;124(4):783-801.
- [6] Medzhitov R, Janeway Jr CA. Innate immunity: impact on the adaptive immune response. *Current Opinion in Immunology*. 1997;9(1):4-9.
- [7] Salzberg M. Vertebrate innate immunity resembles a mosaic of invertebrate immune responses. *Trends in Immunology*. 2001;22(6):285-8.
- [8] Hoskin DW, Ramamoorthy A. Studies on anticancer activities of antimicrobial peptides. *Biochimica et Biophysica Acta (BBA) - Biomembranes*. 2008;1778(2):357-75.
- [9] Wachinger M, Kleinschmidt A, Winder D, von Pechmann N, Ludvigsen A, Neumann M, et al. Antimicrobial peptides melittin and cecropin inhibit replication of human immunodeficiency virus 1 by suppressing viral gene expression. *Journal of General Virology*. 1998;79(4):731-40.
- [10] Stein T, Vater J, Kruff V, Otto A, Wittmann-Liebold B, Franke P, et al. The multiple carrier model of nonribosomal peptide biosynthesis at modular multienzymatic templates. *Journal of Biological Chemistry*. 1996;271(26):15428-35.
- [11] Hancock REW, Chapple DS. Peptide antibiotics. *Antimicrobial Agents and Chemotherapy*. 1999;43(6):1317-23.
- [12] Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC. Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. *Journal of Antimicrobial Chemotherapy*. 1997;40(1):135-6.
- [13] Uttley A, Collins C, Naidoo J, George R. Vancomycin-resistant *enterococci*. *Lancet*. 1988;1(8575-76):57-8.
- [14] deVos WM, Mulders JW, Siezen RJ, Hugenholtz J, Kuipers OP. Properties of nisin Z and distribution of its gene, *nisZ*, in *Lactococcus lactis*. *Appl Environ Microbiol*. 1993;59(1):213-8.
- [15] Brötz H, Sahl H-G. New insights into the mechanism of action of lantibiotics—diverse biological effects by binding to the same molecular target. *Journal of Antimicrobial Chemotherapy*. 2000;46(1):1-6.
- [16] Brogden KA. Antimicrobial peptides: Pore formers or metabolic inhibitors in bacteria? *NATURE REVIEWS MICROBIOLOGY*. 2005;3:238-50.
- [17] Reddy KVR, Yedery RD, Aranha C. Antimicrobial peptides: premises and promises. *International Journal of Antimicrobial Agents*. 2004;24(6):536-47.
- [18] Kawano K, Yoneya T, Miyata T, Yoshikawa K, Tokunaga F, Terada Y, et al. Antimicrobial peptide, tachyplesin I, isolated from hemocytes of the horseshoe crab (*Tachyplesus tridentatus*). NMR determination of the beta-sheet structure. *Journal of Biological Chemistry*. 1990;265(26):15365-7.
- [19] Tamamura H, Kuroda M, Masuda M, Otaka A, Funakoshi S, Nakashima H, et al. A comparative study of the solution structures of tachyplesin I and a novel anti-HIV synthetic peptide, T22 ([Tyr^{5,12}, Lys⁷]-polyphemusin II), determined by nuclear

- magnetic resonance. *Biochim Biophys Acta (BBA) - General Subjects*. 1993;1163(2):209-16.
- [20] Hwang PM, Zhou N, Shan X, Arrowsmith CH, Vogel HJ. Three-dimensional solution structure of lactoferricin B, an antimicrobial peptide derived from bovine lactoferrin. *Biochemistry*. 1998 1998/03/01;37(12):4288-98.
- [21] Zwaal RFA, Schroit AJ. Pathophysiologic Implications of Membrane Phospholipid Asymmetry in Blood Cells. *Blood*. 1997;89(4):1121-32.
- [22] Brown DA, London E. Structure and origin of ordered lipid domains in biological membranes. *Journal of Membrane Biology*. 1998;164(2):103-14.
- [23] Rana F, Macias E, Sultany C, Modzrakowski M, Blazyk J. Interactions between magainin 2 and *Salmonella typhimurium* outer membranes: effect of lipopolysaccharide structure. *Biochemistry*. 1991;30(24):5858-66.
- [24] Contreras I, Shapiro L, Henry S. Membrane phospholipid composition of *Caulobacter crescentus*. *Journal of Bacteriology*. 1978;135(3):1130-6.
- [25] Tucker AN, White DC. Heterogeneity of phospholipid composition in the bacterial membrane. *Journal of Bacteriology*. 1970;102(2):508-13.
- [26] Rana FR, Blazyk J. Interactions between the antimicrobial peptide, magainin 2, and *Salmonella typhimurium* lipopolysaccharides. *FEBS Letters*. 1991;293(1-2):11-5.
- [27] Hancock REW. Peptide antibiotics. *The Lancet*. 1997;349(9049):418-22.
- [28] Schweizer F. Cationic amphiphilic peptides with cancer-selective toxicity. *Eur J Pharmacol*. 2009;625(1-3):190-4.
- [29] Utsugi T, Schroit AJ, Connor J, Bucana CD, Fidler IJ. Elevated expression of phosphatidylserine in the outer membrane leaflet of human tumor cells and recognition by activated human blood monocytes. *Cancer Research*. 1991;51(11):3062-6.
- [30] Matsuzaki K. Why and how are peptide-lipid interactions utilized for self defence? *Biochemical Society Transactions*. 2001;29:598-601.
- [31] Matsuzaki K, Harada M, Handa T, Funakoshi S, Fujii N, Yajima H, et al. Magainin 1-induced leakage of entrapped calcein out of negatively-charged lipid vesicles. *Biochimica et Biophysica Acta (BBA) - Biomembranes*. 1989;981(1):130-4.
- [32] Ntwasa M, Goto A, Kurata S. Coleopteran Antimicrobial Peptides: Prospects for Clinical Applications. *International Journal of Microbiology*. 2012;In press.
- [33] Shai Y. Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by α -helical antimicrobial and cell non-selective membrane-lytic peptides. *Biochim et Biophys Acta* 1999;1462 55-70.
- [34] Shai Y. Mode of action of membrane active antimicrobial peptides. *Peptide Science*. 2002;66(4):236-48.
- [35] Yang L, Harroun TA, Weiss TM, Ding L, Huang HW. Barrel-stave model or toroidal model? A case study on melittin pores. *Biophysical Journal*. 2001;81(3):1475-85.
- [36] Oren Z, Shai Y. A class of highly potent antibacterial peptides derived from pardaxin, a pore-forming peptide isolated from Moses sole fish *Pardachirus marmoratus*. *European Journal of Biochemistry*. 1996;237(1):303-10.
- [37] Zagorski MG, Norman DG, Barrow CJ, Iwashita T, Tachibana K, Patel DJ. Solution structure of pardaxin P-2. *Biochemistry*. 1991 1991/08/01;30(32):8009-17.

- [38] Matsuzaki K, Murase O, Fujii N, Miyajima K. An antimicrobial peptide, magainin 2, induced rapid flip-flop of phospholipids coupled with pore formation and peptide translocation. *Biochemistry*. 1996 1996/01/01;35(35):11361-8.
- [39] Ludtke SJ, He K, Heller WT, Harroun TA, Yang L, Huang HW. Membrane pores induced by magainin. *Biochemistry*. 1996 1996/01/01;35(43):13723-8.
- [40] Wu M, Maier E, Benz R, Hancock REW. Mechanism of interaction of different classes of cationic antimicrobial peptides with planar bilayers and with the cytoplasmic membrane of *Escherichia coli*. *Biochemistry*. 1999 1999/06/01;38(22):7235-42.
- [41] Takeuchi K, Takahashi H, Sugai M, Iwai H, Kohno T, Sekimizu K, et al. Channel-forming membrane permeabilization by an antibacterial protein, sapecin. *Journal of Biological Chemistry*. 2004;279(6):4981-7.
- [42] Matsuyama K, Natori S. Mode of action of sapecin, a novel antibacterial protein of *Sarcophaga peregrina* (Flesh Fly). *Journal of Biochemistry*. 1990;108(1):128-32.
- [43] Arbuzova A, Schwarz G. Pore-forming action of mastoparan peptides on liposomes: a quantitative analysis. *Biochimica et Biophysica Acta (BBA) - Biomembranes*. 1999;1420(1-2):139-52.
- [44] Whiles JA, Brasseur R, Glover KJ, Melacini G, Komives EA, Vold RR. Orientation and effects of mastoparan X on phospholipid bicelles. *Biophysical Journal*. 2001;80(1):280-93.
- [45] Wimley W, Hristova K. Antimicrobial peptides: Successes, challenges and unanswered questions. *J Membrane Biol*. 2011;239(1):27-34.
- [46] Nakamura T, Furunaka H, Miyata T, Tokunaga F, Muta T, Iwanaga S, et al. Tachyplesin, a class of antimicrobial peptide from the hemocytes of the horseshoe crab (*Tachyplesus tridentatus*). Isolation and chemical structure. *Journal of Biological Chemistry*. 1988;263(32):16709-13.
- [47] Matsuzaki K, Fukui M, Fujii N, Miyajima K. Interactions of an antimicrobial peptide, tachyplesin I, with lipid membranes. *Biochimica et Biophysica Acta (BBA) - Biomembranes*. 1991;1070(1):259-64.
- [48] Yonezawa A, Sugiura Y. Tachyplesin I as a model peptide for antiparallel beta-sheet DNA binding motif. *Nucleic Acids Symp Ser* 1992;27:161-2.
- [49] Yonezawa A, Kuwahara J, Fujii N, Sugiura Y. Binding of tachyplesin I to DNA revealed by footprinting analysis: significant contribution of secondary structure to DNA binding and implication for biological action. *Biochemistry*. 1992 1992/03/01;31(11):2998-3004.
- [50] Allen MD, Yamasaki K, Ohme-Takagi M, Tateno M, Suzuki M. A novel mode of DNA recognition by a [beta]-sheet revealed by the solution structure of the GCC-box binding domain in complex with DNA. *EMBO J*. [10.1093/emboj/17.18.5484]. 1998;17(18):5484-96.
- [51] Raumann BE, Rould MA, Pabo CO, Sauer RT. DNA recognition by β -sheets in the Arc repressor-operator crystal structure. *Nature*. [10.1038/367754a0]. 1994;367(6465):754-7.
- [52] Powers J-PS, Martin MM, Goosney DL, Hancock REW. The antimicrobial peptide polyphemusin localizes to the cytoplasm of *Escherichia coli* following treatment. *Antimicrobial Agents and Chemotherapy*. 2006;50(4):1522-4.

- [53] Hirakura Y, Kobayashi S, Matsuzaki K. Specific interactions of the antimicrobial peptide cyclic β -sheet tachyplesin I with lipopolysaccharides. *Biochimica et Biophysica Acta (BBA) - Biomembranes*. 2002;1562(1–2):32–6.
- [54] Epand RM, Vogel HJ. Diversity of antimicrobial peptides and their mechanisms of action. *Biochimica et Biophysica Acta (BBA) - Biomembranes*. 1999;1462(1–2):11–28.
- [55] Matsuzaki K, Yoneyama S, Fujii N, Miyajima K, Yamada K-i, Kirino Y, et al. Membrane permeabilization mechanisms of a cyclic antimicrobial peptide, tachyplesin I, and its linear analog. *Biochemistry*. 1997 1997/08/01;36(32):9799–806.
- [56] Park CB, Kim HS, Kim SC. Mechanism of action of the antimicrobial peptide buforin II: Buforin II kills microorganisms by penetrating the cell membrane and inhibiting cellular functions. *Biochem Biophys Res Commun* 1998;244(1):253–7.
- [57] Kobayashi S, Chikushi A, Tougu S, Imura Y, Nishida M, Yano Y, et al. Membrane translocation mechanism of the antimicrobial peptide buforin 2. *Biochemistry*. 2004 2004/12/01;43(49):15610–6.
- [58] Lee HS, Park CB, Kim JM, Jang SA, Park IY, Kim MS, et al. Mechanism of anticancer activity of buforin IIb, a histone H2A-derived peptide. *Cancer Letters*. 2008;271(1):47–55.
- [59] Cho JH, Sung BH, Kim SC. Buforins: Histone H2A-derived antimicrobial peptides from toad stomach. *Biochimica et Biophysica Acta (BBA) - Biomembranes*. 2009;1788(8):1564–9.
- [60] Biswas M, Voltz K, Smith JC, Langowski J. Role of histone tails in structural stability of the nucleosome. *PLoS Comput Biol*. 2011;7(12):e1002279.
- [61] Hsu C-H, Chen C, Jou M-L, Lee AY-L, Lin Y-C, Yu Y-P, et al. Structural and DNA-binding studies on the bovine antimicrobial peptide, indolicidin: evidence for multiple conformations involved in binding to membranes and DNA. *Nucleic Acids Research*. 2005;33(13):4053–64.
- [62] White S, Szewczyk JW, Turner JM, Baird EE, Dervan PB. Recognition of the four Watson-Crick base pairs in the DNA minor groove by synthetic ligands. *Nature*. [10.1038/35106]. 1998;391(6666):468–71.
- [63] Kielkopf CL, White S, Szewczyk JW, Turner JM, Baird EE, Dervan PB, et al. A structural basis for recognition of A·T and T·A base pairs in the minor groove of B-DNA. *Science*. 1998;282(5386):111–5.
- [64] Wyman TB, Nicol F, Zelphati O, Scaria PV, Plank C, Szoka FC. Design, synthesis, and characterization of a cationic peptide that binds to nucleic acids and permeabilizes bilayers. *Biochemistry*. 1997 1997/03/01;36(10):3008–17.
- [65] Kichler A, Mason AJ, Bechinger B. Cationic amphipathic histidine-rich peptides for gene delivery. *Biochimica et Biophysica Acta (BBA) - Biomembranes*. 2006;1758(3):301–7.
- [66] Kragol G, Lovas S, Varadi G, Condie BA, Hoffmann R, Otvos L. The antibacterial peptide pyrrocoricin inhibits the ATPase actions of DnaK and prevents chaperone-assisted protein folding. *Biochemistry*. 2001 2001/03/01;40(10):3016–26.
- [67] Otvos L, O I, Rogers ME, Consolvo PJ, Condie BA, Lovas S, et al. Interaction between heat shock proteins and antimicrobial peptides. *Biochemistry*. 2000 2000/11/01;39(46):14150–9.

- [68] Kay BK, Williamson MP, Sudol M. The importance of being proline: the interaction of proline-rich motifs in signaling proteins with their cognate domains. *The FASEB Journal*. 2000;14(2):231-41.
- [69] Kragol G, Hoffmann R, Chattergoon MA, Lovas S, Cudic M, Bulet P, et al. Identification of crucial residues for the antibacterial activity of the proline-rich peptide, pyrrhocoricin. *European Journal of Biochemistry*. 2002;269(17):4226-37.
- [70] Jang SA, Kim H, Lee JY, Shin JR, Kim DJ, Cho JH, et al. Mechanism of action and specificity of antimicrobial peptides designed based on buforin IIb. *Peptides*. 2012(0).
- [71] Shi J, Ross CR, Leto TL, Blecha F. PR-39, a proline-rich antibacterial peptide that inhibits phagocyte NADPH oxidase activity by binding to Src homology 3 domains of p47 phox. *Proceedings of the National Academy of Sciences*. 1996;93(12):6014-8.
- [72] Lehrer RI, Ganz T. Cathelicidins: a family of endogenous antimicrobial peptides. *Current Opinion in Hematology*. 2002;9(1):18-22.
- [73] Yu H, Chen JK, Feng S, Dalgarno DC, Brauer AW, Schreier SL. Structural basis for the binding of proline-rich peptides to SH3 domains. *Cell*. 1994;76(5):933-45.
- [74] Kavanagh K, Dowd S. Histatins: antimicrobial peptides with therapeutic potential. *Journal of Pharmacy and Pharmacology*. 2004;56(3):285-9.
- [75] Oudhoff MJ, Bolscher JGM, Nazmi K, Kalay H, van 't Hof W, Amerongen AVN, et al. Histatins are the major wound-closure stimulating factors in human saliva as identified in a cell culture assay. *The FASEB Journal*. 2008;22(11):3805-12.
- [76] Edgerton M, Koshlukova SE, Lo TE, Chrzan BG, Straubinger RM, Raj PA. Candidacidal activity of salivary histatins. *Journal of Biological Chemistry*. 1998;273(32):20438-47.
- [77] Edgerton M, Koshlukova SE, Araujo MWB, Patel RC, Dong J, Bruenn JA. Salivary histatin 5 and human neutrophil defensin 1 kill *Candida albicans* via shared pathways. *Antimicrob Agents Chemother*. 2000;44(12):3310-6.
- [78] Nishikata M, Kanehira T, Oh H, Tani H, Tazaki M, Kuboki Y. Salivary histatin as an inhibitor of a protease produced by the oral bacterium *Bacteroides gingivalis*. *Biochemical and Biophysical Research Communications*. 1991;174(2):625-30.
- [79] Slots J, Bragd L, Wikström M, Dahlén G. The occurrence of *Actinobacillus actinomycetemcomitans*, *Bacteroides gingivalis* and *Bacteroides intermedius* in destructive periodontal disease in adults. *Journal of Clinical Periodontology*. 1986;13(6):570-7.
- [80] Slots J, Listgarten MA. *Bacteroides gingivalis*, *Bacteroides intermedius* and *Actinobacillus actinomycetemcomitans* in human periodontal diseases. *Journal of Clinical Periodontology*. 1988;15(2):85-93.
- [81] Breukink E, Wiedemann I, Kraaij Cv, Kuipers OP, Sahl H-G, Kruijff Bd. Use of the cell wall precursor lipid II by a pore-forming peptide antibiotic. *Science*. 1999;286:2361-4.
- [82] Weeks BS, Nomizu M, Otaka A, Weston CA, Okusu A, Tamamura H, et al. The synthetic (Tyr5,12,Lys7)-polyphemusin II peptide (T22) binds to the CD4 cell surface molecule. *Biochemical and Biophysical Research Communications*. 1995;215(2):626-31.
- [83] Marcos JF, Beachy RN, Houghten RA, Blondelle SE, Pérez-Payá E. Inhibition of a plant virus infection by analogs of melittin. *Proceedings of the National Academy of Sciences*. 1995;92(26):12466-9.

- [84] Piantavigna S, Czihal P, Mechler A, Richter M, Hoffmann R, Martin L. Cell penetrating apidaecin peptide interactions with biomimetic phospholipid membranes. *International Journal of Peptide Research and Therapeutics*. 2009;15(2):139-46.
- [85] Castle M, Nazarian A, Yi SS, Tempst P. Lethal effects of apidaecin on *Escherichia coli* involve sequential molecular interactions with diverse targets. *Journal of Biological Chemistry*. 1999;274(46):32555-64.
- [86] Li W-F, Ma G-X, Zhou X-X. Apidaecin-type peptides: Biodiversity, structure–function relationships and mode of action. *Peptides*. 2006;27(9):2350-9.
- [87] Papo N, Shai Y. Host defense peptides as new weapons in cancer treatment. *Cellular and Molecular Life Sciences*. 2005;62(7):784-90.
- [88] Thorburn A. Death receptor-induced cell killing. *Cellular Signalling*. 2004;16(2):139-44.
- [89] Okumura K, Itoh A, Isogai E, Hirose K, Hosokawa Y, Abiko Y, et al. C-terminal domain of human CAP18 antimicrobial peptide induces apoptosis in oral squamous cell carcinoma SAS-H1 cells. *Cancer Lett* 2004;212(2):185-94.
- [90] Chen Y, Xu X, Hong S, Chen J, Liu N, Underhill CB, et al. RGD-tachyplesin inhibits tumor growth. *Cancer Research*. 2001;61(6):2434-8.
- [91] Chen J, Xu X-M, Underhill CB, Yang S, Wang L, Chen Y, et al. Tachyplesin activates the classic complement pathway to kill tumor cells. *Cancer Research*. 2005;65(11):4614-22.
- [92] Sharma S. Melittin resistance: a counterselection for ras transformation. *Oncogene*. 1992;7(2):193-201.
- [93] Sharma S. Melittin-induced hyperactivation of phospholipase A2 activity and calcium influx in ras-transformed cells. *Oncogene*. 1993;8(4):939-47.
- [94] Mader JS, Salsman J, Conrad DM, Hoskin DW. Bovine lactoferricin selectively induces apoptosis in human leukemia and carcinoma cell lines. *Molecular Cancer Therapeutics*. 2005;4(4):612-24.
- [95] Furlong SJ, Mader JS, Hoskin DW. Bovine lactoferricin induces caspase-independent apoptosis in human B-lymphoma cells and extends the survival of immune-deficient mice bearing B-lymphoma xenografts. *Experimental and Molecular Pathology*. 2010;88(3):371-5.
- [96] Mader JS, Smyth D, Marshall J, Hoskin DW. Bovine lactoferricin inhibits basic fibroblast growth factor- and vascular endothelial growth factor165-induced angiogenesis by competing for heparin-like binding sites on endothelial cells. *The American Journal of Pathology*. 2006;169(5):1753-66.
- [97] Schultz G, Clark W, Rotatori DS. EGF and TGF- α in wound healing and repair. *Journal of Cellular Biochemistry*. 1991;45(4):346-52.
- [98] Sørensen OE, Cowland JB, Theilgaard-Mönch K, Liu L, Ganz T, Borregaard N. Wound healing and expression of antimicrobial peptides/polypeptides in human keratinocytes, a consequence of common growth factors. *The Journal of Immunology*. 2003;170(11):5583-9.
- [99] Bals R, Wilson JM. Cathelicidins- a family of multifunctional antimicrobial peptides. *Cell Mol Life Sci*. 2003;60:711-20.

- [100] Yang D, Chertov O, Oppenheim JJ. Participation of mammalian defensins and cathelicidins in anti-microbial immunity: receptors and activities of human defensins and cathelicidin (LL-37). *Journal of Leukocyte Biology*. 2001;69(5):691-7.
- [101] Kurosaka K, Chen Q, Yarovsky F, Oppenheim JJ, Yang D. Mouse cathelin-related antimicrobial peptide chemoattracts leukocytes using formyl peptide receptor-like 1/mouse formyl peptide receptor-like 2 as the receptor and acts as an immune adjuvant. *The Journal of Immunology*. 2005;174(10):6257-65.
- [102] Marr AK, Gooderham WJ, Hancock REW. Antibacterial peptides for therapeutic use: obstacles and realistic outlook. *Current Opinion in Pharmacology*. 2006;6(5):468-72.
- [103] Vlieghe P, Lisowski V, Martinez J, Khrestchatsky M. Synthetic therapeutic peptides: science and market. *Drug Discovery Today*. 2010;15(1-2):40-56.
- [104] Lau YE, Rozek A, Scott MG, Goosney DL, Davidson DJ, Hancock REW. Interaction and cellular localization of the human host defense peptide LL-37 with lung epithelial cells. *INFECTION AND IMMUNITY*. 2005;73(1):583-91.
- [105] Sandgren S, Wittrup A, Cheng F, Jönsson M, Eklund E, Busch S, et al. The human antimicrobial peptide LL-37 transfers extracellular DNA plasmid to the nuclear compartment of mammalian cells via lipid rafts and proteoglycan-dependent endocytosis. *Journal of Biological Chemistry*. 2004;279(17):17951-6.
- [106] Kola I, Landis J. Can the pharmaceutical industry reduce attrition rates? *Nat Rev Drug Discov*. [10.1038/nrd1470]. 2004;3(8):711-6.
- [107] Østergaard C, Sandvang D, Frimodt-Møller N, Kristensen H-H. High cerebrospinal fluid (CSF) penetration and potent bactericidal activity in CSF of NZ2114, a novel plectasin variant, during experimental pneumococcal meningitis. *Antimicrobial Agents and Chemotherapy*. 2009;53(4):1581-5.
- [108] Hara S, Mukae H, Sakamoto N, Ishimoto H, Amenomori M, Fujita H, et al. Plectasin has antibacterial activity and no affect on cell viability or IL-8 production. *Biochemical and Biophysical Research Communications*. 2008;374(4):709-13.
- [109] Risso A, Zanetti M, Gennaro R. Cytotoxicity and apoptosis mediated by two peptides of innate immunity. *Cellular Immunology*. 1998;189(2):107-15.
- [110] Lau YE, Bowdish DME, Cosseau C, Hancock REW, Davidson DJ. Apoptosis of airway epithelial cells. *American Journal of Respiratory Cell and Molecular Biology*. 2006;34(4):399-409.
- [111] Holle L, Song W, Holle E, Wei Y, Wagner T, Yu X. A matrix metalloproteinase 2 cleavable melittin/avidin conjugate specifically targets tumor cells in vitro and in vivo. *Int J Oncol*. 2003;22(1):93-8.
- [112] Rawlins MD. Cutting the cost of drug development? *Nat Rev Drug Discov*. [10.1038/nrd1347]. 2004;3(4):360-4.
- [113] Merrifield R. Solid phase peptide synthesis. I. The synthesis of a tetrapeptide. *J Am Chem Soc*. 1963;85:2149-54.
- [114] Bray BL. Large-scale manufacture of peptide therapeutics by chemical synthesis. *Nat Rev Drug Discov*. [10.1038/nrd1133]. 2003;2(7):587-93.
- [115] Gesell J, Zasloff M, Opella SJ. Two-dimensional ¹H NMR experiments show that the 23-residue magainin antibiotic peptide is an α -helix in dodecylphosphocholine micelles,

- sodium dodecylsulfate micelles, and trifluoroethanol/water solution. *Journal of Biomolecular NMR*. 1997;9(2):127-35.
- [116] Bechinger B. Structure and functions of channel-forming peptides: Magainins, cecropins, melittin and alamethicin. *Journal of Membrane Biology*. 1997;156(3):197-211.
- [117] Ouellette A. Paneth cell α -defensins in enteric innate immunity. *Cellular and Molecular Life Sciences*. 2011;68(13):2215-29.
- [118] Sagisaka A, Miyanoshita A, Ishibashi J, Yamakawa M. Purification, characterization and gene expression of a glycine and proline-rich antibacterial protein family from larvae of a beetle, *Allomyrina dichotoma*. *Insect Molecular Biology*. 2001;10(4):293-302.
- [119] Lee S, Moon H, Kurata S, Natori S, Lee B. Purification and cDNA cloning of an antifungal protein from the hemolymph of *Holotrichia diomphalia* larvae. *Biol Pharm Bull* 1995;18(8):1049-52.
- [120] Rozek A, Friedrich CL, Hancock REW. Structure of the bovine antimicrobial peptide indolicidin bound to dodecylphosphocholine and sodium dodecyl sulfate micelles. *Biochemistry*. 2000 2000/12/01;39(51):15765-74.
- [121] Bruckdorfer T, Marder O, Albericio F. From production of peptides in milligram amounts for research to multi-tons quantities for drugs of the future. *Curr Pharm Biotechnol*. 2004;5(1):29-43.
- [122] Yeung A, Gellatly S, Hancock R. Multifunctional cationic host defence peptides and their clinical applications. *Cell Mol Life Sci*. 2011;68(13):2161-76.
- [123] Imura Y, Nishida M, Ogawa Y, Takakura Y, Matsuzaki K. Action mechanism of tachyplesin I and effects of PEGylation. *Biochimica et Biophysica Acta (BBA) - Biomembranes*. 2007;1768(5):1160-9.
- [124] Brötz H, Bierbaum G, Leopold K, Reynolds PE, Sahl H-G. The lantibiotic mersacidin inhibits peptidoglycan synthesis by targeting lipid II. *Antimicrobial Agents and Chemotherapy*. 1998;42(1):154-60.
- [125] Boman HG, Agerberth B, Boman A. Mechanisms of action on *Escherichia coli* of cecropin P1 and PR-39, two antibacterial peptides from pig intestine. *Infection and Immunity*. 1993;61(7):2978-84.
- [126] Shi J, Ross CR, Chengappa MM, Sylte MJ, McVey DS, Blecha F. Antibacterial activity of a synthetic peptide (PR-26) derived from PR-39, a proline-arginine-rich neutrophil antimicrobial peptide. *Antimicrobial Agents and Chemotherapy*. 1996;40(1):115-21.
- [127] Subbalakshmi C, Sitaram N. Mechanism of antimicrobial action of indolicidin. *FEMS Microbiol Lett*. 1998;160(1):91-6.
- [128] Salomón RA, Farías RN. Microcin 25, a novel antimicrobial peptide produced by *Escherichia coli*. *Journal of Bacteriology*. 1992;174(22):7428-35.
- [129] Patrzykat A, Friedrich CL, Zhang L, Mendoza V, Hancock REW. Sublethal concentrations of pleurocidin-derived antimicrobial peptides inhibit macromolecular synthesis in *Escherichia coli*. *Antimicrobial Agents and Chemotherapy*. 2002;46(3):605-14.
- [130] Lehrer RI, Barton A, Daher KA, Harwig SS, Ganz T, Selsted ME. Interaction of human defensins with *Escherichia coli*. Mechanism of bactericidal activity. *The Journal of Clinical Investigation*. 1989;84(2):553-61.

- [131] Andreu D, Rivas L. Animal antimicrobial peptides: An overview. *Peptide Science*. 1998;47(6):415-33.
- [132] Bellemare A, Vernoux N, Morin S, Gagne S, Bourbonnais Y. Structural and antimicrobial properties of human pre-elafin/trappin-2 and derived peptides against *Pseudomonas aeruginosa*. *BMC Microbiol*. 2010;10(1):253.
- [133] Baranger K, Zani M-L, Chandenier J, Dallet-Choisy S, Moreau T. The antibacterial and antifungal properties of trappin-2 (pre-elafin) do not depend on its protease inhibitory function. *FEBS Journal*. 2008;275(9):2008-20.
- [134] Hong RW, Shchepetov M, Weiser JN, Axelsen PH. Transcriptional profile of the *Escherichia coli* response to the antimicrobial insect peptide cecropin A. *Antimicrobial Agents and Chemotherapy*. 2003;47(1):1-6.
- [135] Chan YR, Gallo RL. PR-39, a syndecan-inducing antimicrobial peptide, binds and affects p130^{Cas}. *Journal of Biological Chemistry*. 1998;273(44):28978-85.
- [136] Woodley J. Enzymatic barriers for GI peptide and protein delivery. *Crit Rev Ther Drug Carrier Syst*. 1994;11(2-3):61-95.