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Self-Assembly of Linear, Natural Antimicrobial Peptides: An Evolutionary Perspective

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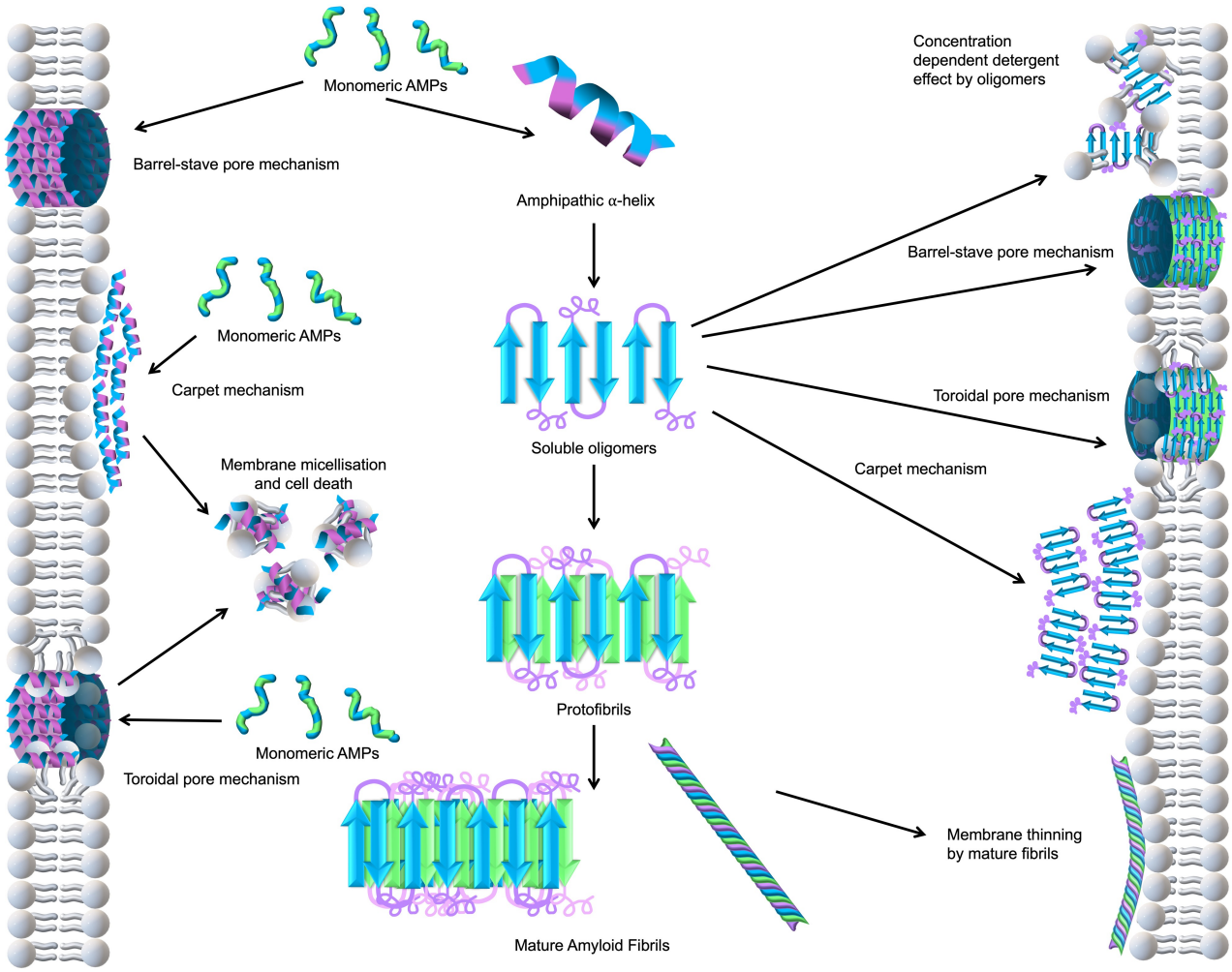

Antimicrobial peptides are an ancient and innate system of host defence against a wide range of microbial assailants. Mechanistically, unstructured peptides undergo a secondary structure transition into amphipathic α-helices, upon contact with membrane surfaces. This leads to peptide binding and removal of the membrane components in a detergent-like manner or via self-organisation into trans-membrane pores (either barrel-stave or toroidal pore) thereby destroying the microbe. Self-assembly of antimicrobial peptides into oligomers and ultimately amyloid has been mostly examined in parallel, however recent findings link diseases, such as Alzheimer's disease as an aberrant activity of a protective neuropeptide with antimicrobial activity. These self-assembled oligomers can also interact with membranes. Here, we review those antimicrobial peptides reported to self-assemble into amyloid, where supported by structural evidence. We consider their membrane activities as antimicrobial peptides and present evidence of consistent self-assembly patterns across major evolutionary groups. Trends are apparent across these groups, supporting the mounting data that self-assembly of antimicrobial peptides into amyloid should be considered as synergistic to the antimicrobial peptide response.

1. Introduction

Antimicrobial peptides (AMPs) are thought to function as an innate form of immune response, and were first reported by Dubos in 1939.[1] AMPs exist as relatively small molecules, with typical sequences of less than 50 amino acids, yet are highly diverse in their sequences and microbial targets.[2] They have also been found in a wide range of prokaryotic and eukaryotic organisms, including humans.[2] Nearly all human tissues which are exposed to pathogens, are able to produce AMPs. AMPs can act as a first line of defence for multicellular organisms against a variety of invading pathogens including; bacteria, fungi, parasites and viruses.[3] As a result of this wide range of activity against foreign microbes, AMPs have been studied for their potential to develop peptide-based antibiotics.[3a,c] In contrast, AMPs have also been investigated for their demonstrated ability to self-assemble into amyloid fibrils. As a number of amyloidogenic peptides have also been found to possess antimicrobial properties,[4] the study of AMPs allows for investigation of the intersection between amyloidogenic proteins and antimicrobial action.

If placed in aqueous solution, AMPs typically present random coil structures.[5] However, if they come into contact with a biological membrane, AMPs transition into an amphipathic conformation, resulting in predominately α-helices, although there are some examples of β-sheet or β-hairpin-type structures.[6] This process is displayed in Figure 1. AMPs typically have a net positive charge and can vary between 12–50 amino acids in length, with their interaction with target cells being mediated primarily by electrostatic attraction.[7] In eukaryotic membranes, the outer leaflet of the lipid bilayer is predominantly zwitterionic in nature.[8] Whereas, bacterial cell membranes have a negative surface charge due to the presence of acidic phospholipids.[9] Hence, AMPs are designed to selectively bind to bacterial membranes, either in a surface-active (carpet) or detergent manner, or via oligomerisation and insertion of pores; both mechanisms cause disruption of the bacteria's pathogenic action. Pores are formed in both mechanisms and the nature of these 'pores' could consist of soluble oligomers which are precursors to amyloid. Notably, a number of amyloidogenic peptides also possess the ability to adopt α-helical conformations if in contact with a biological membrane, presenting the potential for membrane disruption activity.[4] This may promote the notion that the self-assembly properties of AMPs have developed synergistically with their antimicrobial activity.

Amyloid can be defined as insoluble extracellular masses with a highly organised fibrillar morphology,[10] produced by the misfolding of proteins. Amyloid fibrils form after misfolded proteins (or peptides) oligomerise, then undergo an elongation process to form protofibrils,[11] a pathway demonstrated in Figure 2. The thread-like fibrillar structures which arise through the intertwining self-assembly of protofibrils, can then act to cause disruption of normal cell functions.[12] Amyloids can be classified into two major groups, pathogenic amyloids, and non-pathogenic; the latter often termed functional amyloids.[13] Whilst examples from both groups may be found present in the human body, organisms such as bacteria and insects, possess higher amounts of the non-pathogenic form.[14] The role of functional amyloids in the human body continues to expand, as new mechanisms of action are discovered. Known functions of non-pathogenic amyloids in humans include, but are not limited to, cellular responses to stress, storage of peptide hormones and antimicrobial responses.[14–15]

Protein precursors to pathogenic amyloid fibrils can be observed with a variety of sequences, shapes and sizes. Structural differences in fibrils can include parallel vs antiparallel β-sheets,[16] as seen in variants of the amyloid beta (Aβ) peptide (see later). Secondary structure changes, from unstructured to fibrillar peptide structure can be monitored through techniques such as circular dichroism (CD).[17] This method interrogates peptides mostly in aqueous environments, including water or buffer, showing transitions into β-sheet formation, whereas organic environments typically induce an α-helical conformation.[17]

While there are many reviews on AMPs and amyloid-forming peptides, the connection between these two apparently disparate roles for bioactive peptides is rarely made.[18] Specifically, as noted by Häffner et al.,[19] AMPs are a structurally

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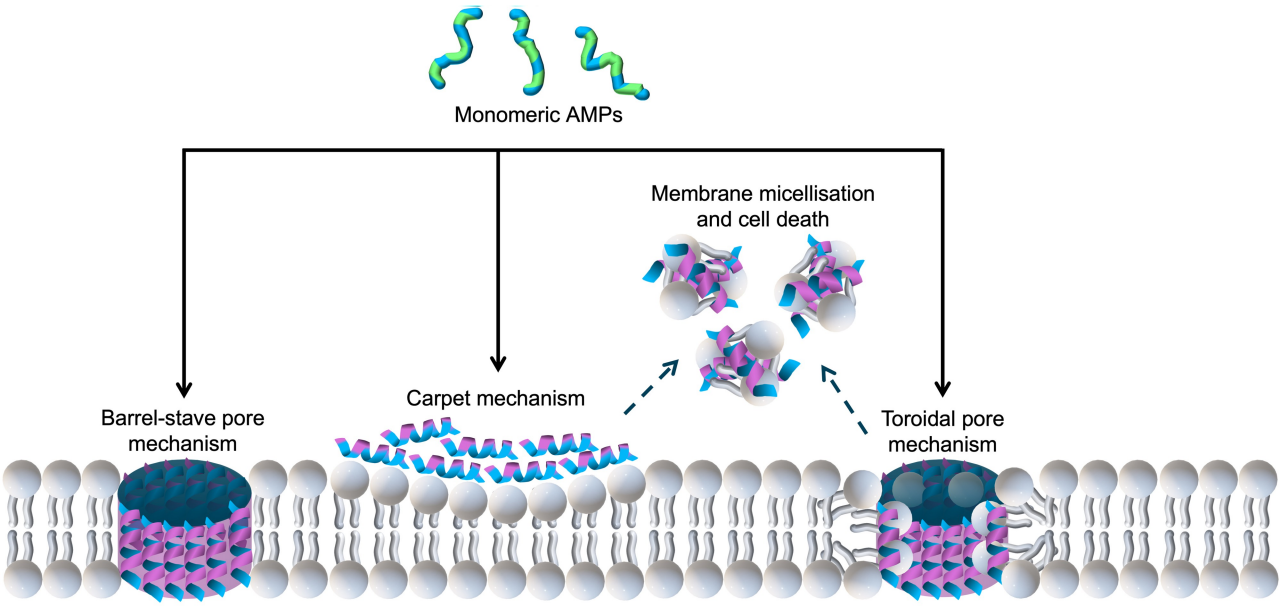

Figure 1. The primary activity of antimicrobial peptides (AMPs) is to effectuate membrane permeabilisation. Illustrated are the soluble (unstructured) AMPs that first contact a membrane (lipid bilayer) to form an α-helical secondary structure. Peptides bind to the membrane which can cause disruption, in a carpet-like manner leading to membrane micelles (detergent action). Alternatively, peptides can self-assemble into trans-membrane pores. Toroidal pores are associated with the lipids, whereas barrel-stave pores assemble their amphipathic helices, so that the hydrophobic surface is in contact with the lipid hydrocarbon chains forming a hydrophilic interior, thereby creating a channel across the membrane bilayer.

and compositionally heterogeneous group, with microbial membrane disruption, regarded as the primary mode-of-action, as their principle association in common. Furthermore, Tian et al.,[20] points out that the property of self-assembly into *super structures* is rare in naturally occuring AMPs, and that this should not be surprising as most AMPs are short, charged, and structurally flexible. Thus, while a few of the enormous number of natural AMPs can self-assemble these AMPs appear to depend on the individual peptide as to the mode-of-action of the amyloid-type structure.[19] It has been demonstrated that

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Paul O'Leary received his PhD from the Department of Pharmacology at The University of Melbourne. His work there involved the design, synthesis and in vitro characterisation of potent, highly constrained peptide mimics of brain-derived neurotrophic factor. Paul continued his career in research by co-founding a start-up company to commercialise his PhD work. He then moved to a peptide-focused clinical-development company, primarily looking at peptide bioanalysis and pharmacokinetics. Paul continued to work in the bioanalytical arena at a GLP-accredited CRO and is now back within an academic environment at Monash University, where he is focusing on drug discovery using molecular modelling and LC–MS/MS techniques.

Lisa Martin was trained in Organic and Inorganic Chemistry at Monash University and received her PhD from The Australian National University on the magnetism and electrochemistry of encapsulated metal ions. Her postdoctoral experience in Switzerland, Germany (Alexander von Humboldt Fellowship) and USA (Fulbright Fellowship), shifted her interests to bioinspired research. She was an academic at Flinders University, before moving to Monash University in 2003. Her current research is at the interface of chemical biology and medicinal chemistry, and she has pioneered methods for studying membrane proteins and peptides at biomimetic membrane interfaces. She is a Fellow of the Royal Society of Chemistry (UK) and the Royal Australian Chemical Institute.

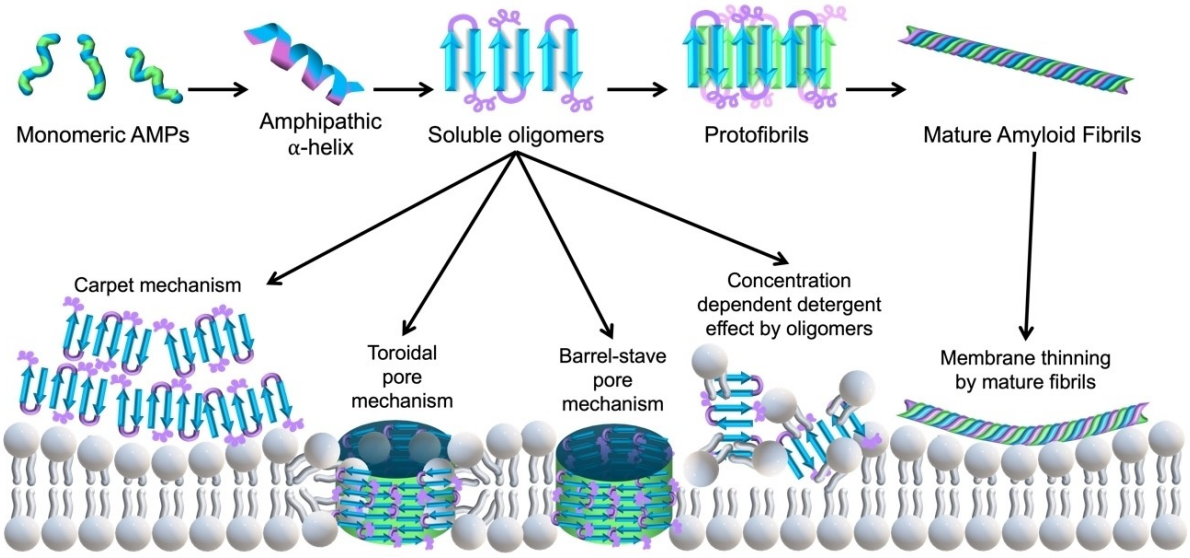

Figure 2. Postulated pathway for amyloid fibril formation, featuring the mode-of-action towards membrane permeabilisation for oligomers and fibrils.

even a small change of a lipid mixture can be sufficient to induce self-assembly of an AMP.[21] Arguably, the most recognised pathological amyloid is linked with dementia, i.e. Aβ the peptide that aggregates in the brain and is a hallmark of Alzheimer's Disease. In their comprehensive treatise, Moir et al.,[18b] presented the "Antimicrobial protection hypothesis" of Alzheimer's disease, in which the pathophysiology of Aβ, associated with abnormal stochastic peptide self-assembly (into amyloid) shifts to a dysregulation of a protective AMP function. Furthermore, as the human Aβ sequence is conserved across most vertebrate species for more than 400 million years,[18b] the role of amyloid as an early innate immune response to microbial challenges of the brain was presented. There are a number of excellent reviews that summarise the factors that determine self-assembly of AMPs, and we direct the reader to reviews by Hamley,[22] Dehsorkhi et al.,[23] Sun et al.,[24] Cui et al.,[25] Häffner et al.[19] and Tian et al.[20] all who address the amphiphilic sequences that are prone to self-assembly as well as the wide range of structures that are formed, although the β-sheet (amyloid) is the most prevalent. This will be discussed in terms of bioinformatic approach to seek consensus data for AMP self-assembly hence providing insight as to whether there is a functional role for amyloid, or is it incidental to the primary mode-of-action as AMPs. In this review, our focus is to highlight the natural AMPs that have been reported to form amyloid either *in vivo* or *in vitro* across a wide evolutionary spread of species, including bacteria through to humans. The natural AMPs to be discussed in this review are listed in Table 1. We consider here only naturally occurring linear AMPs containing <50 amino acids and whose primary function is antimicrobial activity. Also, we limited our review to AMPs where there is structural evidence of self-assembly into amyloid. Thus, we have excluded cyclic peptides, many of which contain disulfide bonds and those of plant origin. Diseases associated with amyloid have been excluded from our focus although we use Aβ as the quintessential amyloid-forming peptide that acts as an AMP. Thus, we highlight the connections between antimicrobial properties (activities) and the self-assembly characteristics associated with amyloid formation.

2. Evolutionary AMP groups

A number of naturally occurring AMPs have been found to also self-assemble to form amyloid (Table 1). These amyloidogenic AMPs will be discussed in this section in terms of their antimicrobial properties, membrane activity and self-assembly processes. The AMPs have been grouped taxonomically in order to discern any potential evolutionary trends.

2.1. Bacteria

2.1.1. Phenol-soluble modulin alpha-3 (PSMα3)

PSMα3 is a 22 residue modulin peptide which is secreted by *Staphylococcus aureus*. PSMα3 has been found to help initiate inflammatory responses, lyse human cells and assist with formation of biofilm structures. Structural analysis of PSMα3 has demonstrated that it forms amphipathic helices in solution. Interestingly, several papers by Tayeb-Fligelman et al.[26] have suggested that PSMα3 maintains its α-helical structure during fibril formation, resulting in the production of α-helical, rather than β-sheet fibrils. Further studies by the same group also found that the α-helical secondary structure of the peptide alone is insufficient to effectuate cytotoxicity.[26a] It was suggested that the cytotoxicity of PSMα3 originates from the self-assembly of the α-helices into carpets of amphipathic sheets across the surface of the membrane. This carpet-like mechanism then causes the deformation of the membrane,

Table 1. Summary of the amyloidogenic AMPs discussed in this review, including details of their sequence, charge, source, and amyloid structural characterisation.

Peptide	Sequence, AA length and net charge, at pH 7	Natural Source	Structural characterisation method
Phenol-soluble modulin alpha-3 (PSMα3)	MEFVAKLFKFFKDLLGKFLGNN-OH (22; 2+)	Golden staph	FT-IR, X-ray, ThT, TEM, NMR
Plantaricin A	KSSAYSLQMGATAIKQVKKLFKKWGW-OH (26; 6+)	Lactic acid bacteria	Phase contrast microscopy, polarizing microscopy, fluorescence microscopy
Longipin	SGYLPGKEYVYKYKGKVF-OH (18; 3+)	Harvestman spider (Daddy longlegs)	FT-IR, ThT
Cecropin A	KWKLFKKIEKVGQNIRDGIIKAGPAVAVVGQATQIAK-NH_2 (37; 7+)	Cecropia moth	TEM – in inducing medium only
Melittin	GIGAVLKVLTTGLPALISWIKRKRQQ-NH_2 (26; 6+)	Honey bee	AFM, ThT
Dermaseptin (S9)	ALWKTMLKKLGTMALHAGKAALGAAADTISQGTQ-OH (34; 3+)	South American hylid frog	TEM, CD, FT-IR, ThT, fluorescence microscopy
Buforin II	TRSSRAGLQFPVGRVHRLLRK-OH (21; 6+)	Stomach tissue of the asiatic toad	small-angle X-ray scattering (SAXS)
Magainin 2	GIGKFLHSAKKFGKAFVGEIMNS-OH (23; 3+)	Skin of the African clawed frog	ThT, TEM, FT-IR, CD
Temporins	Temporin B: LLPIVGNLLKSLL-NH_2 (13; 2+) Temporin L: FVQWFSKFLGRIL-NH_2 (13; 3+)	Skin secretion of the European red frog	Temporin B: Fluorescence spectroscopy, polarised microscopy, bright field microscopy Temporin L: Phase contrast, polarising microscopy, MD
Aurein 3.3	GLFDIVKKIAGHIVSSI-NH_2 (17; 2+)	Skin of the green and golden bell frog, southern bell frog, Australian blue mountains tree frog and frogs from the genus Uperoleia.	Cryo-EM
Uperins	U3.4: GVGDLIRKAVAAIKNIV-NH_2 (17; 3+) U3.5: GVGDLIRKAVSVIKNIV-NH_2 (17; 3+) U3.6: GVIDAAKKVVNVLKNLF-NH_2 (17; 3+)	Skin of the Australian toadlet	ThT, TEM, CD, X-ray, FT-IR, NMR, cryo-EM
Indolicidin	ILPWKWPWWPWRR-NH_2 (13; 4+)	Cytoplasmic granules of bovine neutrophils	CD, phase contrast microscopy, polarizing microscopy
Cathelicidin (LL-37)	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES-OH (37; 6+)	Secondary granules of neutrophils in humans	TEM, cryo-EM, NMR, confocal microscopy, ThT, X-ray diffraction

resulting in cell death. Interactions with the membrane are also thought to be contributed to by the presence of positive charges on the peptide, especially the lysine at position 17.[26a] The membrane activity of PSMα3 is regulated by the inter- and intra-helical electrostatic interactions formed inside the α-helical fibril. Namely, the phenylalanine at position 11 and the leucines at positions 7 and 15 are essential to the formation of the inter-sheet hydrophobic core of the cross-α structure.[26a]

Interestingly, several other phenol-soluble modulin (PSM) variants, including PSMα1 and PSMα4, form highly stable, canonical cross-β amyloid fibrils, which function as part of biofilms.[27] This is in clear contrast to the cross-α structure observed for PSMα3. In addition, PSMα3 is the most toxic member of the PSM family, whilst the other variants confer minimal toxicity. Therefore, as suggested by Salinas et al., the AMP activity of PSMα3 and its amyloid fibrilisation may be functionally linked.[27] This is promoted by Tayeb-Fligelman et al.'s finding that the fibrilisation of PSMα3 increases the host bacteria's toxicity towards human cells.[26b] Crystal structure analysis of segments of PSMα3 also revealed features which were in common with disease-causing amyloids found in humans, such as out-of-register β-sheets and similar interfacing between sheets. The differences between the function of the amyloid structures formed by PSMα3 and other PSM variants suggests, firstly, that cell toxicity is not immediately conferred by fibrilisation. In addition, the reversible, yet distinctive,

formation of cross-α amyloid by PSMα3 may function as a means to regulate the bacteria's cytotoxic activity.

2.1.2. Plantaricin A

Plantaricin A is a peptide pheromone originating from the bacterial species *Lactobacillus plantarum*. Plantaricin A is 26 residues long and is known to be unstructured in aqueous solutions, as well as, when in contact with zwitterionic liposomes. In the presence of a negatively charged membrane plantaricin A displays a right-handed α-helical conformation. In Zhao et al.'s 2006 study on plantaricin A membrane activity,[28] they found that the interaction of the peptide with membranes depends on the lipid composition (e.g. the presence of acidic lipids or sterols). They suggested that plantaricin A's membrane permeabilising activity likely occurs through a leaky slit mechanism. This involves the peptide aggregating onto the surface of the membrane by binding to the membrane lipids. The lipid-bound peptides then assemble into a linear, amphipathic conformation, with the hydrophobic side facing the bilayer and the hydrophilic side causing the contacting lipids to form a highly positive curvature. The observed aggregates of plantaricin A only formed in the presence of a negatively charged lipid bilayer in a solution of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer. Therefore, Zhao et al. proposed that the capability of plantaricin A to form fibril-like structures was responsible for its membrane permeabilisation capabilities.[28] Carpet and toroidal pore mechanisms were also considered possible for plantaricin A, however, the leaky slit model was preferred due to its resulting high solute permeability and difficulty of the cell to repair itself, as well as, the overall toxicity of the fibrils formed by the peptide. Therefore, it may be suggested that the cytotoxic functionality of the fibrils formed by plantaricin A may coincide with a defence mechanism against other bacteria. It is notable that only one early paper studied the amyloid-like fibril forming propensity of plantaricin A, and no further research on such properties has been published.

2.2. Arthropods

2.2.1. Longipin

Longipin is an 18-residue long AMP derived from the hemolymph of the harvestman spider, *Acutisoma longipes*. When in solution, the peptide is mainly unstructured, however, when in the presence of a lipid bilayer longipin demonstrates amyloid-like fibril formations, with β-sheet structures.[29] It has also been shown to preferentially bind to negatively charged membranes only, resulting in membrane permeabilisation. The Tyr-Leu motif of the sequence of longipin is thought to be responsible for its binding capabilities. This was demonstrated by Sayegh et al., through a dye leakage assay, in which binding of longipin to lipid vesicles caused increased membrane permeability. As the minimum inhibitory concentration (MIC) values of longipin were also found to be in the high μM range for AMPs, it was suggested that the peptide's membrane activity is partly due to the presence of the Pro (residue 5) N-terminal peptide bond conformation. Analysis of the secondary structure content of the peptide demonstrated approximately 20–30% β-turn content. Fourier-transform infra-red (FT-IR) spectroscopy of longipin in the presence of POPG:POPC vesicles demonstrated the presence of intermolecular aggregates in the form of amyloid fibrils, these were found to be structured in a β-sheet-like orientation, known as cross-β. Additionally, Thioflavin T (ThT) binding assays performed on longipin in HEPES buffer in the presence of POPG:POPC vesicles also demonstrated the formation of amyloid-like fibrils. As Sayegh et al.'s study is the only paper to detail longipin self-assembly,[29] it is unclear whether the amyloid-like fibrils formed by the peptide possess functionality beyond assisting with membrane disruption.

2.2.2. Cecropins

Cecropin A is a 37-residue long peptide originally identified in the cecropia moth (*Hyalophora cecropia*). Cecropins are short, basic peptides, which act as a form of immune defence to bacterial infection. Cecropins are known to be effective against both gram-negative and gram-positive bacteria. Early research on cecropins demonstrated that when they are in hydrophobic environments they have the tendency to form helical structures. The formation of an amphipathic helix is provided for by the N-terminal half of the peptide's structure. Cecropins have also demonstrated specificity for bacterial cells only, as testing against eukaryotic cell types has resulted in resistance to lysis. In Steiner's 1988 paper,[30] it was noted that cecropin's mechanism of action is primarily via its N-terminal amphipathic helix, which binds to the bacterial membrane through electrostatic interactions. It was suggested that the axis of the peptide remains parallel to the surface of the membrane, as multiple cecropin molecules pack closely on the membrane surface, to cause disruption through a carpet-like mechanism. Other studies, such as Christensen et al.,[31] have favoured a channel formation mechanism of action. Later studies, involving molecular modelling,[32] have supported this, suggesting that this ion channel mechanism is maintained by a helix-bend-helix motif. The positively charged helices of the N-terminal are able to bind to negatively charged head groups of the phospholipid bilayer, whilst the hydrophobic helices of the C-terminal insert into the membrane. The N-terminal helices are pushed into the membrane by application of a positive potential. The transmembrane N-terminal helices form the channel, with the hydrophilic residues forming the pore and the hydrophobic residues being positioned on the outside, in contact with the aliphatic phase of the membrane. Once a number of peptide molecules are bound to the membrane in this fashion, lysis of the bacterial cell may then occur. In a study by Wang et al., it was found that cecropin A is able to form fibrillar aggregates, however, only in the presence of an inducing medium.[33] Similarly, studies on the interaction of cecropin P1 with DMPC/

Chol liposomes demonstrated that cecropin P1 formed large aggregates with solubilised liposome, hence, causing membrane disruption.[34] Lyu et al. suggested that this could point towards cecropin using a carpet-like mechanism to permeabilise membranes.[34]

2.2.3. Melittin

Melittin is a highly studied, 26 residue AMP, derived from the honeybee (*Apis mellifera*). The peptide consists of 26 amino acids and is C-terminal amidated. Melittin has been demonstrated to possess a broad spectrum of antimicrobial activity, including against both gram-positive and gram-negative bacteria. Both molecular dynamics (MD) and *in vitro* experiments have demonstrated that when bound to a lipid membrane, the peptide displays a bent, rod-like conformation with two α-helical sections connected by a non-helical kink section. Near symmetrical distribution of the polar and non-polar residues results in the formation of an amphipathic conformation. Melittin's mechanism of membrane disruption occurs in an asymmetric manner between the two lipid layers.[35] This results in stress being unequally applied between the two layers, causing the mechanical state of the membrane to be highly altered. Thinning and expansion of membrane then occurs, as well as changes to the local curvature. Translocation of melittin into the lipid bilayer may also result in the peptide adopting a U-shaped conformation as an intermediate transition state, with contacting lipids bending around the peptide accordingly. Studies of melittin in the presence of sodium dodecylsulfate (SDS) and heparin demonstrated that helix-rich aggregates formed by the peptide may be amyloidogenic in nature.[36] This promotes the notion that the self-assembly of melittin into oligomers may be increased by the presence of a membrane-mimicking environment.[36] Additionally, in studies using electron microscopy (EM) and atomic force microscopy (AFM) Singh et al. found that melittin is able to form oligomeric species, suggesting that the peptide has the propensity to self-assemble.[36] Whilst, in the presence of heparin, melittin's formation of oligomers is accelerated, with larger oligomers being formed. This may be structurally caused by the sequence of four positively charged amino acids (residues 21–24) which initiate interaction with the anionic polymer, heparin. In the presence of SDS melittin demonstrates immediate oligomerisation and helix formation. The aggregates formed in the presence of SDS demonstrated ThT fluorescence, suggesting that these structures may be amyloidogenic. The oligomers of melittin were also shown to be cytotoxic with haemolytic activity, potentially due to the high accumulation of helix-rich oligomers onto the cell surface. These results suggest that melittin has developed the propensity to form amyloid-like aggregates, without the commonly associated disease-causing capabilities.

2.3. Anurans (Frogs and Toads)

2.3.1. Dermaseptin S9

Dermaseptin S9 exists as a 34 amino acid long AMP, originating from the South American hylid frog, *Phyllomedusa sauvagei*. The peptide is known to form an amphipathic α-helix in solution, with the helix spanning residues 1–27 of the peptide. In an early study on dermaseptin, carried out by Pouny et al.,[37] it was found that the peptide likely acts via a carpet-like mechanism to cause cell lysis. In this paper it was found that the peptide was able to adsorb onto the membrane surface, with the amphiphilic N-terminal component of the protein penetrating into the acyl-chain portion of the membrane. Therefore, the peptide lies mostly parallel to the surface of the membrane, binding to the acidic phospholipid headgroups. As more dermaseptin monomers associate with those already bound to the membrane, a carpet-like structure forms over the surface, increasing membrane stress and eventually resulting in disruption and cell lysis. Subsequent studies have emphasised that the N-terminal region of the peptide is mainly responsible for its membrane disruption activity.[38] Dermaseptins have generally been demonstrated to express lytic activity towards a range of organisms including bacteria, fungi and protozoa, but not against mammalian cells.[38] A number of studies have confirmed the ability of dermaseptin S9, in particular, to form amyloid fibrils. As recorded by Auvynet et al.,[39] dermaseptin S9 is able to form ordered β-sheet aggregates when in aqueous buffers or when bound to negatively charged or zwitterionic phospholipid bilayers. These aggregates are then able to self-assemble into amyloid-like fibrils.[39] The presence of fibrils formed by dermaseptin S9 was confirmed by EM following incubation of the peptides in phosphate buffer at 37 °C for seven days. These results further suggested that as dermaseptin S9 lacks identifiable functionality *in vivo*, and is not associated with any pathological processes, its aggregate structures may demonstrate biological activity *in vitro*. Auvynet et al.'s findings were supported by Caillon et al.'s 2013 paper, in which it was demonstrated that dermaseptin S9 is able to form amyloid-like fibrils when in contact with a lipid membrane, and additionally in an aqueous environment.[40] EM results revealed that the fibrils formed by the peptide were analogous to those of disease-causing amyloidogenic peptides. These assays were performed by adding large unilamellar vesicles (LUVs), Tris/HCl and NaCl to the aqueous peptide solutions. Further, amyloid-like aggregation of the peptide on the surface of a membrane is not required for membrane permeabilisation to occur. It was demonstrated that peptide aggregation on the membrane surface did not cause membrane perturbation, potentially due to a reduction in the surface area of the membrane being in contact with the fibrils. This is in contrast to the majority of amyloidogenic AMPs, which require aggregation at the membrane surface for their cytotoxic properties. Instead, the results suggested that intermediates in the process of amyloid fibril formation were the source of membrane disruption for dermaseptin S9.

2.3.2. Buforin II

Buforin II is a 21-residue peptide, sourced from the stomach tissue of the asiatic toad, *Bufo gargarizans*. The peptide possesses a random coil (unstructured) conformation in water, and adopts an amphipathic α-helical structure when in a mixture of trifluoroethanol-water solution.[41] From residue 12–20 the peptide assumes a regular α-helical structure, whilst this is distorted for residues 7–11. The amphipathic character of the peptide is maintained from residue 5 to the C-terminal.[41] Studies on buforin II have demonstrated that the peptide exhibits potent antimicrobial activity against both gram positive and negative bacteria, which is reliant on its cell-penetrating efficiency. In comparison with other α-helical AMPs, buforin has demonstrated increased and strong antimicrobial activity against a broad spectrum of organisms. However, buforin is unique in that its mechanism of action does not result in cell lysis, instead it is thought to act against intracellular targets, such as nucleic acids including DNA.[42] Buforin's mechanism of action is thought to be caused by its helix-hinge-helix structure, with the helices separated by the proline at residue 11. Buforin II has been found to translocate across bacterial membranes via a mechanism similar to that of magainin 2 (see later).[43] The proline at position 11 causes the residues from 5–21 to become amphipathic, via distortion of the helix. The presence of 5 positive charges in this region then causes the destabilisation of a pore of the lipid bilayers, which results in the peptide being able to traverse the membrane without disruption.[43] In Lee at al.'s 2019 paper,[44] it was suggested that as buforin possesses a hydrophobic face that subtends less than 90°, it is unable to stabilise the hydrophobic core of a protofibril. It was alternatively proposed that it likely forms multimers, hence, emphasising that it may be unable to form amyloid fibrils.[44] The self-assembly properties of buforin have been suggested by Bücker et al.,[45] however, research has yet to be published to support this.

2.3.3. Magainin 2

Magainin 2 is a 23 amino acid long, cationic AMP derived from the skin of the frog species *Xenopus laevis*. Magainin 2 is unstructured in solution, however, when in contact with a membrane it is strongly amphipathic and α-helical.[46] The conformation adopted by the peptide involves the positively charged residues being oriented on the face of the helix. In terms of its function, depending on experimental conditions, magainin 2 is thought to act through toroidal or chaotic pore mechanisms to cause membrane leakage. It binds to the surface of the membrane in a parallel fashion, with the outer hydrophilic face of the peptide interacting with the polar head groups and surrounding solvent. At the same time, the phospholipid acyl chains are interacted with by the hydrophobic regions of the peptide. Interaction of the peptide with the membrane may cause increased tension, which is then relieved by formation of a pore structure by the peptide, resulting in membrane leakage and bacterial death. As demonstrated by Matsuzaki et al.,[47] formation of a toroidal pore by magainin 2 is only observed where the membrane has a low peptide to lipid ratio. At higher peptide to lipid ratios, magainin 2 may accumulate on the surface of the bilayer without resulting in pore formation, inducing a thinning effect on the membrane. The potential ability of magainin to self-assemble into aggregates was first suggested by Urrutia et al.'s 1989 paper.[48] More recently, in a 2020 study by Juhl et al.,[49] Transmission Electron Microscopy (TEM) images of magainin in Mueller-Hinton medium demonstrated the presence of fibrils. These fibrous molecular aggregates formed under physiological conditions in the presence of bivalent anions. The fibrils were long and thin in nature and were not found to undergo any additional self-assembly. Further FT-IR spectroscopy of magainin fibrils formed in phosphate buffer demonstrated secondary structures comparable to that of other amyloids. The aggregation of magainin 2 is thought to be due to a significant number of residues contributing to the peptides hydrophobic character. Additionally, the presence of negative charged ions was suggested to promote close spatial arrangement of the peptides, improving propensity for aggregation. Juhl et al. postulated that the supramolecular aggregate formed by magainin 2 may assist in storing and protecting the AMP from proteolytic degradation, ensuring slow release over a long period of time, much like hormones of the mammalian endocrine system.[49] Therefore, magainin 2 may be characterised similarly to other functional amyloids.

2.3.4. Temporins

Temporin B and temporin L are 13 residues long, amphipathic α-helical AMPs, derived from the European red frog *Rana temporaria*. Temporins are known for their selective ability to bind to the lipids of target cells, rather than host cells. Testing of the activity of temporin B has shown that it is effective against gram-positive bacteria, but is not haemolytic in nature.[50] Whereas, temporin L is effective against both gram-positive and negative bacteria, as well as, fungi and cancer cells.[51] This class of peptides has also been demonstrated to cause cell membrane permeabilisation, via significant perturbations to the structure of the membrane.[52] This process involves conformational alterations induced by the acidic phospholipids, aggregation of the temporin peptides and precipitation of toxic oligomers in the target cell membrane.[3c] The binding of temporins is largely due to hydrophobic interactions, however, the mechanism of temporin membrane disruption is not fully known. It has been suggested that the mechanism is similar to the barrel stave model, involving pore formation by the peptide with the hydrophilic and hydrophobic side chains being situated on the opposite sides of the helix.[3c] In Sood et al.'s 2007 paper,[53] it was found that temporin B forms amyloid-like fibres in the presence of a negatively charged lipid membrane. Such experiments were carried out in a solution of HEPES and EDTA buffer, in the presence of SOPC/POPG LUVs. Amyloid-like fibres formed only in the presence of the negatively charged POPG containing liposomes, whilst the zwitterionic SOPC

containing liposomes lacked similar fibril formation. Sood et al. proposed that temporin B's ability to form amyloid-like fibrils is functionally related to the cytotoxic activity of other AMPs.[53] Similarly, studies carried out by Zhao et al. on temporin L demonstrated that the peptide formed amyloid-like fibres in the presence of acidic phospholipid-containing vesicles.[54] These studies were carried out, in HEPES and EDTA buffer, in the presence of SOPC/brain PS LUVs. Domanov & Kinnunen's 2006 paper focused on the binding of both temporin B and L to supported lipid bilayer (SLB) model membranes, composed of PC and PG, in the presence of HEPES and EDTA buffer.[55] It was identified, using fluorescent microscopy, that the binding of each peptide caused the formation of fibrillar protrusions. These fibrillar structures were suggested to have a tubular structure and contain both lipid and amphiphilic peptide, potentially representing long tubular micelles made by a single cylindrical leaflet of lipids. Whilst, Manzo et al.'s 2019 paper demonstrated through MD simulations that temporin L self-assembled to form aggregates in the presence of a lipid bilayer.[56] Further analysis of temporins has proposed that they possess conformational switches which allow for equal probability of the peptide possessing random coil, α-helical or β-sheet conformations.[3c] As Mahalka & Kinnunen note, these conformational switch regions may possess increased propensity to aggregate and self-assemble into amyloid β-sheet fibrils.[3c] This suggests that temporins B and L may be able to functionally utilise this switching ability to allow for environment dependent cytotoxicity, through formation of fibril structures.

2.3.5. Aurein 3.3

Aurein 3.3 (A3.3) is a 17 amino acid long peptide, reported in a number of frog species, including the Southern bell frog (*Ranoidea raniformis*), Green and golden bell frog (*Litoria aurea*), and the Australian blue mountains tree frog (*Litoria citropa*).[57] Studies on the antimicrobial activity of A3.3 by Rozek et al. have demonstrated that the peptide has wide spectrum activity against gram-positive bacteria.[57] It was suggested that A3.3 forms α-helices when in contact with a lipid bilayer.[58] Furthermore, research suggested that A3.3's mechanism of membrane disruption is through a toroidal pore model, in which peptide molecules would self-assemble into a bundle and insert into the membrane to form a pore.[58] In a study conducted by Bücker et al., the cryo-EM structure of A3.3 was shown to exist as a cross-β fibril with kinked β-sheets, which is a similar structural motif as seen in functional amyloids.[45] The fibrils of A3.3 were demonstrated to exhibit an unusual in-plane arrangement, with six peptide chains per helical layer, and highly dense packing.[45] The fibrils formed by A3.3 are labile and reversible in nature, raising the potential for functionality in a wide range of physiological contexts. It was noted that the fibril forming properties of A3.3 may contribute to its antimicrobial activity, in terms of membrane thinning and permeabilisation. However, further research is necessary to determine the extent of interaction of A3.3 fibrils with various membranes, and potential functionality of these self-assembled structures.

2.3.6. Uperin 3.5

Uperin 3.5 (U3.5) is derived from an Australian toadlet (*Uperoleia mjobergii*), which, like A3.3, is 17 amino acids in length and C-terminal amidated. U3.5 is the most thoroughly studied of the uperin family,[57a] and has recently been reviewed.[59] The peptide has demonstrated antimicrobial activity against a range of gram-positive bacteria, such as *S. aureus*.[60] Studies on the activity of U3.5 have demonstrated that the peptide is stable in water, maintaining random coil configurations.[61] However U3.5 has also been demonstrated to be able to form amyloid fibrils,[3a,57a] as seen in studies conducted in buffer.[61] This is significant as recent studies have suggested that U3.5's propensity to form amyloid is dictated by the peptide's secondary structure.[61] Research has also shown that the introduction of trifluoroethanol (TFE) to peptides with α-helical structures, causes the structures to be locked, preventing transition.[61] Research on the unamidated form of U3.5 (U3.5-OH) found that an alternative cross-α structure may be adopted.[45,62] The interaction of U3.5 with synthetic membranes has also been studied. Testing of fresh solutions and incubated (amyloid containing) suspensions of U3.5 against synthetic DMPC (1,2-dimyristoyl-sn-glycero-3-phosphorylcholine) and DMPC:Chol membranes revealed that the peptide is able to discriminate between bacterial or mammalian membranes (cholesterol containing).[4a] Using Quartz Crystal Microbalance with Dissipation monitoring (QCM-D) data this study showed that the interaction of U3.5 with bacterial mimetic membranes was disruptive.[4a] Also, there was no correlation between amyloid aggregation and the membrane interactions of U3.5, suggesting that amyloidogenic peptides and AMPs are mechanistically similar. Computational studies of the effect of NaCl on early fibril aggregation has revealed that the presence of salt can cause significant changes to U3.5 aggregational structures.[63] Further, a combination of experiments and MD simulations were used to demonstrate that micelles consisting of the membrane mimetic, SDS, induce a coil-to-helix transition of U3.5.[64] Whilst, similar molecular dynamics studies were used to determine the β-sheet forming propensity of U3.x (x = 4, 5, 6) peptides.[65] Such studies revealed that there is an inverse correlation between the β-sheet forming propensity and helical stability of uperin peptides.[65] This structural heterogeneity is highlighted using the uperin 3.5 peptides, in Figure 3.

Bücker et al.'s 2022 paper featured a detailed discussion on the similarities and differences between the structures of U3.5 and A3.3.[45] The two peptides demonstrate dissimilarity in lateral fibril structure and presence of kinked versus extended β-sheets. However, their residue composition is relatively similar, as both contain nine hydrophobic residues, two glycine's and three positively charged residues. This means that the overall percentage of positively charged amino acids is higher compared to that of pathogenic and non-pathogenic amyloids. The high positive residue content of U3.5 and A3.3 is likely responsible for its functional interactions with negatively charged bacterial membranes.

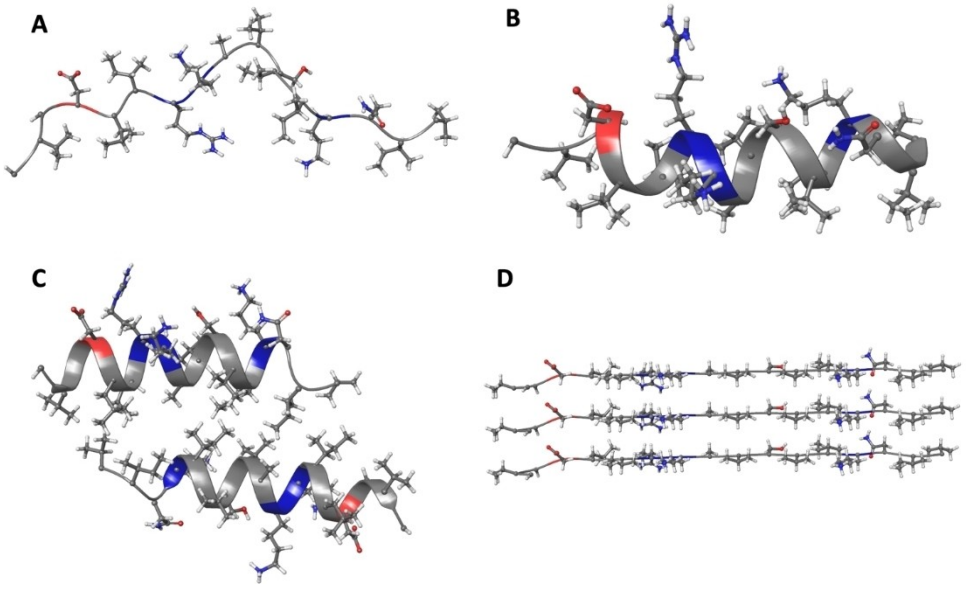

Figure 3. Structural heterogeneity of AMPs: Uperin 3.5. **Panel A**: Theoretically generated random coil structure. Random coils of uperin peptides are often observed in water by circular dichroism.[61,66] **Panel B**: Amphipathic α-helical structure observed in SDS micelles determined by NMR[64] (7S3E.pdb). **Panel C**: Two chains from an X-ray crystal structure of a uperin 3.5-OH cross-α fibril, showing amphipathic α-helices associating via hydrophobic interactions in an anti-parallel arrangement[62] (6GS3.pdb). **Panel D**: Three chains from a Cryo-EM structure of a cross-β fibril, showing quaternary organisation of extended structures[45] (7QV5.pdb). Shown are cartoon representations of the peptide backbone as either a thin tube (for random coiled/extended segments) or ribbon (for helical segments) and coloured according to residue charge (red for negative, blue for positive and grey for neutral). Sidechain and α-carbon atoms are shown.

2.4. Mammals

2.4.1. Indolicidin

Indolicidin is a short 13 amino acid long AMP, derived from bovine species. Testing of the antimicrobial activity of the peptide has demonstrated that it is effective against gram-negative and positive bacteria, as well as, protozoa, fungi, and some viruses. Due to the short length of indolicidin, it is largely linear in structure, forming random coil structures when in solution. The peptide has also been demonstrated to permeabilise membranes without resulting in cell lysis. This is linked to its ability to inhibit DNA synthesis, which contributes to its antimicrobial properties. The specific mode of action of indolicidin is still uncertain, however, recent research by Nielsen et al.[67] suggests that it inserts in the outer leaflet of the lipid bilayer, sitting at the interface between the headgroups and tails of the lipids. When bound to SDS micelles or DPC (dodecylphosphocholine) vesicles the intercalation of indolicidin is thought to be due to the adoption of a wedge shape with a hydrophobic core composed of the proline and tryptophan residues sandwiched by two positively charged regions.[57b] This wedge formation of the peptide is due to the majority of the tryptophan side chains lying flat against the backbone of the molecule. Indolicidin's unique structure and broad activity has made it a popular candidate for therapeutic use. In Falla et al.'s 1996 paper, CD analysis of indolicidin, in sodium phosphate buffer with POPC/POPG liposomes, suggested that the peptide may span bacterial membranes as an aggregate.[68] Zhao et al.'s 2005 study built upon this, demonstrating that indolicidin is able to produce Congo red staining fibres in the presence of phosphatidylserine (PS) containing liposomes.[54] Such studies were carried out in HEPES and EDTA buffer at 37 °C. PS is an acidic phospholipid which is often found on the surface of cancer cells, hence, Zhao et al. hypothesised that indolicidin's formation of fibrils in the presence of PS is likely linked to its cytotoxicity.[54] This could further suggest that the functionality of fibril self-assembly by indolicidin may correspond with inhibition of tumour growth and angiogenesis.

2.5. Humans

2.5.1. LL-37

LL-37 is a human cathelicidin which forms part of the innate immunity system. The peptide consists of a curved amphipathic helix-bend-helix motif which is key to its membrane activity.[7] Studies of the activity of LL-37 have demonstrated that it is toxic towards both eukaryotic and bacterial cells, meaning that it is non-selective in nature. The activity of LL-37 also contrasts most other AMPs, which tend to be selective towards bacterial membranes only. As described by Oren et al. in his 1999 paper,[69] LL-37's mechanism of action against bacterial membranes appears to occur through a carpet-like mode. The peptide first interacts with the membrane in the form of monomers and oligomers, which bind to the membrane surface, causing perturbation. Any existing oligomers then break into monomers which cover the surface, and then diffuse

into the inner membrane. This eventually results in disintegration of the membrane. The ability of LL-37 to oligomerise in solution is mainly due to the hydrophobic N-terminal region of the peptide which is thought to serve as the hydrophobic core of the oligomers. Lee et al.'s 2020 paper states that LL-37 oligomerises into a superhelical amyloid-like fibril when in contact with DNA.[70] Further, a 2020 study by Engelberg & Landau demonstrated that LL-37 is able to self-assemble into a fibril structure in solution consisting of densely packed helices.[71] Whilst EM assays revealed that the fibrils produced by the peptide are stable, the lack of amyloid continuous sheets and stacking of individual peptides perpendicular to the fibril axis prevented binding in the ThT assay performed. These fibrils are wide and ribbon-like in structure, and are thermostable, forming at the surface of a bacterial membrane. The surface of the peptide consists of zigzagged belts, which alternate between being hydrophobic and positively charged, allowing for interaction with negatively charged lipid bilayers to cause their disruption. Similarly, Sood et al.'s study noted that the fibres formed by LL-37 are 'amyloid-like' because they lack the extensive β-sheet structure which is characteristic of amyloid.[72] Engelberg & Landau contended that there is hence a connection between LL-37's biological activity and its fibril forming properties.[71]

3. Evolutionary perspective

The patterns in AMP properties, membrane mechanism and fibril formation demonstrated by the evolutionary groups in this review may suggest that AMP amyloidogenicity is an innate property. The potential connection between peptide antimicrobial properties and the ability to self-assemble to form amyloid has been noted by a number of authors (see review by Häffner et al.).[19] Here we choose to focus on the ideas presented by Moir et al. in his 2018 paper on the role of Aβ in AD. In particular, Moir developed a hypothesis for the role of Aβ in AD, whilst additionally comparing Aβ to LL-37. As Moir suggests, the Aβ protein has been conserved in vertebrates throughout evolution and exists as an ancient effector molecule of innate immune defence. Similarly, as all the evolutionary AMP groups featured in our review form amyloid under near identical conditions, this may suggest that antimicrobial mechanistic specificity developed subsequent to an inherent amyloidogenic property.

As summarised in Table 2, a number of patterns in membrane mechanism and fibril formation are broadly demonstrated by each of the evolutionary groups. The broad trend across the evolutionary groups featured in Table 2, is that the majority of peptides analysed form amyloid in the presence of a negatively charged lipid membrane. This is likely due to bacteria generally possessing a negatively charged membrane, hence, AMPs tend to target bacterial cells as part of their immune defence functionality. This further supports the concept presented in a number of recent papers on amyloidogenic AMPs, that fibril formation is an essential part of the immune defence activity of AMPs and subsequent bacterial cell toxicity. We further this idea by suggesting that as fibril formation across evolutionary groups occurs under largely identical circumstances, production of amyloid by AMPs may be an innate property which has been conserved throughout evolution. It should be noted that a number of AMPs mentioned in this review have other family members with similar properties, however, the majority of these do not form amyloid. Some of these include the aureins, magainins and uperin 2.x family.

Patterns demonstrated in Table 2 in terms of membrane mechanisms are less distinct across the evolutionary groups. The two bacterial AMPs, PSMα3 and plantaricin A both seem to demonstrate a carpet forming mechanism. Whereas, the arthropod AMPs demonstrate more uncertainty and variety, as the mechanism of longipin is yet to be established, and the mechanisms of melittin and cecropin A may be pore or carpet. The amphibian (anurans) AMPs present predominantly pore mechanisms, with dermaseptin and uperin being the exceptions demonstrating carpet-like mechanisms against bacterial membranes (although uperin has been found to form pores in DMPC:Chol). Finally, the mammalian AMPs demonstrate variety and flexibility of mechanisms, including barrel stave-like and carpet-like for indolicidin and LL-37, respectively. Although, a clear pattern in membrane mechanism cannot be elucidated for each evolutionary group, we suggest that AMPs may possess tuneable activities, which result in development of mechanisms which may be specific to the peptide's function and environment. This builds upon the observation that the fibril formation conditions are largely consistent for each evolutionary group. Overall, we suggest that if AMP amyloidogenicity is innate and conserved throughout evolution, specificity of antimicrobial action, in terms of membrane mechanism, is developed subsequent to this amyloid-forming property. Therefore, the variety in mechanisms seen across, and even within, evolutionary groups may be due to these peptide membrane mechanisms being specific to each species, and hence, this feature develops in response to the needs and challenges faced by each individual species. Further development of this concept is limited due to the list of *amyloidogenic* AMPs being so small, largely due to the lack of comparative studies in this area, comparing antimicrobial activity of peptides to their potential to form amyloid.

The ideas we present here largely build upon those presented by Moir in terms of Aβ. Initially, the deposition of β-amyloid plaques by Aβ was thought to be linked to the cause of AD. Moir et al. demonstrated in his 2010 paper, that Aβ also acts as a AMP, suggesting that its role in AD is not as simple as being the cause of the condition.[4b] Moir suggested that Aβ deposition may be triggered by neuroinflammation, however, as Aβ also acts as an AMP in the innate immune system, it may additionally present a normal protective role (Aβ's immune defence activity may lead to the neurodegeneration which results in AD). This led Moir to propose that the involvement of Aβ in AD may be an immune response to infection, rather than a pathological dysregulatory response. Further, in terms of amyloid formation, Moir notes that oligomerisation is a normal function of AMPs, hence, the amyloidogenic propensity of Aβ and similar AMPs is likely to be part of typical antimicrobial

Table 2. Summary of AMP properties, membrane mechanism, and self-assembly conditions for each peptide reviewed.

Peptide	AMP Activity	Mechanism of membrane activity	Self-assembly conditions to form mature fibrils
PSMα3	Gram positive	Carpet-like mechanism	Interaction with negatively charged lipid membrane
Plantaricin A	Gram positive, gram negative	Local carpet or leaky slit mechanism	Interaction with negatively charged lipid membrane
Longipin	Gram positive, gram negative	Yet to be determined	Interaction with negatively charged lipid membrane
Cecropin A	Gram positive, gram negative	Carpet-like or channel formation	Only in the presence of an inducing medium
Melittin	Gram positive, gram negative	Toroidal pore mechanism	In the presence of SDS – membrane-mimicking environment
Dermaseptin (S9)	Gram positive, gram negative, fungi and protozoa	Carpet-like mechanism	When bound to a negatively charged lipid membrane
Buforin II	Gram positive, gram negative	Pore mechanism	May be unable to form fibrils
Magainin 2	Gram positive, gram negative, fungi, protozoa	Toroidal or chaotic pore mechanism	In presence of negatively charged membrane
Temporin B & L	Temporin B: gram positive Temporin L: gram positive, gram negative, fungi and cancer cells	Barrel stave mechanism	Interaction with negatively charged lipid membrane
Aurein 3.3	Gram positive	Toroidal pore mechanism	Interaction with negatively charged lipid membrane
Uperin 3.5	Gram positive	Pore forming in presence of eukaryotic membranes, carpet mechanism in presence of mammalian and bacterial membranes	Interaction with negatively charged lipid membrane
Indolicidin	Gram positive, gram negative, protozoa, fungi, and some viruses	Insertion into the membrane in a wedge formation	In the presence of acidic phospholipids
Cathelicidin (LL-37)	Gram positive, gram negative, enveloped viruses and fungi	Carpet-like mechanism	In the presence of DNA or negatively charged lipid bilayers
Amyloid beta (Aβ)	Gram positive, gram negative, fungi and some viruses	Pore forming mechanism, soluble oligomers may preference a carpet mechanism	Interaction with negatively charged lipid membrane

activity. In the case Aβ, formation of amyloid assists with the entrapment of microbial pathogens.

3.1. Bioinformatic Insights

The AMPs are a diverse group in terms of sequence identity, structural characteristics and gene family.[73] Whilst some peptides arise from the proteolytic cleavage of proteins with established functions (for e.g. buforin II from Histone H2 A[74]) other peptides arise from genes whose apparent primary function is to produce the AMP itself (for e.g. plantaricin A from the gene *plnA*).[75] As a group, they are defined by their microbiocidal activity and they can be further classified based on structural, mechanistic or species-specificity criteria - which has been harnessed to produce several databases[76] (for example APD3[77] and CAMPR3[78]), and computational tools[79] useful for predicting antimicrobial activity (for e.g. AmpGram[80]) or amyloid propensity[81] (for e.g. AmyloGram[82]).

The small selection of peptides covered in this review span a broad range of evolutionary groups and, not surprisingly, when their sequences are aligned they show little overall homology (data not shown). However, alignment of primary sequences in families and subfamilies with highly related members (such as, the PSMα's, cecropins, dermaseptin S's, magainins, temporin 1T's, aureins, and uperins), produces well-defined consensus sequences (Table 3). The consensus sequences in Table 3 all contain an amphipathic helical signature (alternating hydrophobic and hydrophilic residues at N + 3/4[83]) despite overall identity being low. Even from this small selection of AMPs we see indications of convergent evolution whereby sequences with relatively low overall identity, across a wide variety of phyla and genes, have independently evolved to produce peptides with similar structural characteristics which all act with a similar mechanism to selectively disrupt membranes of pathogenic microbes. The defensin and defensin-like families of AMPs provide one of the best examples of convergent AMP evolution, whereby independent origins led to a unique structural-motif consisting of a short helical segment

Table 3. Consensus peptide sequences for selected members of AMP families.

Peptide Family Members	Consensus Sequence[a]
PSMα 1, 2, 3 & 4	MgIIAgIIKfIKsLIEKFtGkx
Cecropin, cecropin 1, 2, A, B, C, D, P1, P2, P3 & P4	xWLxKxxKKLExxxKKxxxxxIxxAVxxxxxAxxxxxxx
Dermaseptin S: 1[b], 2, 3, 4, 5, 6, 7, 8, 11, 12 & 13	xLWxTMLKxVGTxAxxAxKAALxAAxNxxSxxxx
Magainin B1, B2, BM1, BM2, R1[c], R2 & R3	GIxKFLHSAGKFGKAFVGEIMxS
Temporin 1T: a, b[d], c, d, e, f, g, h, I[e] & k	xLPIIGKLLNxIL
Aurein 1.1, 1.2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 3.1, 3.2, 3.3, 4.1, 4.2, 5.1 & 5.2	GLFDIVKKVVGxIxxxIxxxxxxxx
Uperin 2.1, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 4.1, 5.1, 6.1, 6.2 & 7.1	GILDxxKKVVxxIKNVLxixxxxxxxxxxx

[a] Consensus sequence created using EMBOSS Cons (https://www.ebi.ac.uk/Tools/msa/emboss_cons/). x signifies position of low consensus (i.e., high variability between residue types), lower case letters represent a mid-level of consensus. Sequences were obtained from UniProt (https://www.uniprot.org/). [b] Also known as dermaseptin (S9). [c] Also known as magainin 2. [d] Also known as temporin B. [e] Also known as temporin L.

linked via disulfide(s) to a stretch of β-strand with the highly conserved cysteine residues involved in cis- or trans-disulphide arrangement with other structural elements (see review by Shafee et al.[84]). Interestingly, this structural class has provided a basis for evolutionary divergence, with related peptides having diverse activities independent of antimicrobial activity.

Evolutionary divergence is a key mechanism whereby organisms counter new or altered threats from microbial pathogens.[85] In the context of AMP activity, this may arise from genetic mechanisms, such as mutation at specific sites to produce altered structures with refined or new activities.[86] Indeed, it is noteworthy that some AMP families, particularly within the anurans, feature peptides with very high homology, suggesting continual evolutionary refinement.[87] Even relatively subtle changes may have follow-on effects, such as increased protease susceptibility to favour formation of proteolytic degradation products with new antimicrobial activity.[88] Alternatively, they may confer new structural characteristics, such as an increased propensity to dimerise or oligomerise[89] or lead to distinct structural forms such as β-sheet or amyloid, and along the progression pick up refinements to, or additional, activity.

Our review has investigated antimicrobial peptides which can also self-assemble into amyloid structures and considered whether this property has evolved as a synergistic tool for antimicrobial action. There is no doubt that across evolution, self-assembly into amyloid appears to be an advantage for this particular function. However, more work is required to understand fully, how and why just a small subset of AMPs have evolved this capacity.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: amyloids · antimicrobial peptides · evolution · innate immunity · self-assembly

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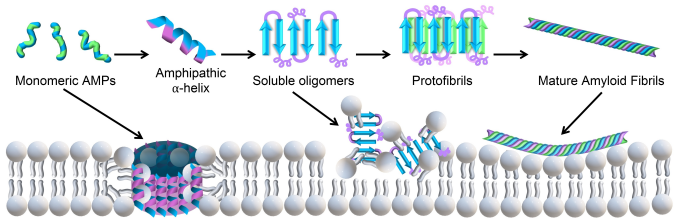

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Antimicrobial peptides are an ancient defence against a wide range of microbes. Some can self-assemble into oligomers and amyloid. Similarities with neurodegenerative diseases support a hypothesis that some diseases are due to aberrant activity of a protective neuropeptide. This review highlights the activities of antimicrobial peptides that can self-assembly to form amyloid in major evolutionary groups.