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REVIEW ARTICLE

An Atlas of Anionic Antimicrobial Peptides from Amphibians

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Abstract: Anionic antimicrobial peptides (AAMPs) with net charges ranging from -1 to -8 have been identified in frogs, toads, newts and salamanders across Africa, South America and China. Most of these peptides show antibacterial activity and a number of them are multifunctional, variously showing antifungal activity, anticancer action, neuropeptide function and the ability to potentiate conventional antibiotics. Antimicrobial mechanisms proposed for these AAMPs, include toroidal pore formation and the Shai-Huang-Matsazuki model of membrane interaction along with pH dependent amyloidogenesis and membranolysis via tilted peptide formation. The potential for therapeutic and biotechnical application of these AAMPs has been demonstrated, including the development of amyloid-based nanomaterials and antiviral agents. It is concluded that amphibian AAMPs represent an untapped potential source of biologically active agents and merit far greater research interest.

Keywords: Anionic antimicrobial peptides, amphibian, amyloid, amyloidogenesis, anticancer, membranolysis.

1. INTRODUCTION

Over the centuries, skin extracts from frogs and toads have been used as traditional medicine in many cultures ranging from that of ancient Egypt to that of China [1]. For example, Chan Su is derived from the dried skin and venom gland secretions of toads from the Bufo genus and is used in Chinese traditional medicines as a remedy for numerous conditions [2]. The earliest recorded use of this preparation appears to be in the Tang Dynasty (618 to 907 AD), and it is now known that the major active ingredients in Chan Su are bufadienolides, which are steroids or cardiac glycosides [3]. It is also now known that a wide variety of bioactive compounds are found in the skin secretions of amphibians [4], including peptides, ranging from those with myotropical and antioxidant properties to those with antimicrobial and anticancer activity (Table 1) [5-6]. Many of these peptides are pleiotropic. For example, some antimicrobial peptides (AMPs) exhibit antibacterial, antifungal, antiviral and anticancer action [7-8]. Amphibian skin is a particularly rich repository of AMPs [5-6] and was the source of one of the first of these peptides to be discovered, namely magainin from *Xenopus laevis* in the early 1980s [9]. Homologues of magainin have since been identified in other species of the Xenopodinae and studies on the phylogenic and evolutionary relationships between amphibian AMPs have estimated that the ancestral genes of these peptides may be up to around 200 million years old [10-11]. Indeed, it is now generally accepted that AMPs are ancient endogenous components of the innate immune system and exert their antimicrobial activity *via* membrane interactions that involve relatively non-specific mechanisms at multiple sites of action [12]. Microbes appear to have a limited capacity to defend them-

Table 1. Major bioactive peptides found in the secretions of amphibian skin. Table 1 was compiled from Xu and Lai [5].

Myotropical peptides	Wound-healing peptides
Opioid peptides	Immunomodulatory peptides
Corticotropin- releasing peptides	Neuronal nitric oxide synthase inhibitors
Angiotensins	Antibacterial peptides
Protease inhibitor peptides	Antifungal peptides
Neuropeptides	Antiviral peptides
Antioxidant peptides	Antiparasitic peptides
Lectins	Anticancer peptides
Insulin releasing peptides	Pheromone peptides
Mast cells degradation / histamine-releasing peptides	Granains

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Table 2. AAMPs from amphibians.

AAMPs	Sequence	Host amphibian	Key references
He-1	DDDKTEEEDDKENETTKVVE	*Hyla eximia*	[35]
Thaulin-4	DDGEEAESEAANPEENTVGG	*Pleurodema thaul*	[36]
Octacidin	DSVASSAAQELSGVLASN	*Osteocephalus taurinus*	[37]
Pleurain-C1	YPELQQDLIARLL	*Rana pleuraden*	[38]
Pleurain-C2	FPELQQDLIARLL	*Rana pleuraden*	[38]
Brevinin-1-AJ3	FLPLAVSLAANFLPKLFCKITKNVETLE-MELEII	*Amolops jingdongensis*	[39]
Jingdongin-2	FLPIVENCSLVCWENNQKC	*Amolops jingdongensis*	[39]
*Popu*Def	GASPALWGCDSFLGYCRIACFA-HEASVGQKDCAEGMICCLPNVF	*Polypedates puerensis,*	[40]
Defensin-TK	SPAIWGCDSFLGYCRLACFA-HEASVGQKECAEGMLCCIPNVF	*Theloderma kwangsiensis*	[41]
CFBD-1	FAVWGCADYRGY-CRAACFAFEYSLGPKGCTE-GYVCCVPNTF	*Cynops fudingensis*	[42]
PYL	ADADDDDDK	*Xenopus laevis*	[31]
XLAsp-P1	DEDDD	*Xenopus laevis*	[33]
XLAsp-P2	DEDLDE	*Xenopus laevis*	[32]
PD-3-7	LLGDLLGQTSKLVNDLTDTVGSIV	*Pachymedusa dacnicolor*	[43]
Maximin H5	ILGPVLGLVSDTLDDVLGIL-NH_2	*Bombina maxima*	[44]

Table 3. Peptide B / enkelytin from amphibians. Table 3 shows the sequences of peptide B / enkelytin, which are encrypted within proenkephalin (PENK) of a variety of amphibians. Also shown are the SwissProt accession codes for these sequences, which are available at the SwissProt database (http://web.expasy.org/docs/swiss-prot).

Host Amphibian	Sequence	Key References and SwissProt Accession Code
Amphiuma means (Salamander)	FTDYSAPSDEDGESYSKEIPEMEKRYGGFMRF	[45] Q6YIR4
Necturus maculosus (Salamander)	FADYSAPSDEDGESYSKEIPEMEKRYGGFMRI	[45] Q6YIR3
Taricha granulose (Newt)	FADSSAPSEEEAESYSKEIPEMEKRYGGFMRY	[46] Q5PZ00
Xenopus laevis (Frog)	FTDSFLPSDEDGESYSKENPDMEKRYGGFMRF	[47] P01212
Xenopus tropicalis (Frog)	FTDSFLPSEEDGESYSKENPDMEKRYGGFMRF	[48] F6Y5F6
Bombina orientalis (Toad)	FADSLLPSDEDGESYSKEVPEVEKRYGGFMRF	[49] Q5Y4B6
Spea multiplicatus (Toad)	FSDSVLPSDEDGESYSKEIPEMDKRYGGFMRF	[50] Q91817

found to possess both antibacterial activity, killing *E. coli*, *S. aureus* and *B. subtilis*, and antifungal activity with action against *C. albicans* [38]. Two AAMPs were identified in the skin of the Chinese torrent frog, *Amolops jingdongensis*, which is also native to South Western China. These AAMPs were designated brevinin-1-AJ3 (net charge -1) and jingdongin-2 (net charge -1) [39]. The latter peptide along with the CAMP, jindongin-1, appeared to constitute a novel family of AMPs with no similarity to known amphibian peptides [5], but both peptides showed the potential to form a C-terminal cyclic region stabilised by a disulphide bond, Cys18-$(Xaa)_4$-Lys-Cys24 (Table **2**) [39], which is known as the 'Rana box' and is characteristic of AMPs from Ranid frogs [5-6]. The 'Rana box' is often associated with the antimicrobial activity of AMPs [74], but jingdongin-2 exhibited no activity against the panel of bacteria and fungi tested, although this panel was small [39]. Many brevinins form a 'Rana box' [5] and in the case of brevinin-1-AJ3, a sequence homologous to this motif, Cys18-$(Xaa)_4$-Lys-Asn24 (Table **2**) was present in the peptide's primary structure, suggesting that Cys24 has been mutated to Asn24 resulting in a loss of ability to form a 'Rana box' [39]. Brevinin-1-AJ3 was not characterised by the latter study and its properties remain unknown but many atypical brevinins with potent antimicrobial activity are known where the 'Rana box' of these peptides has either been lost or mutated [5, 75-77]. Brevinin-1-AJ3 and jingdongin-2 were also notable for the fact that they each contained a free half-cysteine residue [39], which is rare amongst AAMPs, evidenced by the small number of these peptides listed in the APD3 database [22]. These AAMPs have been identified in several sources [78] but most appear to be from plants [22], such as *Cn*-AMP3, which is one of several AAMPs found in the Coconut, *Cocos nucifera* [28, 79]. In general, the role of free cysteine residues in the activity of AMPs is unclear although recent work has suggested that these residues may play a role in facilitating peptide dimerization rather than directly promoting the antimicrobial activity of AMPs [80]. However, it is also interesting to note that an increasing number of multifunctional peptides are being identified in the skin of amphibians, both serving as AAMPs / CAMPs and possessing antioxidant activity, which is promoted by the presence of free half-cysteine residues [38, 81-82]. Indeed, a number of anionic antioxidant peptides have been reported that have yet to be tested for antimicrobial activity, such as andersonin-AOP1 (net charge -1) from Anderson's frog, *Odorrana andersonii* which is found in the Yunnan Province of China and regions of neighbouring countries [83]. The Puer tree frog, *Polypedates puerensis* (*Rhacophorus puerensis*), is primarily known in the Yunnan Province of China, and was recently shown to be the host of the first anionic defensin from amphibians, *Popu*Def (net charge -1). It was found that the expression of *Popu*Def was upregulated in immune related tissues of *P. puerensis*, such as the skin and gut, in response to bacterial challenge. The peptide showed moderate activity against organisms able to induce its expression, including Gram-negative bacteria, such as *P. aeruginosa* and Gram-positive bacteria, including *B. subtilis* [40]. Since the discovery of *Popu*Def, a second amphibian anionic defensin has been identified in skin secretions of the tree frog, *Theloderma kwangsiensis* (*Theloderma kwangsiense*), which is mostly found in Eastern Guangxi Province, China. Designated defensin-TK (net charge -1), the peptide showed broad range antimicrobial activity with efficacy against *C. albicans,* Gram-positive bacteria, such as *S. aureus,* and Gram-negative bacteria, including *B. dysenteriae* [41]. Our own analyses using the SIM - Alignment Tool for protein sequences (http://web.expasy.org/sim/) revealed that there is *circa* 90% sequence homology between defensin-TK and *Popu*Def. Given that these peptides have been identified in frogs from the same taxonomic family [84], this would suggest that defensin-TK and *Popu*Def may have originated from a common gene. Moreover, these latter two AAMPs showed homology of around 50% with CFBD-1, which is a defensin from the fire-bellied newt, *Cynops fudingensis*, a new species recently identified in North Eastern Fujian Province, China [40-41]. This peptide was mildly anionic (net charge - 0.4) and showed moderate activity against Gram-positive bacteria, such as *S. aureus*, and weaker activity against other bacteria and fungi [42].

CFBD-1 would appear to be the first AAMP to be characterised in salamanders, although evidence has been previously presented, which infers the presence of other AAMPs in the brains of these organisms (Table **3**) [26]. In human brains, it is believed that peptide B (net charge -6) and its truncated form, enkelytin (net charge -7), are enzymatically cleaved from the opioid hormone, proenkephalin (PENK), in response shown to microbial challenge [85-86]. PENK processing to produce these AAMPs also generates opioid peptides and it is believed that this strategy helps provide an immediate, coordinated innate immune response to the threat of infection (Fig. **1**) [26, 86]. PENK is highly conserved across the eukaryotic kingdom, and homologues of peptide B / enkelytin are encrypted within the sequence of this opioid hormone in a number of amphibians, as revealed by a search of the SwissProt database (http://web.expasy.org/docs/swiss-prot). Examples include salamanders, newts, frogs and toads, which strongly suggests that these AAMPs are present in the brains of organisms across Amphibia (Table **3**). Recently, the PENK related potential to produce peptide B / enkelytin was reported for the Western clawed frog, *Xenopus tropicalis* (*Silurana tropicalis*), which is found in the West African rainforest belt and is closely related to *X. laevis* (Table **3**) [48]. As a matter of historical interest, the PENK related potential to produce peptide B / enkelytin was identified in the latter organism over five years before it was shown to be the host of PYL, the first of demonstrated amphibian AAMPs [47].

The Yunnan firebelly toad, *Bombina maxima*, is native to the mountainous regions of South Western China and adjacent Northern Vietnam, and the skin secretions of the frog have been found to include maximin H5 (net charge –1) [44], which is probably the best characterised of amphibian AAMPs [29, 54, 87-90]. A number of characterisation studies have shown that maximin H5 is a predominantly α-helical peptide with multiple biological roles, which are described below, and include: antibacterial action [44, 54, 89], anticancer activity [29, 55] and neuropeptide functions [56].

3.1. The Neuropeptide Function of Maximin H5

Phylogenetic studies have shown that maximin H5 and homologous AAMPs belong to a suite of AMPs that has arisen through rapid gene diversification, driven by positive

segment of the peptide. These aspartate residues were distal from the membrane surface and appeared to play no direct role in the lipid interactions of maximin H5, rather, serving a primarily structural role [89]. The amidated C-terminal segment, V16-L20-NH_2, of maximin H5 (Table **2**) was found to play a key role in its membranolytic activity by forming an intra-peptide hydrogen bonding network with the N-terminal region of the peptide that stabilised its tilted α-helical structure (Fig. **2A**). A number of recent studies have shown that the activity of some AAMPs against *S. aureus* are influenced by pH [101], such as that of hebraein (net charge -4), which is produced by *Amblyomma hebraeum* [102] and other ticks [103-104]. This was also found to be the case for maximin H5 when a very recent study showed that low pH enhanced the membranolytic action of the peptide against *S. aureus*, making maximin H5 the only major amphibian AAMP reported to possess pH dependent antibacterial activity [54]. Low pH appeared to have the general effect of enhancing the levels of amphiphilic α-helical structure possessed by maximin H5, thereby maximising its membranolytic ability and facilitating the killing of *S. aureus via* a 'carpet'-type mechanism [54, 89], which is that most commonly used by α-helical AMPs [12, 105]. According to this mechanism, increasing levels of maximin H5 'carpet' membranes of *S. aureus* in a nonspecific manner, thereby inducing progressively greater numbers of lesions and ultimately, membranolysis [54, 89]. It is believed that a number of other amphibian AMPs eradicate bacteria *via* the 'Carpet' mechanism with a tilted α-helical structure incorporated into their lytic action [12, 100]. Examples include aurein 2.3 from Green and Golden Bell frog, *Litoria aurea* [106]. Comparisons were made between the tilted α-helix of maximin H5 [54, 107] and that of the anionic influenza haemagglutinin peptide, HA2 [100, 108], which has a low pH optimum for the adoption of tilted α-helical structure and membranolytic action [109]. These comparisons revealed many compositional and structural similarities between the two molecular architectures and led to the suggestion that low pH enhanced the activity of maximin H5 against *S. aureus* primarily by maximising its level of N-terminal tilted α-helical structure [54]. In combination, these observations indicate that maximin H5 exerts antibacterial activity *via* a pH dependent antimicrobial mechanism that has not yet been reported for AAMPs or, indeed, AMPs in general [101].

3.3. Bacterial Resistance to the Action of Maximin H5

S. aureus has evolved a wide repertoire of resistance mechanisms to counter the action of AMPs and other host defence molecules [113-114] including a number that are influenced by pH [101], as recently shown for the resistance of both *S. aureus* and methicillin-resistant *S. aureus* (MRSA) to analogues of magainin 2 from *X. laevis* [115]. This possibility was investigated for maximin H5, and it was found that *S. aureus* had developed protection from the action of the peptide. This protection was pH dependent and involved lysylated-phosphatidylglycerol (lys-PG) in the membranes of the organism [54]. It is well established that levels of lys-PG in *S. aureus* membranes are elevated at low pH, which enhances the ability of the lipid to attenuate properties of these membranes and thereby enable the organism to resist AMPs [115]. Consistent with these observations, it was shown that the elevated levels of lys-PG present in *S. aureus* membranes at low pH reduced the net negative surface charge carried by these membranes and affected characteristics of their structural order, such as lipid packing and membrane fluidity, thereby inducing rigidification. These effects appeared to inhibit the ability of maximin H5 to target and penetrate membranes of *S. aureus*, which was surprising given that the peptide carries a net negative charge [44] and lys-PG mediated mechanisms of resistance appear to have evolved primarily to protect the organism from CAMPs [19, 114]. To explain this apparent contradiction, it was suggested that the membranolytic form of maximum H5 effectively acted as a CAMP *via* the amidated residues at its two termini, which are the only positively charged residues possessed by the peptide [54]. Consistent with this suggestion, MD simulations showed that these amidated residues were relatively more accessible to external molecules than the internally located, anionic residues possessed by the peptide (Fig. **2**) [88, 90, 110]. These findings appeared to help explain how maximum H5 targeted membranes of *S. aureus* and also why the peptide was able to evade some adaptive mechanisms of resistance possessed by the organism to combat anionic AMPs [114, 116]. Further investigations into the antimicrobial action of maximin H5 showed that with the exception of *S. aureus*, the peptide had no activity against other Gram-positive bacteria, Gram-negative bacteria, fungi or enveloped viruses [44, 88, 90, 117]. A clear difference between these two groups of microbes was the presence of phosphatidylethanolamine (PE) in the membranes of organisms resistant to the action of maximin H5 and the absence of this lipid in the membranes of microbes susceptible to its activity [118-120]. Studies on *E. coli* showed that maximin H5 had no propensity to partition into membranes derived from this organism, or other PE-containing membranes. In addition, the peptide was predicted by MD simulations to remain bound to the surface of these bilayers *via* a variety of peptide-lipid interactions and intra-peptide associations (Figs **2B** and **2C**). The major contributions to this peptide-membrane binding came from hydrogen bonding between phosphate and ammonium groups within the PE head-group and residues in both terminal regions of maximin H5 [88, 110]. Essentially, in relation to the membranolytic form of the peptide (Fig. **2A**) [89-90], maximin H5 appeared to undergo a conformational change in the presence of PE that led to random coil structure in its amidated N-terminal segment, H_2N-I1–S10 and the absence of intra-peptide interactions between this segment and the amidated C-terminal segment, V16-L20-NH_2, of the peptide (Table **2**) (Figs. **2B** and **2C**) [88, 110]. The N-terminal PE-binding sequence of the peptide showed significant homology to those of the bacterial AMPs, cinnamycin and duramycin, which suggested that the affinity of maximin H5 for the lipid may involve an ability to mimic structural properties of the PE-binding pocket formed by these prokaryotic peptides [110]. Consistent with this proposal, a CAMP with potent activity against the enveloped virus, human immunedeficiency virus was produced by substituting arginine residues for the three aspartate residues of maximin H5 [117], thereby destabilising the PE binding conformation of the peptide [88, 110]. Taken in combination, these observations suggested that maximin H5 has high affinity for PE that induces immobilisation of the peptide on the surface of membranes, which include the lipid, thereby inhibiting the ability

Characterisation of the anticancer action of maximin H5 showed that it adopted high levels of α-helical structure that facilitated membranolytic action against cancer cell membranes [55]. These studies also showed that this action was promoted by anionic lipid and required the presence of the peptide's terminal amide moieties for optimal anticancer effect, supporting the view that maximin H5 effectively acts as a CAMP [54, 89]. It is well established that CAMPs interact with cancer and microbial membranes through generally similar mechanisms, targeting the negative surface charge carried by both these membrane types [128-129]. To investigate the interaction of maximin H5 with cancer cell membranes at the molecular level, here we present MD simulations of the peptide interacting with a bilayer composed of PC and PS in a 10:1 molar ratio. This lipid composition corresponds to that used to mimic cancer cell membranes in the experimental work of Dennison *et al.,* [55] and consistent with this work, our MD simulations predicted that native maximin H5 would partition into these bilayers primarily through the formation of high levels of α-helical architecture (Fig. **3A** (i)). Similar to the antibacterial form of the native peptide [89] (Fig. 2A), this α-helical architecture primarily resides in the segments, H_2N-I1–S10 and V16-L20-NH_2, of maximin H5 (Fig. **3A** (i)). These segments of the peptide were predominantly associated with DMPS molecules in the membrane and facilitated penetration of the hydrophobic core of the bilayer, as indicated by the partial density profile of native maximin H5 (Fig. **3A** (ii)). In particular, hydrophobic residues in the terminal segments of this peptide interacted with the acyl chains of DMPS whilst charged and polar residues in these segments associated with moieties in the head-group region of the lipid, such as phosphate groups. The overall lipid interactive conformation of native maximin H5 was stabilised by high levels of hydrogen bonding, which was predominantly between residues within these N-terminal and C-terminal segments and multiple components of the bilayer (Fig. **3A** (i)). These observations are generally consistent with experimental work [55] and indicate that native maximin H5 has a strong preference for anionic lipid over zwitterionic lipid, providing a basis for the observed ability of PS to promote the membranolytic and anticancer action of the peptide.

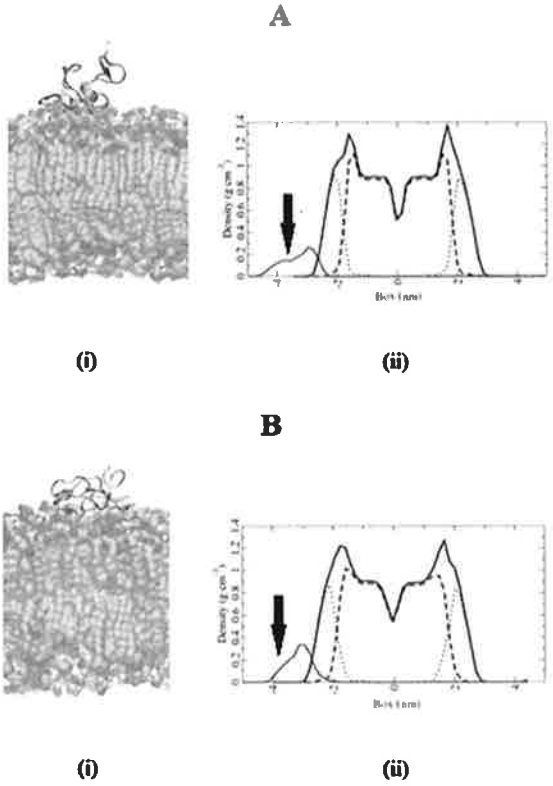

Fig. (3). MD simulations of maximin H5 interacting with cancer cell membranes. Figures 3A (i) and 3B (i) show side elevations of MD simulations for the interaction of maximin H5 and its deaminated isoform, respectively, with bilayers formed from DMPS / DMPC (10:1) and this lipid composition was taken to represent cancer cell membranes as previously described [130-131]. A detailed description of these lipid interactions is provided above but essentially, these simulations predicted that both peptides partitioned into these bilayers *via* an N-terminal region comprising 10 residues and a shorter C-terminal segment. These MD simulations also generated partial density profiles and the graphics in each panel shows partial densities of the components in each peptide / lipid system (Figures 3A (ii) and 3B (ii): overall lipid density (solid black line), lipid head-groups (dotted line), lipid tails groups (dashed line) and peptide (solid line and indicated by arrows). A comparison of the partial density profiles for peptides, predicts that maximin H5 (Figure 3A (ii)) would penetrate the hydrophobic membrane core region of DMPS / DMPC (10:1) membranes more deeply than its deaminated isoform (Figure 3B (ii)). MD simulations were performed as previously described except that lipid bilayers were constructed from lipid mixtures of DMPS / DMPC in a molar ratio of 10:1 [88].

AAMPs [26]. Maximin H5 appeared to be the only peptide reviewed here with neuropeptide function (Table **4**), although it has been predicted that a number of homologous AAMPs may share this property [56]. It would seem that this localisation of antimicrobial and neuropeptide capability within single peptides might be an important strategy for the promotion of a rapid unified neuroimmune response by the amphibian host. This strategy clearly differs fundamentally from that mediated by the processing of PENK where antimicrobial and neuropeptide functions reside separately in AAMPs and opioid peptides (Fig. **1**) [85-86]. Currently, the presence of this opioid hormone in *B. maxima* does not appear to have been investigated, although it has been identified in the brain of the closely related Oriental Fire-bellied Toad, *Bombina orientalis* (Table **3**) [49]. In combination, these observations suggest that the brains of amphibians may have a number of strategies for providing a coordinated neuroimmune response to threats such as microbial challenge, stress, or other stimuli.

This review has shown that some progress has been made in elucidating the mechanisms underpinning the biological activities of amphibian AAMPs. For example, XLAsp-P1 appeared to kill bacteria using a toroidal pore type mechanism, and the action of XLAsp-P2 shows hallmark characteristics of the SHM model of membrane interaction. This latter peptide also showed the ability to potentiate the action of conventional antibiotics against MDR pathogens, and the potential advantages of this form of combination therapy are increasingly being recognised and are viewed as a major, potential strategy for combating microbial pathogens with MDR [137-138]. For example, combination treatment can potentially reduce the required dosage of individual drugs, thereby diminishing side effects, as well as, not only eliminating resistant strains, but also delaying the emergence of drug resistance [139-140]. Indeed, it has been observed that combination therapy to potentiate failing antibiotics could be an interim solution to the global problem of MDR pathogens until sufficient numbers of novel antimicrobial molecules and strategies become available [141].

Several AAMPs reviewed here, namely PD-3-7 and maximin H5, exhibited pH-dependent mechanisms of biological activity, which is relatively rare amongst amphibian AMPs [101]. In the case of PD-3-7, this activity appeared to involve epimerisation and pH-dependent amyloid formation, which led to the proposal that studies on amyloidogenesis by these epimers could help understand the pathogenesis of disease-related amyloid, such as AD [52-53]. It is well established that D-enantiomers of residues are present in the Aβ peptides, and elevated levels of these residues have been described in amyloid plaques and the brain tissue of AD patients [142]. Based on the pH-dependent reversibility of amyloid formation by PD-3-7, it has also been proposed that studies on the peptide could aid the development of amyloid-based nanomaterials [52-53]. The ability of amyloid to reversibly self-assemble has been employed in the design of long-acting drugs where the sustained and controlled release of biologically active peptides occurs from the termini of fibrils [143]. Currently, the number of known amyloidogenic AAMPs is increasing [60], such as the recently reported Cn-AMP2 from the Coconut, *Cocos nucifera* [144]. In addition, functional amyloid nanostructures are being developed for use in a growing number of diverse areas, such as tissue repair / engineering, which is of high technological and medical importance [143, 145].

In the case of maximin H5, there is strong evidence to suggest that the peptide kills *S. aureus* using a pH dependent membranolytic mode of action that encompasses tilted peptide formation. Interestingly, it can be seen from (Fig. **4**) that the hydrophilic face of the tilted α-helix formed by maximin H5 is rich in glycine residues, a feature shared by a number of tilted peptides [112]. It is believed that this segregation of glycine residues promotes a more favourable free energy of membrane insertion because the asymmetric distribution of the bulkier, hydrophobic residues along the other side of the α-helical axis is able to drive penetration of the membrane at a shallow angle [134]. A number of amphibian AMPs with tilted structure incorporated into their lytic action have been previously reported [100, 106, 146] and together, these results have the potential to aid the design of novel AMPs with pH dependent biological activity, which is currently, a major area of research [101]. Recent work has described such peptides for action against bacteria, fungi, biofilms and cancer cells, as well as for biotechnical applications involving the delivery of drugs and genes [101]. *S. aureus* has developed protection from the action of maximin H5 by modulating its membrane properties but the peptide has successfully overcome this defensive shield. It is notable that the membranolytic mode of action used by maximin H5 is enhanced by the low pH conditions that favour the growth of *S. aureus* [54]. This observation tempts the speculation that the secretion of the peptide by *B. maxima* [44], may have developed specifically to combat the organism, particularly in view of the fact that *S. aureus* appears to be the sole microbial target of the peptide [44, 117]. These observations would seem to provide a clear illustration of the co-evolutionary processes of mutual inhibition, evasion and adaptation strategies that characterise host-pathogen interactions [23], a scenario often referred to as the microbial 'arms race' [17-18, 147]. Moreover, given the low levels of haemolysis shown by maximin H5, it would seem that the peptide is worthy of further investigation as a potential template for development as an anti-staphylococcal agent [44, 54, 89]. In contrast to *S. aureus, E. coli* exhibits resistance to the action of maximin H5, and this resistance appears to be based on the ability of the peptide to bind PE with high affinity. It has previously been suggested that some plant AAMPs and other AMPs with a similar affinity for PE may serve as lead compounds for tumour imaging [110, 148-149]. Interestingly, what would appear to be the first pH-dependent anionic, antimicrobial protein identified in amphibians was recently reported when phylogenetic analyses predicted that homologues of human psoriasin would be expressed in a number of amphibians [150]. It is well-established that human psoriasin exhibits Zn^{2+}-dependent and pH-dependent activity in the antimicrobial defence of the skin [101], and a homologue of the protein, designated RtS100A7 (net charge -5) was detected in the skin secretions of the European Common Frog, *Rana temporaria* [150]. In contrast to human psoriasin, RtS100A7 was ineffective against *E. coli* at neutral pH, which appeared to result from an inability to form Zn^{2+} binding sites, a property that underpins activity of human psoriasin against the organism. However, similarly to human psoriasin, RtS100A7

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