BioMol Concepts, Vol. 2 (2011), pp. 27–38 • Copyright © by Walter de Gruyter • Berlin • New York. DOI 10.1515/BMC.2011.006

Review

Antimicrobial peptides from amphibians

Yao Xiao, Cunbao Liu and Ren Lai*

Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, Yunnan, China

*Corresponding author
e-mail: rlai@mail.kiz.ac.cn

Abstract

Increased prevalence of multi-drug resistance in pathogens has encouraged researchers to focus on finding novel forms of anti-infective agents. Antimicrobial peptides (AMPs) found in animal secretions are components of host innate immune response and have survived eons of pathogen evolution. Thus, they are likely to be active against pathogens and even those that are resistant to conventional drugs. Many peptides have been isolated and shown to be effective against multi-drug resistant pathogens. More than 500 AMPs have been identified from amphibians. The abundance of AMPs in frog skin is remarkable and constitutes a rich source for design of novel pharmaceutical molecules. Expression and post-translational modifications, discovery, activities and probable therapeutic application prospects of amphibian AMPs will be discussed in this article.

Keywords: amphibian antimicrobial peptides; design strategy; therapeutic applications.

Introduction

The class Amphibia includes three orders: (i) the order Anura (frogs and toads), 5679 species in 48 families; (ii) order Caudata or Urodela (salamanders, newts), 580 species in 9 families; (iii) order Gymnophiona or Apoda (caecilians), 174 species in 3 families. All of the data above is dynamic with new species continually being discovered.

In the past decades, several bioactive agents including antimicrobial peptides (AMPs), pharmacological peptides or peptides with unknown function have been identified from skin secretions of amphibians (1–3). These agents are important to prevent attack from aggressors (4, 5). For example, batrachotoxin and tetrodotoxin are toxic to predators (6, 7). Some of the components cause a temporary oral movement obstacle to predators and provide the opportunity for amphibians to escape (4).

Compared with the limited predators, widespread microbes are much more threatening to the survival of amphibians. As an ancient creature that metamorphose from a juvenile water-breathing form to an adult air-breathing form which is capable of living both on land and in water, amphibians constantly expose themselves to various harmful pathogens with their moist skins. It has been reported that a chytrid fungus *Batrachochytrium dendrobatidis* is the main disease-causing pathogen to amphibians (8).

During the long history of arm race with pathogens, amphibian skins have evolved several systems to defend themselves: (i) physical barriers: except for the compact skins function as a physical barrier to pathogens similar to other species, mucus glands on the dorsal skins of amphibians secrete copious proteoglycans. These substances aggregate to form polypeptide meshes that cover the entire outer surface of the skin to form a natural barrier against pathogen invasion (9). (ii) Adaptive immunity system: including B cells, antibodies and T cells similar to other vertebrate species (10–14). This system in amphibians share the same deficiency with other species: they respond too slowly to the invasion of pathogens. (iii) Innate immunity system: including phagocytes, complement system proteins, interferon systems, natural killer cells and AMPs. Although all these components play key roles in the swift killing of invasive pathogens, a variety of AMPs existing all over the skin surface could work swiftly and directly (3, 10). In addition, a specific AMP could have broad-spectrum activity against Gram-positive bacteria, Gram-negative bacteria, fungi and protozoa. As the first line to defend themselves from pathogens, amphibian skin is considered as abundant sources for AMPs (8).

The first reported AMP from amphibians is bombinin, a 24-residue peptide found from skin secretions of European frog *Bombina variegata* in 1969 with antimicrobial and hemolytic activity (15). Magainins were identified from skin of *Xenopus laevis* in 1987 (16, 17). Since then, a large number of AMPs with diversified structures and activities have been isolated from amphibians. Until now, more than 500 AMPs were identified from amphibian species. AMPs are almost exclusively found in Anura, less found in Caudata. More than 200 AMPs were isolated from *Ascaphidae*, *Bombinatoridae*, *Hylidae*, *Hyperoliidae*, *Leptodactylidae*, *Myobatrachidae*, *Pipidae* and *Ranidae* (18–32).

Granular glands in Anura skin are rich sources of AMPs but it is not the unique source. Some AMPs are also identified from gastric mucosa (33), stomach (34) and frog eggs (35). For example, buforin I and buforin II were isolated from stomach of the Asian toad *Bufo bufo gargarizans* (36).

Expression and post-translational modifications of amphibian AMPs

There are two main glands in the skin of amphibians (the lipid gland was only found in Phyllomedusa): granular

glands and mucus glands. They mainly locate in the dorsal region, but in *Xenopus* granular glands cover the entire outer surface. These glands are controlled by sympathetic axons. Once stimulated, secretions produced within the cell discharge by the rupture of the plasma membrane, thus releasing the cellular contents into the lumen. AMPs stored in the granular glands in prepropeptide form will remove the signal peptide from the precursor and release into the skin surface in a holocrine manner upon stress and injury (37, 38).

Most AMPs are synthesized as a long protein called precursors (16). In general conditions, these precursors consist of three parts: signal sequence, acidic propeptide and mature AMPs. The signal peptide of a precursor is cleaved off by an endopeptidase. Then they are transported to granular glands on the dorsal surface of the amphibian in an inactive form. Upon appropriate stimulation or injury, the spacer peptide is cleaved by a second endopeptidase and the mature peptide with activity is secreted from granular glands onto the dorsal surface (39–41). A third endopeptidase will deactivate these AMPs when they are no longer needed and this deactivate process takes 5–30 min (42–44). For example, mature AMP amolop with 18 amino acid residues comes from a preproprotein with 62 amino acids which contain a hydrophobic signal peptide of 22 residues followed by an 18 residue acidic propiece which terminates by a typical prohormone processing signal Lys-Arg (45).

To prolong the lifetime of mature AMPs, post-translational modifications such as C-terminal α-amidation and amino acid isomerization usually occur (4). C-terminal amidation is the most common modification in amphibian AMPs. Amidation is a process of C-terminal glycine oxidative decarboxylation which can prevent carboxypeptidase cutting, while providing a hydrogen bond which is required for the formation of α-helix and add positive charge (33), which could be responsible for the enhancement of the antimicrobial activity of AMP (46). This phenomenon is validated by AMPs such as brevinin-1, nigrocin and palustrin (24, 47).

Although L-amino acid is most common in naturally occurring AMPs, some peptides containing D-amino acid have also been found. These two isomers usually exist at the same time on the same position of one class of AMPs. This suggests that conversion of a L-amino acid to its D-isomer is a novel post-translational process (48). Bombinin H was found in the skin secretions of *Bombina varigata* and *Bombina orientalis* (49). Among them, bombinins H3–H5 include a D-alloisoleucine in the second position and leucine is also observed at the same position at the same time, but there is no significant difference between their antibacterial activities. The D-amino acid could enhance the stability of peptides when exposed to enzymes (20).

Identification of amphibian AMPs

There are several ways to stimulate the secretion of AMPs from amphibian skins:

i. injection of adrenaline or noradrenalin: small amounts of adrenaline and noradrenalin occur naturally in skin glands. Adrenergic receptor activation induces the contractions of the glandular myoepithelium and outflow of skin secretion. Injection of adrenaline or noradrenalin can activate adrenergic receptors and stimulate secretion, which is suitable for most amphibians.
ii. Physical stimuli: mild electrical stimulation has been demonstrated to be an effective method to stimulate the release of amphibian skin secretions even in species lacking enlarged compact glands (38). Another simple method to obtain amphibian skin secretions is to press the granular glands gently so as to milk skin secretions, which is suitable for amphibians with enlarged compact glands.
iii. Chemical stimuli: ether has been demonstrated to be somewhat successful in stimulation of amphibian skin secretions. Cotton infiltrated with small amounts of ether loaded in sealed glass beakers will stimulate the secretion of amphibian skins instead of anesthetizing the animals.

After collection of secretions, classic protein purification processes are used to obtain AMPs. In addition, the combination of modern genomics and proteomics was demonstrated to be an effective method to identify amphibian AMPs, especially to identify the family of AMPs (50).

Structural characteristics of amphibian antimicrobial peptides

Except for wood frog (*Rana sylvatica*) whose skin secretions contain only a single AMP (brevinin-1SY) (51), other related species can synthesize and secrete a variety of components with antimicrobial activities. Li et al. (24) combined peptidomics and genomics analyses to study an array of anti-infection peptides from amphibian skins. In total, 372 cDNA sequences of AMPs were characterized from a single individual skin of the frog *Odorrana grahami* that encode 107 novel antimicrobial peptides. Those peptides could be organized into 30 divergent groups, including 24 novel groups. The diversity in AMP coding cDNA sequences described here is the most extreme yet for any animal (24).

Length

Most amphibian AMPs are short in length, containing 9–50 amino acid residues (Table 1), whereas a few of them contain more than 50 residues (52). Temporins, which comprise between 10 and 14 amino acid residues, were firstly identified in the skin of the European frog *Rana esculenta* (18) and then *Rana temporaria* (2). Recently, an AMP (odorranain V) containing only 9 amino acid residues was identified from the frog *O. grahami* (24). Tigerinins which contain 11–12 amino acid residues are identified from skin secretions of Indian tiger frog *Rana tigerina* (53). Some tigerinin-like peptides are also found from the frog *Fejervarya cancrivora*, which lives in sea water (54). The esculentin-1 family of AMPs containing 46 amino acid residues isolated from

Table 1 Length of AMPs.

Peptide	Source	Sequence	Length	Ref.
Odorranain-N1	*Odorrana grahami*	DEKGPKWKR	9	(24)
Temporin K	*Rana temporaria*	LLPNLLKSLL	10	(2)
Temporin H	*Rana temporaria*	LSPNLLKSLL	10	(2)
Aurein 1.1	Southern bell frog	GLFDIIKKIAESI	13	(65)
Aurein 2.2	Southern bell frog	GLFDIVKKVVGALGSL	16	(65)
Aurein 3.2	Southern bell frog	GLFDIVKKIAGHIASSI	17	(65)
Bombinin H4	*Bombina variegate*	LIGPVLGLVGSALGGLLKKI	21	(49)
Brevinin-1	*Rana brevipoda porsa*	FLPVLAGIAAKVVPALFCKITKKC	24	(47)
Maximin 1	*Bombina maxima*	GIGTKILGGVKTALKGALKELASTYAN	27	(21)
Brevinin-2	*Rana brevipoda porsa*	GLLDSLKGFAATAGKGVL QSLLSTASCKLAKTC	33	(47)
Gaegurin-2	*Rana rugosa*	GIMSIVKDVAKNAAKEA AKGALSTLSCKLAKTC	33	(70)
Gaegurin-3	*Rana rugosa*	GIMSIVKDVAKTAAKEA AKGALSTLSCKLAKTC	33	(70)
Esculentin-2A	*Rana esculenta*	GILSLVKGVAKLAGKGLA KEGGKFGLELIACKIAKQC	37	(1)
Esculentin-2B	*Rana esculenta*	GIFSLVKGAAKLAGKGLA KEGGKFGLELIACKIAKQC	37	(1)
Esculentin-1A	*Rana esculenta*	GIFSKLAGKKIKNLLISGLKNVGK EVGMDVVRTGIDIAGCKIKGEC	46	(1)
Esculentin-1B	*Rana esculenta*	GIFSKLAGKKLKNLLISGLKNVG KEVGMDVVRTGIDIAGCKIKGEC	46	(1)

R. esculenta could be the longest amphibian antimicrobial peptides (1).

Charge

Because of their amino acid compositions which are rich in lysine, arginine and histidine, most of the AMPs are positively charged (Table 2). This feature is considered to be essential for antimicrobial activities of AMPs by binding them with the negatively charged phospholipids in the membrane of pathogens (55). Aurein 1.2 from Australian tree frogs has a net positive charge of +1 (43). Temporin A from *R. temporari* and tigerinin 1, 2, 3 from *R. tigerina* have a net positive charge of +2. Temporin L from *R. temporaria*, magainin 2 from *Xenopus laevis*, dermaseptin S1 from *Phylomedusa sauvagil* bears a net positive charge of +3 (56). Brevinin-1 from *Rana brevipoda*, ranateurin 4 from *R. temporaria* and aurein 1.1 from Australian tree frogs have a net positive charge of +4. Gaegurin 5 from *Rana rugosa* and ranalexin from *Rana catesbeiana* have a net positive charge of +5. Esculentin-1 from *R. esculenta* has a net positive charge of +6. Interestingly, owing to the three aspartate residues and lack of basic amino acid residues, Maximin H5 (ILGPVLGLVSDTLDDVLGIL-NH2) belong to the maximins H family which was isolated from the toad *Bombina maxima* and exhibits a unique anionic characteristic. Maximin H5 represents the first example of potential anionic AMPs discovered from amphibians, and it has a limited antimicrobial spectrum. Among all the tested bacteria, only the Gram-positive strain *Staphylococcus aureus* was sensitive to maximin H5. The results provide the first evidence that anionic AMP exist naturally (22).

Amino acid composition

Most of the AMPs contain more than half of the hydrophobic amino acids (57). These amino acid residues are believed to be essential for the membrane-disruptive effects by clustering together. Together with the clustering hydrophilic amino acids, these amino acid compositions usually make the AMPs an amphipathic molecule, which is considered important for their antimicrobial functions (43).

Secondary structure of antimicrobial peptides

Based on secondary structure, amphibian AMPs are classified into three broad families [(3, 19, 37, 58–62), Table 3]:

i. the first group of AMPs contain α-helix structure. Magainins (16), ranatuerin-2, temporin families (63, 64) and dermaseptins (25) adopt an amphipathic α-helial conformation, either in aqueous solution or upon interaction with membranes of microorganisms (64). However, ranatuerin-1, aurein 1.2 and caerin 1.1 from Australian frog adopt random coil arrangement in aqueous solution and an α-helical structure in membrane mimetic environments (65–69). The commonly used membrane mimicking solvent is varying mixtures of water and trifluoroethanol (30–50%) or detergent micelles (44, 69).
ii. The second group of AMPs includes those that contain a single intracellular disulfide bond. In this group, β-hairpin-like peptides were mostly observed. One specific feature of β-hairpin-like peptides is the C-terminal cyclic region which is called 'Rana box' (70). It is formed by two cysteine residues at the C-terminal linked by a disul-

Table 2 Net charge of AMPs.

Peptide	Source	Charge	Ref.
Maximin H5	*Bombina maxima*	-3	(21)
Aurein 2.1	Southern bell frog *Litoria aurea* and *Litoria raniformis*	1	(65)
Aurein 2.2	Southern bell frog *Litoria aurea* and *Litoria raniformis*	1	(65)
Aurein 2.6	Southern bell frog *Litoria aurea* and *Litoria raniformis*	1	(65)
Aurein 2.5	Southern bell frog *Litoria aurea* and *Litoria raniformis*	1	(65)
Aurein 3.3	Southern bell frog *Litoria aurea* and *Litoria raniformis*	2	(65)
Aurein 3.1	Southern bell frog *Litoria aurea* and *Litoria raniformis*	2	(65)
Maximin 3	Chinese red belly toad *Bombina maxima*	3	(21)
Aurein 3.2	Southern bell frog *Litoria aurea* and *Litoria raniformis*	4	(65)
Brevinin-1Ea	Edible frog *Rana esculenta*	4	(1)
Odorranain-B1	*Odorrana grahami*	5	(4)
Brevinin-2TC	European common frog	6	(3)
Odorranain-E1	*Odorrana grahami*	6	(24)
Buforin II	*Bufo bufo gargarizans*	7	(122)
Odorranain-K1	*Odorrana grahami*	8	(4)
Buforin I	*Bufo bufo gargarizans*	13	(26)

fide bridge (33). The size of Rana box is different although heptapeptide ring is common. Tigernins from Indian frog *R. tigerina* have an intracellular disulfide-linked ring containing 9 amino acid residues called nonapeptides (23). Japonicin-2 contains an 8 amino acids ring named octapeptide and ranatuerin-2 family contains hexapeptide formed by 6 amino acid residues (23). There are several AMP families from *O. grahami* containing variable disulfide-bridged segments at the C-terminus. The disulfide-bridged segment in the groups odorranain-A, -J and odorranain-B, -T, is composed of 12 and 11 amino acid residues, respectively, and the size of the disulfide-bridged segment in the group odorranain-U is 13 amino acid residues (24). Main members belonging to this group are brevinins from Japanese pond frog *R. brevipoda porsa* (47), tigerinins from Indian frog *R. tigerina* (53), ranalexins from American pig frog *Rana grylio* and green frog *Rana clamitans* (71, 72), amolopin P1 from skin secretions of the rufous-spotted torrent frog, *Amolops loloensis* (45). Acyclic brevinin-1 peptides isolated from skin of the Ryukyu brown frog *Rana okinavana* lack C-terminal cyclic region but still show antimicrobial activity against *Escherichia coli* and *S. aureus*. It is speculated that the C-terminal region is not essential for bactericidal activity (73).

iii. The third group contains peptides with unusual structure or amino acids compositions (74). A linear, cationic AMP kassinatuerin-1 isolated from the skin of African frog *Kassina senegalensis* exhibits no sequence similarity with previous characterized AMPs from amphibian skins (51). A family of AMPs (amolopin) with unique sequence (NILSSIVNGINRALSFFG) is found in the torrent frog, *A. loloensis* (45). In addition, in skin secretions of frog *Phyllomedusa distincta*, a particular heterodimer AMP with two peptide chains linked by disulfide bonds is also discovered (75).

Table 3 Structure of AMPs.

Peptide	Source	Structure	Ref.
Temporin A	*Rana temporaria*	Helix	(2)
Ranalexin	Bull frog, *Rana catesbeiana*	Helix	(90)
Magainin 2	African clawed frog *Xenopus laevis*	Helix	(16)
Dermaseptin-S1	Sauvage's leaf frog	Helix	(25)
Dermaseptin-S2	Sauvage's leaf frog	Helix	(25)
Temporin D	*Rana temporaria*	Helix	(2)
Temporin H	*Rana temporaria*	Helix	(2)
Odorranain-B1	*Odorrana grahami*	Helix	(24)
Bombinin-like peptide 1	*Bombina orientalis*	Helix	(60)
Brevinin-1Ea	Edible frog *Rana esculenta*	Bridge	(1)
Brevinin-1Eb	Edible frog *Rana esculenta*	Bridge	(1)
Brevinin-1	*Rana brevipoda porsa*	Bridge	(46)
Brevinin-2	*Rana brevipoda porsa*	Bridge	(46)
Gaegurin-1	Korean wrinkled frog *Rana rugosa*	Bridge	(69)
Tigerinin-1	*Rana tigerina*	Bridge	(52)
Tigerinin-2	*Rana tigerina*	Bridge	(52)
Brevinin-1SY	Wood frog *Rana sylvatica*	Bridge	(49)

Therapeutic applications of amphibian AMPs

AMPs are key effectors in innate immunity. They not only kill pathogens directly but also rapidly. AMPs can take effect more than 100 times faster than other protective proteins such as IgM on killing pathogens (48). Mixed peptides secreted from amphibian skins are more effective than individual peptides (14). In other words, AMP reservoirs of amphibian skin contain weaponry with various structures aimed at different pathogen spectra, and act synergistically (76). Some AMPs have a broad spectrum of activity not only on bacteria but also on fungi, protozoa (77) and cancer cells (6, 21, 78, 79). Several positively charged amphipathic AMPs also show antiviral activity *in vitro* on enveloped viruses such as HIV, herpes simplex virus (HSV) and vesicular stomatitis virus.

Antibacterial activities

Many AMPs display broad-spectrum activity on Gram-negative bacteria, Gram-positive bacteria, protozoa and fungi, whereas some AMPs preferentially kill only some of them.

Brevinin-1, brevinin-2, esculentin-1 and esculentin-2 AMP families, which are found in many Ranidae amphibians, exhibit high potency on a wide range of Gram-positive bacteria, Gram-negative bacteria and fungi (1, 23, 64, 80). For example, esculentin-1 showed strong antimicrobial activities against a range of human pathogens such as *E. coli*, *S. aureus*, *Pseudomonas aeruginosa* and *Caenorhabditis albicans*. The minimum inhibitory concentration (MIC) is very low (<1 μM), indicating that the esculentin-1 family have very high sterilization potency.

Ranalexin from *R. catesbeiana* is active against Gram-positive bacteria such as methicillin-resistant *S. aureus*, *Staphylococcus epidermidis* and *Streptococcus pneumoniae* but is inactive against some Gram-negative strains such as *Pseudomonas aeruginosa* and *Proteus mirabilis* (81). Temporin shows preferential activity against Gram-positive bacteria such as *S. aureus* and *Enterococcus faecium* (2). Caerin 4.1 preferentially kills Gram-negative bacteria (43) and the reported MIC against *E. coli* is 20 μg/ml.

Antiviral activity

Anti-herpes activity Magainins I and II exhibit an inhibitory effect towards HSV-1 and HSV-2 at a concentration of 50 μg/ml, which is non-cytotoxic for epithelial cells (82, 83). Modified brevinin-1 also show significant antiviral activity against HSV-1 (84).

Anti-HIV activity Some AMPs are effective on inhibition of growth or replication of enveloped viruses. Maximin 3 from skin secretions of Chinese red belly toad *B. maxima* show anti-HIV activity *in vitro*, which provides the first evidence that some of these linear cationic AMPs can have anti-HIV potency. It is important to note that maximin 3 possesses a unique histidine C-terminus, which might contribute to this unique biological activity (21). Other amphibian AMPs such as caerin 1.9, caerin 1.1, dermaseptin S4 and maculatin 1.1 are found to inhibit human immunodeficiency virus infection at low concentrations (10–20 μM) with limited toxicity to the target T cells (9, 85–87). Caerin 4.1 lyses *Pasteurella haemolytica*, which causes swine fever (43). The AMP database could be a good resource for the discovery of novel anti-HIV drugs (88).

Anticancer activity

Aurein 1.2 first isolated from the Australian bell frog *Litoria raniformis* does not lyse erythrocytes. However, at the same concentration, it kills most human cancer cells; it is also the smallest peptide which has both antibiotic and anticancer activity (65, 89). Caerin 1.1 identified from the magnificent tree frog *Litoria splendida* and green tree frog *Litoria caerulea*, has an IC_{50} value of $<10^{-6}$ M against all the major human cancer types (44). Citropin 1.1, gaegurins, magainin 2 and analog peptides of magainins are selectively cytotoxic to human cancer cells (17, 79, 89, 90).

Temporin L isolated from the skin of the European red frog *R. temporaria* induces necrosis of three different human tumor cell lines (56). Magainins and their analogs have been found to be able to lyse hematopoietic tumor and solid tumor cells with little toxic effect on normal blood lymphocytes (78, 79). The mechanism of anticancer activity of magainins is suggested to be targeting of cell membranes by a non-receptor pathway (79).

Antiprotozoal activity

At concentrations of 10^{-6} M, caerin 1.1 kills nematodes. It is also effective on the malaria parasite *Plasmodium falciparum* (MIC 10 μg/ml) (44). Ranalexin isolated from *R. catesbeiana* tadpoles has lower activity against intestinal parasite *Cryptosporidium parvum*, whereas the synergism between ranalexin and conventional antibiotics can enhance antiprotozoal activity (91, 92). Magainin 2 could disrupt membranes of *Paramecium caudatum*, *Amoeba proteus* and *Euglena gracilis* (16).

Hemolytic activity

Many amphibian AMPs exert hemolytic activity. For example, brevinin-1E from *R. esculenta* has a HC_{50} value (the concentration producing 50% hemolysis) <1 μM and the HC_{50} value of magainin 2 is as high as 1000 μM (1, 93). As a result, discovery of less toxic antimicrobial peptides either from natural resources or designed from current AMP databases is somewhat necessary for the pharmaceutical application of AMPs.

Reproduction toxicity

Sperm immobilization Maximins 1 and 3 isolated from *B. maxima* have sperm immobilization activity. They can inhibit 80% of sperm motility at a concentration 100 μg/ml within 30 min (21). At the same concentration, magainin also show similar sperm immobilization ability (94). In addition, two synthetic magainins, magainin A and magainin G, show

spermicidal activity by altering the plasma membranes of sperms (95).

Embryo-fetal toxicity Mystkowska and colleagues have reported that magainin on its own is highly embryotoxic (96). Its embryotoxicity is enhanced by cyclodextrin, albumin, H_2O_2 and acidification. Magainin-2-amide killed 100% of cells at the minimal concentration of 250 μM within 30 min (96). Magainin-2-amide can exert its embryotoxicity by interacting with negatively charged, non-cholesterol-containing cell membranes of preimplantation embryos.

Mast cell degranulation and histamine release

Mast cells are secretory cells necessary for specific and innate immunity, allergy and inflammation processes. Histamine release is responsible for allergic symptoms and also as a result of mast cell degranulation. Many amphibian AMPs have been found to induce mast cell degranulation. For example, brevinins-ALb and temporins-ALa identified from *A. loloensis* at a concentration of 100 μg/ml were found to promote mast cell degranulation by 17% and 87%, respectively (31). Kassinakinin S, esculentin-1SEa, brevinin-1SE and ranaruerin-2SEa are also reported to be inducers of histamine release (97). Brevinins-Alb and temporins-Ala also show histamine release activity. At a concentration of 100 μg/ml, they induced histamine release by 59.2% and 65.8%, respectively (31).

Amphibian peptides that mimic neurotransmitters and mammalian hormones

Many peptides isolated from frog skin are insulin secretogues. Some of them have potential for development of pharmaceutical agents especially for the treatment of type 2 diabetes. Plylloseptin-L2 isolated from the skin secretion of Lemur leaf frog *Hylomantis lemur* has a significant effect on insulin release from the rat BRIN-BD11 clonal β cell line at a concentration of 3 nM and it does not cause cytolysis at a concentration of 3 μM (98). Brevinin-2GUb which belongs to the Brevinin-2 family can increase the release of insulin from BRIN-BD11 cells (139% of basal rate) at a concentration of 100 nM, whereas at a concentration of 3 μM the basal rate increased to 373% (99).

Gaegurin-6 is isolated from the skin secretion of Korean frog *R. rugosa*. With an α-helical conformation and two cysteine residues it can significantly upregulate insulin secretion in pancreatic β cells in a Ca^{2+} influx-dependent manner. The study also showed that these structures are crucial for its biology activity (100). Ocellatin L2 and plasticin-L1 are devoid of antimicrobial activity but both have the ability to release insulin from BRIN-BD11 cells (101). Pseudin-2 isolated from the skin of the paradoxical frog *Pseudis paradoxa* is a cationic and α-helical peptide that can stimulate insulin release from the BRIN-BD11 clonal β cell line without hemolytic activity (102). Exendin-4 isolated from venom of a reptile has been used as therapeutic agent for type 2 diabetes (103, 104). In addition, Temporin and Brevinin-1 are also well known insulinotropic peptides (105).

Mechanism of action

Some AMPs have a selective cytotoxic effect on bacteria instead of on animal cells. This feature can attribute to the negatively charged phospholipids in the bacterial membrane (106). In animal cell membranes, components such as cholesterol can also facilitate stabling membrane structure (10). As with most other innate immunity effectors, bactericidal effects mediated by amphibian AMPs are rapid. Regardless of how quickly they are, certain steps described below are widely adopted.

i. Attraction. When face microbes, positively charged AMPs are driven by electrostatic attraction and aggregate on the bacterial membrane surface which is negatively charged. In Gram-negative bacteria, the negatively charged phosphate groups of phospholipids and lipopolysaccharides (LPS), which are located in the outer membrane, play key roles in this process, whereas in Gram-positive bacteria negatively charged teichoic acid in cell walls play an important role (107).
ii. Attachment. Before entering cells, AMPs have to cross a variety of envelopes formed by capsular polysaccharides. These envelopes include Gram-negative bacteria LPS, Gram-positive bacteria teichoic acid, as well as the thick peptidoglycan layer. Eukaryocytes are seldom taken as target, as they have less negative charges (107). Although many reports have indicated that AMPs use electrostatic attraction to interact with the bacterial membrane, some of them use the passive transport system to enter microbial cells and complete the membrane rupture process within the cell. Details can be found in a review article by Otvos (108).
iii. Transmembrane pore-forming mechanisms. When the peptide/lipid ratio exceeds a certain threshold value, the AMPs in the bacterial membrane will form pores. Three models including the barrel-stave model (107, 109, 110), carpet-like model (111) and toroidal pore model (93, 107) are proposed for the pore-forming mechanism.
    - Barrel-stave model. AMPs aggregate on the cytoplasm with the hydrophobic part interacting with the cytoplasm and the hydrophilic parts forming penetrating channels. The channel formed by alamethinin has an internal diameter of approximately 1.8 nm and an external diameter of approximately 4.0 nm (112, 113). The thickness of the wall is approximately 1.1 nm, which is equal to the diameter of the helix form of alamethinin. This reminds us that the channel is formed by 3–11 parallel helix form alamethinins penetrating the cytoplasm vertically (114, 115).
    - Carpet-like model. AMPs such as ovispirin rest on the surface of the cytoplasm (116–118). When the concentrations increase, they destroy the cytoplasm in a detergent manner and form micelles. As the concentration increases, temporary holes are formed and AMPs out of the cytoplasm will influx (107). Inner and outer AMPs of the cytoplasm react with each other and make the micelles dissociate from the cytoplasm and make a hole on it (110).

- Toroidal pore model. This model is used to explain how AMPs such as magainins (114), protrgrins (119) and melittins (120, 121) work. The helix form of AMPs slot into the cytoplasm and cause the outer layer of the cytoplasm to bend continually until hydrophilic channels are formed by AMPs, and the hydrophilic parts of the lipids (120). Compared with the channels formed in the barrel-stave model, they are a little bigger and the size is always unstable (107, 114). The inner diameter is approximately 3.0–5.0 nm and the external diameter is approximately 7.0–8.4 nm. Each channel is formed by approximately 4–7 magainins and 90 lipid molecules (122–124).

iv. Intracellular killing. Although pore-forming and disruption of the bacterial cell membranes is considered as the main reason for the direct killing of bacteria by AMPs, some reports show that AMPs can hit multiple targets in the bacterial cytoplasm similar to conventional antibiotics (46). AMPs aimed at cytoplasm targets are likely to inhibit the synthesis of important compounds for bacteria survival, such as DNA, RNA or some crucial intracellular proteins (55). Where AMPs persistently take effects, target cells could not metabolize properly, thus resulting in cell death.

Design strategy of amphibian AMPs

Although some of the AMPs from amphibians have been demonstrated to be powerful on pathogens, especially some clinical strains and resistance by pathogens rarely occurs, they are too sensitive to trypsin-like enzymes and some side effects such as hemolysis or cytotoxic effects usually occur (125). As a result, design of more stable and more target-specific AMPs is somewhat necessary for the application of AMPs. Several strategies will be discussed below.

Modification of amphibian AMPs to resist trypsin

Most of the AMPs from amphibians contain one or more positive charged amino acid residues (Arg or Lys), which are sensitive to trypsin-like proteases (125). Thus, AMPs with trypsin inhibitory capability should be excellent candidates for novel clinical antibiotics.

A disulfide-bridged undecapeptide (CWTKSIPPKPC) loop derived from an AMP (named ORB-O) of *O. grahami*, which is named trypsin inhibitory loop, is found to contain potential trypsin-inhibitory capability. It is considered as the smallest serine inhibitor. Different trypsin inhibitors or AMPs can easily be designed on the basis of such a loop. Small bifunctional peptides that have both trypsin inhibitory and antimicrobial activities could be designed. The application of developing novel oral or other anti-infective agents will soon be the focus of attention. ORB-O without the disulfide-bridged loop in its structure had neither protease inhibition activity nor antimicrobial activity, implying that the disulfide-bridged undecapeptide (CWTKSIPPKPC) loop has an essential role in both activities (80).

Substitution of amino acids in AMPs

Some amino acids play key roles in the biologic activity of AMP. Substitution of certain amino acids could effectively alter the net charge, hydrophobicity or secondary structure of AMPs so as to enhance the antimicrobial activity, or reduce the side effects of hemolysis, etc. In this regard, suitable AMPs applicable for clinical use could be designed (126–129).

Modification of charge Net charges of AMPs have a key effect on their antimicrobial abilities. Increasing positive charge of AMP is a strategy to improve antimicrobial potency. Ranatuerin-1 (SMLSVLKNLG^{10}KVGLGFVACK^{20}INKQC) isolated from the skin of the bullfrog *R. catesbeiana* contains broad-spectrum antimicrobial activity (63). Substitution of Lys to Asn^{8} increased positive charge and potency (between 2-fold and 8-fold) against tested strains (*S. aureus*, *P. aeruginosa* and *C. albicans*) with only a very small increase in hemolytic activity (130). The same situation is also observed in tigerinin. Substitution of threonine by lysine increased both positive charge and antimicrobial potency (131).

Change of hydrophobicity and α-helicity There are three structural domains in ranatuerin-1, including α-helix (residues 1–8), β-sheet (residues 11–16) and β-turn (residues 20–25). The substitution of Asn^{22} by Ala increased its hydrophobicity and α-helicity and resulted in a little change of antimicrobial ability but hemolytic activity was markedly increased (130). The data indicated that hydrophobicity play key roles for hemolytic activity.

Change in the disulfide loop As mentioned above, many amphibian AMPs contain a C-terminal disulfide loop. Some reports suggest that the heptapeptide disulfide loop is not essential for antimicrobial activity. For example, acyclic brevinin-1 containing no disulfide 'Rana box' does not lose antimicrobial activity (73). In contrast to the heptapeptide disulfide loop, the nonapeptide disulfide loop in tigerinins is essential for their antimicrobial abilities; replacement of cysteine residue by leucine results in loss of most of their antimicrobial abilities. This substitution destroys the β-turn structure. It seems that β-turn is essential for antimicrobial activity (131).

Amino acid isomerization Isomerization of amino acids in AMPs can improve the stability of peptides when exposed to enzymes. In addition, some reports indicate that isomerization of amino acids could improve the antimicrobial activities of AMPs (132).

Expert opinion

The growing resistance of pathogens to antibiotics has encouraged researchers to focus on finding novel forms of anti-infective agents. AMPs found in animal secretions are components of host innate immune responses and have survived eons of pathogen evolution. Thus, they are likely to

be active against pathogens and even those that are resistant to conventional drugs. AMPs are considered as powerful and rapid broad-spectrum novel antibiotics effective on drug resistant pathogens. These advantages compared with conventional antibiotics make them perfect candidates for novel therapeutic agents. With the design strategy mentioned above, several promising compounds have been discovered.

Outlook

Magainins are membrane-active peptides that exert antimicrobial activity by forming ion channels in microbial cell membranes that lead to cell death. Based on these features, two magainin mimetics called MSI-751 and MSI-774 are obtained. They are tested with anaerobia oral pathogens such as *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Actinobacillus actinomycetemcomitans*, *Eikenella corrodens*, *Prevotella loescheii* and *Prevotella intermedia*. The results of the antimicrobial assay show that all of the periodontal pathogens are sensitive to MSI-751 and MSI-774 with MIC values of 2.5–40 mg/l and 10–80 mg/l, respectively (133).

MSI-78 (trade name: Pexiganan) is a synthetic amphipathic, α-helical peptide magainin derivative antibiotic, containing 22 amino acid residues (134). MSI-78 has antimicrobial activity against local infections caused by both Gram-positive and Gram-negative bacteria, including some infections caused by pathogens resistant to conventional antibiotics. MSI-78 is considered as a novel antibiotic that is effective on many skin infections, such as impetigo, diabetic foot, surgical wound infections and bed sores. In testing polymicrobial diabetic foot ulcers, MSI-78 showed equal efficacy with oral ofloxacin (121).

In the skin secretions of the *Phyllomedusa* genus, an AMP family named dermaseptins is well studied and has been demonstrated to be a promising candidate for designing novel antimicrobial drugs owing to their great affinity to the plasma membrane of human red blood cells (46). Some analogs of dermaseptin have been demonstrated to be effective on the treatment of infections involved in blood circulation. By reaching the target microorganisms, they could kill them directly but no toxic or only less toxic effects are observed on eukaryotic cells. This is the so-called ‘affinity driven molecular transfer’, which means drugs could be bind to peptides with high affinity and sent directly to the designated location where the effects take place. Obviously, the advantages of these applications are rapid and receptor-independent (52).

Highlights

- AMPs are produced by many living organisms, such as animals and plants. Among them amphibian skin is a rich source of AMPs.
- AMPs have broad-spectrum activities that can defend against pathogens including Gram-positive and Gram-negative bacteria, viruses and fungi.
- Some AMPs have been demonstrated to be effective on pathogens that are drug-resistant to conventional antibiotics and thus are potential candidates for therapeutics.
- Because their fundamental structures have been clearly researched, AMPs could be designed and reformed artificially.
- Currently, some new drugs based on AMPs have been created and take effects in the treatment of human disease.

References

1. Simmaco M, Mignogna G, Barra D, Bossa F. Antimicrobial peptides from skin secretions of Rana esculenta. Molecular cloning of cDNAs encoding esculentin and brevinins and isolation of new active peptides. J Biol Chem 1994; 269: 11956–61.
2. Simmaco M, Mignogna G, Canofeni S, Miele R, Magoni ML, Barra D. Temporins, antimicrobial peptides from the European red frog Rana temporaria. Eur J Biochem 1996; 242: 788–92.
3. Simmaco M, Mignogna G, Barra D. Antimicrobial peptides from amphibian skin: what do they tell us? Biopolymers 1999; 47: 435–50.
4. Bevins CL, Zasloff M. Peptides from frog skin. Annu Rev Biochem 1990; 59: 395–414.
5. Clarke BT. The natural history of amphibian skin secretions, their normal functioning and potential medical applications. Biol Rev Camb Philos Soc 1997; 72: 365–79.
6. Daly JW, Myers CW, Whittaker N. Further classification of skin alkaloids from neotropical poison frogs (Dendrobatidae), with a general survey of toxic/noxious substances in the Amphibia. Toxicon 1987; 25: 1023–95.
7. Witkop B, Gössinger E. Chapter 5: Chemistry and Pharmacology, in: Brossi A, editor. The alkaloids. New York: Academic Press, 1983: 139–251.
8. Rollins-Smith LA, Doersam JK, Longcore JE, Taylor SK, Shamblin JC, Carey C, Zasloff MA. Antimicrobial peptide defenses against pathogens associated with global amphibian declines. Dev Comp Immunol 2001; 26: 63–72.
9. Rollins-Smith LA. The role of amphibian antimicrobial peptides in protection of amphibians from pathogens linked to global amphibian declines. Biochim Biophys Acta 2009; 1788: 1593–9.
10. Barra D, Simmaco M, Boman HG. Gene-encoded peptide antibiotics and innate immunity. FEBS Lett 1998; 430: 130–4.
11. Castell-Rodriguez AE, Hernandez-Penaloza A, Sampedro-Carrillo EA, Herrera-Enriquez MA, Alvarez-Perez SJ, Rondan-Zarate A. ATPase and MHC class II molecules coexpression in Rana pipiens dendritic cells. Dev Comp Immunol 1999; 23: 473–85.
12. Castell-Rodriguez AE, Sampedro-Carrillo EA, Herrera-Enriquez MA, Rondan-Zarate A. Non-specific esterase-positive dendritic cells in epithelia of the frog Rana pipiens. Histochem J 2001; 33: 311–6.
13. Pasquier DL, Flajnik M. Expression of MHC class II antigens during Xenopus development. Dev Immunol 1990; 1: 85–95.
14. Rollins-Smith LA, Reinert LK, O’Leary CJ, Houston LE, Woodhams DC. Antimicrobial peptide defenses in amphibian skin. Integr Comp Biol 2005; 45: 137–42.
15. Csordas A, Michl H. Isolation and structure of a haemolytic polypeptide from the defensive secretion of European Bombina species. Monatsh Chem 1970; 101: 182–9.
16. Zasloff M. Magainins, a class of antimicrobial peptides from

Xenopus skin: isolation, characterization of two active forms, and partial cDNA sequence of a precursor. Proc Natl Acad Sci USA 1987; 84: 5449–53.
17. Zasloff M, Martin B, Chen HC. Antimicrobial activity of synthetic magainin peptides and several analogues. Proc Natl Acad Sci USA 1988; 85: 910–3.
18. Simmaco M, Biase DD, Severini C, Aita M, Erspamer GF, Barra D, Bossa, F. Purification and characterization of bioactive peptides from skin extracts of Rana esculenta. Biochim Biophys Acta 1990; 1033: 318–23.
19. Simmaco M, Barra D, Chiarini F, Noviello L, Melchiorri P, Kreil G, Richter K. A family of bombinin-related peptides from the skin of Bombina variegata. Eur J Biochem 1991; 199: 217–22.
20. Simmaco M, Kreil G, Barra D. Bombinins, antimicrobial peptides from Bombina species. Biochim Biophys Acta 2009; 1788: 1551–5.
21. Lai R, Zheng YT, Shen JH, Liu GJ, Liu H, Lee WH, Tang SZ, Zhang Y. Antimicrobial peptides from skin secretions of Chinese red belly toad Bombina maxima. Peptides 2002; 23: 427–35.
22. Lai R, Liu H, Lee WH, Zhang Y. An anionic antimicrobial peptide from toad Bombina maxima. Biochem Biophys Res Commun 2002; 295: 796–9.
23. Conlon JM, Kolodziejek J, Nowotny N. Antimicrobial peptides from ranid frogs: taxonomic and phylogenetic markers and a potential source of new therapeutic agents. Biochim Biophys Acta 2004; 1696: 1–14.
24. Li JX, Xu XQ, Xu CH, Zhou WP, Zhang KY, Yu HN, Zhang YP, Zheng YT, Rees HH, Lai R, Yang DM, Wu J. Anti-infection peptidomics of amphibian skin. Mol Cell Proteomics 2007; 6: 882–94.
25. Mor A, Nicolas P. Isolation and structure of novel defensive peptides from frog skin. Eur J Biochem 1994; 219: 145–54.
26. Park CB, Kim MS, Kim SC. A novel antimicrobial peptide from Bufo bufo gargarizans. Biochem Biophys Res Commun 1996; 218: 408–13.
27. Duda TF Jr, Vanhoye D, Nicolas P. Roles of diversifying selection and coordinated evolution in the evolution of amphibian antimicrobial peptides. Mol Biol Evol 2002; 19: 858–64.
28. Conlon JM, Al-Ghaferi N, Abraham B, Leprince J. Strategies for transformation of naturally-occurring amphibian antimicrobial peptides into therapeutically valuable anti-infective agents. Methods 2007; 42: 349–57.
29. Conlon JM. Refections on a systematic nomenclature for antimicrobial peptides from the skins of frogs of the family Ranidae. Peptides 2008; 29: 1815–9.
30. Xu XQ, Li JX, Han YP, Yang HL, Liang JG, Lu QM, Lai R. Two antimicrobial peptides from skin secretions of Rana grahami. Toxicon 2006; 47: 459–64.
31. Lu Y, Li JX, Yu HN, Xu XQ, Liang JG, Tian YQ, Ma DY, Lin GQ, Huang GQ, Lai R. Two families of antimicrobial peptides with multiple functions from skin of rufous-spotted torrent frog, Amolops loloensis. Peptides 2006; 27: 3085–91.
32. Lu Y, Ma YF, Wang X, Liang JG, Zhang CX, Zhang KY, Lin GQ, Lai R. The first antimicrobial peptide from sea amphibian. Mol Immunol 2008; 45: 678–81.
33. Bullet P, Stöcklin R, Menin L. Anti-microbial peptides: from invertebrates to vertebrates. Immunol Rev 2004; 198: 169–84.
34. Moore KS, Bevins CL, Brasseur MM, Tomassini N, Turner K, Eck H, Zasloff M. Antimicrobial peptides in the stomach of Xenopus laevis. J Biol Chem 1991; 266: 19851–7.
35. Han YP, Yu HN, Yang XB, Rees HH, Liu JZ, Lai R. A serine proteinase inhibitor from frog eggs with bacteriostatic activity. Comp Biochem Physiol B 2008; 149: 58–62.
36. Kim HS, Park CB, Kim MS, Kim SC. cDNA cloning and characterization of Buforin I, an antimicrobial peptide: a cleavage product of histone H2A. Biochem Biophys Res Commun 1996; 229: 381–7.
37. Barra D, Simmaco M. Amphibian skin: a promising resource for antimicrobial peptides. Trends Biotechnol 1995; 13: 205–9.
38. Tyler MJ, Stone DJ, Bowie JH. A novel method for the release and collection of dermal, glandular secretions from the skin of frogs. J Pharmacol Toxicol Methods 1992; 28: 199–200.
39. Ganz T. Biosynthesis of defensins and other antimicrobial peptides. Ciba Found Symp 1994; 186: 62–71.
40. Amiche M, Aurelia AS, Thierry NP, Nicolas P. The dermaseptin precursors: a protein family with a common preproregion and a variable C-terminal antimicrobial domain. FEBS Lett 1999; 456: 352–6.
41. Bowie JH, Wegener KL, Chia BCS, Wabnitz PA, Carver JA, Tyler MJ, Wallace JC. Host defence antibacterial peptides from skin secretions of Australian amphibians. The relationship between structure and activity. Peptides 1999; 6: 259–69.
42. Resnick NM, Maloy WL, Guy HR, Zasloff M. A novel endopeptidase from Xenopus that recognizes α-helical secondary structure. Cell 1991; 66: 541–54.
43. Boland MP, Separovic F. Membrane interactions of antimicrobial peptides from Australian tree frogs. Biochim Biophys Acta 2006; 1758: 1178–83.
44. Apponyi MA, Pukala TL, Brinkworth CS, Maselli VM, Bowie JH, Tyler MJ, Booker GW, Wallace JC, Carver JA, Separovic F, Doyle J, Llewellyn LE. Host-defence peptides of Australian anurans: structure, mechanism of action and evolutionary significance. Peptides 2004; 25: 1035–54.
45. Wang AL, Wang JY, Hong J, Feng H, Yang HL, Yu XD, Ma YF, Lai R. A novel family of antimicrobial peptides from the skin of Amolops loloensis. Biochimie 2008; 90: 863–7.
46. Papagianni M. Ribosomally synthesized peptides with antimicrobial properties: biosynthesis, structure, function, and applications. Biotechnol Adv 2003; 21: 465–99.
47. Morikawa N, Hagiwara K, Nakajima T. Brevinin-1 and -2, unique antimicrobial peptides from the skin of the frog, Rana brevipoda porsa. Biochem Biophys Res Commun 1992; 189: 184–90.
48. Richter K, Egger R, Kreil G. D-alanine in the frog skin peptide dermorphin is derived from L-alanine in the precursor. Science 1987; 238: 200–2.
49. Mignogna G, Simmaco M, Kreil G, Barra D. Antibacterial and haemolytic peptides containing D-allo-isoleucine from the skin of Bombina variegate. EMBO J 1993; 12: 4829–32.
50. Lai R. Combined peptidomics and genomics approach to the isolation of amphibian antimicrobial peptides. Methods Mol Biol 2010; 615: 177–90.
51. Matutte B, Storey KB, Knoop C, Conlon JM. Induction of synthesis of an antimicrobial peptide in the skin of the freeze-tolerant frog, Rana sylvatica in response to environmental stimuli. FEBS Lett 2000; 483: 135–8.
52. Rinaldi AC. Antimicrobial peptides from amphibian skin: an expanding scenario. Curr Opin Chem Biol 2002; 6: 799–804.
53. Sai KP, Jagannadham MV, Vairamani M, Raju NP, Devi AS, Nagaraj R, Sitaram N. Tigerinins: novel antimicrobial peptides from the Indian frog Rana tigerina. J Biol Chem 2001; 276: 2701–7.
54. Song Y, Lu Y, Wang L, Yang H, Zhang K, Lai R. Purification, characterization and cloning of two novel tigerinin-like peptides from skin secretions of Fejervarya cancrivora. Peptides 2009; 30: 1228–32.

55. Hancock REW, Diamond G. The role of cationic antimicrobial peptides in innate host defences. Trends Microbiol 2000; 8: 402–10.
56. Rinaldi AC, Mangoni MI, Rufo A, Luzi C, Simmaco M, Barra D, Zhao H, Kinnunen PKJ, Bozzi A, Giulio DA. Temporin L: antimicrobial, cytotoxic activities and effects on membrane permeabilization in lipid vesicles. Biochem J 2002; 368: 91–100.
57. Powers JP, Hancock RE. The relationship between peptide structure and antibacterial activity. Peptides 2003; 24: 1681–91.
58. Boman HG. Peptide antibiotics and their role in innate immunity. Annu Rev Immunol 1995; 13: 61–92.
59. Charpentier S, Amiche M, Mester J, Vouille V, Caer JPL, Nicolas P, Delfour A. Structure, synthesis, and molecular cloning of dermaseptins B, a family of skin peptide antibiotics. J Biol Chem 1998; 273: 14690–7.
60. Gabay JE. Ubiquitous natural antibiotics. Science 1994; 64: 229–30.
61. Gibson BW, Tang D, Mandrell R, Kelly M, Spindel ER. Bombinin-like peptides with antimicrobial activity from skin secretions of the Asian toad, Bombina orientalis. J Biol Chem 1991; 266: 23103–11.
62. Nicolas P, Mor A. Peptides as weapons against microorganisms in the chemical defense system of vertebrates. Annu Rev Microbiol 1995; 49: 277–304.
63. Goraya J, Knoop FC, Conlon JM. Ranatuerins: antimicrobial peptides isolated from the skin of the American bullfrog, Rana catesbeiana. Biochem Biophys Res Commun 1998; 250: 589–92.
64. Goraya J, Wang Y, Li Z, O'Flaherty M, Knoop FC, Platz JE, Conlon JM. Peptides with antimicrobial activity from four different families isolated from the skins of the North American frogs, Rana luteiventris, Rana berlandieri and Rana pipiens. Eur J Biochem 2000; 267: 894–900.
65. Rozek T, Wegener KL, Bowie JH, Olver IN, Carver JA, Wallace JC, Tyler MJ. The antibiotic and anticancer active aurein peptides from the Australian Bell Frogs Litoria aurea and Litoria raniformis. The solution structure of aurein 1.2. Eur J Biochem 2000; 267: 5330–41.
66. Chia BCS, Carver JA, Mulhern TD, Bowie JH. Maculatin 1.1, an antimicrobial peptide from the Australian tree frog, Litoria genimaculata – solution structure and biological activity. Eur J Biochem 2000; 267: 1894–908.
67. Wegener KL, Brinkworth CS, Bowie JH, Wallace JC, Tyler MJ. Bioactive dahlein peptides from the skin secretions of the Australian aquatic frog Litoria dahlii: sequence determination by electrospray mass spectrometry. Rapid Commun Mass Spectrom 2001; 15: 1726–34.
68. Michael CJ, Sonnevend A, Jouenne T, Coquet L, Cosquer D, Vaudry H, Iwamuro S. A family of acyclic brevinin-1 peptides from the skin of the Ryukyu brown frog Rana okinavana. Peptides 2005; 26: 185–90.
69. Wang G, Li Y, Li X. Correlation of three-dimensional structures with the antibacterial activity of a group of peptides designed based on a nontoxic bacterial membrane anchor. J Biol Chem 2005; 280: 5803–11.
70. Park JM, Jung JE, Lee BJ. Antimicrobial peptides from the skin of a Korean frog, Rana rugosa. Biochem Biophys Res Commun 1994; 205: 948–54.
71. Kim JB, Halverson T, Basir YJ, Dulka J, Knoop FC, Conlon JM. Purification and characterization of antimicrobial peptides from skin extracts and skin secretions of the North American pig frog Rana grylio. Regul Pept 2000; 90: 53–60.
72. Halverson T, Basir YJ, Knoop FC, Conlon JM. Purification and characterization of antimicrobial peptides from the skin of the North American green frog Rana clamitans. Peptides 2000; 21: 469–76.
73. Conlon JM, Sonnevend A, Jouenne T, Coquet L, Cosquer D, Vaudry H, Iwamuro S. A family of acyclic brevinin-1 peptides from the skin of the Ryukyu brown frog Rana okinavana. Peptides 2005; 26: 185–90.
74. Won HS, Kang SJ, Lee BJ. Action mechanism and structural requirements of the antimicrobial peptides, gaegurins. Biochim Biophys Acta 2009; 1788: 1620–9.
75. Batista CVF, Scaloni A, Rigden DJ, Silva LR, Romero AR, Dukor R, Sebben A, Talamo F, Bloch C. A novel heterodimeric antimicrobial peptide from the tree-frog Phyllomedusa distincta. FEBS Lett 2001; 494: 85–9.
76. Yan H, Hancock REW. Synergistic interactions between mammalian antimicrobial defense peptides. Antimicrob Agents Chemother 2001; 45: 1558–60.
77. Arrighi RB, Nakamura C, Miyake J, Hurd H, Burgess JG. Design and activity of antimicrobial peptides against sporogonic-stage parasites causing murine malarias. Antimicrob Agents Chemother 2002; 46: 2104–10.
78. Cruciani RA, Barker JL, Zasloff M, Chen HC, Colamonici O. Antibiotic magainins exert cytolytic activity against transformed cell lines through channel formation. Proc Natl Acad Sci USA 1991; 88: 3792–6.
79. Baker MA, Maloy WL, Zasloff M, Jacob LS. Anticancer efficacy of magainin 2 and analogue peptides. Cancer Res 1993; 53: 3052–7.
80. Li JX, Zhang C, Xu XQ, Wang J, Yu HN, Lai R, Gong WM. Trypsin inhibitory loop is an excellent lead structure to design serine protease inhibitors and antimicrobial peptides. FASEB J 2007; 21: 2466–73.
81. Giacometti A, Cirioni O, Greganti G, Quarta M, Scalise G. In vitro activities of membrane-active peptides against Gram-positive and Gram-negative aerobic bacteria. Antimicrob Agents Chemother 1998; 42: 3320–4.
82. Aboudy Y, Mendelson E, Shalit I, Bessalle R, Fridkin M. Activity of two synthetic amphiphilic peptides and magainin-2 against herpes simplex virus types 1 and 2. Int J Pept Protein Res 1994; 43: 573–82.
83. Clara A, Manjramkar DD, Reddy VK. Preclinical evaluation of magainin-A as a contraceptive antimicrobial agent. Fertil Steril 2004; 81: 1357–65.
84. Yasin B, Pang M, Turner JS, Cho Y, Dinh NN, Waring AJ, Lehrer RI, Wagar EA. Evaluation of the inactivation of infectious herpes simplex virus by host-defense peptides. Eur J Clin Microbiol Infect Dis 2000; 19: 187–94.
85. Lorin C, Saidi H, Belaid A, Zairi A, Baleux F, Hocini H, Bélec L, Hani K, Tangy F. The antimicrobial peptide dermaseptin S4 inhibits HIV-1 infectivity in vitro. Virology 2005; 334: 264–75.
86. VanCompernolle SE, Taylor RJ, Oswald-Richter K, Jiang J, Youree BE, Bowie JH, Tyler MJ, Conlon JM, Wade D, Aiken C, Dermody TS, KewalRamani VN, Rollins-Smith LA, Unutmaz D. Antimicrobial peptides from amphibian skin potently inhibit human immunodeficiency virus infection and transfer of virus from dendritic cells to T cells. J Virol 2005; 79: 11598–606.
87. Zairi A, Tangy F, Bouassida K, Hani K. Dermaseptins and magainins: antimicrobial peptides from frogs' skin – new sources for a promising spermicides sicrobicides – a mini review. J Biomed Biotechnol 2009; 2009: 452567.
88. Wang G, Watson KM, Peterkofsky A, Buckheit RW Jr. Identification of novel human immunodeficiency virus type 1-inhibitory peptides based on the antimicrobial peptide database. Antimicrob Agents Chemother 2010; 54: 1343–6.

89. Hoskin DW, Ramamoorthy A. Studies on anticancer activities of antimicrobial peptides. Biochim Biophys Acta 2008; 1778: 357–75.
90. Wegener KL, Wabnitz PA, Carver JA, Bowie JH, Chia BCS, Wallace JC, Tyler MJ. Host defense peptides from the skin glands of the Australian Blue Mountains tree frog Litoria citropa. Solution structure of the antibacterial peptide citropin 1.1. Eur J Biochem 1999; 265: 627–37.
91. Clark DP, Durell S, Maloy WL, Zasloff M. Ranalexin A novel antimicrobial peptide from bullfrog (Rana catesbeiana) skin, structurally related to the bacterial antibiotic, polymyxin. J Biol Chem 1994; 269: 10849–55.
92. Giacometti A, Cirioni O, Barchiesi F, Scalise G. Anticryptosporidial activity of ranalexin, lasalocid and azithromycin alone and in combination in cell lines. J Antimicrob Agents 2000; 45: 375–7.
93. Matsuzaki K, Sugishita K, Harada M, Fujii N, Miyajima K. Interactions of an antimicrobial peptide, magainin 2, with outer and inner membranes of Gram-negative bacteria. Biochim Biophys Acta 1997; 1327: 119–30.
94. Wojcik C, Sawicki W, Marianowski P, Benchaib M, Czyba JC, Guerin JF. Cyclodextrin enhances spermicidal effects of magainin-2-amide. Contraception 2000; 61: 99–103.
95. Reddy KV, Shahani SK, Meherji PK. Spermicidal activity of Magainins: in vitro and in vivo studies. Contraception 1996; 53: 205–10.
96. Mystkowska ET, Niemierko A, Komar A, Sawicki W. Embryotoxicity of magainin-2-amide and its enhancement by cyclodextrin, albumin, hydrogen peroxide and acidification. Hum Reprod 2001; 16: 1457–63.
97. Graham C, Richter SC, McClean S, O'Kane E, Flatt PR, Shaw C. Histamine-releasing and antimicrobial peptides from the skin secretions of the Dusky Gopher frog, Rana sevosa. Peptides 2006; 27: 1313–9.
98. Abdel-Wahab YHA, Power GJ, Flatt PR, Woodhams DC, Rollins-Smith LA, Conlon JM. A peptide of the phylloseptin family from the skin of the frog Hylomantis lemur (Phyllomedusinae) with potent in vitro and in vivo insulin-releasing activity. Peptides 2008; 29: 2136–43.
99. Conlon JM, Power GJ, Abdel-Wahab YH, Flatt PR, Jiansheng H, Coquet L, Leprince J, Jouenne T, Vaudry H. A potent, non-toxic insulin-releasing peptide isolated from an extract of the skin of the Asian frog, Hylarana guntheri (Anura: Ranidae). Regul Pept 2008; 151: 153–9.
100. Kim JH, Lee JO, Jung JH, Lee SK, You GY, Park SH, Kim HS. Gaegurin-6 stimulates insulin secretion through calcium influx in pancreatic βRin5mf cells. Regul Pept 2010; 159: 123–8.
101. Conlon JM, Abdel-Wahab YH, Flatt PR, Leprince J, Vaudry H, Jouenne T, Condamine E. A glycine-leucine-rich peptide structurally related to the plasticins from skin secretions of the frog Leptodactylus laticeps (Leptodactylidae). Peptides 2009; 30: 888–92.
102. Abdel-Wahab YH, Power GJ, Ng MT, Flatt PR, Conlon JM. Insulin-releasing properties of the frog skin peptide pseudin-2 and its [Lys18]-substituted analogue. Biol Chem 2008; 389: 143–8.
103. Eng J, Kleinman WA, Singh L, Singh G, Raufman JP. Isolation and characterization of exendin-4, an exendin-3 analogue, from Heloderma suspectum venom. Further evidence for an exendin receptor on dispersed acini from guinea pig pancreas. J Biol Chem 1992; 267: 7402–5.
104. Barnett A. Exenatide. Expert Opin Pharmacother 2007; 8: 2593–608.
105. Abdel-Wahab YH, Marenah L, Flatt PR, Conlon JM. Insulin releasing properties of the temporin family of antimicrobial peptides. Protein Pept Lett 2007; 14: 702–7.
106. Zasloff M. Antimicrobial peptides of multicellular organisms. Nature 2002; 415: 389–95.
107. Brogden KA. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? Nat Rev Microbiol 2005; 3: 238–50.
108. Otvos L. Antibacterial peptides and proteins with multiple cellular targets. J Pept Sci 2005; 11: 697–706.
109. Ehrenstein G, Lecar H. Electrically gated ionic channels in lipid bila. Q Rev Biophys 1977; 10: 1–34.
110. Oren Z, Shai Y. Mode of action of linear amphipathic α-helical antimicrobial peptides. Biopolymers 1998; 47: 451–63.
111. Shai Y. Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by α-helical antimicrobial and cell non-selective membrane-lytic peptides. Biochim Biophys Acta 1999; 1462: 55–70.
112. Spaar A, Munster C, Salditt T. Conformation of peptides in lipid membranes studied by X-ray grazing incidence scattering. Biophys J 2004; 87: 396–407.
113. He K, Ludtke SJ, Huang HW, Worcester DL. Antimicrobial peptide pores in membranes detected by neutron in-plane scattering. Biochemistry 1995; 34: 15614–8.
114. Yang L, Harroun TA, Weiss TM, Ding L, Huang HW. Barrel-stave model or toroidal model? A case study on melittin pores. Biophys J 2001; 81: 1475–85.
115. He K, Ludtke SJ, Worcester DL, Huang HW. Neutron scattering in the plane of membranes: structure of alamethicin pores. Biophys J 1996; 70: 2659–66.
116. Bechinger B. The structure, dynamics and orientation of antimicrobial peptides in membranes by multidimensional solid-state NMR spectroscopy. Biochim Biophys Acta 1999; 1462: 157–83.
117. Yamaguchi S, Huster D, Waring A, Lehrer RI, Kearney W, Tack BF, Hong M. Orientation and dynamics of an antimicrobial peptide in the lipid bilayer by solid-state NMR spectroscopy. Biophys J 2001; 81: 2203–14.
118. Pouny Y, Rapaport D, Mor A, Nicolas P, Shai Y. Interaction of antimicrobial dermaseptin and its fluorescently labeled analogues with phospholipid membranes. Biochemistry 1999; 31: 12416–23.
119. Yamaguchi S, Hong T. Solid-state NMR investigations of peptide-lipid interaction and orientation of a β-sheet antimicrobial peptide, protegrin. Biochemistry 2002; 41: 9852–62.
120. Matsuzaki K, Murase O, Fujii N, Miyajima K. An antimicrobial peptide, magainin 2, induced rapid flip-flop of phospholipids coupled with pore formation and peptide translocation. Biochemistry 1996; 35: 11361–8.
121. Hallock KJ, Lee DK, Ramamoorthy A. MSI-78, an analogue of the magainin antimicrobial peptides, disrupts lipid bilayer structure via positive curvature strain. Biophys J 2003; 84: 3052–60.
122. Yi G-S, Park CB, Kim SC, Cheong C. Solution structure of an antimicrobial peptide buforin II. FEBS Lett 1996; 398: 87–90.
123. Matsuzaki K, Sugishita K, Ishibe N, Ueha M, Nakata S, Miyajima K, Epand RM. Relationship of membrane curvature to the formation of pores by magainin 2. Biochemistry 1998; 37: 11856–63.
124. Matsuzaki K, Sugishita K, Harada M, Fujii N, Miyajima K. Interactions of an antimicrobial peptide, magainin 2, with outer and inner membranes of Gram-negative bacteria. Biochim Biophys Acta 1997; 1327: 119–30.

125. Huang HW, Chen FY, Lee MT. Molecular mechanism of peptide-induced pores in membranes. Phys Rev Lett 2004; 92: 198304.
126. Juretić D, Chen HC, Brown JH, Morell JL, Hendler RW, Westerhoff HV. Magainin 2 amide and analogues. Antimicrobial activity, membrane depolarization and susceptibility to proteolysis. FEBS Lett 1989; 249: 219–23.
127. Maloy WL, Kari UP. Structure-activity studies on magainins and other host defense peptides. Biopolymers 1995; 37: 105–22.
128. Kwon MY, Hong SY, Lee KH. Structure-activity analysis of brevinin 1E amide, an antimicrobial peptide from Rana esculenta. Biochim Biophys Acta 1998; 1387: 239–48.
129. Dathe M, Meyer J, Beyermann M, Maul B, Hoischen C, Bienerta M. General aspects of peptide selectivity towards lipid bilayers and cell membranes studied by variation of the structural parameters of amphipathic helical model peptides. Biochim Biophys Acta 2002; 1558: 171–86.
130. Pukala TL, Bowie JH, Maselli VM, Musgrave IF, Tyler MJ. Host-defence peptides from the glandular secretions of amphibians: structure and activity. Nat Prod Rep 2006; 23: 368–93.
131. Sonnevend A, Knoop FC, Patel M, Pál T, Soto AM, Conlon JM. Antimicrobial properties of the frog skin peptide, ranatuerin-1 and its [Lys-8]-substituted analog. Peptides 2004; 25: 29–36.
132. Sitaram N, Sai KP, Singh S, Sankaran K, Nagaraj R. Structure-function relationship studies on the frog skin antimicrobial peptide tigerinin 1: design of analogs with improved activity and their action on clinical bacterial isolates. Antimicrob Agents Chemother 2000; 46: 2279–83.
133. Mangoni ML, Papo N, Saugar JM, Barra D, Shai Y, Simmaco M, Rivas L. Effect of natural L- to D-amino acid conversion on the organization, membrane binding, and biological function of the antimicrobial peptides bombinins H. Biochemistry 2006; 45: 4266–76.
134. Genco CA, Maloy WL, Kari UP, Motley M. Antimicrobial activity of magainin analogues against anaerobic oral pathogens. Int J Antimicrob Agents 2003; 21: 75–8.