Antimicrobial peptides from animals: focus on invertebrates

Jacopo Vizioli and Michel Salzet

An expensive group of cationic antimicrobial peptides has been isolated from animals and plants during the past two decades. Three novel classes of natural antibiotics (anionic peptides, aromatic dipeptides and processed forms of oxygen-binding proteins) have recently been isolated from different vertebrate and invertebrate species. In this article, we present an overview of natural animal anti-microbials, with an emphasis on those isolated from invertebrates, and discuss their possible use as alternative drugs to chemical antibiotics.

Hospitals worldwide have become literal breeding grounds for some of the most deadly bacteria. Many infections acquired in hospitals are caused by potentially fatal bacteria, such as *Staphylococcus aureus*, that survive for extended periods on medical devices such as intravenous lines and catheter tubes. It is now estimated that half of *S. aureus* strains found at many medical institutions are resistant to antibiotics such as methicillin [1]. The emergence among enterococci of resistance to another useful and widely effective antibiotic, vancomycin [2], might accelerate the spread of vancomycin-resistant genes, via plasmids, among other species, eventually limiting the usefulness of this drug. Indeed, antibiotic resistance might soon leave healthcare professionals with ineffective therapies for bacterial infections. Consequently, the priority for the new century is the development of alternative drugs to fight such pathogens. Innovative and promising sources of candidate molecules are the so-called antibiotic peptides, a series of natural compounds that exhibit antimicrobial activity and have been isolated in the past 20 years from many plant, insect and animal species [3–5] as defense molecules implicated in the innate immune response [6]. Animals, in particular, are the most important producers of antimicrobial compounds and several hundreds of antibiotic peptides have been found in a wide range of invertebrate and vertebrate species [3–5]. Some representatives of the major groups of natural antibiotics are reported here, and classified on the basis of their biochemical and structural features.

Table 1. Cationic antimicrobial peptides

Structure and representative peptides	Organism	Antimicrobial activity	Refs
Linear α-helix peptides			
Cecropins	Insects, pig	Bacteria, fungi, virus, protozoa, metazoa	[3–5,8]
Clavanin, styelin	Tunicates	Bacteria	[5]
Magainin, dermaseptin	Amphibians	Bacteria, protozoa	[3,5]
Buforins	Amphibians	Bacteria, fungi	[3,9,11]
Linear peptides rich in certain amino acids			
Pro-rich:			
drosocin, metchnikowins,	Fruit fly	Bacteria	[4]
pyrrhocoricin, metalnikowin	Hemipteran	Bacteria, fungi	[4]
Gly-rich:			
diptericins, attacins	Dipterans	Bacteria	[4]
His-rich:			
histatin	Human	Bacteria, fungi	[3,5]
Try-rich:			
indolicidin	Cattle	Bacteria	[3,5]
Single disulfide bridge			
Thanatin	Hemipteran	Bacteria, fungi	[3,4,12]
Brevinins	Frog	Bacteria	[3,5]
Two disulfide bridges			
Tachyplesin II	Horseshoe crab	Bacteria, fungi, virus	[3,5,12]
Androctonin	Scorpion	Bacteria, fungi	[3,12]
Protegrin I	Pig	Bacteria, fungi, virus	[3]
Three disulfide bridges			
α-Defensins	Mammals	Bacteria, fungi	[3,5]
β-Defensins	Mammals	Bacteria, fungi	[3,5]
Defensins	Insects	Bacteria, fungi, protozoa	[4,8,12]
Penaeidins	Shrimp	Bacteria, fungi	[12,20]
More than three disulfide bridges			
Tachycitin	Horseshoe crab	Bacteria, fungi	[12]
Drosomycin	Fruit fly	Fungi	[12]
Gambicin	Mosquito	Bacteria, fungi, protozoa	[26]
Heliomicin	Lepidopteran	Bacteria, fungi	[27]
Defensins	Plants	Fungi	[3,13]

Cationic peptides

The largest group of antimicrobial peptides is that of cationic molecules, which are widely distributed in animals and plants. So far, >400 peptides from this group have been characterized and half of them have been isolated from insects [4,5]. On the basis of their structural features, cationic peptides can be divided into three classes (Table 1): (1) linear peptides forming α-helical structures; (2) cysteine-rich open-ended peptides containing single or several disulfide bridges; and (3) molecules rich in specific amino acids such as proline, glycine or histidine. A more exhaustive list of these peptides can be found in [3] or at http://www.bbcm.univ.trieste.it/~tossi/antimic.html.

Cecropins (Table 1) are a family of 3–4-kDa linear amphipatic peptides [3,5] devoid of cysteine residues and containing two α-helical segments (a strongly basic N-terminal domain and a long hydrophobic C-terminal helix) linked by a short hinge. Following the characterization of the first cecropins

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from the hemolymph of lepidopteran *Hyalophora cecropia* at the beginning of 1980s [3], other linear amphipatic peptides were isolated from many invertebrate and vertebrate species [3–5]. One of these molecules, magainin from *Xenopus* skin [3], was characterized and used as a template for pioneering clinical trials of such peptides for biomedical applications [7]. Cecropins and magainins are active against a large spectrum of microorganisms, including bacteria and filamentous fungi in addition to protozoan and metazoan parasites [8]. A novel family of cationic peptides, corresponding to cleaved forms of histones, was isolated recently from toad [9] and fish epithelia [10]. These 2–4-kDa molecules, structurally similar to cecropins, are also active against bacteria and fungi. Interestingly, as demonstrated for buforin II, a histone H2A-derived peptide isolated from the toad *Bufo bufo gargarizans*, these α-helical molecules penetrate bacterial membranes and bind to nucleic acids, interfering with cell metabolism and leading to rapid cell death [11].

The second class of cationic peptides includes a wide range of 2–8-kDa antimicrobial compounds, characterized by the presence of cysteine residues engaged in one or more disulfide bridges. The smallest members of this class are the peptides that contain one (thanatin and brevinin) or two (androctonin, tachyplesin and protegrin I) disulfide bridges (Table 1). These 2-kDa, hairpin-structured peptides have been isolated from both invertebrates and vertebrates and show antibacterial and antifungal activities [12].

More complex structural motifs characterize the defensins, a huge group of 4-kDa open-ended cysteine-rich peptides isolated from insects, mammals and plants. Defensins are arranged in families, based on their structural differences. Invertebrates [4,5,12] and plant [13] defensins are characterized by three and four disulfide bridges, respectively, and show a common structure comprising an α-helix linked to a β-sheet by two disulfide bridges (CSαβ-motif). In mammals, α- and β-defensins are characterized by an antiparallel β-sheet structure, stabilized by three disulfide bridges [3].

The third class of cationic peptides, enriched in specific amino acids (Table 1), includes antibacterial and antifungal compounds isolated from insects (mainly proline- and glycine-rich) [4] and mammals (histidine- and tryptophane-rich) [3].

At present, the mechanism of action is not completely established for all of these cationic peptides. However, the Shai-Matzusaki-Huang model provides a possible explanation for antimicrobial activity of most of these compounds [3]. In this model, antimicrobial compounds such as linear amphipatic α-helical peptides increase membrane permeability by: (1) interaction of their positive charge with anionic lipids; and (2) membrane destabilization by lipid displacements. In some cases, these peptides penetrate into the target cell. A similar mechanism has been proposed for the cysteine-rich peptides such as defensins, which are suggested to form ion-permeable channels in the lipid bilayer. Several hypotheses have been proposed to explain the mechansims by which peptides kill target cells; such hypotheses include induction of hydrolases degrading the cell wall, disturbance of membrane functions, or damaging of crucial intracellular targets after internalization of the peptide [3].

Table 2. Non-cationic antimicrobial peptides

Structure and representative peptides	Organism	Antimicrobial activity	Refs
Anionic peptides			
Neuropeptide derived:			
Enkelytin	Bovine, human,	Bacteria	[14,15]
Peptide B	Bovine, human, leech, mussel	Bacteria	[14,15]
Aspartic acid rich:			
H-GDDDDDD-OH	Ovine	Bacteria	[16]
Dermcidin	Human	Bacteria	[17]
Aromatic dipeptides			
N-β-alanyl-5-S-glutathionyl-3,4-dihydroxyphenylalanine	Flesh fly	Bacteria, fungi	[18]
p-Hydroxycinnamaldehyde	Saw fly	Bacteria, fungi	[19]
Peptides derived from oxygen-binding proteins			
Hemocyanin derived	Shrimp	Bacteria	[20]
Hemoglobin derived	Tick	Bacteria	[21]
Lactoferrin	Human	Bacteria, virus	[25]

Anionic peptides

The anionic peptides are a novel group of antimicrobial molecules that are generally isolated from mammalian epithelia (Table 2). The first class includes phosphorylated compounds (e.g. peptide B and enkelytin) derived from the processing of neuropeptide precursors (e.g. pro-enkephalin-A) [14]. These peptides were recently found in infectious exudates of cattle and humans and are mainly active against Gram-positive bacteria at micromolar concentrations, as are cationic peptides [14]. Similar products have been reported in some invertebrate species [15].

Additional anionic peptides are the aspartic-acid-rich molecules isolated from sheep [16] or cattle pulmonary surfactant. These antibacterial compounds have a structure similar to the charge-neutralizing pro-peptides of Group I serine proteases and could regulate the activity of pulmonary enzyme systems [16].

Recently, a novel anionic 47-amino-acid peptide, dermcidin, has been identified in human sweat, in response to a variety of pathogenic Gram-positive bacteria [17].

Aromatic dipeptides

The aromatic dipeptides comprise low molecular weight antibacterial compounds isolated from dipteran larvae, such as the N-β-alanyl-5-*S*-glutathionyl-3,4-dihydroxyphenylalanine (573 Da), identified in the flesh fly *Sarcophaga peregrina* [18], and *p*-hydroxycinnamaldehyde, isolated from the saw fly *Acantholyda parki* [19] (Table 2). The mode of action of these molecules is, at present, unknown.

Peptides derived from oxygen-binding proteins

Hemocyanin [20] derivatives, the first representatives of the group of peptides

derived from oxygen-binding proteins (Table 2), were recently isolated from the hemolymph of arthropods and annelids species, respectively. A third molecule, detected in tick hemolymph, is a cleaved form of vertebrate hemoglobin, processed by the parasite after blood meal ingestion [21]. These proteins have been reported as bactericide compounds and might be considered as a reservoir of defense molecules to be used as integrative weapons to fight pathogens.

Bactericidal activity of anionic peptides, oxygen-binding protein derivatives and aromatic dipeptides is generally weak, compared with cationic peptides, and their physiological relevance remains to be established. These molecules, whose mode of action could differ from that of cationic peptides and other antibiotics, could complement the activity of other compounds and constitute a useful base to develop novel synthetic derivatives.

Concluding remarks

The appearance in the mid-1980s of multi-resistant bacterial strains has demonstrated the need to search for alternatives to synthetic antibiotics. The discovery of natural antimicrobial peptides provided new therapeutic weapons to fight microorganisms. Recent studies showed that several cationic and non-cationic peptides are expressed in many vertebrate and invertebrate species and it is possible that these compounds act in synergy to improve the immune response against pathogens.

The knowledge acquired in the past two decades and the discovery of new groups of antimicrobial peptides makes these natural antibiotics the basic element of a novel generation of drugs for the treatment of bacterial and fungal infections [3,7,22]. In addition, the wide spectrum of antimicrobial activities reported for these molecules suggests that they could be used in the treatment of cancer [23] and viral [24,25] or parasitic infections [8]. Different therapeutic applications, from topical administration to systemic treatment of infections have been developed by several biotechnology companies [7]. Interestingly, to date, clinical Phase I and II trials have shown a limited resistance for the bacterial strains tested [3]. These features make the antibiotic peptides a powerful arsenal of molecules that could be the antimicrobial drugs of the new century.

Note added in proof

A weak antibacterial activity against *S. aureus* has been reported for a novel anionic peptide, maximin H5, recently isolated from the skin of the toad *Bombina maxima* [28].

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References

1 Roder, B.L. *et al.* (1999) Clinical features of *Staphylococcus aureus* endocarditis: a 10-year experience in Denmark. *Arc. Intern. Med.* 159, 462–469
2 Novak, R. *et al.* (1999) Emergence of vancomycin tolerance in *Streptococcus pneumoniae*. *Nature* 399, 590–591
3 Zasloff, M. (2002) Antimicrobial peptides of multicellular organisms. *Nature* 415, 389–395
4 Bulet, P. *et al.* (1999) Antimicrobial peptides in insects; structure and function. *Dev. Comp. Immunol.* 23, 329–344
5 Andreu, D. and Rivas, L. (1998) Animal antimicrobial peptides: an overview. *Biopolymers* 47, 415–433
6 Hoffmann, J.A. and Reichhart, J.M. (2002) *Drosophila* innate immunity: an evolutionary perspective. *Nat. Immunol.* 3, 121–126
7 Hancock, R.E. (2000) Cationic antimicrobial peptides: towards clinical applications. *Expert. Opin. Investig. Drugs* 9, 1723–1729
8 Vizioli, J. and Salzet, M. (2002) Antimicrobial peptides: new weapons to control parasitic infections? *Trends Parasitol.* 18, 475–476
9 Park, C.B. *et al.* (1996) A novel antimicrobial peptide from *Bufo bufo gargarizans*. *Biochem. Biophys. Res. Commun.* 218, 408–413
10 Park, I.Y. *et al.* (1998) Parasin I, an antimicrobial peptide derived from histone H2A in the catfish, *Parasilurus asotus*. *FEBS Lett.* 437, 258–262
11 Park, C.B. *et al.* (1998) Mechanism of action of the antimicrobial peptide buforin II: buforin II kills microorganisms by penetrating the cell membrane and inhibiting cellular functions. *Biochem. Biophys. Res. Commun.* 244, 253–257
12 Dimarcq, J.L. *et al.* (1998) Cysteine-rich antimicrobial peptides in invertebrates. *Biopolymers* 47, 465–477
13 Garcia-Olmedo, F. *et al.* (1998) Plant defense peptides. *Biopolymers* 47, 479–491
14 Metz-Boutigue, M.H. *et al.* (2000) Antibacterial and antifungal peptides derived from chromogranins and proenkephalin-A. From structural to biological aspects. *Adv. Exp. Med. Biol.* 482, 299–315
15 Salzet, M. (2001) Vertebrate innate immunity resembles a mosaic of invertebrate immune responses. *Trends Immunol.* 22, 285–288
16 Brogden, K.A. *et al.* (1996) Isolation of an ovine pulmonary surfactant-associated anionic peptide bactericidal for *Pasteurella haemolytica*. *Proc. Natl. Acad. Sci. U. S. A.* 93, 412–416
17 Schittek, B. *et al.* (2001) Dermcidin: a novel human antibiotic peptide secreted by sweat glands. *Nat. Immunol.* 2, 1133–1137
18 Leem, J.Y., *et al.* (1996) Purification and characterization of N-β-alanyl-5-*S*-gluthathionyl-3,4-dihydroxyphenylalanine, a novel antibacterial substance of *Sarcophaga peregrina* (Flesh fly). *J. Biol. Chem.* 271, 13573–13577
19 Leem, J.Y. *et al.* (1999) Isolation of p-hydroxycinnamaldehyde as an antibacterial substance from the saw fly, *Acantholyda parki S*. *FEBS Lett.* 442, 53–56
20 Destoumieux-Garzon, D. *et al.* (2001) Crustacean immunity. Antifungal peptides are generated from the C terminus of shrimp hemocyanin in response to microbial challenge. *J. Biol. Chem.* 276, 47070–47077
21 Fogaca, A.C. *et al.* (1999) Antimicrobial activity of a bovine hemoglobin fragment in the tick *Boophilus microp. J. Biol. Chem.* 274, 25330–25334
22 De Lucca, A.J. (2000) Antifungal peptides: potential candidates for the treatment of fungal infections. *Expert. Opin. Investig. Drugs* 9, 273–299
23 Tanaka, K. *et al.* (2001) PI3-kinase p85α is a target molecule of proline-rich antimicrobial peptide to suppress proliferation of ras-transformed cells. *Jpn. J. Cancer Res.* 92, 959–967
24 Chinchar, V.G. *et al.* (2001) Inactivation of frog virus 3 and channel catfish virus by esculentin-2P and ranatuerin-2P, two antimicrobial peptides isolated from frog skin. *Virology* 288, 351–357
25 Andersen, *J.H. et al.* (2001) Lactoferrin and cyclic lactoferricin inhibit the entry of human cytomegalovirus into human fibroblasts. *Antiviral Res.* 51, 141–149
26 Vizioli, J. *et al.* (2001) Gambicin: a novel immune responsive antimicrobial peptide from the malaria vector *Anopheles gambiae*. *Proc. Natl. Acad. Sci. U. S. A.* 98, 12630–12635
27 Lamberty, M. *et al.* (2001) Solution structures of the antifungal heliomicin and a selected variant with both antibacterial and antifungal activities. *Biochemistry* 40, 11995–12003
28 Lai, R. *et al.* (2002) An anionic antimicrobial peptide from toad *Bombina maxima*. *Biochem. Biophys. Res. Commun.* 295, 796–799

Jacopo Vizioli
Michel Salzet*
Laboratoire de Neuroimmunologie des Annélides, UMR CNRS 8017, SN3, Université des Sciences et Technologies de Lille, 59655 Villeneuve d'Ascq, France.
*e-mail: michel.salzet@univ-lille1.fr