Minireview

Cathelicidins: a novel protein family with a common proregion and a variable C-terminal antimicrobial domain

Margherita Zanetti[a,b,*], Renato Gennaro[b], Domenico Romeo[c]

[a]*Laboratorio Nazionale Consorzio Interuniversitario Biotecnologie, AREA Science Park, Padriciano 99, I-34012 Trieste, Italy*
[b]*Dipartimento di Scienze e Tecnologie Biomediche, Università di Udine, I-33100 Udine, Italy*
[c]*Dipartimento di Biochimica, Biofisica e Chimica delle Macromolecole, Università di Trieste, I-34127 Trieste, Italy*

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Abstract A novel protein family, showing a conserved proregion and a variable C-terminal antimicrobial domain, and named cathelicidin, has been identified in mammalian myeloid cells. The conserved proregion shows sequence similarity to members of the cystatin superfamily of cysteine proteinase inhibitors. Cathelicidins are stored in the cytoplasmic granules of neutrophil leukocytes and release the antimicrobial peptides upon leukocyte activation. Some of these peptides can assume an α-helical conformation, others contain one or two disulfide bonds, still others are Pro- and Arg-rich, or Trp-rich. In addition to bacterial killing, some of these peptides exert additional functions related to host defense such as LPS-neutralization and promotion of wound healing.

Key words: Antimicrobial peptide; Cathelicidin; Myeloid cell; Innate immunity

1. Introduction

A rapid and effective response to challenge by pathogens is essential for the survival of all living organisms. Among the several different defense mechanisms which have evolved to meet this necessity is the production of a large variety of microbicidal peptides; over 100 have been isolated so far from both animals and plants [1,2]. These peptides play a major role in the immune defense of invertebrates, while in vertebrates they act as a first line of defense against invasion by pathogens and in the control of the natural flora [2,3]. The importance of these peptides can be inferred from their specific localization at sites which are exposed to microbial invasion as well as in the professional phagocytes. They are in fact produced in the epithelia of amphibia [3], mammals [4–6] and insects [2,7], are secreted into internal body fluids in arthropodes [2,7] and are stored in the cytoplasmic granules of professional phagocytes of mammals and birds [8–11].

Based on the presence or absence of disulfide bonds, these peptides can be divided into two broad classes, each including several peptide families (Table 1). Among the most studied families are those comprising linear peptides like the insect cecropins [2,7], amphibian magainins [3] and Pro- and Arg-rich peptides from mammals [12,13]. Defensins are another well studied family of mammalian myeloid and epithelial peptides, characterized by the presence of three disulfide bridges that maintain the molecule in a compact β-sheet structure [5,10]. In spite of the significant diversity of sequence and structure in the different peptide families, some common features appear to be required for their activity, such as a high content of basic residues and the tendency to adopt an amphipathic conformation, which accounts for their functioning as membrane-disruptive agents.

The spectrum of organisms susceptible to these peptides is broad [2–11], including various bacteria, protozoa, fungi, and in some cases, virally infected and tumor cells. Their antimicrobial and cytotoxic effects are mediated by the ability to bind and permeabilize the surface membrane of the target cells [2,4,7,10,14]. The initial binding is thought to depend on electrostatic interactions between the positively charged residues of the peptides and the negatively charged molecules exposed at the target cell surface. They may then float with their hydrophobic face buried in the lipid bilayer [15], or form transmembrane channels in a voltage-dependent manner [2,3,5,7,10]. These interactions lead to alteration of membrane permeability, with leakage of metabolites [10,14]. The preferential selectivity for prokaryotic and transformed eukaryotic cell membranes seems to depend on the different lipid composition of bacterial and transformed cell membranes with respect to normal eukaryotic membranes [16,17].

Unlike most of the classical antibiotics, which are built in a stepwise manner through a complex enzymatic synthesis, all the known antimicrobial peptides are made from gene-encoded precursors (prepropeptides), from which the mature peptides are derived by the sequential removal of the signal peptide and of a variably extended prosequence. In general the propiece precedes the mature peptide, is anionic and, at least in some cases, has been suggested to play a role in targeting and/or in assisting the correct folding of the antimicrobial peptide [18]. The preproregion is often highly conserved within families of antimicrobial peptides, as deduced from sequence analysis of the precursors at the cDNA level, thus suggesting that members of each family evolved from ancestor genes through duplication and modification.

2. Cathelicidins, a novel family of antimicrobial peptide precursors

A rapidly expanding group of antimicrobial peptide precursors with unique features has recently been identified in myeloid cells. Precursors of this type have highly identical N-terminal preprosequences, followed by highly variable C-terminal sequences which correspond to the antibacterial peptides (Fig. 1).

*Corresponding author. Fax: (39) (40) 398990.

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Table 1
Structure-based classification of animal antimicrobial peptides

Peptides with disulfide bonds		Peptides without disulfide bonds	
Peptides with three disulfide bonds:		*Mostly α-helical peptides*:	
Defensins, cryptdins,	mammals	*PMAP-36 and -37, *CAP18, *FALL-39	mammals
β-defensins	mammals, birds	Magainins, dermaseptins	amphibia
Insect defensins	insects	Cecropins	insects
Peptides with two disulfide bonds:		*Pro- and Arg-rich peptides*:	
*Protegrins	mammals	*Bactenecins, *PR-39, *prophenin	mammals
Tachyplesins, polyphemusins	horse-shoe crabs	Apidaecins, abaecin, drosocin	insects
Peptides with one disulfide bond:		*Trp-rich peptides*:	
*Cyclic dodecapeptide	mammals	*Indolicidin, *PMAP-23	mammals
Brevinins, ranalexin, esculentin	amphibia		
		Gly-rich peptides	
		Hymenoptaecin, coleoptericin	insects

Representative peptides and peptide families are reported (see refs. in [2–5,7,10]). Peptides derived from cathelicidins are indicated by asterisks.

The presence of a conserved 5′ region in the mRNAs of these precursors allowed the amplification of the cDNAs of novel congeners. In these putative precursors the conserved domain is followed by previously unknown sequences with structural features consistent with antibacterial activity. These prepropeptides have molecular masses of 16–26 kDa, and have been identified in bovine [19–22], porcine [23–29], rabbit [30,31] and, more recently, human [32,33] myeloid cells. The sequence is comprised in most cases of a highly conserved preproregion of 128–143 residues including a putative 29–30 residue signal peptide and a propiece of 99–114 residues, and a C-terminal region ranging in length from 12 to 100 residues (Fig. 2). The mature peptides corresponding to the C-terminal sequences have all been given individual names that identify them as such. At present they include the bovine cyclic dodecapeptide [34], Bac5 [35], Bac7 [35] and indolicidin [36]; the porcine PR-39 [13], PMAP-36 [26], PMAP-37 [29], PMAP-23 [27], protegrins [37] and prophenin [38]; rabbit CAP18(106–142) [39] and human FALL-39/CAP18 [32,33].

The prosequence which is present in the precursors of these peptides is highly identical to the sequence of a protein termed cathelin. This protein was isolated from porcine leukocytes [40], and is likely to be the proregion of a processed precursor of this type, from which the C-terminal antimicrobial peptide has been released (Fig. 1). Based on the presence of a common cathelin-like domain, we propose the name cathelicidins for this group

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PMAP-37(P)          QALSYREAVLRAVDRLNEQSSEANLYRLLELDQPPKADEDPGTPKPVSFTVKETVCPRPTWRPPELCDFKENGRVKQCVGTVTLDQIKDPLDITCNEIQSV
PG-1(P)             ----------------------------------------------------------RQ----------------------------------V-G-
PG-2(P)             ----------------------------------------------------------RQ----------------------------------V-G-
PG-3(P)             ----------------------------------------------------------RQ----------------------------------V-G-
PG-4(P)             ----------------------------------------------------------RQ----------------------------------V-G-
PMAP-23(P)          ----------------------------------------------------------RQ--------------------KE-RGNF-----QL---
PMAP-36(P)          ------------------------------------------------------------------------------NPSN-----N-D-----
C12(P)              ----------------------------------------------------------R-----------------------------------..G-
PR-39(P)            ----------------------------------------------------------RQ--------------------NPSIHS---S-------
FALL-39/hCAP18(H)   -V---K------I-GI-QR--D-------D--PR-TM-G--D---------------T-QQS--D----KD-L--R-M-----N-ARGSF--S-DKDNKR
Indolicidin(B)      --------------Q---L-------------P---DN--L--R-------------TIQQ-A-Q-----K------------PSN-QF-LN---L---
Bac7(B)             ---------------I--R-------------P---DV--R-AR--T-----------TSPQ---Q-------L-------I----SD-LF-LN---L---
Bac5(B)             --------------QF--R-------------PT-ND-L----R-----R----D---TSQQ-L-Q-------L-----------PSN-QF--N---L---
Dodecapeptide(B)    --------------Q-------P-I----------QD----DS--R---R------S-T-QQ---Q-------LL-R-E-------VRGNF-----NH--I
CAP18(R)            -D-I----------AF-Q-----------SM-PQQLE-AK-Y--Q---------E---T--KL--Q----D-L--R------RY-AW-SF-R--RA-ES
p15(R)          IPHRR-R-E-V-AQ-LQFY--GQQGQP-F----ATP--SLNSKS..RI-LN-RI-----IFTLD-Q-GN-A-R-G-EERI-R-AFVRRRRVRA-TLR-DRD-RR
Cathelin(P)         E-R---------------------------------------------------------RQ---------....----------NPSIHS---S-------

PMAP-37(P)          GLLSRLRDFLSDRGRRLGEKIERIGQKIKDLSEFFQS
PG-1(P)             RGGRLCYCRRRFCVCVGRG
PG-2(P)             RGGRLCYCRRRFCICVG
PG-3(P)             RGGGLCYCRRRFCVCVGRG
PG-4(P)             RGGRLCYCRGWICFCVGRG
PMAP-23(P)          RIIDLLWRVRRPQKPKFVTVWVR
PMAP-36(P)          GRFRRLRKKTRKRLKKIGKVLKWIPPIVGSIPLGCG
C12(P)              RRFPWWWPFLRRPRLRRQAFPPPNVPGPRFPPPNVPGPRFPPPNFPGPRFPPPNFPGPRFPPPNFPGPPFPPPIFPGPWFPPPPPFRPPPFGPPRFPGRR
PR-39(P)            RRRPRPPYLPRPRPPPFFPPRLPPRIPPGFPPRFPPPRFPGKR
FALL-39/hCAP18(H)   FALLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES
Indolicidin(B)      ILPWKWPWWPWRRG
Bac7(B)             RRIRPRPPRLPRPRPRPLPFPRPGPRPIPRPLPFPRPGPRPIPRPLPFPRPGPRPIPRPL
Bac5(B)             RFRPPIRRPPIRPPFYPPFRPPIRPPIFPPIRPPFRPPLGPFPGRR
Dodecapeptide(B)    RITKQPWAPPQAARLCRIVVIRVCR
CAP18(R)            PEPTGLRKRLRKFRNKIKEKLKKIGQKIQGFVPKLAPRTDY
p15(R)              QPEFPRVTRPAGPTA
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Fig. 1. Alignment of cathelicidin sequences (cathelin-like proregion and C-terminal antimicrobial domain), as deduced from porcine (P) [23–29], human (H) [32,33], bovine (B) [19–22] and rabbit (R) [30,31] myeloid cDNAs. The sequence of the PMAP-37 proregion is in black capital letters. Red dashes denote identical residues in other congeners. C- terminal antimicrobial domains are in blue, while amidation signals are in red. Gaps are indicated by dots. The amino acid sequence of cathelin was determined by Edman degradation [40].

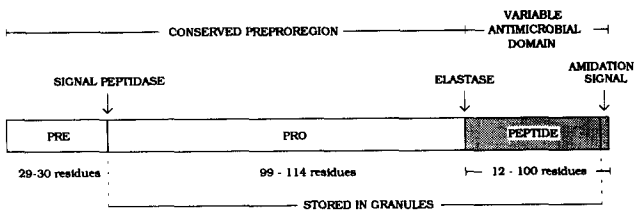

Fig. 2. Schematic representation of prepropeptides of the cathelicidin family.

of precursors, and this term will be used herewith to indicate molecules with a cathelin-like proregion and a C-terminal antimicrobial domain.

Several mismatches in the 5′ regions indicate that, in general, cathelicidins do not originate by posttranscriptional processing. The genes for one porcine [2] and one bovine (Scocchi, M. et al., unpublished) precursor have been sequenced and both have been shown to contain four exons. The preproregions share a high similarity, with an intra-species identity of 90–97% for porcine, and 75–87% for bovine preprosequences. The rabbit congeners show the lowest degree of similarity. In particular, rabbit p15 is the only congener which is not processed to give a free C-terminal peptide [31]. Four invariant cysteines clustered in the C-terminal region of the cathelin-like propiece are arranged to form two intramolecular disulfide bonds (Storici, P. et al., unpublished), which may impose structural constraints on the molecule. When the prosequence is compared with other known proteins, the best alignment scores are with members of the cathelicidin family, immediately followed by members of the cystatin superfamily, proteins known to inhibit cysteine proteinases [41]. The most significant alignments are with the cystatin-like domains of kininogens. For instance, a stretch of 92 residues of the propiece of bovine Bac5 (residues 36–127 of preproBac5 in ref. [21]) shows a 22% identity and a further 37% similarity with the third cystatin-like domain (residues 277–370) of bovine kininogen II [42], including alignment of all four cysteines comprised in this segment. This sequence may thus be considered a modular unit associated to a number of different and rapidly evolving C-terminal domains.

Cathelicidins are expressed early in myeloid differentiation, and their mRNAs are not detectable in mature neutrophils. Human FALL-39 mRNA is also expressed in testis [32], and PR-39 has been isolated from pig intestine [13], suggesting that the peptides derived from these precursors might contribute to the control of microbial growth also in these anatomic compartments. Studies on the biosynthesis and the intracellular processing of the precursors of Bac5 and Bac7, the most extensively characterized antimicrobial peptides of this group, indicate that they are synthesized in bone marrow myeloid cells as prepropeptides. These are processed by the removal of the signal peptide to proforms which are stored in the large granules of bovine neutrophils [43]. Western analysis of bovine and porcine neutrophils, using specific antibodies, has shown that other members of this family are also stored as proforms and some have been purified from bovine neutrophils (Storici, P. et al., unpublished).

As shown for proBac5 and proBac7, cathelicidins can be released extracellularly from granules by neutrophil stimulants [44]. Purified proBac5 and proBac7 do not display antimicrobial activity [45], most likely because the anionic propiece causes inactivation of the cationic C-terminal peptide. ProBac5 has been shown to inhibit the in vitro activity of the cysteine proteinase cathepsin L [46], which provides additional evidence for the evolutionary relatedness of the prosequence with the cystatin superfamily of cysteine proteinase inhibitors. This inhibitory effect is exerted with a Ki value of 60 nM, which is some orders of magnitude higher than those of cystatins (K_i values of 0.23 nM and 0.005 nM for human cystatin B and C, respectively) and kininogens (K_i of 0.017 nM for human L-kininogen) [47]. Whether this trace of a common evolutionary origin also has functional implications in modulating inflammatory responses is not yet clear, and further structural and functional studies are needed in order to assess its physiological significance.

Investigations on possible function(s) of the conserved N-terminal domain may help unravel important aspects of the biology of cathelicidins. Indeed, the evolutionary pressure exerted towards conservation of this prosequence suggests it may have a role in specific functions such as targeting of the antimicrobial peptides to the granules or aiding their correct proteolytic maturation. Studies on the maturation of the bovine Pro- and Arg-rich peptides have shown that the N-terminal conserved region is cleaved off by elastase under conditions that favour concomitant release of the contents of the large granules and the azurophils (i.e. granule discharge into phagocytic vacuoles) [44,45]. This enzyme liberates the mature antimicrobial peptide at a specific valyl residue of the precursor. Most cathelicidins have cleavage sites for elastase at corresponding positions and may undergo a similar processing.

3. Structure and function of the mature C-terminal peptides

The C-terminal sequences are structurally varied (Fig. 1) and in several cases [19,21,23–26,28] show a C-terminal amidation

Table 2
Antibacterial activity of peptides corresponding to the active C-terminal domain of cathelicidins

Organism	Minimal inhibitory concentration (μM)					
	Bac5	Bac7	PMAP-36 (1–20)	PMAP-37	PMAP-23	CAP18 (106–125)
E. coli	2	2	12	1	4	4
S. typhimurium	2	2	48	4	8	2
P. aeruginosa	>40	8	3	2	16	0.5
S. aureus	>40	>40	6	32	4	4
B. megaterium	2	2	3	4	2	4

The minimal inhibitory concentration (MIC) is defined as the lowest peptide concentration preventing visible growth of approximately 1.5×10^5 colony forming units/ml after 18 h incubation at 37°C [35]. Results have been obtained by using natural Bac5 and Bac7 [35] and synthetic PMAP-36(1-20) [26], PMAP-37 [29], PMAP-23 [27] and CAP18(106-125) [51].

signal. Peptides corresponding to all known sequences have either been purified from natural sources [13,34–39], after processing of the respective precursors, or have been obtained by chemical synthesis based on the sequence deduced from cDNA [26,27,29,32,33]. At present they include rabbit CAP18(106–142) [39], human FALL-39/CAP18 [32,33], PMAP-36 [26] and PMAP-37 [29], all of which are mostly α-helical; two Trp-containing peptides, indolicidin [36] and PMAP-23 [27]; the Pro- and Arg-rich Bac5 [12], Bac7 [12], PR-39 [13] and prophenin [38]; the cyclic dodecapeptide [34] and protegrins [37] which are loop-forming molecules with one and two disulfide bonds, respectively. The sequence of the Pro- and Arg-rich peptides (Fig. 1) is peculiar in that several short modules are present, and often arranged in tandem repeats [12,13,38].

The structure of some of these peptides has been analyzed by circular dichroism spectroscopy, showing that PMAP-36 [26], PMAP-37 [29] and FALL-39 [32] undergo a transition from a random coil to an ordered, mainly α-helical conformation on addition of an organic solvent. This behaviour indicates the presence of an amphipathic α-helical conformation, a structure found in many membrane-active peptides. A polyproline type structure has been suggested for PR-39 [48] and, based on sequence similarity, such a structure might also occur in Bac5 and Bac7, although this still requires confirmation.

Peptides derived from cathelicidins exert a broad spectrum antibacterial activity at μmolar concentrations, with a wide overlap in specificity but also with significant differences in potency among each other (Table 2). The Pro- and Arg-rich peptides are substantially more active against gram-negative than gram-positive bacteria [13,35,38]. Conversely, the other linear (helical and Trp-rich) and Cys-containing peptides are in general highly active against both gram-positive and gram-negative microorganisms [26,27,32,34,36,37,49]. Protegrins also exhibit fungicidal activity [37], while Bac5 and Bac7 kill leptospirae [50].

Although several approches have been used to investigate the mode of action of these peptides, our understanding of their killing mechanism(s) is still incomplete. Many of them have been shown to rapidly permeabilize the membranes of susceptible bacteria [14,26,27,29,51]. The Pro- and Arg-rich Bac5 and Bac7 inhibit incorporation of precursor molecules into protein and RNA [14] and PR-39 has been shown to stop protein and DNA synthesis in gram-negative bacteria [52].

In addition to antimicrobial activity, some of these peptides display other functions. Rabbit and human CAP18 bind LPS, inhibit multiple LPS biological activities in vitro, and reduce LPS lethality in murine models of endotoxemia [33,53], whereas PR-39 can induce expression of cell surface heparan sulphate proteoglycans (syndecan-1 and -4), as part of the wound repair process [54]. These molecules thus appear to be capable of performing several functions, such as bacterial killing, LPS-neutralization, inhibition of a tissue-degrading enzyme and promotion of wound healing, which in general are related to the protection of the host.

4. Conclusions

The cathelicidin family was first recognized in 1993 [21] and now counts numerous members (Fig. 1) which are distributed among various mammalian species. Most of these congeners have been identified through molecular biological approaches that are based on the high conservation of their prosequence. These effector molecules of innate immunity are peculiar in that a highly conserved proregion is associated to a highly variable and biologically active unit. An analogous case may be that of the mouse antimicrobial cryptdin mRNAs, which show pre-proregions similar to those of other Cys-rich sequences whose function, however, is as yet unknown [55]. The functional organization of cathelicidins in constant and variable regions in a sense parallels that used in antigen-driven immunity, albeit at a much lower level of complexity.

A number of studies have led to a relatively good characterization of the antibacterial domains, whereas the several putative roles that have been proposed for the conserved proregion remain to be verified. Furthermore, the structural organization of cathelicidins poses interesting questions concerning the genetic mechanisms by which they have been generated. Some indications have come from cDNA sequence analysis of the protegrin gene which appears to have arisen from insertion of a protegrin coding region into a pre-existing C12/prophenin gene [28]. Similar insertional events may have generated bovine Bac7 and porcine PR-39 from a common ancestor gene [22]. However, these mechanisms cannot fully explain the high diversity shown by this family. Thus, research in the future should also aim at obtaining a better knowledge of the various mechanisms which worked at the genomic level to produce this component of innate immunity.

5. Note added in proof

After the submission of this manuscript, the structure of the genes coding for the porcine cathelicidins PR-39 (Gudmundsson, G.H., Magnusson, K.P., Chowdhary, B.P., Johansson, M., Andersson, L. and Boman, H.G. (1995) Proc. Natl. Acad. Sci. USA 92, 7085–7089) and protegrins (Zhao, C., Ganz, T. and Lehrer, R.I. (1995) FEBS Lett. 368, 197–202) were reported.

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