Biochimica et Biophysica Acta 1858 (2016) 546–566

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

The human cathelicidin LL-37 — A pore-forming antibacterial peptide and host-cell modulator☆

Daniela Xhindoli, Sabrina Pacor, Monica Benincasa, Marco Scocchi, Renato Gennaro, Alessandro Tossi *

Department of Life Sciences, University of Trieste, via Giorgeri 5, 34127 Trieste, Italy

ARTICLE INFO

Article history:
Received 7 August 2015
Received in revised form 30 October 2015
Accepted 5 November 2015
Available online 10 November 2015

Keywords:
Cathelicidin
LL-37
hCAP-18
CRAMP
Host defence peptide
Antimicrobial peptide
Innate immunity

ABSTRACT

The human cathelicidin hCAP18/LL-37 has become a paradigm for the pleiotropic roles of peptides in host defence. It has a remarkably wide functional repertoire that includes direct antimicrobial activities against various types of microorganisms, the role of 'alarmin' that helps to orchestrate the immune response to infection, the capacity to locally modulate inflammation both enhancing it to aid in combating infection and limiting it to prevent damage to infected tissues, the promotion of angiogenesis and wound healing, and possibly also the elimination of abnormal cells. LL-37 manages to carry out all its reported activities with a small and simple, amphipathic, helical structure. In this review we consider how different aspects of its primary and secondary structures, as well as its marked tendency to form oligomers under physiological solution conditions and then bind to molecular surfaces as such, explain some of its cytotoxic and immunomodulatory effects. We consider its modes of interaction with bacterial membranes and capacity to act as a pore-forming toxin directed by our organism against bacterial cells, contrasting this with the mode of action of related peptides from other species. We also consider its different membrane-dependent effects on our own cells, which underlie many of its other activities in host defence. This article is part of a Special Issue entitled: Pore-Forming Toxins edited by Mauro Dalla Serra and Franco Gambale.

1. Introduction

Cathelicidins are a family of vertebrate host defence peptides (HDPs) defined by the conserved nature of their pro-region rather than to the sequence or structure of the antimicrobial peptides themselves, which are quite variable. It is one of the two principal families of HDPs in mammals, the other one being the defensins [1–3]. The two families differ significantly in the number of expressed peptides (defensins are present in humans as multiple genes, cathelicidin only as one), structure, mode of action and pro-piece, which is much shorter and unstructured in the defensins [4].

Since their discovery in the late '80s, cathelicidins have demonstrated a remarkably wide functional repertoire. They display direct antibiotic activities against bacterial, fungal, viral and parasitic microorganisms. They can act as 'alarmins', helping to orchestrate the immune response to infection. They can modulate inflammation, both enhancing it to aid in combating infection, and limiting it to prevent damage to the host. They can promote wound healing and angiogenesis, and they have also been implicated in elimination of abnormal cells [2,5–9]. Strictly speaking, the term cathelicidin indicates the pro-form [10], while the active HDP is variously indicated by size and sequence features (e.g. human LL-37 starts with two Leu residues and is 37 residues long), by size and provenance (e.g. its bovine orthologue BMAP-34 stands for Bovine Myeloid Antimicrobial Peptide of 34 residues), by provenance and pro-form (e.g. mouse CRAMP stands for Cathelin Related AMP) or by various other features (see [11]). However, it has become customary to refer to the active HDPs as cathelicidins as well.

hCAP18 is the only human cathelicidin. The HDP it releases, LL-37, has been extensively studied since its discovery in 1995, and is a paradigm for the multiple roles of cathelicidin peptides in host defence. As a consequence, the literature is vast and the interested reader is referred

Abbreviations: AFM, atomic force microscopy; AMP, antimicrobial peptide; ATR, attenuated total reflection; BF, biofilm; CD, circular dichroism; CLD, cathelin-like domain; Ch, cholesterol; CREB, cAMP responsive element; D8PG, dioctanoylphosphatidylglycerol; DPC, dodecylphosphocholine; dPG, di-phosphatidylglycerol; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine; DPPG, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-*rac*-(3-lysyl(1-glycerol)); EGFR, epidermal growth factor receptor; FITC, fluorescein isothiocyanate; FPLC, fast protein liquid chromatography; FPLR, formyl-peptide-like receptor, GPCR, G protein-coupled receptor; hCAP18, human 18 kDa cathelicidin antimicrobial protein; HDP, host defence peptides; LPS, lipopolysaccharide; LTA, lipoteichoic acid; mAb, monoclonal antibody; NET, neutrophil extracellular trap; PAMPs, pathogen associated molecular patterns; PBS, phosphate-buffered saline; PC, phosphatidylcholine; PG, phosphatidylglycerol; PI, propidium iodide; SDS, sodium dodecylsulphate; SM, sphingomyelin; SOPC, 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; SOPG, 1-stearoyl-2-oleoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol); SPR, surface plasmon resonance; TLR, toll-like receptor; VDR, vitamin D receptor; VDRE, vitamin D response element; WTA, wall teichoic acid.

☆ This article is part of a Special Issue entitled: Pore-Forming Toxins edited by Mauro Dalla Serra and Franco Gambale.

* Corresponding author at: University of Trieste, Department of Life Sciences, Building Q, Via Giorgieri 5, 34127 Trieste, Italy.
E-mail address: atossi@units.it (A. Tossi).

http://dx.doi.org/10.1016/j.bbamem.2015.11.003
0005-2736/

to several comprehensive reviews on different aspects of its structure, mode of action and biological functions [3,7,12–23]. The multiplicity of reported functions is reflected in the adjectives often used to qualify LL-37 — 'multifunctional', 'multifaceted', 'pleiotropic', even '*factotum*'. It manages to carry out all its reported activities with only 37 residues arranged in a simple, linear, amphipathic, helical structure, which spans most of its sequence. In this review our intent is to consider how different aspects of its primary and secondary structures, as well as its marked tendency to form oligomers and bind to other molecules or surfaces as such, may explain some of its cytotoxic and immunomodulatory effects. We consider its modes of interaction with biological membranes and subsequent effects, also contrasting this with the mode of action of its fragments, related peptides from other species and artificial analogues.

2. Discovery of cathelicidins and LL-37

The first papers referring to a cathelicidin (not yet known as such at that time), were by our group in Trieste in 1988 [24,25]. They report on a small, cyclic peptide (first generically referred to as bactenecin, then more specifically as dodecapeptide) isolated from granule extracts of bovine neutrophils. A monoclonal antibody obtained against these extracts was co-reactive to this peptide and a larger, non-bactericidal protein, indicating that it might be produced as an inactive precursor. Two considerably longer, linear and proline-rich antimicrobial peptides, Bac5 and Bac7, as they were also referred to as bactenecins, were subsequently also isolated from a total granule extract of bovine neutrophils. As they had no homology to the dodecapeptide they were thought to be unrelated to it [26,27].

By 1990, using anti-peptide antibodies, it had been determined that bactenecins were synthesized in immature bone marrow cells of the myeloid lineage as prepro-forms, targeted to the so called large granules of bovine neutrophils [28]. Biosynthesis occurred at the myelocyte stage, when large granules are assembled, and was then switched off with further myeloid differentiation. Processing was simple and fast, and the active mature HDPs appeared to be released from the pro-form by a neutral serine protease. It was proposed that the pro-part might contain sequences important for sorting and intracellular transport to granules, and also possess toxicity-neutralizing properties, as the pro-forms were inactive. It was then conclusively shown [29,30] that these peptides were *i*) present in the mature neutrophil granules only as pro-forms from which they were released by proteolytic cleavage, *ii*) that the pro-sequence indeed masked the activity of the mature peptide; *iii*) that this provided a temporal coupling between discharge and their activation, *iv*) that release and concomitant activation of the pro-form in phagosomes were early events after phagocytosis, and *v*) that the serine protease responsible for release was elastase. At about this time, a proline-rich peptide, PR-39, was also reported from pig [31].

An attempt was then made to clone the pro-form of Bac5 via a modified RACE protocol, using degenerate primers based on the sequence of the HDP in a primer extension analysis to complete to the 5′-end, and then using primers from this region to extend back to the 3′-end [32]. This resulted in a series of surprises. The 5′ end was very similar to that of rabbit CAP18, a protein with antimicrobial and LPS-binding activity just then reported, which carried an amphipathic helical peptide at its C-terminus [33], and also had significant sequence identity to pig cathelin, a putative inhibitor of the protease cathepsin L [34]. The RACE strategy led to identifying homologous pro-regions also for the dodecapeptide [35], the Trp-rich bovine AMP indolicidin [36,37] and porcine PR-39 [38], and there soon followed a spate of other porcine and bovine HDPs with quite variable structures, all linked to a cathelin-like domain (CLD) (see [10]), as well as a human homologue.

Three different groups independently reported the human cathelicidin, hCAP18, in 1995. One used PCR probes based on porcine PR-39, and identified the pro-form of a peptide they called FALL-39 [39]. The second went looking for the human equivalent of LPS-binding CAP18, using oligonucleotide probes based on the rabbit sequence [40], while the third directly isolated a 19 kDa protein from the specific granules of human neutrophils and then isolated its cDNA from a chronic myeloid leukaemia library [41].

The human cathelicidin HDP, LL-37, an amphipathic α-helical peptide, had a quite medium-sensitive antimicrobial activity [42] and, unusually, the capacity to adopt this structure also in aqueous solutions at physiological salt concentrations [43]. Most other helical AMPs only do so in the presence of biological membranes. It also had a significant capacity as a chemotactic agent for different types of immune cells, could stimulate release of pro-inflammatory chemokines and cytokines [5], could repress the effects of pathogen associated molecular patterns (PAMPs) such as LPS and LTA [44–46] and could stimulate propagation of some cells involved in healing processes [47,48] — it was a truly pleiotropic host defence peptide. Later studies indicated that these capacities likely depend on its particular structural characteristics.

3. Evolution, expression and features of the pro-region

3.1. Evolution

As mentioned in the previous section, cathelicidin peptides were initially identified as apparently unrelated HDPs, and each was brought into the cathelicidin fold only when the sequence of the pro-region was eventually determined. Given the widely different structures of some of these peptides, and broad species distribution, it sometimes came as quite a surprise. An example are curious helical peptides from the hagfish (*myxini*), a basal vertebrate, containing the unusual modified residue bromo-tryptophan [49]. Some years after their discovery, cloning and sequencing of the cDNA unexpectedly revealed them to be linked to a CLD, suggesting that the cathelicidin family was quite widespread among vertebrates and therefore ancient [50]. Several other members of the family were identified in horse, dog, rodents, birds, fish, amphibians and reptiles [51–57]. It has been suggested that cathelicidins evolved in vertebrates from cystatins, as they share significant structural similarity [58].

In a recent review, 148 database entries for cathelicidins were reported from 31 vertebrate species [7]. We have been keeping track of cathelicidins in protein and annotated or unassembled nucleotide sequence databases and have found evidence for them in 133 vertebrate species to date. They are present in lampreys, another basal vertebrate, numerous fish, amphibian and reptile species, birds, and placental and non-placental mammals (see Fig. 1), confirming their role as ancient components of vertebrate immunity. Analysis of the C-terminal HDP domains, when available, suggests that an orthologue of human LL-37 is present in all placental mammals, and indeed this is often the only cathelicidin present (e.g. in primates, glires and other rodents, as well as carnivores). Such an orthologue has not yet been identified in non-placental mammals or non-mammalian vertebrates.

LL-37 orthologues are relatively easy to identify due to a few characteristic sequence features that are conserved, as indicated in Fig. 2A and B. A conserved pattern of hydrophobic and polar residues should contribute to the formation of a long amphipathic helix in all the putative HDPs. Furthermore, a few segments of the sequence are conserved down to the amino acid level (Fig. 2A, shaded grey). In particular, most orthologues of LL-37 have a proline residue close to the C-terminus, often preceding an arginine, which results in the helix ending with a disordered and a polar tail. In orthologues where this feature is missing, a codon for proline can usually be found at the expected position in the 3′-UTR, following premature stop codons that were introduced during the peptides' evolution (e.g. in cetartiodactyls). Considering over 70 likely orthologous sequences, the length of the putative HDPs varies little, with only few being longer than 40 or shorter than 34 (see Fig. 2C). Furthermore, the conservation of hydrophobic