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Review

Antifungal proteins: More than antimicrobials?

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ABSTRACT

Antimicrobial proteins (AMPs) are widely distributed in nature. In higher eukaryotes, AMPs provide the host with an important defence mechanism against invading pathogens. AMPs of lower eukaryotes and prokaryotes may support successful competition for nutrients with other microorganisms of the same ecological niche. AMPs show a vast variety in structure, function, antimicrobial spectrum and mechanism of action. Most interestingly, there is growing evidence that AMPs also fulfil important biological functions other than antimicrobial activity. The present review focuses on the mechanistic function of small, cationic, cysteine-rich AMPs of mammals, insects, plants and fungi with antifungal activity and specifically aims at summarizing current knowledge concerning additional biological properties which opens novel aspects for their future use in medicine, agriculture and biotechnology.

1. Introduction

Small proteins with antimicrobial activity, so called antimicrobial proteins (AMPs), are produced by organisms throughout all kingdoms comprising prokaryotes, lower and higher eukaryotes. AMPs are secreted proteins that efficiently inhibit the growth of viruses, bacteria, fungi and parasites. In unicellular organisms, AMPs might provide their hosts the advantage to successfully compete with organisms that possess similar nutritional and ecological requirements. In multicellular organisms, AMPs constitute a primitive mechanism of innate immunity and form the first line of defence to protect their hosts from microbial attack. The innate immunity represents an evolutionarily ancient and widespread defence mechanism found in plants, insects and vertebrates. In addition, vertebrates developed the adaptive immune system – a sophisticated mechanism that uses antibodies and killer cells to recognize and eliminate invading microorganisms and allows immunological memory and self versus non-self recognition. The innate immune response is fast, and complements the adaptive immunity. Thus, both mechanisms combine to form an optimal and efficient defence system that supports the fitness of the host. The fact that closely related AMPs are widely distributed over different eukaryotic kingdoms, i.e. the class of defensins, suggests that ancestral AMP genes existed in basal eukaryotes even before fungal and insect lineages diverged (Lehrer and Ganz, 1999; Lehrer, 2007; Zhu, 2008).

AMPs are gene-encoded and they are either constitutively expressed or rapidly transcribed upon induction. In higher eukaryotes invading microbes and their products, e.g. lipopolysaccharides (Mendez-Samperio *et al.*, 2007; Amlie-Lefond *et al.*, 2005), or host cellular compounds, such as butyrate (Murakami *et al.*, 2002), cytokines (Wolk *et al.*, 2004; Wilson

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et al., 2007; Lai and Gallo, 2009), and vitamins (Schauber *et al.*, 2006) stimulate AMP production.

Due to the vast variety in function, structure, expression pattern, target organisms and producing hosts, the classification of AMPs is difficult and somewhat arbitrary to date. Mostly, AMPs are classified according to their functional and/or structural properties. Both characteristics are determined by the primary sequence of the protein which very often shows a high number of certain amino acids such as glycine, cysteine, histidine, proline, tyrosine, arginine, lysine and serine.

Although only a defined number of AMPs has been structurally analysed by nuclear magnetic resonance (NMR), calorimetric dichroism (CD) or X-ray crystallography, the secondary and tertiary structure of numerous AMPs has been predicted from primary sequence homologies. The most common classes contain proteins with α-helical, β-sheet or mixed α-helical/β-sheet structures (Zhu, 2008; Dimarcq *et al.*, 1998; Giangaspero *et al.*, 2001; Tossi *et al.*, 2000).

The best functionally characterized AMPs are bactericidal, whereas the properties of antifungal AMPs and their mode-of-action are less well studied. In the online database of AMPs at http://aps.unmc.edu/AP/main.php around 1900 AMPs of different origin are registered. From these, more than 1500 AMPs (79 %) have been assigned antibacterial activity compared to 648 antifungal AMPs (34 %). This classification, however, is redundant and antibacterial AMPs may also show antifungal activity that has not been investigated so far.

Most interestingly, the number of reports that document new additional functions of AMPs beyond their antimicrobial activity is constantly increasing. These features might arise from signalling functions of AMPs that accompany the activity of AMPs to interfere with the cell proliferation of microbes. For example, antimicrobial peptides from bacteria are part of the quorum-sensing mechanism that helps microorganisms to communicate and co-ordinate their behaviour by accumulating signalling molecules in the extracellular environment. This microbial communication system regulates e.g. symbiosis, biofilm formation, conjugation, sporulation, virulence, motility and the production of various secondary metabolites (Raina *et al.*, 2009; Maroti *et al.*, 2011). In fungi, plants and insects, AMPs have been related to development and differentiation (Stotz *et al.*, 2009a,b; Eigentler *et al.*, 2012; Hegedus *et al.*, 2011a), symbiotic interaction (Maroti *et al.*, 2011) and root hair extension (Allen *et al.*, 2008). In vertebrates, especially mammals, most diverse biological effects of AMPs have been reported, such as endotoxin neutralization (Rosenfeld *et al.*, 2010), signalling (Salzet, 2002), regulation of the immune response by chemotactic and immunomodulating activities (Wiesner and Vilcinskas, 2010; Yang *et al.*, 2002), induction of angiogenesis and wound repair (Baroni *et al.*, 2009) and protection against cancer (Yang *et al.*, 2002).

This review focuses on the properties and mode-of-action of small (5–8 kDa), cysteine-rich antifungal AMPs produced in mammals, insects, plants and fungi of related structure and highlights additional biological functions apart from antimicrobial activity. We want to apologize in advance for not being able to refer to all excellent publications available in this field due to space limitation. Readers specifically interested in antibacterial AMPs are directed to other excellent reports and reviews (Lehrer, 2007; Wiesner and Vilcinskas, 2010; Bulet and Stocklin, 2005; Yang *et al.*, 2004; Selsted and Ouellette, 2005; Lay and Anderson, 2005; Wong *et al.*, 2007; Papagianni, 2003; Boman, 2003; Brogden, 2005; Reddy *et al.*, 2004; Guani-Guerra *et al.*, 2010; Taylor *et al.*, 2008; Mygind *et al.*, 2005).

2. A short general overview on the structure and mode-of-action of antifungal cysteine-rich AMPs

The most prominent group within the antifungal AMPs with a close structural relationship constitutes defensins from plants, insects and mammals (Taylor *et al.*, 2008; Aerts *et al.*, 2008). Defensins contain six to eight cysteines which form intramolecular disulfide bonds and stabilize an antiparallel β-sheet conformation flanked by an α-helical segment, also called cysteine stabilized α/β motif (CSα/β) (Bulet and Stocklin, 2005; Selsted and Ouellette, 2005; Taylor *et al.*, 2008). Defensin-like antifungal AMPs of fungal origin are steadily increasing in number and show similar structural features as defensins but lack the α-helix (Campos-Olivas *et al.*, 1995; Batta *et al.*, 2009). The compact structure of defensins and defensin-like AMPs confers resistance towards extreme temperature, pH and protease-mediated degradation (Batta *et al.*, 2009; Bulet *et al.*, 1999; Landon *et al.*, 1997; Hajji *et al.*, 2010).

Based on their cationic character, defensins are thought to interact with negatively charged plasma membrane components of sensitive microorganisms. Two general models try to explain the mechanism of antimicrobial action of defensins: (A) the permeabilization of the cell membrane by (i) the carpet model and (ii) the pore model (Brogden, 2005). Whereas in (i) several protein molecules insert into the membrane forming a pore, in (ii) the protein molecules oligomerize and form a multimeric pore. Both models are primarily based on AMP-bacterial interaction and describe the disintegration of the plasma membrane, cell leakage and cell death by necrosis. (B) Alternatively, the membrane interaction of defensins and defensin-like AMPs may not primarily damage the plasma membrane of target cells. Instead, antifungal protein interaction with specific lipid and/or protein components of the plasma membrane leads to a selective ion permeability of the membrane and to the formation of transient pores and/or results in (active) protein transport into the host cell where these antifungals interact with intracellular targets (Brogden, 2005; Marx *et al.*, 2008; Thevissen *et al.*, 2003a). This antifungal activity increases the intracellular level of reactive oxygen species (ROS) and triggers programmed cell death (PCD) (Leiter *et al.*, 2005; Aerts *et al.*, 2011, 2009). Thus, plasma membrane leakage could occur at a later time point after protein contact with the fungal cell as a secondary effect of extensive intracellular ROS formation, as proposed for plant defensins (Thevissen *et al.*, 2003a).

3. Mammalian AMPs

Among mammalian AMPs, the defensins are best characterized and categorized in three subfamilies, α-, β- and θ-