Biochimie 130 (2016) 132–145

Contents lists available at ScienceDirect

Biochimie

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

Review

Antifungal peptides: To be or not to be membrane active

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ARTICLE INFO

Article history:
Received 11 April 2016
Accepted 20 May 2016
Available online 24 May 2016

Keywords:
Antifungal peptides
Mode of action
Membrane lipids
Cell membrane
Cell wall

ABSTRACT

Most antifungal peptides (AFPs), if not all, have membrane activity, while some also have alternative targets. Fungal membranes share many characteristics with mammalian membranes with only a few differences, such as differences in sphingolipids, phosphatidylinositol (PI) content and the main sterol is ergosterol. Fungal membranes are also more negative and a better target for cationic AFPs. Targeting just the fungal membrane lipids such as phosphatidylinositol and/or ergosterol by AFPs often translates into mammalian cell toxicity. Conversely, a specific AFP target in the fungal pathogen, such as glucosylceramide, mannosyldiinositol phosphorylceramide or a fungal protein target translates into high pathogen selectivity. However, a lower target concentration, absence or change in the specific fungal target can naturally lead to resistance, although such resistance in turn could result in reduced pathogen virulence. The question is then to be or not to be membrane active - what is the best choice for a successful AFP? In this review we deliberate on this question by focusing on the recent advances in our knowledge on how natural AFPs target fungi.

Contents

Abbreviations: AFP, antifungal peptide; AMP, antimicrobial peptide; CL, cardiolipin; GlcCer, glucosylceramide; GPL, glycerophospholipid; GSL, glycerosphigolipid; GPI, glycerophosphatidylinositol; IPC, inositolphosphorylceramide; MIPC, mannosylinositol phosphorylceramide; $M(IP)_2C$, mannosyldiinositol phosphorylceramide; MDR, multi-drug resistance proteins; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phoshatidylethanolamine; PI, phosphatidylinositol; PIP_2, phosphatidylinositol bisphosphate; $PI(4,5)P_2$, phosphatidylinositol(4,5)-bisphosphate; PG, phosphatidylglycerol; PS, phosphatidylserine; ROS, radical oxygen species.

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1. Introduction

The widespread use of broad spectrum antibiotics is believed to be the main culprit behind the rise in microbial resistance [1–4]. Apart from the over- and misuse of antifungals [3–7], the increase in immune-compromised individuals in recent years [7–9] is responsible for the rise in the range, and variety of pathogenic fungal infections [10,11]. In recent years there has been a disturbing rise in fungal pathogen resistance against conventional antifungal

http://dx.doi.org/10.1016/j.biochi.2016.05.013

compounds [12–15]. Candidiasis is caused by *Candida* species such as *Candida albicans*, where these fungi are some of the major causative agents of nosocomial fungal infections [16,17]. With a mortality rate of approximately 40%, invasive candidal infections are a serious medical concern [1,7,18,19]. Aspergilloses, caused by *Aspergillus* species, such as *A. terreus*, *A. niger*, *A. flavus* and *A. fumigates* [20,21], are also a significant medical concern. Invasive *Aspergillus* infections, particularly in high risk individuals, such as organ transplant patients, are very serious as it is associated with near 100% mortality [1,22]. After *Aspergillus* species, *Fusarium* species are the most frequent filamentous pathogens identified in high risk patients. *F. solani*, which is also a plant pathogen, contributes to almost 50% of *Fusarium* infections with the rest of these infections caused by other opportunistic *Fusarium* species such as *F. oxysporum*, *F. moniliforme* and *F. verticilloides* [23,24].

Compared to the broad array of antibacterial drug classes, there are only a few classes of antifungal drugs, which drastically limit the choices for therapy [25]. The development of novel, non-toxic antifungal compounds is much more challenging than for their antibacterial counterparts, given that some antifungal drugs affect common eukaryotic targets and many fungal targets are closely related to the corresponding human protein or cell structure [26]. The current antifungal drug classes are limited to azoles targeting ergosterol synthesis, polyenes targeting ergosterol in the fungal membrane and echinocandins targeting cell wall synthesis [27,28]. This situation is particularly problematic when pathogens are resistant to more than one class of antifungal agent [29,30]. The development of novel antifungals, with novel mode(s) of action, is therefore essential for human health and well-being.

Microorganisms, such as fungi, may be small, but they have fast evolution and adaptation on their side when it comes to antibiotics and antifungal drugs, except for their cell membranes which are slower to evolve. Antimicrobial peptides (AMPs) are natural antibiotics that are produced by almost every organism providing a first line of defence against various infections [31–33]. Most AMPs target the vulnerable cell membranes because these compounds probably evolved with their producing organism in the fight for survival. For the purpose of this review, natural AMPs with potent antifungal activity are sub-classified as antifungal peptides (AFPs). With the escalating problem of microorganisms exhibiting resistance against conventional antibiotics, there is growing interest in the potential of such peptides to serve as novel antibiotics [34]. Membrane interaction of many AMPs and AFPs is integral to their antimicrobial activity, but additional/alternative modes of action for microbial inhibition have been illustrated [35]. Conversely, the resistance of *C. albicans* to antifungal drugs has been linked to membrane remodelling, with a central role of the ergosterol-sphingolipid rich lipid rafts containing membrane bound multidrug resistance (MDR) proteins [36–39]. As a result of the rapid and potent membrane activity together with a wide range of inhibitory mechanisms exhibited by AFPs, they have less likelihood of inducing *de novo* resistance in target microorganisms [40]. Their selectivity, rapid action and low likelihood of inducing resistance make AFPs ideal candidates as templates for novel antifungals [41].

The AFPs are a subset of the larger group of AMPs, consequently, their structures are just as diverse as their multi-kingdom origins: AFPs can be divided into linear peptides which form amphipathic and hydrophobic helices, β-sheet peptides, peptides with a mixture of α-helices and β-sheets, peptides rich in specific amino acids, as well as modified cyclic peptides, depsipeptides and lipopeptides [42–47] (examples are given in Fig. 1). However, within the structural diversity, a substantial number of the AFPs produced by unicellular organisms are small (<1.5 kDa) with a constrained N→C cyclic structure that include non-protein amino acids and/or a fatty acyl moiety in the structure, while the AFPs produced by multicellular organisms are generally larger (>3 kDa) with the majority having either linear α-helical or cystine-stabilised

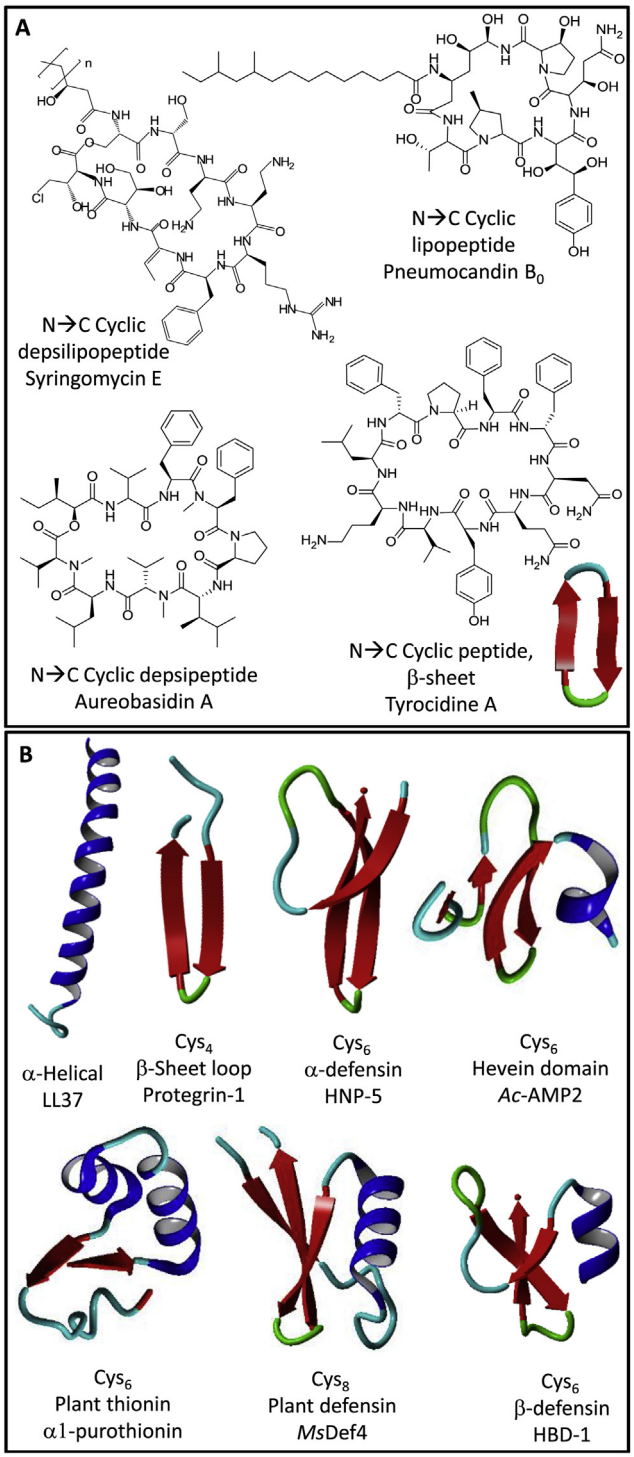

Fig. 1. Examples of AFPs and AMPs to illustrate their structural diversity with the non-ribosomally synthesised peptides shown in panel **A** and ribosomally synthesised peptides shown in panel **B**. For 3D structures β-sheet structures are shown in red, α-helices in dark blue, β-turns in green and light blue is either unstructured or random structures; disulphide bonds are not indicated. The peptide 3D structures were obtained from the Protein Data Bank: *Ac*-AMP2 (DOI: 10.2210/pdb1mmc/pdb) protegrin-1 (DOI: 10.2210/pdb1pg1/pdb) HNP5 (DOI: 10.2210/pdb2lxz/pdb) LL37 (DOI: 10.2210/pdb2k6o/pdb) α1-purothionin (DOI: 10.2210/pdb2plh/pdb) *Ms*Def4 (DOI: 10.2210/pdb2lr3/pdb) HBD1 (DOI: 10.2210/pdb1iju/pdb). Tyrocidine A 3D structure is from [49].