Biochimie 121 (2016) 268–277

Contents lists available at ScienceDirect

Biochimie

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

Research paper

Assessing the potential of four cathelicidins for the management of mouse candidiasis and *Candida albicans* biofilms

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ARTICLE INFO

Article history:
Received 8 June 2015
Accepted 29 November 2015
Available online 2 December 2015

Keywords:
Cathelicidins
Antifungal susceptibility
Oral candidiasis
Advanced structures
Anti-biofilm

ABSTRACT

As the most common fungal pathogen of humans, severe drug resistance has emerged in the clinically isolated *Candida albicans*, which lead to the urgency to develop novel antifungal agents. Here, four our previously characterized cathelicidins (cathelicidin-BF, Pc-CATH1, Cc-CATH2, Cc-CATH3) were selected and their antifungal activities against *C. albicans* were evaluated *in vitro* and *in vivo* using amphotericin B and LL-37 as control. Results showed that all four cathelicidins could eradicate standard and clinically isolated *C. albicans* strains with most MIC values ranging from 1 to 16 μg/ml, in less than 0.5 h revealed by time-kill kinetic assay. Four peptides only exhibited slight hemolytic activity with most HC_{50} > 200 μg/ml, and retained potent anti-*C. albicans* activity at salt concentrations below and beyond physiological level. In animal experiment, 50 mg/kg administration of the four cathelicidins could significantly reduce the fungal counts in a murine oral candidiasis model induced by clinically isolated *C. albicans*. The antibiofilm activity of cathelicidin-BF, the most potent among the five peptides was evaluated, and result showed that cathelicidin-BF strongly inhibited *C. albicans* biofilm formation at 20 μg/ml. Furthermore, cathelicidin-BF also exhibited potent anti-*C. albicans* activity in established biofilms as measured by metabolic and fluorescent viability assays. Structure-function analyses suggest that they mainly adopt an α-helical conformations, which enable them to act as a membrane-active molecule. Altogether, the four cathelicidins display great potential for antifungal agent development against candidiasis.

1. Introduction

Candida albicans, the most common fungal pathogen of humans, colonizes asymptomatically in skin, gastrointestinal tract, oral and vaginal mucosa in about 30–70% of individuals, and its overgrowth often results in candidiasis [1]. A common form of candidiasis restricted to the mucosal membranes in mouth or vagina is thrush, which is usually easily cured in normal people, can develop into very severe mucosal and systemic infections in immunocompromised individuals, such as stomatitis, vaginitis, bloodstream infections and deep tissue infections [1]. So far, although a lot of antifungal agents have been developed and administered to the patients with *candida* infections, however, extensive drug-resistance still has been widely observed in clinically isolated *C. albicans* strains and become a severe challenge for public health nowadays [2]. In most cases, *C. albicans* could attach to a tissue surface and encase themselves in a self-released slimy polysaccharide and protein layer, known as biofilms, which is hardly penetrated by traditional antibiotics and finally prevent fungus from being cleared [3]. Besides, biofilm forming on implant surfaces becomes a more and more serious problem and a major cause of chronic infections worldwide [4]. The antibiotic resistance of biofilm is also owing to the slow growth of biofilm cells, which are tolerant to the antibiotics capable of penetrating the biofilm matrix [5]. Therefore, tight surveillance and novel antifungal agents that could directly target cell membrane are in great needed to manage such pandrug-resistant *C. albicans* infections.

Cathelicidins are a family of multifunctional antimicrobial peptides (AMPs) found in vertebrates, and most abundantly in

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http://dx.doi.org/10.1016/j.biochi.2015.11.028
0300-9084/

Table 1
Structural parameters of the five cathelicidins.

Sample	Amino acid sequence (Length)	Mw/pI	Net charge	Origin	Helix content
cathelicidin-BF	KFFRKLKKSVKKRAKEFFKKPRVIGVSIPF (30)	3637.50/11.79	11	*Bungarus fasciatus*	56.67%
Pc-CATH1	RIKRFWPVVIRTVVAGYNLYRAIKKK (26)	3175.90/11.60	8	*Phasianus colchicus*	38.46%
Cc-CATH2	LVQRGRFGRFLKKVRRFIPKVIIAAQIGSRFG (32)	3715.54/12.70	9	*Coturnix coturnix*	62.50%
Cc-CATH3	RVRRFWPLVPVAINTVAAGINLYKAIRRK (29)	3379.11/12.18	7	*Coturnix coturnix*	65.52%
LL-37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLV PRTES (37)	4493.32/10.61	6	*Homo sapiens*	78.38%

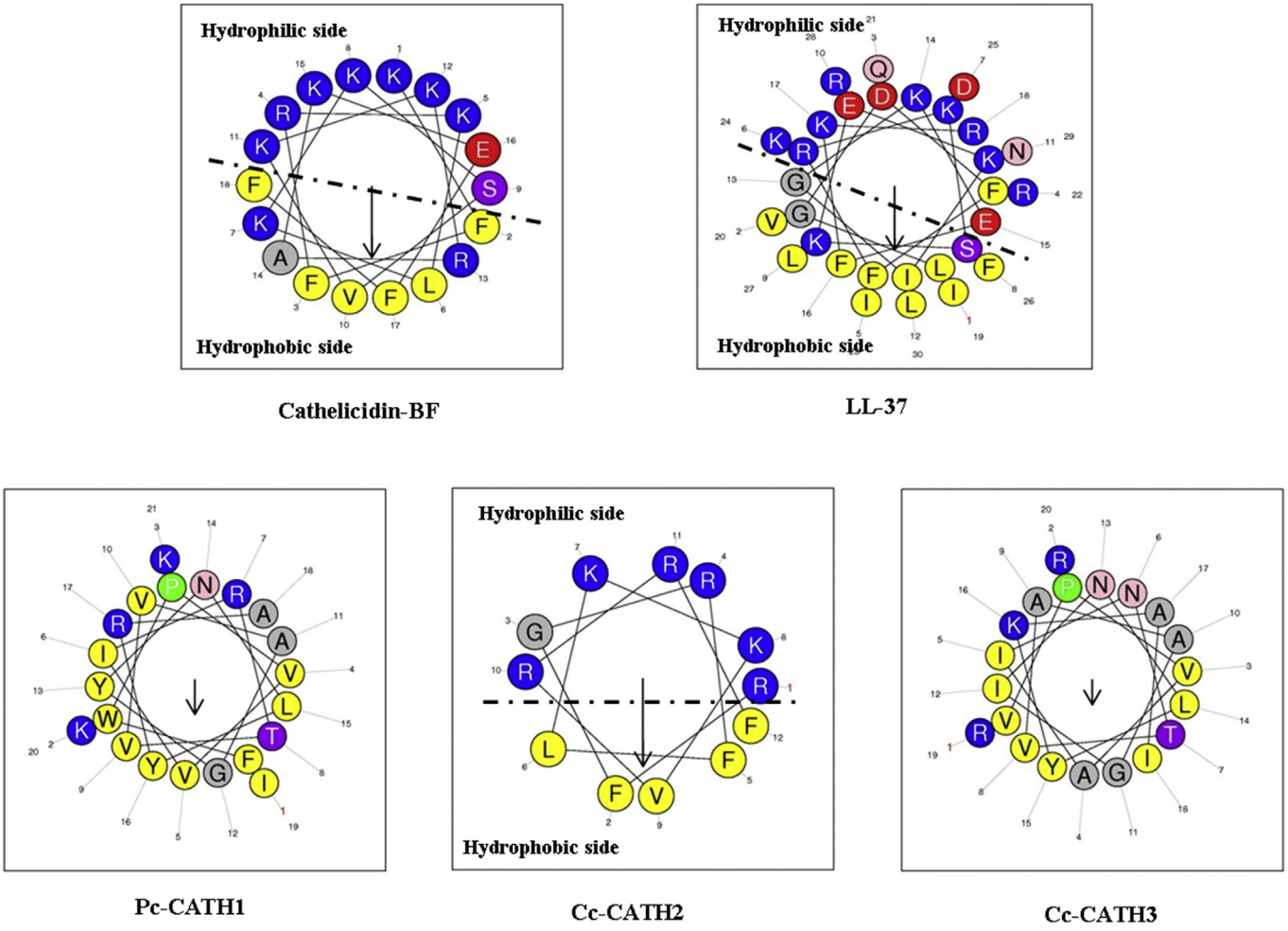

Fig. 1. Helix-wheel plots of the five cathelicidins: cathelicidin-BF, Pc-CATH1, Cc-CATH2, Cc-CATH3 and LL37. The hydrophobic and hydrophilic residues are separated with dash dot line, with the hydrophobic residues being concentrated on upper side of the helix and hydrophilic amino acids on the lower.

circulating neutrophils, myeloid bone marrow cells, and also in mucosal epithelial cells and skin keratinocytes [6,7]. Cathelicidins serve a critical role in mammalian innate immune defense against invasive bacterial infection [6,7]. Most of them possess potent antimicrobial activities against a broad range of microorganisms including bacteria, fungi, protozoa and certain viruses [8]. More interestingly, some of them exhibit great efficacy against a large number of clinically isolated drug-resistant pathogens [7]. Nowadays, some cathelicidin-derived AMPs, such as Iseganan, Omiganan, and MBI 594AN, have been already in clinical trials, suggesting their good prospects for the development of novel peptide antibiotics [9].

In previous works, we have identified a lot of cathelicidins from different species of vertebrates, and some have been proved to possess potent and broad-spectrum antimicrobial activities. In the present study, we selected four of them, cathelicidin-BF from *Bungarus fasciatus* [10], Pc-CATH1 from *Phasianus colchicus* [11], Cc-CATH2 and 3 from *Coturnix coturnix* [12], to examine their anti-*C. albicans* activities *in vitro* and *in vivo* in a murine oral candidiasis model, using human LL-37 and amphotericin B as positive control. Besides, their bacterial killing kinetics, hemolysis, cytotoxicity and salt stability were also examined. The effect of cathelicidin-BF on the *C. albicans* biofilm formation was investigated by XTT assays, and the antifungal activity against *C. albicans* communities in an established biofilms was measured by metabolic and fluorescent viability assays. The current results would provide basic concepts to evaluate the potential of these cathelicidins as novel antifungal agents against superficial and systemic candidiasis caused by *C. albicans*, especially against oral candidiasis and biofilm cultures of oral pathogens.