Biochimie xxx (2013) 1–10

Contents lists available at SciVerse ScienceDirect

Biochimie

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

Research paper

The attack of the phytopathogens and the trumpet solo: Identification of a novel plant antifungal peptide with distinct fold and disulfide bond pattern

Q2 Santi M. Mandal [a], William F. Porto [b], Prabuddha Dey [c], Mrinal K. Maiti [c], Ananta K. Ghosh [c], Octavio L. Franco [b,*]

[a] Central Research Facility, Indian Institute of Technology Kharagpur, Kharagpur 721302, India
[b] Centro de Análises Proteômicas e Bioquímicas, Pós-Graduação em Ciências Genômicas e Biotecnologia UCB, Brasília, DF, Brazil
[c] Department of Biotechnology, Indian Institute of Technology Kharagpur, Kharagpur 721302, India

ARTICLE INFO

Article history:
Received 5 April 2013
Accepted 28 June 2013
Available online xxx

Keywords:
αβ-Trumpet
Antifungal peptides
Pisum sativum
Plant defense
Lectin-like peptides
Molecular dynamics

ABSTRACT

Phytopathogens cause economic losses in agribusiness. Plant-derived compounds have been proposed to overcome this problem, including the antimicrobial peptides (AMPs). This paper reports the identification of Ps-AFP1, a novel AMP isolated from the *Pisum sativum* radicle. Ps-AFP1 was purified and evaluated against phytopathogenic fungi, showing clear effectiveness. *In silico* analyses were performed, suggesting an unusual fold and disulfide bond pattern. A novel fold and a novel AMP class were here proposed, the αβ-trumpet fold and αβ-trumpet peptides, respectively. The name αβ-trumpet was created due to the peptide's fold, which resembles the musical instrument. The Ps-AFP1 mechanism of action was also proposed. Microscopic analyses revealed that Ps-AFP1 could affect the fungus during the hyphal elongation from spore germination. Furthermore, confocal microscopy performed with Ps-AFP1 labeled with FITC shows that the peptide was localized at high concentration along the fungal cell surface. Due to low cellular disruption rates, it seems that the main target is the fungal cell wall. The binding thermogram and isothermal titration, molecular dynamics and docking analyses were also performed, showing that Ps-AFP1 could bind to chitin producing a stable complex. Data here reported provided novel structural–functional insights into the αβ-trumpet peptide fold.

1. Introduction

Phytopathogenic fungi may cause enormous problems in agribusiness, generating severe economic losses, since plants provide the main source of nutrition for these pathogens [1]. However, several plant-derived compounds have been reported as defense mechanisms, activated upon pathogen attack [2]. Among such mechanisms, the antimicrobial peptides (AMPs) have been described as being able to kill or slow the growth of infecting microorganisms and help to develop plant adaptive immunity [3]. In this field, AMPs provide innate immunity by the rapid formation of a "first defense line" against phytopathogens.

Hundreds of different antimicrobial proteins and peptides encoded within the genomes of plants have been described [3–5], and plant seeds have been used as a target for identification of plant AMPs. Several plant AMPs have been isolated from plant seeds, such as Pg-AMP1 [6], Cr-ACP1 [7], Cp-AMP [8] and Cp-thionin [9]. The AMPs can be classified in two major groups, according to the presence or absence of disulfide bridges [10]. The disulfide-free peptides are composed mainly of α-helical and unstructured AMPs; while the cysteine-stabilized AMPs can have a number of different folds. The plant cysteine-stabilized AMPs are classified according to their folds and disulfide patterns, so that the discovery of a novel fold could lead to a re- classification or to the creation of a novel AMP class [2,11,12]. In plants, there are few examples of plant disulfide-free AMPs [6,7,13–15], with most plant AMPs stabilized by disulfide bonds [2,4]. The main plant cysteine-stabilized AMP classes are thionins [16,17], defensins [18,19], cyclotides [20,21], hevein-like peptides [5,22], helical hairpins [23,24] and snakins [25–27]. Indeed, a more accurate classification could lead to a better understanding of structural–functional relations, despite the fact that the multifunctional character of AMPs [28] has made it difficult to acquire a complete understanding of their mechanisms of action.

* Corresponding author. Tel.: +55 6134487167.
E-mail addresses: ocfranco@gmail.com, ocfranco@pos.ucb.br (O.L. Franco).

0300-9084/$ – see front matter © 2013 Published by Elsevier Masson SAS.
http://dx.doi.org/10.1016/j.biochi.2013.06.027

Here, the identification of a novel small antimicrobial peptide with a distinct fold and disulfide bond pattern, Ps-AFP1, is reported. Ps-AFP1 was isolated from the *Pisum sativum* radicle, a very soft tissue, which grows downward in soil and survives on the fungal and bacterial population. Moreover, *in silico* analyses were performed, suggesting an unusual disulfide bond pattern and also an unusual fold. Therefore, the class of αβ-trumpet peptides is proposed here. The Ps-AFP1 mechanism of action was also proposed by using scanning electron and confocal microscopy analyses. Finally, molecular dynamics and docking analyses were also performed in order to better understand the structure–function relationship.

2. Results

2.1. Purification and sequencing of Ps-AFP1

In order to explore the radicle peptide content, an RP-HPLC-based separation strategy was used (Fig. 1(a)), yielding multiple fractions. After lyophilization, all fractions were challenged against fungi and one of them (star-marked in Fig. 1(a)) showed the highest efficiency. This fraction was submitted to rechromatography and after purification was named Ps-AFP1. Pure peptide (>90% after rechromatography, Fig. 1(b)) was obtained at a yield of ~0.18 mg from 100 g of radicle flour. The monoisotopic molecular mass of Ps-AFP1 was *m*/*z* 4198 Da (Fig. 1(c)). The presence of cysteine residues in Ps-AFP1 was confirmed by MALDI-MS analysis of reduced peptide after alkylation, which showed an addition of *m*/*z* 57 Da in each cysteine residue (data not shown). Firstly, the N-terminal sequence of Ps-AFP1 was determined as ^{1}RQLKS^{5}. In order to complete this sequence, a 5′-forward primer obtained from N-terminal sequence primers was constructed and a 3′-RACE-based poly (A) tail containing cDNA was used to amplify the corresponding Ps-AFP1gene sequence. The obtained cDNA sequence of Ps-AFP1 was "CGCCAGCTGAAAAGCAGCCGCCGCGGCGCGCTG GTGTGCGTGCGCCTGAAACTGTGCAGCGCGATTCTGAGCCGCGGCCT-GAGCTGCGGCATGTTTAGCTGCAACGCGCGCCGC" and the corresponding amino acid sequence was ^{1}RQLKSSRRGALVCVRLKLC SAILSRGLSCGMFSCNARR^{38} (Fig. S1).

Table 1
Antifungal activities of Ps-AFP1.

Fungus	EC_{50} (μM)
Aspergillus niger	15.62
Aspergillus terreus	7.81
Fusarium solani	7.81
Fusarium oxysporum	3.91
Pythium sp.	15.62
Colletotrichum gloeosporioides	7.81
Candida albicans	3.91
Glomerella sp.	7.81

2.2. Antifungal activity of Ps-AFP1

The antifungal activity was evaluated against several soil-borne plant pathogens. Ps-AFP1 exhibited different antifungal activities against most of the pathogens used (Table 1). These phytopathogenic fungi mostly cause damping-off diseases of seeds, seedlings and roots. The EC_{50} values were ranged at 0.975–500 μM, where *Fusarium oxysporum* and *Candida albicans* were the most affected

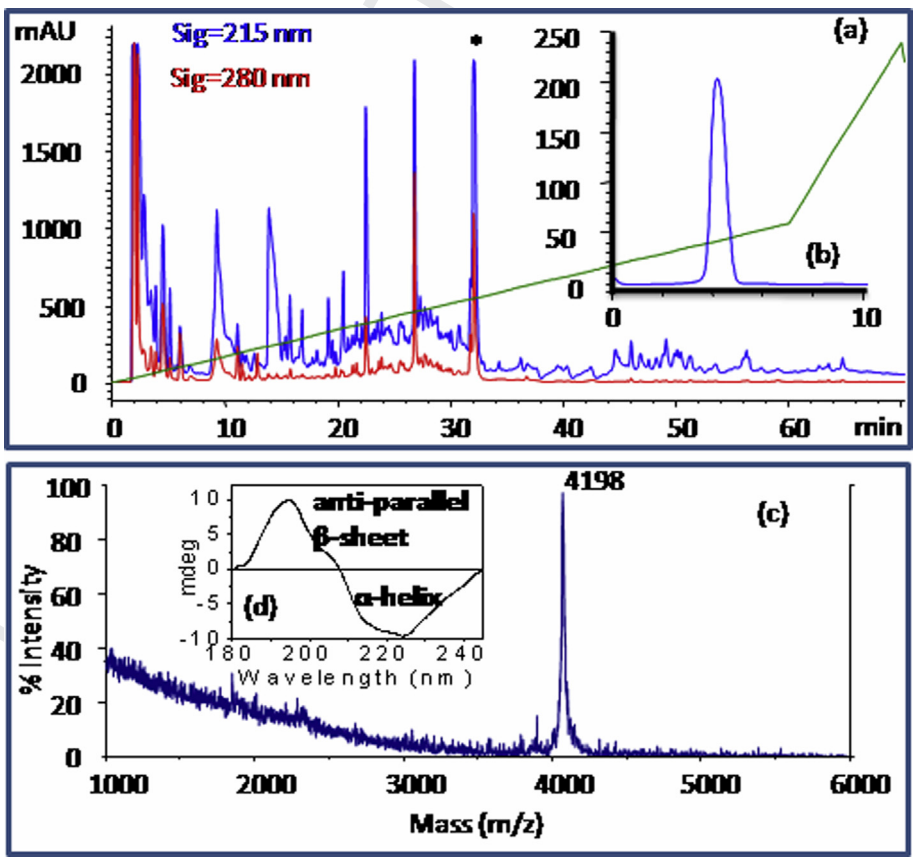

Fig. 1. Purification profile of Ps-AFP1 extracted from radicle of garden pea *Pisum sativum*. Reversed-phase HPLC chromatogram profile of acetic acid extract (a); diagonal line indicates the gradient of solvent B; rechromatogram profile of asterisk-indicated fraction (b, in inset); the mobile phase and other conditions are described in the text. MALDI TOF mass spectrum of asterisk-indicated antifungal active fraction (c), CD spectra of Ps-AFP1 in buffer solution (d).

Please cite this article in press as: S.M. Mandal, et al., The attack of the phytopathogens and the trumpet solo: Identification of a novel plant antifungal peptide with distinct fold and disulfide bond pattern, Biochimie (2013), http://dx.doi.org/10.1016/j.biochi.2013.06.027