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NMR structure, conformational dynamics, and biological activity of *Ps*Def1 defensin from *Pinus sylvestris*

Bulat I. Khairutdinov[a,b,1], Elena A. Ermakova[a,1], Yuri M. Yusypovych[c], Elena K. Bessolicina[a], Nadezhda B. Tarasova[a], Yana Y. Toporkova[a], Valentina Kovaleva[c], Yuriy F. Zuev[a,b,d], Irina V. Nesmelova[e,f,*]

[a] Kazan Institute of Biochemistry and Biophysics, Lobachevsky St. 2/31, Kazan 420111, Russia
[b] Kazan Federal University, Kremlyovskaya Str. 18, Kazan 420008, Russia
[c] Ukrainian National Forestry University, Gen. Chyprunku St. 103, Lviv 79057, Ukraine
[d] Kazan State Power Engineering University, Krasnoselskaya st. 51, Kazan 420066, Russia
[e] Department of Physics and Optical Science, University of North Carolina, 9201 University City Blvd., Charlotte, NC 28223, USA
[f] Center for Biomedical Engineering and Science, University of North Carolina, 9201 University City Blvd., Charlotte, NC 28223, USA

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ABSTRACT

Plants have developed a complex defense response system against pests and pathogens. Defensins, produced by plants as part of their innate immune response, form the family of small, basic, cysteine-rich proteins with activity primarily directed against fungal pathogens. In addition, plant defensins can show antibacterial activity and protease and insect amylase inhibitory activities. However, in gymnosperms, only antifungal activity of defensins has been described thus far. Here, we report antibacterial and insect α-amylase inhibition activities for defensin *Ps*Def1 from *P. sylvestris*, the first defensin from gymnosperms with a broad range of biological activities described. We also report the solution NMR structure of *Ps*Def1 and its dynamics properties assessed by a combination of experimental NMR and computational techniques. Collectively, our data provide an insight into structure, dynamics, and functional properties of *Ps*Def1 that could be common between defensins from this taxonomic group.

1. Introduction

Defense mechanisms of plants against pests and pathogens include the production of antimicrobial peptides (AMPs). The group of plant AMPs that resemble structural and functional features of previously characterized antimicrobial peptides found in insects and mammals, are called defensins [1]. Most plant defensins show activity against fungi [2–4], but they can also act as inhibitors of protein synthesis [5,6], α-amylases [7–9], proteases [10,11], or ion channels [12]. Some plant defensins possess the activity against Gram-positive and Gram-negative bacteria [13,14].

Plant defensins are small, cysteine-rich, cationic peptides of approximately 45–54 amino acid residues [1,15,16]. The amino acid sequence identity between different plant defensins can be < 35% [2,17] and only a small number of residues is conserved. Most plant defensins have six or eight conserved cysteine residues that form three and four disulfide bonds, respectively, although a few plant defensins with ten cysteine residues and five disulphide bonds have been identified [16]. In addition to conserved cysteine residues, a glycine, a serine, and an aromatic residue (F/W/H/Y) that is always followed by another glycine are conserved. Conserved disulfide bonds are thought to define the physico-chemical properties of defensins, such as an extreme resistance to high temperatures and acidic environments [18–21]. The stability of plant defensins makes them attractive as biotechnological tools against phytopathogenic fungi, currently controlled only by chemicals [2].

Despite the large variability of amino acid sequence, all plant defensins with reported three-dimensional structures show a similar three-dimensional fold as monomers, consisting of a triple-stranded antiparallel β-sheet and one α-helix tethered to the β-sheet by disulfide bonds, i.e., a cysteine-stabilized αβ, or CSαβ fold [2]. Two antiparallel β-strands β2 and β3 joined by a loop (loop L3) form a γ-core motif $GXCX_{3-9}C$ (X being any amino acid and G being a conserved glycine residue). The γ-core motif has a net positive charge and is thought to be

* Corresponding author at: Grigg Hall 306, Department of Physics and Optical Sciences, University of North Carolina, 9201 University City Blvd., Charlotte, NC 28223, USA.
E-mail address: Irina.Nesmelova@uncc.edu (I.V. Nesmelova).
[1] Authors contributed equally to this work.

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