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Plant Antibacterial Peptides and their Possible Oligomerization

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Plant antimicrobial peptides form an integral component of the innate immunity of plants and have activities against a broad spectrum of microorganisms. They were classified into various families and most share general features such as the presence of structure-stabilizing disulfide bridges and positive charge. Self-aggregation was suggested as one of the possible mechanisms for the effect of these antimicrobial peptides. Here, we have created a dataset comprising plant peptides with activity against gram positive and gram negative bacteria. We have found that the majority of the antibacterial peptides in this dataset contain at least one amyloidogenic region. We proposed that these amyloidogenic regions could help in the self-aggregation of the peptides and presented a possible ring-like oligomeric structure of the plant antimicrobial peptide NaD1.

Key words: Plant antimicrobial peptide, Oligomerization, Antibacterial peptide, Multiple Sequence Alignment.

Растительные антибактериальные пептиды и их возможная олигомеризация

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Растительные антимикробные пептиды являются неотъемлемой частью наследственного иммунитета растений и обладают активностью против широкого спектра микроорганизмов. Эти пептиды были расклассифицированы на различные семейства, и большинство из них имеют общие характеристики, такие как наличие стабилизирующих структуру дисульфидных мостиков и положительный заряд. Предполагается, что самоагрегация является одним из возможных механизмов воздействия этих противомикробных пептидов. В настоящей работе была создана база данных, включающая в себя растительные пептиды с активностью против грамположительных и грамотрицательных бактерий. На основании этой базы данных было обнаружено, что в большинстве антибактериальных пептидов содержится, по меньшей мере, один амилоидогенный участок. Мы предположили, что наличие амилоидогенных участков может помочь в самоагрегации этих пептидов и представили возможную кольцеобразную олигомерную структуру растительного антимикробного пептида NaD1.

Ключевые слова: растительный антимикробный пептид, олигомеризация, антибактериальный пептид, множественное пространственное выравнивание.

1. Introduction

Plants are constantly exposed to various environmental and/or pathogenic stresses, such as fungi, bacteria, insects, drought etc. To counter these potentially lethal conditions, plants have evolved sophisticated defence mechanisms ranging from cell wall, which forms a physical barrier, to constitutive or inducible expression of chemical compounds including the production of antimicrobial peptides (AMPs). The AMPs are an important component of the innate immunity in fungi, insects, animals and plants [1–5]. AMP expression in plant cells can be either constitutive

Table 1. Number of predicted amyloidogenic regions

	Number of peptides with 1 region	Number of peptides with 2 regions	Number of peptides with 3 regions	Number of peptides with 4 regions	Number of peptides with no region
FoldAmyloid	60	18	1	0	31
PASTA 2.0	17	3	0	0	90
WALTZ	13	1	0	0	96
AGGRESCAN	36	28	8	1	37

(e.g. in specialized tissues or organs) or can be induced (e.g. pathogen challenge) [6–8]. Plant AMPs are cysteine-rich cationic peptides which act against a broad range of organisms [7, 9]. Based on sequence similarity, number of cysteine residues, disulfide bond patterns and tertiary structure, plant AMPs have been divided into several distinct subgroups like thionins, defensins, cyclotides, hevein-like peptides, knottin-type peptides, lipid transfer proteins, α-hairpinin, and snakins [7, 9, 10]. These peptides have been isolated from various plant organs such as seeds, roots, flowers, stems and leaves. As potential candidates for increasing pathogen resistance to engineered crops and leads for new-generation drugs, AMPs generated a lot of interest in recent years [11–17].

Several studies also focused towards the elucidation of their antimicrobial properties and mechanism of action [9, 18]. One common theme observed for the action of most of these antibacterial peptides is self-aggregation upon reaching a threshold concentration [9]. Here, in this study we have collected plant AMPs with antibacterial activity from the Antimicrobial Peptide Database (APD3) [19] and predicted amino acid regions within these antibacterial peptides which could help in their self-aggregation and oligomerization. To find these amino acid regions we have utilized four popular web servers which predict the amyloidogenic regions within a given peptide sequence. Finally, we also tried to suggest an oligomeric form of a plant defensin called NaD1 which is present in tobacco [20].

2. Materials and methods

2.1. Construction of the dataset

We have created a dataset containing 110 antimicrobial peptides from plants. To construct this dataset, we have used the Antimicrobial Peptide database (APD3) [19] where search terms “plants” in combination with “gram positive/negative bacteria” were used to extract antimicrobial peptides. Further, we used an in-house python script to store the data into a tabular and more manageable form with APD ids, sequences and additional information of the corresponding peptides.

2.2. Prediction of amyloidogenic regions

In this study we have used four web servers, namely FoldAmyloid [21], PASTA 2.0 [22], WALTZ [23] and AGGRESCAN [24], to predict the amyloidogenic regions of the antibacterial peptides extracted as mentioned earlier. We further analysed the number of regions predicted by each of these four methods.

2.3. Antibacterial peptide length distribution and amino acid composition

We have calculated the amino acid composition and the distribution of peptide lengths from our dataset using an in-house python script. The plots were created using the R statistical software package [25].

2.4. Multiple sequence alignment

We have isolated three groups of antibacterial peptides from our dataset belonging to the plant ‘defensin’, ‘thionin’ and ‘cyclotide’ families. Sequences from each group were then used to obtain multiple sequence alignment using Clustal Omega [26] and weblogos were generated using WebLogo 3 tool [27, 28].

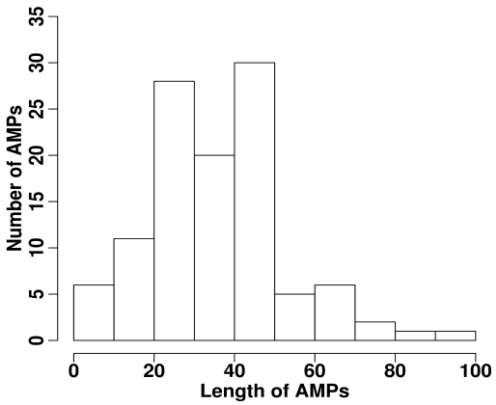

Fig. 1. Length distribution of plant antibacterial peptides.

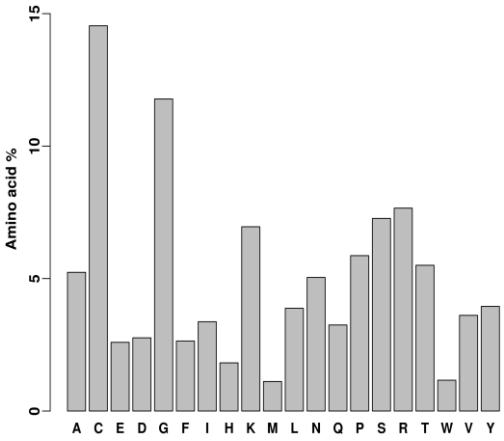

Fig. 2. Amino acid compositions of plant antibacterial peptides.

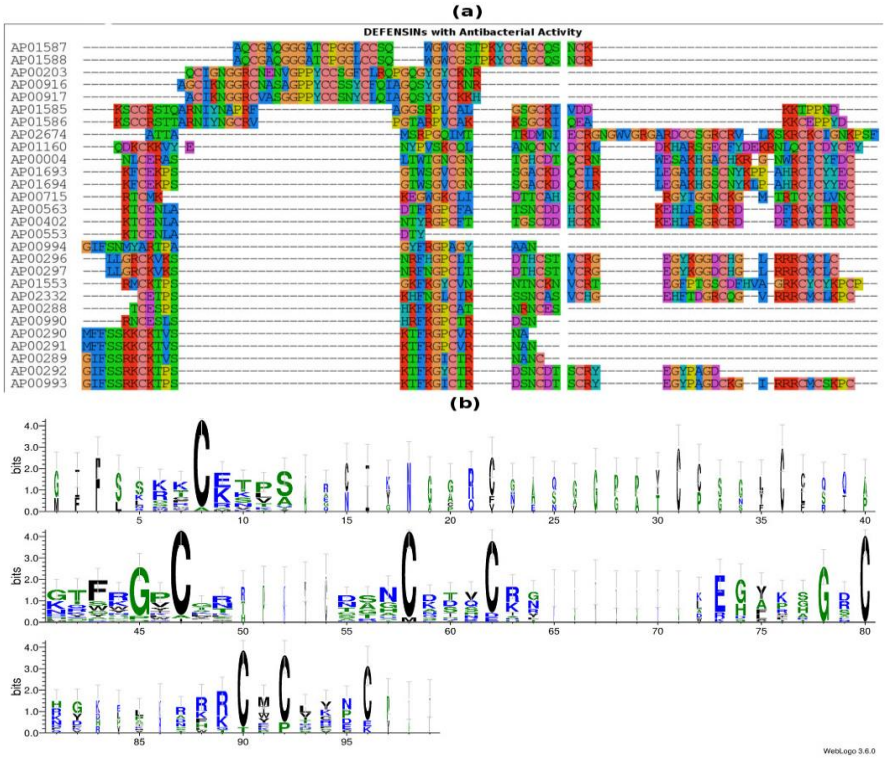

Fig. 3. Multiple sequence alignment of antibacterial defensins and their corresponding (**a**).Weblogo (**b**).

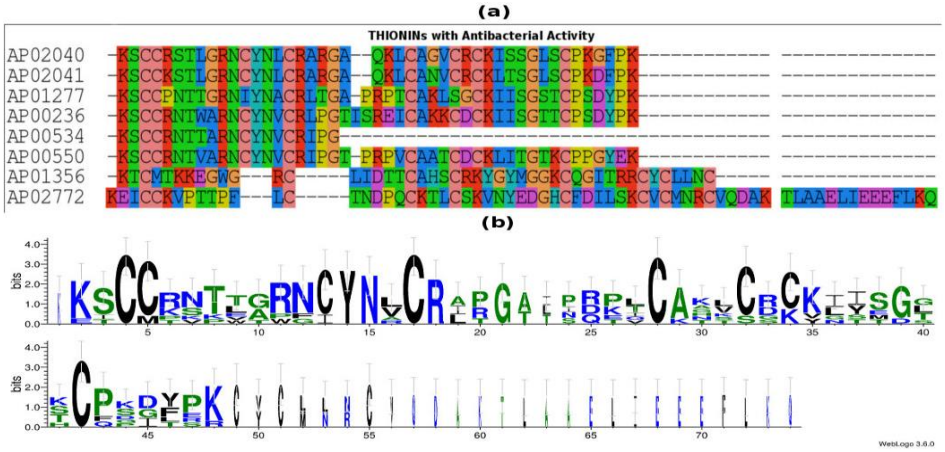

Fig. 4. Multiple sequence alignment of antibacterial thionins and their corresponding (**a**). Weblogo (**b**).

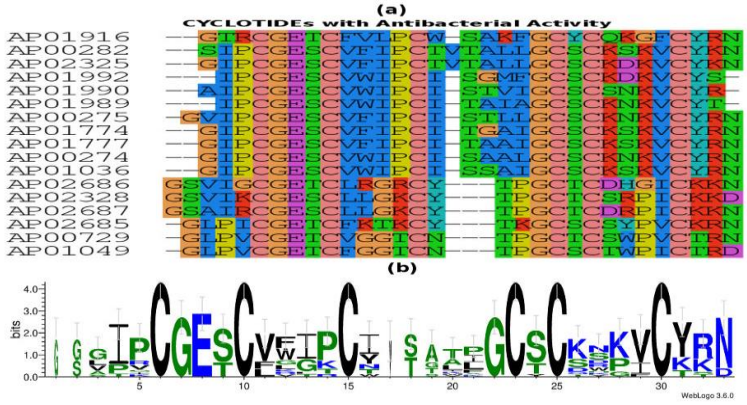

Fig. 5. Multiple sequence alignment of antibacterial cyclotides and their corresponding (**a**). Weblogo (**b**).

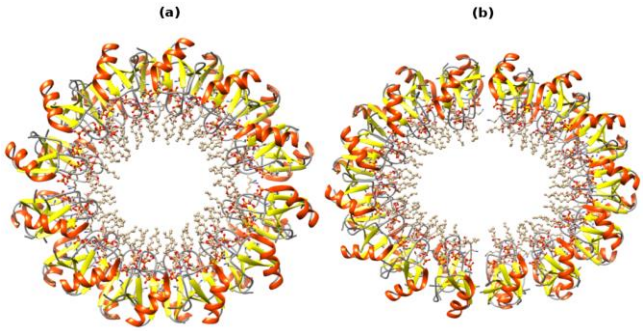

Fig. 6. Proposed oligomeric forms of plant defensin NaD1 containing (**a**) 24 NaD1-PIP_2 units and (**b**) 28 NaD1-PIP_2 units. Diameter of the inner hole is about 30 Å, and the outer one is about 100 Å.

2.5. Construction of oligomer structure

Structure 4CQK (14-mers) [20] from the Protein Data Bank was taken as template to construct a ring-like oligomer structure for plant defensin NaD1. Packing of oligomers was done by the YASARA program [29].

3. Results and discussions

3.1. Predicted amyloidogenic regions

We have analysed the number of peptides with different number of predicted amyloidogenic regions and found that this greatly varies with the choice of web servers (Table 1). We noticed that FoldAmyloid and AGGRESCAN reported the maximum number of peptides with at least one amyloidogenic regions. In our dataset there are only 16 antibacterial peptides for which all of the algorithms used in this study predicted absence of any amyloidogenic region.

3.2. Length distribution of plant antibacterial peptides and amino acid composition

In our dataset the length of the plant antibacterial peptides varies from 7–92 amino acid residues with majority of the peptides having a length of 20–50 amino acid residues (Fig. 1). When we analysed the amino acid composition (Fig. 2) of all the peptides in our dataset, we found that cysteine was the most frequently occurring amino acid, which was expected because of the prevalence of highly conserved cysteine disulfide bridges. We also noticed that glycine was the second most frequent amino acid residue followed by the positively charged arginine and lysine and polar serine residues.

3.3. Multiple sequence alignment

Multiple sequence alignments and weblogo (Figures 3–5) analysis have clearly showed very distinct features among the three groups we studied, namely defensins, thionins and cyclotides. In general, cysteine residues were highly conserved in all the three families. In the case of defensin, we observed the presence of 5 short and disjoint motifs (Fig. 3,b) whereas no such motifs were present in the thionin (Fig. 4,b) and the cyclotide (Fig. 5,b) families. We also observed that defensin and thionin families have more conserved positively charged residues such as lysine and arginine compared to cyclotides.

3.4. Oligomerization of AMPs

For the function of some AMPs, formation of oligomeric structure is necessary. Different authors have suggested several mechanisms for permeabilization of membranes [9, 30]. In recent years some of the oligomeric structures of plant AMPs bound to their membrane target have been reported and their structures were deposited in the Protein Data Bank [20, 31–33]. We found that most of the peptides in our dataset were predicted to have at least one amyloidogenic region which strengthens our belief that these regions could help the AMPs to form oligomers. From protein data bank we extracted a 14-mer crystal structure of plant defensin NaD1 [20] and proposed a ring-like oligomer structure (Figure 6) containing either 24 or 28 NaD1 monomers bound to phosphatidylinositol 4.5-bisphosphate (PIP_2). We proposed that this ring-like oligomeric unit could be the building block of the observed NaD1 fibrils [20]. It should be mentioned that $PIP_2$is important for oligomerization, and the diameter of the fibrils is about100 Å [20], which coincides (or match) with the outer diameter of the oligomers (Fig. 6).

4. Conclusion

Based on the idea that self-aggregation of peptides might be a common theme for the mode of action of plant antibacterial peptides [9], we used four [21–24] popular prediction tools for finding amyloidogenic regions in our antibacterial dataset. We found that only 16 peptides, out of the 110 peptides in our dataset, were predicted to not have any amyloidogenic region. Three families, namely defensins, thionins and cyclotides have highly conserved cysteine residues. We have proposed a possible ring-like oligomeric structure for plant defensin NaD1 which might explain the formation of NaD1 fibrils [20].

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