

Antimicrobial Peptide Protegrins Interact with DPC Micelles by Apolar Hydrophobic Cluster: Structural Studies by High-Resolution NMR Spectroscopy

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Abstract For the disulfide-bonded β -sheet-forming peptides which include protegrins, the presence of positively charged amino acid residues allows their strong interaction with the lipid matrix of the plasma membrane, as opposed to a protein target on the surface of the cell. We used high-resolution NMR spectroscopy for 3D structure determination of several protegrins in a solution with perdeuterated dodecylphosphocholine (DPC) micelles which is a commonly used zwitterionic detergent for the solubilization of membrane peptides and proteins. Structural studies by NMR spectroscopy of protegrins PG-1 (PDB ID: 1PG1), PG-2 (PDB ID: 2MUH), and PG-3 (PDB ID: 2MZ6) shown that the sidechains of Leu5, Phe12, Val14, and Val16 form a relatively well-ordered apolar cluster. Due to the fact that a membrane surface with positive curvature allows the hydrophobic cluster to be buried while the charged residues remain solvated in water, here we can conclude that this area could bind to the bacterial cell walls via hydrophobic and positively charged amphipathic surfaces.

Keywords Antimicrobial peptides · Protegrin · Antibiotic · NMR

1 Introduction

Antimicrobial peptides (AMPs) are small peptides with a strong antibiotic activity which play an important role in the immune system of many different animals. Naturally

occurring AMPs probably represent one of the very first evolved forms of chemical defense of living eukaryotic cells against invasion by bacteria, protozoa, fungi, and virus. AMPs are less susceptible to the development of bacterial resistance because they disrupt the membrane of bacteria through non-specific peptide–lipid interactions. Natural AMPs have such properties as the broad-spectrum antibacterial activity, high selectivity, and the disruption of bacterial cell membranes, which allow to suggest that these molecules are potentially useful as antibiotics; so, it is important to investigate their structure and function at high resolution in order to increase their potency and selectivity. Protegrins are one of the best characterized β -sheet cytolytic peptides isolated from porcine leukocytes that contain 16–18 residues whose structure has been shown to be a β -hairpin stabilized by two disulfide bonds [1–4]. There are five known protegrins: PG-1 (RGGRL⁵CYCRR¹⁰RFCVC¹⁵VGR¹⁸), PG-2 (RGGRL⁵CYCRR¹⁰RFCIC¹⁵V¹⁶), PG-3 (RGGGL⁵CYCRR¹⁰RFCVC¹⁵VGR¹⁸), PG-4 (RGGRL⁵CYCRG¹⁰WICFC¹⁵VGR¹⁸), and PG-5 (RGGRL⁵CYCRP¹⁰RFCVC¹⁵VGR¹⁸) with a high content of cysteine (Cys) and several positively charged arginine (Arg) residues. For the disulfide-bonded β -sheet-forming peptides, which include protegrins, the presence of positively charged amino acid residues allows their strong interaction with the lipid matrix of the plasma membrane, as opposed to a protein target on the surface of the cell.

2 Material and Methods

The protegrin peptides were synthesized by solid-phase peptide synthesis by Dr. Andrey Filippov in the Chemistry of Interfaces Laboratory at the Luleå University of Technology.

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The NMR investigation was done on a Bruker Avance 700 spectrometer equipped with a cryoprobe. The peptide (4 mg) was solubilized in an aqueous solution (H_2O or $^2\text{H}_2\text{O}$, 500 μl) containing 20 mg perdeuterated dodecylphosphocholine (DPC) (molar ratio $\sim 1:12$). 3-(Trimethylsilyl)-propionic-2,2,3,3- $^2\text{H}_4$ acid (TMSP-2,2,3,3- $^2\text{H}_4$) (98 % atom ^2H , Aldrich) was added as an internal chemical shift standard for ^1H NMR spectroscopy. Perdeuterated d_{38} DPC (98 % ^2H) and TSP- d_4 were purchased from Aldrich. Chemical shift assignments of PG-2 and PG-3 in DPC micelles were obtained using standard methods of protein NMR spectroscopy by 2D NMR ^1H - ^1H TOCSY and NOESY (Nuclear Overhauser Effect Spectroscopy) experiments. Interproton NOE distance constraints determined from NOESY experiments were used for the structural calculations by molecular dynamic method calculations of the Xplor-NIH program [5].

3 Results and Discussion

Protegrins are antibacterial peptides of 16–18 residues whose structure has been shown to be a β -hairpin stabilized by two disulfide bonds [3]. The most studied of these is the protegrin-1 (PG-1) which is composed of 18-amino acid residues (PDB IDs: 1PG1 and 1ZY6) and supposed that PG-1 forms ion channels in membranes and kills bacteria involved in oligomeric peptide toroidal pores in anionic lipid bilayer membranes, which mimic the inner membrane of Gram-negative bacteria. However, even for PG-1, there are a lot of experimental and theoretical studies; for other protegrins (PG-2, PG-3, PG-4, PG-5), structural studies are almost have not been investigated and biological functions among the different protegrins are also still limited.

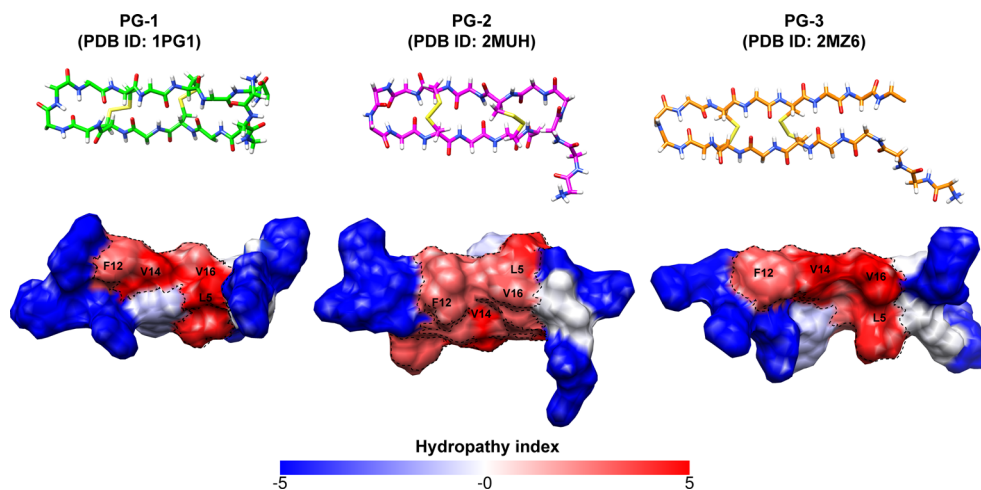
We used high-resolution NMR spectroscopy for 3D structure determination of protegrins PG-2 and PG-3 in a solution with perdeuterated dodecylphosphocholine (DPC) micelles which is a commonly used zwitterionic detergent for the solubilization of

membrane peptides and proteins, and found a tendency for dimerization of PG-3 the same as for PG-1 [6–8]. Protegrins PG-1, PG-2, and PG-3 form a well-defined structure composed of a two-stranded antiparallel β -sheet in the presence of DPC micelles [3, 4, 7, 8]. In present paper, we focused on the protegrin peptide–micelle binding via hydrophobic and positively charged amphipathic surfaces. Structural studies by NMR spectroscopy of protegrins PG-1 (PDB ID: 1PG1), PG-2 (PDB ID: 2MUH), and PG-3 (PDB ID: 2MZ6) shown that the sidechains of Leu5, Phe12, Val14, and Val16 form a relatively well-ordered apolar cluster (Fig. 1). Due to the fact that a membrane surface with positive curvature allows the hydrophobic cluster to be buried while the charged residues remain solvated in water, here we can conclude that this area could bind to the bacterial cell walls via hydrophobic and positively charged amphipathic surfaces. These observations also support the hypothesis of the requirement of hydrophobic/hydrophilic cluster proximity to induce a cytolytic activity of protegrins [1]. Here, we should mentioned that even though solution NMR experiments on DPC micelles provide high-resolution structures, the structural results obtained from a detergent micelle need to be substantiated by studies in lipid bilayers, which do not have a curved surface and are a better approximation to cell membranes. We plan to investigate these problems in our further researches of AMPs.

4 Conclusions

Structural studies by NMR spectroscopy of protegrins PG-1 (PDB ID: 1PG1), PG-2 (PDB ID: 2MUH), and PG-3 (PDB ID: 2MZ6) shown that the sidechains of Leu5, Phe12, Val14, and Val16 form a relatively well-ordered apolar cluster. Due to the fact that a membrane surface with positive curvature allows the hydrophobic cluster to be buried while the charged residues remain solvated in water, here we can conclude that this area could bind to the bacterial cell walls via hydrophobic and positively charged amphipathic surfaces.

Fig. 1 Kyte–Doolittle hydrophobicity surface structural model for PG-1 (PDB ID: 1PG1), PG-2 (PDB ID: 2MUH), and PG-3 (PDB ID: 2MZ6): from blue for the most hydrophilic to white and to red for the most hydrophobic (50). An apolar cluster (Leu5, Phe12, Val14, and Val16) is marked by a dotted line. The structures were drawn using chimera (<https://www.cgl.ucsf.edu/chimera/>)



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