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Antimicrobial activity and membrane selective interactions of a synthetic lipopeptide MSI-843

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Abstract

Lipopeptide MSI-843 consisting of the nonstandard amino acid ornithine (Oct–OOLLOOLOOL–NH_2) was designed with an objective towards generating non-lytic short antimicrobial peptides, which can have significant pharmaceutical applications. Octanoic acid was coupled to the N-terminus of the peptide to increase the overall hydrophobicity of the peptide. MSI-843 shows activity against bacteria and fungi at micromolar concentrations. It permeabilizes the outer membrane of Gram-negative bacterium and a model membrane mimicking bacterial inner membrane. Circular dichroism investigations demonstrate that the peptide adopts α-helical conformation upon binding to lipid membranes. Isothermal titration calorimetry studies suggest that the peptide binding to membranes results in exothermic heat of reaction, which arises from helix formation and membrane insertion of the peptide. ^{2}H NMR of deuterated-POPC multilamellar vesicles shows the peptide-induced disorder in the hydrophobic core of bilayers. ^{31}P NMR data indicate changes in the lipid head group orientation of POPC, POPG and *Escherichia coli* total lipid bilayers upon peptide binding. Results from ^{31}P NMR and dye leakage experiments suggest that the peptide selectively interacts with anionic bilayers at low concentrations (up to 5 mol%). Differential scanning calorimetry experiments on DiPOPE bilayers and ^{31}P NMR data from *E. coli* total lipid multilamellar vesicles indicate that MSI-843 increases the fluid lamellar to inverted hexagonal phase transition temperature of bilayers by inducing positive curvature strain. Combination of all these data suggests the formation of a lipid–peptide complex resulting in a transient pore as a plausible mechanism for the membrane permeabilization and antimicrobial activity of the lipopeptide MSI-843.

Keywords: Lipopeptide; Antimicrobial activity; Membrane permeabilization; Solid-state NMR; Oriented bilayers; Curvature strain

1. Introduction

Antimicrobial peptides are produced as a part of the host defense mechanisms by various organisms that include microbes, insects, plants, vertebrates and mammals [1]. Many of the antimicrobial peptides exert their activity by interacting non-specifically with target biological membranes [2–5]. These peptides are characterized as having hydrophobic, cationic and amphiphathic features even though the exact attributes are not clearly known. Upon binding to the membrane, they assume a parallel or perpendicular or an oblique angular orientation with respect to the membrane normal axis [6–8]. These peptides adopt an

Abbreviations: CSA, chemical shift anisotropy; CD, circular dichroism; DiPoPE, 1,2-dipalmitoleoyl-*sn*-glycero-3-phosphotydylethanolamine; DSC, differential scanning calorimetry; H_I, normal hexagonal phase; H_{II}, inverted hexagonal phase; ITC, isothermal titration calorimetry; L_α, fluid lamellar phase; MIC, minimum inhibitory concentration; MLVs, multilamellar vesicles; NMR, nuclear magnetic resonance; O, ornithine; Oct, octanoyl; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine; POPC-d_{31}, 1-d_{31}-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylglycerol; POPE, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylethanolamine; SUVs, small unilamellar vesicles

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amphiphathic structure in the membrane embedded state in such a way that the hydrophobic side chains are inserted deep into the membrane while the charged and polar residues on the hydrophilic portion interact with the head groups of phospholipids in the membrane.

Lipopeptides are unique among the antimicrobial peptides in that they are relatively smaller in size as compared to other cationic antimicrobial peptides, and yet, exhibit excellent antimicrobial activity. Fatty acid acylation of antimicrobial peptides of bacterial and fungal origin have mostly been limited to non-gene-encoded peptides such as echinocandin [9], polymyxins [10], daptomycin [11] lipopeptaibols [12,13] and syrinomycin, syringotoxin and syringopeptin from *Pseudomonas syringae* [14]. Enzymatic fatty acid acylation is one of the post-translational modifications of peptides involved in functional [15,16] and structural roles [17]. It has been postulated that fatty acylation of proteins are necessary to increase their membrane association and sorting into specific sub-cellular localizations [16,18]. Consequently, several studies to assess the effect of acylation have revealed that conjugation of fatty acid to cationic peptides enhances their antimicrobial activity [19–23]. However, it is not clear if fatty acylation influences the association of hydrophilic portions of peptides with membranes or modulates the orientation of peptides in membranes. Therefore, it would be relevant to study the interactions of antimicrobial lipopeptides in neutral and anionic model membranes as it would help in understanding the biophysical properties governing the association of fatty acylated peptides with biological membranes.

Lactoferricin H, the proteolytic product of human lactoferrin, consists of an amphiphathic α-helical region (residues 21–31) of lactoferrin and has been reported to show enhanced antibacterial activity compared to the intact lactoferrin [24]. Lipophilic modification at the C-terminus of a peptide based on residues 21–31 of human lactoferrin has resulted in enhanced antimicrobial activity against Gram-negative and Gram-positive bacteria [25]. The alkyl chain (6-octanoyl/heptanoyl diaminobutyl group) in the cationic cyclic antimicrobial peptide polymixin B has been shown to be essential for its antimicrobial activity [26]. Removal of 6-octanoyl/heptanoyl diaminobutyl moeity from polymyxin B resulted in the loss of activity [27]. Aliphatic acids can, therefore, be used to increase the hydrophobicity and membrane association of short cationic peptides and consequently improve the antimicrobial activity.

For the present study, a synthetic lipopeptide MSI-843 (Oct–OOLLOOLOOL–NH_2), with a short helical stretch and an octyl chain at the N-terminus, was designed and interactions with lipid bilayers and biological activities were determined to understand the molecular mechanism of membrane permeabilization. CD and ITC experiments were used to determine the energy involved in the secondary structure formation and membrane insertion. ^{31}P NMR experiments were used to determine the peptide-induced changes in the head group conformation of lipids. ^{2}H NMR experiments provided a measure of the peptide-induced disorder in the hydrophobic core of bilayers. DSC and ^{31}P NMR provided information on peptide-induced curvature strain in the membrane. Our results also show that the lipopeptide did not cause hemolysis at concentrations required for antimicrobial activity. Our data from this study suggest that the peptide–lipid complex induces local defects in the membrane that form the basis for the observed antimicrobial activity of the lipopeptide.

2. Materials and methods

2.1. Materials

POPG, POPC, POPC-d_{31}, POPE, DiPoPE and *Escherichia coli* total lipid extract were purchased from Avanti Polar Lipids (Alabaster, AL). Chloroform and methanol were procured from Aldrich Chemical Inc. (Milwaukee, WI), and naphthalene was from Fisher Scientific (Pittsburgh, PA). All the chemicals were used without further purification. The peptide was synthesized by Genaera Corporation (Plymouth Meeting, PA).

2.2. Outer membrane permeabilization assay

The outer membrane permeabilizing ability was investigated using the 8-anilinonapthalene-1-sulfonic acid (ANS) uptake assay [28], using *E. coli* strain L21 (DE3). Bacterial cells from an overnight culture were inoculated into LB medium. Cells from the mid-log phase were centrifuged and washed with buffer (10 mM Tris, 150 mM NaCl, pH 7.4), and then resuspended in the same buffer to an OD_{600} of 0.065. To 3.0 mL of the cell suspension in a cuvette, a stock solution of ANS was added to a final concentration of 5.0 μM. The degree of membrane permeabilization as a function of peptide concentration was observed by the increase in fluorescence intensity at ~ 500 nm.

2.3. High-sensitivity titration calorimetry

The heat of peptide-into-lipid mixing reaction was measured using a high sensitivity titration calorimeter as described elsewhere [29] (Calorimetry Sciences Corporation, Model CSC-4200, Utah, USA). Peptide and lipid solutions were degassed under vacuum prior to use. The calorimeter was calibrated as recommended by the manufacturer. The heats of dilution for successive 10 μL injections of the peptide solution into buffer were insignificant compared to the heats of peptide–lipid reaction. The heat of peptide–lipid binding was determined by integrating the area under each titration curve using the built-in Bindworks software.