Investigations of molecular-level interactions between Piscidins and bacterial lipid membranes

Thulasi Kulasinghe[1] and Ella Mihailescu[2]

[1] *University of Maryland, Shady Grove*

[2] *Institute for Bioscience and Biotechnology Research*

Abstract

In this study, piscidin 1 (p1) and piscidin 3 (p3) are tested with 3:1 phosphatidylethanolamine/phosphatidylglycerol (3:1 POPE/POPG) bilayers to identify molecular features important for membrane destabilization in bacterial cell membranes. Lipid membranes are destructed by P1 and P3 and results were observed through changes in phase transition temperatures in differential scanning calorimetry and changes in X-ray diffraction patterns from lipid bilayers with Piscidins.

Introduction

Piscidins 1 and 3 (P1 & P3) are amphipathic, cationic, antimicrobial peptides with predominantly α-helical secondary structure in the presence of lipids. They are isolated from Hybrid striped bass (*Morone chrysops x Morone saxatilis* and have potent antimicrobial activities against both gram-positive and -negative bacteria.(1-5)

P1 FFHHIFRGIVHVGKTIHRLVTG

P3 FIHHIIFRGIVHVAGRSIGRFLTG

Structures of Piscidins 1 and Piscidins 3

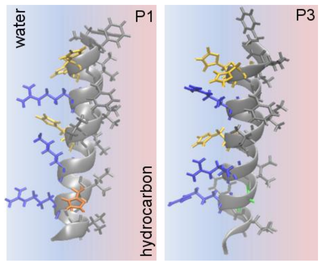


Figure 2. Structural representation of Piscidins 1 and 3. They are unstructured in solution but acquire α-helical secondary structures in a membrane environment.

Figure 1. Hybrid striped bass (*Morone chrysops x Morone saxatilis*

Structures of Phosphatidylethanolamine (POPE) and Phosphatidylglycerol (POPG)

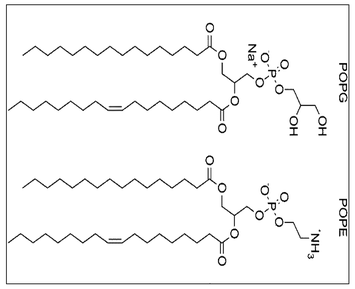


Figure 3. Structural representation of POPE and POPG lipids, the major components of bacterial membranes . They have a polar, hydrophilic headgroup attached to hydrophobic, hydrocarbon chains. The hydrophilic/hydrophobic interactions make them organize into bilayers when on contact with water. POPE has a overall neutral headgroup, while POPG has negative head group which can interact tightly with positively charged P1 and P3.

Materials and Methods

Differential Scanning Calorimetry

X-ray Diffraction

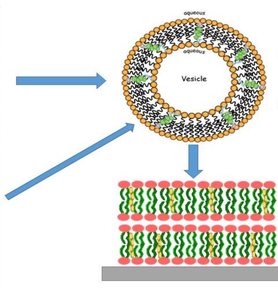


Figure 4. Globular vesicles with peptides in buffer solutions

Figure 5. Films with peptides deposited on glass covers.

Differential Scanning Calorimetry (DSC) heating curve of POPE

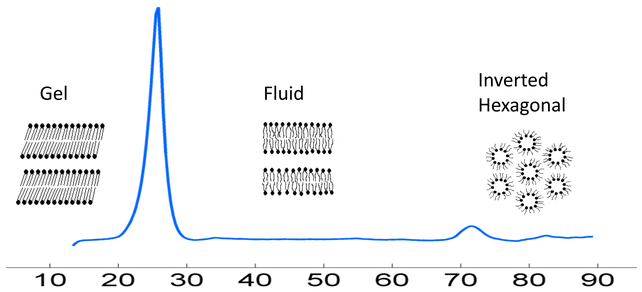
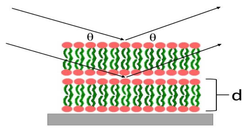


Figure 6. POPE presents two main transitions, a lamellar gel-to-fluid phase transition at 25C, and a lamellar fluid to inverted hexagonal around 75C. By comparison, POPG is in a lamellar fluid state in this temperature range. It has a gel-to-fluid phase transition temperature at -2C, and cannot be accessed with DSC.

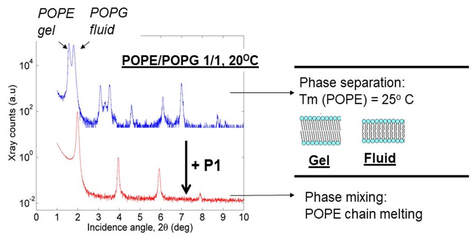
X-ray Diffraction and Braggs Law



$$n * \lambda = 2 * d * sin\theta$$

λ = 1.53 Å

The Effect of P1 on 1:1 POPE/POPE Bilayer



Results -DSC

Heating and Cooling Curves from Differential Calorimetry

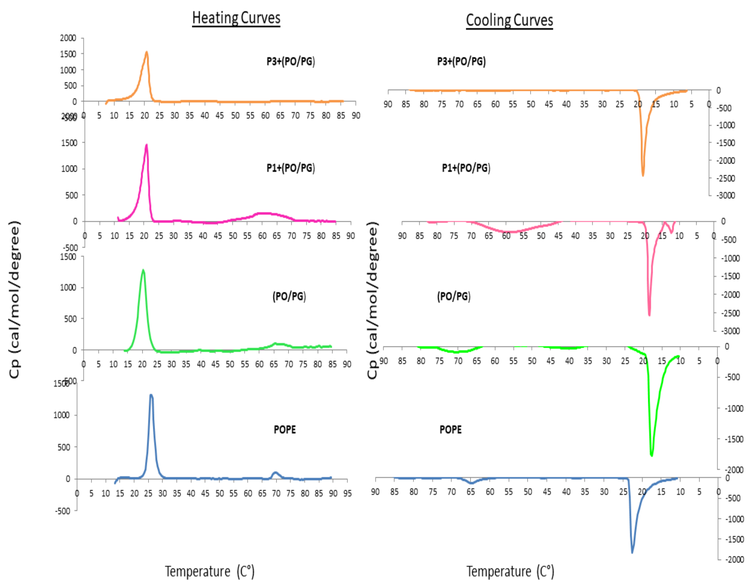


Figure 7. Observing the phase transitions in lipid bilayers with or without P1 and P3.

Results -XRD

Comparison of X-Ray diffraction patterns of 3:1 POPE:POPG with or without P1 Antimicrobial

Without P1: d=49.7 Angstrom (35C°) **With P1**: d=46.8 Angstrom (35C°)

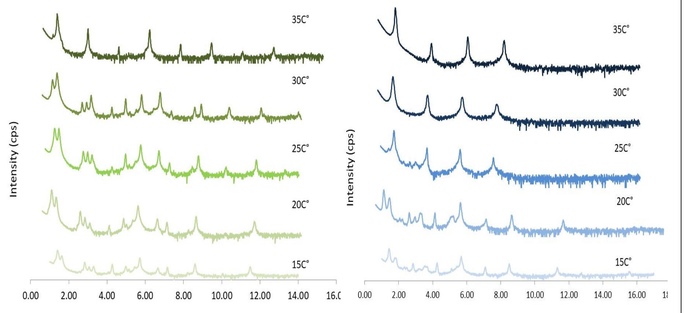


Figure 8: This is a diagram comparing the X-ray diffraction patterns of P1 antimicrobial peptide on 3:1 POPE/POPG In different temperatures. At temperatures 15C° and 20C°, there are more peaks and they combine to become one big peak at 25C°, 30C° and 35C°

Comparison of X-Ray diffraction patterns of 3:1 POPE:POPG with or without P3 Antimicrobial

Without P3: d=49.7 Angstrom (35C°) **With P3**: d=47.4 Angstrom (35C°)

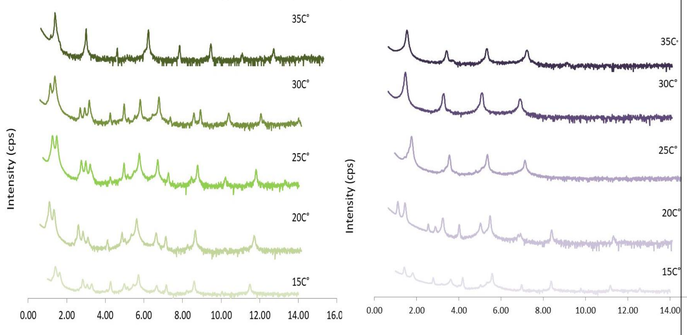


Figure 9. This is a diagram comparing the X-ray diffraction patterns of P3 antimicrobial peptide on 3:1 POPE/POPG In different temperatures. At temperatures 15C° and 20C°, there are more peaks and they are combining to becoming one big peak at 25C°, 30C° and 35C°. Adding P1 shows thinning the membrane as repeat spacing has been decreased.

Conclusions

- Both P1 and P3, bind to lipid membranes that mimic the bacterial membrane compositions (POPE/POPG) and induce structural changes

- DSC results show that P1 interacts more strongly with the lipid headgroup leading to a transition to a non-lamellar structure (i.e inverted hexagonal)

- XRD results also show that P1, the more active form of Piscidins, causes a more pronounced thinning of the membrane than P3, as an initial step in membrane destabilization and bacterial killing.

- Limitations : The lamellar to hexagonal phase transition is difficult to observe, especially in lipids such as POPE, where this occurs close to the boiling point of water.

- Future Ideas: Use a different lipid as an alternative to observe the phase transition of hexagonal shape. (Di-POPE lipid – 41C°transition to hexagonal)

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