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

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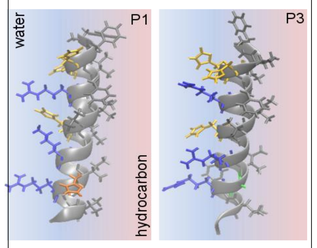
Abstract

Metal-binding Antimicrobial peptides derived from the innate immune systems of various organisms are a promising avenue for designing new and potent antibiotics. In this study, two homologs (P1 and P3) from the Piscidin family of antimicrobial peptides from fish, have been studied. Ni+2 and Cu+2 bound piscidin 1 (p1) and piscidin 3 (p3) are tested at ratios of 1:25 and 1:50 with 3:1 phosphatidylcholine/phosphatidylglycerol (3:1 POPC/POPG) bilayers to identify the effect of metals on membrane destabilization in bacterial cell membranes. Lipid membranes are destructed by Ni+2 and Cu+2 bound P1 and P3 and results were observed through changes in X-ray diffraction patterns from lipid bilayers with Piscidins and changes in secondary structures with Circular Dichroism.

Structures

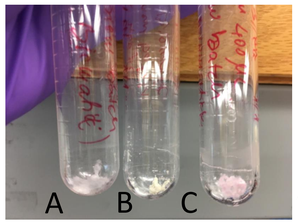


P1 FFHHIFRGIVHVGKTIHRLVTG
P3 FIHHIFRGIVHAGRSIGRFLTG

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). The peptides do not have a structure in solution, but become helical in interaction with lipid membranes (1). It was recently established that Cu+2 and Ni+2 coordinated piscidin 1 and 3 enhances their antimicrobial activity against membranes and DNA. However, their effect on the membrane integrity and the role of metal ions is not fully understood. Here, we are investigating the effect of metal (Cu2+ and Ni2+) to the interaction with lipid bilayers.

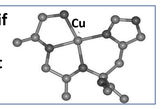
Materials and Samples



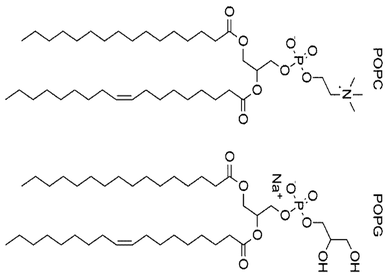
Peptides:
When metals (Cu2+ and Ni2+) are bound to Piscidins 1 and 3 there is a change in color that can be observed. The metals bind to the (amino-terminal Cu/Ni (ATCUN) motif in the N-terminus and modify the electronic structure.

A: P3 with 3:1 POPC/POPG
B: P3-Ni with 3:1 POPC/POPG
C: P3-Cu with 3:1 POPC/POPG

ATCUN motif
XXH
(Histidine at position 3)

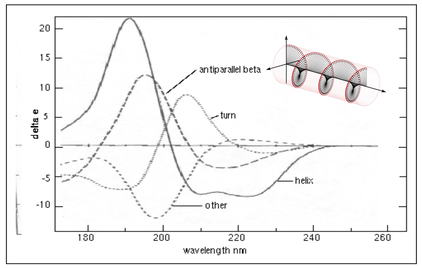


Structures of Phosphatidylethanolamine (POPE) and Phosphatidylglycerol (POPG)



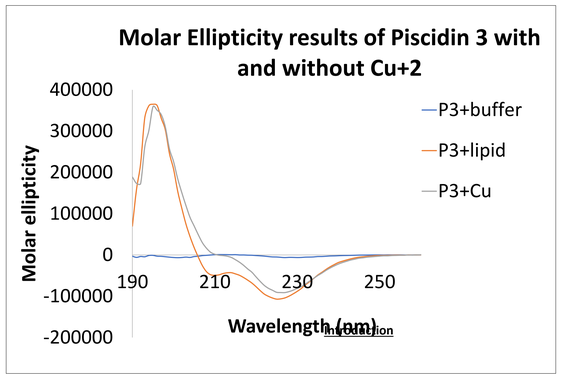
Lipids:
Structural representation of POPC and POPG lipids, major components of cellular membranes. The phospholipids have a polar headgroup and two hydrocarbon tails. POPC has a net neutral character at the head group, while POPG has negative head group which can interact tightly with positively charged P1 and P3.

Results – Circular Dichroism



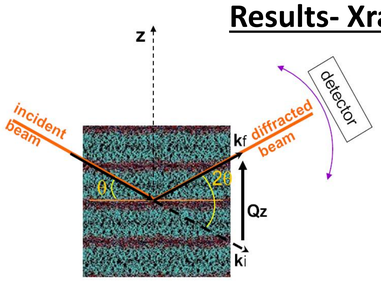
Circular dichroism is dichroism involving circularly polarized light. It is mostly used to:

- Protein secondary structure determination
- Heat, pH, or solvent induced structural change identifications
- Protein folding/unfolding information
- Ligand or ion induced structural changes



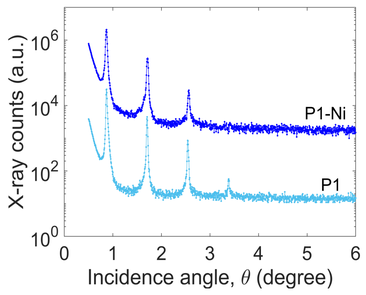
CD spectrum of Piscidin 3 with and without Cu+2. When peptide is added to phosphate buffer, there is almost no signal present in the absence of the lipid vesicles, indicating that the peptide has no secondary structure in solution (random coil), but it acquires secondary structure (mostly helical) in the presence of lipid. The helical content was determined to be (91% without Cu, 75% with Cu) in the range 190-200 nm. The peptide binds a metal ion at the N-terminus, and looses some of the helical structure.

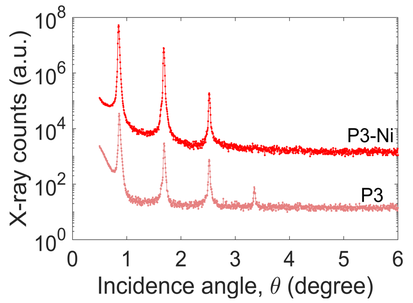
Results- Xray diffraction



Sample :
Lipid films with peptides or with metal ions (controls) are deposited on glass cover slips and from oriented multilayers

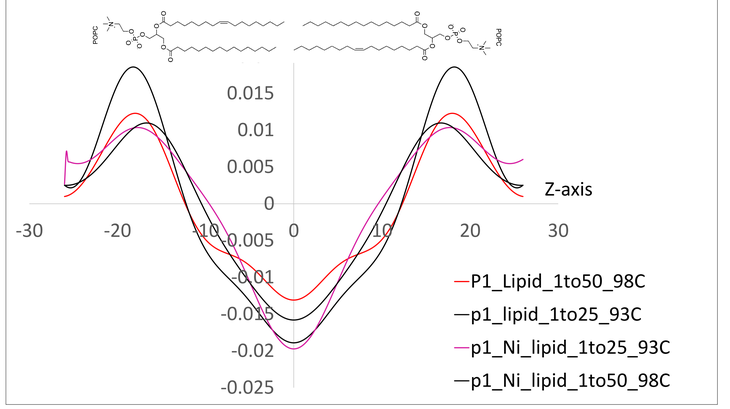
The Effect Copper ion in bilayers
POPC/POPG/Aldo-PC



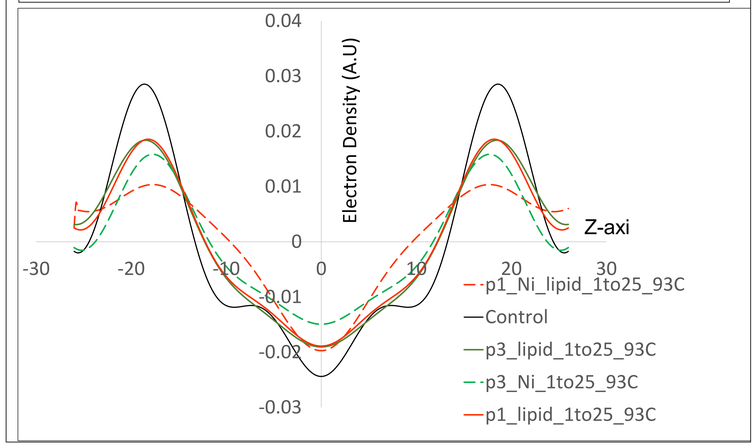


The diffraction shows that the peptides change the structure of the bilayer. The samples were meaured at 98% relative humidity and 25 degrees Celsius.

Results – Electron density profiles



One-dimensional Electron Density profile, projected along the lipid axis, for P1 antimicrobial peptide with 1to25 and 1to50 concentrations of Ni. The high density peaks correspond to the location of the phosphates of the lipid polar headgroups (phosphates), while the low density region in the center of the profile reflects the hydrocarbon chains. Perturbations to the intact bilayer structure due to the presence of P1 peptide can be seen as broadening of the phosphate group regions, and significant thinning of the hydrocarbon region, compared to bilayers without the peptide.



Conclusions

- CD results demonstrate that P1/P3 are unstructured in solution but become alpha-helical in the presence of lipid bilayers. They become less helical in the presence of Cu2+.
- XRD results indicate that while both peptides induce severe bilayer perturbations, P1 is much more efficient. This correlates with the much higher antimicrobial and anticancer potency pf P1 compared to P3 (as found in other research (1)).
- P3-Cu is causes more changes to the bilayer structure than P3.
- Future Ideas: It is not clear what factors (at the molecular level) contribute the most to the bilayer disruption ability for Piscidin-metal complex, compared to non-metallated forms. Hypothesis: Copper-dependent lipid oxidation may play a role. We must investigate this possibility using lipid oxidation analysis for lipid/Piscidin samples in the presence of metals, using other methods (e.g. oxidation assays).

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References (1) Perrin, B.S., …, Cotten , M. *J. Am. Chem. Soc.* 2014,136:3491-3504, (2)