Investigating the Effects of a Charged Residue on Alamethicin's Pore Formation Ability

Emma K. Cheung, Class 2026

Antimicrobial peptides (AMPs) are a promising alternative for current antibiotics in the midst of the rise of antibiotic resistance since they disrupt bacterial membranes through pore formation, a mechanism that targets non-genetic components of cells, and hence less susceptible to resistance development. Alamethicin (ALM) is an AMP that molecular dynamics (MD) simulations predict form different pore types due to its altered charge distribution, and studying ALM pore formation lays the groundwork for future studies on the ALM mutant. The purpose of this project was to investigate the effect of substituting the seventh glutamine (Q) residue of ALM with a charged lysine (K) residue, improving our understanding of how ALM interacts with membranes, which can address the growing issue of antibiotic resistance.

We hypothesize that the distribution of charged residues along an AMP determines the pore mechanism whether they form a barrel-stave versus a toroidal pore. By comparing wild-type ALM versus the ALM-Q7K mutant with polarized attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy, we can determine the orientation of lipids and peptides within membrane bilayers and how changes in their orientation depend on the specific mutation in ALM. I will use methodologies from a previous student researcher to synthesize small unilamellar vesicles (SUVs) and fuse them on the ATR crystal, forming a supported lipid bilayer. By analyzing the differential absorption of polarized light in these bonds (Dichroic ratio) from lipid and peptide bond vibrational intensity, I can determine the tilt angle of both the peptides and lipids within the membrane and therefore how they are oriented in the membrane, helping distinguish between barrel-stave pores, where peptides insert perpendicularly, and toroidal pores, where peptides adopt a more tilted orientation.

I initially encountered issues where the amide I peaks, peaks in the IR spectra between 1700-1600 cm^{-1} that can help us determine the angle at which ALM is inserted into the membrane at, were obscured in my spectra and had to troubleshoot the protocol, trying to determine the problem when I take the peptide spectra. After testing different background spectra, the issue was resolved by collecting a background spectra with DMSO to subtract the DMSO vibrations from the peptide spectra, and I was able to continue with experiments. I tested ALM concentrations of 5.0 μM and 10.0 μM on bilayers of pure POPC to verify the previous honors student's data and increase the number of trials. In pure POPC bilayers, ALM concentrations of 5.0 μM corresponded to an average tilt angle of 64.1˚ (n=3), whereas higher concentrations of 10.0 μM resulted in an average tilt angle of 50.4˚ (n=3), indicating that tilt angle is concentration dependent and that the pore is likely a barrel-stave pore. Future directions for my honors project include exploring a broader range of ALM concentrations on lipid bilayers. Studying higher concentrations may help confirm previous findings regarding ALM's orientation when all peptides adopt a pore-forming conformation, while examining lower concentrations could clarify its orientation in the surface-bound state. Additionally, studying how different ALM concentrations affect bilayers with cholesterol and negatively charged lipids like POPG could simulate how ALM interacts with eukaryotic and prokaryotic mimic membranes, respectively.

Faculty Mentor: Professor Michael Henderson

Funded by the Robert Freedman '87, P'17 and Anne Cirillo P'17 Student Fellowship Program