EPR Analysis of Lipid Ordering Induced by an Antibacterial Lipopeptide

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Introduction

Antimicrobial peptides (AMPs) have become a promising research area due to their ability to slow and prevent the growth of bacteria that would otherwise be resistant to antibiotics. AMPs more effectively prevent the development of bacterial resistance due to the differing mechanisms of action between AMPs and antibiotics. While antibiotics generally target specific cell functions, most AMPs disrupt the lipopolysaccharide layer of the cell membrane. Their amphipathic structure allows them to bind to the membrane and interact with both the polar head groups and non-polar lipid tails to facilitate membrane disruption. Because this membrane structure is ubiquitous among microorganisms, it is harder for bacteria to develop resistance.

Natural AMPs can be found in the human body and most other living organisms, but synthetic AMPs can be designed to be more effective due to lower toxicity or higher bacterial sensitivity. Learning about the mechanisms of these peptides and how those mechanisms differentiate between mammalian and bacterial membranes is essential to put these peptides to clinical use. This study utilized EPR spectroscopy to detect fluidity changes in membranes with or without cholesterol upon the addition of an antibacterial lipopeptide, LP1, in order to better understand the selectivity of this and other peptides.

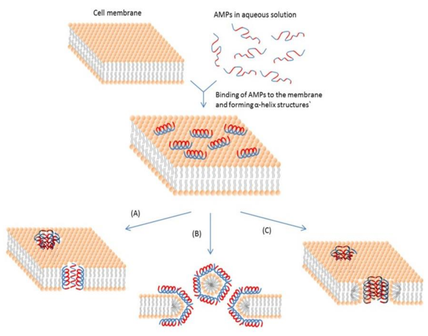


Figure 1: Three commonly proposed mechanisms of AMP action. A) Barrel-Stave Model B) Carpet Model C) Toroidal Pore Model [1]

Electron Paramagnetic Resonance

Electron Paramagnetic Resonance (EPR) spectroscopy was used to measure changes in the membrane fluidity of liposomes samples. This method is able to detect the absorption of microwaves by unpaired electrons. Because the lipid membranes do not contain unpaired electrons, spin labels are used to mark a small percentage of the lipids and allow detection of the sample. The resulting EPR spectrum can then convey information about the motion and local environment of the spin labels and the overall membrane.

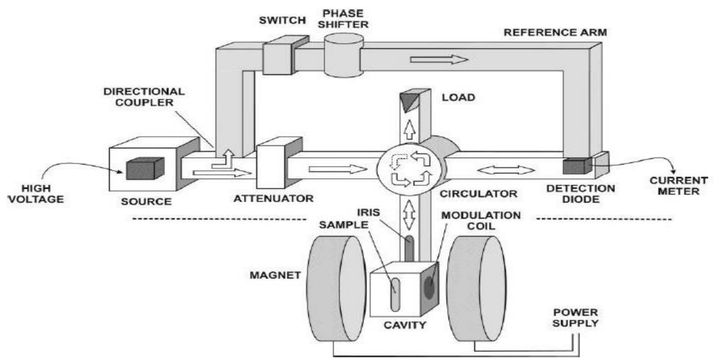
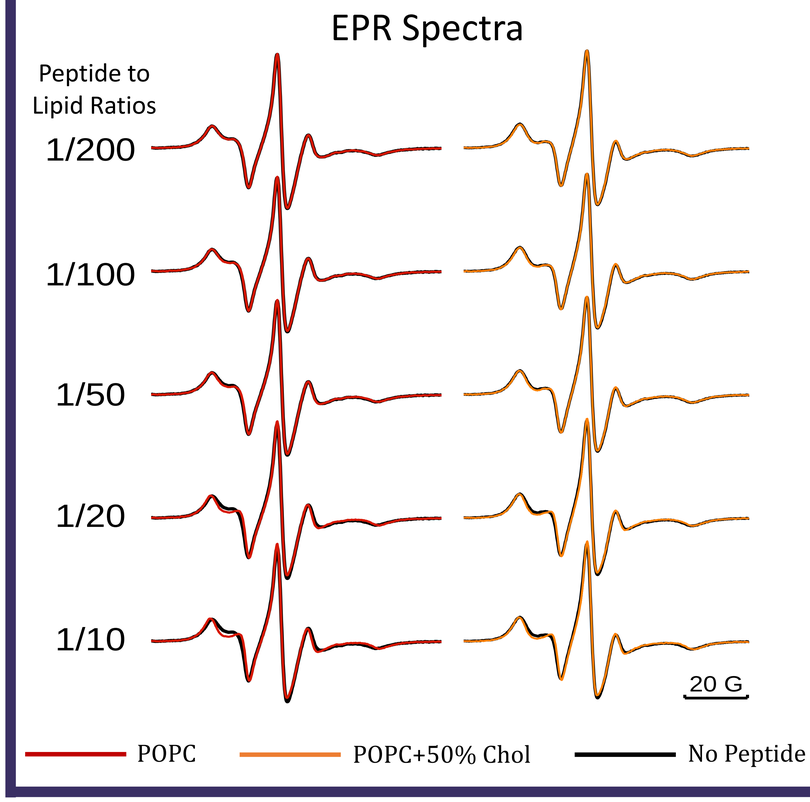
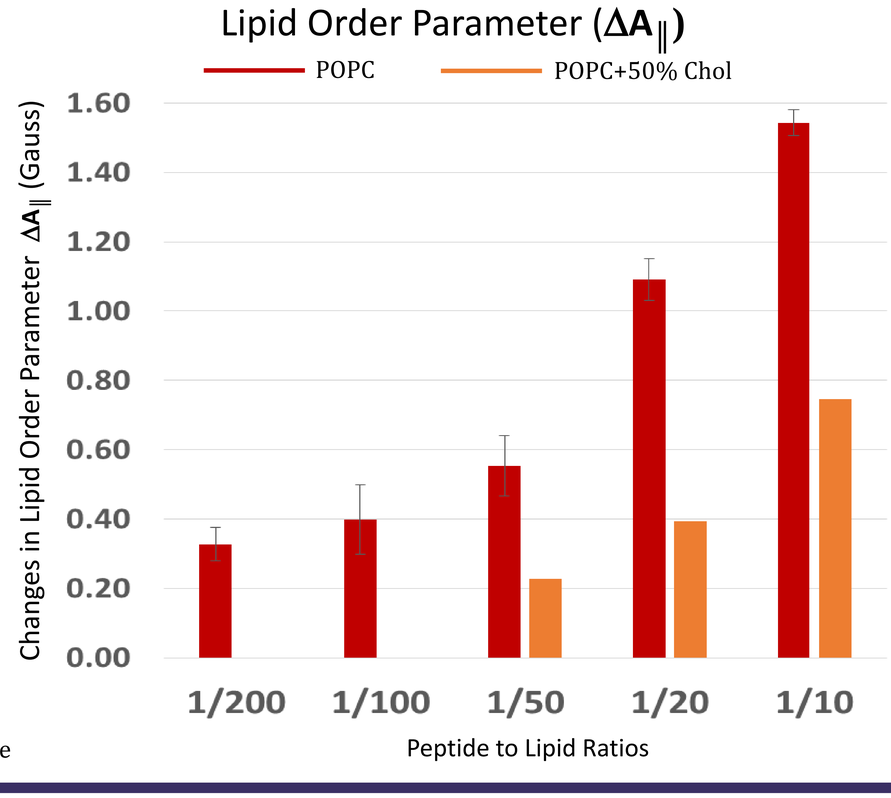


Figure 2: Example of the set up for a general EPR spectrometer similar to the one used for this experiment[2].

Data and Results





Methods

When placed in aqueous environments, lipids organize in a spherical bilayer membrane called a liposome. Based on the structure of the lipids used to form the membrane, the resulting liposome can mimic a bacterial or mammalian membrane. POPC and POPC plus cholesterol composed the membranes used for this study. The spin label 5-SASL was used to tag a trace amount of the lipids in the membranes. The lipids, once rehydrated in buffer, were sonicated, put through 6 freeze-thaw cycles, and extruded. LP1 was then added to the liposome samples in the following lipid/peptide ratios: 1/200, 1/100, 1/50, 1/20, and 1/10. EPR spectra of the liposome samples were then collected at X-band frequency and analyzed to detect changes in the lipid fluidity of the liposomes after the addition of the peptide. A decrease in fluidity is indicated by a widening of the spectrum.

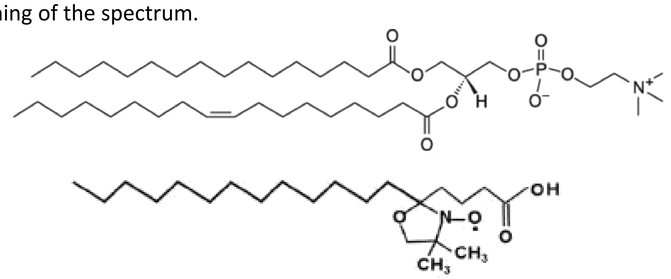


Figure 3: Structures for POPC phospholipid (top) and 5-SASL spin labeled phospholipid (bottom)[3].

Discussion

The resulting spectra above (colored trace) are shown overlaying the spectra obtained before adding the LP1 peptide (black trace). With increasing amounts of peptide, the two spectra show an increasing difference in shape. In particular, an increased distance between the two outermost peaks can be detected, which indicates a decrease in the fluidity of the lipid membrane. The measure of the peak-to-peak distance reveals that the changes were less significant for the cholesterol-containing membranes, indicating that the presence of cholesterol is a factor that influences the antimicrobial activity of this peptide. Bacterial membranes generally do not contain cholesterol while mammalian membranes do, so this peptide would likely have higher antimicrobial effect on a cholesterol-free membrane than on a mammalian membrane.

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References

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[3]Kaur P, Li Y, Cai J, Song L. Selective Membrane Disruption Mechanism of an Antibacterial γ-AApeptide Defined by EPR Spectroscopy. *Biophysical Journal*. 2016;110(8):1789-1799. doi:10.1016/j.bpj.2016.02.038.