Electron Paramagnetic Resonance (EPR)

EPR has found wide applications in the study of lipid membranes and membrane proteins. EPR measures the absorption of microwaves by molecules with an unpaired electron spin. Proteins and lipid membranes lack unpaired electrons and therefore, groups with stable free radicals, called spin labels, are used to label proteins and lipid membranes. EPR spectrum is sensitive to molecular motion and the local environment of electron spins. Hence, EPR can be applied to characterize lipid membrane properties, and protein-membrane interaction.

Introduction

Resistance to conventional antibiotics is a significant threat to public health. Host defense peptides (HDPs) have emerged as promising antibiotic agents. HDPs’ amphiphilic structures allow them bactericidal properties as they bind and disrupt inherently anionic bacterial membranes through electrostatic and hydrophobic interactions [1]. α-AA peptides are synthetic antibacterial peptides that disrupt the bacterial membrane. This study employs electron paramagnetic resonance (EPR), to look at the binding of a cyclic α-AA peptide with membrane bilayers of varied lipid compositions. Lipid fluidity, polarity, and membrane ordering were studied using multi-frequency EPR. This study will help in providing a clearer picture of the peptide’s membrane interaction and understanding the mechanism that leads to membrane disruption and hence, antimicrobial activity.

Sample prep:
- Unlabeled phospholipids, mixed with a fraction of spin-labeled lipids, were dissolved in chloroform, and subsequently dried under nitrogen to produce thin layers. Dried lipid were rehydrated with buffer and extruded to form uniform liposomes with 100nm in diameter.
- α-AA peptides were added in different molar ratios w.r.t phospholipids (Protein : Lipid = 1:5, 1:10, 1:20, & 1:40)
- EPR spectra of bare liposomes and protein-bound liposomes were obtained at X-band frequency (9.75 GHz) and W-band frequency (94.0 GHz). Polarity changes were measured by microwave power saturation technique.

Lipid Fluidity

X-band (9.75 GHz) at room temp.
Spin label: 5-SASL

<table>
<thead>
<tr>
<th>P:L=1:5</th>
<th>POPC-POPG</th>
<th>POPC</th>
<th>POPC-30%Chol</th>
</tr>
</thead>
<tbody>
<tr>
<td>P:L=1:10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P:L=1:20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P:L=1:40</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Fluidity changes with membrane composition: POPC-POPG > POPC > POPC-30%Chol
- Fluidity changes at varied α-AA peptide concentrations: 1.5 > 1.10 > 1.20 > 1.40

Lipid Polarity

Oxygen Accessibility in host membrane mimic

- Lipid polarity increases at higher peptide to lipid ratio

Summary

- Lipid fluidity changes in the presence of α-AA peptide and the change is largest for P:L=1:5.
- Fluidity changes for bacterial mimic membranes are larger than host mimic membranes.
- Lateral ordering of membrane also changes in the presence of α-AA peptide as seen at W-band frequency.
- Changes in accessibility indicates changes of membrane polarity. Polarity change is largest for P:L= 1:5.
- Changes in polarity indicates that bacterial membrane might be disrupted by α-AA peptide.

References


Acknowledgements

This work would not have been possible without the profoundly instructive mentorship of Dr. Likai Song & Pavanjeet Kaur, the remarkable support of the National High Magnetic Field Laboratory and its Center for Integrating Research & Learning, as well as the National Science Foundation (DMR1157490).