Abstract

With the advent of many immuno-compromising medicines as well as drug-resistant strains of fungi there is an increasing need for new classes of antifungal compounds. The lipopeptide fengycin (FE), produced by Bacillus subtilis, has demonstrated potent antifungal activity while having little inhibitory effect towards mammalian cells and bacteria. Experiment indicates fengycin acts as a toxic agent via cytoplasmic membrane disruption, resulting in permeabilization of the cell membrane. Here we explore this hypothesis using coarse-grained molecular dynamics simulations. While the insertion of multiple fengycins into our model bilayers causes notable thinning there is no significant difference in average thinning between the three bilayer types. This is likely due to fengycin clustering in the different types of membranes. Membranes with exponential susceptibility to fengycin demonstrated more clustering in our simulations. We hypothesize that fengycin-resistant membranes disperse fengycin about the membrane eliminating radial pockets of curvature.

Fengycin

• Potent anti-fungal agent
  - acts by disrupting outer membrane
  - little toxicity towards mammalian and bacterial cells
  - hypothesized to make pores at moderate concentrations and dissolve bilayers at high conc.
• Structural characteristics
  - net charge equals -2
  - amphipathic molecule with 10 amino acid cyclic peptide and 14 carbon tail
  - hydrophobic tail promotes insertion into lipid bilayers
• FE Has Distinct Ring States
  - Torsion Calculations
    - used Ala-6, Pro-7, Ile-10, and Tyr-3 ring beads (highlighted purple in fengycin introduction)
    - 13 FE calculation run after 400ns equilibration
  - Fengycin-fengycin contacts modulate ring structure in two possible ways:
    - slowing of ring transition due to stabilization of the transition state
    - increased oscillation b/w. the two main ring states
• FE Binding Bends Membrane
  - Heat map of phosphate heights in the upper and lower bilayer leaflets
  - representative image of PO4 curve when FE is inserted in DPPC, POPC, or POPE/POPG bilayers
  - curvature occurs in the upper and lower leaflets where fengycin is inserted
  - no average curvature in neat bilayer

FE Binding Bends Membrane

POPC Lowers FE Ring Relative to DPPC and POPE/POPG

• Location of phosphates relative to height of fengycin ring
  - centered about 1 fengycin
  - Fengycin ring location differs in each bilayer
  - in POPC FE ring is inserted further
  - in POPE/POPG and DPPC FE ring sits atop lipid heads

FE Has Distinct Ring States

POPC Lowers FE Ring Relative to DPPC and POPE/POPG

Simulation Details

• Simulations carried out in physiologic salt (100mM NaCl) concentration plus neutralizing salt for fengycin/lipid charges
- DPPC, POPC, POPE/POPG (2:1) membrane models were used
- 256 lipids per leaflet
• Pure bilayer, 1 fengycin, and 13 fengycin systems constructed
- 8,000-12,000 waters and 15,000-20,000 total 'atoms' per system
- 16 replicas per system type for a total of 144 trajectories
- each trajectory at >1us

Clustering of Fengycin Varies Between Bilayer Types

Analysis of FE clustering
- criteria for cluster was at least 2 contacts at a distance less than 7Å
- Discreet data shown right
- data was combined from 16 trajectories with 13 fengycin inserted each
• Each bilayer had distinct ordering of fengycin
  - In POPC fengycin remains largely clustered
  - In POPE/POPG fengycin tends to disperse

Conclusions

Model supports hypothesis that fengycin acts through bilayer disruption. Fengycin clusters in POPC and disperses in POPE/POPG and DPPC. Fengycin appears to have affinity for self-self interaction.

Future Directions

• Test whether the fengycin clustering properties in POPC are a result of the FA tail length, fengycin ring structure (specifically charged beads) or a combination of the two
• new coarse-grained fengycin with additional/decreased carbons on the FA tail
• Lack of distinct ring conformation of single fengycin in each bilayer type
• Favors ring-lipid interactions are not as important
• FE Has Distinct Ring States
  - Torsion Calculations
    - used Ala-6, Pro-7, Ile-10, and Tyr-3 ring beads (highlighted purple in fengycin introduction)
    - 13 FE calculation run after 400ns equilibration
  - Fengycin-fengycin contacts modulate ring structure in two possible ways:
    - slowing of ring transition due to stabilization of the transition state
    - increased oscillation b/w. the two main ring states
  - Fengycin ring might not determine if clustering or dispersal occurs