Understanding the function of the cyclic antifungal lipopeptide fengycin using all-atom simulation

Sreyoshi Sur, Tod D. Romo, Alan Grossfield
University of Rochester Medical School, Rochester, NY, USA

Abstract
Fengycin is one of a class of cyclic lipopeptides synthesized by the bacterial genus Bacillus. Many bacteria synthesize similar cyclic peptides, some of which have antifungal or even antibacterial properties, so studying how they interact with membranes is a promising path for drug development. Previously, we ran a series of coarse-grained molecular dynamics simulations using MARTINI force field exploring the interactions of fengycin with models for bacterial and fungal membranes. The results suggested that the peptide’s ability to aggregate and deform the membrane depends on the nature of the surrounding lipid headgroups, and that these interactions might be the origins of its selectivity. However, coarse-grained models, by definition, lack atomic-level resolution, so all-atom simulations are needed to confirm and expand on these results. First, we developed parameters for several unusual chemical moieties found in fengycin, such as the cyclization between the C-terminus and a Tyr side chain, as well as the amide linkage between Glu and Gly residues in the cyclic peptide. We validated these parameters via simulations of isolated and clustered fengycin molecules in water, as well as simulations of 3 bilayer membranes: POPC (model mammalian/fungal membranes) and 2:1 POPE:POPG (model bacterial membrane). The results show that the specificity of fengycin aggregation and its ability to deform lipid bilayers is not based only on electrostatic interactions. Moreover, fengycin disturbs the POPE:POPG membrane more than the POPC model bilayer.

Simulation Details

Fengycin

- Structure: 16-carbon acyl chain
- Hydroxyl group
- Cyclic ring with eight amino acids
- Ester bond between Tyr-3 and Ile-10
- D-α-amino acids
- Net charge = -2

- Physiological salt: 100mM NaCl
- POPC, POPE:POPG (2:1) membrane models
- 90 lipids and 10 fengycins per isoleaflet
- 7,500 waters and 50,000 total atoms per system
- POPC has 1 change
- Box size: 90 Å x 90 Å x 70 Å
- Forcefield: CHARMM22
- Ensemble: NpT
- Temperature: 310 K
- Pressure: 1 bar
- Electrostatics: PME
- VDW cutoff: 10 Å
- Time step: 2 fs
- RATTLE
- Software: NAMD 2.8
- Computer resource: BlueGene/Q

Fengycin forms aggregates in water

- Fraction of contacts between fengycin hydrocarbon tails
- Tail-tail
- Tail-water
- Initial aggregation is fast
- Peptide portion remains hydrated

Fengycin forms aggregates in POPC

- Radial distribution in membrane plane
- No change in the lipid-lipid packing
- Fengycin packs tighter against POPE and POPG

Fengycin decreases chain order

- Molecular order parameter for lipid palmitoyls
  - Average 2nd and 3rd principal axes
  - Chains significantly disordered relative to neat bilayer
  - Effect strongest at short range, r < 10 Å

Fengycin forms aggregates in POPC

- Aggregation stronger in POPC
- Specific interactions drive formation of aggregates

Fengycin forms tube-like structures

- Number of fengycins in contact with each fengycin
- Equilibrates rapidly usually having 2-3 neighbors
- Not spherical micelles

Future directions

- Compute the free energy of fengycin binding to model membranes
- Effects of cholesterol, ergosterol

Work done in LOOS (Lightweight Object Oriented Structure analysis library), an open source C++ library designed and maintained by the Grossfield lab. LOOS provides a concise, adaptable framework for designing analysis tools that interfaces with native the formats of most simulation engines.

http://loos.sourceforge.net