Towards Finding the Antimicrobial Mechanism of Action of Bombina maxima’s Maximin 3, Using GROMACS

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Introduction

- Antibiotic resistance is on the rise and only two new classes of antibiotics have been approved by the FDA in the past 25 years, so alternatives to antibiotics may be worth finding.
- Antimicrobial peptides (AMPs) have broad-spectrum activity against microbes. AMPs exist in all kingdoms of life and are essential to normal immune response and resistance to bacterial skin infection (Figure 1).
- The AMP Maximin 3 is a proposed alpha helix 27 amino acids long and is part of Bombina maxima’s epithelial defenses, but its mechanism of action is unknown.
- Hypothesis: the amphipathic nature of Maximin 3 means that it might follow a model in Figure 2.
- This research used GROningen MAchine for Chemical Simulations (GROMACS) version 5.0.1 to simulate the interaction of Maximin 3 with a cell membrane, following the KALP15 in DDPC tutorial (3) explained in Method. The change to the tutorial was using Maximin 3 instead of KALP15.

Method

1. Prepare the Topology
2. Modify the Topology
3. Defining the Unit Cell and Adding Solvent
   - Insert Maximin 3 into the DPPC membrane
   - Pack the lipids around the protein
   - Solvate with water
4. Adding Ions
5. Energy Minimization
6. Equilibration to stabilize T at 323 K (NVT ensemble)
7. Equilibration to stabilize pressure (NPT ensemble)
8. Production Molecular Dynamics (MD)
   - Simulation of how the system evolves over time
9. Analysis (Future Work)

Results

Figure 1: Partial list of the layered antimicrobial peptides of human skin, not including the AMPs produced by prokaryotes that inhabit the skin surface that also contribute to immune defense (1).

Figure 2: Five models of AMP activity by formation of ion channels or pores (2). Red represents a hydrophilic surface, while blue represents a hydrophobic surface, so the red and blue AMPs are amphipathic. (A) barrel-stave model, (B) carpet model, (C) toroidal pore model, (D) molecular electroporation model, (E) sinking raft model

Figure 3: Helical wheel model of Maximin 3 (previous work). The start (N-terminus) is G1 and the end (C-terminus) is H27. Hydrophilic residues are circles, hydrophobic residues are diamonds, potentially negatively charged are triangles, and positively positively charged are pentagons.

Figure 5: Maximin 3 on 0% charged lipid membrane (previous work in MC Pep)

Discussion

- Dr. Middleton’s previous in vitro work used vesicles of 75% POPC and 25% cholesterol (mammalian-type neutral membrane) and 75% POPC and 25% POPG (bacterial-type negatively charged membrane) to test Maximin 3’s effectiveness at inducing membrane leakage. Future GROMACS simulations should use those membranes instead of the neutrally charged DPPC membrane used in the tutorial.
- Ways to improve GROMACS simulation:
  - Increase the MD simulation time from 1 nanosecond to more than 50 to 100 nanoseconds
  - Use a better force field: CHARMM36.
  - Try to improve the lipid packing (eg. use em.tpr properly during lipid shrinking and deletion)
  - Simulate several Maximin 3 at once and consider multiple starting/assembly states and perhaps consider coarse graining so the Maximin 3 might aggregate into one of the assemblies in Figure 2.
- Future research: minimize the toxic effects of Maximin 3 on mammals so that Maximin 3 might be a good topical, injectable, or pill form antimicrobial.

References


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