Specific binding of an anionic bacterial ceramides to BamA in model membranes

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Abstract

BamA is a beta-barrel outer membrane protein (OMP) that assists in folding other OMPs in Gram-negative bacteria. Because of its key role, high conservation, and location at the cell's surface, it is a promising target for therapeutic intervention and is worth further investigation. The exact mechanism that modulates the open and closed state of BamA's lateral gate is currently not well understood. Recently, a novel anionic ceramide, ceramide polyphosphoglycerate (CPG2), was found in the Gram-negative bacterium *Caulobacter crescentus*. Knocking down BamA or anionic ceramide synthesis yields similar phenotypes, including increased antibiotic susceptibility and induction of the unfolded-protein response, suggesting the two may be linked. Here, we computationally study anionic ceramide interactions with BamA using coarse-grained molecular dynamics. We found several superficial binding sites for anionic ceramide and measured their binding affinity to BamA in ternary mixed POPC, neutral ceramide and anionic ceramide model membranes using the density threshold affinity method.

Approach

- Calculate the density distribution of lipids around BamA
- Qualitatively determine sites of high lipid density enrichment
- Quantitatively rank binding sites using density threshold affinity method[3]

$$\Delta G_{bind} = -RT \ln \frac{P_{occ,site}}{P_{unocc,site}} + RT \ln \frac{P_{occ,bulk}}{P_{unocc,bulk}}$$

- $P_{occ,site}$: Occupancy probability of lipid in the site
- $P_{occ,bulk}$: Occupancy probability of lipid in bulk patch

Methods

- System composition: BamA (PDBID: 5EKQ), 4% anionic ceramide, 4% neutral ceramide, 92% POPC
- Simulation software: Gromacs 2022[4]
- Force Field: Martini 2.2[5]
- Membrane Building Tool: Insane[6]
- Protein Coarse-Graining Tool: Martinize[7]
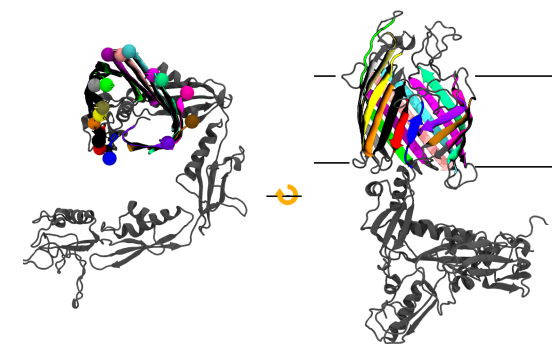- Simulation Time: 40 μs
- Replicas: 10

BamA

Fig 2. Visualization of BamA (PDBID: 5EKQ) topdown (left) and side view (right), each strand of the beta-barrel colored.

Background

- *Caulobacter Crecentus* is a gram negative bacteria that was found to elongate in phosphate starved conditions (Fig 1), which normally limits lipid production.
- Under lipid A deficiency, *Caulobacter Crecentus* can survive using a novel anionic ceramide[1,2].
- The role of anionic ceramide in the outer membrane of *C.Crecentus* has not been determined.
- The Bam complex fold proteins in the outer membrane.
- Preliminary data shows that knocking out anionic ceramide or BamA cause similar phenotypic response.
- It is unclear if anionic ceramide has specific binding to BamA.

Results

Fig 3. Lipid density enrichment within 40 angstroms of BamA center of mass. Strands of the beta-barrel are colored as in Fig 1. High density anionic ceramide sites are outlined and labeled. Density enrichments are averaged over 10 replicas

Fig 4. Contact Frequency between the anionic ceramide head group region and BamA residues in the beta-barrel. High contact frequency in red and low contact frequency in blue. Residues with intermediate contact are colored white.

Fig 5 Occupancy probability for anionic ceramide in site 1 for 10 replicas (top). Occupancy probability for anionic ceramide in a patch of bulk membrane with the same area as site 1 (bottom).
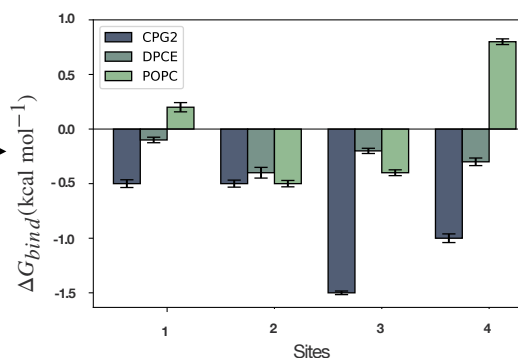
Fig 6. The free energy of binding for each lipid species in sites as in Fig 3. Error bars are the standard error between 10 replicas

Summary

- Here we show that anionic ceramide has specific interactions with the beta barrel regions of BamA
- Anionic ceramide binds to site 3 and 4 with the highest affinity
- Anionic ceramide binds to positively charged residues in the loop regions
- POPC interacts indiscriminately with BamA
- Neutral ceramide does not compete with anionic ceramide

Research Questions

1. Does anionic ceramide bind to BamA?
2. What is the binding affinity for anionic ceramide sites on BamA?
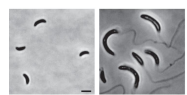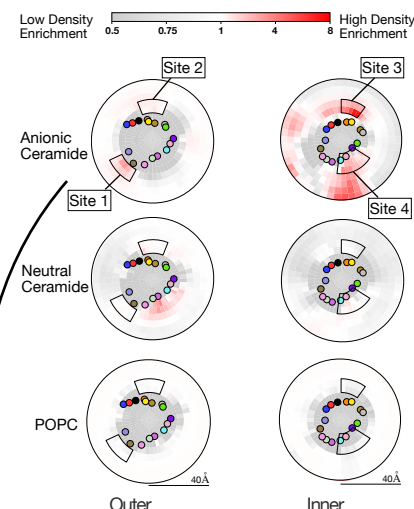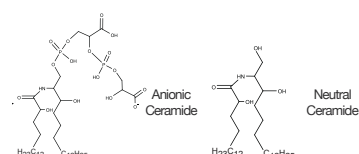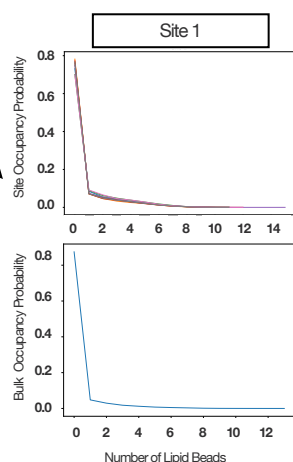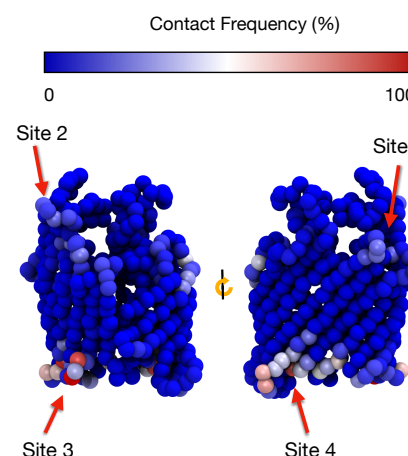3. What residues of BamA contact anionic ceramide?

Fig 1. Image of *C.crecentus* in phosphate rich (left) and starved (right) conditions[1]

Acknowledgments

References

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