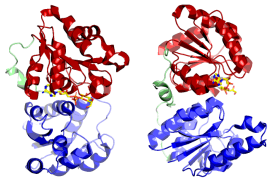

Exploring Simulated Molecular Dynamics Between Heptosyltransferase I Domains

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Introduction

Heptosyltransferase I (HepI) dynamics are critical to successful addition of a heptose moiety to lipopolysaccharides, a component of the Gram-negative bacterial cell membrane. Without this addition, a truncated lipopolysaccharide is synthesized, and survival of bacteria treated with hydrophobic antibiotics decreases. Previous investigations, including tryptophan fluorescence studies, and molecular dynamics (MD) simulations, indicate that HepI undergoes a large-scale closing motion upon substrate binding. MD simulations characterizing enzyme interconversion between open and closed states identified negatively correlated motions between residues in flexible active site loops, and residues in the C-terminal domain. Mutagenesis of amino acids hypothesized to modulate chain flexibility within the C-terminal loops, increased HepI enzymatic efficiency, indicating that these dynamic regions play a role in the formation of the Michaelis complex. However, simulated dynamics of these mutants have not yet been studied. Root mean-square deviation, root mean-square fluctuation, and dynamic cross correlational analyses were performed and revealed that, with one exception, mutagenesis of these residues modified correlated motion between the N- and C-terminal domains, and eliminated the strong negatively corelated motions within the N-terminal domain. The findings support previous kinetic and simulation data indicating the importance of the dynamic modes of HepI, and contribute to the growing body of work seeking to characterize this enzyme with a view to increasing bacterial antibiotic sensitivity.

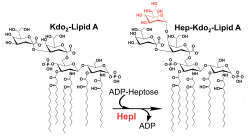

Fig 1. HepI in LPS Biosynthesis

Previously Identified Correlations Between Domains

- HepI MD simulations identified negatively correlated motion between the C-terminal region and residues in flexible active site loops (including R63, R98, and R120 which are known to be involved in ligand binding.)
- Mutagenesis of prolines and glycines in these corresponding C-terminal regions decreased K_m and did not alter k_{cat}, suggesting that an overall closing motion is critical for substrate binding, rather than catalytic chemistry.

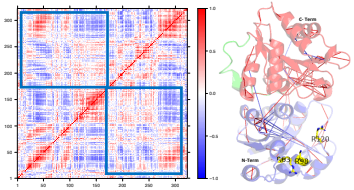

Fig 2. Visualizing large-scale negatively correlated motion
Graphical and cartoon representation of DCC analysis performed on 2 ns MD simulation trajectory of wildtype HepI. Negative correlations shown in red and positive correlations in blue. Substrate binding residues shown in yellow. Negatively correlated motions between domains boxed in blue.

Glycine to Proline	Proline to Glycine
G280P	P195G
G285P	P216G
G288P	P240G
G289P	P281G
G301P	P284G

Fig 3. P to G and G to P mutants
Proline to glycine and glycine to proline mutants studied, based on wildtype MD simulations.

References

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Examining Molecular Dynamics Of Proline and Glycine Mutants

Simulated Molecular Dynamics

- Protein structures were constructed using PDB crystal structure coordinates and PyMOL mutation tools.
- Trajectories were computed using the amber99 forcefield and GROMACS 2020.1, after equilibrating the system at 300K and 1 atm, using a three point water model and sodium and chloride counter-ions.

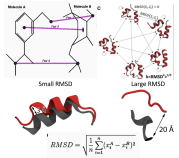

Fig 4. Root Mean-Square Deviation

- RMSD and RMSF analyses performed to examine structural fluctuation.
- DCC analysis performed to examine intra-protein molecular dynamics.
- RMSD for proline and glycine mutants was found to be near 2 Å and comparable between mutant and wildtype simulations.

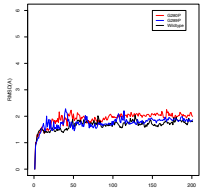

Fig 5. RMSD G280P and G289P Compared to Wildtype

Proline and Glycine Mutants and Changes in Flexibility and Dynamics

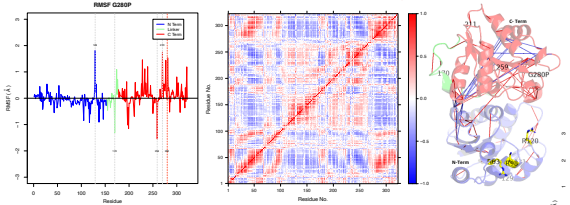

Fig 6. RMSF and DCC analysis G280P
Difference between wildtype and G280P RMSF (negative value indicates larger wildtype value.) Graphical and cartoon representation of correlated motion within G280P (correlation cutoff value of 0.6 for negative values and 0.8 for positive values in cartoon visualization.)

- DCC analysis revealed fewer negatively correlated motions between the N- and C- terminal domains overall.
- Seven out of ten mutants (G280P, G285P, P216G, G288P, G289P, P281G, G301P) exhibited fewer negatively correlated motions, with correlation value stronger than -0.4 between substrate binding residues and the C-terminus.
- The strong negatively correlated motion within the N-terminal domain was also eliminated.
- Comparison between wildtype and mutant RMSF revealed changes in chain flexibility.
- These differences in fluctuation were typically of the largest magnitude in the C-terminal region, the domain containing the proline and glycine mutations.

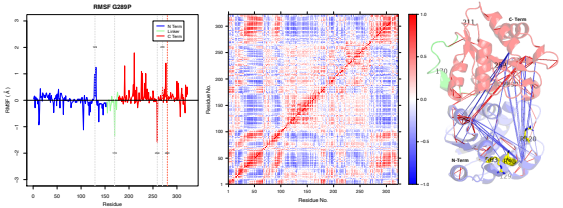

Fig 7. RMSF and DCC analysis G289P
Difference between wildtype and G289P RMSF values, and graphical and cartoon representation of correlated motion within G289P (correlation cutoff value of 0.6 for negative values and 0.8 for positive values in cartoon visualization.)

Summary

- A negatively correlated closing motion between the N- and C- domains of HepI is critical in catalyzing the addition of a heptose moiety to lipopolysaccharides.
- MD simulations of mutants targeting amino acids hypothesized to modulate chain flexibility in the C- terminal domain, showed fewer negatively corelated motions both between domains and within the the N-terminal domain.

Acknowledgements

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