Understanding the thermostability and catalytic activity of Bacillus subtilis lipase: Insights from Molecular Dynamics Simulations

by

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Understanding the thermostability of *Bacillus subtilis* lipase: Insights from Molecular Dynamics Simulations

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Introduction

- Lipases are water-soluble enzymes that catalyze the hydrolysis, esterification and transesterification reactions involving water insoluble esters.
- Lipases are very important as industrial biocatalysts and in detergent industry.
- *Bacillus subtilis* lipase (LipA), is a 19.34 kDa monomeric protein and possess a common fold known as alpha/beta hydrolase fold.
- LipA has broad substrate specificity but preferentially hydrolyzes C-8 fatty acid esters.
- It is a typical mesophilic enzyme having temperature optima of activity at 37 °C.
- Five thermostable mutants of wild type LipA have been produced through directed evolution\(^1\),\(^2\), without affecting its activity at mesophilic temperature.
- These five mutants are as follows:

<table>
<thead>
<tr>
<th>Mutant</th>
<th>No. of Mutations</th>
<th>Mutation: location</th>
<th>Implications from crystal structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1T4M</td>
<td>2</td>
<td>A132D: Loop N166Y: Helix</td>
<td>Improved solvent interaction, stacking interaction</td>
</tr>
<tr>
<td>1T2N</td>
<td>2+1</td>
<td>L114P: Loop</td>
<td>Anchoring of C-ter to rest of the protein</td>
</tr>
<tr>
<td>3D2A</td>
<td>2+1+1</td>
<td>I57A: 1Helix</td>
<td>Improved hydrophobic packing</td>
</tr>
<tr>
<td>3D2B</td>
<td>2+1+2</td>
<td>P178: 3Helix</td>
<td>Favourable contacts with solvents, Stabilization of C-ter of helix</td>
</tr>
<tr>
<td>3D2C</td>
<td>2+1+2+3</td>
<td>A155: Loop A20E: N-ter Helix G111D: Loop</td>
<td>H-bonds and salt bridge formation, loss of intrinsic flexibility</td>
</tr>
</tbody>
</table>

Results and Observations

- The average values of RMSD and Radius of gyration for wild type (WT) and different mutants respectively do not appear to correlate with their experimentally observed thermal stabilities. This is especially true for trajectories at lower temperature.
- At 450K however the most thermostable mutant(3D2C) shows least values for average RMSD and Radius of gyration. Interestingly a moderately thermostable mutant (1T2N) also shows similar values, lower than the other stabler mutants.
- Similar trends were also observed with SASA (hydrophobic) and H-bonds.
- The RMSF graph show variation in relative values for different regions, both for all the mutants at different temperatures as well as for different mutant at given temperature.
- The 2-d free energy landscape along the first two principal components show a more structured landscape for the most thermostable mutant (3D2C).
- PCA analysis show significant motions within the core regions for wild type (WT), whereas no such motions were observed in thermostable mutants.
- Analysis of trajectory dynamics did not significantly substantiate the implications drawn from crystal structure for explaining thermostability of less thermostable mutants.
- There can be different thermostabilization mechanisms operative on the less stable mutants.

Objectives

- To understand the molecular mechanism of thermostability and assess the effect of point mutations on the thermostability of mutants through molecular dynamics simulations.

Computational details

- Software: GROMACS-4.5.3; Forcefield: AMBER99sb-ildn; Temperature: 328, 339 and 450K; Ensemble: NPT
- Explicit solvent simulations using TIP3P water molecules inside a cubic box with periodic boundary condition were carried out
- Energy minimization: Steepest descent; Equilibration: NVT, 1ns NPT;
- Production run: 100 ns at 328 and 339K and 50ns at 450K

Conclusions

- The average values of RMSD and Radius of gyration for wild type (WT) and different mutants respectively do not appear to correlate with their experimentally observed thermal stabilities. This is especially true for trajectories at lower temperature.
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References: