Allosteric Regulation and Structural Dynamics of Thermostable L-Lactate Dehydrogenase: Insights from Molecular Dynamics Simulations and Experimental Analysis

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Understanding the allosteric regulation of *Geobacillus stearothermophilus* L-lactate dehydrogenase (GsLDH), a homotetrameric enzyme, is crucial for its application in industrial biocatalysis due to its role in lactate-pyruvate interconversion and NAD^+/NADH cofactor regeneration. In this study, we combined experimental and computational approaches to elucidate the mechanisms underlying the enzyme's allosteric activation by fructose 1,6-bisphosphate (FBP), which stabilizes tetramerization and enhances substrate affinity for pyruvate. We compared the wild-type (wt) enzyme with a triple mutant and a single mutant to assess the contributions of oligomerization and allosteric modulation. Our findings reveal that the triple mutant retains its tetrameric structure and high substrate affinity regardless of FBP. In contrast, the single mutant maintains tetramerization but lacks the enhanced substrate affinity, suggesting that oligomerization alone is insufficient to propagate the allosteric signal. Both oligomerization and allosteric modulator binding are necessary to establish communication pathways that effectively modulate substrate affinity, as demonstrated by significant changes in K_m [1]. To investigate dynamic conformational changes, we employed molecular dynamics (MD) simulations and applied our newly developed tool, MDavocado [2]. This computational tool visualizes MD trajectories using time-resolved Ramachandran plots, effectively aggregating millions of data points into interpretable visualizations. This approach enables rapid identification of flexible regions and global motion patterns across all amino acids (Figure 1). The application of MDavocado highlighted how FBP-induced oligomerization correlates with coordinated backbone dynamics, reinforcing the necessity of dual structural and allosteric inputs for optimal enzyme function. Our findings advance the understanding of structure-allostery relationships in industrially relevant enzymes and underscore the utility of MDavocado in resolving complex protein dynamics.

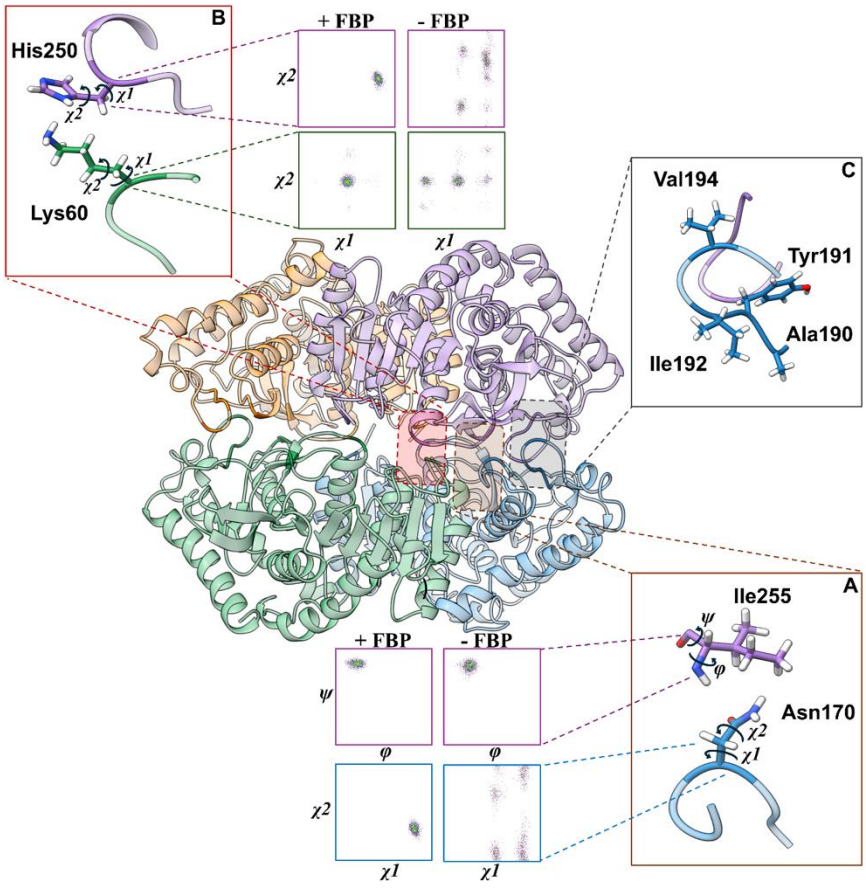

Figure 1. Analysis of conformational changes modulated by FBP. Three regions exhibiting distinct dynamics in MD simulations, identified using the MDavocado tool, are highlighted. (A) The region involves Asn170, located within the FBP-binding site and oriented toward Ile255 of the P-axis-related subunit. (B) The region includes Lys60, situated at the junction between helix αC and strand βC, as well as His250 on the α3G helix of the diagonal subunit. (C) The region consists of a predominantly hydrophobic loop formed by Ala190, Tyr191, Ile192, and Val194, which connects βC and αT at the P-axis dimer interface. The inner panels display Ramachandran plots (ϕ-ψ diagrams) and Janin plots (χ1-χ2 diagrams) for the identified residues, with their corresponding dihedral angles marked.

References:

[1] H. Cai, S. Shulami, Z. Štefanić, T. Hrenar, A. Maršavelski, A. Fishman, *Protein Sci.* submitted.

[2] B. Gomaz, A. Pandini, A. Maršavelski, Z. Štefanić, *J. Chem. Inf. Model.* **64** (2024) 5742–5748.