Utilizing pH-driven time-resolved crystallography to elucidate the reaction mechanism in bacterial HMG-CoA reductase.

Content

HMG-CoA reductase in Pseudomonas mevalonii (pmHMGR) uses mevalonate as a carbon source and produces 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) using the cofactors coenzyme A and NAD. The mevalonate pathway is critical for the survival of multiple gram-positive bacteria, making HMGR a novel target for antibacterial development. Previous studies propose that the enzyme’s reaction mechanism involves the production of intermediates mevaldehyde and mevaldyl-CoA with structural evidence only for mevaldyl-CoA (Steussy et. al, 2013). Structural and chemical features of the enzyme explaining the formation of mevaldehyde and the changes leading up to the formation of Mevaldyl-CoA remain unknown.

Our studies have identified a pH range within a modified crystal environment that can be used to transition the enzyme from an inactive to active state. Using a pH-jump as a reaction triggering method, we have observed ligand-bound pmHMGR structures leading up to the formation of Mevaldyl-CoA. The technique used for this study involves the flash-freezing of ligand-bound pmHMGR crystals after being soaked at a higher pH. The structures obtained from crystals flash-frozen at shorter time points following the pH-jump have shown changes in the electron densities of the ligands that are associated with the hydride transfer step converting mevalonate into mevaldehyde. We have also observed the hydride transfer using UV-absorbance measurements in pmHMGR crystals. Subsequently, at longer time points, we have identified movements in the enzyme’s C-terminal region that position catalytic residues after the hydride transfer to allow the formation of mevaldyl-CoA.

The pH-jump method allows us to adjust the catalytic rate of the enzyme in crystals. It is therefore being utilized to not only develop methods of obtaining time-resolved structures accessible with freeze-trap methods, but those that could be applied at faster timescales and can capture short-lived changes along the reaction pathway.

Primary author(s) : Mr. PUROHIT, Vatsal (Department of Biological Sciences, Purdue University)

Co-author(s) : Dr. STEUSSY, Calvin (Department of Biological Sciences, Purdue University); Mr. SCHMIDT, Tim (Department of Biological Sciences, Purdue University); Dr. DUNCAN, Chandra (Department of Biological Sciences, Notre Dame); Dr. ROSALES, Tony (Department of Chemistry and Biochemistry, University of Notre Dame); Dr. HELQUIST, Paul (Department of Chemistry and Biochemistry, University of Notre Dame); Dr. WIEST, Olaf (Department of Chemistry and Biochemistry, University of Notre Dame); Dr. STAUFFACHER, Cynthia (Department of Biological Sciences, Purdue University; Purdue Institute of Inflammation, Immunology and Infectious Diseases; Purdue Center for Cancer Research)

Presenter(s) : Mr. PUROHIT, Vatsal (Department of Biological Sciences, Purdue University)

Comments:

2 of the co-authors (Tony Rosales and Chandra Duncan) have moved on to other non-academic positions after their work on this project and hence, I have not included their work address and phone number.

Submitted by PUROHIT, Vatsal on Saturday 31 October 2020