Poster Presentation

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Structural basis of Iridoid synthase mediated cyclization of 10-oxogeranial

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Iridoids are the structurally diverse class of bi-cyclic monoterpene indole alkaloids (MIA's), which act as important secondary metabolites in plants and insects. These natural products are the major synthetic biological source for microbial production of bio- fuels, medicines, and numerous chemical commodities. C.Roseus is the major source of iridoid derived MIA'S and is known to contain over 2000 alkaloids in various tissues. Among these, there are several important compounds such as vinblastine, vincristine that are being used in the preparation of anticancer, anti-malarial and antihypertensive. The crucial step in the synthesis of plant iridoids is NAD(P)H-dependent reduction and subsequent cyclization of 10-oxogeranial, involving either a Diels–Alder cycloaddition or a Michael addition. This step is catalyzed by Iridoid synthase (IDS), a short chain Dehydrogenase/Reductase (SDR) belonging to Progesterone 5β-reductase (DlP5βR) family. To understand the structural basis this specific problem, the crystal structure of IDS-NAHPH complex has been determined. The structure of IDS is similar to that of DlP5βR. However, divergent residues at the catalytic pocket and a loop present at the opening of the catalytic pocket plays a critical role in conferring substrate specificity. Furthermore, previous studies have shown that incubation of 10-hydroxygeraniol with 10-Hyroxygeranial dehydrogenase (10HGO) and IDS in the presence of NADP+ yielded cis-trans nepetalactol as a major product unlike, IDS producing a mixture of cis-trans nepetalactol and Iridoids, when acting sequentially. To delineate the "hand-shake" interaction between 10HGO and IDS, a chimera of 10HGO and IDS was cloned, Mass Spectrometer studies and biochemical assays were also performed. Our results indicate that 10HGO and IDS interact in a spatiotemporal manner to yielded cis-trans nepetalactol as a major product. To understand the molecular mechanism of 10HGO and IDS enzyme cascade at an atomic level, X-ray crystallography and Cryo-electron microscopy (EM) studies have been performed. The crystallization attempts for the chimera of 10HGO and IDS were not successful and hence, Cryo-EM Single Particle reconstruction was performed. Preliminary 3D reconstruction of the EM map shows the existence of two molecules. A better resolution is needed for complete understanding of the interaction of 10HGO and IDS for which data is being optimized.

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