Poster Presentation

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Structural studies on the catalytic mechanism of Diaminopropionate ammonia lyase

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Diaminopropionate ammonia lyase (DAPAL) is a non-stereo specific fold-type II pyridoxal 5' phosphate (PLP) dependent enzyme that catalyzes the conversion of both D/L isoforms of the nonstandard amino acid Diaminopropionate (DAP) to pyruvate and ammonia. DAP is important for the synthesis of nonribosomal peptide antibiotics such as viomycin and capreomycin. Earlier structural studies on EcDAPAL bound to a reaction intermediate (aminoacrylate) suggested that the enzyme follows a two base mechanism, where Asp120 and Lys77 function as general bases to abstract proton from D-DAP and L-DAP respectively. A novel disulfide was observed near the active site, although its functional significance was not clear. In the present study, structural and biochemical characterization of active site mutants Asp120 (Asp120Asn/Ser/Thr/Cys) and Lys77 (Lys77His/ Thr/Ala/Val) of EcDAPAL has been carried out. Reduction of catalytic efficiency (Kcat/Km) of D120N EcDAPAL for D-DAP by 140 fold and presence of the uncatalyzed ligand at the active site in the crystal structure suggested that Asp120 indeed abstracts proton from D-DAP. Lys77, which was speculated to be important for proton abstraction from L DAP, however seemed to be crucial for PLP binding only. Presence of non-covalently bound PLP in the L77W mutant and occurence of both the ketoenamine, enolimine forms of internal aldimine in L77R mutant provided an in depth insight into the unique chemistry of internal aldimine formation in PLP dependent enzymes. To investigate the role of the novel disulfide bond near the active site, C265 and C291 were mutated to Serine. Studies on these mutants show that this disulfide bond gives additional stability to the protein and might regulate the entry of substrates to the active site. Thus, these studies provide deeper insights into the reaction mechanism of EcDAPAL, representing the overall reaction mechanism followed by several other fold-type II PLP pendent enzymes.

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