Structural and functional studies on Pyridoxal 5' phosphate-dependent lyases and aminotransferases

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The thesis describes structural and functional studies of two PLP-dependent enzymes, diaminopropionate (DAP) ammonia lyase (DAPAL) and N-acetylornithine aminotransferase (AcOAT). The main objective of this work was to understand the structural features that control and impart specificity for PLP-dependent catalysis.

DAPAL is a prokaryotic enzyme that catalyzes the degradation of D and L forms of DAP to pyruvate and ammonia. The first crystal structure of DAPAL was determined from *Escherichia coli* (*Ec*DAPAL) in holo and apo forms, and in complex with various ligands. The structure with a transient reaction intermediate (aminoacrylate-PLP azomethine) bound at the active site was obtained from crystals soaked with substrate, DL-DAP. Apo and holo structures revealed that the region around the active site undergoes transition from disordered to ordered state and assumes a conformation suitable for catalysis only upon PLP binding. A novel disulfide was found to occur near a channel that is likely to regulate entry of ligands to the active site. Based on the crystal structures and biochemical studies, as well as studies on active site mutant enzymes, a two base mechanism of catalysis involving Asp^{120} and Lys^{77} is suggested.

AcOAT is an enzyme of arginine biosynthesis pathway that catalyses the reversible conversion of N-acetylglutamate semialdehyde and glutamate to N-acetyl ornithine and α-ketoglutarate. It belongs to subgroup III of fold type I PLP dependent enzymes. Many clinically important aminotransferases belong to the same subgroup and share many structural similarities. We have carried out extensive comparative analysis of these enzymes to identify the unique features important for substrate specificity. Crystal structures of AcOAT from *Salmonella typhimurium* were determined in presence of two ligands, canaline and gabaculine, which are known to act as general inhibitors for most of the enzymes of this class. There structures provided important insights into the mode of binding of the substrates. The structures illustrated the switching of conformation of an active site glutamate side chain on binding of the two substrates. In addition to that, structural transitions involving three loop regions near the active site were observed in different ligand bound structures. Kinetics of single turnover fast reactions and multiple turnover steady state reactions indicated that N-AcOAT dimer might follow a

mechanism involving sequential half site reactivity for efficient catalysis. The changes observed in loop conformation that resulted in asymmetric forms of the dimer enzyme might form the structural basis for half site reactivity. Single site mutants were designed to understand the significance of these structural transitions and the specific role of active site residues in determining substrate specificity and catalysis. Biochemical characterization of wild type and mutant enzymes by steady state and fast kinetic studies, along with their crystal structures provided detailed insights into subtlety of active site features that manifest substrate specificity and catalytic activity.

The thesis also describes the investigations on fold type II enzymes directed towards analyses of polypeptide folds of these enzymes, features of their active sites, nature of interactions between the cofactor and the polypeptide, oligomeric structure, catalytic activities with various ligands, origin of specificity and plausible regulation of activity. Analysis of the available crystal structures of fold type II enzymes revealed five different classes. The dimeric interfaces found in these enzymes vary across the classes and probably have functional significance.

Contributions made towards structural and functional studies of three other PLP-dependent enzymes, serine hydoxymethyltransferase (SHMT), D-serine deaminase (DSD) and D-cysteine desulfhydrase (DCyD) are described in an appendix.