Structural and Biochemical Studies of Channeling of Unstable Intermediates in Biological Pathways

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In this work we use biochemical analysis in conjunction with X-ray crystallography to understand the mechanism of catalysis and channeling in the mulitdomain bi-functional enzyme PurL (StPurL). StPurL, is a 140 kDa four subunit protein in the purine de nova biosynthetic pathway. PurL catalyzes the fourth step in this pathway and is responsible for conversion of formylglycinamide ribonucleotide (FGAR) and glutamine into formylglycinamidine ribonucleotide (FGAM) and glutamate in an ATP assisted fashion. The bifunctional PurL enzyme has a gene duplication in it, one of these gene duplicated subunits contain a potential allosteric adenosine di-phosphate (ADP) regulatory site and the other site is where the ammonia is channeled from the glutaminase domain and FGAR is converted to FGAM. Here in an effort to identify the path traversed by ammonia and to understand the role of structural ADP molecule, we engineered mutants along the proposed ammonia channel using site-directed mutagenesis (overlap PCR method) and subsequently probe the transfer using biochemical assay and X-ray Crystallography. We are also trying to identify the xenon binding residues by passing the xenon into the StPurL which will give an idea to find out ammonia channel. We have solved the StPurL-AMPPNP (analogue of ATP) complex structure at resolution of 3.3Å. This complex shows structural similarity to TmPurL-AMPPCP/TmPurL-ATP complexes and also has some differences in ligand binding site.