USE OF SITE-DIRECTED MUTAGENESIS IN THE UNDERSTANDING OF THE CATALYTIC ACTIVITY OF THE HUMAN SACCHAROPINE DEHYDROGENASE (SDH) ENZYME.

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The Saccharopine pathway is the main degradation pathway of Lysine in plants and animals. The first gene of the pathway, AASS, encodes the bifunctional enzyme Aminoadipate-Semialdehyde Synthase, responsible for the conversion of Lysine in Aminoadipate Semialdehyde (AASA) via the intermediate Saccharopine. AASA is then oxidized to Aminoadipic Acid by AASA Dehydrogenase enzyme (ALDH7A1). Defects in this enzyme causes Piridoxine Dependent Epilepsy, resulted from toxic accumulation of AASA and its cyclic form piperideine-6-carboxylate (P6C). Recently our group have solved the structure of the Saccharopine Dehydrogenase (SDH) domain of human AASS enzyme co-crystallized with NAD+. The current project aims to unravel the catalytic mechanism of SDH and to understand its biochemistry by performing site-directed mutagenesis of residues potentially related to catalysis. Firstly we used the tridimensional structure of human SDH to select, by computational biology tools, residues which were establishing contacts with NAD and/or Saccharopine molecules and which were found as conserved in different organisms. The selected residues (Y105, Y127, D153, W201 and R275) were mutated to Alanine using Q5® Site-Directed Mutagenesis Kit. Potential mutant clones were screened by Sanger sequencing using the ABI3730 platform. Final contig sequences were obtained using the softwares Phred, Phrap and Consed. Mutations were validated by alignment of the potential mutant and wild type SDH sequences and manual curation of the chromatograms. The mutated SDH sequences are cloned in the pNIC28 plasmid, which allows heterologous expression of 6x-his tagged proteins in E. coli BL21(DE3). We have chosen the strain Rosetta which has proven to be successful in expression of the pNIC28-SDH wild type protein. Next steps will be expression and purification of mutant constructs. We expect to see reduced activity in case the mutated residue is important for catalysis. This project will provide basis for the understanding of the activity and catalytic site of Saccharopine Dehydrogenase (SDH).