Structure-function studies with Δ1-pyrrolin-5-carboxylate synthetase (P5CS), a key bifunctional player in amino acid biosynthesis, inborn disease and stress resistance.

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Δ^1-Pyrrolin-5-carboxylate synthetase (P5CS), a bifunctional enzyme, catalyzes in multicellular eukaryotes the first two steps of proline synthesis. These steps are also the first of ornithine synthesis in animals. In plants, P5CS is very important for stress resistance and is feed-back inhibited by proline. In mammals one isoform is inhibited by ornithine. In humans, P5CS deficiency (P5CSD) is a rare inborn error associated with either a cutis laxa/developmental delay/metabolic phenotype or with a presentation of complicated spastic paraplegia, and exhibits both recessive or dominant inheritance depending on the mutation. We determined the crystal structure of the enzyme that corresponds in bacteria to the glutamate-5-kinase domain and the structural mechanism of its inhibition by proline. We identified the first dominant human mutation in P5CS deficiency and were centrally involved in reporting the spastic paraplegia phenotype. We now try to understand the double phenotype and dual type of inheritance of the human deficiency on the basis of a hypothesis of negative dominance.

We have produced recombinantly the human and the *Arabidopsis thaliana* enzymes, developed assays to determine their enzyme activities and substrate and inhibition kinetics, characterized the oligomeric structure of the human enzyme by showing that it is a basic dimer that associates at least into tetramers in equilibrium with higher oligomers in a concentration-dependent way, and made site directed mutagenesis studies to test the effects of the clinical mutations. Attempts at structure determination have failed thus far from the crystallographic side, but they are yielding clear-cut results by single particle electron microscopy. Our findings (still in progress at the time of writing) will be used to discuss the negative dominance hypotyhesis for explaining the dual inheritance of the deficiency. *Grants BFU-2014-58229-P and PrometeoII/2014/029 (Spanish and Valencian Govertments).*