Marco Malatesta XXXIII Cycle
Biotechnologies and life sciences

Research project

"Characterization of the complete taurine biosynthesis pathway in Sauropsida"

Research activity

During my second year of PhD I continued my research activity on taurine biosynthesis pathway of *Gallus gallus.* Last year I have identified the gene encodes cysteine lyase (CL), a PLP-dependent enzyme discovered sixty years ago, when its activity was caught in the yolk sac of developing chicken embryos. CL acts with a beta replacement of H_2S of L-cysteine with sulphite ion to form cysteic acid (CA), an amino sulfonate compound. This enzymatic activity suggests a new alternative pathway in *Gallus gallus* and likely in the other sauropsids, that probably has a second reaction (decarboxylation) leading to taurine production. In these months I have wondered which enzyme could be responsible for this final reaction of sauropsids taurine biosynthesis. So far there isn't a known specific enzyme that catalyses this kind of reaction in BRENDA or other enzymes database. There's only one enzyme almost ubiquitous in Amniota that also require PLP as a cofactor called CSAD (Cysteine Sulphinic Acid Decarboxylase) that has cysteine sulphinic acid (CSA) as substrate (a very similar compound compared to CA with a sulphinic group ($C\text{-}SO_2$) instead a sulfonic one ($C\text{-}SO_3$))with formation of hypotaurine, involved in the classical pathway for taurine biosynthesis in mammals, including *Homo sapiens.* Precisely HsCSAD has a decarboxylating activity also with CA as substrate, according to previous studies that show a slighter CAD activity than CSAD one. Since there isn't any gene in *Gallus gallus* that could be a putative GgCAD, I have decided to express, purify and assay the corresponding CSAD ortholog present in *Gallus gallus* as best candidate of CAD enzyme. I've obtained a fairly pure protein to do a 1H NMR assay to test the activity. Surprisingly, GgCSAD converts CA into taurine after few minutes, while the conversion of CSA in hypotaurine is very low and it stopped after few minutes. Reaction with both substrates causes a very high inhibition of CA decarboxylation due to the presence of CSA.

Evidently, GgCSAD has undergone a change which allows it to decarboxylate CA with higher specificity than CSA; it's less clear which mutations are responsible to this different activity between *Gallus* and *Homo*. I decided to confirm HsCSAD activity through the same strategies used for GgCSAD previously optimized, and to understand better the phylogenesis of this gene, I decided to express also CSAD of

Danio rerio, an organism that belongs to an evolution line that seceded before separation between Sauropsida (reptils and birds) and Synapsida (mammals). HsCSAD and DrCSAD purified enzymes show a specificity for CSA instead CA. They are both able to convert CA in taurine with less efficiency than CSA in hypotaurine. It confirms that GgCSAD has undergone some mutations after the separation of Sauropsida and Synapsida, more or less at the same time of CBS duplication that gave rise to CL in sauropsids. Analysing the aminoacidic sequences of these three orthologs, there isn't apparently any substitution of the residues which coordinate PLP or take part to the active site cave. Therefore, I have extended the analysis of other amino acids that are close by active site residues. There are two substitutions that are specifically conserved in two distinct groups of sauropsids and non-sauropsids (mammals including *Danio rerio* that has the same activity as demonstrated). These are residues surrounding the arginine which recognizes sulphur group of CA and CSA. I have expressed three different mutants of GgCSAD (Q467V, T470A, double mutant) that replace its native residues with corresponding HsCSAD ones. The activity of these replacements justifies the substrate specificity change only to some extent, especially double mutant can convert CSA and CA with comparable efficiency. Thus, we can't with these substitutions change completely the specificity of *Gallus gallus* for CA into CSA as in *Homo sapiens*, but they can explain only partly the different activities.