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Molecular characterization and docking dynamics simulation prediction of cytosolic OASTL switch cysteine and mimosine expression in *Leucaena leucocephala*

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Abstract

Out of twenty common protein amino acids, there are many kinds of non protein amino acids (NPAAs) that exist as secondary metabolites and exert ecological functions in plants. Mimosine (Mim), one of those NPAAs derived from *L. leucocephala* acts as an iron chelator and reversely block mammalian cell cycle at G1/S phases. Cysteine (Cys) is decisive for protein and glutathione that acts as an indispensable sulfur grantor for methionine and many other sulfur-containing secondary products. Cys biosynthesis includes consecutive two steps using two enzymes-serine acetyl transferase (SAT) and O-acetylserine (thiol)lyase (OASTL) and appeared in plant cytosol, chloroplast, and mitochondria. In the first step, the acetylation of the β-hydroxyl of L-serine by acetyl-CoA in the existence of SAT and finally, OASTL triggers α, β -elimination of acetate from OAS and bind H_2S to catalyze the synthesis of Cys. Mimosine synthase, one of the isozymes of the OASTLs, is able to synthesize Mim with 3-hydroxy-4-pyridone (3H4P) instead of H_2S for Cys in the last step. Thus, the aim of this study was to clone and characterize the cytosolic (Cy) OASTL gene from *L. leucocephala*, express the recombinant OASTL in *Escherichia coli*, purify it, do enzyme kinetic analysis, perform docking dynamics simulation analysis between the receptor and the ligands and compare its performance between Cys and Mim synthesis. Cy-OASTL was obtained through both directional degenerate primers corresponding to conserved amino acid region among plant Cys synthase family and the purified protein was 34.3KDa. After cleaving the GST-tag, Cy-OASTL was observed to form mimosine with 3H4P and OAS. The optimum Cys and Mim reaction pH and temperature were 7.5 and 40^0C, and 8.0 and 35^0C respectively. Michaelis constant (Km) values of OAS from Cys were higher than the OAS from Mim. Inter fragment interaction energy (IFIE) of substrate OAS-Cy-OASTL complex model showed that Lys, Thr81, Thr77 and Gln150 demonstrated higher attraction force for Cys but 3H4P-mimosine synthase-OAS intermediate complex showed that Gly230, Tyr227, Ala231, Gly228 and Gly232 might provide higher attraction energy for the Mim. It may be concluded that Cy-OASTL demonstrates a dual role in biosynthesis both Cys and Mim and extending the knowledge on the biochemical regulatory mechanism of mimosine and cysteine.

Keywords: non protein amino acids, mimosine, cysteine, O-acetylserine (thiol) lyase

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