Title

Molecular Characterization of Cysteine synthase in *Mimosa pudica* and *Leucaena leucocephala*

By

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Abstract

In the cysteine (Cys) and mimosine biosynthesis process, *O*-acetyl-L-serine (OAS) is the common substrate. In the presence of *O*-acetylserine(thiol)lyase (OASTL, Cys synthase) the reaction of OAS with sulfide produces Cys, while with 3-hydroxy-4-pyridone (3H4P) produces mimosine. The enzyme OASTL can either catalyze Cys synthesis or both Cys and mimosine. A cDNA for cytosolic OASTL was cloned from *M. pudica* for the first time containing 1410 bp nucleotides.

The purified protein product from overexpressed bacterial cells produced Cys only, but not mimosine, indicating it is Cys specific. Kinetic studies revealed that pH and temperature optima for Cys production were 6.5 and 50 °C, respectively. The measured K_m, K_{cat}, and $K_{cat}\ K_m^{-1}$ values were 159 ± 21 μM, 33.56 s^{-1}, and 211.07 $mM^{-1}s^{-1}$ for OAS and 252 ± 25 μM, 32.99 s^{-1}, and 130.91 $mM^{-1}s^{-1}$ for Na_2S according to the *in vitro* Cys assay. The Cy-OASTL of *M. pudica* is

specific to Cys production, although it contains sensory roles in sulfur assimilation and the reduction network in the intracellular environment of *M. pudica*.

In higher plants, multiple copies of the Cys synthase gene are present for Cys biosynthesis. Some of these genes also have the potential to produce various kinds of *β*-substitute alanine. In the present study, we cloned a 1275 bp cDNA for Cy-OASTL from *L. leucocephala*. The purified protein product showed a dual function of Cys and mimosine synthesis. Kinetics studies showed pH optima of 7.5 and 8.0, while temperature optima of 40 ^{0}C and 35 ^{0}C, respectively for Cys and mimosine synthesis. The kinetic parameters such as K_m, k_{cat}, and k_{cat} K_m^{-1} were determined for both Cys and mimosine synthesis with substrates OAS and Na_2S or 3H4P. From the results with the common substrate OAS, k_{cat} for Cys production is over six-fold higher than mimosine synthesis and the K_m is 3.7 times lower, suggesting Cys synthesis is the favored pathway.

On the basis of molecular modeling and simulation, a plausible mimosine biosynthetic pathway is proposed. Using the known crystal structures of *A. thaliana O*-acetylserine sulfhydrylase K46A mutant and *β*-cyanoalanine synthase K95A mutant in soybeans as a model, we have predicted the *α*-aminoacrylate-complex in the active site of mimosine synthase. A three-dimensional structure of mimosine synthase's active site harboring the *α*-aminoacrylate intermediate was created, and the complex was docked with 3H4P. The PAICS calculation between *α*-aminoacrylate and the mimosine synthase-3H4P complex showed strong attractive forces between amino acids Lys 49, Asn 80, Arg 303, Arg 108 and Arg 51. In addition, the interaction calculation between 3H4P and the mimosine synthase- *α*-aminoacrylate complex also revealed strong attractive forces between amino acids Gly230, Tyr227, Ala231 and Gly 228 which reside within 3Å.

Cysteine biosynthesis is directed by the successive commitments of serine acetyltransferase, and *O*-acetylserine(thiol)lyase (OASTL) compounds, which subsequently frame the decameric cysteine synthase complex. The isoforms of OASTL are found in three compartments of the cell: the cytosol, plastid, and mitochondria. In this investigation, we first isolated chloroplastic OASTL (Ch-OASTL) from *Leucaena leucocephala*, and the Ch-OASTL was then expressed in BL21-competent *E. coli*. The Ch-OASTL cDNA clone had 1,543 base pairs with 391 amino acids in its open reading frame and a molecular weight of 41.54 kDa. The purified protein product exhibited cysteine synthesis ability, but not mimosine synthesis activity. However, they both make the common α-aminoacrylate intermediate in their first half reaction scheme with the conventional substrate *O*-acetyl serine (OAS). Hence, we considered Ch-OASTL a cysteine-specific enzyme. Kinetic studies demonstrated that the optimum pH for cysteine synthesis was 7.0, and the optimum temperature was 40 °C. In the cysteine synthesis assay, the K_m and K_{cat} values were 838 ± 26 μM and 72.83 s^{-1} for OAS, respectively, and 60 ± 2 μM and 2.43 s^{-1} for Na_2S, respectively. We can infer that Ch-OASTL is necessary for the survival of *L. leucocephala* plants. Its regulatory role is considered a sensor for sulfur constraint conditions, and it acts as a forerunner of various metabolic compound molecules.

In conclusion, these outcomes impart real perception on the unique nature of cysteine synthase on the biosynthesis process of cysteine and mimosine in *M. pudica* and *L. leucocephala* and give us a new outlook of S-assimilation network of plants.