（学位第３号様式）

# 学 位 論 文 要 旨

| | |
|---|---|
| 氏　名 | Shigeki Oogai |
| 題　目 | Studies on synthesis and degrading enzymes of non-protein amino acid mimosine<br>(非タンパク性アミノ酸ミモシンの合成・分解系酵素に関する研究) |


Mimosine is a non-protein amino acid that occurs only in the species *Leucaena leucocephala* (*Ll*) and *Mimosa pudica* (*Mp*), which belong to the subfamily Mimosoideae. The enzymes of mimosine synthesis and degrading activity requires pyridoxal-5′-phosphate (PLP) as cofactor thereby are classified as members of PLP dependent enzymes. However, there are scarece information on their reaction mechanism and molecular evolution. Furthermoe, various toxic biological activities of mimosine have been reported, which include the following: eukaryotic cell cycle arrest at the G1/S phase by the inhibition of DNA polymerase; chelation of bivalent metal ions, thereby limiting the availability of ionic cofactors for a number of enzymes and thus resulting in the inhibition of several important biological processes ; and inactivation of PLP-dependent enzymes by the formation of a stable complex with PLP. Because of these toxicities of mimosine, the use of *Ll* foliage for livestock feed has been hindered. Thus the object of this study was twofold: to get into molecular mechanism of the enzyme reactions and evolution; to screen an bacteria possessing strong mimosinase activity and to eliminate toxic mimosine in the *Ll* foliage.

Isoforms of cysteine synthase are found in three compartments of the cell: the cytosol, plastid, and mitochondria. Our previous study demonstrated that cytosolic cysteine synthase showed dual function of cysteine and mimosine synthesis, and is responsible for the biosynthesis of mimosine. In this investigation, we isolated putative chloroplastic cysteine synthase from *Ll*, and found that the purified recombinant enzyme exhibited cysteine synthesis ability, but not mimosine synthesis activity. The results showed that the chloroplastic enzyme is not involved in mimosine biosynthesis in this plant.

Mimosinase is thought to have evolved from cystathionine β-lyase (CBL) via gene duplication. However, no study has, to date, examined the molecular evolution of mimosinase from CBL. This study cloned mimosinase and CBL from the Mimosoideae subfamily member *Mp* and explored the molecular evolution of mimosinase. The homology modeling and molecular simulation of these enzymes predicted variations in the residues that interact with substrates and a closer coordination of the residues that interact with mimosine at the active site compared with cystathionine. This result suggested that alteration in active site pocket size is responsible for the diversification from CBL into mimosinase.

The screening of soil of *Ll* tree surroundings led to isolation of *Arthrobacter* sp. Ryudai-S1 that can degrade and assimilate mimosine as nitrogen source. Mimosine degrading enzyme in this strain showed strong activity in the ever-known mimosinase. Inspection of docking model and calculation of interaction energy between mimosine-PLP complex found several amino acid residues stabilizing the binding and playing an critical role in guiding the substrate to proper positions to accomplish high catalytic efficacy and selectivity.

These results provide new insights into the molecular mechanism of PLP-dependent enzyme reaction and evolution and also into the development of *Ll* feed with reduced mimosine content.