

STUDIES ON ENZYMES INVOLVED IN CANNABINOID BIOSYNTHESIS

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Marijuana contains unique secondary metabolites called cannabinoids. Among them, THC, the psychoactive component, and CBD, an isomer of THC, are regarded as medicines in USA and some European countries, because they show valuable pharmacological activities. Previous research has demonstrated that THC and CBD generate from their precursors, THCA and CBDA, via non-enzymatic decarboxylation in post-harvest materials of *Cannabis sativa*, and that *Cannabis* plants are classified into two phenotypes, THCA strain and CBDA strain. In this study, I have investigated the biosynthetic mechanisms of cannabinoids including THCA and CBDA.

Purification of biosynthetic enzymes : It had long been believed that THCA is biosynthesized from CBDA by isomerization, however I could not detect enzyme activity catalyzing this reaction. Contrary, THCA synthase, a novel enzyme that catalyzes oxidative cyclization of CBGA, has been successfully purified from THCA strain. In addition, further study using CBDA strain has identified and purified CBDA synthase that produces CBDA from CBGA by reaction similar to that of THCA synthase. Biochemical characterization of purified cannabinoid synthases has revealed that these enzymes can complete the biosynthetic reactions without the need for any coenzymes.

Molecular characterization : Degenerate PCR primers have designed from the partial amino acid sequences of purified THCA synthase and CBDA synthase. RT-PCR using degenerate and specific primers has amplified the two cDNAs encoding respective cannabinoid synthases. Sequence analysis has demonstrated that these cannabinoid synthases have 84 % amino acid identity to each other, suggesting that these enzymes have evolved from common ancestor via changes in amino acids regulating the cyclization reactions. Biochemical analyses with the recombinant enzymes over-expressed in insect cell cultures have proved that both enzymes oxidize the substrate CBGA using FAD that is covalently bound to them as a coenzyme.

Localization of biosynthetic enzyme : This study has shown that THCA synthase localizes in the secretory cavity of glandular trichomes that cover the surface of *C. sativa*, and that THCA is a cytotoxic metabolite. Therefore, it is reasonable that *Cannabis* plants secrete THCA synthase into secretory cavity, a non-cellular compartment, to prevent the cellular damage as well as for the self-defense to predators.

As described, I have studied enzymes involved in cannabinoid biosynthesis. I thank to Profs. Yukihiro Shoyama and Satoshi Morimoto, the supervisors of this study. I also thank to Dr. Hiroyuki Tanaka, and co-workers in the lab. of plant resources regulation, Kyushu University.

