【Grant-in-Aid for Scientific Research(S)】
Biological Sciences (Agricultural sciences)

Title of Project : Comprehensive elucidation and application of the function of enzymes involved in cleavage and synthesis of a carbon-nitrogen bond

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Research Area : Applied Biochemistry, Applied Microbiology
Keyword : Enzyme, Reaction

【Purpose and Background of the Research】

A lot of studies on proteases, which act on a peptide bond, have widely been carried out. But there have been less studies on non-proteolytic enzymes involved in the cleavage and synthesis of a carbon-nitrogen bond. At the protein and gene levels, we have investigated the non-proteolytic enzymes involved in the cleavage of a carbon-nitrogen triple bond or a carbon-nitrogen single bond. We discovered several new enzymes from microorganisms and identified active sites (e.g., active amino acid residues) in the enzymes. So far unknown carbon-nitrogen-bond-cleaving enzymes still exist in nature.

In the present study, focusing on our original carbon-nitrogen-bond-cleaving enzymes, new carbon-nitrogen-bond-cleaving enzymes and carbon-nitrogen-bond-synthesizing enzymes, we aim at biochemical elucidation of their functions and structures. We also try to develop a basis for the production of useful compounds, utilizing the information obtained through the above fundamental studies.

【Research Methods】

Not only so far known carbon-nitrogen-bond-cleaving and synthesizing enzymes (e.g., aldoxime dehydratase, isonitrile hydratase, and *N*-substituted formamide deformylase) but also unknown enzymes will be investigated in detail. Analyses of their enzymological and physiochemical properties by means of spectral measurements, etc will be carried out. By utilization of the structural information obtained and analysis of mutant enzymes, enzymes with new function can be constructed and developed for the production of useful compounds through versatile enzyme reactions, which are different from the original reaction.

【Expected Research Achievements and Scientific Significance】

The functions of many biologically active molecules require the presence of carbon-nitrogen bonds at strategic positions. The carbon-nitrogen bond is of considerable importance in life. Identification of the active site in non-proteolytic enzymes involved in the cleavage and formation of this bond will certainly facilitate elucidating its unique specificity for the substrate as well as its reaction mechanism, and subsequently help us to understand its physiological functions. This study will make a significant contribution to the body of basic knowledge on these unique enzymes and will allow us to design a new process for the production of useful compounds.

【Publications Relevant to the Project】

1) Abe, T., Hashimoto, Y., Hosaka, H., Tomita-Yokotani, K & Kobayashi, M. "Discovery of amide (peptide) bond synthetic activity in acyl-CoA synthetase" *J. Biol. Chem.*, 283, 11312-11321 (2008)
2) Konishi, K., Ohta, T., Oinuma, K-I., Hashimoto, Y., Kitagawa, T. & Kobayashi, M. "Discovery of a reaction intermediate of aliphatic aldoxime dehydratase involving heme as an active center" *Proc. Natl. Acad. Sci. USA*, 103, 564-568 (2006)
3) Goda, M., Hashimoto, Y., Shimizu, S. & Kobayashi, M. "Discovery of a novel enzyme, isonitrile hydratase, involved in nitrogen-carbon triple bond cleavage" *J. Biol. Chem.*, 276, 23480-23485 (2001)

【Term of Project】 FY2011-2015

【Budget Allocation】 148,500 Thousand Yen

【Homepage Address and Other Contact Information】

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