SUMMARY OF Ph.D. DISSERTATION

School	Student Identification Number	SURNAME, First name
Fundamental Sciences and Technology		TERAO Yosuke

Title

Modification of the function of arylmalonate decarboxylase

Abstract

Enzymes and microorganisms are becoming more useful and important for biotransformation and bioprocesses in not only industry but also many other fields as the solution for the recent environmental problems, because enzymes have high selectivity and are friendly to the environment.

Arylmalonate decarboxylase (AMDase) is a unique and useful enzyme originated from a microorganism in soil, and catalyzes enantioselective decarboxylation of α-arylmalonate derivatives to give optically pure α-arylpropionates.

In this thesis, I would like to show the results of modification of the enzymatic functions of AMDase by the introduction of mutation(s) utilizing biotechnology based on the comparison of the reaction mechanism and homology with other enzymes. Also, the promiscuity of AMDase was examined via mutagenesis studies and evaluation of the properties of the resulting enzyme.

In the first section, the synthesis of both of the enantiomers of biologically active compound flurbiprofen using AMDase and its mutant were reported.

In section 2, the details of the studies on the inversion of the enantioselectivity of AMDase were described. It was established by the introduction of only two mutations at the amino acid residues located in the active site, based on the comparison of homology and the consideration of the reaction mechanism.

In section 3, it was reported that the enzymatic activity of the above double mutant AMDase was improved by random mutagenesis coupled with high-throughput screening and the resulting triple mutant exhibited ten-fold higher activity than compared to the original double mutant.

In section 4, the enzymatic promiscuity of AMDase was described. When single mutation was introduced at the amino acid residue in the active site, AMDase was endowed a new catalytic activity, *i.e.*, racemase activity, which catalyzes racemization of α-arylcarboxylic acids.

In the final section, another catalytic promiscuity was demonstrated that AMDase could catalyze aldol-type reaction of the well-designed substrate.