SUMMARY OF Ph.D. DISSERTATION

School Fundamental Science and Technology	Student Identification Number	SURNAME, First name KATO, Dai-ichiro

Title

Microbial Deracemization of α-Substituted Carboxylic Acids

Abstract

Deracemization is a novel and unique method for the preparation of optically active compounds starting from the corresponding racemates. The equal amounts of two enantiomers in the reaction mixture result in shifting to 100:0 ratio by the progress of the reaction. In addition, no separation steps of the product and starting material are necessary because the chemical structure of the product is the same except for its absolute configuration. Theoretically, this reaction is capable of giving the desired enantiomer in 100% yield and it means that the synthesis of racemate is almost equal to the preparation of the optically active compounds. Deracemization is the novel and useful method to overcome the drawback of kinetic resolution technique; i.e., the maximum yield of the desired enantiomer is theoretically limited to 50% and the tedious separation steps are required after the reaction.

In the dissertation in the candidacy for the degree of Doctor of Philosophy, I have investigated the deracemization of two types of α-substituted carboxylic acids, i.e., α-methyl carboxylic acids and α-amino acids, aiming at establishing the efficient method for the preparation of optically active compounds and clarifying the reaction mechanism.

In the research field of the deracemization of α-methyl carboxylic acids, *Nocardia diaphanozonaria* JCM 3208 and *Brevibacterium ketoglutamicum* KU 1073 were used mainly. I have established the reaction conditions to increase the deracemization activity in preference to side reactions. In addition, through the mechanistic investigations using inhibitors and deuterated compounds, I have disclosed that deracemization process is realized by the combination of three enzymatic reactions. This type of reaction is involved a racemization reaction of an intermediate which was derived from one enantiomer. Thus I would like to propose to call this type of biotransformation as enantioselective racemization. In the case of *B. ketoglutamicum*, I could purify an enzyme which was estimated to catalyze the enantioselective thioesterification.

In the research field of the deracemization of α-amino acids, many kinds of microorganisms having the deracemization activity toward phenylalanine could be obtained through efficient screening techniques. From the investigation using whole cell systems as well as cell-free systems of *N. diaphanozonaria*, *Sinorhizobium meliloti* ATCC 51124, and *Pseudomonas* sp. No. 9, it was revealed that deracemization was achieved by the enantioselective inversion of configuration process by way of α-keto acid as an intermediate. I could also establish the reaction conditions utilizing cell free systems to proceed the deracemization of phenylalanine and phenylglycine in the presence of artificial cofactors. In addition, I have constructed the recombinant *E. coli* having the deracemization activity. This transformant had a foreign aminotransferase gene derived from *S. meliloti*, and had the ability to deracemize DL-4-chlorophenylalanine in high efficiency and enantiomerically pure L-form was obtained in a quantitative yield.