NISR RESEARCH GRANT
2002 Research Grant

Analysis of Mechanism for Regulation of Novel Lysine Biosynthesis in *Thermus* for Development of Novel Lysine Production System

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Research aims

We found that the extremely thermophilic bacterium, *Thermus thermophilus* HB27 synthesizes lysine via non-diaminopimelate pathway (α-aminoadipate pathway). α-Aminoadipate pathway is known to be present only in lower eukaryotes, such as fungi and yeast, and *Thermus* lysine pathway proceeds in the same way until α-aminoadipate synthesis. However, the synthesis from α-aminoadipate to lysine in *Thermus* pathway is different from the pathway in lower eukaryote with saccharopin as a biosynthetic intermediate, and therefore the pathway is novel as a whole. The reactions from 2-oxoglutarate to α-aminoadipate in the pathway is similar to those from 2-oxoisovalerate to leucine in leucine biosynthesis and from oxaloacetate to 2-oxoglutarate in the tricarboxylic acid cycle. Furthermore, the reactions from α-aminoadipate to lysine proceeds in a way similar to those from glutamate to ornithine in arginine biosynthesis, and some reactions in biosynthesis of proline and aspartate group amino acids. Thus, the lysine biosynthesis is evolutionarily related to other biosynthetic or metabolic pathways. These observations suggest that the lysine biosynthesis or its ancestral pathway had a definitive role in developing biological system in the early stage of evolution. This study aims at i) elucidation of the substrate specificity, reaction mechanism, and feedback regulation system of the newly found lysine biosynthesis of *T. thermophilus*, and ii) establishment of the base for new lysine fermentation process.

Methods

We have developed the system to express the genes involved in the lysine biosynthesis from *T. thermophilus* HB27 in *Escherichia coli* cells. After purification of the enzymes, their catalytic properties were analyzed by using several related compounds; those are intermediates in arginine and leucine biosynthesis and in the tricarboxylic acid cycle. Based on the information on the primary and tertiary structures of related enzymes, amino acid residues that determine substrate specificity were chosen as candidates, and their contribution was analyzed by site-directed mutagenesis.

Results

Our previous studies revealed that lysine is synthesized from 2-oxoglutarate through ten steps of the reactions. In the present study, we have tried to clone two genes, homoisocitrate dehydrogenase converting homoisocitrate to α-ketoadipate and α-aminoadipate aminotransferase catalyzing the following reaction to produce α-aminoadipate, which have not yet been cloned. Since the former gene was expected to have the amino acid sequence homologous to those of isocitrate dehydrogenase and isopropylmalate dehydrogenase, we used degenerate primers designed to correspond to internal sequences for PCR cloning. The latter gene was also cloned by PCR based on the primary sequence of the mammalian enzyme that was reported to have ability to use α-aminoadipate as a amino donor. We generated *Thermus* mutants carrying disruption of both the genes and examined their auxotrophy. Disruptant of homoisocitrate dehydrogenase gene exhibited complete lysine-auxotrophic phenotype and disruptant of α-aminoadipate aminotransferase gene showed partial lysine-auxotrophic phenotype, on minimal medium. Homoisocitrate dehydrogenase produced in *E. coli* cells were characterized by using homoisocitrate or its structurally related compounds. We have succeeded in identification of amino acid residues that determine the substrate specificity by site-directed mutagenesis based on the three dimensional structures of isocitrate dehydrogenase and isopropylmalate dehydrogenase, both of which share reaction mechanism and substrate recognition mechanism with homoisocitrate dehydrogenase. As to α-aminoadipate aminotransferase, we are obtaining preliminary results that the enzyme has branched chain amino acid aminotransferase activity, though no significant identity in amino acid sequence is observed between *Thermus* α-aminoadipate aminotransferase and branched chain amino acid aminotransferase.

It is well known that an enzyme responsible for the first reaction in amino acid biosynthetic pathway is regulated via feedback inhibition by end product of the pathway. We therefore analyzed homocitrate synthase that catalyzes the first reaction in the lysine biosynthesis through α-aminoadipate, and found that the enzyme was inhibited by lysine with high sensitivity (K_i=9.4 μM). Homocitrate synthase could not recognize 2-oxoisovalerate as a substrate though the synthase has the amino acid sequene similar to

that of isopropylmalate dehydrogenase. However, the enzyme could utilize oxaloacetate as a substrate, although no sequence homology was found between homocitrate synthase and citrate synthase. This suggests that homocitrate synthase also serves as citrate synthase in *Thermus* cells under some circumstance.

Conclusion

We have succeeded in cloning all the genes involved in lysine biosynthesis from *T. thermophilus* HB27, and have developed their efficient expression system in *E. coli*. Several information on the enzymatic properties including substrate recognition is being accumulated. We therefore think that we have successfully developed the base for lysine production through the studies. We will move on to establishment of lysine fermentation process based on the accumulated information.

Reference

Miyazaki, J., Kobashi, N., Nishiyama, M., and Yamane, H. Characterization of homoisocitrate dehydrogenase involved in lysine biosynthesis of an extremely thermophilic bacterium, *Thermus thermophilus* HB27, and evolutionary implication of β-decarboxylating dehydrogenase. *J. Biol. Chem.*, **278**, 1864–1871 (2003)