Studies on function and regulation of amino acid biosynthetic enzymes

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In this study, we focused on the biosynthetic pathways of amino acids and revealed the mechanisms of enzyme regulation and the novel biosynthetic machinery by using X-ray crystallography and biochemical analyses.

Aspartate kinase (AK) is an enzyme catalyzing the first step of lysine biosynthetic pathway and is regulated by the end-product in a feedback manner. AK from *Corynebacterium glutamicum* (CgAK), which has been used in the industrial production of lysine, is inhibited in the presence of both lysine and threonine. Since this regulation of CgAK is a disadvantage for the over-production of lysine, the mutant strain insensitive to the feedback inhibition has been developed and used. However, the mechanism of feedback inhibition had not been elucidated for a long time. Then, we determined the crystal structures of CgAK in an inhibitory form with inhibitors, and an active form with threonine, and also a structure of the mutant resistant to the feedback inhibition. The structural comparison clarified that the binding of inhibitors stabilizes the “closed” inhibitory form and that inhibitor-binding could not stabilize the “closed” conformation in a feedback-resistant mutant. The information of the regulatory mechanism of CgAK obtained in this study will contribute to more efficient production of lysine.

In a thermophilic bacterium, *Thermus thermophilus* uses a unique pathway for lysine biosynthesis, which uses an amino-group carrier protein named LysW. The amino group of α-aminoadipate (AAA), an intermediate in this pathway, is protected by the formation of an isopeptide bond with C-terminal glutamate residue in LysW. We determined the crystal structures of LysW conjugated with AAA, and LysZ, which is an amino acid kinase, in complex with LysW. As a result, negatively charged LysW interacted with the positively charged area of LysZ, showing that LysW functions as a protecting-group of the amino-group of AAA and also as a carrier protein, which is electrostatically recruited to each enzyme, enabling the efficient conversion of AAA to lysine. We also found that not only lysine but also ornithine could be produced by using LysW system in *Thermococcus kodakarensis* where a single set of bifunctional biosynthetic enzymes are involved. This result provided the experimental evidence for the evolutionary hypothesis, patchwork hypothesis, in metabolic pathways, suggesting that a pathway composed of multifunctional enzymes was used for the production of multiple compounds in an ancestral organism with a limited size of genome.

Recently, we are interested in the protein lysine acetylation on the metabolic enzyme, which is one of the posttranslational modifications of protein known as a new regulatory mechanism of metabolism. We conducted the proteomic analysis for *T. thermophilus* and determined the 208 acetylated proteins. Among the acetylated proteins identified, we found that 2-isopropylmalate synthase (IPMS) involved in a leucine biosynthesis is inhibited by the acetylation on a specific lysine residue. Our observation that the deacetylation of IPMS restored the activity suggested that IPMS is regulated by both the feedback inhibition by leucine and the protein acetylation. From this study, we provide a novel regulatory mechanism by the protein acetylation in amino acid biosynthesis.