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Crystallographic studies to elucidate of mechanism of substrate specificity in homoisocitrate dehydrogenase from an extreme thermophile, Thermus thermophilus.

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Introduction

There are two pathways for lysine biosynthesis known to date; diaminopimelate pathway and α-aminoadipate pathway. In the former pathway, lysine is biosynthesized from aspartate through L,L-diaminopimelate, while in the latter pathway, lysine is biosynthesized from 2-oxoglutarate through α-aminoadipate. It was known that most of bacteria and plants biosynthesize lysine by the former pathway, and only yeasts and fungi use the latter pathway for lysine biosynthesis. However, we showed that lysine is biosynthesized not through diaminopimelate but through α-aminoadipate in an extremely thermophilic bacterium, *Thermus thermophilus* [1]. In lysine biosynthesis in the bacterium, conversion of 2-oxoglutarate to α-aminoadipate is similar to that of 2-oxoisovalerate to leucine in leucine biosynthesis and similar to that of oxaloacetate to glutamate in glutamate biosynthesis, and conversion of α-aminoadipate to lysine is similar to that of glutamate to ornithine in arginine biosynthesis [2, 3]. Studies on the whole lysine biosynthesis in the bacterium leads us to elucidate not only biochemical characterization in the lysine biosynthesis, but also evolution of these related metabolic pathways.

Homoisocitrate dehydrogenase (HICDH) is the 3rd enzyme involved in lysine biosynthesis through α-aminoadipate. Although HICDH from *T. thermophilus* HB27 (TtHICDH) is essential for lysine biosynthesis and actually catalyzes β-decarboxylating dehydrogenation of the native substrate, homoisocitrate, the enzyme can utilize isocitrate which is the substrate of HICDH paralog, isocitrate dehydrogenase (ICDH), as a substrate with the efficiency about twenty-fold higher than that with homoisocitrate as a substrate [4]. We carried out mutational analysis and demonstrated that arginine 85 in TtHICDH is the most important residue for recognizing isocitrate as a substrate and replacement of the Arg85 to Val altered the enzyme to show the activity for 3-isopropylmalate that is a substrate of 3-isopropylmalate dehydrogenase (IPMDH) [4]. These results indicate that Arg85 directs substrate specificity in TtHICDH. Since we are interested in how TtHICDH recognizes substrate, we carried out crystallization of TtHICDH and the mutants. The data for X-ray diffraction was obtained using ADSC Quantum4R CCD detector at BL6A, PF and Q210 CCD detector in NW12 beamline, PF-AR.

Results

X-ray data collection

We obtained crystals of TtHICDH and the mutant, 7ScHICDH which utilizes only homoisocitrate as a substrate [4]. All data were collected at 100 K. Diffraction images were indexed, integrated, and scaled using the HKL2000 program. The crystal of TtHICDH belonged to space group $C222_1$ with unit cell dimensions of a = 60.96 Å, b = 143.24 Å, c = 177.85 Å. The crystal of 7ScHICDH belonged to space group $C222_1$ with unit cell dimensions of a = 61.25 Å, b = 143.59 Å, c = 178.51 Å. The data collected at the wavelength of 1.85Å and 1.91Å were used for subsequent molecular replacement and crystallographic refinement of TtHICDH and 7ScHICDH, respectively.

Crystal structure of TtHICDH and the mutant, 7ScHICDH

The crystal structure of TtHICDH and the mutant, 7ScHICDH were determined at 1.85Å and 1.91Å, respectively. Although we soaked homoisocitrate or isocitrate to a crystal, the determined structures did not contain the compounds. However, by comparing the crystal structure in the apo-form with those of substrate-bound IPMDH and ICDH, we could dipict outline of the mechanism showing substrate specificity in TtHICDH. Now, we are trying to obtain crystals which contained a substrate for the future crystallographic analysis.

References

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