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Analysis of mechanism for substrate recognition of homoisocitrate dehydrogenase from *Thermus thermophilus* HB27

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Introduction

Homoisocitrate dehydrogenase (HICDH) is the fourth enzyme involved in lysine biosynthesis through α-aminoadipate and is member of a family of β-decarboxylating dehydrogenases that includes isocitrate dehydrogenase (ICDH) in the tricarboxylic acid cycle, 3-isopropylmalate dehydrogenase (IPMDH) in leucine biosynthesis, and tartarate dehydrogenase in vitamin production. It has been suggested that those enzymes diverged from a common ancestral β-decarboxylating dehydrogenase.

We previously cloned, expressed the gene product as recombinant protein, and demonstrated that HICDH has HICDH activity. A knockout mutant of the gene showed an AAA-auxotrophic phenotype, indicating that the gene product is involved in lysine biosynthesis throuth AAA. We therefore named this gene hicdh, the gene product, did not catalyze the conversion of 3-isopropylmalate to 2-oxoisocaproate, a leucine biosynthetic reaction, but it did recognize isocitrate, related compound in the tricarboxylic acid cycle, as well as homocitrate as a substrate. It is of interest that HICDH catalyzes the reaction with isocitrate about 20 times more efficiently than the reaction with the putative native substrate, homoisocitrate. Site-directed mutagenesis study reveals that replacement of Arg85 with Val in HICDH causes complete loss of activity with isocitrate but significant activity with 3-isopropylmalate and retains activity with HICDH. These results indicate that Arg85 is a key residue for both substrate specificity and evolution of β-decarboxylating dehyrogenases.

Material and Methods

Overexpression of TtHICDH were performed as described previously (1). Crystallization was carried out by the hanging-drop method. Three microliters of 10-mg/ml purified TtHICDH was added to an equal volume of reserior solution containing 24% polyethylene glycol 400 (PEG400) and 0.1 M citrate, pH 4.8. Then, 3 μl of solution containing 100 mM $MgCl_2$, 5 mM isocitrate or homocitrate, and 1 μl of 50 mM of $CdCl_2$ was added to the drop. The mixture was equilibrated against 500 μl of reservoir solution at 20°C. Crystals were grown in 5 days, with a typical dimension of 0.5 mm by 0.5 mm by 0.05 mm.Before data collection, to avoid freezing, the crystals were transferred to a cryoprotectant solution containing 25% PEG400, 0.1 M $MgCl_2$, 5 mM $CdCl_2$, 0.1 M citrate, and 5 mM isocitrate or homoisocitrate.

Diffraction data were collected with an ADSC-Q210 charge-coupled-device camera on the NW12 station of PF-AR. Data were indexed, integrated, and scaled using the HKL2000 program. The structure were determined by molecular replacement method using the structure of the A172V mutant of IPMDH from *T. thermophilus* as a model. Model correction in the electron density map was performed with the XtalView protram. Refinement was carried out with CNS1.1.

Results and Discussion

The structure of HICDH was determined at 1.85-Å resolution. Arg85, which was shown to be a determinant for substrate specificity in our previous study, is positioned close to the putative substrate binding site and interacts with Glu122. Glu122 is highly conserved in the equivalent position in the primary sequence of ICDH and archeal 3-isopropylmalate dehydrogenase (IPMDH) but interacts with main- and side-chain atoms in the same domain in those paralogs. In addition, a conserved Tyr residue (Tyr125 in TtHICDH) which extends its side chain toward a substrate and thus has a catalytic function in the related β-decarboxylating dehydrogenases, is flipped out of the substrate-binding site. These results suggest the possibility that the conformation of the region containing Glu122-Tyr125 is changed upon substrate binding in TtHICDH. The crystal structure of TtHICDH also reveals that the arm region is involved in tetramer formation via hydrophobic interactions and might be responsible for the high thermotolerance. Mutation of Val135, located in the dimer-dimer interface and involved in the hydrophobic interaction, to Met alters the enzyme to a dimer (probably due to steric perturbation) and markedly decreases the thermal inactivation temperature. Both the crystal structure and the mutation analysis indicated that tetramer formation is involved in the extremely high thermotolerance of TtHICDH.

References

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