No. 55

Thermus thermophilus 由来ホモクエン酸合成酵素のフィードバック制御機構の解析

Mechanism of Feedback Inhibition of Homocitrate Synthase from *Thermus thermophilus*

岡田卓也 [1], 富田武郎 [1], 葛山智久 [1], 西山真 [1,2]

Takuya Okada[1], Takeo Tomita[1], Tomohisa Kuzuyama[1], Makoto Nishiyama[1,2]

([1]東京大学生物生産工学研究センター, [2]理研SPring-8)

([1]Biotechnology Research Center, The University of Tokyo, [2]RIKEN SPring-8)

e-mail: aa096093@mail.ecc.u-tokyo.ac.jp

Lysine is known to be biosynthesized by two completely different pathways. Fungi and yeast synthesize lysine through the α-aminoadipate (AAA) pathway, whereas most bacteria and plants synthesize lysine through the diaminopimelate (DAP) pathway. We found that *Thermus thermophilus* biosynthesizes lysine through the AAA pathway [1]. Homocitrate synthase (HCS), responsible for the first reaction in lysine biosynthesis through AAA, transfers the acetyl group from acetyl-CoA to α-ketogluterate (α-KG) to yield homocitrate and CoA. As commonly observed in enzymes responsible for the first reaction of amino acid biosynthesis, HCS from *T. thermophilus* (TtHCS) is feedback-inhibited by the end product, lysine. In this study, we analyzed the mechanism of feedback inhibition of TtHCS by crystallography.

α-Isopropylmalate synthase (IPMS), responsible for the first reaction of leucine biosynthesis, is a HCS paralogue. IPMS is composed of two domains, N-terminal catalytic domain and C-terminal regulatory domain. While leucine inhibits IPMS by binding to the dimer interface between its C-terminal regulatory domains, HCS lacks most of the C-terminal portion corresponding to the regulatory domain of IPMS, suggesting that the regulatory mechanism of HCS is different from that of IPMS. Indeed, the enzyme kinetic analysis of TtHCS showed that lysine inhibited TtHCS competitively with α-KG, which indicates that lysine is bound to the substrate binding site of the catalytic domain of HCS. However, there remains a problem that the characteristics of chemical structure of these two ligands, lysine and α-KG, are so different that they seem unlikely to share the same binding site (Figure 1). Here, we determined the crystal structure of the TtHCS/lysine complex in addition to the previously determined structure of the TtHCS/α-KG complex, which reveals the regulatory mechanism of HCS at atomic level [2].

We determined the crystal structure of the HCS/lysine complex at 1.80 Å resolution（Figure 2）. In the TtHCS/lysine complex a lysine molecule occupies the active site in a manner similar to that of α-KG in the TtHCS/α-KG complex. Comparing the structure between TtHCS/lysine and TtHCS/α-KG complexes, the recognition of two structurally different ligands, lysine and α-KG, is found to be accompanied by various conformational changes. First, side chains of amino acid residues surrounding the active site is rearranged upon binding each ligand. The C5-carboxyl group (negative charge) of bound α-KG, for example, is surrounded by basic amino acid residues such as His72 and Arg133, while the ε-amino group (positive charge) of bound lysine is stabilized by acidic residues such as Asp92 and Glu193. For lysine recognition, concerted conformational change of a set of amino acid residues connected by a series of ionic bonds is observed along the central axis of TIM barrel structure (Figure 3). Second, conformational changes are also found in C-terminal small domains. TtHCS forms a dimer structure with C-terminal small domain I covering the active site of the other subunit to provide the catalytic residue His292*. The region of C-terminal small domain I covering the active site forms an

α-helix in the TtHCS/α-KG complex, while it is deformed in the lysine complex. In addition, small domain II which cannot be seen in the structure of the α-KG complex turns visible in that of the lysine complex. The comparison of structures of the TtHCS/lysine complex and *Schizosaccharomyces pombe* HCS binding α-KG also indicates the large dislocation of small domain II [3]. As described above, HCS enables the recognition of two ligands with different structural characteristics, a 2-oxo acid (α-KG) and a basic amino acid (lysine), at the same site by dynamic reconstitution of its conformation. This study provides an interesting case, considered that generally strict substrate selectivity of enzymes which is often compared to "lock-and-key."

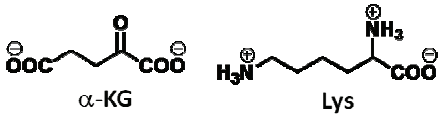

Figure 1. Chemical structures of substrate (α-KG) and inhibitor (lysine)

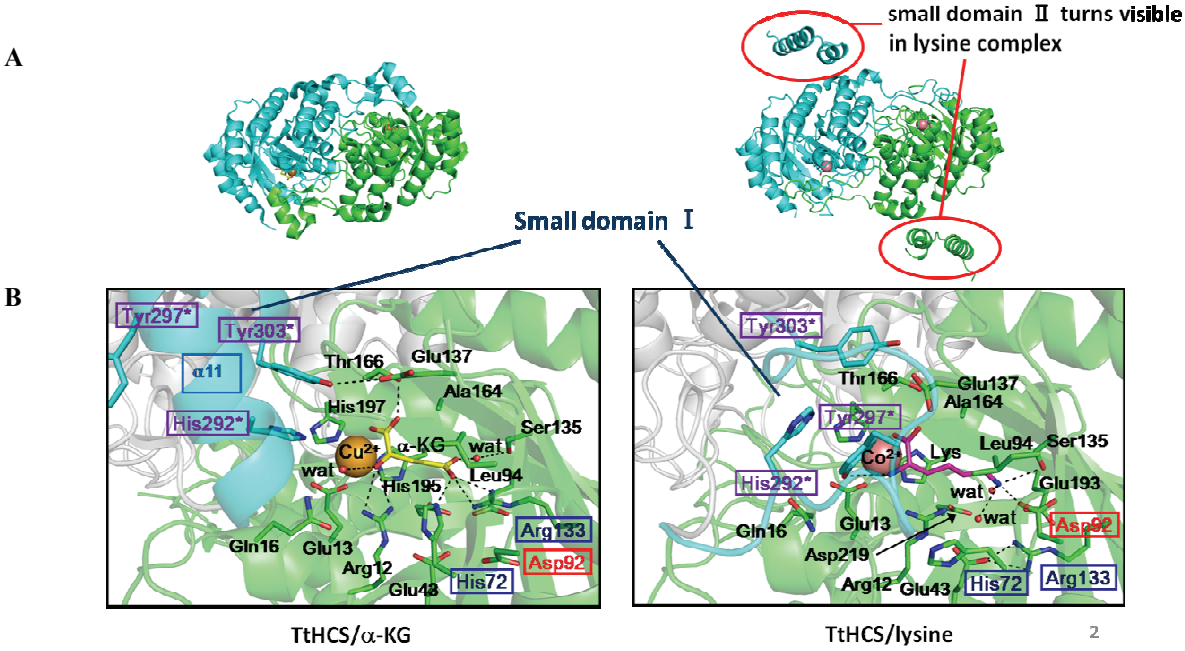

Figure 2. Crystal structures of TtHCS/α-KG and TtHCS/lysine complexes

(A : overall structures, B : structures of active site)

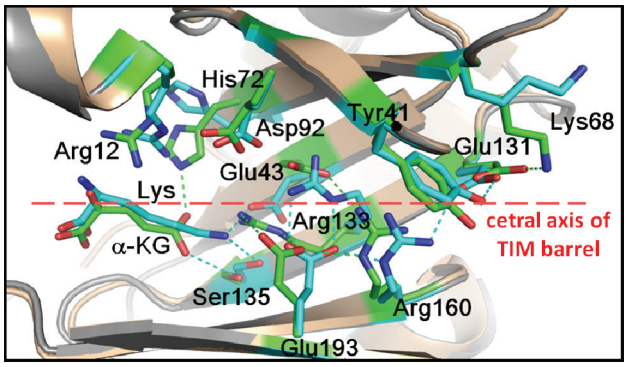

Figure 3. Concerted conformational change along the central axis of TIM barrel

Reference

[1] Kobashi N. et al. (1999) *J.Bacteriol*, 181, 1713-1718

[2] Okada T et al. *J Biol Chem*. (2010) 285(6), 4195-205

[3] Bulfer SL et al. *J Biol Chem*, (2009) 284(51), 35769-80