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Mechanim of feedback inhitibion of aspartate kinases

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Introduction

Aspartate kinase (AK) is the enzyme that catalyzes the first step, phosphorylation of the γ-carboxyl group of aspartate, of the biosynthetic pathway of aspartic acid group amino acids, Lys, Thr, Ile, and Met, in microorganisms and plants. As seen in other enzymes involved in the first step in amino acid biosynthesis, AK is regulated via feedback inhibition by end products. For example, AK I, which is an isoform of AK-homoserine dehydrogenase, and AK III, from *Escherichia coli* both are inhibited by Thr and Lys plus Leu, respectively, AK from *Corynebacterium glutamicum* (CgAK) is inhibited by Lys and Thr in a concerted manner, and AK from *Thermus thermophilus* (TtAK) is Thr-sensitive and involved in biosynthesis of Thr and Met but not of Lys. To date, crystal structure of AK from *E. coli*, *Methanococcus janaschii* and *Arabidpsis thaliana* are determined. Although, these AKs all take α_x-type homooligomeric structures. In contrast this CgAK and TtAK take $\alpha_2\beta_2$-type heterooligomeric structure, indicating that this type AKs has different regulatory mechanism. Thus, CgAK and TtAK is a attractive target for scientific interests. In the other hand, *C. glutamicum* is high amino acids producer used for industrial amino acid fermentations. CgAK mutation which densitizes the regulation sigfinicantly improves the productivity of lysine. Therefore, the mechanism of feedback inhibition is thought to lead the understanding of high lysine producing mechanism and the design of more efficient lysine fermentation system.

Materials and Methods

Preparation of crystals

To obtain crystal of CgAK/Thr,Lys complex, CgAK was expressed as hexahistidyl-tag fusion manner. The Ni-NTA affinity chromatography and following gel filtration were performed to purify. To maintain the $\alpha_2\beta_2$ oligomeric structure, Thr and Lys were added through the purification steps. The crystal was obtained by vapor diffusion method with a reservoir solution which contains 100 mM Tris-HCl (pH8.5) and 1.2 M tri-sodium citrate, at 20 °C in about 1 week.

To obtain TtAKβ, recombinant TtAK was expressed in *E.coli*. The purification was performed by heat-treatment, Ni-NTA affinity purification, and gel filtration. The crystal was obtained by vapor diffusion method with a reservoir solution containing 100 mM sodium acetate (pH5.0) and 1.2-2.0 M NaCl.

Results and Discussion

CgAK/Lys, Thr complex

The structure of CgAK was determined by molecular replacement (MR) method using the model of catalytic domain of MjAK (PDB ID 2HMF) and regulatory domain of CgAK (PDB ID 2DTJ) (1) to 2.5 Å resolution. As predicted, CgAK takes $\alpha_2\beta_2$-type heterooligomeric structure, and forms subunit interactions not only between α and β subunit but also between catalytic domains of two α subunits. In one regulatory domain two Thr molecules and one Lys molecule were buried in the site between distinct subunits. Comparing with the previously determined CgAKβ/Thr structure, we found that Lys binding induces the conformational change of regulatory domain. Comparing with active form of EcAK, the active site of CgAK/Thr, Lys form takes a structure unsuitable for catalysis, ATP binding, and Asp binding, which is induced by reroganization of the structure of the region between regulatory domain and catalytic domain.

TtAKβ- free form

The structure of TtAKβ-free at 2.98 Å resolution was determined by MR method using the model of TtAKβ-Thr structure (PDB ID 2DT9). In the absence of Thr, outward shift of β strands near the Thr-binding site and concomitant loss of the electron density of the loop region between β3 and β4 near the Thr binding site is observed (Fig.1). This result indicates that the bindint of Thr is important in subunit assembly to regulate the enzyme activity.

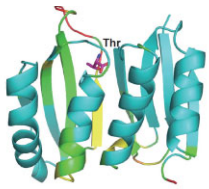

Fig. 1. Movement of Cα atoms by Thr binding mapped on the effector-binding unit of TtAKβ/Thr. Cyan, <1 Å; green, <2 Å; yellow, <3 Å ; orange, <4 Å ; red, >4 Å

Reference

[1] A. Yoshida et al., J. Mol. Biol. 521-36, (2007).

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