

## Regulatory mechanisms of enzymes involved in amino acid metabolism

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### Introduction

Amino acid is recently as supplemental diet. Several amino acids such as glutamate, lysine, are industry produced. To supply increased needs of amino acids, the approaches for effective fermentation system have been done. To achieve this purpose, the engineering of substrate specificity and regulatory mechanism is important.

Aspartate kinase (AK) is an enzyme that catalyzes the committed step, the phosphorylation of the  $\gamma$ -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. AK from *Corynebacterium glutamicum* (CgAK) is inhibited by Thr and Lys in a concerted manner. Meanwhile, *C. glutamicum* is a high amino acids producer used for industrial fermentation. CgAK mutation which desensitizes the regulation improves the productivity of lysine. Therefore, the understanding of the mechanism of feedback inhibition in CgAK could lead to the design of more efficient lysine fermentation system.

We have already determined the crystal structures of  $\beta$  subunit dimer of CgAK<sup>1</sup> and AK from *Thermus thermophilus*<sup>2,3</sup>, which also takes a similar  $\alpha_2\beta_2$ -type structure. To elucidate the mechanism of concerted inhibition in CgAK, we tried to determine the crystal structures of CgAK both in active and inactive forms.

### Materials and Methods

**Preparation of crystals** - CgAK was expressed with (His)<sub>6</sub>-tag in *E. coli* and purified with Ni<sup>2+</sup>-NTA affinity chromatography and gel filtration. To maintain the ratio of  $\alpha$  and  $\beta$  subunit, Thr and Lys were added throughout the purification step. To prepare the CgAK/Thr complex, Lys was removed by dialysis after all of the purification steps. The crystals of CgAK/Lys/Thr and CgAK/Thr complexes were obtained by vapor diffusion method. The crystal of CgAK/Lys/Thr complex was obtained under the condition of 0.1 M HEPES pH 7.5, 2.1% PEG400, and 2.2 M ammonium sulfate, at 20°C. The crystal of CgAK/Thr complex was obtained under the condition 0.1 M Tris-HCl (pH8.5) and 1.2 M sodium citrate.

### Results and Discussion

**Overall structure of CgAK** - The structure of CgAK/Lys/Thr complex was determined at 2.5 Å resolution. CgAK takes an  $\alpha_2\beta_2$ -type heterotetramer structure (Fig. 1). Two Thr and one Lys molecules are bound at two effector-binding units composed of  $\beta$ -

subunit and regulatory domain of  $\alpha$ -subunit, which has the sequence identical to that of  $\beta$ -subunit (Four Thr molecules and two Lys molecules in whole structure). Thr is bound to the effector-binding unit a manner the same as that in CgAK $\beta$  structure. Among the two possible Lys-binding sites per  $\alpha\beta$  dimer, a Lys molecule is present in the binding site that is away from the active site. The analyses of Lys/Thr inhibition profiles of the CgAK mutants carrying single amino acid replacement at either of the two possible binding sites showed that the only the Lys-binding site found in CgAK structure was functional in allosteric regulation.

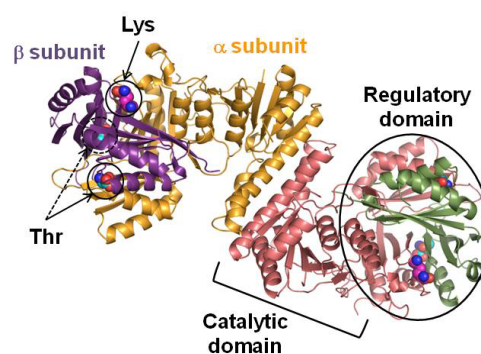


Fig. 1. Structure of CgAK/Lys/Thr complex. The bound Lys and Thr molecules are shown in sphere representations.  $\alpha$ - and  $\beta$ -subunits are shown in yellow and purple ribbon, respectively. The catalytic domain and regulatory domain is indicated by bar and circle, respectively.

**Implication for conformational change during active to inactive state transition** - The structure of CgAK/Thr complex was determined at 2.6 Å resolution. Two Thr molecules are bound at the two binding unit. CgAK/Thr complex has the essentially same architecture to that of CgAK/Lys/Thr complex. The regulatory domain was rotated by 5 degree when the catalytic domain was superposed between the two structures. Concomitantly dislocation of the C $\alpha$  and side chains of active site residues for Asp binding (Arg207, Gly208, and Thr46) was observed. From these structural analyses, the mechanism of the concerted inhibition by Lys and Thr through interdomain interaction is being elucidated

### References

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