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Mechanism of concerted inhibition of aspartate kinase from *Corynebacterium glutamicum*

Takeo TOMITA

Biotechnology Research Center, The University of Tokyo

Research aims

A large amount of lysine is used for the food additives for the livestock today, and lysine is produced by fermentation of high lysine-producing *Corynebacterium glutamicum* mutants. The mutants were obtained by selecting for resistance to (S)-(aminoethyl)-L-cysteine (AEC), which is an analogue of lysine, and the mutations are found in the regulatory domain of aspartate kinase (CgAK). The wild-type CgAK is known to be susceptive to allosteric inhibition by simultaneous addition of lysine and threonine in a concerted manner. Although CgAK is an attractive target in industrial and scientific fields, the regulatory mechanism of CgAK remains to be elucidated. Thus, aim of this research is to elucidate the mechanism of concerted inhibition of CgAK and to obtain basic knowledge for the higher production of lysine or other amino acids.

Methods

Full length CgAK with $\alpha_2\beta_2$ structure and its β-subunit (CgAKβ) were expressed in *E. coli* BL21 RIL-Codon Plus (DE3) and those were purified with Ni-NTA affinity chromatography followed by gel filtration chromatography. Through extensive screening for crystallization conditions, crystals with good diffraction were successfully obtained for both proteins. The structure determination of CgAKβ was performed by molecular replacement method (MR) using the structure of AKβ from *Thermus thermophilus*, which we determined previously by MAD method, as a search model. The structure of CgAK was determined by MR using the structure of AK from *Methanococcus jannaschii* and CgAKβ as search models.

Results

The crystal structure of CgAKβ was determined at 1.58 Å resolution. CgAKβ consists of a homodimer of polypeptides each containing two ACT (aspartate kinase, chorismate mutase, TyrA) domain (Fig. 1). CgAKβ has a unique dimeric form in which two ACT domains each from different chain form a single effector-binding unit. As seen in the regulatory domain of AKs, a single subunit has a unique β-$\beta\alpha\beta\beta\alpha$ topology. Two Thr molecules are found in the dimer interface in a manner where each of two effector-binding unit binds a single Thr molecule. However, Lys was not observed in the crystal structure in spite that it was added to the sample.

The crystal structure of CgAK was determined at 2.50 Å resolution. CgAK has a $\alpha_2\beta_2$-type heterotetramer structure (Fig. 2). Two Thr and one Lys molecules are bound in two effector-binding units composed of β-subunit and regulatory domain of α-subunit, which has the sequence identical to that of β-subunit (Four Thr molecules and two Lys molecules in whole structure). Thr is bound to the effector-binding unit in a manner very similar to that seen in CgAKβ 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 site-directed CgAK mutants carrying single amino acid replacement at either of the two possible binding sites showed that the only the Lys-binding site

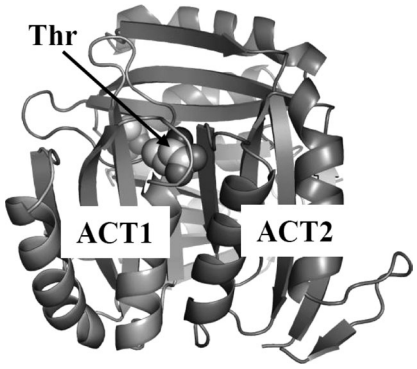

Fig. 1. Structure of CgAKβ.
CgAKβ forms a dimer of the two repeat peptide of ACT domain which is consisted of two repeat of $\beta\alpha\beta\beta\alpha\beta$-fold. The distinct unit (ACT1 and ACT2) forms a interface of ligand binding which is novel manner Thr was buried in the interface between the monomers.

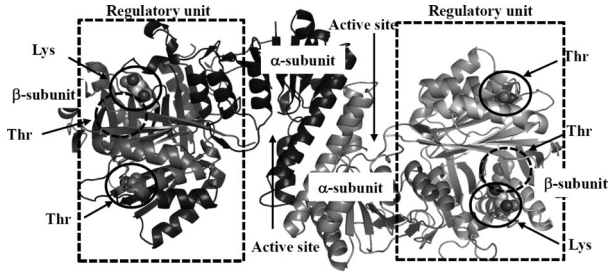

Fig. 2. Structure of CgAK.
CgAK forms a interface between two Structure of α-subunits at the center of the overall structure and β-subunits forms interfaces with distinct α-subunits.

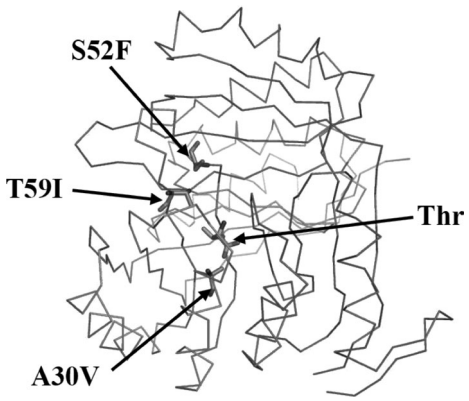

Fig. 3. Mapping of AEC resistant mutation.

found in CgAK structure was functional in allosteric regulation. Comparison of the structures between *E. coli* AK in an active form and CgAK revealed that not only residues involved in aspartate binding and but also those in ATP binding are hampered in CgAK structure, suggesting that Thr/Lys binding induces allosteric conformational change of the active site. Based on a structural comparison between CgAK and CgAKβ, it was suggested that the allosteric regulation proceeds with three steps; (i) Thr-induced association between of β-subunit and C-terminal regulatory domain of α-subunit, (ii) change in domain interaction by Lys-binding, (iii) conformational change of the active site residues, which is transmitted through the new interactions between regulatory domain and catalytic domain.

The amino acid replacements which provide AEC resistance are mapped in CgAKβ structure. Unexpectedly, those mutations (A30V, S52F, and T59I) are clustered around the Thr-binding site in spite of the fact that AEC is an analog of Lys (Fig. 3). Detailed analyses of the Lys/Thr inhibition profiles indicated that A30V and T59I are responsible for Thr-resitance and that S52F is involved in Lys-resistance, suggesting that former two residues are involved in the direct interaction with Thr and the latter is involved in the transduction of the signal of Thr-binding to active site through Lys-binding.

Conclusion

The structures of AK from *C. glutamicum* complexed with Lys and Thr and β-subunit of CgAK complexed with Thr were determined. The structures shed a light to elucidate the mechanism of feedback inhibition of AK. This study would provide the basic knowledge for the design of new feedback-resistant enzymes.

References

1) Yoshida, A., Tomita, T., Kurihara, T., Fushinobu, S., Kuzuyama, T., and Nishiyama, M. (2007) Structural insight into concerted inhibition of $\alpha_2\beta_2$-type aspartate kinase from *Corynebacterium glutamicum*. *J. Mol. Biol.* **368**, 521–536.