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Structural insight into concerted inhibition of $\alpha_2\beta_2$-type aspartate kinase from *Corynebacterium glutamicum*

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Introduction

Aspartate kinase (AK) catalyzes the first step of the biosynthesi of the aspartic acid family amino acids, and is regulated via feedback inhibition by end-products including Thr and Lys. To elucidate the mechanism of this inhibition, we determined the crystal structure of the regulatory subunit of AK from *Corynebacterium glutamicum* at 1.58 Å resolution in the Thr-binding form, the first crystal structure of the regulatory subunit of $\alpha_2\beta_2$-type AK.

Material and Methods

Preparation of crystals

The purified protein was concentrated to 10 mg/ml for crystallization. Crystallization was conducted at 293 K by the hanging-drop vapor-diffusion method. The crystals formed in 0.2 M ammonium sulfate, 0.1 M citrate (pH5.0), 36% (w/v) polyethyleneglycol 4000. The SeMet derivative of TtAK β (β-subunit of AK from *Thermus thermophilus* HB27) was expressed in the methionine autotrophy *E.coli* strain B834 (DE3) (Novagen, Darmstadt, Germany) in medium containing SeMet. The native and SeMet-labeled proteins were purified and crystallized as described[1].

Data collection, structure determination and refinement

Before data collection, crystals were soaked briefly in a cryoprotectant, flash-cooled in a nitrogen gas stream at 95 K, and stored in liquid nitrogen. Diffraction data were collected with a CCD camera on the NW12 station of the Photon Factory AR. The MAD data using SeMet TtAK β crystal was recorded to 2.4 Å resolution, and data for CgAKβ were recorded from the monoclinic native crystal to 1.58 Å resolution. Diffraction data were indexed, integrated, and scaled using the HKL2000 program suite. The crystal of SeMet labeled TtAKβ belongs to the space group $P4_332$ with unit cell parameters of a=b=c=141.3 Å. The MAD method was utilized to carry out the structure determination using SOLVE. Eight Se atoms can be identified from the 12 Se atoms in the primary sequence. Automated model building was performed by cycling with RESOLVE. The subsequent structure refinement was performed by using the program CNS1.1 and model correction in the electron density map was performed with the XtalView program suite. The crystal of native CgAK β belongs to the space group C2 with unit cell parameters of a=79.9 Å, b=59.01 Å, C=75.78 Å, β=116.2º. The structure determination of CgAKβ by molecular replacement was conducted with MOLREP using the model of TtAKβ and the subsequent refinement steps were the sama as those used for TtAKβ.

Results and Discussion

The structure of regulatory subunit of CgAK was determined to 1.58 Å resolution in Thr-binding form. That contains two ACT domain motifs per monomer and is arranged as a dimer. Two non-equivalent ACT domains from different subunit from an effector-binding unit that binds a single Thr molecule, and the resulting two effector-binding units of the dimer associate perpendicularly in a face-to-face manner (Fig. 1). The regulatory subunit is a monomer is a monomer in the absence of Thr but becomes a dimer by adding Thr. The dimerization induced by Thr binding is a key step in the inhibitory mechanism of AK from *C. glutamicum*. A putative Lys-binding site and the inhibitory mechanism of CgAK are discussed using the 3-D structural and mutational informations[2].

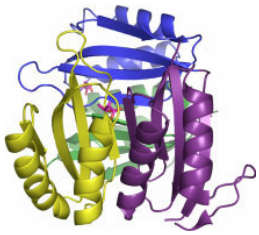

Fig.1 Overall structure of CgAKβ. Blue and purple are ACT1 and ACT2 domains of chain A. Yellow and green are ACT1 and ACT2 domains of chain B. Thr molecules are shown in magenta.

References

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