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# Crystallographic analysis of LysN, α-aminoadipateaminotransferase, from *T. thermophilus* HB27

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## Introduction

We previously cloned lysN gene from *Thermus thermophilus* HB27 as a homolog of mammalian Kynurenine aminotransferase II (KatII) and characterized as a gene playing a major role in the fourth reaction (transamination reaction using 2-oxoadipate as an amino acceptor to yield a-aminoadipate) of AAA pathway for lysine biosynthesis [1]. LysN recognizes not only 2-oxoadipate, an intermediate of lysine biosynthesis but also 2-oxoisocaproate, 2-oxovalerate and 2-oxo-methylvalerate, intermediates of leucine, valine and isoleucine biosynthesis, respectively, along with oxaloacetate, a compound in the TCA cycle, as an aminoacceptor. Althouth these results suggests multiple roles in LysN in several cellular metabolic pathways including lysine and branched-chain amino acid biosynthesis, structural basis for the broad range substrate specificity of LysN remained to be elucidated. Therefore, we conducted crystallographic analysis of LysN.

## Materials and Methods

*E.coli* BL21-Codon-Plus (DE3)-RIL cells harbouring pETLysN7 were cultured in 800 ml of 2x YT medium containing 50 µg/ml kanamycin and 30 µg/ml chloramphenicol. When the culture had reached an OD600 of 0.5, IPTG (final concentration, 0.1 mM) was added. The culture was continued at 25°C for an additional 12 h after the induction. *E. coli* (pETLysN7) cells (wet weight, 4.5 g) collected from the 800 ml culture were suspended in 27 ml buffer I (20 mM potassium phosphate buffer, pH6.5, 0.5 mM EDTA ) and disrupted by sonication. The supernatant prepared by centrifugation at 40 000 g for 20 min was heated at 80°C for 20 min, and denatured proteins from *E.coli* cells were removed by centrifugation at 40 000 g for 20 min. Supernatant fractions were applied to an anion-exchange column (DE-52; Whatman), pre-equilibrated with buffer I and eluted with buffer I containing 0.1 M NaCl. After the addition of ammonium sulfate to a final concentration of 65% saturation to active fractions, the resultant precipitate was collected by centrifugation at 40 000 g for 30 min. The precipitated proteins were solubilized with buffer I and applied onto a Hi-load 26/60 Superdex 200 prep-grade column (Amersham-Pharmacia Biotech) equilibrated with buffer I containing 0.1 M NaCl to yield the purified preparation. Protein concentration was determined by the method of Bradford using a protein assay kit (Nippon Bio-Rad).

## Results and Discussion

*Crystallization*

Following the purification, LysN was concentrated upto 5 mg/ml. Crystals of LysN were obtained by the vapor diffusion method with a reservoir solution containing 8-13% (w/v) polyethylene glycol (PEG) 6000 and 100 mM MES-NaOH (pH6.0-6.5) within 1-2 day at 20°C.

*Data collection and processing*

Before data collection, the crystals were transferred to a reservoir solution finally supplemented with a cryoprotectant, 25% PEG 400 (w/v) by increasing the concentration of cryoprotectant to 25% by 5% in each step, with equilibration for 1 minute between the steps. Crystals were flash-frozed in a cold N2 stream from a liquid-nitrogen cryostat (Rigaku). All the data collection were carried out at 100 K. Diffraction dataset was collected with an ADSC Quantum CCD detector on the BL5A station at Photon Factory in High Energy Accelerator Research Organization (KEK). Data processing and scaling were performed using HKL2000. Statistics of data collection was summerized in Table1.Crystal structure determination is now underway.

Table1: Statistics of data collection

| Crystal | A |
|---|---|
| Space group | $P2_1$ |
| Cell dimensions (Å) | a=55.95, b=167.64, c=97.87 |
| Resolution (Å) (outer shell) | 2.26 (2.26-2.34) |
| $R_{merge}$ (%) | 12.2 |
| I/σ (I) | 19.4 (4.9) |
| Completeness (%) | 99.3 (100.0) |

## References

[1] T. Miyazaki et al., Microbiology, 150, 2327 (2004).

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