

## Crystal structures of enzymes and transporters involved in amino acid metabolism

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### Introduction

Genome analysis of various organisms has revealed that members of the Lrp (Leucine responsive regulatory protein) family of transcriptional regulators are widely distributed among prokaryotes, both bacteria and archaea and are responsible for various regulatory systems via global transcriptional regulation. For example, the Lrp from *E. coli* is a global regulator involved in modulating a variety of metabolic functions, including the catabolism and anabolism of amino acids. Lrp consists of the N-terminal DNA binding domain and the C-terminal RAM (Regulation of Amino acid Metabolism) domain. Although *Thermus thermophilus* does not possess homologues of Lrp gene on its genome, it possesses a gene encoding stand-alone RAM domain protein (TTC0493; SraA), which has similarity to C-terminal domain of Lrp. Thus we hypothesized that SraA senses amino acids as a signal and mediates global regulation of metabolism through interaction with other proteins. In this study, we analysed the function and structure of SraA to reveal the signal transduction system of amino acids in *T. thermophilus*. The crystal structure of SraA from *T. thermophiles* HB8 has already been determined as a ligand-free form. RAM domain of SraA can binds to amino acids, and we hypothesized that amino acids are ligand of SraA and tried to determine the crystal structure in complex with amino acids.

### Materials and Methods

#### *Purification of SraA*

Strep-SraA was overexpressed in *E. coli* BL21-Codon-Plus (DE3)-RIL as a host using 0.1 mM isopropyl- $\beta$ -D-1-thiogalactopyranoside (IPTG) for induction. From cell lysate prepared by sonication, SraA was purified through heat treatment, Streptactin affinity chromatography and Superdex 200 gel filtration chromatography.

#### *Elucidation of the oligomeric state of SraA*

We performed gel filtration chromatography to elucidate the oligomeric state of SraA. We mixed SraA (2.0 mg/mL) with amino acid (5 mM) and applied to gel filtration chromatography.

#### *Crystallization of SraA*

Screening of crystallization condition was initially performed with Crystal screen I and II, Wizard classic I, II, and III, and PEG/ION screening kits by hanging drop

vapor diffusion method. crystallization conditions were further optimized by changing pH and concentration of precipitant.

#### *Data collection and processing.*

The X-ray diffraction data of native proteins were collected using the beamline, NW12, NE3 and 5A at PF. The image sets were integrated and scaled using HKL2000.

### Results and Discussion

We examined the effect of amino acids on the oligomeric state of SraA with gel filtration chromatography. In the absence of amino acids, SraA was present in an equilibrium between dimer and decamer, whereas in the presence of some amino acids, SraA decamer was much induced. This suggests that some amino acids binds SraA and changes its conformation.

Furthermore, these results may suggest that SraA senses various amino acids as signals to regulate a variety of metabolism by changing its conformation.

We tried to performed screening of crystallization condition of SraA with amino acids and obtained micro- or needle like- crystals from several conditions. Now we are trying to optimize the crystallization conditions.

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