# Crystal structure of aspartase from *Bacillus* sp. YM55-1

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## Introduction

Aspartase (EC 4.3.1.1) plays an important role in bacterial nitrogen metabolism by catalyzing the reversible conversion of L-aspartate to fumarate and ammonium ion. Although the crystal structure of the enzyme from *Escherichia coli*, which is allosterically activated in the presence of $Mg^{2+}$ ion at alkaline pH, has been elucidated, there are little known about the catalytic residues and detailed reaction-mechanism of the enzyme [1].

Recently, a novel thermostable aspartase from *Bacillus* sp. YM55-1 was isolated and characterized using biochemical and biophysical methods [2]. The *Bacillus* sp. YM55-1 aspartase forms a homotetramer and the subunit consists of 468 amino acid residues ($M_r$ = 51,627). The enzyme has a high specific activity without any allosteric behavior and displays much higher resistance to thermal and chemical denaturation than other mesophilic aspartases. In order to obtain structural information on the reaction mechanism of aspartase and the thermostability of the present enzyme, we have determined the crystal structure of the *Bacillus* sp. YM55-1 aspartase at 2.5 Å resolution by X-ray crystallography.

## Experimental

The crystals of the enzyme were obtained by a sitting drop vapor diffusion method using PEG4000 and 2-propanol as precipitants at 30°C. Two sets of diffraction data for two crystals (NAT1 and NAT2) were collected on a screenless Weissenberg camera with a imaging plate (400 mm × 200 mm size) at the BL18B station of the Photon Factory. The data collection of NAT1 and NAT2 were performed at 291K with one crystal sealed in a glass capillary and at 100K with a flash-cooled crystal in the liquid nitrogen gas stream, respectively. Both the data sets were processed, scaled and merged with the programs DENZO and SCALEPACK. The crystals belonged to space group $P2_12_12$ with unit cell dimensions of $a$ = 76.21 Å, $b$ = 140.5 Å and $c$ = 101.9 Å for NAT1 with $R_{merge}$ 11.8%, and $a$ = 74.90 Å, $b$ = 139.5 Å and $c$ = 100.2 Å for NAT2 with $R_{merge}$ 10.2%, respectively.

The structure of the present aspartase was determined by a molecular replacement method using coordinates of the *E. coli* aspartase as a starting model, with a technique of searching *R*-factor along the crystallographic *c*-axis using the program X-PLOR. The NAT1 data was used at the first stage of the analysis. One of three pattern-combinations of two subunits selected from four subunits in the molecule shows an apparently high peak for the solution of the rotation function. The model of the subunit dimer rotated based on the solution was translated at intervals of 1.0 Å along the crystallographic *c*-axis over overlaying the molecular two-fold axis on the crystallographic axis. After each translation, the model was refined as a rigid body and the *R*-factor and $R_{free}$-factor values were calculated in 10–4 Å resolution range. The subunit dimer was transferred to the location giving the minimum values. Calculations of the positional refinement, the solvent flattening, and the density averaging by NCS, and the model building of polypeptide were repeated at 3.0 Å resolution. After the model of the structure was constructed up to about 80%, the NAT2 data set was used instead of the NAT1 data set, and the program CNS with a maximum likelihood target function was used for the refinement. Finally, the refinement of the current model was converged to an *R*-factor of 22.2% and $R_{free}$-factor of 26.9% at 2.5 Å. The final model of two independent subunits consists of 468 amino acid residues for A- and D-chains and 225 water molecules.

## Results and discussion

The *Bacillus* YM55-1 aspartase consists of four subunits arranged by the 222 point symmetry, and each subunit is composed of three domains. The N-terminal large domain (residues 1-139) consists of five helices. The central helix domain (residues 140-393) is composed mainly of six long α-helices. The C-terminal small domain (residues 394-468) is composed of several short helices. The secondary-structure location and the folding pattern in the subunit of the *Bacillus* YM55-1 aspartase are quite similar to those of the *E. coli* aspartase and the *E. coli* fumarase C. However, the overall structure of the subunit of the present enzyme resembles that of the citrate-complexed fumarase C from *E. coli* rather than that of the ligand-free aspartase from *E. coli*.

The main reasons for the thermostability of the *Bacillus* YM55-1 aspartase are the increase of especially intersubunit hydrogen-bonds and ion-pairs, the slight increase of interface area between subunits, and the shortening of turns.

## References

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