Acta Cryst. (1998). D**54**, 1032–1034

Crystallization and preliminary X-ray characterization of aspartate aminotransferase from an extreme thermophile, *Thermus thermophilus* HB8

Tadashi Nakai,[a] Kengo Okada,[a] Shin-ichi Kawaguchi,[b] Ryuichi Kato,[b] Seiki Kuramitsu[b] and Ken Hirotsu[a]* at [a]*Department of Chemistry, Faculty of Science, Osaka City University, Sugimoto, Sumiyoshi-ku, Osaka 558, Japan, and* [b]*Department of Biology, Graduate School of Science, Osaka University, Toyonaka, Osaka 560, Japan. E-mail: hirotsu@sci.osaka-cu.ac.jp*

(*Received 24 December 1997; accepted 20 March 1998*)

Abstract

Recombinant aspartate aminotransferase from an extremely thermophilic bacterium, *Thermus thermophilus* HB8, has been crystallized in two different crystal forms. The crystals of both forms are orthorhombic and belong to space group $P2_12_12_1$ with cell dimensions a = 124.3, b = 113.6 and c = 61.6 Å for form I and a = 197.3, b = 109.7 and c = 80.3 Å for form II. The crystals of form I and II diffract to 2.1 and 2.5 Å resolution, respectively, on a conventional laboratory rotating-anode source. Two heavy-atom derivatives have been identified for form I.

1. Introduction

The aminotransferases, which require a pyridoxal 5′-phosphate as a cofactor, are the key enzymes in amino-acid metabolism and have been widely investigated (Christen & Metzler, 1985). Aspartate aminotransferase (AspAT), one of the most important of these enzymes, catalyzes a reversible transamination reaction between the dicarboxylic α-amino acid and α-keto acid. AspATs from many species were classified into aminotransferase subgroup I (Mehta *et al.*, 1993), which was further subdivided into subgroups Ia and Ib (Okamoto *et al.*, 1996). AspATs from *Escherichia coli*, chicken, pig, *etc.* belong to subgroup Ia and those from thermophilic bacterium, *Thermus thermophilus* HB8 (Okamoto *et al.*, 1996) and *Bacillus* sp. YM-2 (Sung *et al.*, 1991), and thermoacidophilic archaebacterium, *Sulfolobus solfataricus* (Cubellis *et al.*, 1989) belong to subgroup Ib. *T. thermophilus* HB8 AspAT has 46 and 29% amino-acid sequence homology with *Bacillus* sp. YM-2 and *S. solfataricus* AspATs, respectively, but less than 16% sequence homology with AspATs of subgroup Ia. Although extensive X-ray studies on AspATs of subgroup Ia have been performed (McPhalen *et al.*, 1992; Malashkevich *et al.*, 1995; Jäger *et al.*, 1994; Okamoto *et al.*, 1994; Miyahara *et al.*, 1994; Rhee *et al.*, 1997), the three-dimensional structure of AspAT of subgroup Ib has not yet been determined. Many of the important active-site residues for catalysis seem to be conserved between the AspATs of subgroups Ia and Ib, but the residue which recognizes the distal carboxylate of the substrate (Arg292 in AspATs of subgroup Ia) is unknown in the AspATs of subgroup Ib. It is well known that the AspATs of subgroup Ia show a large conformational change in the small domain against the large domain to close the active-site upon binding of the substrate. The driving force for this small domain movement has not been determined.

Structure determination of *T. thermophilus* HB8 AspAT should help to clarify the role of the active-site residues, the mechanism of substrate recognition and the function of a mobile small domain in AspAT, and to give some insight into the thermostability of the protein folding. In this communication, we report the crystallization and preliminary X-ray diffraction studies of *T. thermophilus* HB8 AspAT, which contains 385 residues per monomer, with a calculated molecular weight of 42 051. The enzyme is stable up to about 353 K at neutral pH.

2. Experimental

T. thermophilus HB8 AspAT was expressed in *E. coli* and purified by a previously described method (Okamoto *et al.*, 1996). Preliminary crystallization conditions were determined using the sparse-matrix method (Jancarik & Kim, 1991) with the hanging-drop vapor-diffusion method (McPherson, 1982). Two crystal forms were obtained and the crystallization conditions were optimized.

A 5 µl protein solution (10 mg ml^{-1} protein, 100 m*M* KCl and 2 m*M* HEPES, pH 7.0) was mixed with an equal volume of reservoir solution (300 m*M* ammonium phosphate, pH 4.3) and equilibrated against 400 µl of reservoir solution at 293 K to give crystals of form I. Meanwhile, a droplet of 5 µl protein solution (10 mg ml^{-1} protein, 40 m*M* maleate, 100 m*M* KCl and 2 m*M* HEPES buffer, pH 7.0) was mixed with an equal volume of reservoir solution (200 m*M* sodium acetate, 100 m*M* citrate, 16% polyethylene glycol 6000, pH 6.5) and equilibrated against 400 µl of reservoir solution at 293 K to give crystals of form II. For preliminary characterization, crystals were mounted in glass capillaries with a small amount of mother liquor. Still and precession photographs were taken at room temperature with an Enraf–Nonius precession camera using Ni-filtered Cu $K\alpha$ radiation, which was generated with a Mac

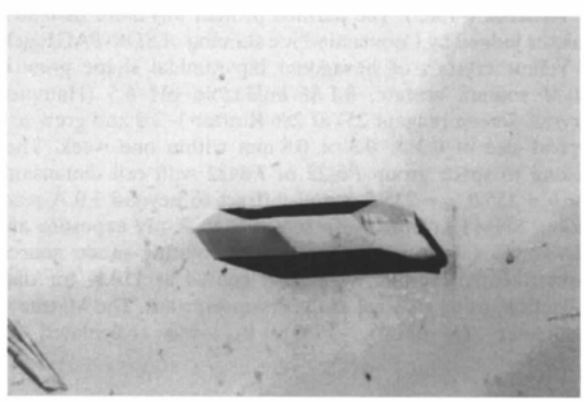


Fig. 1. Crystals of aspartate aminotransferase from an extremely thermophilic bacterium, *T. thermophilus* HB8. Crystal of form I was grown in a hanging drop using ammonium phosphate as precipitant.

ISSN 0907-4449

Science X-ray generator operating at 50 kV and 25 mA. The crystal-to-film distance was set at 100 mm.

Data collection was performed at 293 K on a Rigaku R-AXIS IIc imaging-plate detector with graphite-monochromated Cu $K\alpha$ X-rays produced from a Rigaku RU-200 rotating-anode X-ray generator operating at 40 kV, 100 mA. The data were processed using the *DENZO* and *SCALEPACK* packages (Otwinowski, 1993). The self-rotation function was calculated with the *CCP*4 program *POLARRFN* (Collaborative Computational Project, Number 4, 1994).

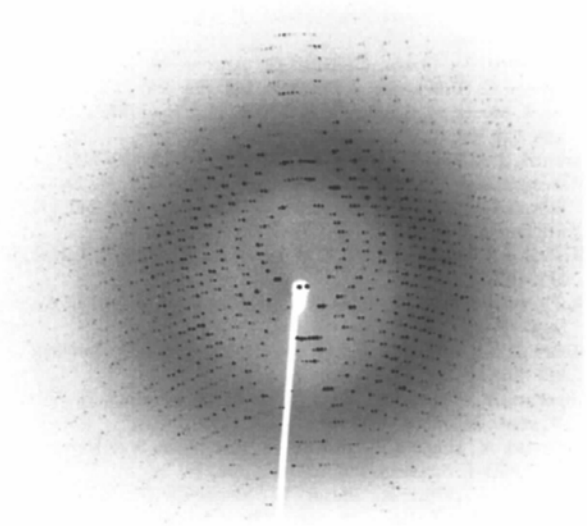


Fig. 2. 25 min exposure with 1.3° oscillation from a crystal of form I. The crystal-to-detector distance is 100 mm and the plate is 200 mm wide.

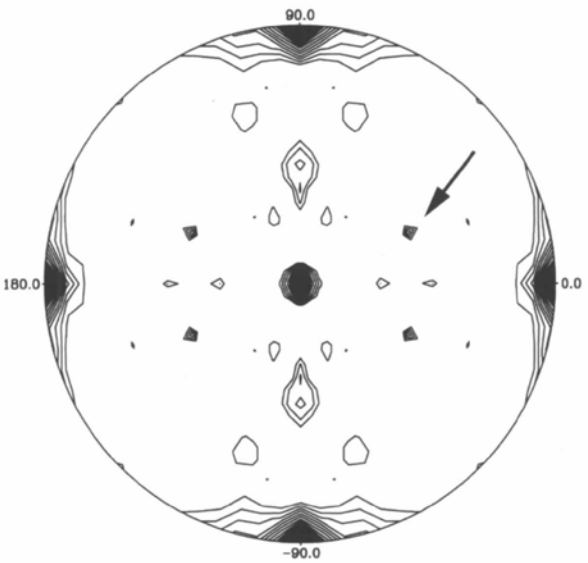
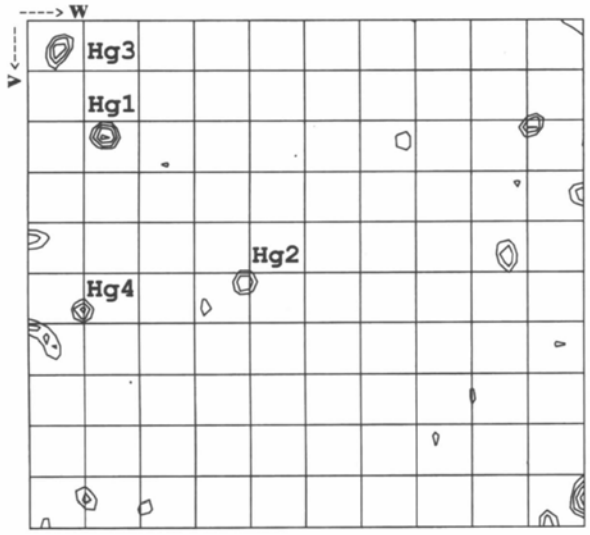


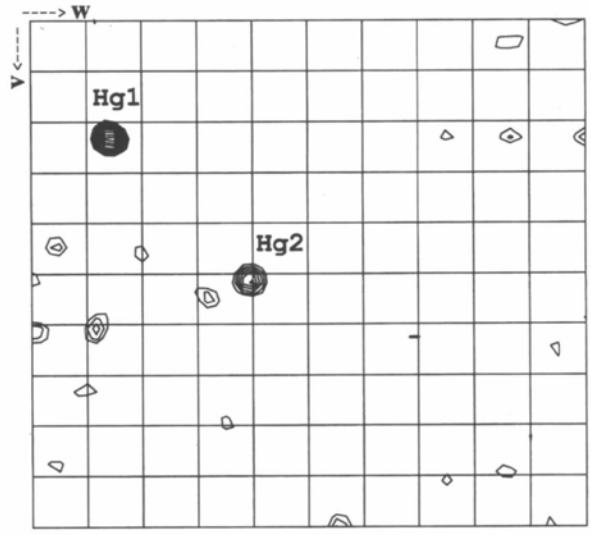
Fig. 3. $\kappa = 180°$ section of the self-rotation function calculated on the native data set of a crystal form I. The resolution of the data used was 8–4 Å with a radius of integration of 25 Å. The highest non-crystallographic peak shown by an arrow corresponds to the direction of the non-crystallographic twofold axis of the dimer and its height is 19% of the origin.

3. Results

The yellow crystals of form I appeared after a few days incubation and grew to a maximum size of 0.60 × 0.15 × 0.15 mm (Fig. 1). From precession photographs, the space group of the crystal was determined to be orthorhombic $P2_12_12_1$. The unit-cell dimensions were $a = 124.3$, $b = 113.6$ and $c = 61.6$ Å with a



(*a*)



(*b*)

Fig. 4. Harker section $u = 1/2$ of the isomorphous difference Patterson maps of (*a*) the $HgCl_2$ derivative and (*b*) the mercurochrome derivatives. The mercury sites of Hg1 and Hg3 are related to those of Hg2 and Hg3, respectively, around the non-crystallographic twofold axis found through the calculation of self-rotation function.

unit-cell volume of 8.70×10^5 Å^3. Assuming one dimer in the asymmetric unit, the V_m value was calculated as 2.57 Å^3 Da^{-1} indicating a solvent content of approximately 52% in the unit cell. These values are within the ranges of those for typical protein crystals (Matthews, 1968). The crystal diffracts to approximately 2.1 Å resolution on a rotating Cu-anode generator operating at 40 kV, 100 mA (Fig. 2). The yellow crystals of form II appeared after a few days incubation and grew to a maximum size of $1.00 \times 0.15 \times 0.15$ mm. From precession photographs, the space group of this crystal form was determined to be orthorhombic $P2_12_12_1$. The unit-cell dimensions were a = 197.3, b = 109.7 and c = 80.3 Å, with a unit-cell volume of 17.4×10^5 Å^3. Assuming two dimers in the asymmetric unit, the V_m value was calculated as 2.58 Å^3 Da^{-1} indicating a solvent content of approximately 52% in the unit cell. The crystal diffracts to approximately 2.5 Å resolution.

A native data set with 44 171 unique reflections has been collected from a crystal of form I, giving a data set completeness of 98.1% at 50.0–2.2 Å with R_{merge} = 8.3%. These data indicate the good quality of the crystals for the X-ray structural analysis. The crystals showed no significant decay upon exposure. The self-rotation function computed with the native data indicates a non-crystallographic twofold axis consistent with the presence of one dimer in the asymmetric unit (Fig. 3). For form II, a native data set has been collected, giving a data set completeness of 93.5% at 50.0–2.5 Å with an R_{merge} of 10.9%.

The data sets for the form I crystals soaked in 1 m*M* $HgCl_2$ and 1 m*M* mercurochrome were also collected to 3.0 Å (R_{merge} = 6.8%) and 3.5 Å (R_{merge} = 8.6%) resolution, respectively, on the same system used for the native crystal. Four mercury sites of the $HgCl_2$ derivative and two mercury sites of the mercurochrome derivative were located using the difference Patterson maps (Fig. 4). We are now in the process of refining the heavy-atom parameters for the two derivatives and searching for more heavy-atom derivatives for determination of the high-resolution three-dimensional structure of *T. thermophilus* HB8 AspAT.

This study was supported by a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science, Sports and Culture of Japan (08214212), and a grant on Research for the Future Program from the Japan Society for the Promotion of Science.

References

Christen, P. & Metzler, D. E. (1985). Editors. *Transaminases*. New York: John Wiley.

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.

Cubellis, M. V., Rozzo, C., Nitti, G., Arnone, M. I., Marino, G. & Sannia, G. (1989). *Eur. J. Biochem.* **186**, 375–381.

Jäger, J., Moser, M., Sauder, U. & Jansonius, J. N. (1994). *J. Mol. Biol.* **239**, 285–305.

Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.

Malashkevich, V. N., Strokopytov, B. V., Borisov, V. V., Dauter, Z., Wilson, K. S. & Torchinsky, Y. M. (1995). *J. Mol. Biol.* **247**, 111–124.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

McPhalen, C. A., Vincent, M. G. & Jansonius, J. N. (1992). *J. Mol. Biol.* **225**, 495–517.

McPherson, A. (1982). *Preparation and Analysis of Protein Crystals*. New York: John Wiley.

Mehta, P. K., Hale, T. I. & Christen, P. (1993). *Eur. J. Biochem.* **214**, 549–561.

Miyahara, I., Hirotsu, K., Hayashi, H. & Kagamiyama, H. (1994). *J. Biochem.* **116**, 1001–1012.

Okamoto, A., Higuchi, T., Hirotsu, K., Kuramitsu, S. & Kagamiyama, H. (1994). *J. Biochem.* **116**, 95–107.

Okamoto, A., Kato, R., Masui, R., Yamagishi, A., Oshima, T. & Kuramitsu, S. (1996). *J. Biochem.* **119**, 135–144.

Otwinowski, Z. (1993). *Data Collection and Processing, Proceedings of the CCP4 Study Weekend*, pp. 56–62. Warrington, England: Daresbury Laboratory.

Rhee, S., Silva, M. M., Hyde, C. C., Rogers, P. H., Metzler, C. M., Metzler, D. E. & Arnone, A. (1997). *J. Biol. Chem.* **272**, 17293–17302.

Sung, M., Tanizawa, K., Tanaka, H., Kuramitsu, S., Kagamiyama, H., Hirotsu, K., Higuchi, T. & Soda, K. (1991). *J. Biol. Chem.* **266**, 2567–2572.