Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray analysis of the Mj0684 gene product, a putative aspartate aminotransferase, from *Methanococcus jannaschii*

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Received 10 October 2002
Accepted 2 January 2003

A putative aspartate aminotransferase from the hyperthermophilic archaeon *Methanococcus jannaschii* encoded by the Mj0684 gene has been overexpressed in *Escherichia coli* and crystallized at 296 K using the sitting-drop vapour-diffusion method. The crystals belong to space group $P4_12_12$ (or $P4_32_12$), with unit-cell parameters $a = b = 111.87$, $c = 60.86$ Å. They diffract to 2.2 Å resolution using Cu $K\alpha$ X-rays. The asymmetric unit contains a single subunit of the recombinant Mj0684 gene product, giving a corresponding V_M of 2.25 Å^3 Da^{-1} and a solvent content of 45.3% by volume. An X-ray diffraction data set has been collected to 2.2 Å at 295 K.

1. Introduction

Aminotransferases constitute a large superfamily of enzymes that catalyze the reversible transfer of the α-amino group from an amino acid to a 2-keto acid in a pyridoxal 5′-phosphate (PLP) dependent manner. They have been grouped into four families (Mehta *et al.*, 1993; Jensen & Gu, 1996) and family I can be further divided into subfamilies (Jensen & Gu, 1996). It has been suggested that the common family I aminotransferase ancestor was probably a broad-specificity enzyme that could function as an aspartate aminotransferase (AspAT), an aromatic aminotransferase (ArAT) and an alanine aminotransferase (Jensen & Gu, 1996). During the evolution of aminotransferase, the substrate specificity appears to have been narrowed down. For instance, the contemporary members of subfamily Iα are all AspATs that accept aromatic amino acids to a negligible extent (*e.g.* vertebrate enzymes), to a moderate extent (*e.g. Escherichia coli* AspC) or very well (*e.g. E. coli* TyrB). Structural studies on a number of aminotransferases belonging to subfamily Iα such as AspATs from *E. coli*, chicken, pig and yeast (Jäger *et al.*, 1994; McPhalen *et al.*, 1992; Malashkevich *et al.*, 1995; Rhee *et al.*, 1997; Jeffery *et al.*, 1998) have been reported. AspATs in subfamily Iα use a conserved arginine residue (Arg292*, according to the numbering for pig cytosolic AspAT; Rhee *et al.*, 1997) to recognize a distal carboxyl group of a dicarboxylic substrate, while those in subfamily Iγ use the conserved Lys109 (Nobe *et al.*, 1998; Nakai *et al.*, 1999). The asterisk after the residue number indicates that the residue is supplied by the other subunit of the dimer. In comparison with the extensive structural data available on subfamily Iα, structural information on other subfamilies of the family I aminotransferases is relatively limited. For subfamily Iγ, for instance, the crystal structures of AspAT from *Thermus thermophilus* HB8 (Nakai *et al.*, 1999), tyrosine aminotransferase from *Trypanosoma cruzi* (Blankenfeldt *et al.*, 1999) and ArAT from *Pyrococcus horikoshii* (Matsui *et al.*, 2000) have been reported.

The Mj0684 (aspB2) gene from *Methanococcus jannaschii* encodes a 370-residue protein (calculated M_r = 42 347) and has been annotated as an AspAT. The results of a BLAST search (Altschul *et al.*, 1997) indicate that the Mj0684 gene product belongs to the aminotransferase subfamily Iγ, since it bears significant sequence similarity to AspATs of subfamily Iγ (34–39% sequence identity in a 330–370-residue overlap). In contrast, the Mj0684 protein neither shows any significant sequence similarity to family Iα AspATs nor possesses the conserved key residue Arg292* of family Iα AspATs. However, the Mj0684 protein lacks in its primary sequence the conserved Lys109 residue which is characteristic of subfamily Iγ AspATs. Thus, it may prefer other substrates such as tyrosine, phenylalanine or alanine. Alternatively, it may possibly recognize the distal carboxylate group of the dicarboxylic substrate in a distinctive manner. In order to provide insights into the mechanism of substrate recognition, structural diversity and evolution of aminotransferases, it will be interesting to solve the three-dimensional structure of the Mj0684 protein from *M. jannaschii* and to compare it with the previously reported crystal structures of

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aminotransferases. As a first step toward its structure determination, we report here its overexpression, crystallization and preliminary X-ray crystallographic studies.

2. Materials and methods

2.1. Protein expression and purification

The Mj0684 gene encoding a putative AspAT was amplified by the polymerase chain reaction using the *M. jannaschii* genomic DNA as a template. The amplified DNA was inserted into the *Nde*I/*Bam*HI-digested expression vector pET-22b (Novagen). The complete nucleotide sequence of the insert was confirmed by dideoxy-DNA sequencing. The enzyme was overexpressed in soluble form in BL21(DE3) cells. The cells were grown in Luria–Bertani medium to an OD_{600} of 0.6 at 310 K and the expression of the recombinant enzyme was induced by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 0.5 m*M*. Cell growth continued at 310 K for 4 h after IPTG induction and cells were harvested by centrifugation at 7000 rev min^{-1} (Sorvall GSA rotor) for 10 min at 277 K. The cell pellet was resuspended in ice-cold lysis buffer (20 m*M* Tris–HCl pH 8.0, 1 m*M* phenylmethylsulfonyl fluoride) and was then homogenized by sonication. The crude lysate was centrifuged at 36 000*g* (18 000 rev min^{-1}, Hanil Supra 21K rotor) for 30 min at 277 K and the cell debris was discarded.

We purified the recombinant protein in the supernatant fraction by heat treatment and four chromatographic steps. Firstly, the cell extract was heated and kept at 353 K for 10 min. After centrifugation at 35 000*g* for 30 min, the supernatant was subjected to ion-exchange chromatography on a Q-Sepharose column (Amersham-Pharmacia), employing a linear gradient of 0–1.0 *M* sodium chloride in buffer *A* (20 m*M* Tris–HCl pH 8.0). Next, gel filtration was performed on a HiLoad XK 16 Superdex 200 prep-grade column (Amersham-Pharmacia), which was previously equilibrated with buffer *A* containing 100 m*M* sodium chloride. The next purification step was hydrophobic interaction chromatography on a phenyl-Sepharose column (Amersham–Pharmacia), which was previously equilibrated with buffer *A* containing 1.0 *M* ammonium sulfate. Before loading the sample onto this column, 3.0 *M* ammonium sulfate was added to the sample to a final concentration of 1.0 *M*. While most impurities bound to this column, the Mj0684 protein did not. The sample was dialyzed against buffer *A* and was subjected to further purification on a Mono Q column (Amersham–Pharmacia), employing a linear gradient of 0–1.0 *M* sodium chloride in buffer *A*. The purified Mj0684 protein was highly homogeneous as judged by SDS–PAGE) Laemmli, 1970). Finally, the purified sample was dialyzed against storage buffer (20 m*M* sodium phosphate pH 7.5, 2 m*M* β-mercaptoethanol), concentrated to 10 mg ml^{-1} using an YM10 ultrafiltration membrane (Amicon) and stored at 253 K. The protein concentration was estimated by measuring the absorbance at 280 nm, employing the calculated extinction coefficient of 38 260 M^{-1} cm^{-1} (SWISS-PROT; http://www.expasy.ch/).

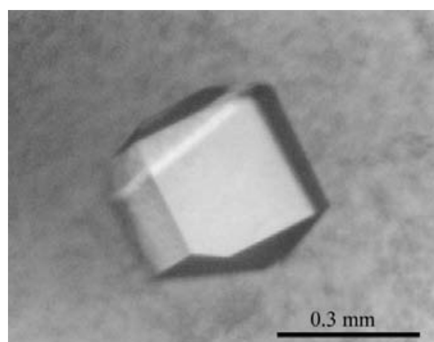

Figure 1
A crystal of the Mj0684 protein from *M. jannaschii*. Its approximate dimensions are 0.3 × 0.3 × 0.2 mm.

2.2. Dynamic light-scattering studies

A dynamic light-scattering experiment was performed on a Model DynaPro-801 instrument from Protein Solutions (Lakewood, New Jersey). The measurements were made at 297 K on the purified protein at ~2 mg ml^{-1} in buffer *A* containing ~400 m*M* sodium chloride.

2.3. Crystallization

Crystallization was achieved by the sitting-drop vapour-diffusion method at 296 K using 24-well VDX plates and Micro-Bridges (Hampton Research). A sitting drop was prepared by mixing equal volumes (4 µl each) of the protein solution and the reservoir solution. The protein solution contained the Mj0684 protein at 9 mg ml^{-1}, 0.4 m*M* pyridoxal phosphate and 0.5 m*M* α-ketoglutarate before mixing with the reservoir solution. Each sitting drop was placed over 0.5 ml reservoir solution. Initial crystallization conditions were established by sparse-matrix sampling (Jancarik & Kim, 1991).

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell (2.24–2.20 Å).

X-ray wavelength (Å)	1.5418
Temperature (K)	295
Space group	$P4_12_12$ (or $P4_32_12$)
Unit-cell parameters (Å)	*a* = 111.87, *b* = 111.87, *c* = 60.86
Resolution range (Å)	50.0–2.20
Total/unique reflections	120856/20121
R_{merge}† (%)	7.0 (40.7)
Data completeness (%)	99.6 (98.9)
Average $I/\sigma(I)$	36.1 (4.3)
Redundancy	6.1 (4.6)

† $R_{merge} = \sum_h \sum_i |I(h)_i - \langle I(h)\rangle| / \sum_h \sum I(h)_i$, where $I(h)$ is the intensity of reflection *h*, $\sum_h$ is the sum over all reflections and $\sum_i$ is the sum over *i* measurements of reflection *h*.

2.4. X-ray diffraction experiments

A crystal was mounted in a thin-walled glass capillary and the capillary was sealed with wax after filling both ends with the mother liquor. X-ray diffraction data were collected using Cu $K\alpha$ radiation on an R-AXIS IV image-plate system attached to a Rigaku rotating-anode generator (RU-300) running at 50 kV and 90 mA with a 0.3 mm focus cup. The data set was processed and scaled using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

3. Results

We have overexpressed a putative aspartate aminotransferase from *M. jannaschii* encoded by the Mj0684 gene with a yield of ~10 mg of the intact protein from a 4 l culture. Dynamic light-scattering analysis showed that the purified Mj0684 protein was highly monodisperse, with a polydispersity of 10%, and that it has a native molecular mass of ~85 kDa. Therefore, the recombinant Mj0684 protein exists as a dimer. Well diffracting crystals were obtained with the reservoir solution 0.1 *M* sodium acetate, 0.2 *M* ammonium sulfate, 6–7%(*w*/*v*) PEG 4000 at a final pH of 4.5, which was prepared by mixing appropriate volumes of 1.0 *M* sodium acetate pH 4.6, 4.0 *M* ammonium sulfate, 50%(*w*/*v*) PEG 4000 and adjusting the final volume with water. The crystals grew to maximum dimensions of 0.3 × 0.3 × 0.2 mm within one week (Fig. 1). The crystals diffracted to 2.2 Å resolution using Cu $K\alpha$ X-rays from a rotating-anode source and were very stable in the X-ray beam. A complete set of diffraction data to 2.2 Å was collected at 295 K from a crystal using Cu $K\alpha$ radiation. A total of 20 121 unique reflections were measured with a redun-

dancy of 6.1. The space group was determined to be $P4_12_12$ (or $P4_32_12$) on the basis of systematic absences and the unit-cell parameters are a = b = 111.87 (0.01), c = 60.86 (0.05) Å, where the estimated standard deviations are given in parentheses. Table 1 summarizes the statistics of data collection. The asymmetric unit contains one subunit of the Mj0684 protein, giving a $V_{\rm M}$ of 2.25 $Å^3\,Da^{-1}$ and a solvent content of 45.3% by volume. These values are within the frequently observed ranges for protein crystals (Matthews, 1968). An interpretable electron-density map was calculated at 2.5 Å using the single isomorphous replacement data with anomalous differences from a mercury derivative crystal. The phasing step revealed that the correct space group is $P4_32_12$. Refinement of the model is under way.

This work was supported by a grant from the Korea Ministry of Science and Technology (NRL-2001, grant No. M1-0104-00-0132). JKY is a recipient of the BK21 Fellowship.

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