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Purification, crystallization and preliminary X-ray analysis of the fumarylacetoacetase family member TTHA0809 from *Thermus thermophilus* HB8

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Fumarylacetoacetase catalyzes the final step of tyrosine and phenylalanine catabolism. A recombinant form of the fumarylacetoacetase family member TTHA0809 from *Thermus thermophilus* HB8 has been crystallized by the oil-microbatch method using sodium chloride as a precipitating agent. The crystals belong to the monoclinic space group $P2_1$, with unit-cell parameters a = 93.3, b = 73.4, c = 122.6 Å, β = 111.8°. The crystals are most likely to contain two dimers in the asymmetric unit, with a V_M value of 3.32 Å^3 Da^{-1}. Diffraction data were collected at 2.2 Å resolution using synchrotron radiation at beamline BL26B1 of SPring-8, Japan.

1. Introduction

The TTHA0809 protein from *Thermus thermophilus* HB8 belongs to the fumarylacetoacetase (FAA) family. FAA is a metabolic enzyme catalyzing the last step of tyrosine and phenylalanine catabolism: the hydrolysis of fumarylacetoacetate into acetoacetate and fumarate. Acetoacetate is further degraded to acetyl-CoA and fumarate joins the citric acid cycle. The FAA family contains members from bacteria to mammalian species. In humans, deficiency of this activity is associated with the metabolic disease hereditary tyrosinaemia type I, which is also known as hepatorenal tyrosinaemia (Lindblad *et al.*, 1977).

The crystal structure of mouse FAA in complex with its physiological products has been reported previously (Timm *et al.*, 1999). Mouse FAA is a homodimer of 46 kDa monomeric protomers. The protomer, which consists of 419 amino-acid residues, folds into a small α/β N-terminal domain and a large catalytic C-terminal domain with a mixed β-sandwich roll fold. Based on the crystal structure, a reaction mechanism involving a Glu–His–water catalytic triad that is distinct from those of other hydrolases was proposed (Bateman *et al.*, 2001). Recently, the crystal structure of the FAA family member human FLJ36880 protein has been reported (Manjasetty *et al.*, 2004). This protein is a homodimer of 25 kDa monomeric protomers. The FLJ36880 protomer consists of 224 amino-acid residues and is similar to the C-terminal domain of mouse FAA. The TTHA0809 protein studied in this work consists of 264 amino-acid residues. The C-terminal 200 amino-acid residues of TTHA0809 share 43% sequence identity to FLJ36880 and 30% sequence identity to the C-terminal domain of mouse FAA. The N-terminal domain of TTHA0809 has no sequence homology to other FAA family members. The structure–thermostability relationship of FAA family members also remains to be elucidated, as studies have mostly focused on enzymes from mesophilic organisms.

In the present paper, we describe the purification, crystallization and preliminary X-ray diffraction study of the TTHA0809 protein from the extreme thermophile *T. thermophilus* HB8, which has an optimum growth temperature of 348 K. The determination of the crystal structure of TTHA0809 and structural comparison with

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

X-ray wavelength (Å)	1.0
Space group	$P2_1$
Unit-cell parameters	
a (Å)	93.3
b (Å)	73.4
c (Å)	122.6
β (°)	111.8
Resolution range (Å)	30.0–2.20 (2.28–2.20)
Unique reflections	78745 (7869)
Redundancy	4.5 (4.6)
Completeness (%)	100 (100)
$I/\sigma(I)$	11.8 (4.29)
$R_{\rm merge}$† (%)	8.3 (33.4)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i \langle I(hkl)\rangle$, where $I_i(hkl)$ and $\langle I(hkl)\rangle$ are the observed intensity of measurement i and the mean intensity of the reflection with indices hkl, respectively.

homologous enzymes may provide insight into functional aspects and the thermostabilization mechanism of the FAA family enzymes.

2. Experimental

2.1. Expression and purification

The gene coding for TTHA0809 protein (residues 1–264; 29.4 kDa) was amplified by the polymerase chain reaction (PCR) using *T. thermophilus* HB8 genomic DNA (Yokoyama *et al.*, 2000) as a template. The PCR product was inserted into the expression vector pET11a (Novagen). Protein expression and purification were performed as follows by the Structurome Research Group at RIKEN SPring-8 Center. *Escherichia coli* BL21 (DE3) cells were transformed with the recombinant plasmid and grown without IPTG induction at 310 K in LB medium containing 50 µg ml^{-1} ampicillin for 20 h. The cells were harvested by centrifugation at 20 000*g* for 5 min, suspended in 20 m*M* Tris–HCl pH 8.0 (buffer *A*) containing 0.5 *M* NaCl, 5 m*M* 2-mercaptethanol and 1 m*M* phenylmethanesulfonyl fluoride and disrupted by sonication. The cell lysate was heated at 363 K for 13 min. After heat treatment, denaturated proteins were removed by centrifugation (21 600*g*, 30 min) and the supernatant solution was used as the crude extract for purification. The crude extract was desalted using a HiPrep 26/10 desalting column (Amersham Biosciences) and applied onto a Super Q Toyopearl 650M column (Tosoh) equilibrated with buffer *A*. Proteins were eluted with a linear gradient of 0–0.3 *M* NaCl in buffer *A*. The fractions containing TTHA0809 were desalted with a HiPrep 26/10 desalting column with buffer *A* and subjected to a Resource Q column (Amersham Biosciences) equilibrated with buffer *A*. Proteins were eluted with a linear gradient of 0–0.3 *M* NaCl in buffer *A*. The buffer of the fractions containing TTHA0809 was exchanged to 10 m*M* phosphate–NaOH pH 7.0 using a HiPrep 26/10 desalting column and they were applied onto a Bio-Scale CHT-10-I column (Bio-Rad) equilibrated with the same buffer. Proteins were eluted with a linear gradient of 10–200 m*M* phosphate–NaOH pH 7.0. The fractions containing TTHA0809 were pooled, concentrated by ultrafiltration using a Vivaspin concentrator (Vivascience; 5 kDa molecular-weight cutoff) and loaded onto a HiLoad 16/60 Superdex 200 pg column (Amersham Biosciences) equilibrated with buffer *A* containing 0.2 *M* NaCl. The purified protein showed a single band on SDS–PAGE. The concentration of the protein was estimated from the absorbance at 280 nm, assuming $E^{1\%}_{1\,\rm cm} = 15.3$ as calculated from the modified absorption coefficient of the residues (Pace *et al.*, 1995). The purified protein was concentrated to 15.0 mg ml^{-1} using a Vivaspin concentrator (10 kDa molecular-weight cutoff) and stored at 277 K.

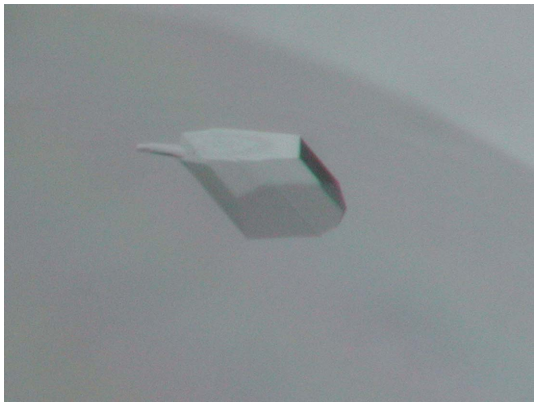

Figure 1
Optical micrograph of a TTHA0809 crystal obtained by the oil-microbatch method. Its approximate dimensions were 0.30 × 0.30 × 0.05 mm.

2.2. Dynamic light-scattering study

The oligomerization state of the purified TTHA0809 protein was examined by a dynamic light-scattering experiment using a DynaPro MS/X instrument (Protein Solutions), which was performed at a protein concentration of 15.0 mg ml^{-1} in 20 m*M* Tris–HCl pH 8.0 with 200 m*M* NaCl. Several measurements were taken at 291 K and analyzed using the *DYNAMICS* software v.3.30 (Protein Solutions). The result showed a sharp monomodal profile centered at 3.25 nm hydrodynamic radius, corresponding to a molecular weight of 53 kDa and suggesting a dimeric state of TTHA0809 in solution.

2.3. Crystallization

Preliminary sparse-matrix screening of crystallization conditions (Jancarik & Kim, 1991) was performed on the purified TTHA0809 protein using the oil-microbatch method (Chayen *et al.*, 1990). Several crystal forms were obtained and one of the most promising crystallization conditions was optimized. Each crystallization drop was prepared by mixing equal volumes (1.0 µl) of precipitant solution (2.5 *M* sodium chloride, 0.2 *M* lithium sulfate, 0.1 *M* sodium citrate buffer pH 5.4) and protein solution (0.2 *M* sodium chloride, 20 m*M* Tris–HCl pH 8.0). The crystallization drop on a NUNC HLA plate (Nalge Nunc International) was overlaid with a 3:7 mixture of silicon and paraffin oils, allowing slow evaporation of water in the drop, and stored at 297 K.

2.4. Data collection

X-ray diffraction data were collected on a Rigaku R-AXIS V image-plate detector using synchrotron radiation at beamline BL26B1 of SPring-8, Japan (Ueno *et al.*, 2006). For data collection under cryogenic conditions, crystals were soaked for a few seconds in a cryoprotectant solution consisting of 20%(*v*/*v*) glycerol, 2.5 *M* sodium chloride, 0.2 *M* lithium sulfate and 0.1 *M* sodium citrate buffer pH 5.4. Crystals were mounted in a nylon loop and flash-cooled in a nitrogen-gas stream at 100 K. Each frame was exposed for 30 s with a 1° oscillation at a crystal-to-detector distance of 310 mm. Data were processed and scaled using *HKL*-2000 (Otwinowski & Minor, 1997).

3. Results

We have established the expression, purification and crystallization of the TTHA0809 protein. Crystals appeared within 3 d of incubation and grew to approximate dimensions of 0.30 × 0.30 × 0.05 mm in one week (Fig. 1). The crystals belonged to the primitive monoclinic system and the systematic absences of reflections ($k = 2n + 1$ at $0k0$) indicated space group $P2_1$. The statistics of the intensity data are shown in Table 1. A dynamic light-scattering experiment and the crystal structures of other FAA family members (PDB codes 1hyo, 1i7o, 1qqj, 1qcn, 1qco and 1saw) suggested a dimeric state of TTHA0809 in solution (see §2). Assuming the presence of two dimers per asymmetric unit, the calculated Matthews coefficient V_M (Matthews, 1968) was 3.32 Å^3 Da^{-1}, which corresponds to a solvent content of 62.9%. Phasing by the molecular-replacement method using the coordinates of human FLJ36880 protein (PDB code 1saw) was successful and details of the refined structure will be published elsewhere.

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References

Bateman, R. L., Bhanumoorthy, P., Witte, J. F., McClard, R. W., Grompe, M. & Timm, D. E. (2001). *J. Biol. Chem.* **276**, 15284–15291.
Chayen, N. E., Shaw Stewart, P. D., Maeder, D. L. & Blow, D. M. (1990). *J. Appl. Cryst.* **23**, 297–302.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Lindblad, B., Lindstedt, S. & Steen, G. (1977). *Proc. Natl Acad. Sci. USA*, **74**, 7641–7645.
Manjasetty, B. A., Niesen, F. H., Delbruck, H., Gotz, F., Sievert, V., Bussow, K., Behlke, J. & Heinemann, U. (2004). *Biol. Chem.* **385**, 935–942.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Pace, C. N., Vajdos, F., Fee, L., Grimsley, G. & Gray, T. (1995). *Protein Sci.* **4**, 2411–2423.
Timm, D. E., Mueller, H. A., Bhanumoorthy, P., Harp, J. M. & Bunick, G. J. (1999). *Structure*, **7**, 1023–1033.
Ueno, G., Kanda, H., Hirose, R., Ida, K., Kumasaka, T. & Yamamoto, M. (2006). *J. Struct. Funct. Genomics*, **7**, 15–22.
Yokoyama, S., Hirota, H., Kigawa, T., Yabuki, T., Shirouzu, M., Terada, T., Ito, Y., Matsuo, Y., Kuroda, Y., Nishimura, Y., Kyogoku, Y., Miki, K., Masui, R. & Kuramitsu, S. (2000). *Nature Struct. Biol.* **7**, 943–945.