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Purification, crystallization and preliminary X-ray crystallographic study of the L-fuculose-1-phosphate aldolase (FucA) from *Thermus thermophilus* HB8

Jeyaraman Jeyakanthan,[a]* Junichiro Taka,[a] Akihiro Kikuchi,[a] Chizu Kuroishi,[b] Katsuhide Yutani[b] and Yoshitugu Shiro[a]

[a]Biometal Science Laboratory, RIKEN Harima Institute, 1-1-1 Kouto, Mikazuki-cho, Sayo-gun, Hyogo 679-5148, Japan, and [b]Advanced Protein Crystallography Research Group, RIKEN Harima Institute, 1-1-1 Kouto, Mikazuki-cho, Sayo-gun, Hyogo 679-5148, Japan

Correspondence e-mail: kanthan@spring8.or.jp

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Fuculose phosphate aldolase catalyzes the reversible cleavage of L-fuculose-1-phosphate to dihydroxyacetone phosphate and L-lactaldehyde. The protein from *Thermus thermophilus* HB8 is a biological tetramer with a subunit molecular weight of 21 591 Da. Purified FucA has been crystallized using sitting-drop vapour-diffusion and microbatch techniques at 293 K. The crystals belong to space group $P4$, with unit-cell parameters $a = b = 100.94$, $c = 45.87$ Å. The presence of a dimer of the enzyme in the asymmetric unit was estimated to give a Matthews coefficient (V_M) of 2.7 $Å^3$ Da^{-1} and a solvent content of 54.2%(v/v). Three-wavelength diffraction MAD data were collected to 2.3 Å from zinc-containing crystals. Native diffraction data to 1.9 Å resolution have been collected using synchrotron radiation at SPring-8.

1. Introduction

Aldolases have been divided into two groups of enzymes on the basis of their method of enzyme catalysis (Rutter, 1964). Class I aldolases are found predominantly in animals, higher plants and green algae, while class II aldolases are found in bacteria, yeasts and fungi. Class I aldolases have an essential lysine residue that forms a protonated Schiff base with the substrate carbonyl carbon to stabilize the intermediate (Horecker *et al.*, 1972; Littlechild & Watson, 1993), whereas the class II aldolases are metal-containing enzymes in which the substrate is coordinated to a divalent metal cation such as zinc or magnesium (Rutter, 1964; Morse & Horecker, 1968). The potential of aldolases is widely recognized in synthetic chemistry (Wong *et al.*, 1995; Whitesides & Wong, 1985), in particular the class II aldolases, which are more stable. Aldolases useful for organic synthesis can be classified into four groups based on the accepted donor substrate, namely (1) dihydroxyacetone phosphate as the donor to produce 2-keto-3,4-dihydroxy adducts, (2) pyruvate or phosphoenol pyruvate as the donor to form 3-deoxy-2-keto acids, (3) acetaldehyde as the donor to form 3-hydroxy aldehydes and (4) glycine as the donor to produce β-hydroxy-α-amino acids. These aldolases are the targets of inhibitors that may have antibacterial properties. Structures of class I aldolases have been previously determined from rabbit muscle (Sygusch *et al.*, 1987; Blom & Sygusch, 1997), human muscle (Gamblin *et al.*, 1991; Dalby *et al.*, 1999), *Drosophila melanogaster* (Hester *et al.*, 1991) and *Plasmodium falciparum* (Kim *et al.*, 1998). Other aldolase structures have been determined from *Pseudomonas putida* and of *N*-acetylneuraminate aldolase from *Escherichia coli* (Izard *et al.*, 1994; Lawrence *et al.*, 1997), 7,8-dihydroneopterin aldolase from *Staphylococcus aureus* (Hennig *et al.*, 1998), L-rhamnulose-1-phosphate aldolase (Kroemer & Schulz, 2002), fructose-1,6-bisphosphate aldolase from the hyperthermophile *Thermoproteus tenax* (Lorentzen *et al.*, 2003) and 2-deoxyribose-5-phosphate aldolase from *Thermus thermophilus* HB8, *E. coli* and *Aeropyrum pernix* (Lokanath *et al.*, 2004; Heine *et al.*, 2001; Sakuraba *et al.*, 2003). Class II aldolases contain a divalent metal ion (usually zinc). The known structures of this class include L-fuculose-1-phosphate aldolase from *E. coli* (Dreyer & Schulz, 1993, 1996*a*,*b*) and fructose-1,6-bisphosphate aldolase from *E. coli* (Blom *et al.*, 1996; Cooper *et al.*, 1996; Hall *et al.*, 1999). L-Fuculose-1-phosphate from *T. thermophilus* HB8 (FucA) is a class II aldolase that catalyzes the reversible cleavage of L-fuculose-1-phosphate (Fuc1P), leading to dihydroxyacetone phos-

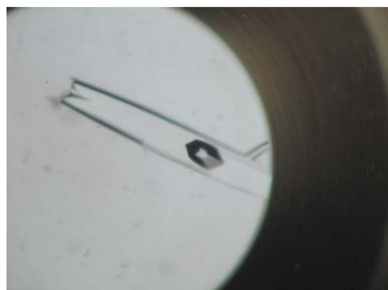

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Table 1
Experimental conditions and data-collection statistics.

Values in parentheses are for the highest resolution shell.

Data collection	Native	Zn peak	Edge	Remote
X-ray source	BL26B1, SPring-8	BL26B1, SPring-8		
Wavelength (Å)	1.0000	1.28230	1.28275	1.3330
Detector	Jupiter 210cs CCD	Jupiter 210cs CCD		
Temperature (K)	100	100		
Crystal-to-detector distance (mm)	180	150		
Space group	$P4$	$P4$		
Unit-cell parameters (Å)	$a = b = 100.944$, $c = 45.870$	$a = b = 100.860$, $c = 45.959$		
Resolution range (Å)	30.0–1.90 (1.97–1.90)	50–2.30 (2.38–2.30)		
Total reflections	245580	105929	100444	102657
Unique reflections	36580	20641	20603	20593
Completeness (%)	99.3 (99.2)	98.8 (99.2)	98.8 (99.3)	98.7 (99.4)
$R_{\rm sym}$†	6.6 (24.6)	10.8 (26.0)	10.6 (25.3)	11.6 (28.5)
Average $\langle I/\sigma(I)\rangle$	18.4 (3.8)	17.9 (5.8)	17.1 (5.2)	16.8 (5.0)
Redundancy	6.7	5.1	4.9	5.0
Anomalous signal (asn‡)		1.69		
$V_{\rm M}$ (Å^3 Da^{-1})	2.7			
Z	2			
Solvent content (%)	54.2			

† $R_{\rm sym} = \sum I_{hkl} - \langle I\rangle|/\sum I_{hkl}$. ‡ Average anomalous signal-to-noise ratio.

phate (DHAP) and L-lactaldehyde, which is a crucial step in the bacterial L-fucose metabolism. This reaction constitues one of the most important methods for forming carbon–carbon bonds (Ghalambor & Heath, 1962) in synthetic organic chemistry. We report here the purification, crystallization and preliminary X-ray analysis of fuculose-L-phosphate aldolase (FucA) from *T. thermophilus* HB8.

2. Material and methods

2.1. Protein expression and purification

The gene was amplified by the polymerase chain reaction (PCR) using *T. thermophilus* HB8 genomic DNA as a template. The recombinant plasmid was constructed using the super-rare-cutter system (Kanagawa *et al.*, manuscript in preparation). *E. coli* BL21-CodonPlus (DE3)-RIL cells were transformed with the recombinant plasmid and grown at 310 K in LB medium containing 50 µg ml^{-1} ampicillin for 20 h. The cells were harvested by centrifugation at 6500 rev min^{-1} for 5 min at 277 K and were suspended in 20 m*M* Tris–HCl pH 8.0 (buffer *A*) containing 0.5 *M* NaCl and 5 m*M* 2-mercaptoethanol and disrupted by sonication. The cell lysate was clarified by centrifugation (15 000 rev min^{-1}, 30 min) and the supernatant was heated at 363 K for 11.5 min. After heat treatment, denaturated proteins were removed by centrifugation (15 000 rev min^{-1}, 30 min) and the supernatant solution was used as the crude extract for purification. The crude extract was desalted using HiPrep 26/10 desalting column (Amersham Biosciences) and applied onto a Super Q Toyopearl 650M column (Tosoh) equilibrated with buffer *A*. The protein was eluted with a linear gradient of 0–0.3 *M* NaCl in buffer *A*. The protein was desalted using a HiPrep 26/10 desalting column with buffer *A* and subjected to a Resource Q column (Amersham Biosciences) equilibrated with buffer *A*. The protein was eluted with a linear gradient of 0–0.3 *M* NaCl in buffer *A*. The fractions containing proteins were desalted using a HiPrep 26/10 desalting column with 10 m*M* sodium phosphate pH 7.0 and applied onto a Bio-Scale CHT-20-I column (Bio-Rad) equilibrated with the same buffer. The protein was eluted with a linear gradient of 10–300 m*M* sodium phosphate pH 7.0. The fractions containing protein were pooled, concentrated by ultrafiltration (Vivaspin, 5 kDa cutoff) and loaded onto a HiLoad 16/60 Superdex 200 prep-grade column (Amersham Biosciences) equilibrated with buffer *A* containing 0.2 *M* NaCl. The purified protein was homogeneous on native PAGE.

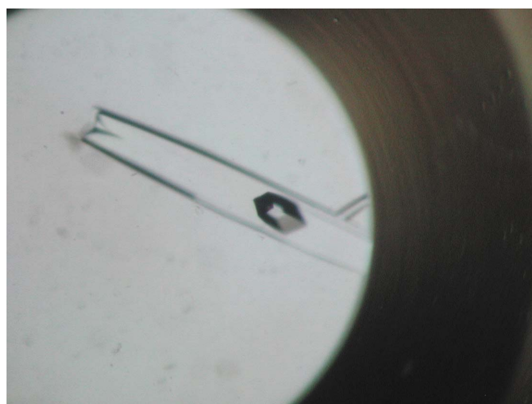

Figure 1
A crystal (two crystals are inside the well) of L-fuculose-1-phosphate aldolase from *T. thermophilus* HB8 (FucA). The largest crystal has approximate dimensions of 0.6 × 0.3 × 0.1 mm.

2.2. Crystallization

Crystallization was performed by the sitting-drop vapour-diffusion method at 293 K using Linbro multiwell plates. Each drop consisting of 1 µl 20 mg ml^{-1} protein solution in 0.1 *M* Tris–HCl buffer pH 7.9 and 1 µl reservoir solution was allowed to equilibrate against 100 µl reservoir solution. Preliminary screening was performed using Hampton Research Crystal Screens I and II (Jancarik & Kim, 1991). Small crystals appeared from a condition with reservoir solution consisting of 1.5 *M* lithium sulfate monohydrate in 0.1 *M* HEPES pH 7.5. Slightly larger crystals were obtained in the range 1.4–1.5 *M* lithium sulfate monohydrate, 0.1 *M* Na HEPES pH 7.0–7.5, followed by refinement of this condition through variation of protein concentration, pH and drop volume. To improve the crystal size and quality, further optimization of the conditions was carried out using the microbatch (diffusion through paraffin oil) method with Nunc HLA plates (Nalge Nunc International) at 293 K. Crystals suitable

for X-ray data collection appeared (Fig. 1) within 10–15 d and reached final dimensions of 0.6 × 0.3 × 0.1 mm.

2.3. Data collection and processing

Diffraction data were collected using a Jupiter 210cs CCD detector at the BL26B1 beamline, SPring-8, Japan. The crystals were flash-frozen in a nitrogen-gas stream at 100 K directly from a drop containing 30%(*w*/*v*) glycerol as a cryoprotectant and were maintained at 100 K during data collection. For the Zn-containing crystal, MAD (multiple anomalous diffraction) data sets were collected corresponding to the maximum f'' (peak), the minimum f' (edge) and a reference wavelength (remote), chosen on the basis of the absorption spectrum of the metal. Based on the absorption spectrum, two energy levels were chosen for data collection, both of which were near the absorption *K* edge of the Zn atom: 9.669 and 9.666 keV. The third energy level was set to 9.301 keV as a remote point. Native diffraction data were collected to 1.9 Å. The diffraction data were processed with the *HKL*2000 package (Otwinowski & Minor, 1997). The crystallographic data and the MAD data statistics for zinc-containing crystals are summarized in Table 1.

2.4. Dynamic light-scattering studies

A dynamic light-scattering experiment was performed using a DynaPro MS/X instrument from Protein Solutions (Lakewood, New Jersey, USA). The measurements were made at 291 K on the purified protein at 0.5–1.0 mg ml^{-1} in buffer solution containing 20 m*M* Tris–HCl and 200 m*M* sodium chloride.

3. Discussion

The crystals belonged to space group *P*4, with unit-cell parameters $a = b = 100.94$, $c = 45.87$ Å. A total of 245 580 measured reflections in the resolution range 30–1.9 Å were merged into 36 580 unique reflections with an R_{merge} of 6.6%. Details of data collection and processing are given in Table 1. The value of the Matthews coefficient (Matthews, 1968) is 2.7 $Å^3 Da^{-1}$ and the solvent content is 54.2%(*v*/*v*) assuming the presence of a dimer in the asymmetric unit. Dynamic light-scattering experiments showed the presence of a tetramer in solution, suggesting biological significance of the tetramer. The present enzyme shows 33% sequence identity with L-fuculose-1-phosphate aldolase from *E. coli* (PDB code 1dzw; Dreyer & Schulz, 1993). The molecular-replacement method using L-fuculose-1-phosphate aldolase as a search model, which was attempted using *AMoRe* (Navaza, 1994) and *PHASER* (Read, 2001; Storoni *et al.*, 2004), did not yield a clear solution. Class II aldolases contain a divalent metal ion (usually zinc) in the active site. On the basis of this, we measured the X-ray fluorescence scans of the divalent metal atoms Fe^{2+}, Ni^{2+}, Co^{2+} and Zn^{2+}. X-ray fluorescence scans of the crystals had a strong absorption edge corresponding to Zn. The diffraction data recorded at wavelengths near the Zn X-ray absorption edge were used in MAD phasing (Hendrickson *et al.*, 1990). The peak data set was used for the analysis of Bijvoet and difference Patterson maps did not clearly show the position of the anomalous scatter. The average value of the anomalous signal-to-noise ratio in the high-energy data set is 1.69 in the resolution range 30–2.3 Å and is similar to that in the edge and remote data sets. Therefore, reliable initial phases could not be obtained from the Zn MAD data sets. A selenomethionyl derivative of the protein has now been obtained under the same conditions as used for the native crystals and appears to have similar diffraction properties. Further attempts to determine the structure using Zn data as well as SeMet data are in progress.

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