*Journal of Biochemistry and Molecular Biology*, Vol. 31, No. 6, September 1998, pp. 600–603

JOURNAL OF
Biochemistry
and
Molecular Biology



**Short communication**

# A Thermostable Xylose Isomerase from *Thermus thermophilus*: Biochemical Characterization, Crystallization, and Preliminary X-ray Analyses

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*Received 1 June 1998, Accepted 30 June 1998*

**A highly thermostable xylose isomerase from *Thermus thermophilus* has been expressed in *Escherichia coli* and crystallized. The purified enzyme shows its optimum temperature at 90°C. It has been crystallized at room temperature using polyethylene glycol 4000 as the precipitant. The crystal belongs to the orthorhombic space group $P2_12_12_1$, with unit cell parameters of $a$ = 73.34 Å, $b$ = 144.05 Å, $c$ = 155.07 Å. The presence of one molecule of tetrameric xylose isomerase in the asymmetric unit gives a crystal volume per protein mass ($V_m$) of 2.32 Å$^3$/Da and the solvent content of 47.0% by volume. The diffraction pattern extends to 1.9 Å Bragg spacing with synchrotron radiation and a set of native data has been collected to 2.3 Å.**

**Keywords:** Crystallization, Thermostable enzyme, *Thermus thermopilus*, Xylose isomerase.

## Introduction

Xylose isomerase (XI; E.C. 5.3.1.5) catalyses the reversible isomerization between an aldo sugar D-xylose and a keto sugar D-xylulose in the first step of xylose metabolism. It also catalyzes the isomerization between D-glucose and D-fructose (Meng *et al.*, 1991; Rangarajan and Hartley, 1992). The isomerization reaction catalyzed by xylose isomerases is an equilibrium reaction and the ratio between D-glucose and D-fructose in the product depends on the temperature. The current isomerization process operated at 58°C yields a 40–42% fructose syrup and an additional chromatographic step is necessary to produce the 55% syrup concentration. Performing the isomerization reaction at 95°C would achieve a high fructose corn syrup without the chromatographic step (Zeikus, 1996). Therefore, there is much commercial interest in developing highly thermostable xylose isomerases for the industrial production of high fructose corn syrup.

Crystal structures of type I XIs from several mesophilic bacteria have been reported (Carrell *et al.*, 1984; Rey *et al.*, 1988; Carrell *et al.*, 1989; Farber *et al.*, 1989; Henrick *et al.*, 1989; Dauter *et al.*, 1990; Whitlow *et al.*, 1991; Cha *et al.*, 1994; Lavie *et al.*, 1994; Rasmussen *et al.*, 1994; Allen *et al.*, 1995). They share the common fold of TIM barrel with an extended domain for each subunit and a similar tetrameric arrangement of subunits. However, there has been no report of the structure for any type I XIs with an optimum temperature near 90°C. When compared with type I XIs, type II XIs have extended N-terminal amino acid residues. Highly thermostable type II XIs from *Thermoanaerobacterium thermosulfurigenes* and *Thermotoga neapolitana* have been crystallized (Lloyd *et al.*, 1994; Chayen *et al.*, 1997), but their crystal structures have yet to be reported.

*Thermus thermophilus* HB8, a thermophilic bacterium, produces a highly thermostable type I XI, which is a homotetrameric enzyme of 387-residue subunits (subunit $M_r$ = 43,849 Da; Dekker *et al.*, 1991). In this study, its optimum reaction temperature is determined to be 90°C. Therefore, XI from *T. thermophilus* (TthXI) is potentially useful for the production of high fructose corn syrup. It will be interesting to compare the structure of this thermostable XI with those of less thermostable XIs to delineate the structural basis of the thermostability. As a first step, we report here the crystallization and preliminary X-ray analysis of the crystals.

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## Materials and Methods

**Expression, purification, and biochemical characterization** The gene encoding XI (*xylA*) was obtained by the polymerase chain reaction (PCR) using the chromosome of *Thermus thermophilus* HB8 as the template and two primers, 5′-CATARGTACGAACCCAAACCGGAGCACAGG-3′ and 5′-TCANCCNCKNACNCCNRNA RRTA-3′ (where K is G or T, N is A or C, and R is A or G). The PCR product was initially inserted into the *Sma*I site of the vector pUC19. Then, the cloned plasmid was digested with *Eco*RI–*Sal*I and the fragment of 1.2 kb size was inserted into the same site of pKK223-3, in which the protein was expressed under the control of *tac* promoter without any extra amino acids at either C- or N- terminus.

Cells were harvested by centrifugation of a 10 l culture (4200 × *g*, 10 min, Sorvall GS3, Newtown, USA). The pellet was resuspended in 100 ml of the lysis buffer (50 mM potassium phosphate at pH 7.2, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 mM β-mercaptoethanol, 0.1% pepstatin A), and then homogenized with an ultrasonic processor (Branson sonifier model 350, Danbury, USA). The solution was centrifuged (Sorvall GS3, 4200 × *g*, 1 h) and the cell debris was discarded. The cell extract was heated and kept between 75 and 80°C for 10 min, taking 5 min to reach 75°C from room temperature, in order to denature contaminating heat-labile proteins. Upon being chilled in an ice bath, it was centrifuged (Sorvall GS3, 4200 × *g*, 1 h). The supernatant was subject to column chromatography using DEAE-Sephacel employing a linear gradient of 0 to 500 mM NaCl in buffer P (50 mM potassium phosphate, pH 7.0), and subsequently on a hydrophobic interaction column (phenyl-Toyopearl TSK, Montgomeryville, USA) with a linear gradient of 0.8 to 0.4 M ammonium sulfate in buffer P.

The optimum reaction temperature was determined by incubating a mixture of the substrate (100 mM D-fructose), buffer (100 mM HEPES, pH 7.5), metal ion (2.5 mM $MnCl_2$), and xylose isomerase at different temperatures for 10 min. Then, the reaction mixture was chilled on ice to stop the reaction and D-glucose in the solution was quantified by using the peroxidase-glucose oxidase (PGO) assay kit from Sigma (St. Louis, USA).

**Crystallization** The purified enzyme was concentrated to about 10 mg/ml concentration using a YM30 ultrafiltration membrane (Amicon) and then dialyzed against 50 mM sodium phosphate, pH 7.2, for crystallization. The protein concentration was estimated by measuring the absorbance at 280 nm, assuming a correspondence of 1.0 mg/ml concentration to the unit absorbance for 1.0 cm pathlength. Crystallization was performed by the hanging-drop vapor diffusion method using 24-well tissue culture plates (Linbro, Aurora, USA) at room temperature. Hanging drops were prepared by mixing 4 μl each of the protein and the reservoir solutions. The protein solution contained TthXI at 10 mg/ml concentration in 50 mM sodium phosphate buffer at pH 7.2. Initial searches for crystallization was performed using SCREEN I (Jancarik and Kim, 1991) and SCREEN II conditions (Hampton Research).

**X-ray data collection** X-ray data of TthXI were collected from one crystal that was mounted with the longest axis being tilted by 30° from the rotation axis. The X-ray data were collected at 14°C using a Weissenberg camera for macromolecular crystallography, at the BL-$6A_2$ experimental station of Photon Factory, Tsukuba, Japan (Sakabe, 1991). The wavelength of synchrotron X-rays was 1.00 Å and a 0.1 mm collimator was used. A Fuji image plate (type BAIII, 20 × 40 cm) was placed at a distance of 429.7 mm from the crystal and the coupling constant was 1.0°/mm. The oscillation range per one image plate was 3.5° with a speed of 2.0°/sec, allowing an overlap of 0.5° between two contiguous image plates.

The diffraction patterns recorded on the image plates were digitized by a Fuji BA100 scanner. The raw data were processed using the program DENZO (Otwinowski, 1993) and scaled with SCALA and AGROVATA in the CCP4 package (SERC Daresbury, 1993)

## Results and Discussion

**Expression and purification** The expression of recombinant XI was induced by 0.3 mM IPTG when the *E. coli* MV1184 cells were grown up to $OD_{600}$ of 0.6. The expression level reached about 10% of total soluble cellular protein after 6 h of induction. Heat treatment and two chromatographic purification steps yielded highly purified XI. The homogeneity was about 99% as estimated by SDS-PAGE (Fig. 1). The final yield was 20 mg of the purified protein per liter of culture.

**Optimum temperature** The temperature-dependence of the activity of TthXI is shown in Fig. 2. The activity increases as the temperature is raised and it reaches the maximum at 90°C. The optimum reaction temperature of 90°C for this XI is the highest among type I XIs.

**Crystallization and X-ray experiments** Rod-shaped crystals grew up to typical dimensions of 0.15 mm × 0.15 mm × 0.5 mm within one wk, under the optimized

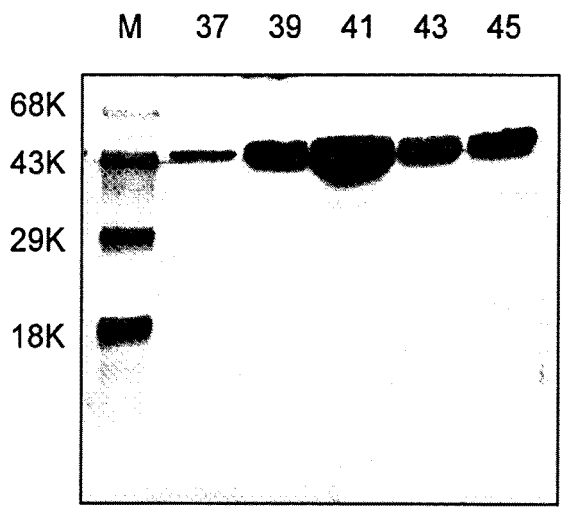


**Fig. 1.** SDS-PAGE of purified TthXI. The lane marked M at the top is molecular weight markers with indicated sizes and all other lanes are fractions from the phenyl-toyopearl TSK column.

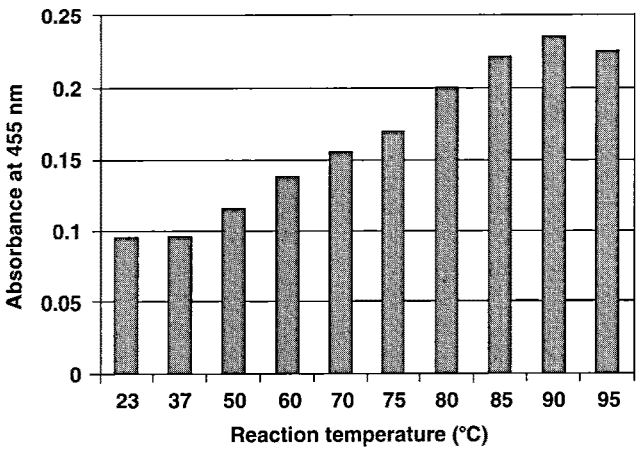


**Fig. 2.** The reaction temperature-activity profile of TthXI.

condition of 20% (w/v) polyethylene glycol 4000, 10 mM CDTA, 30 mM lithium sulfate, and 100 mM sodium borate (final pH 8.81) for the reservoir solution.

Upon exposure to synchrotron X-rays, the crystal initially showed the diffraction pattern to be of 1.9 Å Bragg spacing. Since the crystal was somewhat sensitive to X-rays, the crystal was translated along the rotation axis (Z-translation) after 3 h exposure. Sixty-seven frames of oscillation data from one crystal were collected and processed. The data consist of 295,294 measurements of 66,746 unique reflections with $R_{merge}$ of 7.8%. The completeness of merged data set is 74% in the resolution range of 8.0 to 2.3 Å and 51.6% for the outer shell between 2.35 Å and 2.3 Å for reflections with $F_o > 2s$. The intensity distribution of the data indicates that the space group is $P2_12_12_1$, and the unit cell dimensions are $a = 73.34$ Å, $b = 144.05$ Å, $c = 155.07$ Å. The presence of one tetrameric XI in the asymmetric unit gives a crystal volume per protein mass ($V_M$) of 2.32 Å$^3$/Da and a solvent content of 47.0% by volume (Matthews, 1968).

**Acknowledgements** This work was supported by the Korea Science Engineering Foundation through the Center for Molecular Catalysis, Seoul National University and the Korea Ministry of Education (BSRI-97-3418). We thank Prof. N. Sakabe, Dr. Watanabe, and Dr. Z. M. Suzuki for assistance during data collection at BL-6A2 of Photon factory, Japan.

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