

Shankar Prasad Kanaujia,^a
Chellamuthu Vasuki Ranjani,^a
Jeyaraman Jeyakanthan,^b
Masami Nishida,^b Yoshiaki
Kitamura,^b Seiki Baba,^{b,c} Akio
Ebihara,^b Nobutaka Shimizu,^d
Noriko Nakagawa,^{b,d} Akeo
Shinkai,^b Masaki Yamamoto,^{b,d}
Seiki Kuramitsu,^{b,c} Yoshitsugu
Shiro,^b Kanagaraj Sekar^{a,e*} and
Shigeyuki Yokoyama^{b,f,g}

^aBioinformatics Centre (Centre of Excellence in Structural Biology and Biocomputing), Indian Institute of Science, Bangalore 560 012, India,

^bRIKEN SPring-8 Center, Harima Institute, 1-1-1 Kouto, Sayo, Hyogo 679-5148, Japan,

^cDepartment of Biology, Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan, ^dJapan Synchrotron Radiation Research Institute, 1-1-1 Kouto, Sayo, Hyogo 679-5198, Hyogo, Japan,

^eSupercomputer Education and Research Centre, Indian Institute of Science, Bangalore 560 012, India, ^fRIKEN Genomic Sciences Center,

1-7-22 Suehiro-cho, Tsurumi, Yokohama 230-0045, Japan, and ^gDepartment of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

Correspondence e-mail:
sekar@physics.iisc.ernet.in

Received 24 December 2006

Accepted 27 March 2007

Preliminary X-ray crystallographic study of glucose dehydrogenase from *Thermus thermophilus* HB8

Thermus thermophilus is an aerobic chemoorganotroph that has been found to grow anaerobically in the presence of nitrate. Crystals of glucose dehydrogenase (GDH) from *T. thermophilus* HB8 belong to space group $P2_1$, with unit-cell parameters $a = 36.90$, $b = 132.96$, $c = 60.78$ Å, $\beta = 97.2^\circ$. Preliminary studies and molecular-replacement calculations reveal that the asymmetric unit contains two monomers.

1. Introduction

Thermophiles are heat-loving organisms that grow at an average temperature of 323 K and to a maximum of 343 K or greater. These ancient organisms have been found to prefer the extreme conditions that were typical of the incipient earth. Researchers believe that the study of these organisms will provide details pertaining to the origin of life on earth (Guilio, 2003). Most thermophiles that live in geothermal environments are strict anaerobes. Exceptions include the *Thermus* species, especially *T. thermophilus*, which are aerobic chemoorganotrophs (Ramírez-Arcos *et al.*, 1998). *T. thermophilus* HB8, however, has been found to grow anaerobically in the presence of nitrate. Proteins from thermophilic organisms typically remain active without denaturation even at high temperatures.

In recent years there has been an intense focus on the study of proteins present in *T. thermophilus*, as similar proteins have been found to occur in certain mesophilic organisms. Many new enzymes of potential interest for biotechnological applications have been found in *T. thermophilus*. These include various proteases and key enzymes for other fundamental biological processes such as DNA replication, DNA repair and RNA maturation (Pantazaki *et al.*, 2002). Taq polymerase from the same genus is extensively used in the polymerase chain reaction (PCR). Glucose dehydrogenase (GDH; EC 1.1.1.47) is an enzyme found in *T. thermophilus* that catalyses the oxidation of D-glucose without prior phosphorylation to D-β-gluconolactone using NAD or NADP as a coenzyme (Pauly & Pfeleiderer, 1975). The opportunistic uptake of glucose and galactose by *T. thermophilus* GDH suggests that it represents an early state of the evolution of GDH enzymes in hyperthermophilic species (Schafer, 1996; Albers *et al.*, 1999; Theodossis *et al.*, 2004). The purpose of the work reported in this paper is to generate a better understanding of the structural features that are responsible for the catalytic activity of GDH.

2. Materials and methods

2.1. Cloning, expression and purification

The *TTHA0570* gene, which encodes GDH, was amplified by polymerase chain reaction from the *T. thermophilus* HB8 genome and cloned into the expression plasmid pET-11a (Novagen). *Escherichia coli* BL21(DE3) cells were transformed by the expression plasmid and the transformants were grown at 310 K overnight in a medium containing 1.0% polypeptone, 0.5% yeast extract, 0.5% NaCl and 50 µg ml⁻¹ ampicillin pH 7.0. The cells were collected by centrifugation and were disrupted by sonication in 20 mM Tris-HCl



buffer pH 8.0 containing 50 mM NaCl. The soluble fraction obtained after centrifugation at 15 000g for 20 min was heated at 343 K for 10 min. After the denatured proteins had been removed by centrifugation at 15 000g for 20 min, the sample was applied onto a Resource ISO column (GE Healthcare Biosciences) pre-equilibrated with 50 mM sodium phosphate buffer pH 7.0 containing 1.5 M (NH₄)₂SO₄. The bound proteins were eluted with a linear gradient of 1.5–0 M (NH₄)₂SO₄. The fractions containing TTHA0570 were collected, desalted against 20 mM MES buffer pH 6.0 containing 0.1 M NaCl and then applied onto a Resource S column (GE Healthcare Biosciences), which was eluted with a linear gradient of 0.1–0.5 M NaCl. The fractions containing TTHA0570 were collected and then applied onto a HiLoad 16/60 Superdex 75pg column (GE Healthcare Biosciences) pre-equilibrated with 20 mM Tris–HCl buffer pH 8.0 containing 0.15 M NaCl. Finally, the purified protein was applied onto a HiPrep 26/10 Desalting column (GE Healthcare Biosciences) pre-equilibrated with 20 mM Tris–HCl buffer pH 8.0 containing 150 mM NaCl and 1 mM DTT. The sample was concentrated to 3.76 mg ml⁻¹ with a VivaSpin 6 concentrator (5 kDa molecular-weight cutoff, Sartorius AG). The fraction containing TTHA0570 protein was identified by SDS–PAGE in each case. The N-terminal amino-acid sequence of the purified protein is LLGLG, which means that nine N-terminal amino acids have been deleted.

2.2. Crystallization experiments

Preliminary screening of crystallization conditions was carried out using the Hampton MembFac kit. Crystals were obtained by the sitting-drop vapour-diffusion method with drops consisting of 1 µl protein solution and 1 µl reservoir solution equilibrated against 100 µl well solution at 293 K. Using a reservoir solution composed of 12% (w/v) PEG 6000, 0.1 M NaCl and 0.1 M sodium acetate pH 4.6 (condition No. 10), small crystals (Fig. 1) appeared in about 80 d. Further optimization of promising crystallization conditions [12–15% (w/v) PEG 6000, 0.1 M NaCl and 0.1 M sodium acetate pH 4.6] yielded good diffraction-quality crystals. Prior to immersion in liquid nitrogen for storage and data collection, the crystals were transferred to reservoir solution containing 30% (v/v) trehalose for cryoprotection.

2.3. Data collection

Diffraction data were collected at 100 K using the Structural Biology I beamline BL41XU at SPring-8 (Hyogo, Japan), which was equipped with an ADSC Q315 detector. The distance between the crystal and the detector was maintained at 250 mm. The crystal

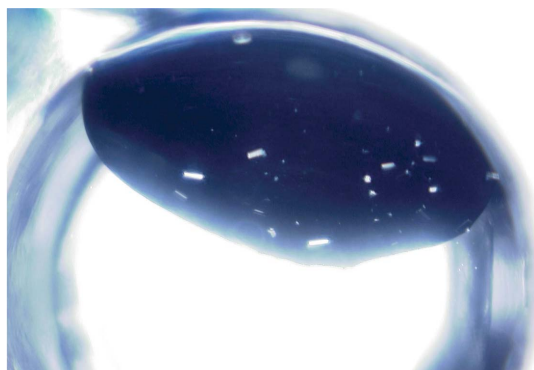


Figure 1
Crystals of glucose dehydrogenase from *T. thermophilus* HB8.

Table 1

Crystal data and X-ray data-collection statistics of glucose dehydrogenase from *T. thermophilus* HB8.

Values in parentheses are for the last resolution shell.

Crystal symmetry	Primitive monoclinic
Wavelength (Å)	0.9790
Temperature (K)	100
Space group	<i>P</i> 2 ₁
Unit-cell parameters (Å, °)	<i>a</i> = 36.90, <i>b</i> = 132.96, <i>c</i> = 60.78, β = 97.2
Matthews coefficient (Å ³ Da ⁻¹)	1.9
Solvent content (%)	35.2
Resolution range (Å)	50.0–1.90 (1.97–1.90)
No. of observed reflections	330344
No. of unique reflections	45627 (4548)
Completeness (%)	100 (99.7)
<i>R</i> _{sym} [†] (%)	7.8 (32.0)
Multiplicity	7.2 (6.3)
Average <i>I</i> /σ(<i>I</i>)	23.23 (3.5)
Wilson <i>B</i> factor (Å ²)	19.5

[†] $R_{\text{sym}} = \sum_h \sum_i |I(h)_i - \langle I(h) \rangle| / \sum_h \sum_i I(h)_i$, 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*.

(0.04 × 0.025 × 0.025 mm) diffracted to 1.77 Å resolution. The *HKL* program suite (Otwinowski & Minor, 1997) was used to process the X-ray diffraction data. The BL41XU beamline was specially designed to collect intensity data from micro-sized crystals. A portion of the diffraction pattern is shown in Fig. 2. The micro-sized beam (25 × 25 µm) leads to high signal-to-noise ratio data collection from such a small crystal. The pertinent details of diffraction data are provided in Table 1.

3. Results and discussion

The diffraction data from the crystal indicate that it belongs to space group *P*2₁, with unit-cell parameters *a* = 36.90, *b* = 132.96, *c* = 60.78 Å, β = 97.2°. The presence of a dimer in the asymmetric unit gives a *V*_M value of 1.9 Å³ Da⁻¹, with a solvent content of 35.2% (Matthews, 1968), suggesting that this is the correct packing. The molecular weight of the protein is 39 kDa for 352 amino-acid residues.

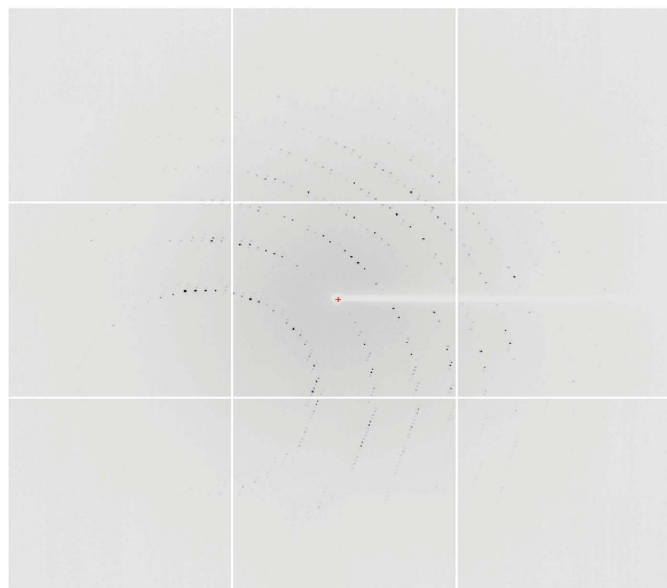


Figure 2
Portion of the X-ray diffraction frame of the glucose dehydrogenase crystal.

3.1. Molecular replacement

The crystal structure of the *T. thermophilus* GDH was solved by molecular replacement using the program *CNS* v.1.1 (Brünger *et al.*, 1998). The search-model coordinates were those of aldose sugar dehydrogenase (PDB code 2g8s; Southall *et al.*, 2006), which shares 34% sequence identity with GDH from *T. thermophilus*. Nonprotein atoms were removed from the search model used in the molecular-replacement calculations and the monomer was used as a search model. Molecular-replacement calculations confirmed the presence of two monomers in the asymmetric unit, with a crystal-packing value of 0.6697 for the best solution. The molecules were subjected to rigid-body refinement using *CNS* v.1.1 (Brünger *et al.*, 1998). A total of 5% of the reflections were used for the calculation of R_{free} (Brünger, 1992). The R_{work} and R_{free} values of the partially refined structures were 40.3% and 42.1%, respectively.

SPK, CVR and KS thank the Bioinformatics Centre (DIC) and the Supercomputer Education and Research Centre (SERC). We thank Dr Koji Takio and Naoko Takahashi for the N-terminal amino-acid sequence analysis of the recombinant protein. This work was supported by the RIKEN Structural Genomic/Proteomics Initiative (RSGI), the National Project on Protein Structural and Functional Analyses, Ministry of Education, Culture, Sports, Science and Tech-

nology of Japan. The diffraction experiments were performed at the SPring-8 with the approval of the Japan Synchrotron Radiation Research Institute (Proposal No. 2006A1414).

References

- Albers, S. V., Elferink, M. G. L., Charlebois, R. L., Sensen, C. W., Driessen, A. J. M. & Konings, W. N. (1999). *J. Bacteriol.* **181**, 4285–4291.
- Brünger, A. T. (1992). *Nature (London)*, **355**, 472–474.
- Brünger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Gross-Kunstleve, R. W., Jiang, J.-S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T. & Warren, G. L. (1998). *Acta Cryst.* **D54**, 905–921.
- Guilio, M. D. (2003). *J. Theor. Biol.* **221**, 425–436.
- Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
- Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
- Pantazaki, A. A., Pritsa, A. A. & Kyriakidis, D. A. (2002). *Appl. Microbiol. Biotechnol.* **58**, 1–12.
- Pauly, H. E. & Pfeleiderer, G. D. (1975). *Hoppe-Seyler's Z. Physiol. Chem.* **356**, 1613–1623.
- Ramírez-Arcos, S., Fernández-Herrero, L. A., Marín, I. & Berenguer, J. (1998). *J. Bacteriol.* **180**, 3137–3143.
- Schafer, G. (1996). *Biochem. Biophys. Acta*, **1277**, 163–200.
- Southall, S. M., Doel, J. J., Richardson, D. J. & Oubrie, A. (2006). *J. Biol. Chem.* **281**, 30650–30659.
- Theodossis, A., Walden, H., Westwick, E. J., Connaris, H., Lamble, H. J., Hough, D. W., Danson, M. J. & Taylor, G. L. (2004). *J. Biol. Chem.* **279**, 43886–43892.