Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Lactate dehydrogenase from the hyperthermophilic archaeon *Methanococcus jannaschii*: overexpression, crystallization and preliminary X-ray analysis

Byung Il Lee,[a] Changsoo Chang,[a] Seung-Je Cho,[a] Gye Won Han,[a] Yeon Gyu Yu,[b] Soo Hyun Eom[c] and Se Won Suh[a]*

[a]Department of Chemistry, College of Natural Sciences, Seoul National University, Seoul 151-742, South Korea, [b]Structural Biology Center, Korea Institute of Science and Technology, PO Box 131, Cheongryang, Seoul 130-650, South Korea, and [c]Department of Life Science, Kwangju Institute of Science and Technology, Kwangju 500-712, South Korea

Correspondence e-mail: sewonsuh@snu.ac.kr

Received 6 August 1999
Accepted 27 October 1999

L(+)-Lactate dehydrogenase (LDH) is a key enzyme in anaerobic metabolism which converts pyruvate to lactate. LDH from the hyperthermophilic archaebacterium *Methanococcus jannaschii* has been overexpressed in *Escherichia coli* and crystallized in two crystal forms at 297 K using 2-methyl-2,4-pentanediol as precipitant. Type I crystals grew rapidly and diffracted to at least 2.8 Å Bragg spacing upon exposure to Cu $K\alpha$ X-rays. X-ray diffraction data to 2.9 Å have been collected from a native crystal. The type I crystal is tetragonal, belonging to the space group $P4_22_12$, with unit-cell parameters $a = b = 99.74$, $c = 170.00$ Å. The asymmetric unit contains two LDH subunits, with a corresponding crystal volume per protein mass (V_m) of 3.05 Å^3 Da^{-1} and a solvent content of 59.7%. Type II crystals, which grew more slowly, diffracted to at least 1.8 Å Bragg spacing upon exposure to Cu $K\alpha$ X-rays. X-ray diffraction data to 1.9 Å have been collected from a native crystal. The type II crystal is orthorhombic, belonging to the space group $P2_12_12$, with unit-cell parameters $a = 47.65$, $b = 125.10$, $c = 58.08$ Å. The asymmetric unit contains a single LDH subunit, with a corresponding crystal volume per protein mass (V_m) of 2.50 Å^3 Da^{-1} and a solvent content of 50.8%. Therefore, the type II crystal is more suitable for high-resolution structure determination than the type I crystal.

1. Introduction

L(+)-Lactate dehydrogenase (LDH; E.C. 1.1.1.27) is a key enzyme in anaerobic metabolism which converts pyruvate to lactate using NADH as a cofactor (Holbrook *et al.*, 1975). LDHs from different sources have been extensively characterized for their catalytic mechanism, protein evolution, stability, folding and three-dimensional structure (Rossmann *et al.*, 1975; Jaenicke, 1987). Crystal structures of LDHs from mesophilic organisms (Adams *et al.*, 1970; Buehner *et al.*, 1974; White *et al.*, 1976; Grau *et al.*, 1981; Abad-Zapatero *et al.*, 1987; Hogrefe *et al.*, 1987; Iwata *et al.*, 1994; Dunn *et al.*, 1996), thermophilic organisms (Piontek *et al.*, 1990; Wigley *et al.*, 1992) and the hyperthermophilic bacterium *Thermotoga maritima* (Auerbach *et al.*, 1998) have been determined. However, no structure of any LDH from archaebacteria has been reported.

LDH from the hyperthermophilic bacterium *T. maritima* was found to represent the most stable LDH isolated so far (Ostendorp *et al.*, 1996). The major fraction of the *E. coli* expressed enzyme existed as homotetramers, like other LDHs, but a small fraction existed as homooctamers. The two species were indistinguishable in terms of spectral properties, but the octamer exhibited reduced specific activity. The crystal structure of the tetramer revealed possible strategies for protein thermostabilization (Auerbach *et al.*, 1998).

In order to provide more structural information on LDH from hyperthermophilic organisms, we have initiated the structure determination of LDH from the hyperthermophilic archaeon *M. jannaschii*. Its polypeptide chain comprises 313 amino-acid residues (M_r = 34 609). Its amino-acid sequence (Bult *et al.*, 1996) shows 38.1% identity with LDH from *T. maritima*. As the first step toward its structural elucidation, we report here its gene cloning, overexpression, crystallization and preliminary X-ray crystallographic analysis.

2. Experimental

2.1. Cloning, overexpression and purification

The gene encoding *M. jannaschii* LDH (MJ0490) was amplified by the polymerase chain reaction (PCR) using its chromosomal DNA as the template. The forward and reverse oligonucleotide primers were designed using the published sequence (Bult *et al.*, 1996). The PCR product was inserted into *Nde*I/*Bam*HI-digested pET-22b (Novagen). The protein was overexpressed in *E. coli* C41(DE3) cells

© 2000 International Union of Crystallography
Printed in Denmark – all rights reserved

(Miroux & Walker, 1996). The cells were grown to an OD_{600} of 0.6 at 310 K and the expression of the recombinant enzyme was induced using 1 m*M* IPTG. The expression level reached about 30% of total soluble cellular protein after 18 h of induction. Cultured cells were resuspended in the lysis buffer (50 m*M* Tris–HCl pH 8.3, 1 m*M* EDTA, 1 m*M* phenylmethylsulfonyl fluoride) and were homogenized with an ultrasonic processor (Branson sonifier model 350). The solution was centrifuged at 35 000*g* for 30 min and the cell debris was discarded. The cell extract was heated and kept between 353 and 358 K for 10 min. This enzyme was found to be stable at this temperature (unpublished data). It was then centrifuged again at 35 000*g* for 30 min. The supernatant was subjected to column chromatography using DEAE–Sepharose (Pharmacia), employing a linear gradient of 0–1.0 *M* NaCl in buffer *A* (20 m*M* Tris–HCl pH 8.3, 1 m*M* EDTA). The enzyme was dialyzed against buffer *A* and was further purified using a blue-Sepharose column (Pharmacia), eluting with 1.0 *M* NaCl in buffer *A*. The purified protein was homogeneous on SDS–PAGE. Prior to crystallization, the protein buffer was changed to 20 m*M* sodium phosphate pH 6.6 containing 0.1 m*M* dithiothreitol using a gel-filtration column (Superdex-200 prep grade, Pharmacia).

2.2. Crystallization

The purified enzyme was concentrated to about 15 mg ml^{-1} concentration using a YM30 ultrafiltration membrane (Amicon). The protein concentration was estimated by measuring the absorbance at 280 nm, employing the calculated extinction coefficient $\varepsilon_{280nm,\,0.1\%,\,1\,cm}$ of 0.236 $cm^2\,mg^{-1}$. Crystallization was performed by the hanging-drop vapour-diffusion method using 24-well tissue-culture VDX plates (Hampton Research) at 297 K. Each hanging drop was prepared by mixing 4 µl each of the protein solution and the reservoir solution. It was placed over 0.5 ml of the reservoir solution. Initial searches for crystallization conditions were performed using Screen I (Jancarik & Kim, 1991), Screen II and MembFac screening solutions (Hampton Research).

2.3. X-ray diffraction experiments

X-ray diffraction data were collected from a type I crystal at 293 K using graphite-monochromated Cu $K\alpha$ X-rays from a rotating-anode generator (Rigaku RU-200BH) on the FAST area detector (Enraf–Nonius, Delft) using the *MADNES* software (Messerschmidt & Pflugrath, 1987). The unit-cell parameters were determined by the autoindexing and parameter-refinement procedure of the *MADNES* software. The reflection intensities were obtained using the profile-fitting procedure (Kabsch, 1988) and the data were scaled using the Fourier scaling program (Weissman, 1982). Native data were collected from a type II crystal at 293 K using Yale/MSC mirror-focused Cu $K\alpha$ X-rays on a Rigaku R-AXIS IV image-plate detector. Recorded diffraction images were processed using the *HKL* package (Otwinowski & Minor, 1997).

Table 1
Data-collection statistics.

Crystal type	I	II
Space group	$P4_22_12$	$P2_12_12$
Unit-cell parameters (Å)		
a	99.74	47.65
b	99.74	125.10
c	170.00	58.08
Total/unique reflections	57998/18314	75794/26843
R_{merge}† (%)	9.7	4.9
Completeness (%)	93.0 (8.0–2.91 Å), 84.5 (3.13–2.91 Å)	95.2 (100–1.90 Å), 80.4 (1.93–1.90 Å)

† $R_{merge} = \sum_h \sum_i |I(h,i) - \langle I(h)\rangle| / \sum_h \sum_i I(h,i)$, where $I(h,i)$ is the intensity of the *i*th measurement of reflection *h* and $\langle I(h)\rangle$ is the mean value of $I(h,i)$ for all *i* measurements.

3. Results

Recombinant LDH from *M. jannaschii* has been overexpressed in *E. coli* as a soluble fraction under the control of the T7 promoter to the level of about 70 mg per litre of culture (Fig. 1). It has been crystallized under the reservoir conditions 0.10 *M* sodium acetate pH 5.6, 20–30%(*v*/*v*) 2-methyl-2,4-pentanediol, 200 m*M* sodium chloride. Two types of crystals were obtained in the same hanging drop (Fig. 2). Type I crystals appeared first and grew to dimensions of 0.4 × 0.3 × 0.15 mm within 3 d, but these crystals deteriorated within two weeks. Type II crystals appeared later and grew to dimensions of 0.3 × 0.3 × 0.2 mm within one month and were stable for at least several months.

Type I crystals showed diffraction to 2.8 Å when exposed to Cu $K\alpha$ X-rays. Native X-ray diffraction data have been collected to 2.9 Å Bragg spacing from a type I crystal. The systematic absences indicate that the space group is $P4_22_12$. The unit-cell parameters are $a = b = 99.74$, $c = 170.00$ Å. The asymmetric unit contains two monomers of LDH, giving a crystal volume per protein mass (V_m) of 3.05 $Å^3\,Da^{-1}$ and a solvent content of 59.7%. Type II crystals diffracted to 1.8 Å and native data were collected to 1.9 Å. The final merged data set consisted of 75 794 measurements of 26 843 unique reflections, with an R_{merge} (on intensity) of 4.9%. The merged data set is 95.2% complete to 1.9 Å. The space group was determined to be $P2_12_12$ on the basis of systematic absences. Unit-cell parameters

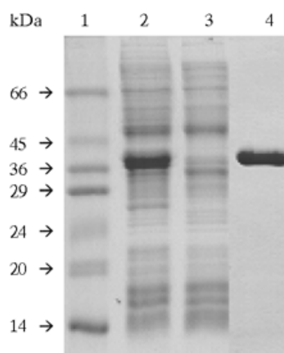
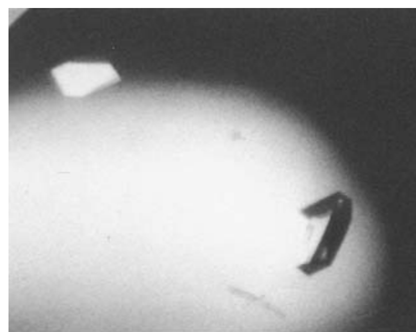


Figure 1
Expression and purification of *M. jannaschii* LDH. SDS–PAGE of the cell extracts of *E. coli* C41(DE3) cells transformed with the plasmid pET-22b containing the LDH gene (lane 2, after IPTG induction; lane 3, before induction). Lane 1, molecular-weight markers. Lane 4, purified enzyme.



(*a*)

(*b*)

Figure 2
(*a*) Tetragonal crystals, typical dimensions 0.4 × 0.3 × 0.15 mm. (*b*) Orthorhombic crystals, typical dimensions 0.3 × 0.3 × 0.2 mm.

were a = 47.65, b = 125.10, c = 58.08 Å. The presence of one monomer of LDH in the asymmetric unit gives a crystal volume per protein mass (V_m) of 2.50 $Å^3\,Da^{-1}$, with a corresponding solvent content of 50.8%. The statistics for data collection are summarized in Table 1. The structure determination of this LDH from the hyperthermophile *M. jannaschii* will provide additional information necessary to enhance our understanding of the possible determinants of protein thermostabilization.

We thank the Inter-University Center for Natural Science Research Facilities, Seoul National University, for providing the X-ray equipment. This work was supported by grants from the Korea Research Foundation made in the programme year of 1998 and from KOSEF through the Center for Molecular Catalysis. GWH was supported by a Postdoctoral Fellowship from the Ministry of Education.

References

Abad-Zapatero, C., Griff, J. P., Sussman, J. L. & Rossmann, M. G. (1987). *J. Mol. Biol.* **198**, 445–467.

Adams, M. J., Ford, G. C., Koekoek, R., Lentz, P. J., McPherson, A. Jr, Rossmann, M. G., Smiley, I. E., Schevitz, R. W. & Wonacott, A. J. (1970). *Nature (London)*, **227**, 1098–1103.

Auerbach, G., Ostendorp, R., Prade, L., Korndörfer, I., Dams, T., Huber, R. & Jaenicke, R. (1998). *Structure*, **6**, 769–781.

Buehner, M., Ford, G. C., Olsen, K. W., Moras, D. & Rossmann, M. G. (1974). *J. Mol. Biol.* **82**, 563–585.

Bult, C. J., White, O., Olsen, G. J., Zhou, L., Fleischmann, R. D., Sutton, G. G., Blake, J. A., FitzGerald, L. M., Clayton, R. A., Gocayne, J. D., Kerlavage, A. R., Dougherty, B. A., Tomb, J. F., Adams, M. D., Reich, C. I., Overbeck, R., Kirkness, E. F., Weinstock, K. G., Merrick, J. M., Glodek, A., Scott, J. L., Geoghagen, N. S. M., Weidman, J. F., Fuhrman, J., Nguyen, D., Utterback, T. R., Kelley, J. M., Peterson, J. D., Sadow, P. W., Hanna, M. C., Cotton, M. D., Robert, K. M., Hurst, M. A., Kaine, B. P., Borodovsky, M., Klenk, H. P., Fraser, C. M., Smith, H. O., Woese, C. R. & Venter, J. C. (1996). *Science*, **273**, 1058–1073.

Dunn, C. R., Banfield, M. J., Barker, J. J., Higham, C. W., Moreton, K. M., Turgut-Balik, D., Brady, R. L. & Holbrook, J. J. (1996). *Nature Struct. Biol.* **3**, 912–915.

Grau, U. M., Trommer, W. E. & Rossmann, M. G. (1981). *J. Mol. Biol.* **151**, 289–307.

Hogrefe, H. H., Griffith, J. P., Rossmann, M. G. & Goldberg, E. (1987). *J. Biol. Chem.* **262**, 13155–13162.

Holbrook, J. J., Liljas, A., Steindel, S. J. & Rossmann, M. G. (1975). *The Enzymes*, Vol. 11, edited by P. D. Boyer, 3rd ed., pp. 191–292. New York: Academic Press.

Iwata, S., Kamata, K., Yoshida, S., Minowa, T. & Ohta, T. (1994). *Nature Struct. Biol.* **1**, 176–185.

Jaenicke, R. (1987). *Prog. Biophys. Mol. Biol.* **49**, 117–237.

Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.

Kabsch, W. (1988). *J. Appl. Cryst.* **21**, 916–924.

Messerschmidt, A. & Pflugrath, J. W. (1987). *J. Appl. Cryst.* **20**, 306–325.

Miroux, B. & Walker, J. E. (1996). *J. Mol. Biol.* **260**, 289–298.

Ostendorp, R., Auerbach, G. & Jaenicke, R. (1996). *Protein Sci.* **5**, 862–873.

Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–325.

Piontek, K., Chakarabarti, P., Schär, H.-P., Rossmann, M. G. & Zuber, H. (1990). *Proteins*, **7**, 74–92.

Rossmann, M. G., Liljas, A., Brändén, C.-I. & Banaszak, L. J. (1975). *The Enzymes*, Vol. 11, edited by P. D. Boyer, 3rd ed., pp. 61–102. New York: Academic Press.

White, R. H., Hackert, M. L., Buehner, M., Adams, M. J., Ford, G. C., Lentz, P. J. Jr, Smiley, I. E., Steindel, S. J. & Rossmann, M. G. (1976). *J. Mol. Biol.* **102**, 759–779.

Weissman, L. (1982). *Computational Crystallography*, edited by D. Sayre, pp. 56–63. Oxford University Press.

Wigley, D. B., Gamblin, S. J., Turkenburg, J. P., Dodson, E. J., Piontek, K., Muirhead, H. & Holbrook, J. J. (1992). *J. Mol. Biol.* **223**, 317–335.