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Crystallization and preliminary X-ray analysis of human liver α-enolase

Juan Wang,[a] Yan-Feng Zhou,[a] Lan-Fen Li[a]* and Xiao-Dong Su[a,b]

[a]The National Laboratory of Protein Engineering and Plant Genetic Engineering, College of Life Sciences, Peking University, Beijing 100871, People's Republic of China, and [b]Peking University Shenzhen Graduate School, Shenzhen 518055, People's Republic of China

Correspondence e-mail: lilf@pku.edu.cn

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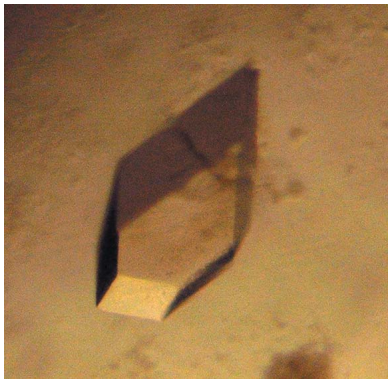

Enolase is a multifunctional enzyme that plays important roles in many biological and disease processes. α-Enolase from human liver (hENO1) was expressed as a soluble protein and purified by affinity column chromatography and gel filtration. Crystals were obtained by the hanging-drop vapour-diffusion method and diffracted to 2.5 Å resolution. The crystals belonged to space group $P2_1$, with unit-cell parameters a = 72.85, b = 66.02, c = 79.43 Å, β = 94.54°.

1. Introduction

Enolase (2-phospho-D-glycerate hydrolyase; EC 4.2.1.11) is a key enzyme that catalyzes the reversible dehydration of 2-phosphoglycerate (PGA) to form phosphoenolpyruvate (PEP) in the glycolytic and gluconeogenesis pathways (Reed *et al.*, 1996). In mammals, three isozymes of enolase, α, β and γ, are expressed by different genes in a tissue-specific manner. α-Enolase is found in a variety of tissues, while β-enolase is muscle-specific and γ-enolase is neuron-specific (Chai *et al.*, 2004). The isozymes form heterodimers or homodimers to exhibit enzymatic activity (Liu & Shih, 2007).

The crystal structure of yeast apo enolase (Lebioda & Stec, 1988; Stec & Lebioda, 1990) has been reported and was followed by studies of catalytic and inhibitory complexes (Zhang *et al.*, 1997; Stec & Lebioda, 1990; Wedekind *et al.*, 1995; Larsen *et al.*, 1996), contributing to the understanding of the catalytic mechanism of enolase in general. Crystal structures of bacterial enolase (Ehinger *et al.*, 2004; Hosaka *et al.*, 2003), *Trypanosoma brucei* enolase (Navarro *et al.*, 2007) and human γ-enolase (hNSE) have also been determined (Chai *et al.*, 2004). hNSE is a major brain protein that is used as clinical marker for neuronal and neuroendocrine cells. Recent research has revealed that human α-enolase (hENO1) is a multifunctional protein that takes part in cancer metastasis, infection and autoimmune diseases (Liu & Shih, 2007; Kim & Dang, 2005). The sequence identity between hENO1 and hNSE is 83% (361 of 430 residues). Structural comparisons between hENO1 and hNSE will contribute to an improved understanding of their differences in function. The protein residues that are conserved between hENO1 and other enolases have been determined by multiple sequence alignment (Fig. 1). It is thus important to determine the structure of hENO1 and compare it with other published enolase structures. We have been working towards the structural determination of human liver hENO1. Although the structure of hENO1 has been reported very recently (Kang *et al.*, 2008), our crystal was obtained in different conditions and belonged to space group $P2_1$, which is very different from the published results. Here, we present the expression, purification, crystallization and preliminary crystallographic studies of hENO1 from human liver.

2. Materials and methods

2.1. Cloning and expression

The Matchmaker two-hybrid system human liver cDNA library from Clontech (Takara Bio, USA) was used as a source for PCR amplification of the target gene (gi:16507965) and the primers for

PCR were 5′-CGC**GGATC**CATGTCTATTCTCAAGATCCATGC-3′ and 5′-CCG**CTCGAG***TTA*CTTGGCCAAGGGGTTTCTG-3′; the *Bam*HI and *Xho*I sites for restriction enzyme digestion and ligation are shown in bold and the stop codon is shown in italics. The double-digested DNA fragment was purified using agarose gel and then inserted into expression vector pET28a(+) (Novagen) containing an N-terminal His_6 tag. The recombinant plasmid containing the target fragment was confirmed by nucleotide sequencing and transformed into *Escherichia coli* strain BL21 (DE3) for protein expression. A single colony was selected and cultured in 10 ml Luria–Bertani (LB) medium at 310 K overnight; the overnight culture was added to 1.0 l fresh LB containing 50 µg ml^{-1} kanamycin and grown until an OD_{600} of 0.6–0.8 was reached. 0.5 m*M* isopropyl β-D-1-thiogalactopyranoside was then added to induce protein expression. After growth for a further 6 h at 303 K, cells were harvested by centrifugation at 4000*g*.

2.2. Protein purification

The cell pellet was resuspended in buffer *A* (20 m*M* Tris–HCl, 500 m*M* NaCl pH 7.5) and lysed by ultrasonication on ice. The cell debris was removed by centrifugation for 30 min at 39 000*g* twice and the supernatant was loaded onto a 5 ml HiTrap Ni^{2+}-chelating column (GE Healthcare, USA). The bound target protein was eluted by applying a linear gradient of 50–500 m*M* imidazole in buffer *A*. The eluate containing target protein from the nickel column was then loaded onto a Superdex 75 XK 16/60 gel-filtration column (GE Healthcare, USA) for homogenous separation. A Superose 6 HR10/30 column (GE Healthcare, USA) was used to analyze the protein further. Gel-filtration column chromatography was carried out at 277 K in buffer *C* (20 m*M* Tris–HCl, 200 m*M* NaCl pH 7.5); the concentration of the protein sample solution applied to the column was about 2 mg ml^{-1}. The purity of the protein was checked using SDS–PAGE at each step.

2.3. Crystallization

The purified protein was concentrated to 16 mg ml^{-1} using a Millipore centrifugal filter device with a 10 kDa cutoff. The concentrated protein solution contained 20 m*M* Tris–HCl and 200 m*M* NaCl pH 7.5. Crystallization experiments were performed by the hanging-drop vapour-diffusion method at 289 K. 1 µl protein solution was mixed with an equal volume of reservoir solution and equilibrated against 500 µl reservoir solution. Several commercially available crystallization screening kits from Hampton Research such as Crystal Screen, Crystal Screen 2 and Index Screen were used as initial screens for crystallization conditions.

2.4. Data collection

X-ray diffraction data were collected on a Bruker SMART 6000 CCD detector using Cu $K\alpha$ radiation from a Bruker–Nonius FR591 rotating-anode generator operated at 45 kV and 100 mA. During data collection, the crystal was maintained at 100 K using cooled nitrogen gas. The crystal-to-detector distance was set to 6 cm. A total of 1250 frames were collected with 60 s exposures, with a 0.3° φ scan per frame. Diffraction data were processed using the online *PROTEUM* software suite (Bruker).

3. Results

When expressed in *E. coli* BL21 (DE3), hENO1 showed good solubility. The molecular weight of the target protein (434 amino-acid residues) was calculated to be 47 kDa from the sequence and the

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ENOA_HUMAN MSILKIHAREIFDSRGNPTVEVDLFTSKGLFRAAVPSGASTGIYEALELRDNDKTRYMGKGVSKAVEHINKTIAPALVSKKLNVTEQEKI  90
ENOB_HUMAN MAMQKIFAREILDSRGNPTVEVDLHTAKGRFRAAVPSGASTGIYEALELRDGDKGRYLGKGVLKAVENINNTLGPALLQKKLSVVDQEKV  90
ENOG_HUMAN MSIEKIWAREILDSRGNPTVEVDLYTAKGLFRAAVPSGASTGIYEALELRDGDKQRYLGKGVLKAVDHINSTIAPALISSGLSVVEQEKL  90
ENO1_YEAST MAVSKVYARSVYDSRGNPTVEVELTTEKGVFRSIVPSGASTGVHEALEMRDGDKSKWMGKGVLHAVKNVNDVIAPAFVKANIDVKDQKAV  90
     ruler 1.......10........20........30........40........50........60........70........80........90

          *.::::.*** ****:***********..:*.*.*: :***:*:*:*: .  .  :**** :**:*******. **:***** *.** .*
ENOA_HUMAN DKLMIEMDGTENKSKFGANAILGVSLAVCKAGAVEKGVPLYRHIADLAGN--SEVILPVPAFNVINGGSHAGNKLAMQEFMILPVGAANF 178
ENOB_HUMAN DKFMIELDGTENKSKFGANAILGVSLAVCKAGAAEKGVPLYRHIADLAGN--PDLILPVPAFNVINGGSHAGNKLAMQEFMILPVGASSF 178
ENOG_HUMAN DNLMLELDGTENKSKFGANAILGVSLAVCKAGAAERELPLYRHIAQLAGN--SDLILPVPAFNVINGGSHAGNKLAMQEFMILPVGAESF 178
ENO1_YEAST DDFLISLDGTANKSKLGANAILGVSLAASRAAAAEKNVPLYKHLADLSKSKTSPYVLPVPFLNVLNGGSHAGGALALQEFMIAPTGAKTF 180
     ruler .......100.......110.......120.......130.......140.......150.......160.......170.......180

           :*:*:*:**** **.: * :** .* *******.****   .*.*:*:  **  **:  *: **:* *:***::.********.*  *
ENOA_HUMAN REAMRIGAEVYHNLKNVIKEKYGKDATNVGDEGGFAPNILENKEGLELLKTAIGKAGYTDKVVIGMDVAASEFFRSGKYDLDFKSPD-DP 267
ENOB_HUMAN KEAMRIGAEVYHHLKGVIKAKYGKDATNVGDEGGFAPNILENNEALELLKTAIQAAGYPDKVVIGMDVAASEFYRNGKYDLDFKSPD-DP 267
ENOG_HUMAN RDAMRLGAEVYHTLKGVIKDKYGKDATNVGDEGGFAPNILENSEALELVKEAIDKAGYTEKIVIGMDVAASEFYRDGKYDLDFKSPT-DP 267
ENO1_YEAST AEALRIGSEVYHNLKSLTKKRYGASAGNVGDEGGVAPNIQTAEEALDLIVDAIKAAGHDGKVKIGLDCASSEFFKDGKYDLDFKNPNSDK 270
     ruler .......190.......200.......210.......220.......230.......240.......250.......260.......270

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ENOA_HUMAN SRYISPDQLADLYKSFIKDYPVVSIEDPFDQDDWGAWQKFTASAGIQVVGDDLTVTNPKRIAKAVNEKSCNCLLLKVNQIGSVTESLQAC 357
ENOB_HUMAN ARHITGEKLGELYKSFIKNYPVVSIEDPFDQDDWATWTSFLSGVNIQIVGDDLTVTNPKRIAQAVEKKACNCLLLKVNQIGSVTESIQAC 357
ENOG_HUMAN SRYITGDQLGALYQDFVRDYPVVSIEDPFDQDDWAAWSKFTANVGIQIVGDDLTVTNPKRIERAVEEKACNCLLLKVNQIGSVTEAIQAC 357
ENO1_YEAST SKWLTGPQLADLYHSLMKRYPIVSIEDPFAEDDWEAWSHFFKTAGIQIVADDLTVTNPKRIATAIEKKAADALLLKVNQIGTLSESIKAA 360
     ruler .......280.......290.......300.......310.......320.......330.......340.......350.......360

          : :   ************************ *********.******* ***:**** **.:* ***.:*::
ENOA_HUMAN KLAQANGWGVMVSHRSGETEDTFIADLVVGLCTGQIKTGAPCRSERLAKYNQLLRIEEELGSKAKFAGRNFRNPLAK 434
ENOB_HUMAN KLAQSNGWGVMVSHRSGETEDTFIADLVVGLCTGQIKTGAPCRSERLAKYNQLMRIEEALGDKAIFAGRKFRNPKAK 434
ENOG_HUMAN KLAQENGWGVMVSHRSGETEDTFIADLVVGLCTGQIKTGAPCRSERLAKYNQLMRIEEELGDEARFAGHNFRNPSVL 434
ENO1_YEAST QDSFAAGWGVMVSHRSGETEDTFIADLVVGLRTGQIKTGAPARSERLAKLNQLLRIEEELGDNAVFAGENFHHGDKL 437
     ruler .......370.......380.......390.......400.......410.......420.......430.......
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Figure 1
Multiple sequence alignment of enolases. Asterisks indicate strictly conserved residues. The alignment was performed using the program *ClustalX* (Thompson *et al.*, 1997). ENOA_HUMAN, human α-enolase; ENOB_HUMAN, human β-enolase; ENOG_HUMAN, human γ-enolase; ENO1_YEAST, *Saccharomyces cerevisiae* enolase 1. All protein sequences are from the SWISS-PROT database (Watanabe & Harayama, 2001).

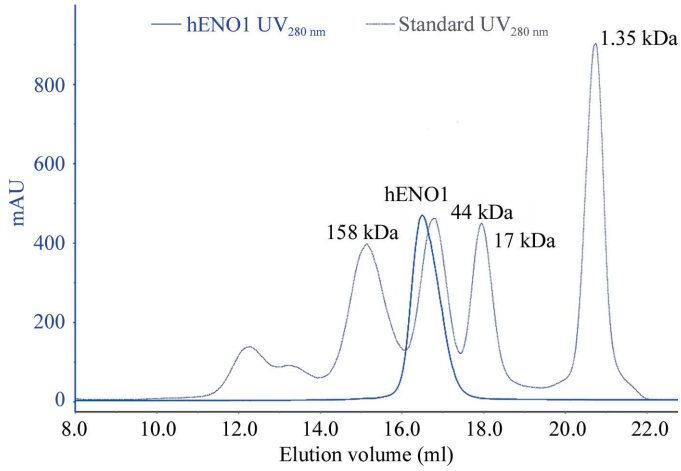

Figure 2
Chromatogram of hENO1 on a Superose 6 HR 10/30 column (GE Healthcare, USA). $UV_{280\,nm}$ absorption curves of gel-filtration standard proteins (Bio-Rad Laboratories, USA) and hENO1 are shown as dashed and solid lines, respectively.

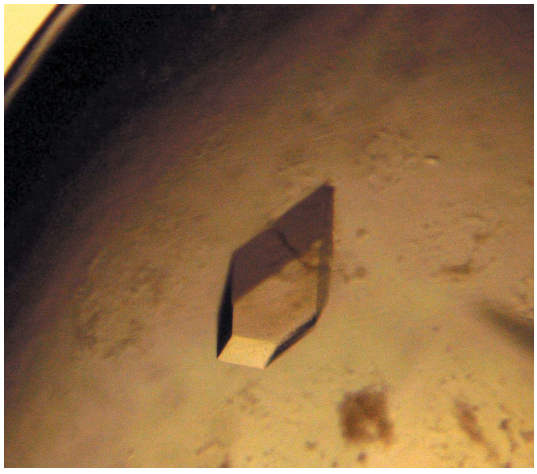

Figure 3
Crystal of hENO1 grown in 0.1 *M* ammonium acetate, 0.1 *M* bis-tris, 20%(*w*/*v*) polyethylene glycol monomethyl ether 2000 pH 5.5 using the hanging-drop vapour-diffusion method. The dimensions of the crystal are approximately 0.07 × 0.07 × 0.2 mm.

protein was detected on SDS–PAGE as ∼51 kDa, including the His_6 tag added to the N-terminal position which had a molecular weight of 4 kDa (MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGS). After two-step chromatographic purification, homogenous samples suitable for crystallization trials were obtained. The Superose 6 HR 10/30 gel filtration gave a peak at ∼50 kDa, indicating that hENO1 is a monomer in solution (Fig. 2). Crystals were optimized from Index Screen condition No. 78 (Hampton Research) containing 0.2 *M* ammonium acetate, 0.1 *M* bis-tris pH 5.5, 25%(*w*/*v*) polyethylene glycol (PEG) 3350. Crystals suitable for data collection were obtained at 289 K under conditions containing 0.1 *M* ammonium acetate, 0.1 *M* bis-tris pH 5.5, 20%(*w*/*v*) polyethylene glycol monomethyl ether 2000 (Fig. 3). Using 15%(*v*/*v*) PEG 400 and 5%(*v*/*v*) sucrose as the cryo-protectant, the crystal diffracted to 2.5 Å resolution and belonged to space group $P2_1$, with unit-cell parameters a = 72.85, b = 66.02, c = 79.43 Å, β = 94.54°. We have determined the hENO1 structure by molecular replacement using the coordinates of PDB entry 3b97 (Kang *et al.*, 2008) as a search model. The result confirms that there are two molecules per asymmetric unit, which gives a V_M value of 2.0 $Å^3$ Da^{-1} (Matthews, 1968) corresponding to a solvent content of 38.6%. The crystallographic parameters and data-collection statistics are listed in Table 1.

Table 1
Data-collection statistics for hENO1.

Values in parentheses are for the highest resolution shell.

Resolution range (Å)	50–2.5 (2.62–2.5)
Data completeness (%)	99.2 (95.6)
R_{merge}† (%)	7.5 (47.6)
Average $\langle I/\sigma(I)\rangle$	7.5 (1.63)
Space group	$P2_1$
Unit-cell parameters (Å, °)	a = 72.85, b = 66.02, c = 79.43, β = 94.54
No. of observed reflections	145275
No. of unique reflections	23747
Molecules per ASU	2
V_M ($Å^3$ Da^{-1})	2.0
Solvent content (%)	38.6

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where the summation is over all Bijvoet-equivalent observations.

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References

Chai, G., Brewer, J. M., Lovelace, L. L., Aoki, T., Minor, W. & Lebioda, L. (2004). *J. Mol. Biol.* **341**, 1015–1021.
Ehinger, S., Schubert, W. D., Bergmann, S., Hammerschmidt, S. & Heinz, D. W. (2004). *J. Mol. Biol.* **343**, 997–1005.
Hosaka, T., Meguro, T., Yamato, I. & Shirakihara, Y. (2003). *J. Biochem.* **133**, 817–823.
Kang, H. J., Jung, S.-K., Kim, S. J. & Chung, S. J. (2008). *Acta Cryst.* D**64**, 651–657.
Kim, J. W. & Dang, C. V. (2005). *Trends Biochem. Sci.* **30**, 142–150.
Larsen, T. M., Wedekind, J. E., Rayment, I. & Reed, G. H. (1996). *Biochemistry*, **35**, 4349–4358.
Lebioda, L. & Stec, B. (1988). *Nature (London)*, **333**, 683–686.
Liu, K.-J. & Shih, N.-Y. (2007). *J. Cancer Mol.* **3**, 45–48.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Navarro, M. V. de A. S., Gomes Dias, S. M., Mello, L. V., da Silva Giotto, M. T., Gavalda, S., Blonski, C., Garratt, R. C. & Rigden, D. J. (2007). *FEBS J.* **274**, 5077–5089.
Reed, G. H., Poyner, R. R., Larsen, T. M., Wedekind, J. E. & Rayment, I. (1996). *Curr. Opin. Struct. Biol.* **6**, 736–743.
Stec, B. & Lebioda, L. (1990). *J. Mol. Biol.* **211**, 235–248.
Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). *Nucleic Acids Res.* **25**, 4876–4882.
Watanabe, K. & Harayama, S. (2001). *Tanpakushitsu Kakusan Koso*, **46**, 80–86.
Wedekind, J. E., Reed, G. H. & Rayment, I. (1995). *Biochemistry*, **34**, 4325–4330.
Zhang, E., Brewer, J. M., Minor, W., Carreira, L. A. & Lebioda, L. (1997). *Biochemistry*, **36**, 12526–12534.