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Purification, crystallization and preliminary crystallographic study of the putative enolase MJ0232 from the hyperthermophilic archaeon *Methanococcus jannaschii*

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Enolase is a glycolytic enzyme that catalyzes the interconversion of phosphoenolpyruvate and 2-phosphoglycerate. In order to gain insight into the biological significance of the oligomeric state of this enzyme, the putative enolase MJ0232 from the hyperthermophilic archaeon *Methanococcus jannaschii* was cloned, overexpressed and purified. Crystals were obtained by the oil-microbatch method at 291 K using PEG 4000 as a precipitant. A native data set was collected to 1.85 Å resolution. The crystal belonged to the tetragonal space group $I4$, with unit-cell parameters a = 148.8, c = 91.2 Å. An initial model was obtained by molecular replacement, which revealed an octameric subunit association (a tetramer of dimers). This result is consistent with that from a dynamic light-scattering experiment, suggesting biological relevance of the octameric state of MJ0232 in solution.

1. Introduction

Enolase, a glycolytic enzyme, catalyzes the reversible dehydration of 2-phosphoglycerate to phosphoenolpyruvate in the eighth step of the glycolytic pathway. The substrate/product phosphoenolpyruvate (PEP) is located at a crucial point in this pathway. In glycolysis, PEP is converted to pyruvate *via* the physiologically irreversible pyruvate kinase reaction. In contrast, in glyconeogenesis oxaloacetate as a carbon source is converted to PEP by phosphoenolpyruvate carboxykinase and flows into the reverse sequence of reactions to glycolysis. Thus, the balance between the forward (glycolysis) and reverse (glyconeogenesis) reactions catalyzed by these enzymes needs to be strictly controlled according to physiological demand. Genes for enolase orthologues are widely distributed in all three domains of organisms: eukarya, bacteria and archaea. In most cases, the enolase orthologues share high full-length (about 430 amino acids) identities of more than 40%, suggesting the conservative nature of this enzymatic function. In addition to the primary structural similarity, a striking difference in quaternary structure has been found between enzymes of eukaryal and bacterial origins. It has long been well established that eukaryotic enolases are dimeric (Brewer & Weber, 1968; Brewer *et al.*, 1970; Pancholi, 2001), while some bacterial enolases have been reported to be octameric in solution (Brown *et al.*, 1998; Schurig *et al.*, 1995; Pawluk *et al.*, 1986). Several structures of enolases have been reported to date: those from human (Chai *et al.*, 2004), yeast (Lebioda & Stec, 1988), lobster (Duquerroy *et al.*, 1995), *Trypanosoma brucei* (Giotto *et al.*, 2003), *Escherichia coli* (Kühnel & Luisi, 2001), *Streptococcus pneumoniae* (Ehinger *et al.*, 2004) and *Enterococcus hirae* (Hosaka *et al.*, 2003). The structures of the enolases from eukaryotic sources are dimeric, while the crystal structures of the bacterial enolases from *S. pneumoniae* and *En. hirae* show a similar octameric association comprising a tetramer of dimers. However, the biological significance of their oligomeric state has not been fully discussed to date from a structural viewpoint. One possible role of the octameric state of these bacterial enolases is to contribute to the thermo-adaptation of the enolase function. Therefore, structural study of this enzyme from a thermophile would provide insight into the biological role of the oligomeric state of this evolutionary

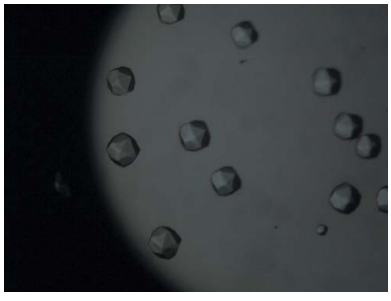


Table 1
Data-collection statistics.

Values in parentheses are for the outermost shell.

Space group	*I*4
Unit-cell parameters (Å)	a = 148.8, c = 91.2
Wavelength (Å)	1.54178
No. of reflections	807214
Observed unique reflections	85037
Resolution (Å)	1.85 (1.92–1.85)
Completeness (%)	100.0
$\langle I/\sigma(I)\rangle$	15.4 (4.8)
$R_{\rm merge}$† (%)	7.8 (52.9)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle|/\sum_{hkl}\sum_i I_i(hkl)$, where $\langle I(hkl)\rangle$ is the mean intensity of the unique reflection hkl and $I_i(hkl)$ is the intensity of its ith observation.

conserved enzyme. Here, we report the purification, crystallization and preliminary crystallographic study of the putative enolase MJ0232 from *Methanococcus jannaschii*, which can grow at temperatures of up to around 363 K. This is the first report of a preliminary crystallographic study of this enzyme from a hyperthermophilic archaeal source.

2. Experimental

2.1. Sample preparation

The 46.8 kDa monomeric protomer of MJ0232 consists of 427 amino-acid residues. The plasmid encoding MJ0232 (residues 1–427) was digested with *Nde*I and *Bgl*II and the fragment was inserted into the expression vector pET-21a (Novagen Inc.) linearized with *Nde*I and *Bam*HI. 2.5 l Luria–Bertani medium, supplemented with ampicillin at a concentration of 100 µg ml^{-1}, was inoculated with a single colony of *E. coli* BL21-Codon Plus (DE3)-RIL (Novagen Inc.) carrying the recombinant plasmid and grown at 310 K for 20 h with vigorous shaking. Cells were harvested, resuspended in 20 ml buffer solution containing 20 m*M* Tris–HCl pH 8.0, 500 m*M* NaCl, 5 m*M* 2-mercaptoethanol and 1 m*M* PMSF and sonicated. The cell lysate was incubated at 363 K for 10 min. The heat-treated lysate was centrifuged at 21 600*g* for 30 min at 277 K. The protein solution was desalted using a HiPrep 26/10 column (GE Healthcare Biosciences) equilibrated with 20 m*M* Tris–HCl pH 8.0 (buffer *A*) and then applied onto a Super Q Toyopearl 650M (Tosoh Inc.) anion-exchange column (30 ml) equilibrated with buffer *A*. The protein was eluted with a 120 ml linear gradient of 0–0.4 *M* NaCl. The fractions were analyzed by SDS–PAGE and those containing a 46.8 kDa protein were collected. The protein solution was desalted with a HiPrep 26/10 column equilibrated with buffer *A* and applied onto a Resource Q (GE Healthcare Biosciences) anion-exchange column equilibrated with buffer *A*. The protein was eluted with a 90 ml linear gradient of 0–0.4 *M* NaCl. The fractions were analyzed and collected as described above. The protein solution was desalted using a HiPrep 26/10 column equilibrated with 10 m*M* potassium phosphate pH 7.0 (buffer *B*) and applied onto a CHT 20-I (Bio-Rad Inc.) hydroxyapatite column equilibrated with buffer *B*. The protein was eluted with a 300 ml linear gradient of 0.01–0.5 *M* potassium phosphate pH 7.0. The fractions were analyzed and collected as described above. The protein solution was concentrated by ultrafiltration (Vivaspin, 30 kDa cutoff, Vivascience) and applied onto a HiLoad 16/60 Superdex 200 prep-grade column equilibrated with buffer *A* containing 200 m*M* NaCl. The fractions were analyzed and collected as described above. The purified protein solution was concentrated to 0.14 ml by ultrafiltration. The protein concentration was determined to be 27.4 mg ml^{-1} by measuring the UV absorption at 280 nm, using a molar absorption coefficient of 26 410 M^{-1} cm^{-1}.

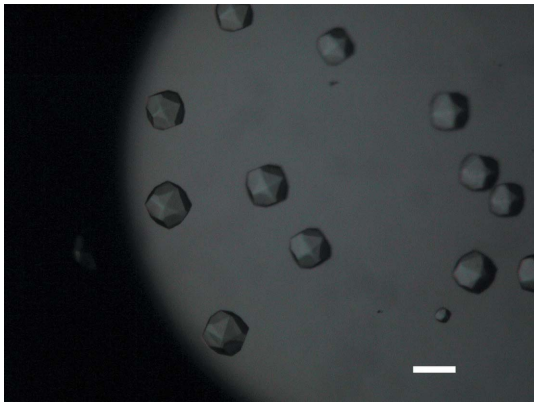


Figure 1
Optical micrograph of MJ0232 crystals. The white bar represents 100 µm.

2.2. Dynamic light scattering

The oligomerization state of MJ0232 was analyzed by dynamic light scattering using a DynaPro MS/X instrument (Protein Solutions Inc.). MJ0232 at a concentration of 1.0 mg ml^{-1} in 20 m*M* Tris–HCl pH 8.0 and 200 m*M* NaCl was centrifuged at 18 800*g* for 30 min and the supernatant was used for data collection. More than ten sets of scattering data were obtained at 289 K and were analyzed using the *DYNAMICS* software v.5.26.60 (Protein Solutions Inc.). The hydrodynamic radius ($R_{\rm h}$) and polydispersity were calculated to be 6.2 nm and 12.7%, respectively. This indicates a monodisperse distribution of a single species with an apparent molecular mass of 410 kDa, which was estimated from a calibration curve with catalase ($R_{\rm h}$ and molecular mass of 4.8 nm and 232 kDa, respectively) and ferritin (6.4 nm and 440 kDa, respectively). The apparent molecular mass and low polydispersity suggest that most of MJ0232 molecules exist as octamers in solution.

2.3. Crystallization

Crystals of MJ0232 protein were grown by the oil-microbatch method (Chayen *et al.*, 1990) using Nunc HLA plates (Nalge Nunc

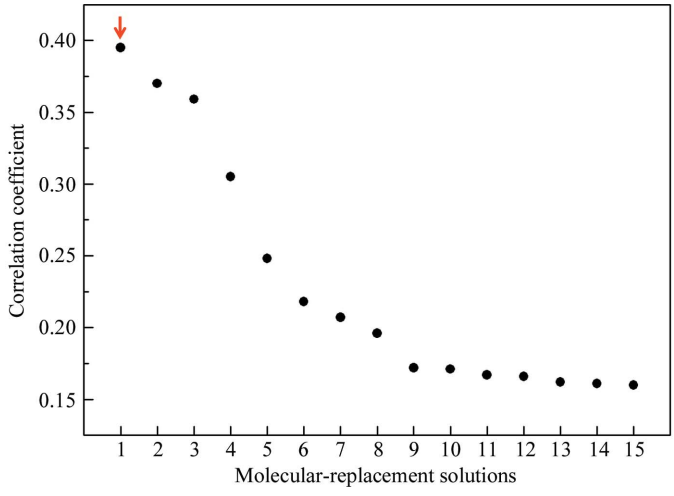


Figure 2
Correlation coefficients of the top 15 solutions in the molecular-replacement calculation. The red arrow indicates the true solution with the best coefficient of 0.395.

Table 2
Unit-cell parameters and oligomers in the crystals of enolases from various sources.

Source organism (PDB code)	Length (amino acids)	Identity/similarity† (%)	Space group/unit-cell parameters (Å, °)	Oligomers in the crystal
M. jannaschii (2pa6)	427	—	$I4$; a = 148.8, b = 148.8, c = 91.2, $\alpha = \beta = \gamma$ = 90.0	8‡
S. pneumoniae (1w6t)	434	50/68	$I4$; a = 143.7, b = 143.7, c = 100.6, $\alpha = \beta = \gamma$ = 90.0	8‡
En. hirae (1iyx)	433	54/72	$I4$; a = 153.5, b = 153.5, c = 90.7, $\alpha = \beta = \gamma$ = 90.0	8‡
Homo sapiens (2akz)	434	53/70	$P2_12_12$; a = 109.0, b = 118.5, c = 67.2, $\alpha = \beta = \gamma$ = 90.0	2
Homarus gammarus (1pdz)	434	51/67	$P3_121$; a = 110.8, b = 110.8, c = 73.4, $\alpha = \beta$ = 90.0, γ = 120.0	2
T. brucei (1oep)	429	56/69	$C222_1$; a = 74.0, b = 110.5, c = 109.1, $\alpha = \beta = \gamma$ = 90.0	2
Saccharomyces cerevisiae (2one)	437	54/69	$P2_1$; a = 63.1, b = 110.0, c = 66.2, $\alpha = \gamma$ = 90.0, β = 113.0	2
S. cerevisiae (1p48)			$P2_12_12$; a = 107.8, b = 114.5, c = 73.1, $\alpha = \beta = \gamma$ = 90.0	2
S. cerevisiae (1els)			$P4_22_12$; a = 124.1, b = 124.1, c = 66.9, $\alpha = \beta = \gamma$ = 90.0	2
S. cerevisiae (1one)			$C2$; a = 121.9, b = 73.2, c = 93.9, $\alpha = \gamma$ = 90.0, β = 93.3	2
E. coli (2fym)	432	53/67	$P2_1$; a = 77.1, b = 124.2, c = 96.1, $\alpha = \gamma$ = 90.0, β = 90.6	2
E. coli (1e9i)			$C2$; a = 107.9, b = 150.0, c = 127.4, $\alpha = \gamma$ = 90.0, β = 109.2	2

† The values were calculated between MJ0232 and each enolase. ‡ The octamers were generated by a crystallographic symmetry operation.

International). The crystallization drop was prepared by mixing 0.5 µl protein solution comprising 13.7 mg ml^{-1} MJ0232, 200 m*M* NaCl and 20 m*M* Tris–HCl pH 8.0 and 0.5 µl precipitant solution comprising 8%(*w*/*v*) polyethylene glycol (PEG) 4000 and 0.1 *M* sodium acetate buffer pH 4.6. The crystallization drop was overlaid with paraffin oil, allowing slow evaporation of the water in the drop, and stored at 291 K. Crystals grew within a few days.

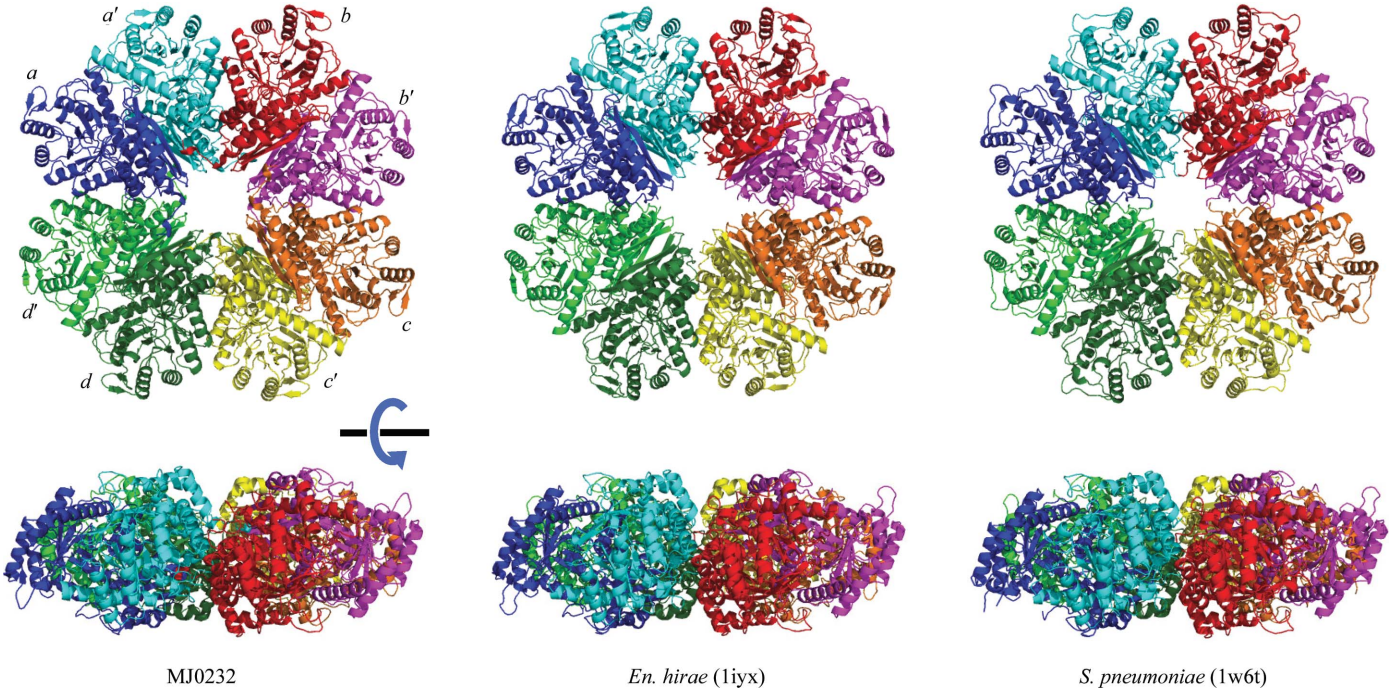
2.4. X-ray diffraction study

Crystals were flash-cooled in a nitrogen-gas stream at 100 K using a cryoprotectant consisting of 5.6%(*w*/*v*) PEG 4000, 70 m*M* sodium acetate buffer pH 4.6 and 30%(*w*/*v*) glycerol. A native data set was collected to 1.85 Å resolution using a Rigaku R-AXIS IV image-plate detector and an UltraX18 rotating-anode tube generator with a copper target. The *HKL*-2000 software package (Otwinowski & Minor, 1997) was used for data reduction and scaling. The statistics of data collection are summarized in Table 1.

3. Results

We have established the expression, purification and crystallization of the putative enolase MJ0232 from the hyperthermophilic archaeon *M. jannaschii*. After a few days at 291 K, tetragonal crystals grew with typical dimensions of 93 × 93 × 93 µm (Fig. 1). The crystals diffracted X-rays to 1.85 Å resolution at 100 K using an in-house diffractometer (Table 1). The crystals belonged to space group $I4$, with unit-cell parameters a = 148.8, c = 91.2 Å. Assuming the presence of two chains of MJ0232 in the asymmetric unit gives a reasonable value of 2.7 Å^3 Da^{-1} for the Matthews coefficient (V_M; Matthews, 1968), corresponding to a solvent content of 54.4%.

We obtained a molecular-replacement solution using *MOLREP* (Vagin & Teplyakov, 1997) with the coordinates of PDB entry 1oep as a starting model. Fig. 2 shows the distribution of correlation coefficients for the top 15 solutions. The coefficient for the best solution and the average coefficient for the solutions were 0.395 and 0.230, respectively. The initial molecular-replacement result confirmed that the asymmetric unit contained a similar dimer with an overall conserved fold as found in other enolase structures. Furthermore, a



MJ0232 *En. hirae* (1iyx) *S. pneumoniae* (1w6t)

Figure 3
Ribbon representations of the ring-like octameric structure (a tetramer of dimers) found in the crystals of three enolases. The dimers are distinguished by labelling with letters. A letter with a prime indicates the cognate protomer in a dimer. The chains are distinguished by colour. This figure was produced using *PyMOL* (DeLano, 2002).

crystallographic symmetry operation can generate a similar ring-like octamer (a tetramer of dimers) to those found in the crystal structures of the *S. pneumoniae* (PDB code 1w6t) and *En. hirae* (PDB code 1iyx) enolases (Fig. 3). This is consistent with the DLS experiment result showing an octameric state in solution (see §2.2). Because they share high full-length sequence identities (>50%) and their crystal forms are closely related (Table 2), the octamer found in the MJ0232 crystal may be the biologically relevant form of this enzyme. A detailed discussion of the refined structure will be published elsewhere.

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References

Brewer, J. M., Fairwell, T., Travis, J. & Lovins, R. E. (1970). *Biochemistry*, **9**, 1011–1016.
Brewer, J. M. & Weber, G. (1968). *Proc. Natl Acad. Sci. USA*, **59**, 216–223.
Brown, C. K., Kuhlman, P. L., Mattingly, S., Slates, K., Calie, P. J. & Farrar, W. W. (1998). *J. Protein Chem.* **17**, 855–866.
Chai, G., Brewer, J. M., Lovelace, L. L., Aoki, T., Minor, W. & Lebioda, L. (2004). *J. Mol. Biol.* **341**, 1015–1021.
Chayen, N. E., Shaw Stewart, P. D., Maeder, D. L. & Blow, D. M. (1990). *J. Appl. Cryst.* **23**, 297–302.
da Silva Giotto, M. T., Hannaert, V., Vertommen, D., Navarro, M. V. A. S., Rider, M. H., Michels, P. A., Garratt, R. C. & Rigden, D. J. (2003). *J. Mol. Biol.* **331**, 653–665.
DeLano, W. L. (2002). *The PyMOL Molecular Graphics System.* http://www.pymol.org.
Duquerroy, S., Camus, C. & Janin, J. (1995). *Biochemistry*, **34**, 12513–12523.
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.
Kühnel, K. & Luisi, B. F. (2001). *J. Mol. Biol.* **313**, 583–592.
Lebioda, L. & Stec, B. (1988). *Nature (London)*, **333**, 683–686.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Pancholi, V. (2001). *Cell. Mol. Life Sci.* **58**, 902–920.
Pawluk, A., Scopes, R. K. & Griffiths-Smith, K. (1986). *Biochem. J.* **238**, 275–281.
Schurig, H., Rutkat, K., Rachel, R. & Jaenicke, R. (1995). *Protein Sci.* **4**, 228–236.
Vagin, A. & Teplyakov, A. (1997). *J. Appl. Cryst.* **30**, 1022–1025.