

Cloning, purification, crystallization and preliminary crystallographic analysis of human phosphoglycerate mutase

Yanli Wang,^{a,b} Zhongjun Cheng,^b
Lin Liu,^{a,b} Zhiyi Wei,^{a,b} Mao
Wan^b and Weimin Gong^{a,b*}

^aNational Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, People's Republic of China, and ^bSchool of Life Sciences, Key Laboratory of Structural Biology, University of Science and Technology of China, Hefei, Anhui 230026, People's Republic of China

Correspondence e-mail: wgong@sun5.ibp.ac.cn

Human B-type 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase (dPGM-B) has been cloned, overexpressed and purified, with a yield of 30% of the total protein. Crystals of human dPGM-B were obtained using the hanging-drop vapour-diffusion technique. X-ray diffraction data were collected to 2.8 Å resolution. The human dPGM-B crystals belong to space group $P2_1$, with unit-cell parameters $a = 130.5$, $b = 75.9$, $c = 187.0$ Å, $\beta = 94.4^\circ$. There could be between 9 and 18 monomers per asymmetric unit, with 12 molecules being the most likely.

Received 19 April 2004

Accepted 3 August 2004

1. Introduction

Phosphoglycerate mutase (EC 5.4.2.1) catalyses the interconversion of 3-phosphoglycerate (3-PGA) and 2-phosphoglycerate (2-PGA) in the glycolytic and gluconeogenic pathways. There are two main types of PGMs: dPGM and iPGM. dPGM requires 2,3-bisphosphoglycerate (2,3-BPG) as a cofactor, while iPGM does not (Fothergill-Gilmore & Watson, 1989). In addition to mutase activity, PGM also possesses synthase and phosphatase activities. The synthase activity involves the conversion of 1,3-bisphosphoglycerate (1,3-BPG) to 2,3-BPG. The phosphatase activity catalyzes the hydrolysis of 2,3-BPG to 2-PGA or 3-PGA and phosphate (Fothergill-Gilmore & Watson, 1989). Two genes encoding dPGM exist in mammals. In tissues in which both genes are active, three isoenzymes are formed (Omenn & Cheung, 1974). The B-type isoenzyme of dPGM has been found in brain, liver, kidney, erythrocytes and early foetal skeletal muscle, whereas the M-type is present in adult skeletal and cardiac muscle (Chen *et al.*, 1974; Prehu *et al.*, 1984). The MB heterodimer form of dPGM occurs in heart and in late foetal or neonatal muscle, where the M- and B-types are also both present (Omenn & Cheung, 1974). In mammals the expression of the two genes encoding dPGM depends on tissue type, the state of differentiation of the tissue and whether the tissue has undergone neoplastic transition. DPGM can therefore be exploited as a convenient marker to study and monitor tissue differentiation (Fothergill-Gilmore & Watson, 1989). Several human deficiencies have been described involving the MM and BB dPGM isoenzymes (Toscano *et al.*, 1996; Tsujino *et al.*, 1995; Repiso *et al.*, 2003).

The best-studied dPGM is that from *Saccharomyces cerevisiae*. It has been subjected to biochemical and structural studies

(White & Fothergill-Gilmore, 1992; White *et al.*, 1993a,b; Rigden *et al.*, 1998, 1999). Recently, the crystal structures of dPGMs from *Escherichia coli* and *Bacillus stearothermophilus* dPGM have been reported (Bond *et al.*, 2001, 2002; Rigden *et al.*, 2002, 2003). However, no structure of dPGM from higher organisms is yet available.

Human dPGM-B shares approximately 50% sequence identity with the dPGMs from *S. cerevisiae* and *E. coli*. The structure of human dPGM-B may increase our understanding of the mechanism of dPGM and its relationship to disease. This paper describes the gene cloning, protein expression, purification, crystallization and preliminary X-ray analysis of human dPGM-B protein.

2. Materials and methods

2.1. Expression and purification

The human dPGM-B gene was amplified from the human brain cDNA library (Clontech) by a polymerase chain reaction using the 5'- and 3'-end special primers 5'-CATATGGCCGCCTACAAACTGGTGCTG-3' and 5'-CTCGAGCTTCTTGGCCTTGCCCTG-3' (Sangon) designed based on the mRNA sequence of human dPGM-B (GenBank Accession No. J04173). These oligonucleotides were introduced with *NdeI* and *XhoI* restriction sites, respectively. Taq polymerase, T-vector, DNA ligase and the relevant restriction enzymes were obtained from Takara. The polymerase chain reaction product (~800 bases) was purified with a Gel Extraction Mini Kit (Watson BioTechnologies) and cloned into a T-vector. The positive clones were identified by restriction digest. The DNA fragment was ligated into the *NdeI/XhoI*-cleaved plasmid pET22b (Novagen) to give the pET22b-dPGM-B construction, which was amplified in

Table 1
Data-processing statistics for human dPGM-B.

Values in parentheses are for the highest resolution shell.	
Resolution (Å)	2.8
Space group	$P2_1$
Unit-cell parameters (Å, °)	$a = 130.5, b = 75.9,$ $c = 187.0, \beta = 94.4$
Resolution range (Å)	50–2.8 (2.87–2.8)
No. measured reflections	1603227
No. unique reflections	85016
$I/\sigma(I)$	8.6 (2.2)
R_{merge} (%)	12.1 (45.4)
Completeness (%)	93.9 (93.8)

E. coli BL21 (DE3). The recombinant protein contains eight non-native residues at the C-terminus. The integrity of the gene was confirmed by DNA sequencing. Single colonies were cultured in Luria–Bertani broth medium with ampicillin ($100 \mu\text{g ml}^{-1}$) for expression. Cells were induced with 1 mM isopropyl- β -D-thiogalactopyranoside (IPTG) after attaining an A_{600} of 0.4 and grown for an additional 4 h at 310 K. The cells were harvested by centrifugation, resuspended in lysis buffer containing 0.5 M NaCl, 20 mM Tris–HCl pH 7.5 and lysed by sonication on ice. After centrifugation, the supernatant was loaded onto an Ni–NTA column (Qiagen) and eluted using a step gradient of 0.05 – 0.5 M imidazole. The purity of the fractions was tested by SDS–PAGE. The purified human dPGM-B protein was desalted and concentrated to 28 mg ml^{-1} , as determined by Bio-Rad protein assay. The sample purity and molecular weight were verified by SDS–PAGE and mass spectrometry, respectively.

2.2. Crystallization and data collection

Crystallization attempts were made using Hampton Crystal Screens I and II. Trials were performed using the hanging-drop vapour-diffusion method at 277 K. The drop contained $2 \mu\text{l}$ protein solution (28 mg ml^{-1} in 20 mM Tris–HCl pH 7.5, 20 mM NaCl) and an equal volume of precipitant. X-ray diffraction data were collected on a MAR CCD detector at Beijing Synchrotron Radiation Facility at the Institute of High Energy Physics, Chinese Academy of Science. A total of 366 frames of data to 2.8 \AA were collected with oscillation range 0.5° at a wavelength of 0.90 \AA . The exposure time for each frame was 30 s, with a crystal-to-detector distance of 180 mm. Data were processed with *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

3. Results and discussion

The overexpression level of human dPGM-B was estimated to be approximately 30% of the total soluble cell protein by SDS–PAGE, which facilitated the subsequent purification process. The purity of human dPGM-B determined by SDS–PAGE was approximately 90%, with a yield of about 42 mg protein from 5 g cell paste as estimated by the method of Bradford (1976) using the Bio-Rad protein assay reagent.

Small crystals in the form of square plates grew in less than a month in a drop containing 20% (v/v) 2-propanol, 20% (w/v) PEG 4000, 100 mM sodium citrate pH 5.6 and 0.2 mM ammonium sulfate. After systematic variation of pH values and precipitant concentrations, the best conditions were found to be 20% (v/v) 2-propanol, 18% (w/v) PEG 4000, 50 mM sodium citrate pH 5.7 at 297 K. The crystals appeared after 3–4 weeks and continued growing for three months. Crystals of human dPGM-B grew with a cuboid-shaped morphology and had maximal dimensions of $0.8 \times 0.6 \times 0.6 \text{ mm}$.

The crystals were transferred into a cryoprotecting solution before flash-freezing in liquid nitrogen to 100 K. Two types of cryoprotecting solution were used: one contained 30% (w/v) polyethylene glycol 4000, 20% (v/v) 2-propanol and 0.1 M sodium citrate pH 5.6 and the other was the mother liquor containing 20% (v/v) glycerol. Both methods were unsuccessful, giving very smudgy diffraction patterns and very poor resolution. However, the diffraction quality was improved in terms of spot shape and resolution when the crystals were flash-frozen in liquid nitrogen without any cryoprotecting solution. Diffraction data were collected to 2.8 \AA with a directly flash-frozen crystal. The systematic absences in the data suggested the space group to be $P2_1$. Analysis of the diffraction data is shown in Table 1. Crystal-packing considerations indicated the presence of 9–18 chains in the crystallographic asymmetric unit, with 12 chains being most likely ($V_M = 2.57 \text{ \AA}^3 \text{ Da}^{-1}$, solvent content of 52%; Matthews, 1968) as human dPGM-B is a dimer in solution (Omenn & Cheung, 1974) and the dPGM from *S. cerevisiae* is a tetramer of identical subunits (Fothergill-Gilmore & Watson, 1989).

The three-dimensional structure of human dPGM-B is presently under investigation in our laboratory. Solving the structure, with its large number of molecules in

the asymmetric unit, may be a challenge for molecular replacement.

This work is supported by the Foundation for Authors of National Excellent Doctoral Dissertation of the People's Republic of China (project No. 200128), the National Foundation of Talent Youth (grant No. 30225015), the National High Technology Research and Development Program of China (grant No. 2001AA233021), the 863 Special Program of China (grant No. 2002BA711A13), the Key Important Project and other projects from the National Natural Science Foundation of China (grant Nos. 30121001, 30070170, 30130080 and 30121001) and the Chinese Academy of Sciences (grant No. KSCX1-SW-17). We thank Professor Peng Liu and Yuhui Dong for synchrotron data collection.

References

- Bond, C. S., White, M. F. & Hunter, W. N. (2001). *J. Biol. Chem.* **276**, 3247–3253.
- Bond, C. S., White, M. F. & Hunter, W. N. (2002). *J. Mol. Biol.* **316**, 1071–1081.
- Bradford, M. M. (1976). *Anal. Biochem.* **72**, 248–254.
- Chen, S.-H., Anderson, J., Giblett, E. R. & Lewis, M. (1974). *Am. J. Hum. Genet.* **26**, 73–77.
- Fothergill-Gilmore, L. A. & Watson, H. C. (1989). *Adv. Enzymol.* **62**, 227–313.
- Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
- Omenn, G. S. & Cheung, S. C.-Y. (1974). *Am. J. Hum. Genet.* **26**, 393–399.
- Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
- Prehu, M.-O., Calvin, M. C., Prehu, C. & Rosa, R. (1984). *Biochim. Biophys. Acta*, **787**, 270–274.
- Repiso, A., Perez de la Ossa, P., Aviles, X., Oliva, B., Junca, J., Oliva, R., Garcia, E., Vives-Corrans, J. L., Carreras, J. & Climent, F. (2003). *Haematologica*, **88**, ECR07.
- Rigden, D. J., Alexeev, D., Phillips, S. E. V. & Fothergill-Gilmore, L. A. (1998). *J. Mol. Biol.* **276**, 449–459.
- Rigden, D. J., Littlejohn, J. E., Henderson, K. & Jedrzejas, M. J. (2003). *J. Mol. Biol.* **325**, 411–420.
- Rigden, D. J., Mello, L. V., Setlow, P. & Jedrzejas, M. J. (2002). *J. Mol. Biol.* **315**, 1129–1143.
- Rigden, D. J., Walter, R. A., Phillips, S. E. V. & Fothergill-Gilmore, L. A. (1999). *J. Mol. Biol.* **286**, 1507–1517.
- Toscano, A., Tsujino, S., Vita, G., Shanske, S., Messina, C. & Dimauro, S. (1996). *Muscle Nerve*, **19**, 1134–1137.
- Tsujino, S., Shanske, S., Sakoda, S., Toscano, A. & Dimauro, S. (1995). *Muscle Nerve*, **3**, S50–S53.
- White, M. F. & Fothergill-Gilmore, L. A. (1992). *Eur. J. Biochem.* **207**, 709–714.
- White, M. F., Fothergill-Gilmore, L. A., Kelly, S. M. & Price, N. C. (1993a). *Biochem. J.* **291**, 479–483.
- White, M. F., Fothergill-Gilmore, L. A., Kelly, S. M. & Price, N. C. (1993b). *Biochem. J.* **295**, 743–748.