**Ritcha Mehra-Chaudhary,
Carolyn E. Neace and Lesa J.
Beamer***Department of Biochemistry, University of
Missouri, Columbia, MO 65211, USA

Correspondence e-mail: beamerl@missouri.edu

Received 26 May 2009

Accepted 17 June 2009

Crystallization and initial crystallographic analysis of phosphoglucosamine mutase from *Bacillus anthracis*

The enzyme phosphoglucosamine mutase catalyzes the conversion of glucosamine 6-phosphate to glucosamine 1-phosphate, an early step in the formation of the nucleotide sugar UDP-*N*-acetylglucosamine, which is involved in peptidoglycan biosynthesis. These enzymes are part of the large α -D-phosphohexomutase enzyme superfamily, but no proteins from the phosphoglucosamine mutase subgroup have been structurally characterized to date. Here, the crystallization of phosphoglucosamine mutase from *Bacillus anthracis* in space group $P3_221$ by hanging-drop vapor diffusion is reported. The crystals diffracted to 2.7 Å resolution under cryocooling conditions. Structure determination by molecular replacement was successful and refinement is under way. The crystal structure of *B. anthracis* phosphoglucosamine mutase should shed light on the substrate-specificity of these enzymes and will also serve as a template for inhibitor design.

1. Introduction

Peptidoglycan is a major component of the cell wall of both Gram-negative and Gram-positive bacteria. The molecule UDP-*N*-acetylglucosamine (UDP-GlcNAc) is one of the key building blocks in peptidoglycan biosynthesis (Barreteau *et al.*, 2008). In bacteria, the second step in the formation of UDP-GlcNAc is the interconversion of glucosamine 6-phosphate to glucosamine 1-phosphate, which is catalyzed in the cytoplasm by the enzyme phosphoglucosamine mutase (the *glmM* gene product). The glucosamine 1-phosphate product is subsequently converted to *N*-acetylglucosamine 1-phosphate and finally to UDP-GlcNAc, which is then further modified for incorporation into the peptidoglycan heteropolymer (Barreteau *et al.*, 2008). Bacterial enzymes that participate in the biosynthesis of UDP-GlcNAc are potential targets for antibacterial compounds, as its biosynthetic pathway differs between prokaryotes and eukaryotes.

Phosphoglucosamine mutases from various bacteria, including *Escherichia coli*, *Helicobacter pylori*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus gordonii*, have been characterized functionally and/or kinetically. In Gram-negative organisms these enzymes appear to be essential for viability (Mengin-Lecreux & van Heijenoort, 1996; De Reuse *et al.*, 1997). In Gram-positive organisms, gene-deletion studies have shown that the organisms have reduced virulence, increased susceptibility to antibiotics and decreased biofilm formation (Wu *et al.*, 1996; Jolly *et al.*, 1997; Shimazu *et al.*, 2008). The phosphoglucosamine mutases are a subgroup of a large enzyme superfamily known collectively as the α -D-phosphohexomutases. All enzymes in this superfamily catalyze the reversible conversion of 1- and 6-phosphosugars, but differ in their hexose specificity and hence also in their biosynthetic roles. In general, enzymes in this superfamily share key catalytic residues and a similar four-domain architecture but have relatively low overall sequence identities (typically <30% between subgroups; Whitehouse *et al.*, 1998; Shackelford *et al.*, 2004). Although enzymes from the other three major subgroups of the superfamily (*i.e.* phosphoglucomutases, phosphomannomutase/phosphoglucomutases and *N*-acetylglucosamine-phosphate mutases) have been structurally characterized (Liu *et al.*, 1997; Regni *et al.*, 2002, 2004; Nishitani *et al.*, 2006), no

© 2009 International Union of Crystallography
All rights reserved

proteins from the phosphoglucosamine mutase subgroup have yet been crystallized.

Here, we report the expression, purification and crystallization of phosphoglucosamine mutase from the human pathogen and category A bioterrorism agent *Bacillus anthracis*.

2. Materials and methods

2.1. Expression and purification

The *glmM* gene for phosphoglucosamine mutase from *B. anthracis* Ames (residues 1–448) was obtained from the Pathogen Functional Genomics Resource Center as a Gateway entry clone in the pDONR221 vector. The Gateway system (Invitrogen) was used to subclone the gene into the pDEST17 vector, which includes an N-terminal histidine tag of sequence MSYYHHHHHHLESTSL-YKKAG. The resulting construct (molecular weight 50 963 Da) was used to transform *E. coli* BL21 (DE3) cells. Cells were grown in LB medium with 100 µg l⁻¹ ampicillin at 310 K to an optical density at 600 nm of ~0.7 and protein expression was induced by the addition of IPTG to a final concentration of 0.4 M. Cells were grown overnight at 294 K and centrifuged and the cell pellet was stored at 193 K until use. For purification, cells were thawed, resuspended in 20 mM sodium phosphate pH 7.8, 0.3 M NaCl and 14.3 mM β-mercaptoethanol (plus 2 mM each of the following reagents: PMSF, TLCK, CaCl₂ and MgCl₂) and lysed with a French press. Insoluble debris was removed by centrifugation and the protein was purified using His-Select Ni-Affinity gel (Sigma). It was dialyzed into 50 mM MOPS pH 7.4 and 1 mM MgCl₂ and concentrated to 12 mg ml⁻¹. The protein was flash-frozen in liquid nitrogen and stored at 193 K. For crystallization, the protein was further concentrated to 24 mg ml⁻¹ after thawing at 277 K. The protein concentration was determined *via* Bradford assays using Bio-Rad Protein Assay reagent (Bio-Rad).

2.2. Crystallization

Initial crystallization screens employed conditions found in Crystal Screen kits 1 and 2 (Hampton Research) and Wizard screen kits 1 and 2 (Emerald BioSystems Inc.). Crystals were grown by hanging-drop vapor diffusion at 277 K in drops containing 2 µl protein solution and 2 µl well solution sealed over 1 ml well solution (2.0 M sodium/potassium phosphate and 0.1 M sodium acetate pH 4.5). Crystals were grown from clear drops; the *de novo* growth time was approximately two months. Macroseeding was used to speed up the

Figure 1
Crystal of *B. anthracis* phosphoglucosamine mutase grown by macroseeding.

Table 1

Data-collection statistics for native crystals of *B. anthracis* phosphoglucosamine mutase.

Values in parentheses are for the outer resolution shell (2.80–2.70 Å). No sigma cutoff was applied to the reflection data.

Resolution (Å)	43.03–2.70
$R_{\text{merge}}^{\dagger}$ (%)	7.4 (52.1)
$\langle I/\sigma(I) \rangle$	9.1 (2.0)
Completeness (%)	99.0 (99.7)
Multiplicity	5.83 (6.01)
No. of observed reflections	187105 (18967)
No. of unique reflections	32103 (3157)
Mosaicity (°)	0.71

$$\dagger R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)} \times 100.$$

crystal-growth time and to increase the crystal size (Fig. 1) and was performed by transferring a medium-sized *de novo* crystal into a drop that had been pre-equilibrated for 5–7 h. Drop conditions for seeding were the same as given above. Seeded crystals grew to maximum size within three weeks.

A solution of well buffer containing 20% glycerol, 2.4 M sodium/potassium phosphate and 0.1 M sodium acetate pH 4.5 was used to cryoprotect the crystals for low-temperature data collection. Crystals were mounted in nylon loops, flash-frozen in liquid nitrogen and stored until needed for data collection.

2.3. Data collection and processing

A native data set for phosphoglucosamine mutase was collected to 2.7 Å resolution from a single crystal on beamline 4.2.2 at the Advanced Light Source of Berkeley National Laboratory using a NOIR-1 CCD detector (Table 1). Data were collected at approximately 103 K using the cryoprotectant described above. The X-ray wavelength was 1.0 Å, the crystal-to-detector distance was 185 mm, the oscillation angle was 0.5° and 360 images were collected. The crystals belonged to space group *P*₃₁₂1 or *P*₃₂₁, with unit-cell parameters *a* = *b* = 86.06, *c* = 266.84 Å. Diffraction data were processed using *d*TREK* (Pflugrath, 1999).

3. Results and discussion

Packing-density calculations indicate a *V*_M of 2.8 Å³ Da⁻¹ assuming the presence of two molecules of protein per asymmetric unit. This corresponds to a solvent fraction of about 56.1%, a typical value for protein crystals (Matthews, 1968).

The structures of several enzymes from the α-D-phosphoglucosaminidase superfamily were used as models for molecular-replacement attempts [PDB codes 1p5d (Regni *et al.*, 2004), 1wqa (T. Kawamura, M. Tsuge, N. Watanabe & I. Tanaka, unpublished work) and 2f7l (T. Kawamura, N. Sakai, J. Akutsu, Z. Zhang, N. Watanabe, Y. Kawarabayashi & I. Tanaka, unpublished work)]. Despite the low sequence identity between the proteins (30%), a clear molecular-replacement solution was obtained using a polyalanine version of 2f7l, locating two molecules in the asymmetric unit and confirming the space group of the crystals as *P*₃₂₁. Refinement of the model is under way.

The authors gratefully acknowledge Dale Karr of the MU Structural Biology Core for assistance with construct preparation and protein purification and Dr Jay Nix of Advanced Light Source beamline 4.2.2 for help with data collection. This work was supported by a grant from the MU Children's Miracle Network to LJB. The Advanced Light Source is supported by the Director, Office of

Science, Office of Basic Energy Sciences of the US Department of Energy under Contract DE-AC02-05CH11231.

References

- Barreteau, H., Kovac, A., Boniface, A., Sova, M., Gobec, S. & Blanot, D. (2008). *FEMS Microbiol. Rev.* **32**, 168–207.
- De Reuse, H., Labigne, A. & Mengin-Lecreulx, D. (1997). *J. Bacteriol.* **179**, 3488–3493.
- Jolly, L., Wu, S., van Heijenoort, J., de Lencastre, H., Mengin-Lecreulx, D. & Tomasz, A. (1997). *J. Bacteriol.* **179**, 5321–5325.
- Liu, Y., Ray, W. J. & Baranidharan, S. (1997). *Acta Cryst. D* **53**, 392–405.
- Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
- Mengin-Lecreulx, D. & van Heijenoort, J. (1996). *J. Biol. Chem.* **271**, 32–39.
- Nishitani, Y., Maruyama, D., Nonaka, T., Kita, A., Fukami, T. A., Mio, T., Yamada-Okabe, H., Yamada-Okabe, T. & Miki, K. (2006). *J. Biol. Chem.* **281**, 19740–19747.
- Pflugrath, J. W. (1999). *Acta Cryst. D* **55**, 1718–1725.
- Regni, C., Naught, L. E., Tipton, P. A. & Beamer, L. J. (2004). *Structure*, **12**, 55–63.
- Regni, C., Tipton, P. A. & Beamer, L. J. (2002). *Structure*, **10**, 269–279.
- Shackelford, G. S., Regni, C. A. & Beamer, L. J. (2004). *Protein Sci.* **13**, 2130–2138.
- Shimazu, K., Takahashi, Y., Uchikawa, Y., Shimazu, Y., Yajima, A., Takashima, E., Aoba, T. & Konishi, K. (2008). *FEMS Immunol. Med. Microbiol.* **53**, 166–177.
- Whitehouse, D. B., Tomkins, J., Lovegrove, J. U., Hopkinson, D. A. & McMillan, W. O. (1998). *Mol. Biol. Evol.* **15**, 456–462.
- Wu, S., de Lencastre, H., Sali, A. & Tomasz, A. (1996). *Microb. Drug Resist.* **2**, 277–286.