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

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

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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-Lecreulx & 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

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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 m*M* sodium phosphate pH 7.8, 0.3 *M* NaCl and 14.3 m*M* β-mercapto-ethanol (plus 2 m*M* each of the following reagents: PMSF, TLCK, $CaCl_2$ and $MgCl_2$) 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 m*M* MOPS pH 7.4 and 1 m*M* $MgCl_2$ 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 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.

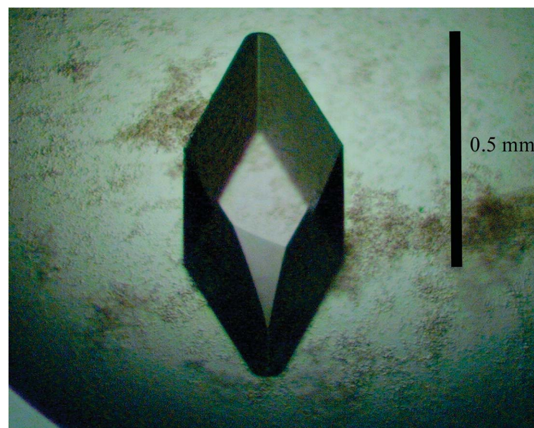

Figure 1
Crystal of *B. anthracis* phosphoglucosamine mutase grown by macroseeding.

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_{merge}† (%)	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

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

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 $P3_121$ or $P3_221$, 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 $Å^3$ 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-phosphoglucomutase 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 $P3_221$. Refinement of the model is under way.

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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.