Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Crystallization and preliminary crystallographic analysis of PimA, an essential mannosyltransferase from *Mycobacterium smegmatis*

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Received 14 March 2005
Accepted 19 April 2005
Online 22 April 2005

Phosphatidylinositol mannosyltransferase (PimA) is an essential enzyme for mycobacterial growth that catalyses the first mannosylation step in phosphatidyl-*myo*-inositol mannoside (PIM) biosynthesis. The enzyme belongs to the large GT4 family of glycosyltransferases, for which no structure is currently available. Recombinant purified PimA from *Mycobacterium smegmatis* has been crystallized in the presence of GDP and *myo*-inositol. The crystals belong to space group $P2_12_12_1$, with unit-cell parameters $a = 37.2$, $b = 72.4$, $c = 138.2$ Å, and diffract to 2.4 Å resolution.

1. Introduction

Phosphatidyl-*myo*-inositol mannoside (PIM), lipomannan (LM) and lipoarabinomannan (LAM) are glycolipids/lipoglycans interspersed in the cell wall of mycobacteria (Brennan & Nikaido, 1995). Linked to the hydrophobic cell envelope through their phosphatidyl-*myo*-inositol anchor, these molecules play important roles both in mycobacterial physiology and in the modulation of the host immune response in the course of tuberculosis and leprosy (Briken *et al.*, 2004).

PimA (MW 43.3 kDa) is an α-retaining glycosyltransferase that catalyses the first mannosylation step in PIM biosynthesis. The enzyme transfers a Man*p* residue from GDP-Man to the 2-position of the *myo*-inositol ring of phosphatidyl-*myo*-inositol (PI) to form phosphatidyl-*myo*-inositol monomannoside (PIM_1; Kordulakova *et al.*, 2002). The *pimA* gene is the fourth of a cluster of five genes conserved in all known mycobacterial genomes (*Rv2613c–Rv2609c* in *Mycobacterium tuberculosis*) that are involved in the early steps of PIM biosynthesis (Cole *et al.*, 1998*a*,*b*; Jackson *et al.*, 2000). The first gene of the cluster encodes a protein of unknown function, the second and third, respectively, encode the phosphatidyl-*myo*-inositol synthase PgsA (Jackson *et al.*, 2000) and an acyltransferase responsible for the acylation of the Man*p* residue linked to the 2-position of the inositol ring in PIM_1 and PIM_2 molecules (Kordulakova *et al.*, 2003), and the last encodes a putative GDP-Man hydrolase (Frick *et al.*, 1995). Both PgsA and PimA have been shown to be essential for the growth of *M. smegmatis*; the acyltransferase *Rv2611c* mutants of *M. smegmatis* exhibit reduced rates of acylation of PIM molecules and show severe growth defects (Kordulakova *et al.*, 2003).

Subsequent mannosylation steps in PIM biosynthesis are catalysed by PimB, which transfers a second Man*p* residue to the 6-position of the *myo*-inositol ring of PIM_1 to form PIM_2 (Schaeffer *et al.*, 1999), and PimC, which transfers the third Man*p* residue to the growing molecule (Kremer *et al.*, 2002). In contrast to PimA, however, PimB and PimC appear to be non-essential genes, suggesting the existence of alternative pathways for the synthesis of PIM_2 and higher PIMs (Schaeffer *et al.*, 1999; Kremer *et al.*, 2002). The three mannosyltransferases PimA, PimB and PimC belong to the GT4 family of glycosyltransferases (see http://afmb.cnrs-mrs.fr/CAZY/index.html; Coutinho *et al.*, 2003; Abdian *et al.*, 2000), for which there is no structure available, and their amino-acid sequences contains the GPGTF (glycogen phosphorylase/glycosyl transferase) motif (Wrabl & Grishin, 2001), a signature present in enzymes of the GT-B fold superfamily (Coutinho *et al.*, 2003).

The involvement of PimA in an essential pathway restricted to mycobacteria and a few other actinomycetes makes this enzyme an attractive drug target for anti-tuberculosis chemotherapy. The crystallographic characterization of PimA will shed light on the catalytic mechanism of the GT4 family of glycosyltransferases and will provide a structural framework for the development of specific inhibitors.

2. Results and discussion

2.1. Expression and purification

Escherichia coli BL21(DE3)pLysS cells transformed with pET-*pimA* (Kordulakova *et al.*, 2002) were grown in 1000 ml of 2×YT medium supplemented with 100 µg ml^{-1} ampicillin and 34 µg ml^{-1} chloramphenicol at 310 K. When the culture reached an A_{600} value of 0.6, PimA expression was induced by adding 0.5 m*M* isopropyl *β*-thiogalactopyranoside (IPTG). After 12 h at 293 K, cells were harvested and resuspended in 40 ml 25 m*M* Tris–HCl pH 7.5 (solution *A*) containing protease inhibitors (Complete EDTA-free, Roche). Cells were then disrupted by three compression–decompression cycles in a French press and the suspension was centrifuged for 20 min at 15 000*g*. The supernatant was subjected to Ni^{2+}-affinity chromatography using a HiTrap Chelating column (5 ml, Amersham Biosciences) equilibrated with 25 m*M* Tris–HCl pH 7.5, 500 m*M* NaCl (solution *B*). The column was washed with solution *B* until no absorbance at 280 nm was detected. Elution was performed with a linear gradient of 0–250 m*M* imidazole in 50 ml solution *B* at 2 ml min^{-1}. Fractions showing enzymatic activity were pooled and dialyzed overnight against solution *A* containing 5 m*M* dithiothreitol (DTT; solution *C*). The dialyzed solution was filtered through a Millipore 0.22 µm filter and applied onto a Mono Q HR 5/5 (Amersham Biosciences) column equilibrated in solution *C*. The enzyme was eluted at 200 m*M* NaCl with a linear gradient of 0–500 m*M* NaCl in 30 ml solution *C* at 1 ml min^{-1}. The resulting preparation displayed a single protein band when run on 10% SDS–PAGE stained with Coomassie brilliant blue. The purified enzyme was stored at 193 K for further use in crystallization trials.

2.2. Crystallization

The protein was concentrated to 10 mg ml^{-1} using a Centricon YM-10 (Millipore) in 10 m*M* Tris–HCl pH 7.5 and 1 m*M* DTT. A broad screening of crystallization conditions using Crystal Screens I and II (Hampton Research), Structure Screens I and II (Molecular Dimensions Ltd) and JBScreens 1–10 (Jena Biosciences) was performed using a Cartesian Technologies workstation by the sitting-drop vapour-diffusion method. Crystallization conditions resulting in small crystals were manually reproduced and further optimized in terms of pH, precipitant concentration and drop volume using the hanging-drop vapour-diffusion method. All experiments were carried out at 291 K. The best crystals were obtained by mixing 8 µl protein (10 mg ml^{-1}) preincubated with 10 m*M* guanosine 5′-diphosphate (GDP, Sigma) and 10 m*M* *myo*-inositol (Sigma) with 2 µl well solution consisting of 18%(*w*/*v*) PEG 8000, 200 m*M* calcium acetate and 50 m*M* HEPES pH 7.5. Crystals appeared after 2–3 d and grew as rods, reaching 0.4 × 0.06 × 0.06 mm (Fig. 1). Prior to data collection, crystals were transferred to a cryoprotectant solution [25%(*v*/*v*) glycerol in the well solution] for 1 min and flash-frozen in liquid nitrogen.

Table 1
Data-collection statistics.

Values in parentheses are for the last resolution shell.

Space group	$P2_12_12_1$
Crystal system	Orthorhombic
Unit-cell parameters (Å)	a = 37.2, b = 72.4, c = 138.2
Matthews coefficient (Å^3 Da^{-1})	2.2
Molecules per AU	1
Resolution range (Å)	50–2.4 (2.53–2.4)
Mosaicity (°)	0.66
Total observations	51389
Unique reflections	17229
Completeness (%)	99.7 (100)
R_{sym}† (%)	5.8 (31.3)
Multiplicity	3.4
$\langle I/\sigma(I)\rangle$ (top shell)	18.5 (2.4)

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

2.3. Preliminary crystallographic characterization

Crystals of PimA belong to the orthorhombic space group $P2_12_12_1$ and have one molecule per asymmetric unit, corresponding to a Matthews coefficient of 2.2 Å^3 Da^{-1} and a solvent content of 43%.

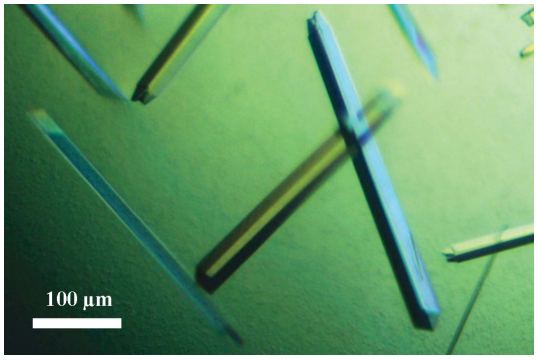

Figure 1
Crystals of PimA from *M. smegmatis* in the presence of GDP and *myo*-inositol grown in 16–18%(*w*/*v*) PEG 8000, 200 m*M* calcium acetate and 50 m*M* HEPES pH 7.5.

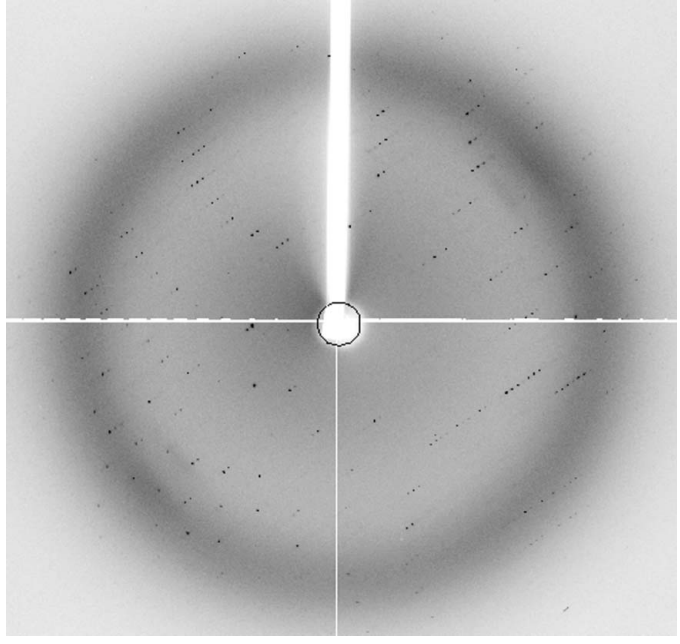

Figure 2
X-ray diffraction image of a *M. smegmatis* PimA crystal.

X-ray diffraction data from a single crystal (Fig. 2) were collected at 2.4 Å on the ID29 beamline (λ = 0.9330 Å) at the ESRF (Grenoble, France) equipped with an ADSC Q210 two-dimensional detector and were processed with the programs *MOSFLM* v.6.2.3, *SCALA* v.3.2.0-3 and *TRUNCATE* from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994). Data-collection statistics are shown in Table 1. Preliminary attempts to determine the crystal structure using molecular-replacement methods with other known GT-B glycosyltransferases as probe models were unsuccessful. The selenomethionine-labelled protein is thus being produced for structure determination using single- or multiple-wavelength anomalous diffraction (SAD/MAD) methods.

We thank A. Haouz (PF6, Institut Pasteur) and W. Shepard (ID29, ESRF, Grenoble, France) for help with data collection. This work was partially supported by the Institut Pasteur (GPH-5), the Ministere de la Recherche, France (contract 01-B-0095) and the European Commission, contracts QLG2-CT-2002-00988 (SPINE) and QLK2-CT-2001-02018 (X-TB).

References

Abdian, P. L., Lellouch, A. C., Gautier, C., Ielpi, L. & Geremia, R. A. (2000). *J. Biol. Chem.* **275**, 40568–40575.
Brennan, P. J. & Nikaido, H. (1995). *Annu. Rev. Biochem.* **64**, 29–63.
Briken, V., Porcelli, S. A., Besra, G. S. & Kremer, L. (2004). *Mol. Microbiol.* **53**, 391–403.
Cole, S. T. *et al.* (1998*a*). *Nature (London)*, **393**, 537–544.
Cole, S. T. *et al.* (1998*b*). *Nature (London)*, **396**, 190.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Coutinho, P. M., Deleury, E., Davies, G. J. & Henrissat, B. (2003). *J. Mol. Biol.* **328**, 307–317.
Frick, D. N., Townsend, B. D. & Bessman, M. J. (1995). *J. Biol. Chem.* **270**, 24086–24091.
Jackson, M., Crick, D. C. & Brennan, P. J. (2000). *J. Biol. Chem.* **275**, 30092–30099.
Kordulakova, J., Gilleron, M., Mikusova, K., Puzo, G., Brennan, P. J., Gicquel, B. & Jackson, M. (2002). *J. Biol. Chem.* **277**, 31335–31344.
Kordulakova, J., Gilleron, M., Puzo, G., Brennan, P. J., Gicquel, B., Mikusova, K. & Jackson, M. (2003). *J. Biol. Chem.* **278**, 36285–36295.
Kremer, L., Gurcha, S. S., Bifani, P., Hitchen, P. G., Baulard, A., Morris, H. R., Dell, A., Brennan, P. J. & Besra, G. S. (2002). *Biochem. J.* **363**, 437–447.
Schaeffer, M. L., Khoo, K. H., Besra, G. S., Chatterjee, D., Brennan, P. J., Belisle, J. T. & Inamine, J. M. (1999). *J. Biol. Chem.* **274**, 31625–31631.
Wrabl, J. O. & Grishin, N. V. (2001). *J. Mol. Biol.* **314**, 365–374.