Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray diffraction data of *Mycobacterium tuberculosis* FbpC1 (Rv3803c)

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Received 25 July 2003
Accepted 16 September 2003

The heterotrimeric antigen 85 complex (Ag85) is a major component of the cell wall of *Mycobacterium tuberculosis* and consists of three abundantly secreted proteins (FbpA, FbpB and FbpC2). These play key roles in the pathogenesis of tuberculosis and in maintaining cell-wall integrity. A homologue of the Ag85 subunits (~40% identity) was recently annotated in the *M. tuberculosis* genome as FbpC1. Unlike the Ag85-complex components, FbpC1 lacks mycolyltransferase activity and its function remains to be established. In order to aid functional characterization, FbpC1 has been crystallized. At room temperature, tetragonal crystals of FbpC1 were obtained belonging to space group $P4_12_12$ (unit-cell parameters $a = b = 109.9$, $c = 61.8$ Å), yet when frozen the crystals underwent a phase transition to orthorhombic symmetry, space group $P2_12_12_1$ ($a = 59.9$, $b = 108.9$, $c = 109.9$ Å). Diffraction data complete to 1.7 Å resolution were recorded at 100 K at the synchrotron.

1. Introduction

Mycobacterium tuberculosis, the primary agent of tuberculosis, is responsible for three million deaths annually worldwide (Dye *et al.*, 1999). Existing antitubercular drugs are directed against biosynthesis of the cell wall of *M. tuberculosis*, which is composed of covalently linked mycolic acids, arabinogalactan and peptidoglycan. The plethora of enzymes participating in mycobacterial cell-wall biosynthesis offer a wealth of potential novel drug targets to counter the recent emergence of multi-drug resistant strains of *M. tuberculosis*. One promising target is the antigen 85 (Ag85) complex, a heterotrimeric complex of three dominant exported fibronectin-binding proteins, FbpA (or Ag85A), FbpB (or Ag85B) and FbpC2 (or Ag85C2). Ag85 and its components have recently been suggested to play a critical role in the final assembly of the mycobacterial cell wall (Harth *et al.*, 2002). All three Ag85 components catalyse the formation of α,α'-trehalose dimycolate (TDM or cord factor), which is essential for cell-wall integrity, by mycolyl transfer from α,α'-trehalose monomycolate (TMM). Conversely, the trehalose analogue 6-azido-6-deoxytrehalose inhibited mycolyltransferase activity of all three Ag85 subunits *in vitro* and inhibited growth of *M. aurum in vivo* (Belisle *et al.*, 1997). Moreover, FbpC2 has been shown to be important for the covalent attachment of mycolic acids to arabinogalactan (Jackson *et al.*, 1999). Recent crystal structures of *M. tuberculosis* FbpB and FbpC2 (Anderson *et al.*, 2001; Ronning *et al.*, 2000) revealed a canonical α/β hydrolase fold with a Ser-His-Glu catalytic triad (Heikinheimo *et al.*, 1999). Both structures feature a 20 Å long hydrophobic channel in the core of the protein, which probably accommodates the mycolyl α-chain. This channel opens to a largely hydrophobic spacious cleft that in the crystal structure of FbpB has been shown to form the binding site for trehalose (Anderson *et al.*, 2001). The completion of the *M. tuberculosis* genome sequence (Cole *et al.*, 1998) revealed a gene, *fbpC1*, whose translation product is highly related to the Ag85 components (~40% sequence identity). This finding prompted speculation that *fbpC1* may encode a potential fourth member of the Ag85 complex. It has subsequently been shown that FbpC1 does not exhibit mycolyltransferase activity, which would be characteristic of a component of the Ag85 complex (Kremer *et al.*, 2002). In order to aid the functional characterization of FbpC1, we have crystallized this peculiar Ag85 homologue and report here preliminary X-ray crystallographic data.

2. Experimental

2.1. Bacterial strains and culture conditions

All cloning steps were performed in *Escherichia coli* XL1-Blue cells (New England Biolabs). Liquid cultures of recombinant *E. coli* C41(DE3) strains were grown in LB broth at 310 K with 150 mg ml^{-1} ampicillin. Plasmids pUC18 and pET23b were purchased from New England Biolabs and Novagen, respectively. Restriction enzymes and T4 DNA ligase were purchased from Roche Molecular Biochemicals, whilst Vent DNA polymerase was

purchased from New England Biolabs. All DNA manipulations were performed using standard protocols as described elsewhere (Sambrook *et al.*, 1998).

2.2. Cloning, expression and purification of *fbpC1* in *E. coli*

The mature *fbpC1* was amplified by PCR using *M. tuberculosis* H37Rv chromosomal DNA. The forward primer was 5′-**CAT ATG** GCA GAA CCC ACC GCA AAG GCC GCC CCA TAC-3′ (*Nde*I site in bold) and the reverse primer was 5′-**CTC GAG** GCG GAT CGC ACC GAC GAT ATC GCC CGA CAT A-3′ (*Xho*I site in bold). The resulting 831 bp fragment was ligated into the *Sma*I site of pUC18, isolated by digestion with *Nde*I and *Xho*I and directionally ligated into pET23b to generate pET23b-*fbpC1*. The fidelity of the construct was determined by sequence analysis. The plasmid was then used to transform *E. coli* C41(DE3) cells. Bulk cultures of Terrific Broth (Sambrook *et al.*, 1998) were inoculated with overnight pre-cultures, grown at 310 K to an optical density of 1.2 and induced with 1 m*M* isopropylthiogalactopyranoside (IPTG). Post-induction, the cultures were incubated for 12–16 h at 289 K and harvested by centrifugation (27 000*g*, 10 min, 277 K). The cell pellet was resuspended in buffer (20 m*M* phosphate buffer pH 7.4, 0.5 *M* NaCl), disrupted by sonication and the resulting extract centrifuged (100 000*g*, 60 min, 277 K). The supernatant was applied onto an Ni^{2+}-charged His-Trap column (1 ml, Pharmacia), washed extensively with 20 m*M* phosphate buffer pH 7.4, 0.5 *M* NaCl and eluted with a stepwise concentration gradient of imidazole (150, 200, 300 and 500 m*M*). 1 ml fractions were collected and analyzed by 10% SDS–PAGE. Pure fractions were pooled, dialyzed against 20 m*M* phosphate buffer pH 7.4, 100 m*M* NaCl and stored at 277 K prior to concentration.

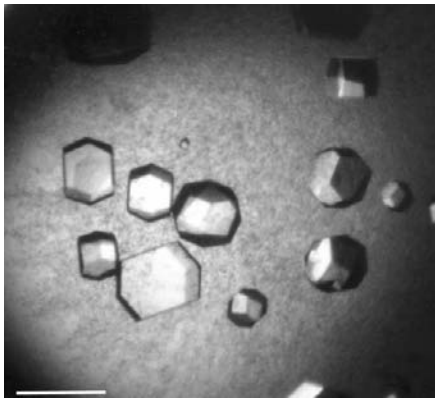

Figure 1
Crystals of FbpC1. The growth of these crystals was accompanied by a slight amorphous precipitate. The scale bar is 300 µm in length.

Table 1
Statistics of X-ray data collection.

Values in parentheses are for the highest resolution shell.

	Room temperature	100 K
Space group, molecules in AU	$P4_12_12$, 1	$P2_12_12_1$, 2
Unit-cell parameters		
a (Å)	109.7	59.9
b (Å)	109.7	108.9
c (Å)	61.8	109.9
Mosaicity (°)		0.86
Source†, wavelength (Å)	Cu $K\alpha$1, 1.54	ID14-3, 0.933
Resolution range (Å)	30–2.8 (2.9–2.8)	30–1.7 (1.76–1.70)
Observations [$I/\sigma(I) > 0$], overall	126286	493963
Observations [$I/\sigma(I) > 0$], unique	9643	78268
R_{sym}‡ (%)	13.5 (60.0)	7.7 (48.9)
$\langle I/\sigma(I)\rangle$	22 (5.04)	22 (3.0)
Completeness (%)	99 (100)	99.7 (99.9)
Redundancy	12.9 (13.7)	6.2 (5.7)

† Station ID refers to ESRF, Grenoble, France. ‡ $R_{sym} = 100\sum(|I - \langle I\rangle|)/\sum|I|$.

2.3. Crystallization

The protein was concentrated to above 70 mg ml^{-1} by ultrafiltration (Centricon 10, Amicon) and initial crystals were obtained by hanging-drop vapour diffusion overnight using a sparse-matrix screen (Jancarik & Kim, 1991; Structure Screen 1, Molecular Dimensions Ltd) as showers of tiny needles. Systematic optimization employing central composite and limited factorial screens (Carter, 1992) showed that nucleation required a pH between 4 and 5 and the presence of acetate, while citrate was required to reduce nucleation. The crystal morphology markedly improved in the presence of up to 10% 2-propanol and in order to reproducibly obtain crystals of a size amenable for X-ray diffraction experiments, 8–10%(*v*/*v*) 2-methyl-2,4-pentanediol was required. The crystals eventually used in X-ray diffraction experiments were grown over a reservoir containing 8–10%(*w*/*v*) polyethylene glycol 8000, 8–10%(*v*/*v*) 2-methyl-2,4-pentanediol, 2.5%(*v*/*v*) 2-propanol, 200 m*M* sodium acetate pH 4.6 and 100 m*M* sodium citrate pH 5.0. 3 µl drops containing a 50:50 mixture of reservoir and protein solution yielded crystals of dimensions typically between 0.2 and 0.35 mm (Fig. 1).

2.4. X-ray diffraction analysis at room temperature

An exceptionally large crystal of FbpC1 (∼0.5 mm in diameter) was mounted in a 1 mm diameter glass capillary and X-ray diffraction images were recorded at room temperature using a SMART6000 CCD detector mounted on a sealed-tube generator with MonoCap capillary optics (Bruker AXS). The crystal system was determined to be tetragonal (Table 1). Diffraction spots could be observed to a maximum resolution of 2.5 Å and the crystal was sufficiently resistant to radiation damage to allow the recording of a data set complete to 2.8 Å, covering a total rotation angle of 180° in increments of 0.3° (crystal-to-detector distance 60 mm, exposure time 20 s) (Table 1). The data were integrated using *SAINT* 6.36b (Bruker AXS) and scaled with *SADABS* 2.10 (G. M. Sheldrick, University of Göttingen, Germany). The symmetry of the intensity data and molecular-replacement calculations established the space group to be $P4_12_12$.

2.5. X-ray diffraction analysis at 100 K

Because of the presence of ample amounts of cryoprotective agents in the crystallization conditions, we initially attempted to transfer the FbpC1 crystals directly from the crystallization drop into either a 100 K nitrogen-gas stream or liquid nitrogen. The resulting diffraction patterns in both cases displayed extremely streaky peak shapes and strong anisotropy in terms of resolution, but no ice rings. Irrespective of the poor quality, the patterns could be indexed using *MOSFLM* (Collaborative Computational Project, Number 4, 1994) indicating an orthorhombic lattice with approximate unit-cell parameters a = 60, b = 108, c = 110 Å. The fact that crystals were frozen without additional cryoprotection suggested that the switch in symmetry from tetragonal to orthorhombic was the result of a temperature-induced structural phase transition. Equilibration of the FbpC1 crystals in up to 10% glycerol plus artificial

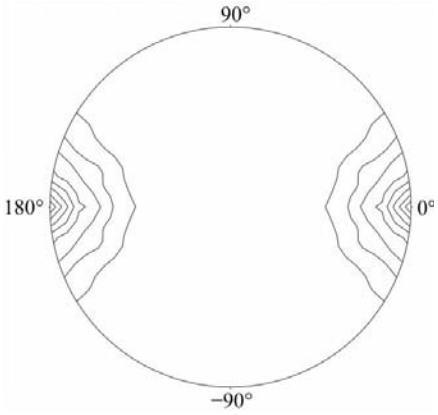

Figure 2
$\kappa = 90^\circ$ section of the self-rotation function calculated on the basis of the orthorhombic low-temperature data set, including reflections between 15 and 3.5 Å, with the integration radius set to 20 Å. The peak height of the apparent non-crystallographic fourfold axis corresponds to 0.95 times the origin peak.

mother liquor [10% polyethylene glycol 8000, 200 m*M* sodium acetate pH 4.6, 100 m*M* sodium citrate, 2.5%(*v*/*v*) 2-propanol and 10% 2-methyl-2,4-pentanediol] prior to flash-freezing dramatically improved the peak shape. The resulting diffraction patterns were still orthorhombic; however, the spontaneous strain $[(b - c)/0.5(b + c)]$ with respect to the pseudo-tetragonal axes was reduced to a minimum of 0.5%. Diffraction data complete to 1.7 Å (Table 1) were recorded on station ID14-3 at ESRF Grenoble, France using a MAR CCD (165 mm) detector and 0.5° oscillations covering a total oscillation range of 180°. Data were integrated and scaled using *DENZO*/*SCALEPACK* (Otwinowski & Minor, 1997). Based on the Matthews volume (V_M = 2.7 Å^3 Da^{-1}, 54% solvent content), we expected to find two molecules in the asymmetric unit of the orthorhombic crystal lattice. A molecular-replacement search (*AMoRe*; Collaborative Computational Project, Number 4, 1994), using the crystal structure of FbpC2 (PDB code 1dqz; Ronning *et al.*, 2000) as a probe and data in the resolution range 10–4 Å displayed two peaks in the cross-rotation function that were consistent with a non-crystallographic twofold axis parallel to $b + c$ in the self-rotation function (*POLARRFN*; Collaborative Computational Project, Number 4, 1994). This non-crystallographic twofold in combination with the crystallographic twofold along the *a* axis gives rise to an apparent non-crystallographic fourfold along *a* (Fig. 2). Systematic extinctions and the translation function unequivocally identified $P2_12_12_1$ as the correct space group. Refinement of this structural model is currently in progress.

We thank the staff at ESRF for support during data collection.

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