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# Crystallization and preliminary X-ray crystallographic analysis of a GroEL1 fragment from *Mycobacterium tuberculosis* H37Rv

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Full-length GroEL1 from *Mycobacterium tuberculosis* H37Rv was cloned, overexpressed and purified. Crystals were obtained by the hanging-drop vapor-diffusion method and contained a 23 kDa GroEL1 fragment. A complete native data set was collected from a single frozen crystal that belonged to the orthorhombic space group $P2_12_12$, with unit-cell parameters $a = 75.47$, $b = 78.67$, $c = 34.89$ Å, $\alpha = \beta = \gamma = 90^\circ$, and diffracted to 2.2 Å resolution on a home X-ray source.

## 1. Introduction

Molecular chaperonins are found in all domains of life. The best characterized member is *Escherichia coli* GroEL, which facilitates the folding of several essential proteins (Houry *et al.*, 1999) and is therefore required for bacterial growth at all temperatures (Fayet *et al.*, 1989). The crystal structure of *E. coli* GroEL revealed a symmetrical 800 kDa double-toroid structure consisting of two heptameric rings that are stacked back to back (Braig *et al.*, 1994; Bartolucci *et al.*, 2005). As part of the folding cycle, asymmetric binding of the GroES co-chaperonin to the *cis* ring of GroEL triggers a major conformational change in the chaperonin and creates an enlarged chamber in which polypeptides can fold into their native conformation (Xu *et al.*, 1997).

Many mycobacteria, such as the human pathogen *Mycobacterium tuberculosis*, are unusual in that they possess more than one copy of the GroEL chaperonin (Kong *et al.*, 1993). *M. tuberculosis* GroEL1 and GroEL2 share 61% amino-acid sequence identity and are up-regulated in response to both heat shock (Stewart *et al.*, 2002) and oxidative stress (Dosanjh *et al.*, 2005). While GroEL2 is essential in actinobacteria (Lund, 2009), GroEL1 is dispensable for viability (Barreiro *et al.*, 2005). *M. tuberculosis* GroEL1 has been proposed to be a nucleoid-associated protein (Basu *et al.*, 2009) and may play a crucial role in biofilm formation (Ojha *et al.*, 2005), but its exact roles in these processes remain unknown.

The distinct biological activities ascribed to GroEL1 and GroEL2 suggest functional differences that might be reflected in their three-dimensional structures. While the crystal structure of *M. tuberculosis* GroEL2 has been determined (PDB code 1sjp; Qamra & Mande, 2004), no high-resolution structural information is available for *M. tuberculosis* GroEL1. Here, we report the cloning, overexpression, purification and crystallization screening of full-length GroEL1 from *M. tuberculosis* H37Rv. Crystals were obtained by the hanging-drop vapor-diffusion technique and diffracted to 2.2 Å resolution on a home X-ray source. A complete native data set was collected from a single frozen crystal that belonged to the orthorhombic space group $P2_12_12$, with unit-cell parameters $a = 75.47$, $b = 78.67$, $c = 34.89$ Å, $\alpha = \beta = \gamma = 90^\circ$. Our preliminary X-ray crystallographic and biochemical analyses suggested that the crystals contained a 23 kDa fragment of *M. tuberculosis* GroEL1.

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## 2. Materials and methods

### 2.1. Cloning and expression

An *Eco*RI/*Xba*I fragment encoding full-length GroEL1 (gene *Rv3417c*) was amplified from *M. tuberculosis* H37Rv genomic DNA (a kind gift from Edward A. Graviss, Baylor College of Medicine). The fragment was inserted into the *Eco*RI/*Xba*I site of the bacterial expression vector pProEX Htb (Invitrogen) to generate plasmid pGroEL1 consisting of full-length *M. tuberculosis* GroEL1 with a tobacco etch virus (TEV) protease-cleavable N-terminal His tag. *E. coli* BL21 (DE3) RIL cells (Agilent Technologies) harbouring pGroEL1 were grown in Luria–Bertani medium at 310 K to an $OD_{600\,nm}$ of 0.6. Cultures were cooled to 298 K, induced with 0.5 m*M* isopropyl $\beta$-D-1-thiogalactopyranoside and incubated at 298 K for a further 4 h. Cells were harvested by centrifugation at 6000*g* for 20 min and the cell pellet was stored at 193 K.

### 2.2. Purification

Cells were thawed, resuspended in buffer *A* (25 m*M* Tris pH 7.5, 300 m*M* NaCl and 30 m*M* imidazole pH 7.5) and disrupted by three passages through a Microfluidizer processor (Microfluidics) at 103 MPa. After centrifugation at 43 000*g* for 30 min, the supernatant was loaded onto an Ni–NTA agarose column (Qiagen) equilibrated with buffer *A*. After washing the column with ten column volumes of buffer *A*, bound proteins were eluted with a linear gradient from 30 to 300 m*M* imidazole in buffer *A*. Fractions containing full-length GroEL1 were pooled and 400 m*M* (final concentration) ammonium sulfate was added prior to loading the protein onto a Butyl-650S column (Tosoh) equilibrated with buffer *B* (50 m*M* Tris pH 7.5 and 400 m*M* ammonium sulfate). GroEL1 was eluted with a linear gradient from 400 to 0 m*M* ammonium sulfate in buffer *B*. The pooled fractions were treated with recombinant TEV protease for 1 h at 298 K and subsequently diluted tenfold into 50 m*M* Tris pH 7.5. Removal of TEV protease and any remaining contaminants was accomplished using a Q-Sepharose HP (GE Healthcare) column and eluting GroEL1 with a linear gradient from 0 to 500 m*M* NaCl in 50 m*M* Tris pH 7.5. Purified GroEL1 was dialyzed into 10 m*M* Tris pH 7.5 and 80 m*M* NaCl, concentrated to 12 mg ml$^{-1}$ and stored at 193 K. The protein concentration was estimated using the Bio-Rad Protein assay (Bio-Rad) with bovine serum albumin as standard.

### 2.3. Crystallization

Crystallization screening experiments of full-length GroEL1 were performed by the hanging-drop vapor-diffusion method at 294 K. 1 µl protein solution (12 mg ml$^{-1}$) in 10 m*M* Tris pH 7.5 and 80 m*M* NaCl was mixed with an equal volume of reservoir solution. A cluster of multiple rod-shaped crystals was obtained after more than 12 months in a single nearly dried drop. The reservoir solution consisted of 20% PEG 6000 and 0.1 *M* Bicine pH 9.0.

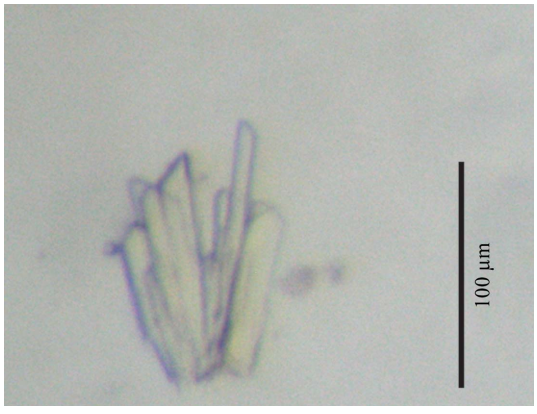


**Figure 1**
Cluster of rod-shaped crystals of a *M. tuberculosis* GroEL1 fragment.

**Table 1**
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

| | |
|---|---|
| Source | Rigaku FR-E+ |
| Wavelength (Å) | 1.5417 |
| Detector | R-AXIS HTC |
| Space group | $P2_12_12$ |
| Resolution (Å) | 40.00–2.20 (2.28–2.20) |
| Unit-cell parameters (Å, °) | $a$ = 75.47, $b$ = 78.67, $c$ = 34.89, $\alpha = \beta = \gamma$ = 90 |
| Total No. of reflections | 47084 |
| No. of unique reflections | 10994 |
| Completeness (%) | 98.7 (91.9) |
| Redundancy | 4.3 (2.6) |
| $I/\sigma(I)$ | 12.3 (2.4) |
| $R_{merge}$† (%) | 10.1 (36.7) |

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $\langle I(hkl)\rangle$ is the mean of the symmetry-equivalent reflections $I_i(hkl)$.

### 2.4. Data collection and processing

The crystal cluster was coated with perfluoropolyether PFO-X175/08 (Hampton Research) and carefully separated. A single crystal was suspended in a small nylon loop at the end of a Hampton mounting pin (Hampton Research) and flash-frozen in a gaseous nitrogen stream at 100 K. A total of 240 frames were collected on a home X-ray source with an oscillation range of 0.5° and an exposure time of 5 min per frame. The distance between the detector and the crystal was 135 mm. The diffraction data were processed and scaled using the *HKL*-2000 software package (Otwinowski & Minor, 1997) and were analyzed using the *CCP*4 suite of programs (Collaborative Computational Project, Number 4, 1994).

### 2.5. Limited proteolysis

50 µg GroEL1 was incubated with 0.2 µg trypsin (Sigma) in 20 µl 50 m*M* Tris pH 7.5 at 294 K for 60 min. The reaction was stopped by the addition of 1% SDS and incubation of the reaction mixture at 373 K for 5 min. A 15 µl sample was run on a 15% SDS–PAGE gel and subsequently electro-blotted on a PVDF membrane (Immobilon-PSQ; Qiagen). The membrane was stained with Coomassie Brilliant Blue R-250. A 23 kDa band was excised from the membrane and submitted for N-terminal sequencing by Edman degradation.

## 3. Results and discussion

Full-length *M. tuberculosis* GroEL1 was cloned and overexpressed in *E. coli*. The protein was purified to near-homogeneity using standard column chromatography and used for crystallization screening. After more than 12 months, a cluster of rod-shaped crystals with dimensions of 0.1 × 0.02 × 0.01 mm (Fig. 1) was obtained from a nearly dried drop. A complete 2.2 Å resolution native data set was collected from a single frozen crystal. The crystal belonged to the orthorhombic space group $P2_12_12$, with unit-cell parameters $a$ = 75.47, $b$ = 78.67, $c$ = 34.89 Å, $\alpha = \beta = \gamma = 90°$. The final data-collection and processing statistics are shown in Table 1.

The calculated Matthews coefficient (Matthews, 1968) indicated that the crystals obtained could not contain full-length GroEL1 (the $V_M$ was 0.92 Å$^3$ Da$^{-1}$ assuming the presence of one molecule of

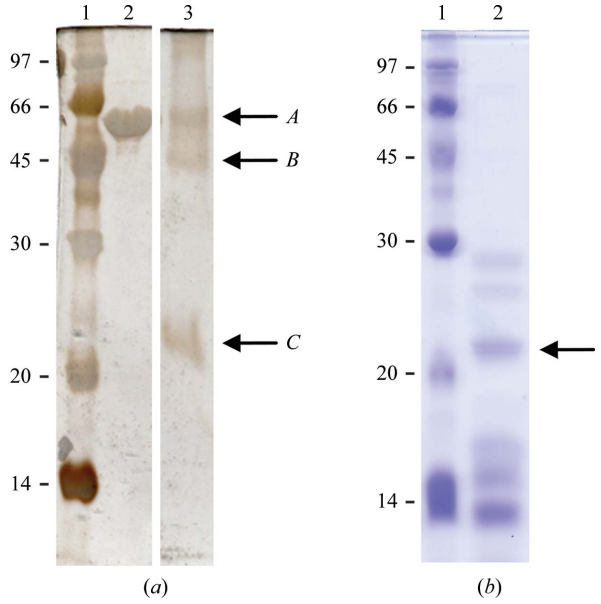


**Figure 2**
(*a*) Silver-stained 12% SDS–PAGE gel showing molecular-weight markers (lane 1), purified full-length *M. tuberculosis* GroEL1 (lane 2) and the crystallization mother liquor (lane 3). The masses of the molecular-weight standards are given in kDa. The arrows indicate full-length GroEL1 (*A*) and the protein fragments of 43 kDa (*B*) and 23 kDa (*C*), respectively. (*b*) Coomassie-stained 15% SDS–PAGE gel showing molecular-weight markers (lane 1) and trypsin-treated GroEL1 (lane 2). The arrow indicates the 23 kDa fragment.

full-length GroEL1 in the asymmetric unit). Unfortunately, mass-spectrometric analyses of crystals by either MALDI-MS or ESI-MS were unsuccessful in identifying the mass:charge ratio of the GroEL1 fragment present in the crystal (data not shown). Analysis of the crystallization mother liquor on a silver-stained 12% SDS–PAGE gel revealed two additional bands corresponding to 43 and 23 kDa proteins as determined from their relative mobility (Fig. 2*a*, lane 3). These two protein bands were not observed in the purified full-length GroEL1 sample used in the initial crystallization setup (Fig. 2*a*, lane 2), suggesting that the full-length protein underwent proteolysis during crystallization.

To test this possibility, a limited tryptic digest of full-length GroEL1 was performed, producing a stable protein fragment of molecular weight 23 kDa as well as several other species (Fig. 2*b*, lane 2). An N-terminal sequence analysis revealed that the sequence of this 23 kDa fragment started with Val-Gly-His-Asp-Gly-Val-Val-Ser-Val-Glu, which matched the sequence of residues 167–176 of *M. tuberculosis* GroEL1. According to the relative mobility and the predicted trypsin cut sites present in *M. tuberculosis* GroEL1, the 23 kDa fragment consists of residues 167–378 of *M. tuberculosis* GroEL1, comprising the apical domain and part of the intermediate domain of GroEL1. Assuming that this fragment is present in our crystal with one molecule in the crystallographic asymmetric unit, the calculated $V_M$ would be 2.25 Å$^3$ Da$^{-1}$ (Matthews, 1968), which is reasonable.

To determine whether our crystallized fragment matched the predicted 23 kDa GroEL1 fragment, we used the corresponding fragment (residues 166–377) of *M. tuberculosis* GroEL2 (PDB code 1sjp; Qamra & Mande, 2004) as a search model for molecular replacement using *MOLREP* (Vagin & Teplyakov, 1997) from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994). A clear solution was found for one molecule in the asymmetric unit which had a correlation coefficient of 0.461 and an *R* factor of 0.556, confirming the identity of the crystallized species.

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