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Crystallization and preliminary X-ray studies of the C-terminal domain of *Mycobacterium tuberculosis* LexA

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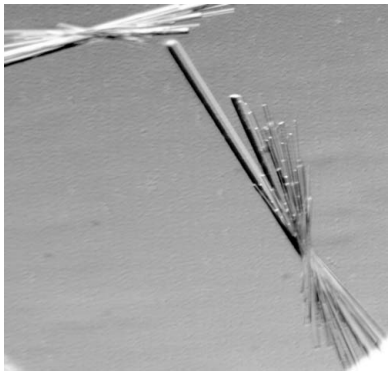


The C-terminal domain of *Mycobacterium tuberculosis* LexA has been crystallized in two different forms. The form 1 and form 2 crystals belonged to space groups $P3_121$ and $P3_1$, respectively. Form 1 contains one domain in the asymmetric unit, while form 2 contains six crystallographically independent domains. The structures have been solved by molecular replacement.

1. Introduction

In *Escherichia coli*, about 40 unlinked genes involved in DNA repair, recombination, error-prone DNA replication and cell division are negatively regulated by LexA (Butala *et al.*, 2009). Under normal conditions, LexA binds to a 20 bp stretch of DNA called an SOS box to prevent the transcription of the genes that it regulates. RecA–ssDNA–ATP filaments formed in response to DNA damage promote the cleavage of the LexA repressor, resulting in derepression of the SOS regulon. Although the nucleotide sequences of the SOS boxes are not conserved (Erill *et al.*, 2007), this phenomenon is conserved in many bacterial species (Eisen & Hanawalt, 1999). One interesting feature of LexA is that both the cleavage site and the catalytic site are present in the same molecule. The known structure of *E. coli* LexA has an amino-terminal domain which binds DNA and a carboxy-terminal domain which contains the catalytic site (Luo *et al.*, 2001). The two domains are separated by a hinge region containing the scissile bond.

In a continuation of our efforts on the structural biology of mycobacterial proteins (Datta *et al.*, 2000; Saikrishnan *et al.*, 2003; Saikrishnan, Kalapala *et al.*, 2005; Saikrishnan, Manjunath *et al.*, 2005; Singh *et al.*, 2006; Selvaraj *et al.*, 2007; Krishna *et al.*, 2007; Roy *et al.*, 2008; Prabu *et al.*, 2009; Chetnani *et al.*, 2010), in particular RecA and single-stranded DNA-binding protein (SSB), we have undertaken structural studies on *Mycobacterium tuberculosis* LexA (MtLexA). MtLexA is 217 amino acids long with a molecular weight of 23 095 Da (UniProt accession No. Q50765). It is homologous to *Escherichia coli* LexA (EcLexA), with a larger hinge region. It has been suggested that the SOS response is involved in the development of drug resistance (Cirz *et al.*, 2005) and therefore LexA has additional importance as a platform for the fight against pathogens. Here, we report the crystallization and preliminary X-ray studies of the C-terminal domain of MtLexA.

2. Materials and methods

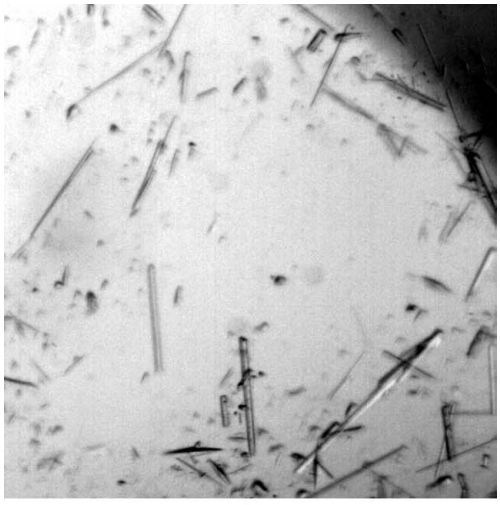
2.1. Purification of *M. tuberculosis* LexA

MtLexA was purified essentially by following the procedure described by Movahedzadeh *et al.* (1997). The cells containing the cloned LexA gene (GenBank reference X91407) were induced with 0.5 m*M* IPTG. The cells were collected by centrifugation and resuspended in 50 m*M* Tris–HCl pH 8.0 containing 20% sucrose. They were then lysed by sonication, which was followed by centrifugation. The lysate containing the His-tagged protein was loaded onto a 5 ml Ni–NTA column equilibrated with a buffer containing 20 m*M* Tris–HCl pH 8.0, 0.2 m*M* NaCl, 10% glycerol and 0.5 m*M* 2-mercapto-

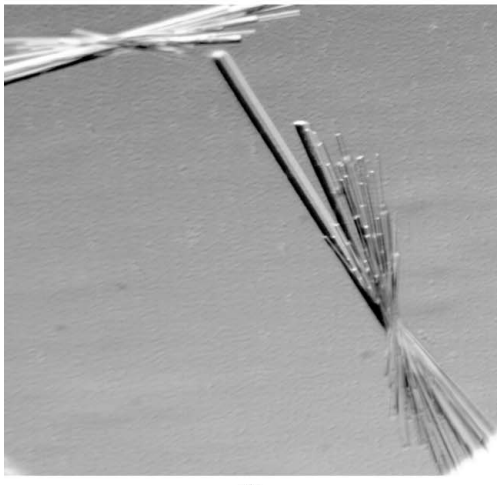
ethanol. The protein was eluted with a gradient of 20–200 m*M* imidazole. The samples were pooled, dialyzed against the same buffer containing 50% glycerol and stored at 253 K. In the protein, the MtLexA sequence was preceded by a peptide stretch with sequence MGSSHHHHHHSSGLVPRGSH. The molecular weight of the protein including this stretch is 25 259 Da.

2.2. Crystallization

Crystallization conditions were screened using commercially available screening kits from Hampton Research employing the microbatch-under-oil method and the hanging-drop vapour-diffusion method. In the microbatch experiments, diffraction-quality crystals (Fig. 1*a*) grew in about three months from a drop consisting of 2 µl 6 mg ml^{-1} protein solution in 0.1 *M* bis-tris pH 6.5, 50 m*M* sodium chloride, 5% glycerol and 1 m*M* 2-mercaptoethanol and 2 µl precipitant solution consisting of 25% polyethylene glycol 3350 in the same buffer at 298 K. The successful vapour-diffusion experiment involved a drop made up of 2 µl 6 mg ml^{-1} protein solution in 0.1 *M* Tris–HCl buffer pH 8.5, 0.2 *M* magnesium chloride, 5% glycerol and 1 m*M* 2-mercaptoethanol and 2 µl 25% polyethylene glycol 3350 in the same buffer at 298 K. The precipitant in the reservoir was 400 µl in volume. Crystals appeared in about three months (Fig. 1*b*).



(*a*)



(*b*)

Figure 1
Crystals of the C-terminal domain of MtLexA: (*a*) form 1, (*b*) form 2.

Table 1
Data-collection and processing statistics.

Values in parentheses are for the highest resolution shell.

	Form 1	Form 2
Wavelength (Å)	1.54179	1.54179
Space group	$P3_121$	$P3_1$
Unit-cell parameters (Å)	$a = b = 40.13$, $c = 116.17$	$a = b = 74.89$, $c = 116.05$
Resolution (Å)	35–2.9	30–2.75
$R_{\rm merge}$† (%)	13.4 (53.6)	13.8 (45.6)
Total No. of observations	27245 (3856)	119526 (17230)
Total No. of unique observations	2629 (356)	18928 (2768)
Mean $I/\sigma(I)$	18.6 (4.8)	14.4 (4.2)
Completeness (%)	98.9 (98.7)	99.9 (100.0)
Multiplicity	10.4 (10.8)	6.3 (6.2)
Matthews coefficient $V_{\rm M}$ (Å^3 Da^{-1})	2.58‡	2.99‡
Solvent content (%)	52	58
No. of domains in the asymmetric unit	1	6

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith observation of reflection hkl and $\langle I(hkl)\rangle$ is the weighted average intensity for all i observations of reflection hkl. ‡ For residues 120–217 of the C-terminal domain.

2.3. Data collection, processing and structure solution

Data were collected from crystals of dimensions 0.04 × 0.04 × 0.4 mm (form 1) and 0.06 × 0.06 × 0.5 mm (form 2) at 100 K (Oxford Cryosystems) on a MAR Research image plate (diameter 345 mm) mounted on a Bruker Microstar Ultra 2 rotating-anode generator. The polyethylene glycol 3350 in the precipitant solution acted as a cryoprotectant. The crystal-to-detector distance was kept at 250 and 175 mm, respectively, for crystal forms 1 and 2. An oscillation of 1° was used throughout data collection. The intensity data were processed and merged using *MOSFLM* (Leslie, 2006) and *SCALA* (Evans, 1993) in the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994). Structure-factor amplitudes were obtained from intensities using *TRUNCATE* (French & Wilson, 1978) from the *CCP*4 suite.

Data-collection statistics are given in Table 1. The solvent content of the crystals was estimated using the method of Matthews (1968). Sequence alignments prior to structure solution were performed using the *EMBOSS* tool from EBI (http://www.ebi.ac.uk/Tools/emboss/align/). *Phaser* (McCoy *et al.*, 2007) from *CCP*4 was used for structure solution employing molecular replacement. Preliminary refinement was carried out using *REFMAC* (Murshudov *et al.*, 1997) from the *CCP*4 suite.

3. Results and discussion

Symmetry and systematic absences indicated the space group of form 1 to be $P3_121$ or $P3_221$, with unit-cell parameters $a = 40.13$, $b = 40.13$, $c = 116.17$ Å. Subsequent structure solution confirmed the space group to be $P3_121$. Data from form 2 could be processed in point groups 3 and 6. Systematic absences indicated the space group to be $P3_1$, $P3_2$, $P6_2$ or $P6_4$. Eventually, the structure was solved in $P3_1$. The unit-cell parameters are $a = 74.89$, $b = 74.89$, $c = 116.05$ Å.

Calculation of the Matthews coefficient showed that the asymmetric unit of form 1 could not accommodate a full-length LexA molecule. Acceptable values for the solvent content (∼50%) could be obtained if the asymmetric unit was made up of an N-terminal or a C-terminal domain. Acceptable values for the Matthews coefficient could be obtained for two or three full-length LexA molecules in the case of form 2. LexA protein is known to undergo autocleavage at alkaline pH. Form 1 was grown at pH 6.5, while form 2 was obtained at pH 8.5. Thus, if the molecule has undergone autocleavage under

the conditions from which form 1 was obtained, it is unlikely that the molecule would remain intact in the conditions under which form 2 was grown. Polyacrylamide gel electrophoresis of the crystals gave single bands corresponding to a molecular weight of about 12 kDa. It was not possible to ascertain which of the two domains the bands correspond to as the two domains have a nearly equal molecular weight of roughly this value. However, it was clear that the crystals did not contain the full-length protein.

EcLexA and MtLexA have a sequence identity of 31%. The N-terminal and C-terminal domains of MtLexA share 24% and 37% identity, respectively, with the corresponding domains of EcLexA. Attempts to solve the structure of form 1 LexA using the N-terminal domain and the C-terminal domain of EcLexA as search models did not succeed. In the meantime, it was noticed that another protein from *E. coli* involved in the SOS response, called UmuD′ (PDB code 1umu; Peat *et al.*, 1996), has a sequence identity of 35% to the C-terminal domain of MtLexA. LexA and UmuD′ belong to the peptidase S24 family of proteins that use a common Ser-Lys mechanism of catalysis (Merops Database; Rawlings *et al.*, 2010; http://merops.sanger.ac.uk). Both proteins undergo autoproteolysis mediated by RecA active filaments. Although their sequence identity is only moderate, the overall fold and catalytic residues are conserved in these proteins. However, attempts at structure solution using the structure of UmuD′ (PDB code 1umu) were also unsuccessful. It was observed that the N-terminal region of UmuD′ did not form an integral part of the structure of the protein. Therefore, in the next set of MR calculations using *Phaser*, models starting at residue 48 were used in the search. Calculations in space group $P3_121$ then led to a unique solution with LLG = 60 and a Z score of 8.4. The structure solution obtained could be readily refined with replacement of side chains as appropriate for MtLexA. The structure of the C-terminal domain of MtLexA obtained from form 1 was used to solve the structure of form 2. MR calculations yielded a unique solution for six domains in the asymmetric unit of space group $P3_1$. Refinement of the structures is in progress. Electron-density maps calculated during the course of the refinement did not indicate the presence of any other major features, confirming that the crystals contained only the C-terminal domain.

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