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Cloning, purification, crystallization and preliminary crystallographic analysis of LsrR from *Escherichia coli*

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In *Escherichia coli*, the *lsr* operon is composed of six genes *lsrACDBFG* which regulate uptake and modification of the signalling molecule AI-2. LsrR is a repressor of the *lsr* operon and itself, which can bind phospho-AI-2 and be released from the promoter region of the operon and thus activate gene expression. LsrR fused with an HHHHHH sequence at the C-terminus was expressed, purified and crystallized in order to determine its structure and elucidate the molecular mechanism of repression. The crystal belonged to space group *I*222, with unit-cell parameters $a = 79.84$, $b = 116.65$, $c = 186.04$ Å, and was estimated to contain two protein molecules per asymmetric unit.

1. Introduction

Quorum sensing is a process that is used by bacteria to communicate with each other in order to determine the population density, triggering the expression shift of a wide range of genes. In the quorum-sensing process, small signalling molecules called autoinducers are used. Autoinducer-2 (AI-2) is a 'universal' signalling molecule with the ability to promote population-density-based gene-expression shifts in multiple species (Bassler, 1999; Miller & Bassler, 2001). In *Escherichia coli*, AI-2 is produced and taken up by cells to regulate communication between cells. Six genes, *lsrACDBFG*, involved in the import and modification of AI-2 constitute the *lsr* operon. The first four genes, *lsrACDB*, encode the ATP-binding cassette transporter responsible for the internalization of AI-2. The other two, *lsrFG*, modify AI-2 after uptake into cells (Xavier & Bassler, 2005*a*; Xavier *et al.*, 2007). The regulation of AI-2 uptake requires *lsrR* and *lsrK*. LsrR binds to the promoters of the *lsr* operon and its own promoters to repress their expression. LsrK is the kinase of AI-2, catalyzing the reaction of AI-2 and ATP to produce phospho-AI-2 and ADP (Xavier & Bassler, 2005*b*). After binding phospho-AI-2, LsrR is released from the promoters and expression of the *lsr* operon is activated. However, the binding of LsrR to promoters is unaffected by the presence of AI-2 both *in vitro* and *in vivo*. This confirms that LsrR and LsrK are important components of the regulation network of AI-2 quorum sensing. Furthermore, the DNA sequence recognized by LsrR has recently been identified (Xue *et al.*, 2009). LsrR regulates not only the expression of the *lsr* operon but also of hundreds of other genes participating in many other biological processes such as mobility, biofilm formation and so on (Li *et al.*, 2007). Serving as an important global regulator of the AI-2 quorum-sensing system, LsrR is a potential drug target for inhibiting cell–cell communication in the treatment of bacterial infections (Suga & Smith, 2003). With this aim, LsrR from *E. coli* was cloned, overexpressed, purified and crystallized.

2. Materials and methods

2.1. Cloning

The DNA of LsrR (gene ID 5594898), which contains 317 amino acids with a molecular weight of 33 825 Da, was amplified by PCR

from genomic DNA of *E. coli* using the primers 5′-CATGCCATGGGTATGACAATCAACGATTCGGCAATTT-3′ (forward) and 5′-CCGCTCGAGACTACGTAAAATCGCCGCTGCTG-3′ (reverse) (Sangon). The product was digested with *Nco*I and *Xho*I and ligated into the pET-28a vector with an HHHHHH sequence fused at the C-terminus (Novagen). The insertion of LsrR into the vector was confirmed by DNA-sequence analysis (Amphipound Biotech).

2.2. Protein expression and purification

The recombinant plasmid was transformed into *E. coli* BL21 (DE3) cells. Cells were grown at 310 K in LB medium containing 50 µg ml^{-1} kanamycin and were induced with 0.1 m*M* isopropyl β-D-1-thiogalactopyranoside (IPTG) at an $OD_{600\,nm}$ of 0.6. After 20 h induction at 289 K, the cells were harvested, resuspended in lysis buffer (50 m*M* Tris–HCl pH 8.0, 1 *M* NaCl, 1 m*M* DTT and 5% glycerol) and lysed by sonication. The lysate was centrifuged at 22 000*g* for 30 min at 277 K. The supernatant was passed over an Ni–NTA column (GE Healthcare, USA) pre-equilibrated with lysis buffer and the column was washed with 20 m*M* imidazole. The target protein was eluted with elution buffer (50 m*M* Tris–HCl pH 8.0, 1 *M* NaCl, 1 m*M* DTT, 5% glycerol and 400 m*M* imidazole). The eluate was loaded onto a Superdex 200 16/60 column (GE Healthcare, USA) pre-equilibrated with column buffer (50 m*M* Tris–HCl pH 8.0, 1 *M* NaCl, 1 m*M* DTT and 5% glycerol). The purified protein was concentrated to 5 mg ml^{-1} for further use. All steps were performed at 277 K and the homogeneity of LsrR was determined by SDS–PAGE.

2.3. Crystallization

Initial screening was performed by the sitting-drop vapour-diffusion method using Crystal Screen I and II kits from Hampton Research and Wizard I and II kits from Emerald BioSystems at 289 K. 1 µl protein solution (5 mg ml^{-1} in buffer consisting of 50 m*M* Tris–HCl pH 8.0, 1 *M* NaCl, 1 m*M* DTT and 5% glycerol) was mixed with 1 µl well solution and the mixture was equilibrated against 100 µl well solution. After several days, microcrystals appeared from condition No. 37 of Crystal Screen II [0.1 *M* HEPES pH 7.5, 10%(*w*/*v*) PEG 8000, 8%(*v*/*v*) ethylene glycol]. The crystallization condition was optimized using the hanging-drop vapour-diffusion method at 283 K. 1 µl protein solution was mixed with 1 µl well solution and the mixture was equilibrated against 500 µl well solution. After several rounds of refinement, well diffracting single crystals were obtained.

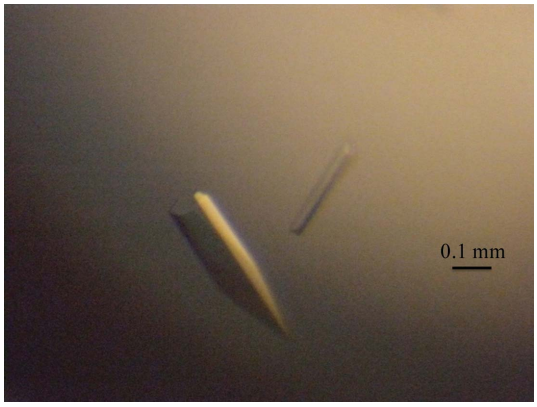

Figure 1
Crystal of LsrR.

Table 1
Crystal parameters and data-collection statistics for the LsrR crystal.

Values in parentheses are for the highest resolution shell.

No. of crystals	1
Beamline	SSRF BL17U
Wavelength (Å)	0.9795
Detector	Rayonix MX-225
Crystal-to-detector distance (mm)	250
Rotation range per image (°)	1
Total rotation range (°)	120
Exposure time per image (s)	1.2
Resolution range (Å)	44.73–3.05
Space group	*I*222
Unit-cell parameters (Å, °)	a = 78.2, b = 114.3, c = 182.2, $\alpha = \beta = \gamma$ = 90
Mosaicity (°)	0.99
Total No. of measured intensities	75340
Unique reflections	15595
Multiplicity	4.8 (4.9)
Mean $I/\sigma(I)$	10.4 (2.4)
Completeness (%)	98.2 (98.2)
R_{merge}† (%)	9.5 (55.2)
R_{meas} or $R_{r.i.m.}$‡ (%)	10.6 (61.6)
Overall B factor from Wilson plot (Å^2)	92.1

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith observation of reflection hkl and $\langle I(hkl)\rangle$ is the weighted average intensity of all observations i of reflection hkl. ‡ $R_{meas} = R_{r.i.m.} = \sum_{hkl}[N/(N-1)]^{1/2}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the ith observation of reflection hkl and $\langle I(hkl)\rangle$ is the weighted average intensity of all observations i of reflection hkl. N is the multiplicity.

2.4. Diffraction data collection and processing

The crystals of LsrR were harvested and soaked in a cryoprotectant solution consisting of 0.1 *M* HEPES pH 8.0, 6% PEG 8000, 8% ethylene glycol and 10% glycerol for several seconds. The crystal was flash-cooled in liquid nitrogen and used for X-ray diffraction data collection using synchrotron radiation at 100 K on beamline BL17U at SSRF (Shanghai). A complete diffraction data set consisting of 120 diffraction images was collected from one crystal with an oscillation angle of 1° per image. Diffraction data were indexed, integrated and

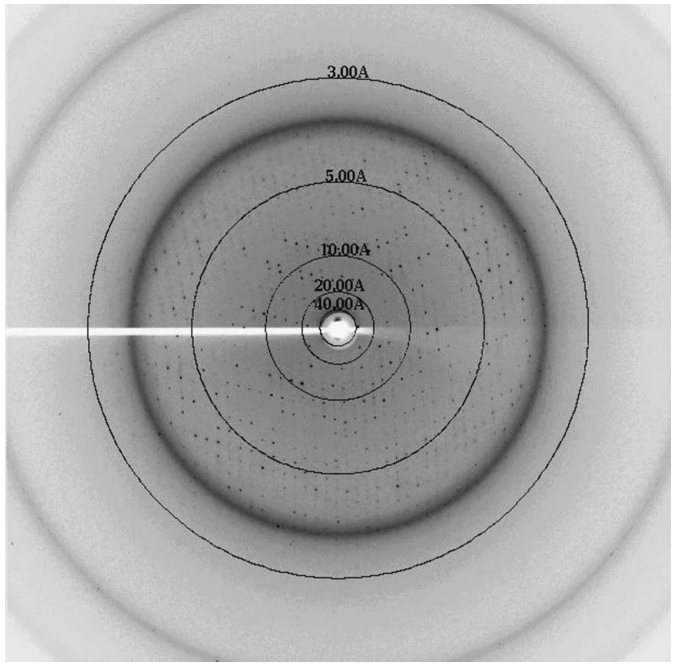

Figure 2
X-ray diffraction map obtained from the LsrR crystal.

scaled using the program *MOSFLM* (Leslie, 1992). Data-collection and processing statistics are listed in Table 1.

3. Results and discussion

The purified LsrR was concentrated to 5 mg ml^{-1} for initial crystal screening experiments. Several days after the crystallization experiments had been set up, microcrystals appeared in a condition consisting of 0.1 *M* HEPES pH 7.5, 10%(*w*/*v*) PEG 8000, 8% ethylene glycol. The crystals obtained from this condition were needle-like and diffracted X-rays poorly. The initial conditions were optimized in order to yield crystals that were suitable for X-ray diffraction. After several rounds of refinement, large crystals appeared at 283 K in a condition consisting of 0.1 *M* HEPES pH 8.0, 6%(*w*/*v*) PEG 8000, 8% ethylene glycol. Typical crystal dimensions were 0.10 × 0.10 × 0.40 mm (Fig. 1) and the crystals diffracted to about 3 Å resolution (Fig. 2). The crystals belonged to space group *I*222. The calculated Matthews coefficient (V_M) of 3.01 Å^3 Da^{-1}, with a solvent content of 59.13%, suggests the presence of two molecules per asymmetric unit (Matthews, 1968). Based on sequence alignment, LsrR has 43% sequence homology to the sorbitol operon regulator SorC. The structure of LsrR will be solved by the molecular-replacement method using the structure of SorC (PDB code 2w48; De Sanctis *et al.*, 2009) as a template.

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