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Crystallization and preliminary crystallographic studies of the D59A mutant of MicA, a YycF response-regulator homologue from *Streptococcus pneumoniae*

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RR02 (MicA) is an essential bacterial protein that belongs to the YycF family of response regulators and consists of two domains: an N-terminal receiver domain and a C-terminal effector domain. *Streptococcus pneumoniae* RR02 (MicA; residues 2–234) has been crystallized using the sitting-drop vapour-diffusion technique. The crystals belong to space group $P2_1$, with unit-cell parameters $a = 46.46$, $b = 32.61$, $c = 63.35$ Å, $\beta = 90.01^\circ$. X-ray diffraction data have been collected to 1.93 Å resolution.

1. Introduction

Streptococcus pneumoniae is a bacterial pathogen responsible for diseases such as pneumonia, bacteraemia and meningitis and is a leading cause of illness and death in infants, the elderly and immunocompromised patients (AlonsoDeVelasco *et al.*, 1995). Many strains of *S. pneumoniae* are becoming resistant to a wide range of antibiotics, including vancomycin and erythromycin, and new targets for antimicrobial agents are being sought.

Among such targets are the two-component signal-transduction systems, 13 of which exist in *S. pneumoniae* (Lange *et al.*, 1999). The majority of two-component systems (TCS) consist of two distinct protein components: a histidine protein kinase (HPK) and a cytoplasmic response regulator (RR). The latter has two distinct domains: a receiver domain, which accepts the phosphoryl group from the HPK on an aspartic acid, and a DNA-binding domain, which controls gene expression (Stock *et al.*, 1995). RR02 (MicA), a 26 kDa response-regulator protein, has been shown to be essential for bacterial growth. Knockout mutations in these genes lead to non-viable bacterial strains, thus making this a prime target for new antibacterial agent design (Brown *et al.*, 2000; Lange *et al.*, 1999; Throup *et al.*, 2000). It has been shown that the two-component signal-transduction system HK02/RR02 is involved in competence repression under oxygen-limiting environments and was thus named Mic (microareobiosis control; Echenique & Trombe, 2001). It was proposed that the histidine kinase HK02 system contains a PAS domain, classically involved in redox reactions and in the protection of the cell against oxidative stress, in particular by repression of competence (Echenique *et al.*, 2000). Under oxygen-limiting conditions, the HK02 histidine kinase sensor phosphorylates RR02 (MicA) on a highly conserved aspartate residue in the presence of magnesium ions, which in turn regulates the transcription of ComCDE (Echenique & Trombe, 2001) by an uncharacterized mechanism. The transfer of a phosphate group from HK02 to both the wild-type and mutant proteins was possible. However, mutation of Asp59 to Ala59 appears to destabilize the bond between Asp52 and phosphate: in the wild-type protein the half-life of RR02-PO_4 was 20 min, whilst for the mutant (RR02-D59A-PO_4) the half-life was 12 min (Echenique & Trombe, 2001).

In this paper, we report the cloning, purification, crystallization and preliminary crystallographic analysis of the RR02-D59A (MicA-D59A) mutant from *S. pneumoniae*.

2. Materials and methods

2.1. Protein expression and purification

The DNA coding for residues 2–234 of the mutated response regulator RR02 (MicA) was cloned into the pET33b vector (Novagen), which utilizes the T7lac promoter to drive recombinant protein overexpression (Echenique & Trombe, 2001). Expression plasmids were transformed into *Escherichia coli* BL21 (DE3) host strain (Stratagene) using a heat-shock technique. Several 1 l batches of LB-enriched growth media (Sigma) containing 100 μg ml^{-1} ampicillin were inoculated with 10 ml of an overnight culture. The cultures were shaken at 200 rev min^{-1} at 310 K until an OD_{600} of 0.5 was reached. IPTG (Melford) was then added to a final concentration of 1 m*M* in each culture to initiate overexpression of the RR02 (MicA) mutant. After induction, cells were allowed to grow for an additional 3 h at 310 K, harvested by spinning the cells at 5000*g* for 15 min at 277 K and resuspended in a buffer containing 25 m*M* Tris pH 7.5, 4 m*M* imidazole and 200 m*M* NaCl. Cells were lysed by sonication on ice (6 × 30 s) and centrifuged at 30 000*g* for 1 h to remove the insoluble fraction. The RR02 (MicA) mutant was puri-

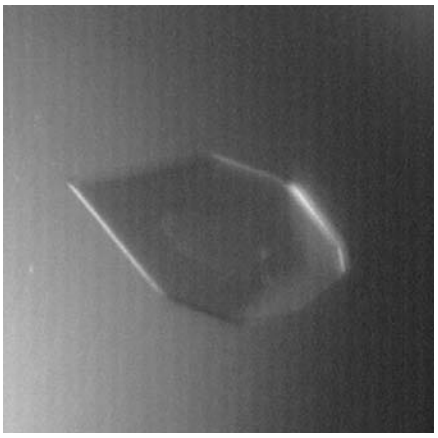

Figure 1
Single crystal of RR02-D59A grown from condition No. 23 of the PEG/Ion screen (Hampton Research).

fied by Ni-affinity chromatography. The protein was loaded slowly (overnight at 277 K) onto a column previously equilibrated with buffer *A* (4 m*M* imidazole, 25 m*M* Tris, 200 m*M* NaCl pH 7.5) before elution at room temperature using a linear gradient of 0–100% buffer *B* (1 *M* imidazole, 25 m*M* Tris, 200 m*M* NaCl pH 7.5). The protein was further purified by gel filtration using a Superdex-75 column previously equilibrated in buffer *C* (25 m*M* Tris, 300 m*M* NaCl pH 7.5). Fractions containing the pure His-tagged protein were concentrated to between 5 and 10 mg ml^{-1} in an Amicon pressure cell before being used in crystallization trials.

2.2. Crystallization and data collection

Trials were set up using sitting-drop and hanging-drop vapour-diffusion techniques at both 277 and 293 K. The protein was mixed with the reservoir solution in a 1:1 ratio to produce 4 or 6 µl drops. Crystals unsuitable for diffraction were observed in a number of conditions after several days. A single crystal was observed after six months in condition No. 23 of the PEG/Ion screen; the reservoir contained 200 m*M* ammonium formate, 20%(*w*/*v*) PEG 3350 pH 6.6 (Fig. 1). Owing to the length of time taken to grow the initial crystal, no optimization was possible prior to data collection. The crystal was flash-cooled using dried paraffin oil as the cryoprotectant (Riboldi-Tunnicliffe & Hilgenfeld, 1999) and an almost complete data set was collected from a native crystal (Fig. 1), which diffracted to beyond 2.0 Å on beamline XRD-1 at ELETTRA, Trieste with a fixed wavelength of 1.0 Å. Data were collected at a detector distance of 140 mm from the crystal, with each frame being 1° in width. Owing to a fall in the intensity of the beam during data collection, the data were collected in three batches. Data were processed to a maximum resolution of 1.93 Å using *MOSFLM* (v.6.2.2; Leslie, 1992) and scaled using *SCALA* (Evans, 1993) and *TRUNCATE* (Collaborative Computational Project, Number 4, 1994). A summary of the crystallization conditions and X-ray data-collection statistics is given in Table 1.

Table 1
Data-collection statistics for RR02-D59A.

Values in parentheses are for the highest resolution shell.

Source	XRD-1, ELETTRA, Trieste
Wavelength (Å)	1.0
Resolution (Å)	63.0–1.93
Space group	$P2_1$
Unit-cell parameters (Å, °)	a = 46.46, b = 32.61, c = 63.35, β = 90.01
Molecules per AU	1
Solvent content (%)	36.05
$R_{\rm sym}$	0.087 (0.361)
$\langle I/\sigma(I)\rangle$	5.3 (2.0)
Total No. reflections	152223
No. unique reflections	14491
Average redundancy	5.1 (4.2)
Completeness (%)	98.7 (95.4)

3. Results and discussion

Crystals of RR02 (MicA) measured 0.1 × 0.1 × 0.08 mm (Fig. 1) and diffracted to 1.93 Å resolution. With one monomer in the asymmetric unit, the determined Matthews coefficient is 1.94 Å^3 Da^{-1} (Matthews, 1968) and the solvent content is 36.05%. A data set with a completeness of 98.7% and an $R_{\rm sym}$ of 0.087% was collected (full data-processing statistics can be found in Table 1).

As the β angle is very close to 90°, the data were processed in several space groups including the orthorhombic space groups $P222$, $P222_1$, $P2_12_12$, $P2_12_12_1$, $C222$ and $C222_1$ and the primitive monoclinic space groups $P2$ and $P2_1$. The merging statistics after *SCALA* (Evans, 1993) were much better in $P2_1$ than for the other space groups. Attempts are now under way to determine the structure by molecular replacement using as starting models RR02-Rec determined recently in our laboratory and the *E. coli* OmpR effector domain (Kondo *et al.*, 1997; PDB code 1odd).

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