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Crystallization and preliminary X-ray diffraction analysis of full-length spr1814, a response regulator of *Streptococcus pneumoniae*, in complex with a phosphoryl analogue

Spr1814 of *Streptococcus pneumoniae* is a signal transduction response regulator belonging to the NarL/FixJ subfamily, which has a helix–turn–helix DNA-binding effector domain. To understand how the phosphorylation of the conserved aspartic acid residue induces conformational changes in spr1814 allowing binding to the target promoter, recombinant spr1814 expressed in *Escherichia coli* was crystallized with the phosphoryl analogue beryllium fluoride BeF₃[−] by the hanging-drop vapour-diffusion method. The crystals diffracted to 1.9 Å resolution and belonged to space group *P*2₁, with unit-cell parameters *a* = 40.2, *b* = 114.5, *c* = 50.1 Å, β = 92.1°. Structure determination by the SAD method using the bromine derivative 5-amino-2,4,6-tribromoisophthalic acid (B3C) is under way.

1. Introduction

Two-component systems (TCS) are regulatory mechanisms that are ubiquitous among bacteria (Parkinson & Kofoed, 1992; Stock *et al.*, 1989). These systems regulate many processes, including cell–cell communication, adaptation to the environment and, in the case of pathogens, pathogenesis (Stock *et al.*, 2000). Because of their absence in humans and other mammals, TCS proteins are considered to be suitable targets for the development of new antibiotics. In their simplest form, TCS consist of two modular proteins: a sensory histidine kinase (HK) and a response regulator (RR) (Parkinson & Kofoed, 1992). The HK senses specific environmental signals which ultimately lead to activation of the kinase activity and autophosphorylation of a conserved histidine residue (Gao & Stock, 2009). The phosphoryl group is subsequently transferred to a conserved aspartic acid residue in the receiver domain of the cognate RR for activation. Most RRs are transcription factors which activate or repress a particular set of genes (Gao & Stock, 2009).

Spr1814, the RR studied here, consists of two domains: a conserved N-terminal receiver domain and a C-terminal effector domain which can bind to DNA. According to the structure of the DNA-binding domain, spr1814 belongs to the NarL/FixJ subfamily, which is characterized by a helix–turn–helix (HTH) DNA-binding domain (Baikalov *et al.*, 1996; Kurashima-Ito *et al.*, 2005). Structural information regarding RRs belonging to the NarL/FixJ subfamily has suggested that they may be activated by domain rearrangements, disrupting the interdomain contacts, upon phosphorylation (Hoch, 2000; Baikarov *et al.*, 1996). The conformational change induced by phosphorylation results in dimerization of the RR and binding of the dimer to the promoter of target genes.

Our previously reported structure of the full-length unphosphorylated spr1814 (PDB entry 4hye, Park *et al.*, 2013) shows that there are two monomers displaying distinct conformations in the asymmetric unit. While one monomer adopts an inactive conformation, the other exhibits an intermediate conformation. The receiver domain of the monomer in the inactive conformation showed two conserved switch residues, Thr81 and Tyr100, oriented away from the phosphorylation site. On the other hand, the active-like conformation was observed in the intermediate state, in which the two residues point toward the phosphorylation site. In terms of domain contacts, when in the inactive state the receiver and effector domain display interdomain contacts resulting in inhibition of the binding of the

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effector domain to its target promoter, while the effector domain was somewhat released from the receiver domain in the intermediate state. This movement of the effector domain in the intermediate state was achieved by a flexible linker region which connects the two domains and is made up of an extended loop and short α -helix.

The fact that the linker region plays an important role in domain reorganization was further supported by an earlier structural study of the receiver domain of spr1814 in the presence and absence of the phosphoryl analogue beryllium fluoride BeF_3^- (PDB entries 4e7o and 4e7p), which demonstrated conformational changes of the linker region upon phosphorylation (Park *et al.*, 2012). However, to understand how these conformational changes allow RRs to bind to and regulate the target promoter, it is necessary to determine the full-length structure of phosphorylated RRs.

Here, we report the crystallization and preliminary X-ray crystallographic analysis of full-length spr1814 from *Streptococcus pneumoniae* in complex with the phosphoryl analogue BeF_3^- (Yan *et al.*, 1999), providing a first step towards the elucidation of the regulatory mechanism employed by spr1814. The experimental phasing was obtained by single-wavelength anomalous dispersion (SAD) using 5-amino-2,4,6-tribromoisophthalic acid (B3C; Beck *et al.*, 2009). B3C contains three functional groups (two carboxylate groups and one amino group) that interact with proteins *via* hydrogen bonds, and three Br atoms suitable for anomalous dispersion phasing (Beck *et al.*, 2010). The B3C molecules bound to the proteins provide a strong anomalous signal which can be used for MAD (multiwavelength anomalous diffraction) phasing (Beck *et al.*, 2010).

2. Materials and methods

2.1. Protein expression and purification

Full-length spr1814 (formerly known as DesR) was overexpressed and purified using previously described methods (Park *et al.*, 2009). Briefly, the full-length spr1814 gene (UniProtKB/TrEMBL accession No. Q8DNC2) was amplified from *S. pneumoniae* genomic DNA by polymerase chain reaction (PCR). Recombinant plasmid bearing the spr1814 gene was transformed into the *Escherichia coli* BL21 (DE3)

strain and the cells were grown in Luria–Bertani medium. Expression was then induced with 0.2 mM isopropyl β -D-1-thiogalactopyranoside (IPTG). After centrifugation, the cell pellets were resuspended in buffer A (20 mM Tris–HCl pH 7.9, 500 mM NaCl, 5 mM imidazole) and lysed by sonication. The supernatant was loaded onto an Ni^{2+} -chelated HiTrap chelating HP column (GE Healthcare, USA) and the proteins were eluted with a linear gradient of buffer A containing 1 M imidazole. The protein was further purified by gel filtration on a HiLoad 16/60 Superdex 200 column (GE Healthcare, USA) which was pre-equilibrated with buffer B (20 mM Tris–HCl pH 7.9, 200 mM NaCl, 5 mM MgCl_2 , 2 mM DTT, 10% glycerol). The purified protein was concentrated to 18 mg ml⁻¹ using an Amicon Ultra-15 ultra-filtration device (Millipore, USA), yielding 20 mg protein per litre of medium (Fig. 1).

2.2. Crystallization

For co-crystallization with BeF_3^- , the protein was incubated with 30 mM NaF and 5 mM BeCl_2 for 30 min on ice and then centrifuged at 20 000g for 10 min to remove the precipitated protein. The protein concentration was 15 mg ml⁻¹ after centrifugation. Initial crystallization screening was performed in 96-well Intelli-Plates (Art Robbins Instruments, USA) at 295 K by the sitting-drop vapour-diffusion method (0.5 μ l protein solution and 0.5 μ l reservoir solution equilibrated against 70 μ l reservoir solution), which was performed by hand using electronic pipettes. Commercial screening kits such as

Figure 1

Expression and purification of spr1814 protein as judged by SDS–PAGE. Lane M, molecular-mass protein marker (labelled in kDa); lane 1, uninduced cell lysate; lane 2, induced cell lysate; lane 3, purified spr1814 protein.

(a)

(b)

Figure 2

Crystals of the spr1814 protein from *S. pneumoniae*. (a) BeF_3^- -complexed crystals. (b) BeF_3^- -B3C-spr1814 ternary-complex crystals. The crystal sizes of the BeF_3^- and ternary-complex crystals were $0.1 \times 0.1 \times 0.3$ and $0.2 \times 0.1 \times 0.6$ mm, respectively.

Table 1

Data-collection statistics.

Values in parentheses are for the highest resolution shell.

	BeF ₃ ⁻ -complexed crystal	BeF ₃ ⁻ -B3C-spr1814 ternary-complex crystals
Space group	<i>P</i> 2 ₁	<i>P</i> 2 ₁
Unit-cell parameters (Å, °)	<i>a</i> = 40.2, <i>b</i> = 114.5, <i>c</i> = 50.1, β = 92.1	<i>a</i> = 40.9, <i>b</i> = 115.5, <i>c</i> = 50.1, β = 92.4
Resolution range (Å)	50.0–1.9 (1.97–1.90)	50.0–2.0 (2.03–2.00)
Total reflections	237789	156743
Unique reflections	35851	29492
Completeness (%)	97.6 (94.8)	96.7 (78.0)
<i>R</i> _{merge} † (%)	5.4 (22.6)	9.1 (25.9)
Multiplicity	6.6 (5.0)	5.4 (3.0)
<i>I</i> /σ(<i>I</i>)	24.2 (4.1)	4.7 (28.7)

† $R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ represents the observed intensity, $\langle I(hkl) \rangle$ represents the average intensity and i counts through all symmetry-related reflections.

Crystal Screen, Crystal Screen 2, PEG/Ion, PEG/Ion 2, PEGRx 1, PEGRx 2, Index (Hampton Research, USA) and Morpheus (Molecular Dimensions, UK) were utilized. Initial crystals were obtained using the PEGRx 1 screen from Hampton Research [0.1 *M* sodium citrate tribasic dihydrate pH 5.0, 30% (v/v) Jeffamine ED-2001 pH 7.0]. However, processing of the diffraction data acquired from the crystals was problematic because of twinning. In order to optimize the crystals, additive screening was carried out using Additive Screen (Hampton Research, USA). For the additive screen, 0.2 μl of the additives were added to 1 μl protein solution and 1 μl reservoir solution and the drops were equilibrated against 500 μl reservoir solution in 24-well VDX hanging-drop plates. After the first round of the additive screen, additive 31 [30% (w/v) 1,5-diaminopentane dihydrochloride] was found to improve the crystal morphology, but the crystals formed were still twinned. For further optimization, 3% (w/v) 1,5-diaminopentane dihydrochloride was added to the protein solution, along with 30 mM NaF and 5 mM BeCl₂. The original additive screen was then repeated. Additive 85 [10% (v/v) 1,2-butanediol] yielded suitable crystals for collection of the diffraction data within one week under the following conditions: 0.1 *M* sodium citrate tribasic dihydrate pH 4.7, 18% (v/v) Jeffamine ED-2001 pH 7.0 (Fig. 2a).

For SAD phasing, a 1 *M* stock solution of B3C was obtained by dissolving the solid material in 2 *M* lithium hydroxide solution (Beck *et al.*, 2010). The B3C stock solution was added to the protein solution to produce a final concentration of 0.2 *M* B3C along with 30 mM NaF, 5 mM BeCl₂, 3% (w/v) 1,5-diaminopentane dihydrochloride. The incorporation of the B3C molecule in the proteins allowed the formation of single crystals without a second additive screen. Crystals were obtained under the same conditions used to produce the BeF₃⁻ complex crystals (Fig. 2b).

2.3. X-ray data collection

Both the BeF₃⁻ and BeF₃⁻-B3C-spr1814 ternary-complex crystals were cryoprotected by the introduction of 20% (v/v) ethylene glycol. X-ray diffraction data for the BeF₃⁻ complex crystals were collected at a resolution of 1.9 Å using an ADSC Quantum 210 CCD detector on beamline AR-NW12 at the Photon Factory, Tsukuba, Japan. The wavelength of the synchrotron X-rays was 1.0000 Å. For the BeF₃⁻-B3C-spr1814 ternary-complex crystals, X-ray diffraction data were collected at a resolution of 2.0 Å using an ADSC Quantum 210 CCD detector on beamline 7A at the Pohang Light Source, Pohang, Republic of Korea. The subsequent SAD data were collected at the peak wavelength (λ = 0.9198 Å) of the Br K-edge absorption profile.

A total range of 360° was covered with 1.0° oscillation for both crystals. All data sets were indexed, processed and scaled using the *HKL*-2000 software package (Otwinowski & Minor, 1997). Table 1 provides a summary of the data-collection statistics.

3. Results and discussion

The full-length spr1814 was successfully cloned, overexpressed and obtained at high purity by affinity and size-exclusion chromatography. To obtain spr1814 crystals in the phosphorylated state, crystallization trials were carried out in which spr1814 was complexed with the phosphoryl analogue beryllium fluoride BeF₃⁻. Crystals of spr1814 complexed with BeF₃⁻ were obtained after subsequent additive screening and they diffracted to 1.9 Å resolution. Initial diffraction analysis of the optimized crystals showed that they belonged to space group *P*2₁, with unit-cell parameters *a* = 40.2, *b* = 114.5, *c* = 50.1 Å, β = 92.1°. The Matthews coefficient (Matthews, 1968) calculated for a crystal containing a dimer in the asymmetric unit was 2.59 Å³ Da⁻¹, with a solvent content of 52.47%.

All attempts to solve the structure by the molecular-replacement method using the full-length structure of unphosphorylated spr1814 (PDB entry 4hye; Park *et al.*, 2013) as a search model failed. In addition, the selenomethionine-substituted protein did not provide crystals of sufficient quality to allow the collection of diffraction data. In order to solve the phase problem by the single-wavelength anomalous diffraction method, 5-amino-2,4,6-tribromoisophthalic acid (B3C; Beck *et al.*, 2009) was used for co-crystallization with the proteins and the structure is currently being refined. Initial diffraction analysis of the BeF₃⁻-B3C-spr1814 ternary-complex crystals showed that they belonged to space group *P*2₁, with unit-cell parameters *a* = 40.9, *b* = 115.5, *c* = 50.1 Å, β = 92.4°. Assuming the presence of a dimer in the asymmetric unit in the crystals, the Matthews coefficient (Matthews, 1968) was calculated to be 2.66 Å³ Da⁻¹, with a solvent content of 53.67%.

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References

- Baikalov, I., Schröder, I., Kaczor-Grzeskowiak, M., Grzeskowiak, K., Gunsalus, R. P. & Dickerson, R. E. (1996). *Biochemistry*, **35**, 11053–11061.
- Beck, T., Gruene, T. & Sheldrick, G. M. (2010). *Acta Cryst.* **D66**, 374–380.
- Beck, T., Herbst-Irmer, R. & Sheldrick, G. M. (2009). *Acta Cryst.* **C65**, o237–o239.
- Gao, R. & Stock, A. M. (2009). *Annu. Rev. Microbiol.* **63**, 133–154.
- Hoch, J. A. (2000). *Curr. Opin. Microbiol.* **3**, 165–170.
- Kurashima-Ito, K., Kasai, Y., Hosono, K., Tamura, K., Oue, S., Isogai, M., Ito, Y., Nakamura, H. & Shiro, Y. (2005). *Biochemistry*, **44**, 14835–14844.
- Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
- Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
- Park, A. K., Bong, S. M., Moon, J. H. & Chi, Y. M. (2009). *Acta Cryst.* **F65**, 727–729.
- Park, A. K., Moon, J. H., Lee, K. S. & Chi, Y. M. (2012). *Biochem. Biophys. Res. Commun.* **421**, 403–407.
- Park, A. K., Moon, J. H., Oh, J. S., Lee, K. S. & Chi, Y. M. (2013). *Biochem. Biophys. Res. Commun.* **434**, 65–69.
- Parkinson, J. S. & Kofoed, E. C. (1992). *Annu. Rev. Genet.* **26**, 71–112.
- Stock, A. M., Robinson, V. L. & Goudreau, P. N. (2000). *Annu. Rev. Biochem.* **69**, 183–215.
- Stock, J. B., Ninfa, A. J. & Stock, A. M. (1989). *Microbiol. Rev.* **53**, 450–490.
- Yan, D., Cho, H. S., Hastings, C. A., Igo, M. M., Lee, S.-Y., Pelton, J. G., Stewart, V., Wemmer, D. E. & Kustu, S. (1999). *Proc. Natl Acad. Sci. USA*, **96**, 14789–14794.