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Crystal structure of receiver domain of putative NarL family response regulator spr1814 from *Streptococcus pneumoniae* in the absence and presence of the phosphoryl analog beryllofluoride

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ABSTRACT

Spr1814 of *Streptococcus pneumoniae* is a putative response regulator (RR) that has four-helix helix-turn-helix DNA-binding domain and belongs to the NarL family. The prototypical RR contains two domains, an N-terminal receiver domain linked to a variable effector domain. The receiver domain functions as a phosphorylation-activated switch and contains the typical doubly wound five-stranded α/β fold. Here, we report the crystal structure of the receiver domain of spr1814 (spr1814$_R$) determined in the absence and presence of beryllofluoride as a phosphoryl analog. Based on the overall structure, spr1814$_R$ was shown to contain the typical fold similar with other structures of the receiver domain; however, an additional linker region connecting the receiver and DNA-binding domain was inserted into the dimer interface of spr1814$_R$, resulting in the formation of unique dimer interface. Upon phosphorylation, the conformational change of the linker region was observed and this suggests that domain rearrangement between the receiver domain and effector domain could occur in full-length spr1814.

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1. Introduction

Bacteria sense and respond to a wide variety of signals through a complex network of signaling systems, many of which are two-component phosphotransfer pathways. These systems regulate many processes, including nutrient uptake, sporulation, chemotaxis, virulence, quorum sensing, and cell adhesion [1]. In the simplest form, a two-component system consists of two modular proteins, a sensory histidine kinase (HK) and a response regulator (RR) [2]. The HK phosphorylates at a specific His residue by itself, then the phosphoryl group is transferred to a conserved Asp residue in the N-terminal regulatory domain of the RR. The phosphorylation event induces the activation of the C-terminal effector domain of the RR, which binds to specific DNA sequences in most two-component systems, and regulates transcriptional initiation [3]. The transcription factor RRs are further divided into subfamilies, including the OmpR/PhoB winged-helix domain [4,5], the NarL/FixJ four-helix helix-turn-helix domain [6,7], the NtrC/DctD AAA + ATPase domain fused to a factor of inversion (Fis)-type helix-turn-helix domain [8], and the recently characterized LytTR domain, which contains an unusual, predominantly β fold [9]. In diverse RRs, different types of conformational change are found to occur upon phosphorylation. Phosphorylation can promote the dimerization (StyR [10] and PhoB [11]), and induce a higher oligomerization state (NtrC [12]). Moreover, it has been suggested that the NarL/FixJ family is activated by phosphorylation-induced domain rearrangements disrupting the inter-domain contacts [13,14].

The spr1814 from *Streptococcus pneumoniae* belongs to the NarL/FixJ family based on similarities of the effector domains, and it has been predicted to regulate the neighboring ABC transporter, which regulates antibiotic transport with its cognate histidine kinase. The structures of receiver domains have been solved for several RRs and the structures exhibit a doubly wound five-stranded α/β fold. The conserved Asp residue located in the C-terminal edge of the β3 strand is the phosphorylation site of the receiver domain and the phosphoryl group is stabilized by the divalent cation, Mg^{2+}.

In this report, we present the crystal structures of the receiver domain of response regulator spr1814 from *Streptococcus pneumoniae* (spr1814$_R$) complexed with and without the phosphoryl analog beryllofluoride (BeF_3^-) at a resolution of 2.2 and 1.9 Å, respectively. Although the structure of spr1814$_R$ had a similar overall fold with other receiver domain structures, there was a unique additional linker region (residues 121–130) connecting the receiver and effector domains. The comparison between

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Table 1
Data collection and refinement statistics.

	Native	BeF_3^- complex
Data collection		
Space group	$P2_1$	$P2_12_12_1$
Cell dimension (Å)	a = 52.87, b = 44.95, c = 59.3, β = 98.98°	a = 75.84, b = 83.98, c = 49.51
Resolution range (Å)	50–2.2 (2.20–2.24)	50–1.9 (1.90–1.97)
Total reflections	68.349	248.110
Unique reflections	13.021	25.704
Redundancy	5.2 (3.0)	9.7 (5.9)
Completeness (%)	91.6 (64.6)	99.6 (98.4)
[a] R_{merge} (%)	4.0 (12.3)	8.4 (32.5)
<I/σ(I)>	47.5 (6.9)	19.0 (2.5)
Refinement		
[b] R/R_{free} (%)	21.4/27.3	18.6/22.6
RMS deviation of		
Bond length (Å)	0.009	0.008
Bond angle (°)	1.2	1.1
No. of atoms		
Protein/waters	261/33	262/274
In Ramachandran plot		
Most favored (%)	95.3	97.3
Additionally allowed (%)	3.9	1.9

Values in parentheses are for the highest resolution shells.
[a] $R_{merge} = \sum_{hkli}|I_{hkli} - \langle I_{hkl}\rangle|/\sum_{hkli} I_{hkli}$, where I represents the observed intensity,$\langle I\rangle$ represents the average intensity, and i counts through all symmetry-related reflections.
[b] $R = \sum||F_{obs}| - |F_{calc}||/\sum|F_{obs}|$, where R_{free} is calculated for a randomly chosen 5% of reflections, which were not used for structure refinement.

inactivated and activated states of $spr1814_R$ shows the structural perturbations at the dimer interface, including linker region, and this result indirectly indicates that rearrangement between two domains could be triggered to activate the effector domain of spr1814.

2. Materials and methods

2.1. Cloning, overexpression and purification

Residues 1–130 of spr1814 were cloned from *S. pneumoniae* genomic DNA by the polymerase chain reaction (PCR) and inserted into the NdeI/XhoI-digested expression vector, pET28a vector (Novagen). The expression construct introduced a hexa-histidine tag at the N-terminus. The constructed plasmid was transformed into *Escherichia coli* strain BL21 (DE3) cells for expression. Transformed cells were cultured in Luria–Bertani (LB) medium containing 50 μg ml^{-1} kanamycin at 37 °C. Protein expression was induced by the addition of 0.5 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG) once the cells had reached an optical density at 600 nm of about 0.45. The cells were then grown for an additional 12 h at 18 °C and harvested. Cells were resuspended in buffer A (20 mM Tris–HCl, pH 7.9, 500 mM NaCl, 5 mM imidazole) and lysed by sonication. Lysate was centrifuged for 45 min at 20,000*g*. the soluble fraction was loaded onto a Ni^{2+}-charged chelated HiTrap chelating HP column (GE Healthcare) equilibrated with buffer A. The protein was eluted with a linear gradient of buffer A containing 1 M imidazole. The protein was purified to its final state by gel filtration on a HiLoad 16/60 Superdex 200 column (GE Healthcare,) that had previously been equilibrated with buffer B (20 mM Tris–HCl, pH 7.9, 200 mM NaCl, 5 mM $MgCl_2$, 10% glycerol). The protein was finally concentrated using an Amicon Ultra-15 ultrafiltration device (Millipore) to 10 mg ml^{-1}. The protein concentration was determined using a Bradford assay.

2.2. Crystallization, data collection and structure determination

$Spr1814_R$ was subjected to screening for crystal growth using the sitting-drop vapor-diffusion method (0.5 μl protein solution and 0.5 μl reservoir solution equilibrated against 50 μl reservoir solution). All crystallization trials were performed at 22 °C. Crystals were obtained in buffers consisting of 0.1 M Tris–HCl, pH 8.0 and 28% PEG 4000. Crystal growth was scaled up using the hanging-drop vapor-diffusion method in 24-well VDX plates (Hampton Research, USA). Each hanging drop was prepared by mixing 1 μl protein solution and 1 μl reservoir solution and equilibrated over a 500 μl reservoir solution. For cocrystallization with BeF_3^- the protein solution was mixed with 30 mM NaF and 5 mM $BeCl_2$. The BeF_3^- complex crystals were obtained under the same conditions that produced native crystals.

X-ray diffraction data were collected on beamline 6C at the Pohang Light Source (Pohang, South Korea). Both crystals were cryo-protected by the introduction of 20% (v/v) ethylene glycol, and frozen in liquid nitrogen for data collection. X-ray diffraction data were collected to a resolution of 2.2 Å from native crystals and 1.9 Å from BeF_3^- complex crystals. All data sets were indexed, processed and scaled using the HKL-2000 software package [15].

The crystal structure was solved by the molecular-replacement method using the *MOLREP* program in the *CCP4* package [16] with the putative DNA binding response regulator of *Staphylcoccus aureus* (PDB ID: 3B2N) [17] as a search model. The space group of native structure was a monoclinic $P2_1$ and the space group of $BeFk_3^-$ complex structure was $P2_12_12_1$. The refinement was performed by *phenix.refine* [18] and the model was rebuilt with the *Coot* [19]. The final model of the native structure contained one dimer and two Mg^{2+} ions and the final R_{cryst} and R_{free} values were 21.4% and 27.3%, respectively. The final model of the BeF_3^- complex structure also contained one dimer, two Mg^{2+} ions, and two BeF_3^- and the final R_{cryst} and R_{free} values were 18.6% and 22.6%, respectively. The stereochemical quality of all the final models as assessed by *PROCHECK* [20] was excellent. The data-collection and final refinement statistics are given in Table 1.

2.3. Protein data bank accession number

The atomic coordinates and structure factors of native and BeF_3^- complex structure have been deposited in the Protein Data Bank, http://www.rcsb.org/pdb (PDB ID codes: 4E7O and 4E7P).

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