Biochemical and Biophysical Research Communications xxx (2016) 1–5

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Structural characterization of the full-length response regulator spr1814 in complex with a phosphate analogue reveals a novel conformational plasticity of the linker region

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ARTICLE INFO

Article history:
Received 18 March 2016
Accepted 29 March 2016
Available online xxx

Keywords:
Two-component system
Response regulator
NarL subfamily
Phosphorylation
Streptococcus pneumoniae

ABSTRACT

Spr1814 of *Streptococcus pneumoniae* is a response regulator (RR) that belongs to the NarL/FixJ subfamily and has a four-helix helix-turn-helix DNA-binding domain. Here, the X-ray crystal structure of the full-length spr1814 in complex with a phosphate analogue beryllium fluoride (BeF_3^-) was determined at 2.0 Å. This allows for a structural comparison with the previously reported full-length unphosphorylated spr1814. The phosphorylation of conserved aspartic acid residue of N-terminal receiver domain triggers a structural perturbation at the α4–β5–α5 interface, leading to the domain reorganization of spr1814, and this is achieved by a rotational change in the C-terminal DNA-binding domain.

1. Introduction

Organisms that live in a changing environment must constantly respond and adapt to a wide range of stimuli. Such organisms sense environmental conditions using diverse systems that include secondary metabolites, ions and regulatory proteins [1]. Of these, two-component systems (TCSs) comprised of histidine kinases (HKs) and response regulators (RRs) are the predominant means through which bacteria respond to environmental signals [2]. In the simplest form, a HK catalyzes its own auto-phosphorylation of conserved histidine residue, followed by the transfer of the phosphoryl group to the conserved aspartic acid residue of RR [3]. Then, phosphorylated RRs undergo a conformational change that triggers the corresponding cellular response. The majority of RRs are transcription regulators, and they can be classified into three major groups based on the structures of their C-terminal DNA-binding effector domain that is attached to a conserved N-terminal receiver domain [4]. Spr1814, the RR that is studied here, belongs to the NarL/FixJ subfamily, which is characterized by a helix-turn-helix (HTH) DNA-binding domain, and accounts for approximately 19% of all response regulators [4–6].

To date, the NarL/FixJ subfamily has had five crystal structures of unphosphorylated full-length RRs and one structure of phosphorylated full-length RR [5,7–10]. Although these structures are similar overall in terms of their receiver and effector domains, different contacts between domains have been observed from all five unphosphorylated structures of full-length RRs [5,7–10]. Despite of the different domain arrangements in the RRs, the NarL/FixJ subfamily RRs have been suggested to release the blockage of the effector domain from the receiver domain upon phosphorylation [5,10,11]. A previous report presented a structural comparison of full-length VraR in the absence and presence of phosphoryl analog beryllium fluoride (BeF_3^-) and revealed that domain rearrangement occurred, allowing DNA binding of the effector domain upon phosphorylation [10].

Furthermore, our previous structures of the receiver domain of spr1814 in the presence and absence of BeF_3^- showed that there was a conformational change of the linker region that connects two domains upon phosphorylation [12]. This suggests that the domain rearrangement of full-length spr1814 could occur upon phosphorylation, and this is further supported by the structure of unphosphorylated full-length spr1814, which exhibited two distinct conformations: inactive and intermediate conformations [9]. The superposition of the structures of inactive and intermediate states indicated that there was a rotational change in the effector domain

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http://dx.doi.org/10.1016/j.bbrc.2016.03.144
0006-291X/

in the intermediate state, showing the release of the effector domain from the receiver domain to some extent [9]. In this respect, structural information for phosphorylated spr1814 is valuable to fill the gaps remaining in our understanding of the conformational changes that are accompanied by phosphorylation.

In this report, we determined the crystal structure of full-length spr1814 with a phosphate analog beryllium fluoride (BeF_3^-). This allows us to describe the conformational changes of spr1814 upon phosphorylation. The overall phosphorylated structure indicated that domain separation was accompanied by phosphorylation, allowing complete release of the effector domain from the receiver domain. However, phosphorylated spr1814 exhibits a distinct domain organization when it compared to phosphorylated VraR, suggesting the flexible nature of the domain interaction even when in a phosphorylated state.

2. Materials & methods

2.1. Recombinant protein expression, purification and crystallization

Purification, crystallization and data collection statistics for full-length spr1814 in a complex with BeF_3^- have been previously published [13]. Briefly, recombinant spr1814 was produced in *Escherichia coli* BL21 (DE3). Following purification, the protein was incubated with 30 mM NaF and 5 mM $BeCl_2$ for co-crystallization with BeF_3^-. The spr1814 crystals in a complex with BeF_3^-, belonging to the space group P21, were obtained by applying the hanging-drop vapor diffusion method at 22 °C, with each drop consisting of 1 ul protein solution (15 mg/ml) [20 mM Tris−HCl pH 7.9, 200 mM NaCl, 2 mM DTT, 5 mM $MgCl_2$, 10% glycerol] and a 1 ul reservoir solution [0.1 M sodium citrate tribasic dehydrate pH 4.7, 18% (v/v) Jeffamine ED-2001 pH 7.0]. 0.2 ul of two additives [3% (w/v) 1,5-Diaminopentane dihydrochloride and 1% v/v 1,2-Butanediol] were added into the corresponding drops. The crystals were then cryoprotected in 20% (v/v) ethylene glycol prior to flash-cooling in liquid nitrogen. Diffraction data were collected on a beamline AR-NW12 at the Photon Factory (Tsukuba, Japan) and were processed using the HKL-2000 software package [14].

For single-wave length anomalous dispersion (SAD) phasing, a 1 M 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 $BeCl_2$, and 3% (w/v) 1,5-Diaminopentane dihydrochloride. Crystals were obtained under the same conditions that were used to produce the BeF_3^- complex crystals and were cryoprotected with 20% (v/v) ethylene glycol and flash-cooled in liquid nitrogen. Subsequent single-wavelength anomalous diffraction (SAD) data were collected at the peak wavelength ($\lambda = 0.9198$ Å) of the Br *K*-edge absorption profile on a beamline 7A at the Pohang Light Source (Pohang, South Korea) and were processed as described above.

2.2. Structure solution and refinement

The structure of the spr1814 in a complex with BeF_3^- was determined using the single-wavelength anomalous dispersion (SAD) method with B3C, a bromine derivative. The bromine positions, initial phase calculations, phase improvement through density modification, and initial maps were calculated using the *PHENIX* program suite [15]. A total of 3 Br sites were determined in the asymmetric unit. The overall figure of merit (FOM) was improved to 0.28, and 298 of 398 residues were automatically built. Building and refinement were performed in cycles of model building with *Coot* [16] and refinement with *PHENIX* [15]. At this stage, a native dataset continued to be used for further refinement. However, we continued to finish the refinement of the BeF_3^--B3C-spr1814 ternary complex structure. After several cycles of model building, simulated annealing, positional refinement, and individual *B* factor refinement, the final R_{work} and R_{free} values of spr1814 in complex with BeF_3^- were 18.0% and 22.7%, respectively. The final BeF_3^- complexed structure contains one protein dimer 407 amino acid residues, 257 water molecules, two BeF_3^- that are covalently bound to Asp53, and two Mg^{2+} ions. The stereochemical qualities for all final models were excellent, as assessed by *MolProbity* [17], and the refinement statistics are summarized in Table 1.

2.3. Protein data bank accession number

The coordinates of the structure together with the structure factors have been deposited in the Protein Data Bank (http://www.rcsb.org/pdb) with the accession code of 4ZMR and 4ZMS.

3. Results and discussion

3.1. Overall structure

The structure of full-length spr1814 in a complex with BeF_3^- was solved by the SAD using B3C and was refined to 1.9 Å. Two molecules are found in the asymmetric unit (chain A and chain B) that form a dimer (Fig. 1A). Two Mg^{2+} ions and two BeF_3^- that were bound to the conserved Asp residue were observed in the phosphorylation site. In addition, one B3C compound was bound to the spr1814 structure. Like most RRs, spr1814 is composed of two domains: the N-terminal receiver domain exhibiting the classic α/β fold was observed in all RRs (residues 1−119), and the C-terminal effector domain with a helix-turn-helix DNA-binding motif (residues 141−199). The two domains are connected by a flexible linker region (residues 120−140) that is composed of short α-helix connected by a long loop.

The unphosphorylated full-length structures of RR in the NarL/FixJ subfamily indicated extensive contacts between their N- and C-terminal domains in a diverse way [5,8,10]. In our previous work, we have also shown that unphosphorylated spr1814 displays unique interdomain contacts [9]. Recently, both unphosphorylated and phosphorylated structures were reported for VraR [10], and these structures have shown that domain rearrangement occurred upon phosphorylation, resulting in the separation of two domains. In spr1814, the binding of phosphorylation mimic BeF_3^- to the conserved Asp53 residue triggers domain separation, which was achieved through the hinge-bending motion of the flexible linker region. When the spr1814 structures for unphosphorylated and phosphorylated specimens are compared with each other, there is a rotational change of the C-terminal domain that keeps it apart from N-terminal domain by more than 20 Å upon phosphorylation. However, even if spr1814 and VraR were both in a phosphorylated state, the structural comparison of the domain organization between both revealed that they adopt different domain contacts. This emphasized the dynamic nature of the interactions between the receiver and effector domains, even if they were in a phosphorylation state.

Specifically, one B3C compound that is used for SAD phasing is observed in the structure. The B3C compound has three functional groups (two carboxylic acid groups and one amino group) that are able to interact with the protein. In spr1814, the backbone nitrogen atom of Lys84 of the receiver domain is hydrogen bonded to one of the carboxylic acid groups of the B3C compound. However, no remarkable change was observed in the spr1814 structure by the binding of B3C.

Please cite this article in press as: A.K. Park, et al., Structural characterization of the full-length response regulator spr1814 in complex with a phosphate analogue reveals a novel conformational plasticity of the linker region, Biochemical and Biophysical Research Communications (2016), http://dx.doi.org/10.1016/j.bbrc.2016.03.144