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Structure analysis of *Bacillus cereus* MepR-like transcription regulator, BC0657, in complex with pseudo-ligand molecules☆

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ABSTRACT

The MarR family of transcriptional regulatory proteins in bacteria and archaea respond to environmental changes and regulate transcriptional processes by ligand binding or cysteine oxidation. MepR belongs to the MarR family, and its mutations are associated with the development of multidrug resistances, causing a growing health problem. Therefore, it has been of great interest to locate the ligand binding site of MepR and reveal the ligand-mediated transcriptional regulation mechanism. Here, we report on the crystal structure of *Bacillus cereus* MepR-like transcription factor, BC0657, at 2.16 Å resolution. Interestingly, BC0657 was complexed with fortuitous pseudo-ligands, which were assessed to be lipid molecules containing a long fatty acid, rather than phenolic compounds previously observed in other MarR proteins. The BC0657-ligand interaction provides the first molecular view of how MepR recognizes ligands to respond to toxic chemicals. Moreover, our comparative structure analyses of ligand binding sites on BC0657 and its homologs suggest that transcriptional repression by MepR would be relieved by ligand-induced changes in dimerization organization.

1. Introduction

Bacteria have developed a variety of molecular protection systems against harmful environmental chemicals and stresses. Multiple antibiotic resistance regulator (MarR) family proteins are transcriptional regulators that modulate the expression of genes involved in oxidative stress responses as well as degradation or export of toxic chemicals, such as phenolic compounds, antibiotics, and detergents [1–6]. The MarR protein was first reported in *Escherichia coli* and is now one of the largest transcription regulator families, including over 15,800 members (http://www.ebi.ac.uk/interpro/entry/IPR000835/proteins-matched). *E. coli* MarR negatively regulates gene expression of the *marRAB* operon by binding to the operator DNA upstream of the transcription start site and inhibiting RNA polymerase-mediated transcription initiation. MarR binding to its ligand, such as sodium salicylate, attenuates the MarR–operator interaction, allowing RNA polymerase to initiate transcription of the resistance gene [7,8].

Structural and biochemical studies have been performed on numerous MarR family members to date. Despite the large variation in primary sequences, MarR proteins adopt the triangular shape of a homodimer with the similar structural fold of the winged helix-turn-helix (wHTH) domain and the homodimerization domain [6,9]. It is well documented that the two wHTH domains of the triangular homodimer recognize palindromic sequences of double-stranded DNA in a symmetrical manner [10–15]. However, for the transcriptional regulation mechanism, each MarR protein appears to have developed a unique ligand recognition mode and, consequently, undergoes different conformational changes upon ligand binding. Several complex structures with ligand/drug molecules have independently allowed for the identification of up to eight discrete, ligand-binding sites for one MarR protomer, indicating multiple regulatory mechanisms in the MarR family [9,16–18].

MepR from *Staphylococcus aureus* belongs to the MarR family and is a multidrug-binding transcription regulator for expression of *mepA* and *mepR* [19,20]. MepA is a multidrug efflux pump that confers resistance to a wide range of toxic compounds, including biocides, fluoroquinolones, glycylcycline, tigecycline, and dyes.

☆ **Data deposition**: The atomic coordinates and structural factors for BC0657 (PDB ID xxxx) have been deposited in the Protein Data Bank, www.pdb.org.

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Mutations of the *mepR* gene [replacements of glutamine for proline residue at 18 (Q18P), of phenylalanine for leucine at 27 (F27L), and of alanine for valine at 103 (A103V)] have been associated with multidrug-resistance of clinical *S. aureus* isolates [21]. A recent structural study revealed that the MepR mutations were located in the dimerization interface and caused severe distortions in quaternary conformations of the MepR dimer [15]. However, due to the absence of the MepR-ligand complex structure, it is currently unclear how the MepR transcription factor recognizes ligand molecules to function as a transcriptional regulator.

Here we present the crystal structure of *Bacillus cereus* MepR-like transcription regulator, BC0657, at 2.16 Å resolution. BC0657 is homodimeric and contains the canonical wHTH domain, as is found in other MarR transcriptional regulators. Interestingly, extra electron density was observed inside a hydrophobic cavity in the dimerization interface and was assessed as fortuitous lipid-like pseudo-ligands. Furthermore, our comparative structural analyses suggest an allosteric mechanism for ligand-mediated disruption of the MepR–operator interaction.

2. Materials and methods

2.1. Preparation of the recombinant BC0657 protein

BC0657 expression plasmid was prepared and recombinant BC0657 protein was expressed and purified as described elsewhere (paper in preparation). Briefly, the BC0657 gene (residues 1–152) was amplified by PCR from the genome DNA of *B. cereus* and inserted into a modified pET49b vector (pET49bm) that contains the N-terminal His_6 tag and thrombin cleavage site. The resulting ligation product was transformed into *E. coli* DH5α cells and nucleotide sequences were confirmed by DNA sequencing.

Recombinant BC0657 protein was overexpressed in the *E. coli* strain, BL21 (DE3) in the presence of 1 mM isopropyl β-D-1-thio-galactopyranoside at 18 °C for ~16 h. Cells were harvested and lysed by sonication. The lysate was cleared by centrifugation (~25,000 × g) and the supernatant was incubated with Ni-NTA resin (Qiagen). The soluble BC0657 protein was eluted using PBS containing 250 mM imidazole, and dialyzed against 20 mM Hepes, pH 7.4/150 mM NaCl/5 mM β-mercaptoethanol (βME). The N-terminal His_6 tag was removed by thrombin and was further purified by gel filtration chromatography using a Superdex 200 16/600 column (GE Healthcare) in 20 mM Hepes, pH 7.4/150 mM NaCl/5 mM βME. Fractions for BC0657 were pooled and concentrated to ~34 mg/ml for crystallization.

2.2. BC0657 crystallization and X-ray diffraction data collection

The BC0657 protein was crystallized by the sitting drop vapor diffusion method at 18 °C. BC0657 crystals were obtained in 0.1 M phosphate citrate, pH 4.0/1.8 M ammonium sulfate. For X-ray diffraction data collection, BC0657 crystals were cryoprotected in a solution of 0.1 M phosphate citrate, pH 4.0/2.4 M ammonium sulfate/30% glycerol. A single crystal was flash-frozen under the cryostream at −173 °C. X-ray diffraction was performed at beamline 7A of the Pohang Accelerator Laboratory (PAL). Diffraction data were indexed, integrated, and scaled using the HKL2000 package [22]. X-ray diffraction statistics are shown in Table S1.

2.3. Structure determination of BC0657

The BC0657 structure was determined by molecular replacement with the *PHASER* program [23] using structure coordinates (PDB ID IS3J) of a MarR protein, YusO, as a search model. The BC0657 structure was iteratively built and refined using the *COOT* and *REFMAC5* programs, respectively [24,25]. The final model includes residues 5–146, 69 waters, and putative lipid-like pseudo-ligand molecules. The final model has excellent stereochemistry, with no Ramachandran outliers. Structure refinement statistics are shown in Table S1.

3. Results

3.1. The overall crystal structure of BC0657

The BC0657 structure was solved by molecular replacement and refined to 2.15 Å resolution (Table S1). The asymmetric unit (ASU) contains one chain of BC0657 (Fig. 1A). The BC0657 structure contains 142 residues (residues 5–146) from the entire molecule (residues 1–152). The overall fold of BC0657 is similar to those of other MarR family members consisting of six helices and three β strands with a topology of H1 (residues 8–33) – H2 (residues 37–49) – β1 (residues 51–53) – H3 (residues 54–61) – H4 (residues 65–77) – β2 (81–85) – β3 (93–97) – $H5^a$ (99–111) – $H5^b$ (114–121) – H6 (126–143) (Fig. 1A and B). The non-helical nature of the BC0657 at residue 113 divides helix 5 into two separate helices, $H5^a$ and $H5^b$. Single-turn 3_{10} helices are also observed in residues 59–61, 65–57, 76–78, 108–110, and 125–127. As for other MarR protein structures, the BC0657 monomer is divided into two functional domains, the dimerization domain and the wHTH domain. The dimerization domain involves the N- and C-terminal helices (H1, H2, H5, and H6) and mediates BC0657 homodimerization. The wHTH domain consists of β1, H3, H4, β2, and β3, and is a putative DNA binding domain.

DALI analyses indicate that the BC0657 structure is closely related to other MarR family proteins, including *S. aureus* MepR Q18P mutant (PDB ID 4LD5; RMSD value of 2.9 Å for 137 residues; 21% sequence identity), *Bacillus subtilis* YusO (PDB ID 1S3J; RMSD value of 3.9 for 129 residues; 32% sequence identity), *B. subtilis* OhrR (BsOhrR, PDB ID 1Z9C; RMSD value of 4.0 for 137 residues; 18% sequence identity), and *Streptomyces coelicolor* PcaV (PDB ID 4FHT; RMSD value of 3.3 for 137 residues; 16% sequence identity) (Fig. 1B). The wHTH domains of MarR homologs superpose well on that of BC0657, whereas dimerization domains showed severe structural deviations (Fig. 1C).

3.2. The dimerization interface of the BC0657 protein

MarR family proteins function as homodimers. Consistently, BC0657 forms a crystallographic dimer (Fig. 1D). The dimerization interface of BC0657 is mainly constituted by helices H1, H2, H5, and H6 of the dimerization domain and supplemented by additional residues including residue 60 of helix H3 and residues 61-62 of an H3–H4 linker. Dimerization of BC0657 buries an accessible surface area of 2180 $Å^2$ per subunit mostly by hydrophobic interactions, as is found in other MarR members [9,10,16].

In the BC0657 structure, it is noteworthy that the N-terminal helix H1 is simultaneously engaged in various interactions, including intra-subunit H1–H5 interactions, homodimeric assembly, and BC0657-ligand recognition (Fig. 2). A C-terminal part (residues 21–33) of H1 makes extensive intra-subunit interactions with H5 (Arg104, Glu107, Ala108, Ala111, Arg112, Asn113, His115, Ile116, and Tyr119), suggesting that the C-terminal H1 is essential in the formation of the globular monomeric fold (Fig. 2C). In contrast to the C-terminal H1, the N-terminal region of H1 (residues 8–20) protrudes out of the monomer without significant contribution to intra-subunit interactions (Fig. 1A). Instead, the N-terminal H1 is plugged inside a bundle of helices of the other subunit, including H2′, H3′, H5′, and H6′ (the prime symbol denotes a 2-fold symmetry-related molecule) and plays a primary role in