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Crystal structure of the C-terminal domain of *Bacillus subtilis* GabR reveals a closed conformation by γ-aminobutyric acid binding, inducing transcriptional activation

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ABSTRACT

Bacillus subtilis GabR (BsGabR) is involved in the γ-aminobutyric acid (GABA) catabolism as a transcriptional regulator, consisting of an N-terminal helix-turn-helix DNA-binding domain and a C-terminal aminotransferase-like (AT-like) domain. Research on the C-terminal AT-like domain of BsGabR (BsGabR-CTD) has focused on the interaction with GABA as an effector, but most its functional details remain unclear. To understand the underlying mechanism, we report the crystal structure of BsGabR-CTD in complex with pyridoxal 5'-phosphate (PLP) and GABA at 2.0 Å resolution. The structure of ligand-bound BsGabR-CTD revealed two distinct monomeric states in a homodimer. One subunit is a closed-form containing the PLP-GABA adduct, and the other subunit is a PLP-bound open-form. Our structural studies provide a detailed mechanism indicating that the open-to-closed transition by the binding of GABA induces the conformational rearrangement of BsGabR-CTD, which may trigger the activation of transcription.

1. Introduction

One of the most abundant and widely distributed groups of helix-turn-helix (HTH) transcription factors is the GntR family of metabolite-responsive regulators [1]. Bacterial proteins of the GntR family have a characteristic structure, which is composed of an N-terminal domain containing a conserved HTH module to recognize the specific DNA motifs and a C-terminal effector-binding and oligomerization domain [2]. Several subfamily members have been identified depending on the heterogeneous structure of the C-terminus domain and on its nature [2]. Among them, the MocR/GabR subfamily is distinguished by a C-terminal domain homologous to a fold type-I pyridoxal 5′-phosphate (PLP)-binding putative aminotransferase (AT). Only a few members of these subfamily have been structurally and functionally characterized [3].

Bacillus subtilis GabR (BsGabR) is one of the most extensively studied proteins among the MocR/GabR subfamily members, and regulates the transcription of the *gab* gene cluster, which is involved in the γ-aminobutyric acid (GABA) catabolism [4,5]. The GABA-inducible *gabTD* pathway in *B. subtilis* allows utilization of extracellular GABA as nitrogen and carbon sources [4]. BsGabR functions as a negative autoregulator of *gabR* regardless of the presence or absence of PLP and GABA, but enhances the transcription of *gabT* (encoding GABA aminotransferase) and *gabD* (encoding succinic semialdehyde dehydrogenase) genes required for the GABA degradation in the presence of PLP and GABA [4]. Structural studies of BsGabR have confirmed the presence of a fold type-I AT-like domain at the C-terminus and contributed to deciphering its mechanism of action [6,7]. The crystal structure of BsGabR showed a head-to-tail domain swapped homodimer, in which each the N-terminal winged HTH domain interacts with the C-terminal AT-like domain of its dimeric partner [6]. Previous studies suggested that the binding of GABA to the C-terminal AT-like domain might convert from a repressor to an activator of the *gabTD* operon [4]. However, the function of the C-terminal AT-like domain remains unclear, even though its three-dimensional structure is known.

Comprehensive structural analyses of BsGabR have been focused on understanding the functional characteristics of C-terminal AT-like domain and shedding new light on its structure-functional relationship as a transcriptional regulator. More detailed investigations based on the molecular structure of BsGabR

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in complex with PLP and GABA are required to improve the understanding of their C-terminal AT-like domain role as bacterial transcriptional regulator. In this study, we constructed the truncated BsGabR containing the C-terminal AT-like domain (BsGabR-CTD) to elucidate its role interacting with the GABA. The crystal structure of BsGabR-CTD was determined at 2.0 Å resolution in the form of a homodimer composed of two monomers with different conformations induced by binding of ligand. Our results provide structure-based molecular insights into the interaction of BsGabR-CTD with GABA, including the conformational change of the open-to-closed transition caused by the formation of an external Schiff base in the ligand-binding site.

2. Materials and methods

2.1. Recombinant protein expression and purification

The gene encoding the C-terminal AT-like domain (residue 101–479) was amplified by polymerase chain reaction (PCR) from *B. subtilis* genomic DNA using the forward primer 5′-ATA **GGA TCC** ATG GAG ATT CAC ATC GAC CAG-3′ and the reverse primer 5′-ATA **CTC GAG** TCA ATC CCC TGT AAC GGG GAT-3′. The primers contained modification to add appropriate restriction endonuclease for insertion into the vector, where *BamH*I site in the forward primer and the *Xho*I site in the reverse primer are shown in bold. The PCR-amplified DNA fragments and the expression vector pET-28a (Novagen) were digested with *BamH*I and *Xho*I, and were then ligated with T4 ligase (Enzynomics), to generate six conservative histidines (His_6)-tagged at the N-terminus of protein. The recombinant plasmids were transformed into *Escherichia coli* BL21 (DE3) strain (Novagen). Transformed cells were grown in Luria–Bertani medium with 50 μg/ml kanamycin at 37 °C. Protein expression was induced by adding 0.5 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG) once the cells had reached an optical density of 0.6 at 600 nm. The cells were then grown for an additional 18 h at 18 °C and harvested. The harvested cell pellets were suspended in buffer A (30 mM Tris-HCl pH 8.5, 500 mM NaCl, 5 mM β-mercaptoethanol and 10% glycerol) containing 1 mM phenylmethylsulfonylfluoride, and were disrupted by sonication. The lysate was centrifuged at 25,000 *g* for 30 min at 4 °C. The supernatant 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 0.5 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 (30 mM Tris-HCl pH 8.5, 150 mM NaCl, 2 mM dithiothreitol and 10% glycerol). Following purification, the soluble fractions containing protein were pooled together and concentrated to 6.5 mg/ml using an Amicon Ultra-15 centrifugal filter device (Millipore). The protein concentration was estimated using the Bradford assay and the purity was confirmed by 12% SDS-PAGE to be >95%.

2.2. Crystallization, X-ray data collection and processing

Prepared BsGabR-CTD protein (residue No. 101–479) was crystallized by the hanging-drop vapor diffusion method at 22 °C. Each hanging drop was prepared by mixing 1 μl protein solution with 1 μl reservoir solution, and was equilibrated over a 500 μl reservoir solution. The apo-crystals of BsGabR-CTD were obtained in reservoir solution containing 0.1 M HEPES pH 7.5 and 1–1.5% (*w*/*v*) polyethylene glycol 8000. The crystal of BsGabR-CTD in a ternary complex with PLP and GABA was obtained by soaking the apo-form crystals in the reservoir solution containing 1 mM PLP and 10–30 mM GABA. For cryogenic experiments, the crystals were transferred into a cryoprotection solution consisting of 30% (*v*/*v*) ethylene glycol in reservoir solution, and were flash-frozen in a stream of nitrogen gas. X-ray diffraction data of BsGabR-CTD crystals in a ternary complex with PLP and GABA were collected on beamline 7A at the Pohang Light Source (Pohang, South Korea) using an ADSC Quantum 270r CCD detector. All data sets were indexed, integrated and scaled using the *HKL-2000* software package [8].

2.3. Structure refinement

The crystal structure of BsGabR-CTD was solved by the molecular-replacement (MR) method using *MOLREP* program [9] in the *CCP4* package [10] with the GabR of *B. subtilis* (PDB ID: 4MGR) [6] as the search model. The crystals of BsGabR-CTD in a ternary complex with PLP and GABA belonged to the tetragonal $P4_1$ with one dimer per asymmetric unit. The values of R_{factor} and R_{free} obtained from preliminary refinement using the program *PHENIX* program [11], were approximately 31% and 35%, respectively. Unfortunately, further refinement could not lead the R_{free} value to be below 30%, although the electron-density map showed a good consistency with the model structure. In order to solve this problem, we were carried out the analysis of diffraction data using the program of *phenix.xtriage* [11]. As a result, the crystal in a ternary complex appeared to be a merohedral twin, indicating a twin fraction of 0.29 and a twin law (*h*, -*k*, -*l*). After the detwinning process, the structural refinements including the rigid body refinement and simulated annealing refinement were performed with *PHENIX* program [11], and the model was rebuilt using *COOT* [12]. The final model of BsGabR-CTD contained either the PLP-GABA adduct or PLP in each subunit, and the final values of R_{factor} and R_{free} were 17.3% and 21.6%, respectively. Structural validations of the complex structure was analyzed with the *PROCHECK* program [13] and no residue was detected in the disallowed region of Ramachandran plot [14]. All structure figures were prepared using

Table 1
Summary of data collection and refinement statics.

	BsGabR-CTD-PLP-GABA (PDB ID: 5X03)
Data collection	
Space group	$P4_1$
Cell dimension (Å)	a = 118.497, b = 118.497, c = 75.862
Wavelength (Å)	1.0000
Resolution (Å)	50.5–2.0 (2.07–2.00)[a]
Total/unique reflections	69052/6399
Completeness (%)	96.5 (89.7)
R_{merge}[b] (%)	8.7 (29.6)
Redundancy	4.6 (2.5)
$I/\sigma(I)$	14.6 (3.0)
Refinement statistics	
R/R_{free}[c] (%)	17.3/21.6
No. of atoms (B-factor, $Å^2$)	
Protein	5891 (30.8)
Ligands (PLP, GABA)	37 (30.3)
Water	248 (31.0)
RMS deviation of	
Bond length (Å)	0.009
Bond angle (°)	1.1
Ramanchandran plot	
Most favored region (%)	99.1
Allowed region (%)	0.9

[a] Values in parentheses refer to data in the highest resolution shell.

[b] $R_{merge} = \sum_{hkli}|I_{hkli}-\langle I_{hkl}\rangle|/\sum_{hkli}I_{hkli}$, where *I* represents the observed intensity, $\langle I\rangle$ represents the average intensity, and *i* counts through all symmetry-related reflections.

[c] $R = \sum||F_{obs}|-|F_{calc}||/\sum|F_{obs}|$, where R_{free} is calculated for a randomly selected 5% of reflections, which were not used for structure refinement.

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