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Crystal structure of a substrate-binding protein from *Rhodothermus marinus* reveals a single α/β-domain

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ABSTRACT

Substrate-binding proteins (SBPs) bind to specific ligands and are associated with membrane protein complexes for transport or signal transduction. Most SBPs recognize substrates by the hinge motion between two distinct α/β domains. However, short SBP motifs are often observed in protein databases, which are located around methyl-accepting chemotaxis protein genes, but structural and functional studies have yet to be performed. Here, we report the crystal structure of an unusually small SBP from *Rhodothermus marinus* (named as RmSBP) at 1.9 Å. This protein is composed of a single α/β-domain, unlike general SBPs that have two distinct domains. RmSBP exhibits a high structural similarity to the C-terminal domain of the previously reported amino acid bound SBPs, while it does not contain an N-terminal domain for substrate recognition. As a result of the structural comparison analysis, RmSBP has a putative SBP that is different from the previously reported SBP. Our results provide insight into a new class of substrate recognition mechanism by the mini SBP protein.

1. Introduction

The ATP-binding cassette (ABC) transporters are found in all kingdoms, which use the hydrolysis of ATP to translocate solutes across biological membranes [1–3]. In this system, the substrate-binding protein (SBP) or covalently bonded substrate-binding domain (SBD) capture the solute and deliver the molecule for transfer by the transmembrane domain of the ABC transporter [4,5]. Among them, the SBP is a key determinant of substrate specificity and has a high affinity towards ABC uptake systems [6]. Many SBP-dependent ABC transporters recognize a broad range of ligands such as metal ions, amino acids, sugars, and peptides [6]. In general, substrate-bound SBPs are involved in the activation of the ABC transporter through interactions with the ABC transporter [7,8].

SBPs have low or no amino acid sequence similarities but have highly conserved three-dimensional structural folds [7]. These SBPs commonly consist of two α/β domains and are connected by a hinge region, which comprises one to three interconnecting strands [5]. The two domains have an open conformation and a closed conformation when a specific substrate is bound between the two domains [8]. Based on the available structures from the Protein Data Bank (PDB), SBP shows four distinct structural states of (i) open-unliganded (ii) open-liganded (iii) closed-unliganded, and (iv) closed-liganded [7]. In the absence of substrates, most SBPs have open-unliganded states and some have closed-unliganded states [9,10], whereas the equilibrium between the open and closed conformation shifts towards the closed-liganded state in the presence of a substrate [7]. Based on the features of the three-dimensional structures, SBPs were divided into seven structural classes (cluster A–G) [5]. In these clusters, the SBPs have various sizes, ranging between 25 and 70 kDa and commonly have two α/β domains [7].

During a protein domain analysis using Pfam and InterPro, we found a 17.96 kDa (164 amino acids excluding the signal peptide) SBP domain from *Rhodothermus marinus*. This protein is smaller than previously known SBP family members. Although the analysis

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of the primary structure predicted that this protein is part of the SBP family, there was a lack of information for understanding the functional relationship with general SBP proteins.

To better understand the unusually small SBP, we have determined the crystal structure of the small SBP from *Rhodobacter marinus* (named as RmSBP) with a resolution of 1.9 Å. This protein has a single α/β-domain, which was structurally completely different compared with previously reported SBPs with two domains. Our structural data provided here for mini SBP now reveals more structural and functional diversity of the SBP family.

2. Materials and methods

2.1. Protein preparation

The gene encoding RmSBP (UniProt entry: D0MDR1, residues 22–185) was cloned between the *Nde*I and *Xho*I sites of the pET28a vector (Novagen). The recombinant DNA was transformed into the *Escherichia coli* BL21 (DE3) strain. Expression of the recombinant protein was induced by 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at 18 °C for overnight. The cells were harvested by centrifugation and then resuspended in a buffer containing 50 mM Tris-HCl, pH 8.0, 200 mM NaCl, and 20 mM imidazole. After sonication, cell debris was removed by centrifugation. The lysate supernatant was loaded onto a Ni-NTA column (QIAGEN) and washed by buffer A. The proteins were eluted by a buffer containing 50 mM Tris-HCl, pH 8.0, 200 mM NaCl, and 300 mM imidazole. The protein was incubated overnight at 4 °C with thrombin protease to remove the N-terminal hexahistidine-tag. Furthermore, this protein was concentrated and purified by size-exclusion chromatography on a HiLoad Sephacryl 100 16/60 column (GE Healthcare) via AKTA start (GE Healthcare) with buffer containing 10 mM Tris-HCl, pH 8.0 and 200 mM NaCl. The selenomethionine (Se-Met)-substituted RmSBP protein was expressed in *E. coli* BL21 (DE3) (Novagen) using the Se-Met expression kit. The Se-Met protein was purified using a protocol as described for native protein purification.

2.2. Crystallization

The recombinant RmSBP was crystallized by the hanging drop vapor diffusion method at 22 °C. The protein solution (20 mg/ml) was mixed with an equal volume of a reservoir solution containing 0.1 M sodium acetate, pH 4.5, 0.2 M $MgCl_2$, and 8–13% (w/v) PEG3350. Crystals were grown to $0.2 \times 0.2 \times 0.4$ mm within a month.

2.3. Data collection

Crystals were soaked in reservoir solution containing an additional 20% (v/v) glycerol and flash cooled in liquid nitrogen. X-ray diffraction datasets for the crystals were collected at 100 K on the beamline 7A at PLS-II (Pohang, Republic of Korea) using an ADSC Quantum Q270 CCD detector at a wavelength of 0.9794 Å. The diffraction datasets were processed and scaled using the HKL2000 program [11].

2.4. Structure determination

Initial phases were obtained with a Se-Met crystal diffracting to 1.9 Å by the Se single-wavelength anomalous dispersion (SAD) method using PHENIX AutoSol [12]. The structure model was automatically built using PHENIX AutoBuild [13], followed by manual model building using COOT [14]. The built model was refined by the program PHENIX [12]. The structural quality was verified by MolProbity [15]. The statistics of the refined model are summarized in Table 1.

2.5. Protein structure analysis

Short length SBP protein sequences were retrieved from the Pfam [16] and InterPro [17] databases. The signal peptide and cellular location were identified by SignalP 4.1 [18]. For the *in silico* analysis, the protein-protein interaction was analyzed by STRING [19]. Sequence searches for close homologs were performed with

Table 1
Data collection and refinement statistics for RmSBP.

Data collection	
Space group	C2
Cell dimensions	
a, *b*, *c* (Å)	63.90, 63.32, 34.67
α, β, γ (°)	90.00, 97.22, 90.00
Resolution (Å)	50.0–1.90 (1.93–1.90)
Completeness	98.0 (97.8)
Redundancy	6.6 (7.2)
I/σ(I)	48.38 (31.85)
R_{merge}[a]	13.3 (38.1)
Refinement statistics	
Resolution (Å)	44.80–1.90
R_{work}/R_{free} (%)[b]	15.37/19.84
B-factor (Averaged)	
Protein	12.74
Water	23.80
R.m.s deviations	
Bond lengths (Å)	0.023
Bond angles (°)	1.139
Ramachandran plot (%)	
favored	98.7
allowed	1.3

Highest resolution shell is shown in parentheses.

[a] $R_{merge} = \Sigma_h\Sigma_i|Ii(hkl)\_<I(hkl)>|/\Sigma_h\Sigma_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the 'ith' measurement of reflection hkl and $<I(hkl)>$ is the weighted mean of all measurements of hkl.

[b] $R_{work} = \Sigma||F_{obs}|-|F_{calc}||/\Sigma|F_{obs}|$, where F_{obs} and F_{calc} are the observed and calculated structure-factor amplitudes respectively. R_{free} was calculated as R_{work} using a randomly selected subset (5%) of unique reflections not used for structure refinement.