Published in final edited form as:

Proteins. 2011 April ; 79(4): 1352–1357. doi:10.1002/prot.22965.

Structural Basis for a Ribofuranosyl Binding Protein: Insights into the Furanose Specific Transport

Ashima Bagaria¹, Desigan Kumaran¹, Stephen K. Burley², and Subramanyam Swaminathan^{1,*}

¹Biology Department, Brookhaven National Laboratory, Upton, New York, 11973, USA

²Eli Lilly & Company, San Diego, California, 92121, USA

Introduction

The ATP-binding cassette transporters (ABC-transporters) are members of one of the largest protein superfamilies, with representatives in all extant phyla¹. These integral membrane proteins utilize the energy of ATP hydrolysis to carry out certain biological processes, including translocation of various substrates across membranes and non-transport related processes such as translation of RNA and DNA repair². Typically, such transport systems in bacteria consist of an ATP binding component, a transmembrane permease, and a periplasmic receptor or binding protein.

Soluble proteins found in the periplasm of gram-negative bacteria serve as the primary receptors for transport of many compounds, such as sugars, small peptides, and some ions. Ligand binding activates these periplasmic components, permitting recognition by the membrane spanning domain, which supports for transport and, in some cases, chemotaxis³⁻⁵. Transport and chemotaxis processes appear to be independent of one another, and a few mutants of bifunctional periplasmic components reveal the absence of one or the other function⁶.

Previously published high-resolution X-ray structures of various periplasmic ligand binding proteins include Arabinose binding protein (ABP)⁷, Allose binding protein (ALBP)⁸, Glucose-galactose binding protein (GBP)^{9,10} and Ribose binding protein (RBP)¹¹. Each of these proteins consists of two structurally similar domains connected by a three-stranded hinge region, with ligand buried between the domains. Upon ligand binding and release, various conformational changes have been observed¹²⁻¹⁴. For RBP, open (apo)¹⁵ and closed (ligand bound)^{14, 11} conformations have been reported and so for MBP¹⁶. The closed/active form of the protein interacts with the integral membrane component of the system in both transport and chemotaxis¹⁷.

Herein, we report 1.9Å resolution X-ray structure of the R_fBP periplasmic component of an ABC-type sugar transport system from *Hahella chejuensis* (UniProt Id Q2S7D2) bound to the unusual furanose form of ribose.

*Corresponding author swami@bnl.gov.

Materials and Methods

Gene cloning and protein purification

The target gene (Gene ID 3841438) for the full-length target protein from *Hahella chejuensis* (residues 38-313) was cloned into *E. coli* using primers, 5'-CAGCCGAAACTGCTGCTGGTCC-3' (forward) and 5'-CCTGCAGCAGGCGAACACCCGTG-3' (reverse) in the pSGX (3) vector. Protein expression/purification utilized previously published protocols, which are described in detail in PepcDB (pepcdb.pdb.org). Mass spectrometry analyses documented that none of the purified proteins had undergone degradation or post-translational modification (data not shown).

Crystallization and data collection

Diffraction-quality crystals of Se-Met protein were obtained at room temperature *via* sitting drop vapor diffusion against a reservoir solution containing 0.1M HEPES pH 7.5, 5% (v/v) MPD, 10% (w/v) PEG 6000 (1 μ l of reservoir solution plus 1 μ l of protein solution at a concentration of $\sim$ 7.5 mg/ml). Crystals were flash frozen by direct immersion in liquid nitrogen using mother liquor supplemented with 20% (v/v) glycerol. Crystals grew in the triclinic system, in space group P1 with two molecules/asymmetric unit. Diffraction data were obtained to 1.9Å resolution using NSLS Beamline X25 (National Synchrotron Light Source, Brookhaven National Laboratory) and processed with HKL2000¹⁸. Crystal parameters and data collection statistics are given in Table 1.

Structure determination

The crystal structure of R_fBP was determined *via* single wavelength anomalous dispersion (SAD) with Se-Met crystals. Selenium positions in the asymmetric unit were located using SHELXD¹⁹. Heavy atom phase refinement was carried out with SHARP²⁰ and the phases were further improved by density modification²¹. About 85% of the polypeptide chain was built automatically by ARP/wARP²². Subsequent model building was performed manually using COOT²³. Rigid-body and restrained refinement were performed using REFMAC²⁴. A continuous residual electron density feature was modeled as β -D-ribofuranosyl. The refined atomic model with R-factor of 0.16 was evaluated using the RCSB AUTODEP²⁵ validation tool (www.pdb.org) and atomic coordinates and structure factor amplitudes have been deposited in the Protein Data Bank (PDB ID: 3KSM). Final refinement statistics for the liganded form of R_fBP are provided in Table 1.

Results

Protomer Structure

R_fBP consists of two structurally similar Rossmann fold domains as shown in Figure 1 (a). Each α/β fold domain (domain 1: residues 39-143 and 279-308; domain 2: residues 144-278 and 309-314) consists of a parallel β -sheet flanked by α -helices. The β -sheets in the two domains are arranged such that the C-termini of the strands are close to one another. A three-stranded hinge region connects the two domains. The bound sugar is completely buried in a binding cleft formed by approximation of the two domains. In contrast to other sugar binding proteins like GBP and ABP, R_fBP does not have any metal binding site.

Crystal Structure

Although our crystals of R_fBP have two molecules per asymmetric unit, there is no experimental evidence suggesting that the periplasmic sugar binding proteins function as dimers¹⁴. The solvent accessible surface area buried by the crystalline protein-protein

interface area $\sim 348 \text{ \AA}^2$ falls below the established ranges for dimeric proteins^{26,27}. The quaternary structure suggested by PISA²⁸ is monomeric, which is consistent with the results of analytical gel filtration (data not shown). Interactions between the two crystallographically-independent monomers are stabilized by three hydrogen bonds (via Ala-202, Arg-172, Gln-150 and Tyr-146), salt bridges (Arg-198 and Asp-131, and Arg-218 and Asp-189), and a few hydrophobic interactions.

Furanose Recognition

The structure presented herein represents another example of the closed (active) conformation of a sugar binding protein. The ligand-binding site occurs at the interface of the two domains, where β -D-ribofuranosyl (BDR) is entirely solvent accessible. The bound sugar occurs in β -anomeric form of the five membered furanose ring of the ribose sugar rather than the more common 6-membered pyranose ring. This finding differs from the prediction of Aksamit and Koshland²⁹. To the best of our knowledge, this work provides the first example of the furanose form of ribose bound to an ABC sugar transporter. Binding of BDR is stabilized by an extensive hydrogen bonded network within in the interdomain cleft (Figure 1 b).

Like other α/β proteins with parallel β -sheets³⁰, the ligand binding cleft is formed by residues from interstrand loops. Sidechains of seven residues (domain 1: Lys46, Asp129 and Gln279; domain 2: Asn177, Ser179, Arg187 and Asp259) form an intricate network of hydrogen bonds ($\leq 3.2 \text{ \AA}$) with sugar hydroxyl groups. An elaborate network of hydrogen bonds involving other residues derived from the two domains buttress the sugar binding residues. It is remarkable that the oxygen atom of the furanose ring does not appear to engage in hydrogen bonds with either the protein or water molecules. All other periplasmic sugar-binding proteins have their sugar oxygen atom bonded to one or more of the residues within the ligand-binding site. For R_fBP, the sugar oxygen atom is oriented in opposite direction as compared to other binding proteins. The nearest residue, Asn233, is $\sim 3.5 \text{ \AA}$ from the sugar oxygen atom. A number of water molecules are observed in the cleft, although only one of them, WAT19, forms hydrogen bond with the O5-hydroxyl group of the sugar. The position of this water molecule is stabilized *via* hydrogen bonds with Asp279-OD2, WAT391, and WAT38. Asp129 and Arg183 both exhibit bidentate interactions, with their two polar atoms interacting with two groups at neighboring positions on the sugar (Fig. 1 b).

In addition to these polar residues, two aromatic rings (Tyr52 and Trp53 from domain 1) make hydrophobic contacts with the bound furanose (Fig. 1 c). Placement of the aromatic rings in the binding pocket of periplasmic sugar binding proteins is critical for specificity⁸, by distinguishing various anomeric sugars that differ at positions 2, 3, 4 or 5⁸. In R_fBP, Tyr52 lies on one side of the sugar almost parallel, while Trp53 ring sits nearly perpendicular to the sugar ring (Figs. 1 c). Another feature of our co-crystal structure of R_fBP that differs from other sugar binding proteins is the absence of the third aromatic sidechain in the ligand binding site (Fig. 1 c).

Interdomain Interactions

In addition to the protein-sugar interactions described above, the R_fBP -ribose complex is stabilized by interactions between the two structurally similar domains. For example, Asn177 from domain 2 makes hydrogen bonds with the sidechain of Lys46 from domain 1, and the O5 hydroxyl group of the sugar. Among these interactions stabilizing the closed conformation of R_fBP, Gln279 found within the connecting segment appears unique. This residue makes hydrogen bonds with both domains and with the bound sugar (see below).

Conformational Flexibility

Given the structures of the two domains making up R_fBP, it appears likely that the hinge region is responsible for the conformational flexibility of the system. HingeProt³¹ predicted three hinge regions (data not shown), Asp144-Asn145 and Gln279-Asn280 in domain 1 and Gly308-Val309 in the segment connecting domain 1 and 2 (Fig. 2). Domain closure probably represents coordinated movements involving all three predicted hinge regions. Gln279 plays a unique role in the closed conformation by making hydrogen bonds with sidechains derived from each domain and with the bound sugar.

Two well-ordered water molecules provide additional interdomain stabilization. WAT12 (Fig. 2), which is entirely buried within the ligand binding cleft makes a hydrogen bond with Asp129-O, Thr143-N, and Gln279-OE1. Another solvent molecule, WAT3 (Fig. 2), makes hydrogen bonds with Thr143-OG1, Asn280-N, Thr307-OG1, and Gly308-O and is also completely buried within the interdomain cleft.

Discussion

The periplasmic sugar-binding proteins belong to a subgroup (pentose/hexose sugar receptors) within the larger family of periplasmic receptors. Crystal structures of several members have been reported in both closed and open forms. The structure is conserved among the family and consists of two similar Rossmann fold domains linked by a three-stranded connecting segment. The domain-domain interface encompasses the sugar binding site and extensive hydrogen bonding and hydrophobic interactions of the ligand with both domains are responsible for the stabilization of the protein's closed conformation. R_fBP is evolutionarily related to various sugar binding proteins, including Ribose binding protein (RBP), Allose binding protein (ABP), Galactofuranose binding protein (G_fBP) and Galactose binding protein (GBP). From the sequence alignment provided (Supplementary material S1), it is clear that some of the residues, Arg, Asp, and Gln, involved in ligand binding are invariant. The aromatic rings responsible for stacking interactions with the sugar ring are almost entirely conserved, with the exception of R_fBP and G_fBP, both of which are selective for the more extended furanose form of the ligand. The Gln residue within the connecting segment that makes contact with both domains and with the ligand is also highly conserved throughout the family (GBP and G_fBP excepted).

Detailed analyses of the sugar binding pocket of R_fBP revealed some features that are distinct from those observed in previously published structures of sugar binding proteins. The most striking difference occurs at position 207 in R_fBP (corresponding to position 164 in RBP), where a phenylalanine in RBP that stacks with the sugar ring is replaced by an aspartate residue in R_fBP. The presence of Thr180, in the R_fBP (Ala138 in RBP) precludes a bulkier aromatic residue at position 207 (Fig. 1 c). Asp207 is present in lieu of the aromatic ring and is actually flipped out of the ligand-binding site, thereby accommodating a more extended form of the ribose sugar, the furanose form. Thus, unlike other periplasmic sugar binding proteins, only two stacked aromatic rings are present in R_fBP within the binding cleft. This feature unique to R_fBP probably explains furanose-binding specificity. A similar feature is observed in galactofuranose-binding protein³² (G_fBP) with only two aromatic rings. Asp90 in G_fBP (Asp 129 in R_fBP) is also flipped out of the ligand binding site, making a salt bridge with Arg17, thereby supporting recognition of the extended furanose form of galactose.

In order to facilitate the ligand entry, it is necessary that the opening motion should be restricted to the short hinge regions that connect the two domains. The two conserved water molecules in the hinge region of R_fBP provide an intricate network of hydrogen bonds at the rendezvous of the two domains. These water molecules are absent in the open

conformation¹⁵. Accordingly, these two water molecules may be recruited when the sugar enters the site to allow for intricate hydrogen bonding network with both domains facilitating closed conformation. Another possibility that can be attributed to the opening motion of the domains is the helix sliding movements, where the helices that are involved may slide as the domains move apart³³. The hinge residue Gln279 is conserved within the family (except GBP) and makes hydrogen bonds with residues from both domains and may play a functional role.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Research was supported by a U54 award from the National Institute of General Medical Sciences to the NYSGXRC (GM074945, PI: S.K.B.) under DOE Prime Contract No. DEAC02-98CH10886 with Brookhaven National Laboratory. We thank Protein Crystallography Research Resource (PXRR) of Brookhaven National Laboratory for providing data collection facilities (X25) at the National Synchrotron Light Source.

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Figure 1.

(a) Cartoon representation of an RfBP monomer. Structurally similar domains are shown in yellow and pink while the hinge region is shown in green. (b). Stereo view of the ligand-binding site. β -D-Ribofuranosyl is modeled into the $|F_o|-|F_c|$ difference electron density map contoured at 3σ . Hydrogen bonds with surrounding residues are seen in dashed lines (yellow and black). (c) Superposition of the ligand binding sites of RfBP (green) and RBP (yellow) shown as stick figures with BDR, β -D-ribofuranosyl (green) and RIP, ribopyranose (yellow) and a well ordered water molecule (WAT19, red).

Figure 2.
Hinge region of R_fBP, including three well ordered water molecules (WAT3, WAT12, and WAT19, red) all of which are seen in both asymmetric units

Table 1**Data Collection and Refinement Statistics**

Unit Cell Dimensions	$a = 37.5\text{\AA}$, $b = 47.7\text{\AA}$, $c = 79.7\text{\AA}$ $\alpha = 103.5^\circ$, $\beta = 90.5^\circ$, $\gamma = 91.1^\circ$
Space Group	P 1
Data Collection Statistics	
Resolution limit ($\text{\AA}$)	29.44-1.9 (1.96-1.9)
Unique reflections	38369 (2028)
Completeness, %	95.7 (90.6)
Rmerge ¹	0.063 (0.178)
Number of molecules/AU.	2
Phasing Statistics	
Phasing power ² (ano)	0.737
FOM ³ : (centric/acentric)	0.000/0.247
FOM after density modification	0.92
Refinement Statistics	
No. of protein atoms	4216
No. of ligand atoms	20
No. of solvent atoms	349
Rcryst	0.162
Rfree	0.203
Mean B-factors ($\text{\AA}^2$)	14.3
Root Mean Square Deviations	
Bond distance ($\text{\AA}$)	0.014
Bond angles ($^\circ$)	1.478
Ramachandran Plot Statistics (%)	
Residues in allowed regions	92.1
Residues in additionally allowed regions	7.1
Average B-factors ($\text{\AA}^2$) for chains A and B	
Main chain (A, B)	12.2, 12.0
Sidechain (A, B)	15.2, 14.8
Solvent (A, B)	23.9, 23.4
Ligand-BDR (A, B)	13.0, 12.2

Values for the highest resolution shell are given within parentheses.

¹ $R_{\text{merge}} = \sum |I_i - \langle I \rangle| / \sum I_i$ where I_i is the intensity of the i^{th} measurement, and $\langle I \rangle$ the mean intensity for that reflection.

² Phasing power

³ FOM (Figure of merit) as defined in SHARP