Crystal Structures of RbsD Leading to the Identification of Cytoplasmic Sugar-binding Proteins with a Novel Folding Architecture*

Min-Sung Kim§, Joon Shin¶, Weontae Lee¶, Heung-Soo Lee¶, and Byung-Ha Oh§**

From the §Center for Biomolecular Recognition and Division of Molecular and Life Science, Department of Life Science, Pohang University of Science and Technology, Pohang, Kyungbuk 790-784, the ¶Department of Biochemistry, College of Science, Yonsei University, Seoul 120-749, and the ¶Pohang Accelerator Laboratory, Pohang, Kyungbuk 790-784, Korea

RbsD is the only protein whose biochemical function is unknown among the six gene products of the rbs operon involved in the active transport of ribose. FucU, a paralogue of RbsD conserved from bacteria to human, is also the only protein whose function is unknown among the seven gene products of the l-fucose regulon. Here we report the crystal structures of Bacillus subtilis RbsD, which reveals a novel decameric toroidal assembly of the protein. Nuclear magnetic resonance and other studies on RbsD reveal that the intersubunit cleft of the protein binds specific forms of D-ribose, but it does not have an enzyme activity toward the sugar. Likewise, FucU binds l-fucose but lacks an enzyme activity toward this sugar. We conclude that RbsD and FucU are cytoplasmic sugar-binding proteins, a novel class of proteins whose functional role may lie in helping influx of the sugar substrates.

D-Ribose is the most abundant and important sugar that is a component of nucleic acids and many other biomolecules as well as an energy source. The Escherichia coli high affinity ribose transport system consists of six proteins encoded by the rbs operon (rbsA, -D, -E, -F, -K, and -R). Ribose-binding protein (RBP) 1 encoded by rbsB binds D-ribose at the periplasmic space (1). The sugar-bound form of RBP interacts with the membrane-bound permease (encoded by rbsC) that transports ribose across the inner membrane (2) coupled with the action of the ATP-binding cassette component (encoded by rbsA) (3). The transported D-ribose is phosphorylated into D-ribose 5-phosphate by ribokinase (encoded by rbsK), which is believed to be the first step for the utilization of the sugar in biosynthetic or metabolic pathways (4). RbsR is the repressor that binds to the transcriptional start site of the rbs operon. Among the six components, RbsD is the only protein whose function is unknown. The only clue about the function of RbsD is that it enhances the ribose uptake and growth of a mutant E. coli strain lacking the rbs operon but containing plasmid-encoded RbsK and mutated glucose transporter ptsG on its genome, which allows transport of D-ribose at low affinity (5). MCD-Ribose in solution exists as multiple forms: β-pyranose (58.9%), α-pyranose (20.3%), β-furanose (13.3%), α-furanose (7.4%), and open chain aldehyde form (0.13%) (6). In contrast, D-ribose in biomolecules is exclusively in the furanose forms, the five-membered ring forms. The cyclic forms of the sugar interconvert spontaneously via the least populated acyclic aldehyde. Surprisingly, ribokinase binds and phosphorylates the α-furanose form of D-ribose, the least populated ring form (7). For an efficient supply of the α-furanose form of D-ribose to ribokinase, an enzymatic activity of increasing the interconversion between the different forms of the sugar may be required, or the spontaneous conversion of the sugar at physiological conditions is fast enough so that such an enzyme activity is unnecessary.

A sequence alignment shows that RbsD is found in a variety of bacteria and that an evolutionarily conserved protein FucU (Fucose, Unknown) is a close paralogue of RbsD, with any compared pair of RbsD and FucU homologues sharing about 20% amino acid sequence identity with each other. Prokaryotic FucU exists as a component of fucose regulon (8–10), whose gene products are involved in the uptake and utilization of L-fucose (6-deoxy-l-galactose) (11). The fucose regulon encodes the following seven components: fucose permease (l-fucose H+ symporter), fucose isomerase, fuculose kinase, fuculose-1-phosphate aldolase, 1,2-propanediol oxidoreductase, regulatory protein, and FucU (9). Of these, FucU is the only protein whose function is unknown. L-Fucose is the major component in various oligo- and polysaccharides and glycosides in mammals as well as bacteria and plant (12). The isomerization of l-fucose into l-fuculose, which is subsequently phosphorylated into fuculose 1-phosphate, is the first step in the degradation of the sugar (13). Or l-fucose is converted into l-fuculose 1-phosphate by fuculose kinase as the first step in the synthesis of fucose-containing oligo- and polysaccharides (11). The conservation of FucU in higher organisms including human highlights the functional significance of the protein. Both FucU and RbsD, containing no signal sequence, must be cytoplasmic proteins. We sought to elucidate the biochemical function of RbsD and FucU on the basis of the three-dimensional structure of RbsD or FucU to provide a complete picture of the energy-driven transport of ribose and fucose. We determined the structure of Bacillus subtilis RbsD, which reveals a novel protein fold that associates into a homodecameric assembly. Crystallographic and other physicochemical studies led to the conclusion that...
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RbsD binds specifically the β-anomeric forms of D-ribose and FucU binds L-fucose, revealing the existence of cytoplasmic sugar-binding proteins. The name is coined reminiscent of the periplasmic sugar-binding proteins that are one component of periplasmic sugar substrates into cells.

MATERIALS AND METHODS

Expression and Purification of RbsD—E. coli RbsD and B. subtilis RbsD were cloned from respective genomes and expressed in E. coli BL21(DE3) (Novagen). The purification and crystallization of RbsD were reported (14). The cells were grown in Luria-Bertani media containing 0.1 mg ml⁻¹ ampicillin. The expression of the protein was induced by 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) at an optical density of 0.6 at 37 °C for 7 h. Both RbsDs were produced without a tag, which was the right choice since the both N and C termini are involved in the oligomerization of the proteins. B. subtilis RbsD was purified by ammonium sulfate precipitation (50–60% fractionation) and column chromatographic separation employing a Hitrap Q (Amersham Biosciences) and a HiLoad 26/60 Superdex 200 size-exclusion column (Amersham Biosciences). The purified protein was concentrated to 10 mg ml⁻¹ in 20 mM Tris-HCl buffer (pH 7.4) containing 0.2 M NaCl and 2 mM dithiothreitol. The crystals of B. subtilis RbsD were soaked into dry vapor diffusion method against reservoir solution containing 20% polyethylene glycol 4000, 19% isopropyl alcohol, and 100 mM sodium cacodylate (pH 6.5).

Overexpression and Purification of E. coli FucU—The E. coli FucU was amplified by the PCR technique from E. coli cell lysate. The PCR products were ligated into pET21a vector (Novagen). The resulting vector was introduced into E. coli BL21(DE3) strain. The expression of recombinant FucU protein was induced by 1 mM IPTG at an optical density of 0.4 at 37 °C for 7 h. Bacterial lystate was prepared by sonication in buffer containing 20 mM Tris-HCl buffer (pH 7.4), 0.1 M NaCl, and 1 mM dithiothreitol. The protein was purified by ammonium sulfate precipitation (50% fractionation) and using a Q-Sepharose fast-flow column (Amersham Biosciences). The purified FucU was dialyzed against 20 mM sodium phosphate buffer (pH 7.6) containing 1 mM β-mercaptoethanol.

Overexpression and Purification of E. coli Ribokinase—The E. coli RbsK gene was amplified by the PCR method from E. coli cell lysate. The PCR products were ligated into pPROEX HTa vector (Invitrogen) and introduced into E. coli BL21(DE3) strain. The protein was expressed as a fusion protein containing a His₅ tag at the N terminus. The cells were grown in Luria-Bertani media containing 0.1 mg ml⁻¹ of ampicillin. The expression of the protein was induced by 1 mM IPTG at an optical density of 0.6 at 18 °C for 7 h. Bacterial lystate was prepared by sonication in buffer A containing 20 mM Tris-HCl buffer (pH 7.4) and 0.1 M NaCl. The expression protein-bound nickel-nitrilotriacetic acid column (Qiagen) was eluted with buffer A containing 200 mM imidazole after washing the column with buffer A containing 20 mM imidazole. The eluted fractions containing RbsK were dialyzed against buffer containing 30 mM Tris-HCl buffer (pH 8.0).

Structure Determination of RbsD in Complex with Glycerol—Initially, we tried to solve the structure of E. coli RbsD. Although we obtained the crystals of the protein (14), difficulty with cryocooling of the crystals hampered the determination of the structure. Subsequently, we switched to B. subtilis RbsD, whose crystals were easier to handle. The crystals belong to the space group C2 with unit cell dimensions of a = 123.52, b = 108.66, c = 83.31 Å, and β = 128.68°. The asymmetric unit of the crystal contained a pentameric ring of RbsD molecules. A data set at three different wavelength was collected with a synchrotron beamline 11-2 at the Canadian Light Source (CLS). A total of 17,358 reflections were collected within the space group C2 with unit cell dimensions of a = 123.52, b = 108.66, c = 83.31 Å, and β = 128.68°. The asymmetric unit of the crystal contained a pentameric ring of RbsD molecules. A data set at three different wavelengths was collected with a synchrotron beamline 11-2 at the Canadian Light Source (CLS).

The crystallographic data were collected at a resolution of 0.1 M NaCl. The fusion protein bound to a nickel-nitrilotriacetic acid exclusion column (Amersham Biosciences). The purified protein was expressed as a fusion protein containing a His₆ tag at the N terminus. The fusion protein was produced without a tag, which was the right choice since the both N and C termini are involved in the oligomerization of the proteins. B. subtilis RbsD was purified by ammonium sulfate precipitation (50–60% fractionation) and column chromatographic separation employing a Hitrap Q (Amersham Biosciences) and a HiLoad 26/60 Superdex 200 size-exclusion column (Amersham Biosciences). The purified protein was concentrated to 10 mg ml⁻¹ in 20 mM Tris-HCl buffer (pH 7.4) containing 0.2 M NaCl and 2 mM dithiothreitol. The crystals of B. subtilis RbsD were soaked into dry vapor diffusion method against reservoir solution containing 20% polyethylene glycol 4000, 19% isopropyl alcohol, and 100 mM sodium cacodylate (pH 6.5).

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Table I

| Parameters                          | Peak   | Edge   | Remote |
|------------------------------------|--------|--------|--------|
| Wavelength (Å)                     | 0.97936| 0.97950| 0.97168|
| Resolution range (Å)               | 20.0-2.30| 20.0-2.30| 20.0-2.30|
| Completeness, %                    | 94.1 (88.5) | 93.6 (88.0) | 94.5 (89.9) |
| Rfuran,%                           | 4.4 (18.4) | 4.5 (15.9) | 4.6 (17.8) |
| lσ                                | 13.9 (3.5) | 12.2 (2.9) | 12.5 (2.9) |
| FOM (centric/acentric)             | 0.63/0.65 |

Refinement statistics

| Parameters                          | Uncomplexed | Ribose | Ribose 5-phosphate |
|------------------------------------|-------------|--------|-------------------|
| Resolution (Å)                     | 30.0-2.0    | 30.0-1.95 | 30.0-2.05         |
| Rfuran,%                           | 4.9 (24.9)  | 6.7 (36.2) | 4.8 (22.5)       |
| Total reflections                   | 278,205     | 295,120 | 236,173           |
| Unique reflections                  | 57,573      | 62,466 | 48,339            |
| Completeness, %                    | 94.3 (85.0)| 97.6 (92.8)| 90.3 (80.7)      |
| R-factor/Rfuran,’%                 | 20.223.1    | 19.8/21.1| 19.4/22.7      |
| r.m.s.d. bond length (Å)           | 0.0055      | 0.0055 | 0.0056            |
| r.m.s.d. bond angle (°)            | 1.288       | 1.342  | 1.268             |
| Ramachandran plot, %               | 92.3        | 93.0   | 91.8              |
| Most favored region                | 7.5         | 7.0    | 8.0               |
| Generously allowed region          | 0.2         |        | 0.2               |

* Completeness for lσ/σ > 1.0.
* Rfuran = \( \sum I_{\text{obs}} - I_{\text{avg}} \), where \( I_{\text{obs}} \) is the observed intensity of individual reflection, and \( I_{\text{avg}} \) is average over symmetry equivalents.
* Figure of merit, defined as \( \sum P(\alpha) e^{-2/2} P(\alpha) \), where \( P(\alpha) \) is the phase probability distribution.
* R-factor = \( \sum F_o - F_c \sum F_o \), where \( F_o \) and \( F_c \) are the observed and calculated structure factor amplitudes, respectively. Rfuran was calculated with 5% of the data.
* r.m.s.d., root mean square deviation.

bility and stability of the decameric structure. A search for analogous folding architectures in the PDB data base using the program Dali (21) revealed that the structure of RbsD is distinct from any other known protein folds. The structure of a hypothetical trimeric protein YjgF (Protein Data Bank code 1Q99; Z-score 3.5), listed as the closest match, is obviously different from that of RbsD. Unlike the structure of RbsD, one side of the central β-sheet of YjgF has no peptide segment and serves as the trimeric interface of the protein.

We found that the clefts between the RbsD subunits bind glycerol (data not shown) when the crystals were immersed in the cryoprotectant containing 7% glycerol before data collection. It has been commonly observed that enzymes with a toroidal structure have the active site at the interface between adjacent subunits (for examples, 2-Cys peroxiredoxin, ribulose-bisphosphate carboxylase/oxygenase, cyanase, muconolactone isomerase, and glutamine synthetase), indicating that the oligomer formation is vital for the enzyme activity. The interaction between RbsD and glycerol is weak when assessed by isothermal titration calorimetry (ITC). It was not possible to measure the dissociation constant \( K_D \) by this method. The high concentration of glycerol present in the cryoprotectant presumably allowed the observation of the bound glycerol. Glycerol may be one of the polyol compounds that are able to bind fortuitously to the binding pocket of RbsD that is designed to interact most strongly with D-ribose (discussed below). The interaction of RbsD with glycerol is presumably physiologically irrelevant.

RbsD Lacks an Enzyme Activity toward Ribose—We recorded \( ^{13}C \) NMR spectra of D-[1,\(^{13}C \)]ribose in the presence and absence of RbsD. The addition of RbsD did not give rise to new peaks, demonstrating that the protein lacks an enzyme activity to convert D-ribose into other compounds. Instead, it caused significant broadening of the resonances arising from the \( ^{13}C \)-1 atoms of the β-pyranose and β-furanose forms but not those of the α-furanose and α-pyranose forms (Fig. 2). These spectroscopic data indicate that RbsD binds both the β-anomeric forms of D-ribose but not the α-anomeric forms of the sugar. Because the NMR spectrum does not indicate binding of the α-anomeric D-ribose, RbsD is not an anomerase, which catalyzes the interconversion between the α- and β-anomeric forms of the sugar. We considered the possibility of RbsD being an enzyme that could increase the interconversion between the β-anomeric forms and the open chain form of D-ribose. If RbsD has this enzyme activity, the line broadening of the two NMR peaks would be due to chemical exchange process between the β-furanose and β-pyranose forms of D-ribose with the open chain aldhyde. Such an enzyme activity could enhance the availability of the least populated α-furanose form of D-ribose for ribokinase. For example, when the α-furanose form is depleted (a non-equilibrium situation), rapid conversion of β-anomeric forms into the open chain form should increase the appearance of the α-furanose form through the spontaneous conversion of the open chain form into the α-furanose form. We cloned and overexpressed E. coli ribokinase and measured the enzyme activity of phosphorylating ribose in the presence and absence of RbsD according to the method reported in the literature (22). RbsD did not enhance the enzyme activity of ribokinase (data not shown), indicating that the protein does not have this hypothetical enzyme activity. We also compared the rates of the change in the optical rotation of freshly dissolved solid D-ribose, which is exclusively in the β-furanose form, in the presence and absence of RbsD. Consistent with the enzymatic assay of ribokinase, the protein did not enhance the rate.

Binding of the β-Pyranose Form of D-Ribose—The exposure of the intersubunit clefts to the bulk solvent in the RbsD crystals facilitated the determination of the structures of RbsD in complex with D-ribose or D-ribose 5-phosphate using the crystals soaked with each of the two compounds. In order to avoid competition of glycerol for binding to the clefts, data were collected at room temperature. With a D-ribose-soaked crystal, less featured “fat” electron density was found at each of the 10 intersubunit clefts (Fig. 3). The poorly defined electron density, despite the high resolution of the data (1.96 Å), suggested the binding of ribose molecules in different configurations. Given the NMR data demonstrating the binding affinity of RbsD for the both β-pyranose and β-furanose form of D-ribose, we first interpreted the density with the most populated β-pyranose...
The 2.05-Å resolution structure of RbsD in complex with a negatively charged ion (in the order of appearance in the primary sequence) bound to the intersubunit clefts are shown as an assembly of RbsD shown in two different orientations. Ribose molecules density indicated that RbsD binds selectively the of the fat electron density with the superimposes the pentameric rings. The symmetry-related pairs are shown in different colors. Water molecules are in red. Two histidine residues hydrogen-bonded to the bound water molecules are shown.

form of D-ribose. The modeled β-pyranose in a standard chair conformation mostly accounted for the electron density (Fig. 3a). The sugar-binding mode reveals that one of the two adjacent RbsD molecules provides a predominant contribution over the other. Asp-28, His-98, Lys-102 (via a water molecule), Tyr-120, and Asn-122 of one subunit and only His-20 of an adjacent subunit are in contact with the bound ribose. The aromatic ring of Tyr-120 is packed against the hydrophobic part of the bound sugar, a typical pattern frequently observed in the protein-sugar interactions. The bound ribose molecule fits tightly into the intersubunit cleft and leaves almost no room (Fig. 3b).

Binding of the β-Furanose Form of D-Ribose—Interpretation of the fat electron density with the β-furanose form of D-ribose was impossible. The electron density did not show the feature of the –CH₂OH group of the furanose ring. We determined the binding mode of the β-furanose form indirectly by elucidating the 2.05-Å resolution structure of RbsD in complex with D-ribose 5-phosphate, whose ring form is exclusively the furanose form. As expected, the electron density for the five-membered ring mostly accounted for the electron density (Fig. 3c). The phosphorylated sugar that we introduced D-[1-13C]ribose. Spectra were collected at 25 °C. In the experimental conditions, we could not observe the peak from the acyclic aldehyde form (0.13% of the total population), which requires a highly concentrated sample and prolonged acquisition time for its detection.

Fig. 1. Structure and ribose-binding site of RbsD. a, decameric assembly of RbsD shown in two different orientations. Ribose molecules bound to the intersubunit clefts are shown as ball-and-sticks. b, ribbon diagram of RbsD monomer. The secondary structures are numbered in the order of appearance in the primary sequence. c, buried ion cage. A negatively charged ion (in cyan), which is putatively a Cl⁻, is bound between two RbsD subunits related by the molecular 2-fold axis that superimposes the pentameric rings. The symmetry-related pairs are shown in different colors. Water molecules are in red. Two histidine residues hydrogen-bonded to the bound water molecules are shown.

missing (methylene part) or diffused (phosphate group). As the phosphate group of the bound sugar barely interacts with the protein, the β-furanose form of D-ribose would bind to the protein in the same mode observed for D-ribose 5-phosphate. Notably, Asn-122 does not interact with the bound ribose 5-phosphate. In order to confirm the correctness of the crystallographically deduced sugar-binding modes, we substituted His-98 with alanine, whose imidazole ring provides a hydrogen bond to both β-anomeric forms of the sugar (Fig. 3). The NMR spectrum of D-[1-13C]ribose in the presence of the mutant RbsD exhibits significantly less broadening of the two peaks corresponding to the β-anomers of D-ribose (Fig. 2) than in the presence of the wild-type RbsD, indicating that the sugar-binding affinity of the mutant is reduced. This is consistent with the deduced sugar-binding modes.

Sugar-binding Residues Are Virtually Invariant—A sequence alignment reveals that Asp-28, His-98, Asn-122, and His-20 are invariant, whereas Lys-102 and Tyr-120 are 100% homologously conserved, among the 40 deposited sequences of RbsD homologues except for three entries. The conservation of the sugar-binding residues is significant in that Pro-32 and Gly-105 are the only two residues that are more than 97% conserved among the RbsD homologues. One of the three exceptions is NP_326431 (annotated as Mycoplasma pulmonis RbsD, GenBank™), which contains two substitutions of His-20 with threonine and His-98 with asparagine (Fig. 4). The other two entries in GenBank™ are NP_407075 and ZP_00060076, which contain a substitution of Lys-102 with histidine and a substitution of Tyr-120 with asparagine, respectively. Interestingly, two of the three genes are not a component of a canonical rbs operon but are associated with genes coding for putative phosphotransferase enzyme II, A and B (NP_326431, GenBank™), or araC family transcriptional regulatory protein (NP_407075, GenBank™), which does not share sequence homology with RbsR. The two genes are likely to be RbsD paralogues whose gene products have binding specificity for sugars different from ribose. The observation raises a possibility that the energy-driven transport of neutral sugars other than ribose...
and fucose may also require biochemical activity similar to that of RbsD in some organisms.

**FucU Lacks an Enzyme Activity toward L-Fucose—** FucU, a close relative of RbsD, is a highly conserved protein; *E. coli* FucU shares 50% sequence identity with human FucU (Fig. 4). We cloned and purified *E. coli* FucU, which shares 21% sequence identity with RbsD. The elution times of the two proteins from a size-exclusion Superdex 200 column were the same, indicating that FucU also adopts a decameric quaternary structure similar to that of RbsD. In FucU, the ribose-binding residues His-20, Asp-28, Tyr-120, and Asn-122 in RbsD are also 100% conserved, whereas His-98 and Lys-102 are substituted with and strictly conserved as arginine and tyrosine, respectively (Fig. 4). L-Fucose is D-ribose containing the 6-methyl group and having a different configuration at the C-2 position. Most likely, these substitutions provide FucU with specificity and affinity for binding L-fucose. We recorded the 1H NMR spectrum of L-fucose in the presence and absence of FucU (Fig. 5). The 1H NMR peaks of L-fucose did not change after a 2-h incubation of the mixture at room temperature, demonstrating that FucU lacks an enzyme activity of converting L-fucose to a product.

**Weak Sugar-binding Affinity of RbsD and FucU—** We analyzed the interactions of RbsD and FucU with sugar molecules by ITC. The isothermal titration curve for ribose 5-phosphate readily indicated that the binding affinity of RbsD for this phosphorylated sugar is quite weak and prevented us from obtaining the dissociation constant by this method (Fig. 6a). However, it was possible to analyze the titration curve obtained for the interaction between RbsD and D-ribose (Fig. 6b). The analysis led to the KD value of 0.93 mM. The data demonstrate that the β-pyranose form binds to RbsD more strongly than the D-ribose. In the data analysis, we assumed that FucU binds pyranose and furanose forms of L-fucose with the same affinity, because the anomeric specificity of FucU is unknown.

The binding affinity between RbsD and D-ribose is quite low.
The differential gain of free energy in the transfer of ribose from the solvent to the binding cleft of RbsD seems small due to the excellent hydrogen-bonding capacity of the sugar molecule in water. The mode of interaction between RbsD and ribose is uniquely distinguished from that between periplasmic RBP and ribose. Although the ribose of binding to RbsD does not induce a noticeable conformational change of the backbone or a side chain of the protein (data not shown), the binding of ribose to periplasmic RBP triggers a large domain movement that leads to the shielding of the bound ribose from the bulk solvent and the formation of new interactions between protein atoms (23). Consequently, the binding of ribose to periplasmic RBP is substantially tight, exhibiting the $K_d$ value of $0.13 \mu M$ (24).

What Would Be the Biological Role(s) of RbsD and FucU?—We demonstrated that the biochemical function of RbsD and FucU is to bind specific forms of D-ribose and fucose, respectively. The residues at the sugar-binding site in both proteins are remarkably conserved. Therefore, the functional role(s) of the proteins must be related to this sugar binding activity. We ruled out an effector function, because the ribose-bound structures of RbsD exhibit no noticeable conformational change of the protein compared with the structure of the uncomplexed RbsD, and the oligomerization state of RbsD is not the result of the ribose binding. RbsD does not appear to be an essential enzyme at the downstream of RbsK, because the E. coli ptsG mutant cells lacking RbsD but containing RbsK can grow on ribose as the only carbon source (5). A 4-fold enhancement of the ribose uptake by expressing RbsD in this mutant (5) is presumably because of the action of the protein
specifically an anomeric form of L-fucose that is not used by mains to be determined whether, like RbsD, FucU may bind difficulties in obtaining suitable crystals of the protein. It relates to RbsC, but the overexpression of RbsD enhances the utilizations the sugar.

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Fig. 6. Isothermal titration calorimetric analysis. a, titration of D-ribose 5-phosphate into RbsD. b, titration of L-fucose into FucU. The ITC measurements were carried out by titrating 5 μl of 29.5 mM D-ribose into 1.4 ml of 281 μM RbsD solution or 10 μl of 10 mM L-fucose 5-phosphate and L-fucose solution into 267 μM RbsD and 61.4 μM FucU solution, respectively.

upstream of RbsK. Noticeably, ribokinase specifically phosphorylates the α-furanose form of D-ribose, the least populated ring form of the sugar (7), whereas RbsD selectively binds the β-anomeric forms of D-ribose that account for about 72% equilibrium population of the sugar. Therefore, without directly competing with ribokinase, RbsD would be able to play a role that requires binding of D-ribose. In playing the role, the low affinity of RbsD for ribose may be required for minimal interference with the availability to ribokinase of the α-furanose form of D-ribose, which is generated through the nonenzymatic conversion of the free β-anomers. We do not know how RbsD would exert its cellular function by binding to the β-anomers of D-ribose. The functional role may lie in facilitating the influx of the sugar substrate, the event upstream of RbsK.

In prokaryotic cells, fucose permease appears primarily responsible for the energy-driven uptake of L-fucose (25). FucU may play a similar role as RbsD by binding L-fucose. We do not know yet the anomeric specificity of FucU in binding L-fucose due to unavailability of commercial C-13-labeled L-fucose and difficulties in obtaining suitable crystals of the protein. It remains to be determined whether, like RbsD, FucU may bind specifically an anomeric form of L-fucose that is not used by fucose isomerase or fucose kinase, the first enzymes in the utilization the sugar.

The membrane-bound fucose permease and ribose permease (RbsC) are unrelated proteins. The mutated ptsG is also unrelated to RbsC, but the overexpression of RbsD enhances the ribose uptake through this transporter (5). These observations suggest that RbsD and FucU do not interact directly with their respective permease component to exert function.

Concluding Remarks—The existence of periplasmic ligand-binding proteins has long been known. The biochemical functions and action modes of these proteins are well characterized. The novel protein fold of RbsD and the sugar binding activity of RbsD and FucU characterized in this study reveal the existence of the cytoplasmic sugar-binding proteins. Although RbsD genes are found only in bacteria, FucU genes are also found in mouse and human genomes, which underscores the functional importance of the protein. The two genes must have been derived from the same ancestral gene and evolved to have different sugar-binding affinities. It remains to be determined whether there are unidentified cytoplasmic sugar-binding proteins with different ligand specificity that act on the transport of other neutral sugars. The two RbsD homologues described above are potential candidates. It is possible that remote homologues of RbsD may play roles in the influx of other sugars. The biochemical function of RbsD and FucU identified in this study is to bind D-ribose and L-fucose, respectively. The sugar binding activity of the proteins should be tightly linked to the utilization of these sugars, because the gene expressions of RbsD and FucU are regulated as a part of the rbs operon or the fucose regulon in prokaryotes. In depth in vitro study should be necessary to elucidate the biological roles of RbsD and FucU. The presented structures and the common biochemical function of the two proteins delineated in this study are a footstep toward gaining a complete picture for the energy-driven transport of the neutral sugars.

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REFERENCES
1. Willis, R. C., and Furlong, C. E. (1974) J. Biol. Chem. 249, 6926–6929
2. Furlong, C. E. (1982) Methods Enzymol. 86, 467–472
3. Buckel, S. D. (1986) J. Biol. Chem. 261, 7659–7662
4. Anderson, A., and Cooper, R. A. (1986) Biochim. Biophys. Acta 854, 163–165
5. Oh, H., Park, Y., and Park, C. (1999) J. Biol. Chem. 274, 14006–14011
6. Drew, K. N., Zajicek, J., Bondo, G., Bose, B., and Serianni, A. S. (1998) Carbohydr. Res. 307, 199–209
7. Sig变现, J. A., Cameron, A. D., Jones, T. A., and Mowbray, S. L. (1998) Structure 6, 183–193
8. Zhu, Y., and Lin, E. C. (1988) J. Bacteriol. 170, 2352–2358
9. Blattner, F. R., Plunkett, G., III, Blech, C. A., Perna, N. T., Burland, V., Riley, M., Collado-Vides, J., Gogol, D. J., Rode, C. K., Mayhew, G. F., Gregor, J., Davis, N. W., Kirkpatrick, H. A., Goeden, M. A., Rose, D. J., Mau, B., and Shao, Y. (1997) Science 277, 1453–1474
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10. McClelland, M., Sanderson, K. E., Spieth, J., Clifton, S. W., Latreille, P., Courtney, L., Porwollik, S., Ali, J., Dante, M., Da, F., Hou, S., Layman, D., Leonard, S., Nguyen, C., Scott, K., Holmes, A., Grewal, N., Mulvaney, R., Ryan, E., Sun, H., Florea, L., Miller, W., Stoneking, T., Nhan, M., Waterston, R., and Wilson, R. K. (2001) *Nature* **413**, 852–856

11. Park, S. H., Pastuszak, I., Drake, R., and Elbein, A. D. (1998) *J. Biol. Chem.* **273**, 5685–5691

12. Flowers, H. M. (1981) *Adv. Carbohydr. Chem. Biochem.* **39**, 279–345

13. Chen, Y. M., Zhu, Y., and Lin, E. C. (1987) *Mol. Gen. Genet.* **210**, 331–337

14. Kim, M. S., Oh, H., Park, C., and Oh, B. H. (2001) *Acta Crystallogr. Sect. D Biol. Crystallogr.* **57**, 728–730

15. Otwinowski, Z., and Minor, W. (1997) *Methods Enzymol.* **276**, 307–326

16. Terwilliger, T. C., and Berendzen, J. (1999) *Acta Crystallogr. Sect. D Biol. Crystallogr.* **55**, 849–861

17. Terwilliger, T. C. (2000) *Acta Crystallogr. Sect. D Biol. Crystallogr.* **56**, 965–972

18. Jones, T. A., Zou, J. Y., Cowan, S. W., and Kjeldgaard, M. (1991) *Acta Crystallogr. Sect. A* **47**, 110–119

19. Brünger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) *Acta Crystallogr. Sect. D Biol. Crystallogr.* **54**, 905–921

20. Collaborative Computational Project Number 4 (1994) *Acta Crystallogr. Sect. D Biol. Crystallogr.* **50**, 760–763

21. Holm, L., and Sanders, C. (1995) *Trends Biochem. Sci.* **20**, 478–480

22. Andersson, C. E., and Mowbray, S. L. (2002) *J. Mol. Biol.* **315**, 409–419

23. Björkman, A. J., and Mowbray, S. L. (1998) *J. Mol. Biol.* **279**, 651–664

24. Björkman, A. J., Binnie, R. A., Zhang, H., Cole, L. B., Hermodson, M. A., and Mowbray, S. L. (1994) *J. Biol. Chem.* **269**, 30206–30211

25. Guns, F. J., Tate, C. G., Sansom, C. E., and Henderson, P. J. (1995) *Mol. Microbiol.* **15**, 771–783

26. Esnouf, R. M. (1999) *Acta Crystallogr. Sect. D Biol. Crystallogr.* **55**, 938–940

27. Hwang, T.-L., and Shaka, A. J. (1995) *J. Magn. Reson.* **112**, 275–279
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