A Mutated PtsG, the Glucose Transporter, Allows Uptake of D-Ribose*

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Mutations arose from an Escherichia coli strain defective in the high (Rbs/ribose) and low (Als/allose and Xyl/xylose) affinity D-ribose transporters, which allow cells to grow on D-ribose. Genetic tagging and mapping of the mutations revealed that two loci in the E. coli linkage map are involved in creating a novel ribose transport mechanism. One mutation was found in ptsG, the glucose-specific transporter of phosphoenolpyruvate:carbohydrate phosphotransferase system and the other in mlc, recently reported to be involved in the regulation of ptsG. Five different mutations in ptsG were characterized, whose growth on D-ribose medium was about 80% that of the high affinity system (Rbs*). Two of them were found in the predicted periplasmic loops, whereas three others are in the transmembrane region. Ribose uptakes in the mutants, competitively inhibited by D-glucose, D-xylose, or D-allose, were much lower than that of the high affinity transporter but higher than those of the Als and Xyl systems. Further analyses of the mutants revealed that the rbsK (ribokinase) and rbsD (function unknown) genes are involved in the ribose transport through PtsG, indicating that the phosphorylation of ribose is not mediated by PtsG and that some unknown metabolic function mediated by RbsD is required. It was also found that D-xylose, another sugar not involved in phosphorylation, was efficiently transported through the wild-type or mutant PtsG in mlc-negative background. The efficiencies of xylose and glucose transport are variable in the PtsG mutants, depending on their locations, either in the periplasm or in the membrane. In an extreme case of the transmembrane change (I283T), xylose transport is virtually abolished, indicating that the residue is directly involved in determining sugar specificity. We propose that there are at least two domains for substrate specificity in PtsG with slightly altered recognition properties.

D-Ribose is a pentose sugar commonly found as a component of nucleic acids. It can be utilized by many bacteria as a sole carbon source. In Escherichia coli, the sugar is transported via the high affinity RbsACB transporter, a member of the ABC-type permeases (1, 2), and phosphorylated by ribokinase (3). Lopilato et al. (4) showed genetically that at least one low affinity ribose transport system exists which shares the kinase activity with the high affinity ribose transporter. Therefore, it has been believed that a specific low affinity transporter for ribose may exist, similar to xylE for D-xylose and araE for L-arabinose. On the other hand, it was recently reported that D-ribose can be transported through another high affinity transport system for D-xylose (5) and D-allose (6), structurally related to D-ribose, by a mutational derepression allowing an elevated level of transport components.

The bacterial phosphoenolpyruvate:carbohydrate phosphotransferase system (PTS) couples translocation and phosphorylation for the PTS sugars, e.g. glucose, mannose, and manitol. Permeases of the PTS system differ in their substrate specificity but share the two general cytoplasmic PTS proteins, the enzymes I encoded by ptsI and HPr by ptsH, which sequentially transfer phosphoryl groups from phosphoenolpyruvate to their specific sugars. The glucose transporter consists of two subunits, IIAGlc encoded by the crr gene and IIBC^Glc encoded by ptsG. IIAGlc is a hydrophilic protein phosphorylated in its His-90 residue (7) and is a central regulatory molecule in catabolite repression. IIBC^Glc (PtsG) consists of the N-terminal domain with eight transmembrane peptides (8) and the hydrophilic C-terminal domain containing a phosphorylation site at Cys-421 (9). The sequence of PtsG protein is homologous to that of N-acetylglucosamine transporter, which has three domains corresponding to IIBC^Glc and IIAGlc in a single polypeptide, and that of malI product, which can complement ptsG when expressed constitutively (10).

In addition to glucose, PtsG is involved in the transport of other sugars that are structurally related to glucose, i.e. methyl α-glucoside, 2-deoxyglucose, glucosamine, mannose, 1-sorbose, and 5-thio glucose. Even though transport does not occur in the PTS system without phosphorylation, there are mutations in ptsG that allow facilitated diffusion of glucose without phosphorylation (11) or vice versa (12). Furthermore, mutation that alters the substrate specificity to allow transport of mannitol, another PTS sugar, was mapped in one of the transmembrane helices of PtsG (13). However, nothing is known about the coupling between phosphorylation and translocation or about the structure determining substrate specificity. The Mlc protein was at first reported as a factor affecting glucose transport, causing an enlarged colony size when overproduced because of a decrease in acetate production as a result of a reduced glucose utilization (14). It was recently reported that it is involved in the regulation of several sugar operons, i.e. manXYZ (14), malT (15), and ptsG (16, 17). Mlc is a 406-amino acid protein containing a putative helix-turn-helix motif at its C-terminal residue that seems to be required for DNA binding involved in a repression.

In searching for secondary D-ribose transporters different from the high affinity system, we discovered that two mutations were simultaneously required for the utilization of ribose, one at the ptsG gene, a glucose-specific PTS permease, and the

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1 The abbreviations used are: ABC, ATP binding cassette; PTS, phosphoenolpyruvate:carbohydrate phosphotransferase system; bp, base pair(s).
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TABLE I
Strains and plasmids

| Strains/plasmids | Genotype | Ref. |
|------------------|----------|------|
| Strains          |          |      |
| CP1035           | MC4100 ΔrbsB4 | —   |
| CP1036           | CP1035 mlc-101 | This work |
| CP1041           | OW1 ΔrbsB4 aics:TpophoA-I | — |
| CP1042           | OW1 aics:TpophoA-I | — |
| MC4100           | FΔargF-lacU169 rpsL relL flhD deoC ptsF rbsR | 30 |
| OW1              | leu thi his rpsK lacY xyl ara thrA tss mlc-101 | 32 |
| W3110            | F’ prototroph | 31 |
| ZSC112ΔG         | ΔptsG:CM manZ gih | 33 |
| Plasmids and phages |          |      |
| pOH106           | pUC19 mlc Ap | This work |
| pOH106           | pUC19 mlc-101 Ap | This work |
| pOH115           | pUC19 PptsG(KpnI/KpnI) Ap | This work |
| pOH116           | pUC19 PptsG(KpnI/PvuII) Ap | This work |
| pYP60            | pACYC184 rbsK | This work |
| pH8415           | lacZYA Ap | 23 |
| ARS45            | blacZ YA bla | 23 |
| ΔTnphoA132       | tnp' tet' phoA | 20 |

* Obtained from our laboratory collection.

FIG. 1. Locations of mutations in the PtsG protein. Mutations transporting D-ribose were found in residues represented by shaded circles. Residues in open circles are mutations allowing facilitated diffusion without phosphorylation (11). Residues in open boxes are mutations that result in poor translocation with phosphorylation (12). The residue in the shaded box has a change with altered substrate specificity toward D-mannitol (13).

EXPERIMENTAL PROCEDURES

Bacterial Strains, Plasmids, and Phages—All strains used are E. coli K12 derivatives that are listed in Table I. MC4100 or ZSC112ΔG was used as a parent for most strains. Cells were grown in Luria-Bertani medium, tryptone broth modified from the preparation according to Miller (18), and H1 minimal medium (19) containing 0.05% ribose, 0.2% glucose, or 0.2% xylose. Antibiotics were added to the growth medium when needed (ampicillin, 100 μg/ml; kanamycin, 25 μg/ml; chloramphenicol, 30 μg/ml; tetracycline, 15 μg/ml), and for minimal medium, they were supplied at 50%. The transposon hopping phage, λ-TnphoA132, was obtained from B. L. Wanner (20).

DNA Manipulation—The recombinant DNA techniques were performed as described by Sambrook et al. (21). A 2.1-kilobase DNA fragment of the chromosome containing the mlc gene was amplified by polymerase chain reaction. The primer complementary to the 5’ end of mlc created an XbaI restriction site at 50 bp upstream of the mlc start codon. The 3’ end region was generated with PvuI, which cuts 600 bp downstream of the mlc stop codon. The resulting 1.9-kilobase DNA fragment was inserted into the pUC19 vector previously cleaved with XbaI and SmalI. The nucleotide sequence was confirmed by DNA sequencing. The clones were named pOH106 (mlc+) and pOH108 (mlc-101).

Genetic Procedures—The P1vir phage was used for transduction experiments (18). To characterize the ptsG mutation, the TnphoA132 transposon was used to obtain an insertion linked to the mutation. Random insertion of the transposon was generated with λ-TnphoA132 for the MC4100 strain from which P1 lysate was prepared to obtain a pool of 20,000 independent insertions. Using the P1 lysate, transduction was carried out for CP1046 showing enhanced growth on ribose, and small colonies that resulted from an exchange of the mutated gene with the wild type were isolated on 0.05% ribose minimal plate containing tetacycline. Auxotrophic mutations were excluded by examining growth on 0.2% glucose minimal medium. The candidate insertions were tested for their cotransduction frequencies (Tetr) with the ribose growth phenotype to measure their linkages. For localization of the insertion sites, the transposons were transferred to the polA mutant strain (22). After appropriate recombination, the flanking regions of the insertions were characterized by DNA sequencing and through a database search with BLAST. All sequencing reactions were carried out with the DNA sequencer version 2.0 (U. S. Biochemical Corp.) and ABI PRISM™ 377 DNA sequencer (Perkin-Elmer).

Construction of the ptsG-lacZ Transcriptional Fusion—A 1074-bp fragment with KpnI ends, comprising part of the ptsG structural gene and the 455-bp upstream sequence, was obtained as a polymerase chain reaction product whose sequence was confirmed by DNA sequencing. The fragment containing the promoter region of ptsG was cloned into pUC19 using KpnI, obtained by EcoRI and BamHI digestions, and
cloned into EcoRI and BamHI sites of pRS451, a lacZ operon fusion plasmid (23), yielding pOH115. The promoter region was transferred to λRS45 and lysogenized into an appropriate host. A single-copy λ prophage was confirmed by polymerase chain reaction with three primers recognizing the attB, attP, and int genes (24). Cells were grown to an optical density of 0.4–0.8 at 30°C in H1 minimal medium (19) containing 0.4% glycerol and 0.2% glucose. β-Galactosidase activity was measured according to the method of Miller (18). All data were averaged from at least three independent experiments.

DNA Mobility Shift Assay—CP1035/pOH106 (mlc·) and CP1036/pOH106 (mlc-101) were grown to late log phase in Luria-Bertani medium containing 100 μg/ml ampicillin. Cells were harvested by centrifugation (5,000 × g) for 10 min, resuspended in 0.05 ml of 10× Tris-HCl (pH 7.0), 0.1 mM EDTA, 1 mM dithiothreitol, and 1 mM of sonicated salmon sperm DNA as a competitor. The reaction mixtures were incubated for 20 min at 25°C and subjected to electrophoresis on 5% polyacrylamide gel (60:1) with 0.5× TBE buffer (45 mM Tris borate, 1 mM EDTA) at room temperature. The gels were exposed to x-ray film (Eastman Kodak Co.) at −70°C.

Uptake Assay—Cultures grown to late log phase were harvested and washed three times with equal volumes of KEP buffer (10 mM KH2PO4 (Eastman Kodak Co.) at optical density of 0.4–0.8 at 70°C.

RESULTS

Screening of Mutations Enhancing Ribose Transport—Even with a defect in the high affinity ribose transport, the ribokinase-proficient E. coli K12 strain can use ribose as a sole carbon source, albeit less efficiently (4). Previous studies demonstrated that this growth deficiency could be partially recovered by a regulatory mutation in the allele (6) or the xylene (5) operons, both of which enhance expression of the low affinity transporter for ribose. When the high affinity transport system as well as the low affinity transporter (ΔptsK-R) and xylG (ΔTnphoA-2) were inactivated in OW1, an E. coli K12 strain, a mutation-enhancing ribose growth still appeared spontaneously at a frequency of 10−8-10−9. This indicates that there are some other loci involved in ribose transport.

To characterize those loci, transposon insertions with TnphoA132 were generated to isolate a tag associated with the mutations. About 20,000 independent insertions in MC4100 were pooled and transduced into the OW1 mutant showing enhanced growth on ribose. Analysis of the mutational locations linked to insertions revealed that two chromosomal loci, one at 36 min and the other at 25 min, are involved in the ribose growth. The high frequency (10−8-10−9) of the original mutation, which lowers the possibility of a double mutation, led us to suspect that one of the mutations might already be present in the parental strain. Indeed, the mutation at 36 min was resident in the OW1 strain, although it was not found in other E. coli strains such as MC4100 and W3110.

Further characterization of the mutations revealed that the mutation at 25 min was found in ptsG, encoding the glucose transporter of the PTS system. A total of seven independent mutants was sequenced, in which five different mutational changes were observed: one for F37Y, G176D, and G281 and two for I283T and L288Q. Repeated occurrence of the mutations indicates an apparent saturation of the changes. F37Y and G176D were found in the predicted periplasmic loops, whereas G281D, I283T, and L288Q are in the transmembrane region (Fig. 1). The mutation at 36 min was characterized to have a change in mlc that has been implicated in the regulation of ptsG (16, 17). This allele in OW1 was named mlc-101 and was sequenced to have C instead of T at the first base of Gln-369, causing an introduction of the UAG stop codon.

Effect of the mlc-101 Mutation on ptsG Expression—To test whether ribose transport through the mutated PtsGs results from an altered level of the transporter, as reported in the Als and Xyl systems (5, 6), we examined a transcriptional effect of Mic-101 on ptsG expression by using DNA mobility shift assay and the ptsG-lacZ fusion. The 568-bp KpnI/PvuII fragment containing the ptsG promoter region was obtained from pOH116 and mixed with crude extract prepared from CP1035/pOH106 (mlc·) and CP1036/pOH108 (mlc-101). A band shift was observed only with the wild-type Mic but not with the truncated Mic-101 (Fig. 2). This observation is consistent with the ptsG-lacZ fusion analysis and uptake assay of D-14C-glucose in which the ptsG transcription was derepressed at about 5-fold, whereas the uptake rates were increased about 33-fold in mlc-101 background (data not shown). Because Mic-101 lacks the C-terminal peptide containing a putative DNA binding motif, it seems likely that the protein loses its function as a repressor.

Effect of the ptsG Mutations on Ribose Transport—Growth of the various ptsG mutants were examined on minimal plate with 0.05% ribose, rates of which were comparable with that of the Rbs− strain and found to be correlated with the uptake of 14C-glucose (Table II). Among them, I283T, located in the transmembrane region, exhibited the highest growth and uptake rates. Although the uptake rates through the mutated PtsG were far less than that of the Rbs transport system, they were considerably better than the transport through the Als or Xyl systems, low affinity transporters for D-ribose (5, 6).

To investigate the effect of PtsG mutations on glucose transport, growth and uptake rates for glucose were measured. Growth showed little differences from that of wild-type PtsG (data not shown), whereas the uptake rates were reduced (Ta-
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**TABLE II**

| Genotype<sup>a</sup> | Ribose | Inhibition<sup>e</sup> of ribose uptake by | Glucose uptake<sup>f</sup> (%) |
|----------------------|--------|------------------------------------------|-----------------------------|
|                      | Plate<sup>b</sup> | Generation time<sup>c</sup> | Uptake<sup>d</sup> | Absolute | Relative | Glucose |
|                      |                 |                          | (relative rate) |
| Wild type            | + + + +         | 1.86                     | ND<sup>g</sup> | ND | ND | ND |
| *ptsG*               | +               | 9.24                     | 0.86 (1) | ND | ND | 308.56 (100) |
| F37Y                 | + + +           | 2.29                     | 5.31 (6.2) | ND | ND | 293.22 (95.0) |
| G176D                | + + +           | 2.32                     | 4.28 (5.6) | ND | ND | 256.82 (80.8) |
| C281D                | + + +           | 2.19                     | 6.52 (7.6) | 14.3 | 31.3 | 321.79 (75.4) |
| I283T                | + + +           | 2.00                     | 10.72 (12.5) | 14.3 | 31.3 | 321.79 (75.4) |
| L289Q                | + + +           | 2.69                     | 5.70 (6.7) | 11.9 | 17.9 | 257.92 (83.6) |
| *ptsG:Cm*            | ND              | ND                       | ND | ND | ND | 40.20 (13) |

<sup>a</sup> Strains for the ribose experiments, except wild type (CP1042), have the mlc-101 and ΔrbsB mutations (CP1041 derivatives). For glucose uptake assay, strains (mlc<sup>c</sup>) with the manZ and glk mutations (ZSC1123G derivatives) were used.

<sup>b</sup> Generation on 0.05% ribose minimal plate was assessed after incubation for 2 days at 37 °C.

<sup>c</sup> Generation time was measured in H1 minimal medium containing 0.05% ribose by determining Δ<sub>gen</sub>.

<sup>d</sup> Cells were grown at 37 °C in H1 minimal medium containing 0.05% ribose. Uptake assay was performed with 20 μM D-[<sup>14</sup>C]ribose as described under "Experimental Procedures." The transport rate was determined using [14C]ribose and expressed in pmol/min/10<sup>7</sup> cells.

<sup>e</sup> Calculated as (ribose uptake rate with sugar competitor/ribose uptake rate) × 100%.

<sup>f</sup>The transport activities rely only on the presence of PtsG because of an introduction of the manZ and glk mutations. Transport rate was determined with n-<sup>14</sup>C-glucose and expressed in pmol/min/10<sup>7</sup> cells.

<sup>g</sup> ND, not determined.

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**TABLE III**

| Requirement of RbsK and RbsD for ribose transport mediated by PtsG |
|-----------------------|---------------------|---------------------|
| Genotype<sup>a</sup> | Plate<sup>b</sup> | Generation time<sup>c</sup> | Uptake rate<sup>d</sup> (pmol/min/10<sup>7</sup> cells) |
|----------------------|--------|---------------------|---------------------|
| *ptsG*               | +      | 9.24                | 0.86                |
| *ptsG*-ΔrbsB4        | +      | 2.29                | 5.31                |
| *ptsG*-ΔrbsB4 Δrbs (D-R) | + + + | 27.72               | 0.23                |
| *ptsG*-Δrbs/ prbsK    | ++     | 4.65                | 2.20                |
| *ptsG*-Δrbs/ prbsK rbsA | +     | 6.54                | 2.01                |
| *ptsG*-Δrbs/ prbsK rbsC | +     | 5.02                | 1.54                |
| *ptsG*-Δrbs/ prbsK rbsD | + + + | 2.21                | 8.56                |
| *ptsG*-Δrbs/ prbsD    | ND<sup>g</sup> | ND                  | 0.25                |

<sup>a</sup> All the strains tested are CP1042 derivatives. The rbs components were supplied on plasmids.

<sup>b</sup> Measured on H1 minimal plates with 0.05% of D-ribose. Symbols represent relative levels of growth.

<sup>c</sup> Cells were grown in 0.05% ribose minimal media at 37 °C, and Δ<sub>gen</sub> was measured.

<sup>d</sup> Midlog phase culture grown in 0.05% H1 ribose minimal medium was used as described under "Experimental Procedures."

<sup>g</sup> ND, not determined.

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**DISCUSSION**

We report here that in addition to Rhs, Als, and Xyl transporters, D-ribose can be transported through PtsG when mutations through these transporters appears to be based on the structural similarities of the sugars to D-ribose as shown in Fig. 3. Interestingly, it was found that xylose can be transported even through the wild-type PtsG only when there is a mutation in mlc, indicating that the xylose transport through PtsG is dose-dependent in terms of the level of transporter (Table IV). The experiment was done in the strain with xylF::TnphoA<sup>-1</sup>, an insertion in the gene coding for the xylene-binding protein that inactivates the high affinity transporter for xylose. The OW1 strain that has xylA (xylene isomerase) and mlc mutations did not grow on xylose minimal plate in the absence of xylFGH (data not shown), indicating that as in ribose transport, xylose is phosphorylated not by the PTS system but by xylene kinase encoded by xylB. The presence of the ribose-specific mutations tends to reduce the xylose uptake rate in both the periplasmic and transmembrane mutants. The impairments of glucose uptake (Table II) are more notable in the membrane mutants than in the periplasmic ones. The degree of reduction, I283T > G281D > L289Q for both xylose and glucose, appears to be correlated with the specificity changes to ribose.
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The differences of configurations found in other sugars that are transported through PtsG are also represented. D-Ribose and D-xylose are shown in their pyranose forms.

Fig. 3. Structure of D-glucose. The differences of configurations found in other sugars that are transported through PtsG are also represented. D-Ribose and D-xylose are shown in their pyranose forms.

TABLE IV
Transport of xylose through PtsG

| Genotype* | ptsG | xylF | mlc | Generation time b | Uptake c (%) |
|-----------|------|------|-----|------------------|-------------|
| +         | +    | 101  |     | 1.50             | 173.51      |
| -         | +    | 101  |     | 1.54             | 176.22      |
| -         | +    | 101  |     | 7.37             | 18.75       |
| F37Y      | -    | +    | 9.12 | 4.72 (2.7)       |             |
| F37Y      | -    | 101  |     | 1.61             | 103.63      |
| G176D     | -    | 101  |     | 1.60             | 88.60 (50.3)|
| G281D     | -    | 101  |     | 1.46             | 151.51 (86.0)|
| I283T     | -    | 101  |     | 5.59             | 6.23 (3.5) |
| L289Q     | -    | 101  |     | 1.49             | 165.62 (94.0)|

* All strains are CP1036 derivatives.

b Strains were grown in H1 minimal medium with 0.2% D-xylose, and their optical densities were measured at 1-h intervals.

c Cells were grown at 37 °C to midlog phase in H1 minimal medium containing 0.2% D-xylose. The uptake assay was performed with 0.2 μM D-[14C]xylose as described under “Experimental Procedures.” The xylose transport rate was measured and expressed in pmol/min/10^9 cells.

tated specifically and derepressed by an inactivation of mlc, encoding a negative regulator of ptcG (16, 17). Moreover, we found that D-xylose, structurally analogous to D-glucose and D-ribose, is transported through PtsG in the absence of Mlc.

Besides glucose, PtsG is involved in the transport of other sugars that are structurally related to glucose such as mannose, 2-deoxyglucose, glucosamine, α-methylglucoside, L-sorbosone, and 5-thioglucoce (Fig. 3). Among these, the first three sugars differ from glucose at their C-2 positions, indicating flexibility in recognizing the configuration around C-2. The same is true for α-methylglucoside at the C-1 position and 5-thioglucoce at C-5. D-Mannitol (1-deoxymannose) was reported to be taken up through PtsG when Gly-320 is mutated to valine and thus modified to recognize the change of D-mannose at C-1 (19). Despite the differences in structures, the glucose analogs have some common features; they can be transported and phosphorylated by PtsG, which is not the case in D-ribose and D-xylose, which require their own kinases for recognizing a furanose form of sugar. The lack of phosphorylation coupling in transport of these sugars might lie in the fact that the hydroxyl group at C-5, normally exposed in the furanose form, is hidden in the transported sugar. The necessity of mlc mutation for the xylose transport, not found in the transport of other phosphorylated glucose analogs, may indicate that an uptake of non-phosphorylated sugar through PtsG requires a higher level of gene expression.

In addition to the C-6 hydroxyl group accepting phosphorylation, D-ribose differs from D-glucose in the hydroxyl group at C-3. Unlike D-ribose, the pyranose form of D-xylose has exactly the same configuration as D-glucose at all carbon positions except for C-6, a site for phosphorylation. Therefore, it seems likely that the configuration at C-5 is a major specificity determinant, more so than the ones at C-1, C-2, or C-6, which is consistent with the fact that an additional mutation is required for the transport of D-ribose and not for D-xylose. The tendency of the ribose-specific mutations toward losing specificities to xylose and glucose is exemplified in the extreme case of I283T. A slight deviation from this general trend is illustrated in the mutations localized in the periplasmic domain in which the glucose specificities were not substantially reduced. This may reflect the differences between the mutations in two locations, which might form two different pores with altered specificities. It is conceivable that a unique feature in the periplasmic pore lies in its discrimination of the C-6 residue. In fact, even though both glucose and xylose are transported, the xylose uptake rate was about half that of glucose, even under a derepressed condition (Table II), which may indicate that the phosphorylation of sugar affects transport, perhaps through an intramolecular signaling. In other words, there might be a coupling between transport and phosphorylation which is likely to be manifested by a structural change in PtsG during sugar phosphorylation.

The possibility of two pores in PtsG could also be discussed in terms of its structural organization. Unlike the ABC transporter, the PTS system has no periplasmic sugar-binding protein, which may explain the fact that PTS has broader substrate specificity than the ABC transporters. It seems likely that in PtsG, the periplasmic region as well as the transmembrane region confers substrate specificities, and the absence of a binding protein may be functionally compensated by the presence of the periplasmic specificity domain, which might be explained by a model proposed here as the “two-sieve mechanism.” A similar mechanism was reported to occur in the potassium channel, in which both the narrow selectivity filter recognizing K⁺ located on the outer surface and the wider, hydrophobic inner pore structure stabilizing a cation exist (28).

It is of interest to note that the D-mannitol-specific mutation of G320V was found in helix VIII (13), and three other mutations involved in facilitated diffusion, including ours, were found in helices VI and VII (11). These observations suggest that these helices form a channel for sugar specificity and transport. It was thought that the hydrophobic pocket plays an important role in sliding of sugar during translocation through the channel of PtsG. Also in the potassium channel, the lining of hydrophobic amino acids in the channel was suggested to...
facilitate a final release of ligand by loosening an association with the substrate (28). In this regard, a change into hydrophilic amino acid as found in our mutations, especially ones located in the transmembrane region, may elicit a negative effect on phosphorylation-mediated glucose transport but a positive effect on ribose transport, perhaps occurring as facilitated diffusion.

During the study of the Rbs component involved in ribose transport though PtsG, we found that RbsD is required in addition to ribokinase. RbsD was originally proposed as a member of the membrane permease associating with RbsC (29). However, it apparently lacks a region predicted to be in the membrane. Furthermore, a mutation in RbsD does not abolish ribose transport, suggesting that it is not directly involved in the permease function. There is no functional homolog found for RbsD based on its sequence similarity. The only clue about its function is that it enhances the utilization of ribose when the sugar is transported through a low affinity transporter. In other words, the role of RbsD is independent of specific types of transporters, implying that RbsD is involved in the step after membrane transport, such as acceleration of the ribose metabolism. The function of RbsD is currently under investigation.

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