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Resistance of the Melibiose Carrier to Inhibition by the Phosphotransferase System Due to Substitutions of Amino Acid Residues in the Carrier of *Salmonella typhimurium**

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The melibiose carrier of *Salmonella typhimurium* is under the control of the phosphoenolpyruvate:carbohydrate phosphotransferase system (PTS). We isolated mutants of the melibiose carrier that showed resistance to inhibition via the PTS. Growth of the mutants on melibiose was not inhibited by 2-deoxyglucose, a nonmetabolizable substrate of the PTS, although growth of the parent strain was inhibited. Transport activity of the melibiose carrier in the mutants was fairly resistant to inhibition by 2-deoxyglucose, although the activity in the parent was sensitive to inhibition. We cloned the mutated *melB* gene that encodes the melibiose carrier, determined the nucleotide sequences, and identified replaced nucleotides. The mutations resulted in substitutions of Asp-438 with Tyr, Arg-441 with Ser, or Ile-445 with Asn. All of these residues are in the COOH-terminal region of the carrier. The secondary structure of this region is predicted to be an α-helix, and the mutated residues were on the same side of the helix. This region showed sequence similarity to a region of the MalK protein, in which substitution of amino acid residues also resulted in PTS-resistant mutants. Thus the COOH-terminal portion of the melibiose carrier is important for the interaction of dephosphorylated III^{Glc}, which is an entity causing reversible inactivation of the carrier.

When cells of *Escherichia coli* or *Salmonella typhimurium* are grown in the presence of glucose and melibiose, the growth curve exhibits two successive growth cycles separated by a lag period (1, 2). This type of growth behavior has been termed diauxie. Glucose is utilized in the first growth cycle and melibiose in the second. In other words, the utilization of melibiose is inhibited by glucose. The two mechanisms responsible for this phenomenon are catabolite repression and inducer exclusion (2, 3). Catabolite repression is a control mechanism for gene expression, and inducer exclusion is a control mechanism for transport. The PTS[1] is involved in both mechanisms (4, 5). We have been investigating membrane transport and are interested in the role and the mechanism of inducer exclusion. We have shown previously that, in *E. coli*, inhibition of melibiose utilization still occurs even if catabolite repression is released by adding cyclic AMP to the growth medium (6).

Transport of substrate not only via the melibiose transport system but also via the lactose, maltose, or glycerol uptake systems is regulated (inhibited) by the PTS. It has been revealed that this inhibition of transport is caused by binding of one of the PTS proteins, dephosphorylated III^{Glc}, to the transport carriers or glycerol kinase (4). III^{Glc} is an important component of the glucose PTS (4), and its phosphorylation and dephosphorylation are crucial not only for activation or inactivation of the carriers but also for inactivation or activation of adenylate cyclase (7), which is involved in the synthesis of cyclic AMP (8, 9). Binding of III^{Glc} to the lactose carrier of *E. coli* has been investigated (10, 11). Mutants possessing an altered lactose carrier resistant to the binding of III^{Glc} (therefore resistant to inhibition by the PTS) have been isolated (12). Replacement of amino acid residues in such mutant carriers has been reported (13). PTS-resistant mutants of the maltose carrier and amino acid substitution in the carrier have been reported (14, 15).

Recently, we cloned the *S. typhimurium melB* gene that codes for the melibiose carrier, determined the nucleotide sequence, and deduced the amino acid sequence of the carrier (16). Thus it became possible to identify substituted amino acid residues in mutant carriers. We isolated mutants of *S. typhimurium* that showed PTS-resistant growth on melibiose and PTS-resistant transport of methyl-β-D-thiogalactoside. We identified the substituted amino acid residues of the carrier of the mutants by cloning and sequencing of the mutated gene. Thus a region of the melibiose carrier that is important for III^{Glc} binding was identified.

EXPERIMENTAL PROCEDURES

Bacteria and Growth—*S. typhimurium* LT2 (wild type) and PP2098, a leaky *ptsI* derivative of LT2, were used. PP2102, PP2103, PP2104, PP2105, PP2106, PP2107, PP2155, and PP2158 are 2-deoxyglucose-resistant derivatives of PP2098. *E. coli* RE16r (*melB*, *recA*, Δ*lac*) (17), DW2 (Δ*melB*, Δ*lac*) (18), PPA172 (*ptsI*-leaky, Δ*lac*), JM83 (19), and GM33 (*dam*) were used. PPA172 was isolated after diethyl sulfate mutagenesis of W3133-2 (6) and penicillin selection as a strain that could not grow on mannitol but was still able to grow on maltose and glycerol but not in the presence of 2-deoxyglucose.

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[1] The abbreviations used are: PTS, phosphoenolpyruvate:carbohydrate phosphotransferase system; III^{Glc}, glucose factor III; TMG, methyl-β-D-thiogalactopyranoside; kbp, kilobase pair(s).

For growth measurement, modified Tanaka medium (20) (Na^+ salts in the original medium were replaced with K^+ salts) supplemented with 10 mM (or 5 mM where indicated) melibiose was used. When necessary, 5 mM (or 10 mM where indicated) 2-deoxyglucose or methyl-α-glucoside (these sugars are nonmetabolizable substrates of the glucose PTS) was added. Cells were grown at 37 °C under aerobic conditions. Growth was measured turbidimetrically at 650 nm. For transport assays, cells were grown in modified Tanaka medium supplemented with 10 mM melibiose and 1% Tryptone (Difco). Cells were harvested at the exponential phase of growth, washed with modified Tanaka medium, and suspended in the same medium. For DNA propagation, L broth (21) supplemented with ampicillin (50–100 μg/ml) was used.

Isolation of Mutants—A mutant PP2098 was isolated in two steps (22): (i) introduction of *cysA1539*::Tn10 (from NK1186) into LT2 by phage P22 transduction, selecting for resistance to tetracycline (resulting strain is PP2092, *cysA1539*::Tn10); (ii) introduction of *ptsI17* from SB1476 into PP2092 by P22 transduction, selecting for growth in the absence of cysteine and no growth on mannitol (resulting strain is PP2098). This strain is hypersensitive to PTS regulation. Cells of PP2098 were unable to grow on melibiose in the presence of 2-deoxyglucose or methyl-α-glucoside. Cells of PP2098 were spread on agar plates containing 10 mM melibiose and 5 mM 2-deoxyglucose and incubated at 37 °C for a few days. Spontaneous mutants grown on the plates were isolated. All mutants used were independent isolates. Each colony obtained was checked for growth on maltose or glycerol in the absence or presence of 2-deoxyglucose. We isolated mutants that grew on melibiose but not on maltose or glycerol in the presence of 2-deoxyglucose. Thus we isolated PP2102, PP2103, PP2104, PP2105, PP2106, PP2107, PP2155, and PP2158.

Preparation of DNA—Chromosomal DNA (23) and plasmid DNA (24, 25) were prepared by published procedures. When necessary, DNA was digested with restriction endonucleases and separated by agarose (or polyacrylamide) gel electrophoresis (26).

Plasmids—Plasmids pBR322 and pBluescriptIIKS(+) were used as cloning vectors. Plasmid pTKK1 was constructed by ligating a *Sma*I-*Sma*I fragment (6 kbp) containing the *melB* region of *S. typhimurium* LT2 to the *Eco*RV site of the pBR322. Three deletion derivatives of pTKK1 were constructed that each lacked a part of the *melB* gene and were used to clone various parts of the mutant *melB* gene. pTKK11 lacks the central part of *melB* (*Eco*RV-*Eco*RV fragment), pTKK12 lacks the downstream region of *melB* (*Nru*I-*Nru*I fragment; the second *Nru*I site is in the vector pBR322), and pTKK13 lacks the upstream region of *melB* (*Kpn*I-*Kpn*I fragment).

Cloning and Subcloning of Mutated melB—The procedure developed by Comeau and Inouye (27) for cloning of a mutated gene was used. Plasmid pTKK1 was used as host plasmid. The plasmid possessing the *melB* region derived from PP2102 is pTKK2102, and so on. Each part of the mutant *melB* (*Eco*RV-*Eco*RV fragment, *Nru*I-*Nru*I fragment, and *Kpn*I-*Kpn*I fragment) was cloned using pTKK11, pTKK12, and pTKK13 as vector. Plasmids carrying each of the three parts derived from pTKK2102 were designated pTKK11-2102, pTKK12-2102, and pTKK13-2102, and so on.

DNA Sequencing—The nucleotide sequences were determined by the dideoxy chain termination method (28, 29).

Southern Blot Analysis—Southern blot analysis was performed by a published method (30), and DNA was detected using the ECL DNA detection kit (Amersham Corp.). An *Eco*RV-*Hae*III fragment derived from the *melB* gene was used as a probe.

Assays—Activity of α-galactosidase was measured as described previously (31). Transport of Na^+ elicited by melibiose influx (32) or transport of [^{14}C]TMG (33) was measured as described previously. Protein contents were determined by the method of Lowry *et al.* (34).

Materials—Restriction endonucleases were from Nippon Gene Co., Takara Shuzo Co., or New England Biolabs. [α-^{35}S]dCTP and [α-^{32}P]dCTP were from Amersham Japan Co., [^{14}C]TMG was from Du Pont-New England Nuclear, and Sequenase (a sequencing kit) was from U. S. Biochemical Corp. Plasmid pBluescriptIIKS(+) was from Stratagene Inc., and KS and SK primers were synthesized by a DNA synthesizer. In some cases, sequencing primers were synthesized based on the nucleotide sequence of wild type *melB* of *S. typhimurium*.

RESULTS

Properties of the Mutants—S. typhimurium PP2098 is a leaky *ptsI* mutant and is hypersensitive to PTS sugars, such as 2-deoxyglucose or methyl-α-glucoside. PP2102 is a mutant that is resistant to such PTS sugars. We tested the effects of the nonmetabolizable PTS sugars on the growth of the mutants. In fact, PP2098 was unable to grow on melibiose as a sole source of carbon in the presence of 2-deoxyglucose (Fig. 1) or methyl-α-glucoside (data not shown). On the other hand, mutant PP2102 grew on melibiose even in the presence of 2-deoxyglucose or methyl-α-glucoside. It is noteworthy that the growth of both PP2098 and PP2102 on maltose or glycerol was strongly inhibited by both 2-deoxyglucose and methyl-α-glucoside (data not shown). We obtained similar results with other mutants, PP2103, PP2104, PP2105, PP2106, PP2107, PP2155, and PP2158 (data not shown). Therefore, the release of growth inhibition in these mutants is specific for melibiose. This suggests that the mutations in these mutants are localized in the melibiose operon but not in the genes of components of the PTS, such as *crr* encoding III^{Glc}. It should be pointed out that *S. typhimurium* does not possess the lactose system, and the melibiose system is the sole system for transport of melibiose.

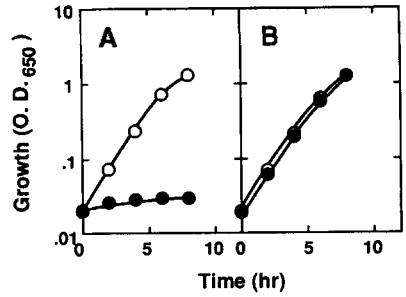

FIG. 1. **Effects of 2-deoxyglucose on growth.** Cells of *S. typhimurium* PP2098 (parent) (*A*) and PP2102 (mutant) (*B*) were grown in modified Tanaka medium supplemented with 10 mM melibiose as the carbon source in the absence (○) or presence (●) of 5 mM 2-deoxyglucose at 37 °C under aerobic conditions. The growth was monitored turbidimetrically at 650 nm.

Although our results indicate that mutations in the mutant PP2102 exist in the melibiose operon, as described above, two types of mutations in the operon seem to be possible. One type is a mutation in the regulatory region of the operon. If overexpression of the operon takes place, a large amount of the melibiose carrier could be synthesized, and the limited amount of III^{Glc} present in cells would not be able to inhibit all of the melibiose carrier. In this case, we should detect elevated activities of α-galactosidase (*melA* product) and the melibiose carrier (*melB* product). In fact, we obtained a number of these mutations in *E. coli*.[2] The second type of mutation is in the *melB* gene. In this case, normal levels of α-galactosidase activity and melibiose transport activity and PTS-resistant transport would be observed. We are interested in the latter type of mutants.

We measured the α-galactosidase activity of the mutants and found that the activity in all of the mutants was almost the same as that of the parent (PP2098) (data not shown). Thus it seemed that the mutations were not in the regulatory region of the operon, but in the *melB* gene.

We then tested the effects of the nonmetabolizable PTS sugars on transport activity of the melibiose carrier by two methods. In the first method, we measured the uptake of [^{14}C]TMG (Table I). The uptake of TMG was inhibited about 70% by 5 mM 2-deoxyglucose in PP2098 (parent), whereas the TMG uptake in the mutants (PP2102–PP2106, PP2155, and PP2158) was very resistant (0–15% inhibition). In the second method, we measured uptake of Na^+, elicited by the addition

[2] M. Kuroda, S. de Waard, K. Mizushima, M. Tsuda, P. Postma, and T. Tsuchiya, unpublished observations.

TABLE I

Effects of 2-deoxyglucose on TMG uptake via the melibiose system in mutants

Cells were grown in modified Tanaka medium supplemented with 10 mM melibiose and suspended in modified Tanaka medium plus 10 mM NaCl. Potassium lactate (5 mM) was added as energy donor. Transport was measured in the absence or presence of 5 mM 2-deoxyglucose under aerobic conditions at 25 °C. The assay was initiated by adding [^{14}C]TMG (final concentration, 1 mM). Samples were taken at 5 min.

Strain	TMG uptake		
	−2-Deoxyglucose	+2-Deoxyglucose	Inhibition
	nmol·mg cell protein^{-1}		%
Experiment 1			
PP2098	30	9	70
PP2102	25	24	4
PP2103	28	31	0
PP2104	35	33	6
PP2105	31	28	10
PP2106	30	29	3
Experiment 2			
PP2098	24	8	67
PP2155	20	18	10
PP2158	20	17	15

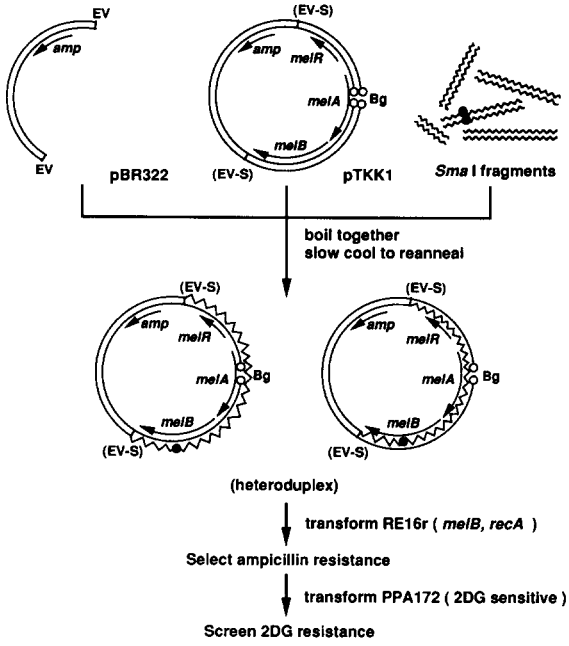

FIG. 2. **Cloning strategy of the mutated *melB* gene.** For details, see "Experimental Procedures." *EV*, *Eco*RV site; *S*, *Sma*I site; *Bg*, *Bgl*II site; *2DG*, 2-deoxyglucose.

of melibiose to a cell suspension, using an Na^+-selective electrode. Since the melibiose carrier mediates cotransport of Na^+ and melibiose, influx of melibiose into cells elicits the Na^+ uptake (35). The Na^+ uptake was inhibited 75% by 2-deoxyglucose in PP2098, whereas it was resistant in PP2102–PP2106, PP2155, and PP2158 (5–25% inhibition) (data not shown).

Thus, all of the mutants we tested seem to possess mutations in the *melB* gene and are suitable for our purpose and for further analysis.

Cloning of the Mutated melB Gene—For cloning of the mutated *melB* gene, we used a procedure developed for the cloning of the mutated *envZ* gene (27) (Fig. 2). Chromosomal DNA of mutant PP2102 was digested with the restriction endonuclease *Sma*I, and vector pBR322 was digested with *Eco*RV. Plasmid pTKK1, which carries the wild type *melB* gene, was digested with *Bgl*II and dephosphorylated. These DNA fragments were mixed, denatured by high temperature, and reannealed. Competent cells of *E. coli* RE16r (*melA*$^+$*B*$^-$) were transformed with the DNA mixture and spread onto agar plates containing L broth and ampicillin (50 μg/ml). Ampicillin-resistant colonies were then checked for growth on melibiose. Plasmids were prepared from the cells that were able to grow on melibiose, and the size of the plasmids was checked. Plasmids of proper size were then introduced into competent cells of *E. coli* PPA172 (*ptsI*), and growth of the transformants on melibiose in the absence or presence of 2-deoxyglucose was tested on agar plates. Plasmids were isolated from cells that showed 2-deoxyglucose-resistant growth, and designated pTKK2102. Plasmid pTKK2102 is expected to possess the *melB* gene derived from PP2102. Plasmids pTKK2103, pTKK2104, pTKK2105, pTKK2106, pTKK2107, pTKK2155, and pTKK2158, which carry the *melB* region of PP2103 to PP2158, respectively, were similarly constructed.

Fig. 3 shows growth of PPA172/pTKK1 and PPA172/pTKK2102 on melibiose in the absence or presence of 2-deoxyglucose. Cells of PPA172/pTKK2102 grew on melibiose even in the presence of 2-deoxyglucose, whereas cells of PPA172/pTKK1, which carry the wild type *melB* gene, did not. Thus it seems clear that pTKK2102 carries the *melB* gene derived from the mutant PP2102. Similar results were obtained with cells harboring plasmids carrying the mutated *melB* gene of other mutants (data not shown).

We tested the effects of 2-deoxyglucose on the activity of the melibiose carrier derived from the pTKK2102. Because PPA172 possesses its own melibiose carrier, pTKK2102 was introduced into *E. coli* strain DW2 that lacks the melibiose carrier. The carrier activity was tested by measuring Na^+ uptake elicited by melibiose influx. The Na^+ uptake in DW2/pTKK2102 was not inhibited significantly, whereas that in DW2/pTKK1 was strongly inhibited (85% inhibition). Thus we confirmed that the 2-deoxyglucose-resistant growth of PPA172/pTKK2102 was due to the *melB* gene carried by the plasmid pTKK2102. Similar results were obtained with cells of DW2 harboring a plasmid carrying the other mutated *melB* gene (data not shown).

Next we tried to do intracistronic mapping of the mutations in the *melB* gene of the mutants. Three types of plasmids (for example, pTKK11-2102, pTKK12-2102, and pTKK13-2102), each carrying a different segment of the mutant *melB* gene, were constructed (Fig. 4). Competent cells of PPA172 were transformed with the plasmids, and growth of PPA172/pTKK11-2102, PPA172/pTKK12-2102, and PPA172/

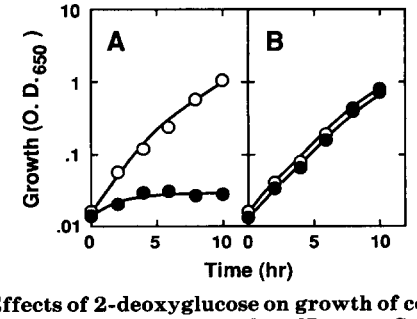

FIG. 3. **Effects of 2-deoxyglucose on growth of cells harboring a plasmid that carries a cloned *melB* gene.** Growth of cells of *E. coli* PPA172 harboring a plasmid carrying wild type *melB* gene (PPA172/pTKK1) (*A*) or mutated *melB* gene (PPA172/pTKK2102) (*B*) derived from *S. typhimurium* was measured in modified Tanaka medium supplemented with 5 mM melibiose in the absence (○) or presence (●) of 10 mM 2-deoxyglucose. Cells were grown at 37 °C under aerobic conditions. The growth was monitored turbidimetrically at 650 nm.

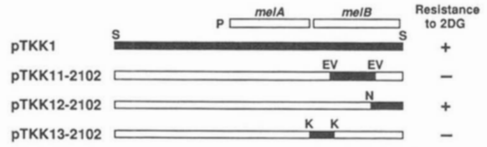

FIG. 4. **Intracistronic mapping of mutations.** Plasmids carrying various portions (*thick black bar*) of the mutant *melB* gene were constructed. Growth of cells of *E. coli* PPA172 harboring each plasmid was tested on a plate containing modified Tanaka medium supplemented with 5 mM melibiose in the presence of 10 mM 2-deoxyglucose (*2DG*) at 37 °C for 24 h. + indicates growth, and – indicates no growth. *P*, promoter; *S*, *Sma*I site; *EV*, *Eco*RV site; *N*, *Nru*I site; *K*, *Kpn*I site.

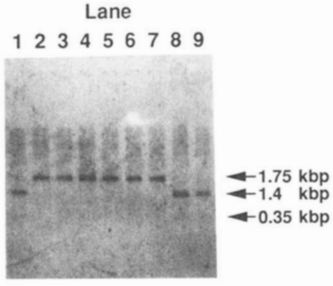

FIG. 5. **Southern blot analysis of chromosomal DNA of wild type and mutants.** Chromosomal DNA of parent and mutants was digested with a restriction endonuclease *Bst*YI and applied to 1% agarose gel. After electrophoresis, Southern blot analysis was performed using an ECL DNA detection kit (Amersham) with *Eco*RV-*Hae*III fragment (489 base pairs) of the *melB* gene as a probe. *Lane 1*, PP2098; *lane 2*, PP2102; *lane 3*, PP2103; *lane 4*, PP2104; *lane 5*, PP2105; *lane 6*, PP2106; *lane 7*, PP2107; *lane 8*, PP2155; *lane 9*, PP2158.

pTKK13-2102 on melibiose was tested in the absence or presence of 2-deoxyglucose. Only PPA172 cells harboring pTKK12-2102 grew in the presence of 2-deoxyglucose (Fig. 4). Thus the mutation of PP2102, responsible for the resistance to the PTS sugar, is localized in the *Nru*I-*Nru*I fragment of the *melB* region, which corresponds to the COOH-terminal portion of the melibiose carrier. We obtained the same result with all other mutants tested (data not shown).

Sequence Analyses—We determined the nucleotide sequences of the *Nru*I-*Nru*I DNA fragment derived from mutated *melB* of PP2102. We found only one nucleotide replacement in the DNA region. The replacement was T with A at position 1,334 starting from A of initiation ATG of the *melB* gene (16). This replacement results in the amino acid substitution of Ile-445 with Asn in the melibiose carrier.

The nucleotide sequence of the region where the mutation was found is AGATCC in the wild type and AGAACC in the mutant. A restriction endonuclease *Bst*YI cleaves the AGATCC site, but not the AGAACC site. To test whether or not we can detect a difference in the digestion pattern between the *melB* region of wild type cells and the mutant cells, the chromosomal DNA from each strain was prepared, digested with *Bst*YI, and analyzed by Southern blot hybridization. In fact, we detected cleavage of wild type *melB* at the expected site (Fig. 5) and DNA bands of 1.4 and 0.35 kbp. But the *melB* region of the mutant PP2102 was not cleaved, and we observed a DNA band of 1.75 kbp (Fig. 5). Surprisingly, we found the same digestion patterns in the *melB* regions derived from PP2103, PP2104, PP2105, PP2106, and PP2107 with *Bst*YI. On the other hand, we observed the same digestion pattern in the *melB* region of PP2155 and PP2158 as that of the parent. These results indicate that PP2103–PP2107 have the same mutation in the *melB* gene as PP2102, and PP2155 and PP2158 have different mutation(s). Consistent results were obtained when plasmids carrying cloned *melB* regions derived from the mutants were propagated in GM33 (*dam*) cells, isolated, digested with *Mfl*I, which cleaves the same site as *Bst*YI, and analyzed by agarose gel electrophoresis (data not shown).

In fact, we found the same replacement of nucleotide in the *melB* gene of PP2103–PP2107 and different replacements in PP2155 and PP2158. The nucleotide replacements were G with T at position 1,312 in PP2155 and C with A at position 1,321 in PP2158. Thus we identified amino acid substitutions Asp-438 with Tyr and Arg-441 with Ser, in addition to Ile-445 with Asn (Table II).

DISCUSSION

E. coli and *S. typhimurium* are closely related microorganisms (36). We introduced the *melB* gene of *S. typhimurium* into *E. coli* cells; and the *melB* gene was functional in *E. coli* cells. The melibiose carrier of *S. typhimurium* synthesized in *E. coli* showed properties similar to those of the carrier synthesized in *S. typhimurium* cells. Furthermore, the wild type melibiose carrier of *S. typhimurium* was sensitive to the PTS of *E. coli*. Roseman and co-workers (37) demonstrated that the lactose carrier of *E. coli* reconstituted into membrane vesicles of *S. typhimurium* was sensitive to the PTS of *S. typhimurium*. In other words, III^{Glc} of *S. typhimurium* is able to regulate the lactose carrier of *E. coli*. It is reported that the sequence homology in III^{Glc} of *E. coli* and *S. typhimurium* is 98% (38, 39).

We isolated mutants of *S. typhimurium* carrying the melibiose carrier that became resistant to inhibition via the PTS. The mutant cells grew on melibiose in the presence of 2-deoxyglucose, a nonmetabolizable PTS sugar, although the parental cells did not. This means that the transport process for melibiose is crucial for cell growth in those cells under such conditions.

Since the *melB* genes, encoding the mutant melibiose carriers, were subsequently cloned on a multicopy plasmid, the resulting strains might become resistant to inhibition by PTS sugars due to overproduction of the melibiose carrier (40). However, from Fig. 3 it is clear that cells containing the pTKK1 plasmid (encoding the wild type melibiose carrier) are still sensitive to inhibition by 2-deoxyglucose. In cells containing this plasmid, the activity of the melibiose carrier is about three times higher compared with control cells (data not shown). Clearly, this level of the melibiose carrier is not sufficient to escape from inhibition via III^{Glc}.

Recently, we determined the nucleotide sequences of the wild type *melB* gene of *S. typhimurium* (16). Thus it became possible to determine altered nucleotides in the *melB* gene of mutants. As a result of cloning and sequencing, we identified the replaced nucleotides in the *melB* gene of the mutants that became resistant to PTS sugars. The deduced amino acid substitutions in the melibiose carrier of the mutants were Asp-438 with Tyr, Arg-441 with Ser, and Ile-445 with Asn.

TABLE II

Replacements of nucleotides and substitutions of amino acid residues

Replacements of nucleotides in the *melB* gene of mutants and substitutions of amino acid residues in the melibiose carrier identified in this study are listed.

Replacement		Mutant
Nucleotide	Amino acid residue	
G-1312 with T	Asp-438 with Tyr	PP2155
C-1321 with A	Arg-441 with Ser	PP2158
T-1334 with A	Ile-445 with Asn	PP2102–PP2107[a]

[a] Six mutants.

All of these residues are in the COOH-terminal region of the carrier. Surprisingly, we found the same amino acid substitution (Ile-445 with Asn) in six mutants that were isolated independently. This suggests that Ile-445 is very important for the regulation of the carrier by III^{Glc}. Since Asp-438, Arg-441, and Ile-445 are all located in the COOH-terminal region of the carrier, it is very likely that this COOH-terminal region is involved in III^{Glc} binding. Judging from the positions of the identified residues (438, 441, and 445), it seemed that these residues could be present on the same side if this region is in an α-helix form. In fact, calculation by the method of Chou and Fasman (41) predicted an α-helix structure in this region (data not shown). A vertical view of the α-helix of this region showed that Asp-438, Arg-441, and Ile-445 are really on the same side of the α-helix (Fig. 6). Therefore, it is likely that these 3 residues are involved in interaction with III^{Glc}. It would be interesting to substitute Arg-452, which is on the same side of the α-helix as Asp-438, Arg-441, and Ile-445 (Fig. 6), with another residue by site-directed mutagenesis. Among the 3 identified residues, 2 residues possess a charge (Asp and Arg) that might be important for binding (or interaction) with III^{Glc}.

We proposed a topological model of the melibiose carrier of *E. coli* based on several types of analysis (42). Since the sequence homology between the melibiose carriers of *E. coli* and of *S. typhimurium* is very high (16), the topology of the two carriers would be very similar. Fig. 7 shows the topological model of *S. typhimurium* melibiose carrier. According to this model, the COOH-terminal portion of the melibiose carrier of *S. typhimurium* is on the cytoplasmic side. Since III^{Glc} is a soluble cytoplasmic protein, this model is convenient for understanding the interaction between the III^{Glc} and the carrier.

Saier and co-workers (14) identified substituted amino acid residues in the MalK protein of PTS-resistant mutants. Two of the substitutions are Gly-278 and Gly-284. They pointed out that this region of the MalK protein showed some similarity of amino acid sequence to the central loop portion of the melibiose carrier of *E. coli*. The amino acid sequences of the melibiose carriers of *S. typhimurium* and of *E. coli* are highly homologous, and the sequences of the central loop portions of the two melibiose carriers are very similar (92% homology including conservative changes) (16). Thus, it is likely that the central loop portion of the melibiose carrier of *S. typhimurium* is involved in the binding of III^{Glc}. The central loop portion is on the cytoplasmic side according to our topological model (Fig. 7). It has been reported that a central loop portion of the *E. coli* lactose carrier is important for binding of III^{Glc} (13). Perhaps the COOH-terminal portion and the central loop portion of the melibiose carrier are in close contact and form a binding site for III^{Glc}. In the case of the lactose carrier, it seems that the NH_2-terminal portion is also involved in the III^{Glc} binding (43). Analysis of more mutants will clarify domains that are involved in III^{Glc} binding.

We also found sequence similarity between the COOH-terminal region that contained Ile-445 of the melibiose carrier and a region of PTS-resistant MalK in which amino acid substitution (Ala-124 with Thr) was found (14) (Fig. 8). Thus it seems reasonable to assume that these regions are really involved in regulation by the PTS, namely binding of III^{Glc}.

Both the melibiose carrier and the lactose carrier mediate the cotransport of H^+ and melibiose (and other galactosides). The two carriers are similarly regulated by the PTS. However, no significant sequence similarity was found between the two carriers (44, 45). Interestingly, hydropathy patterns and topological models of the two carriers are similar and suggest 12 transmembrane segments (42, 46). Although the sequence similarity is low, the three-dimensional structure of the binding site for III^{Glc} in the two carriers could be similar.

The hydrophilic COOH-terminal part of the melibiose carrier of *S. typhimurium* (16) and *E. coli* (44) is almost 30 residues longer than that of the lactose carrier (45). It was not clear whether this long COOH-terminal portion of the melibiose carrier is really necessary for the function of the carrier. It has been reported that introduction of a termination codon in position 434, 440, or 446 of the melibiose carrier of *E. coli* (original length is 469 residues) resulted in a reduction of activity to 25% of that of the intact carrier (47). However, truncation at position 460 or 456 caused no significant reduction in transport activity. These results indicate that the 14 COOH-terminal residues of the carrier are not necessary for carrier activity and that the residues at positions 15–36 from

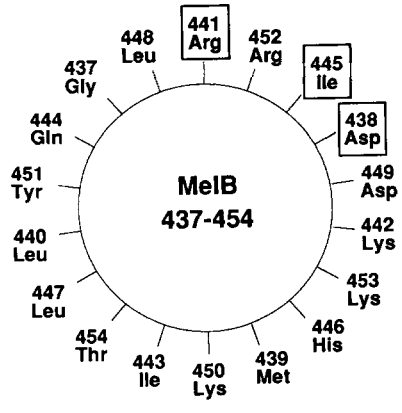

FIG. 6. **Vertical view of a putative α-helix of the COOH-terminal region of the melibiose carrier.** A region from Gly-437 to Thr-454 is shown. Substituted residues in the mutant carriers are *boxed*.

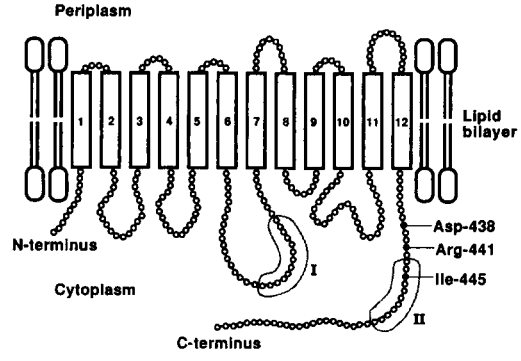

FIG. 7. **Location of substituted amino acid residues in topological model of the melibiose carrier of *S. typhimurium*.** Numbers from *1* to *12* indicate the 12 putative membrane-spanning regions of the melibiose carrier. The substituted residues identified in PTS-resistant melibiose carrier are in the COOH-terminal portion, which is on the cytoplasmic side of the membrane. Two regions (*I* and *II*) surrounded by the *dotted line* are regions that showed sequence similarity to suggested sequences for III^{Glc} binding in MalK, LacY, and GlpK (region *I*) (14) or in MalK (region *II*) (see Fig. 8).

```
MelB (S.t.)   443  Ile Gln Ile  -  His Leu Leu Asp Lys Tyr Arg  -  Lys Thr
                   *   *   *      *   *   *   *   *   *   *      *   *
MelB (E.c.)   439  Ile Gln Ile  -  His Leu Leu Asp Lys Tyr Arg  -  Lys Thr
                   .   *   .      *   *   *   *   .       .      *
MalK (E.c.)   121  Leu Gln Leu Ala His Leu Leu Asp Arg  -  Lys Pro Lys Ala
```

FIG. 8. **Sequence similarity between the COOH-terminal region of MelB and a portion of MalK.** Amino acid sequences of the COOH-terminal region of *S. typhimurium* MelB (*S.t.*) and *E. coli* MelB (*E.c.*) and a central region of *E. coli* MalK (*E.c.*) are shown. Identical residues (*) and conservative changes (•) are indicated.

the COOH terminus are fairly important. The Ile-445 is at position 28 from the COOH terminus. Thus this region is important not only for the binding of III^{Glc} but also for the total function of the carrier.

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