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Altered Sugar Selection and Transport Conferred by Spontaneous Point and Deletion Mutations in the Lactose Carrier of *Escherichia coli*

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Abstract. Spontaneous mutants harboring the *lac*Y gene on an F′-factor were isolated. Those mutants that failed to grow on 5 mM lactose minimal media plates were chosen for further study. The mutants showed striking mutations in the lactose carrier as well as in sugar selection properties during transport assays. DNA sequencing of the *lac*Y gene of the mutants revealed the following mutations: M-1-I, R-144-W, G-370-C and a deletion of residues 387–392, located in helix 12 of the carrier. Transport studies indicated that ONPG transport ranged between 8 and 25% of normal for the M-1-I, G-370-C and Δ387–392 mutants and 51% of normal for the R-144-W mutant. The downhill transport of lactose was 2-fold greater than for melibiose in cells harboring the M-1-I mutation and 3-fold higher for cells with the G-370-C mutation. On the other hand, cells with the Δ387–392-deletion mutation showed no lactose downhill transport, but 47% melibiose transport. Accumulation of TMG, a lactose analog, was 3-fold higher than the accumulation of melibiose in cells with the G-370-C mutation. On the other hand, in cells with the Δ387–392 mutation, TMG accumulation was completely defective, whereas melibiose accumulation was 50-fold higher than that of TMG, indicating that one or more of these residues in helix 12 of the carrier play a role in the active transport of β-galactoside, but not α-galactoside sugars. Initial lactose downhill transport rates were too unreliable to obtain trustworthy kinetic data. TMG and melibiose accumulation activities were present, but severely reduced in the mutant containing the R144W mutation, confirming that Arg-144 is important for active transport. All transport data were normalized for expression levels. The results indicate that the affected residues play a role in dictating sugar specificity and transport in the lactose carrier. The results here are novel in that they represent mutations in unique locations along the lactose carrier protein. For example, the M-1-I mutation was located at the N-terminal cytoplasmic tail of the carrier. Furthermore, G-370-C was located in the periplasmic loop between helices 11 and 12, suggesting a role for residues in this loop in mediating sugar selection.

Key words: Lactose — Melibiose — Sugar — Transport — Bacteria — Specificity — Permease — Mutant

Introduction

Active transport of nutrients, such as sugars, is required for the growth of all cells. The lactose carrier of *Escherichia coli* is a well-studied secondary active transporter that catalyzes the accumulation of the sugar lactose against a concentration gradient using the proton motive force via symport, i.e., sugar-cation cotransport [32, 33]. The *lac*Y gene, a structural gene of the *lac* operon [34], was cloned [46], sequenced in 1977 [7] and found to encode the lactose carrier consisting of a hydrophobic integral membrane protein of 417 amino acids [11] and residing in the inner membrane of *E. coli* [For reviews, *see* 6, 20 and 50]. The LacY protein has been purified to homogeneity [35] and likely functions as a monomer [40, 42]. Evidence for the current structural model of the lactose carrier is based on biochemical and biophysical studies [53, 58–60], experiments with fusions to reporter proteins [8, 47] and second-site revertant analyses from primary mutants with altered charged amino acids [21, 26, 27]. It is widely believed that the lactose carrier has twelve transmembrane α-helices and that the N- and C-termini

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are located in the cytoplasm [9, 19, 44, 52]. The lactose carrier has been extensively studied by site-directed cysteine-scanning mutagenesis [12] and by characterization of spontaneous mutants with alterations in energy coupling [5, 10, 17, 24, 29, 31], kinetics [29, 37, 38] and substrate selection profiles [4, 6, 10, 22, 23, 30, 36, 48]. In addition, experiments exploring the lipid and protein requirements for insertion of LacY into the membrane have been useful [2, 3]. The lactose carrier has also been studied considerably using a variety of biophysical techniques in order to access the orientation of the α-helices within the membrane [18]. For these reasons, the lactose carrier of *E. coli* is an important model system for structural and functional studies of bacterial solute transport.

Considerable effort has been placed on determining the crystal structure of the lactose carrier [39] due to the importance of structure-function analyses of transporters. However, integral membrane proteins are difficult to crystallize due to their hydrophobic nature. To circumvent this, genetic engineering tools have been used to create variants of the lactose carrier that would allow crystallization. The discovery of salt bridges has been tremendously useful in determining the spatial relationships of the α-helices [20, 21, 26, 27]. Unfortunately, a high-resolution structure of the lactose carrier has not been elucidated to date. Thus, it is necessary to study the structure-function relationships of this important model transporter by analysis of mutants with altered transport properties.

We have previously found lactose transporter mutants with more severe defects in the transport of lactose than of melibiose [48]. The mutations were found in the amino acids Tyr-26, Phe-27, Phe-29, Asp-240, Leu-321 and His-322, indicating that these amino acids line the sugar-binding site and may play a role in sugar selection and transport [48]. More recently, we found LacY mutants with severe defects in both sugar recognition and in their ability to actively accumulate sugars [49]. The implicated lactose carrier residues are Met-23, Trp-151, Gly-257, Ala-295, and Gly-377. Two of these mutants were novel in that they harbored the first mutations (G257D and G377V) within periplasmic loops to be implicated in sugar recognition. All previous LacY sugar recognition mutants heretofore had been found within transmembrane domains.

Here, we report spontaneous point and deletion mutations in the lactose carrier of *E. coli*. The mutations involved Met-1-Ile, Arg-144-Trp, Gly-370-Cys and a deletion of residues 387–392, which reside in helix 12 of the lactose carrier. From transport assays, we observed alterations in the substrate specificities in each of the mutants, such as differences in downhill and uphill transport properties between α- versus β-galactoside sugars.

Materials and Methods

Reagents

Lactose, melibiose, methyl β-D-thio-galactoside (TMG), isopropyl-β-D-thio-galactopyranoside (IPTG), methyl-α-D-galactopyranoside (α-MG), *o*-nitro-phenyl-β-D-galactopyranoside (ONPG), β-thio-*o*-nitro-phenyl-β-D-galactopyranoside (TONPG), 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal) and (3-[N-morpholino] propanesulfonic acid) buffer (MOPS) were from Sigma. Reagents for PCR-based DNA amplification and DNA cycle sequencing were from Qiagen and Perkin-Elmer, respectively. DNA was manipulated by using kits purchased from New England Biolabs, Qiagen and Pharmacia Biotech. Radioactive lactose-(glucose-1-[^{14}C]) was purchased from Sigma. Radioactive [^{14}C]-TMG was from DuPont New England Nuclear. Radioactive [^{3}H]-melibiose was the generous gift of Prof. Thomas H. Wilson (Harvard Medical School). Bacteriological media were from Difco.

Bacterial Strains

The bacterial strains in this study are all derivatives of *E. coli* K-12. The relevant genotypes are shown in Table 1. All *E. coli* DP90 cultures were grown at 37°C in LB broth or on LB agar containing 20 μg/ml nalidixic acid (nalidixic acid resistance gene is located on the chromosome). All *E. coli* DW2 cultures were grown at 37°C in LB broth containing 20 μg/ml streptomycin (streptomycin resistance gene is located on the chromosome). Maintenance of the F′ factor containing either the wild-type or mutant *lac*Y genes on the *lac* operon in either *E. coli* DP90 or DW2 strains was performed by selection for blue colonies on LB plates containing either 20 μg/ml nalidixic acid or 20 μg/ml streptomycin, respectively, plus 97 μM X-Gal and 0.5 μM IPTG and grown overnight at 37°C.

Isolation of Mutants

Mutants with defects in the gene encoding the lactose carrier were isolated as described previously [48, 49]. Briefly, *E. coli* strain DP90/F′I^{Q}Z^{+}Y^{+} was plated on minimal media plates containing 0.2% succinate, 3 mM TONPG and 0.5 mM IPTG and incubated for several days at 37°C. Colonies that grew on these plates were plated onto 1% lactose MacConkey agar. Colonies that were white on these plates were used to inoculate plates with 1% lactose MacConkey agar plus 0.5 mM IPTG, which were then incubated overnight at 37°C. Colonies that were red or red center on these plates were chosen, plated onto 5 mM lactose minimal media plates and incubated at 37°C. Bacterial mutants that failed to grow on these plates were chosen for further study and archived at −80°C.

In another case, one mutant designated as AE60 was isolated by Hobson et al. [16] by chemical mutagenesis and selecting for mutants that grew in a *gal*E background *E. coli* strain DCF41Nal by probably surviving the buildup of uridine 5′-diphosphate glucose due to a defective LacY function. This mutant was chosen for further study based on the observation that it failed to grow on 5 mM lactose minimal plates. The F′ factor from the AE60 mutant was transferred by mating into strain DW2 and plating onto LB agar plates containing 20 μg/ml streptomycin (to kill the donor cells), 97 μM X-Gal and 0.5 μM IPTG and selecting for blue colonies. The AE60 was then transferred to strain DP90 by mating and selecting as above in the presence of 20 μg/ml nalidixic acid.

Sequencing of Mutants

E. coli DP90 cells containing the F′ factors were grown in LB broth plus 20 μg/ml nalidixic acid and incubated overnight to saturation

Table 1. Bacterial strains and genotypes used

Strain	Genotype (Chromosome/F′ factor)	Reference or source
DW2	*lac*I$^+$ Δ(ZY) *mel*A$^+$ ΔB *rsp*L/	56
DW2-Y$^+$	*lac*I$^+$ Δ(ZY) *mel*A$^+$ ΔB *rsp*L/ F′*lac*I^{Q}Z$^+$Y$^+$ *pro*AB	16
DW2-ΔY	*lac*I$^+$ Δ(ZY) *mel*A$^+$ ΔB *rsp*L/ F′*lac*I^{Q}Z$^+$ ΔY *pro*AB	48
DW2-M1I	*lac*I$^+$ Δ(ZY) *mel*A$^+$ ΔB *rsp*L/ F′*lac*I^{Q}Z$^+$Y (Met-1→ Ile) *pro*AB	This work
DW2-R144W	*lac*I$^+$ Δ(ZY) *mel*A$^+$ ΔB *rsp*L/ F′*lac*I^{Q}Z$^+$Y (Arg-144→ Trp) *pro*AB	16
DW2-G370C	*lac*I$^+$ Δ(ZY) *mel*A$^+$ ΔB *rsp*L/ F′*lac*I^{Q}Z$^+$Y (Gly-370→ Cys) *pro*AB	This work
DW2-Δ387–392	*lac*I$^+$ Δ(ZY) *mel*A$^+$ ΔB *rsp*L/ F′*lac*I^{Q}Z$^+$Y (Δ387–392) *pro*AB	This work
DP90-Y$^+$	Δ*lac-pro* Nal^r/F′*lac*I^{Q}Z$^+$Y$^+$ *pro*AB	16
DP90-ΔY	Δ*lac-pro* Nal^r/F′*lac*I^{Q}Z$^+$ΔY *pro*AB	48
DP90-M1I	Δ*lac-pro* Nal^r/ F′*lac*I^{Q}Z$^+$Y (Met-1→ Ile) *pro*AB	This work
DP90-G370C	Δ*lac-pro* Nal^r/ F′*lac*I^{Q}Z$^+$Y (Gly-370→ Cys) *pro*AB	This work
DP90-Δ387–392	Δ*lac-pro* Nal^r/ F′*lac*I^{Q}Z$^+$Y (Δ387–392) *pro*AB	This work

at 37°C. Genomic DNA was prepared from these cells according to the method of Wilson [57]. The DNA region coding for lactose carrier gene *lac*Y from the F′ factor DNA was amplified by PCR using primers specific for the flanking regions of the *lac*Y gene and prepared by the Harvard Medical School Oligonucleotide Synthesis Facility. The amplified DNA was isolated and purified with a QIAquick PCR purification kit from Qiagen. The nucleotide sequences were determined for the amplified and purified *lac*Y mutant DNA molecules by cycle sequencing (Perkin-Elmer) [43] using primers synthesized by the Harvard Medical School Oligonucleotide Synthesis Facility and specific for the published *lac*Y nucleotide sequence [7].

Sugar Transport Assays

For the downhill ONPG transport experiments, *E. coli* DP90 cells harboring the F′ factor encoding either wild type or mutant *lac*Y genes (Table 1) were used to perform the transport assay according to Varela et al. [48]. The entry of ONPG into the cell was detected by the yellow color of *o*-nitrophenol, which is a cleavage product of ONPG hydrolysis by β-galactosidase [34]. All data were subtracted from the DP90/F′Z$^+$ΔY control, and the assays were repeated 3 or more times.

For the downhill and uphill sugar transport experiments, either cells of *E. coli* strain DP90 (for transport of lactose or TMG) or DW2 (melibiose) containing F′I^{Q}Z$^+$Y$^+$ and mutant *lac*Y genes were used as previously described [49]. A kinetic analysis for all mutants was attempted with 6 different concentrations of lactose by measuring initial rates of lactose downhill transport as previously described [48, 49]. Initial transport rates were too unreliable to obtain consistent and trustworthy apparent K_m and V_{max} values.

Immunoblot Analysis of the Lactose Carrier

The expression levels of both wild-type and mutant lactose carrier protein in the membrane were determined by the immunoblot method of Lolkema et al. [28]. A polyclonal antibody directed against the C-terminal decapeptide of the lactose carrier was kindly provided by Prof. Thomas Wilson (Harvard Medical School) and used for detection of the lactose carrier. All transport data were normalized for expression levels of each mutant.

Results

Isolation of Lactose Carrier Mutants

Using *E. coli* cells containing the *lac*Y gene on an F′ factor as a parental strain, several mutants were isolated, in which the *lac*Y genes were mutated. Three of the four mutants studied here were isolated for their ability to grow on a TONPG minimal plate. Often mutants isolated in this way show defective energy coupling [16]. The three mutants failed to grow on lactose minimal plates (Table 2). A fourth mutant designated AE60 was isolated by Hobson et al. [16], and it, too, was unable to grow on lactose minimal media (Table 2). All four mutants showed white colonies on either lactose or melibiose MacConkey indicator plates and either red, red center, pink or white colonies on the same MacConkey plates above plus IPTG (Table 2).

Sequencing of Mutants

Determination of the nucleotide sequence of all of the mutants studied here revealed alterations within the *lac*Y structural genes of the F′ factors. The changes in DNA sequences of *lac*Y in the mutants indicated the following amino acid alterations in the lactose

Table 2. Fermentation and growth of lactose carrier mutants

Strain	Colony phenotypes[a]				
	Minimal Media	MacConkey Media			
	Lactose	Lactose		Melibiose	
	+IPTG	−IPTG	+IPTG	−IPTG	+IPTG
Y^+	+	Red	Red	Red	Red
ΔY	−	White	White	White	White
M1I	−	White	Red	White	White
R144W	−	White	Red	White	White
G370C	−	White	Red	White	White
Δ387–392	−	White	Red Center	White	Pink

[a] Colony phenotypes were conducted using minimal media agar plates containing 5 mM lactose plus 1 mM IPTG, MacConkey agar plates containing 30 mM lactose without and with 1 mM IPTG, MacConkey agar plates containing 30 mM melibiose without and with 1 mM IPTG incubated overnight at 37°C.

carrier: M-1-I, R-144-W, G-370-C, and a deletion mutation of residues 387–392. Two mutants that were isolated showed the identical deletion mutation in the carrier.

In all cases, the nature of deduced amino-acid alterations and the locations of the mutations were striking (Fig. 1). The M-1-I mutation was found at the N-terminal end of the lactose carrier. The R-144-W mutation showed a neutralization of a charged arginine residue located within the membrane (helix 5); Arg-144 has been shown to be critical for transport and probably plays a role in forming a salt bridge with Glu-126 (helix 4) [13, 41, 51]. The G-370-C mutation was present in a periplasmic loop between helices 11 and 12. Lastly, the Δ387–392-deletion mutation indicated that 6 residues were missing from the middle of helix 12 of the lactose carrier.

Downhill Transport of ONPG Sugar

In addition to the failure of the mutants isolated in this study to grow on lactose minimal media plates, all of the mutants (except for the mutant with the R-144-W mutation) were selected on the basis of their reduced ability to transport TONPG; this predicted that these mutants would have impaired ONPG transport. The three mutants, M-1-I, G-370-C and Δ387–392, showed severely reduced downhill entry of ONPG, ranging between 8 and 25% of the wild-type carrier (Table 3). On the other hand, the independently isolated R-144-W mutation in the lactose carrier [16] showed 51% ONPG transport activity when compared to the wild-type carrier.

Downhill Transport of β-Galactoside and α-Galactoside Sugars

β-Galactosidase that is expressed from the *lac*Z structural gene in *E. coli* harboring the *lac* operon will cleave lactose into glucose and galactose such that the concentration of lactose inside the cell is continually lower than in the periplasm. Thus, the entry of lactose occurs down its concentration gradient and is thermodynamically "downhill." Three mutants (W-144, C-370 and Δ387–392) showed 15% or below while one mutant (I-1) showed 52% downhill lactose entry when compared to the wild-type lactose carrier (Table 3).

A kinetic analysis of downhill lactose entry was attempted for all of the mutants in this study. However, the initial rates of sugar transport were too unreliable to obtain repeatable data for analysis (*data not shown*). This implies that either the apparent affinities, maximum velocities or both are severely diminished in the mutants and are consistent with the observation that the mutants failed to grow on lactose minimal plates. The positive fermentation phenotype on lactose MacConkey agar containing IPTG suggests that the apparent K_m values are greater than 20 mM.

When placed in a cell containing α-galactosidase, which cleaves the sugar melibiose, the downhill entry of melibiose by the lactose carrier mutants can be measured. The W-144 and C-370 mutations in the lactose carrier resulted in greatly reduced entry of melibiose (6% or less) into the cell (Table 3). On the other hand, cells having the I-1 or Δ387–392 mutations in the carrier showed moderate downhill entry of melibiose (21 and 47%, respectively, of wild-type lactose carrier) into the cell.

Uphill Transport of β-Galactoside and α-Galactoside Sugars

The lactose sugar analog TMG is not a substrate for the enzymatic cleavage by β-galactosidase. Therefore, the entry of TMG occurs against its concentration gradient, i.e., thermodynamically "uphill," and thus TMG accumulates within the cell. Measurement of TMG accumulation in two of the mutants in this study (harboring M-1-I and G-370-C mutations) showed transport activity that was 33% and 54%, respectively, of the normal lactose carrier (Table 4). In addition, the other two mutants (harboring R-144-

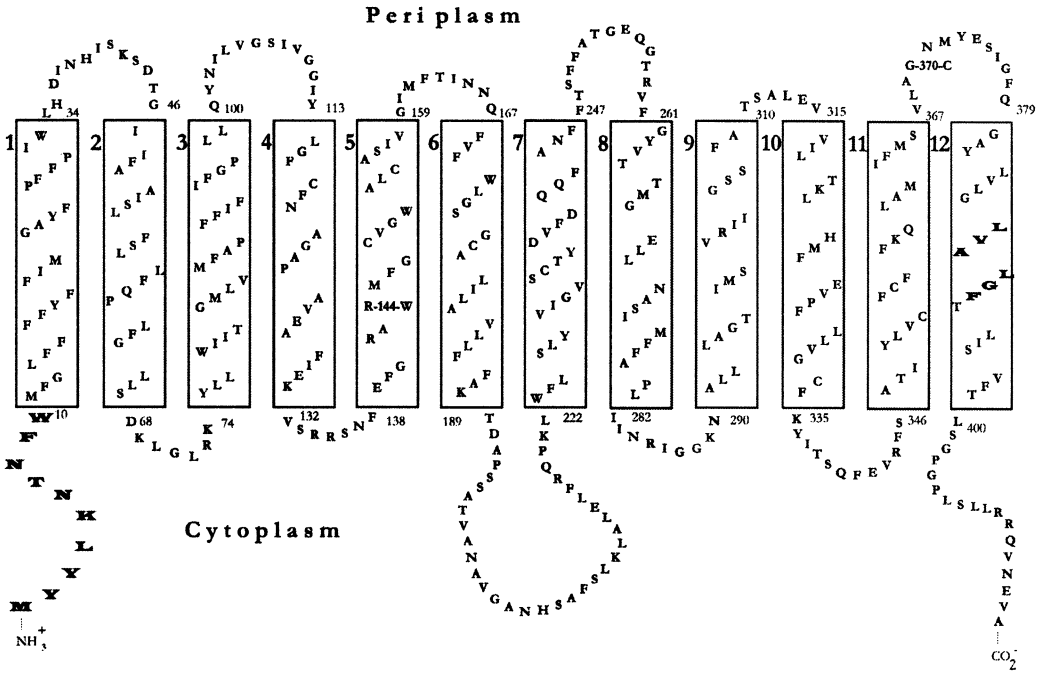

Fig. 1. Secondary structure of the lactose carrier. The lactose carrier in the membrane is depicted as a two-dimensional model indicating the one-letter code for amino-acid residues. The R-144-W and C-370-C mutations are found in helix 5 and the periplasmic loop between helices 11 and 12, respectively, and they are depicted as the wild-type amino acid followed by the number of the residue along the polypeptide chain and the replacement residue, respectively. The deletion mutations are shown in bold and involve residues 1–10, at the N-terminal end, and 387–392, in the middle of helix 12, of the carrier.

Table 3. Downhill transport of ONPG, lactose, and melibiose.

Strain	Percent of normal		
	ONPG	Lactose	Melibiose
Y^+	100	100	100
ΔY	0	0	0
M1I	8 ± 2.4	52 ± 0.7	21 ± 6.4
R144W	51 ± 9	6 ± 0.7	6 ± 4.2
G370C	21 ± 2.7	15 ± 2.5	5 ± 2.3
Δ387–392	25 ± 5	0	47 ± 7.2

To measure the downhill transport of ONPG (2 mM) and lactose (0.4 mM), cells were grown in LB in the presence of 1 mM IPTG (to induce the lactose carrier and β-galactosidase). To measure the downhill transport of melibiose (0.2 mM), cells were grown in LB in the presence of 1 mM IPTG (to induce the lactose carrier) and 1 mM α-MG (to induce α-galactosidase). Cells containing the wild-type lactose carrier transported 24 nmoles lactose/mg protein/min and 17 nmoles melibiose/mg protein/min after incubation for 6 or 12 min. All data were normalized for expression levels, and data represent the averages from 3 or more independent experiments; *t*-test ($P \leq 0.05$).

Table 4. Uphill transport of TMG and melibiose

Strain	Accumulation Ratio In/Out	
	TMG	Melibiose
Y^+	29.1 ± 2.7	29.9 ± 4.8
ΔY	0	0
M1I	9.7 ± 1.2	6.6 ± 1.1
R144W	2.5 ± 0.1	4.1 ± 2.6
G370C	15.8 ± 1.8	5.1 ± 1.9
Δ387–392	0.4 ± .02	21.7 ± 2.7

Cells were grown in LB plus 1 mM IPTG to induce the lactose carrier. To measure TMG transport, cells were exposed to 0.1 mM [^{14}C]-TMG for 10 min. Melibiose accumulation was measured in the presence of 0.2 mM [^{3}H]-melibiose at 25°C after 10 min. All data were normalized for expression and represent the averages of 3 or more independent experiments; *t*-test ($P \leq 0.05$).

W and Δ387–392) showed severely reduced accumulation of TMG (Table 4).

In a striking result, the mutant harboring the Δ387–392 mutation showed accumulation of melibiose, an α-galactoside sugar, that was greatly more pronounced than the accumulation of TMG, a β-galactoside sugar (Table 4). On the other hand, the mutant harboring the G-370-C mutation showed 3-fold greater accumulation of TMG than of melibiose. TMG and melibiose accumulation levels were similar in cells harboring either the M-1-I or the R-144-W mutations.

Immunoblot Analysis of the Lactose Carrier Mutants

The expression levels of the wild-type lactose carrier and mutant derivatives were measured using an antibody directed against the C-terminal end of the

Table 5. Immunochemical assay of lactose carrier mutants

Strains	Percent of Wild-type
Y^+	100 ± 12
Δ387–392	31 ± 19
M1I	62 ± 12
G370C	63 ± 4
R144W	85 ± 11

Lysed cells were filtered and the C-terminal end of the LacY protein was detected with the monoclonal antibody ^{35}S-Protein A. The data represent the averages from 3 or more experiments and were used to normalize transport data; *t*-test ($P \leq 0.05$).

carrier protein according to the method of Lolkema et al. [28]. The amounts of mutant lactose carrier protein ranged between 31 and 85% of the wild-type lactose carrier (Table 5). All transport data above were normalized to account for amounts of carrier expression levels. Therefore, differences in transport activities measured above are not due to alterations in expression or stabilities in the membrane.

Discussion

We have mutants of the lactose carrier of *E. coli* with striking alterations in sugar selection and transport physiology. All of the lactose carrier mutants studied here failed to grow on lactose minimal plates (Table 2). In addition, the mutants grew as white colonies on lactose or melibiose MacConkey indicator plates lacking IPTG, but displayed a negative fermentation phenotype (white or pink colonies) on melibiose MacConkey plates with IPTG (Table 2), indicating alteration of sugar transport properties for the mutants.

Nucleotide sequencing showed mutations in the *lac*Y genes encoding the lactose carriers from the *E. coli* mutants (Fig. 1). Regarding the M-1-I mutation of the carrier, the *lac*Y sequence indicates removal of the original start codon, and during expression, the ribosome probably initiates transcription from the next Met codon, which is found at position 11 along the carrier. Such a mutation would create a deletion of the first 10 residues in the lactose carrier protein, although we have not shown this directly. Alternatively, transcription initiation might occur at the Ile-1 codon. Further studies are needed to distinguish between these two possibilities. Cells harboring this mutation in LacY showed drastically reduced transport of ONPG (8% of normal), consistent with the nature of the mutant isolation conditions, namely TONPG resistance (Table 3). The downhill transport of lactose was over 2-fold higher than of melibiose (Table 3). Thus, the mutant has a lower capacity to bind and transport the sugar melibiose versus lactose, indicating a sugar specificity role for residues 1–10 of the lactose carrier. We also showed that accumulation (uphill transport) of TMG and melibiose, although greatly reduced, was nonetheless present (Table 4). Bibi et al. [1] studied a site-directed mutation in LacY (called "Δ10") where residues 1–10 were deleted and observed significant lactose transport when expressed to very high levels as driven by a strong promoter. Thus, our transport data confirm and extend the work of Bibi et al. [1]. Although we observed expression in the mutant that is 62% of the wild type (Table 5), our *lac*Y mutation resides on an F′ factor, which has a low copy number, and the overall expression was lower than that seen on a higher copy number plasmid or when driven by a strong promoter [1]. We thus confirm the notion that insertion or assembly of LacY into the membrane is independent of residues 1–10 of the carrier [1, 2, 3, 55]. In a proposed structural model for LacY, helix 1 is located in a central position in the water-filled channel [14]. With a few exceptions [25, 49], the vast majority of mutations affecting sugar binding and specificity in the lactose carrier are found within transmembrane domains [13, 50]. Residues in loops are postulated to play a role in gating and in influencing conformational changes associated with the transport cycle [45]. Mutations of residues in helix 1 of the lactose carrier have altered sugar specificity profiles [48, 49]. However, substrate specificity has not previously been considered as a role for residues 1–10 of the lactose carrier. Taken together, we conclude that although the first 10 amino acids of LacY are not absolutely necessary for sugar transport, they are nonetheless important for mediating differences in sugar specificity, possibly through influencing either the gating mechanism or the interaction of the sugar with the binding site in the carrier by affecting the orientation or structure of helix 1. Elucidation of the crystal structure may determine, which of these possibilities is most likely.

Hobson et al. [16] isolated the lactose carrier mutant in which Arg-144 was changed to Trp (R-144-W). Since this mutant was isolated independently of the TONPG resistance method, it is not surprising that ONPG transport was as high as 51% of the wild-type carrier (Table 3). However, we observed that both lactose and melibiose downhill transport were more severely defective (Table 3). Uphill (accumulation) transport of TMG and melibiose were also severely defective in cells harboring the R-144-W mutation (Table 4). Thus, our work here confirms the proposed role of Arg-144 in mediating sugar transport and accumulation [13, 41, 51, 54]. Since the W-144 mutation in the lactose carrier severely affected transport of lactose, TMG and melibiose but not that of ONPG, it is concluded that Arg-144 plays a role in dictating sugar specificity. The mutant is able to bind and transport ONPG to a certain extent. Therefore, we conclude that Arg-144 probably binds the glucose moiety of the lactose molecule during transport.

Since Arg-144 forms a salt-bridge with Glu-126 [41], a charged residue in helix 4, our data implicate involvement of residues in this helix in determining substrate specificity.

In cells harboring the G-370-C mutation in the lactose carrier, we observed downhill transport activities for ONPG, lactose and melibiose that ranged between 5 and 21% of the wild-type control (Table 3). Lactose downhill transport was 3-fold greater than that for melibiose. In addition, the mutant showed 3-fold more uphill TMG transport than melibiose (Table 4), indicating an alteration in sugar specificity. To our knowledge, G-370-C and M-1-I represent the first mutations in LacY that confer profoundly more effects on the transport of melibiose than of lactose. Cysteine scanning mutagenesis of Gly-370 shows 60% lactose transport when compared to the normal C-less carrier [12, 15]. NEM treatment results in a substantial decrease of transport in the mutant containing the C-370 mutation [15], supporting the idea of an important role for Gly-370 in mediating sugar binding and transport. Taken together, the differences in transport rates between lactose [15], TMG and melibiose (Tables 3 and 4) indicate a role in dictating sugar specificity for Gly-370. Since Gly-370 in the wild-type carrier is found in the loop between helices 11 and 12, it is possible that residues in the loop participate in sugar binding and subsequent conformational changes during transport.

In cells harboring the Δ387–392-deletion mutation in the lactose carrier, the downhill transport of lactose was much more severely affected than that of melibiose (Table 3), indicating an altered substrate specificity profile. Furthermore, the uphill transport of TMG was completely defective, while that of melibiose was as high as 72% of normal (Table 4), further confirming a role for residues in helix 12 in mediating sugar specificity. According to the proposed structural model of the lactose carrier, helix 12 is somewhat distal to the helices that form the water-filled channel [14]. Thus, based on this model and our observations of altered sugar selection for cells having the Δ387–392-deletion mutation in LacY, we propose that one or more residues in helix 12 play an indirect role in dictating sugar selection, probably by influencing the interaction of residues in helix 7 with sugar during transport. In support of this notion, Asp-240, which resides in helix 7, has previously been implicated in sugar specificity where the D-240-V mutation shows more melibiose transport than lactose transport [48].

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