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Lactose Permease Mutants Which Transport (Malto-)Oligosaccharides

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Lactose permease mutants, which were previously isolated in sugar specificity studies, were screened for their abilities to transport the trisaccharide maltotriose. Six multiple mutants (e.g., five double mutants and one triple mutant) were identified as forming fermentation-positive colonies on maltotriose MacConkey plates and were also shown to grow on maltotriose minimal plates. All of these multiple mutants contained a combination of two or three amino acid substitutions at position 177, 236, 306, or 322 within the permease. In contrast, none of the corresponding single mutants at these locations were observed to exhibit an enhanced rate of maltotriose transport. In whole-cell assays, the multiple mutants were shown to transport relatively long α-nitrophenylglucoside (αNPG) molecules. In certain cases, αNPG molecules containing up to four glucose residues in addition to the nitrophenyl group were shown to be transported to a significant degree. Overall, the abilities of lactose permease mutants to transport maltotriose and long αNPGs are discussed with regard to the dimensions of the sugar and the mechanism of sugar transport.

The survival of cells requires a selective compartmentalization between the cytoplasm and extracellular environment. In general, the uptake of nutrients and the excretion of waste products are facilitated by membrane proteins which recognize particular substrates and transport them across the hydrophobic barrier of the phospholipid bilayer. In the case of symporters and antiporters, the transport of the relevant solute is coupled to the transport of a cation or anion. In this way, ion electrochemical gradients across the cellular cytoplasmic membrane can be harnessed to enable the active transport of solutes (10, 28).

Cation/solute symporters have been shown to provide an important pathway for nutrient uptake in bacterial (39, 40), fungal (13, 35), plant (20, 24), and animal (11, 12) cells. The lactose permease of *Escherichia coli* has been an extensively studied example of a symporter which cotransports H^+ and lactose into the bacterial cytoplasm (3, 31). The *lacY* gene which encodes the lactose permease has been cloned (37, 38) and sequenced (7) elsewhere. From the DNA sequence, the protein is predicted to contain 417 amino acids with a resulting molecular weight of 46,504. Secondary-structure models are consistent with the idea that the lactose permease contains 12 transmembrane segments that traverse the lipid bilayer in an α-helical conformation (8, 14, 22).

In an attempt to obtain information concerning the molecular architecture of the sugar-binding site within the lactose permease, previous studies have been aimed at the selection and identification of lactose permease mutants which have an alteration in their sugar recognition properties (5, 6, 9, 16, 18, 23, 26). The rationale behind these experiments is that sugar specificity mutations will cause discrete alterations at the sugar-binding domain. In some cases, these could involve changes in amino acids which are directly involved with sugar binding or residues which are close to the sugar-binding site. However, it is also expected that some amino acid substitutions could be relatively far removed from the actual sugar-binding domain but elicit their effects by perturbing secondary structure and folding. To date, sugar specificity studies have involved amino acid substitutions at positions 177, 236, 266, 303, 306, 319, 322, and 389 (5, 6, 9, 16, 18, 23, 26). According to recent secondary-structure models (22), all of these positions are predicted to be on transmembrane segments. This observation is consistent with the idea that the sugar-binding domain is within a hydrophilic channel-like structure (9).

Most previous sugar specificity studies have involved the use of disaccharides (e.g., maltose, cellobiose, sucrose, and thiodigalactoside) to select and identify mutant permeases with altered sugar recognition properties. More recently, however, an alternative approach has been to utilize other sugars for mutant isolation. For example, it has been possible to select for lactose permease mutants which recognize the monosaccharide L-arabinose (18). In the present study, we have taken the opposite approach of identifying mutants which recognize sugars larger than disaccharides. Namely, we have identified mutants that transport the trisaccharide maltotriose. As described below, these mutants were further characterized with regard to their abilities to transport a variety of oligosaccharides.

MATERIALS AND METHODS

Reagents. Lactose (*O*-β-D-galactopyranosyl-[1,4]-α-D-glucopyranose), maltose (*O*-α-D-glucopyranosyl-[1,4]-D-glucopyranose), maltotriose (*O*-α-D-glucopyranosyl-[1,4]-*O*-α-D-glucopyranosyl-[1,4]-D-glucopyranose), melibiose (*O*-α-D-galactopyranosyl-[1,6]-D-glucopyranose), raffinose (*O*-α-D-galactopyranosyl-[1,6]-α-D-glucopyranosyl-[1,2]-β-D-fructofuranoside), stachyose (*O*-α-D-galactopyranosyl-[1,6]-α-D-galactopyranosyl-[1,6]-α-D-glucopyranosyl-[1,2]-β-D-fructofuranoside), and TMG (methyl-β-D-thiogalactopyranoside) were purchased from Sigma. Maltotetraose (tri[α-D-glucopyranosyl-(1,4)]-D-glucopyranose), αNPG_2 (4-nitrophenyl-α-D-glucopyranosyl-(1,4)-α-D-glucopyranoside), αNPG_3 (4-nitrophenyl-di[α-D-glucopyranosyl-(1,4)]-α-D-glucopyranoside),

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant phenotype[a] (chromosome/F'/plasmid) or substitution in lactose permease	Reference or source
Strains		
HS4006/F'$I^QZ^+Y^-$	Δ(Lac-Pro) ΔMalB101/$LacI^q$ $LacO^+$ $LacZ^+$ $LacY^-$/−	6
HS2019	ΔLacU169 RpsL Thi ΔMalE444/−/−	33
HS2019/F'$I^QZ^+Y^-$	ΔLacU169 RpsL Thi ΔMalE444/$LacI^q$ $LacO^+$ $LacZ^+$ $LacY^-$/−	This study
pTE18	−/−/Δ(LacI) $LacO^+$ Δ(LacZ) $LacY^+$ Δ(LacA) Amp^r Tet^r	37
Plasmids[b]		
pB15	Ala-177 to Val	6
pL13	Tyr-236 to Asn	6
pT-D-2	Tyr-236 to His	16
pTG	Ser-306 to Thr	9
pASN322	His-322 to Asn	15
pTL	Ala-389 to Pro	9
pB-8-D	Ala-177 to Val and Tyr-236 to His	16
pBN	Ala-177 to Val and Tyr-236 to Asn	9
pPHE303A	Ala-177 to Val and Ile-303 to Phe	16
pBA	Ala-177 to Val and Ser-306 to Thr	9
pBB	Ala-177 to Val and Ser-306 to Leu	9
pBI	Ala-177 to Val and Lys-319 to Asn	9
pB-5-A	Ala-177 to Val and His-322 to Asn	16
pTG-4-A	Tyr-236 to His and Ser-306 to Thr	16
pBA-1-A	Ala-177 to Val, Tyr-236 to Asn, and Ser-306 to Thr	9

[a] Δ before a symbol indicates a deletion.
[b] The plasmids listed are identical to pTE18, except for substitutions within the *lacY* gene.

αNPG$_4$ (4-nitrophenyl-tri[α-D-glucopyranosyl-(1,4)]-α-D-glucopyranoside), and αNPG$_5$ (4-nitrophenyl-tetra[α-D-glucopyranosyl-(1,4)]-α-D-glucopyranoside) were purchased from Boehringer Mannheim. [^{14}C]lactose was purchased from Amersham, and [^{14}C]TMG was from DuPont-New England Nuclear. The remaining reagents were analytical grade.

Bacterial strains and methods. The bacterial strains and plasmids used in this study are described in Table 1. The strain HS2019/F'$I^QZ^+Y^-$ was created by transferring the F' factor from MAA23 to HS2019.

Plasmid DNA was isolated by the NaOH-sodium dodecyl sulfate method (1) and introduced into the appropriate bacterial strain by the $CaCl_2$ transformation procedure described by Mandel and Higa (25).

Stock cultures of cells were grown in YT medium (27) supplemented with ampicillin (0.1 mg/ml) and tetracycline (0.01 mg/ml). For transport assays, cells were grown to mid-log phase in YT medium containing ampicillin (0.05 mg/ml), tetracycline (0.005 mg/ml), and 0.25 mM isopropyl-β-D-thiogalactopyranoside (IPTG) to induce the synthesis of the lactose permease. In experiments involving *p*-nitrophenyl-α-glucosides (αNPGs), maltose (0.4%) was added to induce the synthesis of maltoporin and α-maltosidase.

MacConkey plates were prepared from MacConkey agar base (Difco) supplemented with ampicillin (0.1 mg/ml) and tetracycline (0.01 mg/ml).

Transport assays. In the experiments involving the transport of αNPGs, mid-log-phase cells of the strain HS2019/F'$I^QZ^+Y^-$ containing the indicated plasmids were washed in phosphate buffer at pH 7.0 containing 60 mM K_2HPO_4 and 40 mM KH_2PO_4 and resuspended in the same buffer to a density of approximately 0.5 mg of protein per ml. Cells were dispensed into 1.5-ml Eppendorf tubes and equilibrated at 37°C for 10 min. The appropriate αNPG was then added to a final concentration of 10 mM, and the cells were allowed to incubate for a period of 15 min. The reaction was quenched by a threefold dilution of an aliquot of cells into 0.6 M Na_2CO_3. The reaction mixture was clarified by centrifugation, and the A_{405} of the supernatant was read in a Beckman DU-64 spectrophotometer to quantitate the amount of *p*-nitrophenol released because of the hydrolysis of the αNPGs by the cytoplasmic enzyme α-maltosidase (30). The value chosen for the molar extinction coefficient under these conditions (25°C, pH > 9.0) was 18,500 liters · mol^{-1} · cm^{-1} (2). Values for the nonspecific hydrolysis of the various αNPGs were obtained by performing parallel experiments with the strain HS2019/F'$I^QZ^+Y^-$/pBR322, which does not possess a functional lactose permease. These background sample values were subtracted from the experimental-sample values to determine the amount of *p*-nitrophenol released from the cells because of transport of the αNPGs via the lactose permease.

In the experiments for which data are given in Table 5, mid-log-phase cells of strain HS2019/F'$I^QZ^+Y^-$ containing the indicated plasmid were washed and resuspended in phosphate buffer, pH 7.0, to a density of approximately 0.75 mg of protein per ml. Cells were dispensed into Eppendorf tubes and equilibrated at 37°C for 10 to 12 min. The transport was initiated by the addition of [^{14}C]lactose to a final concentration of 0.05 mM. For the experiments with nonradioactive glucose and maltose, these two sugars (final concentration, 100 mM) were added at the same time as the radioactive lactose. In the case of maltotriose, this trisaccharide (final concentration, 100 mM) was added during the 37°C equilibration to allow sufficient time for equilibration within the periplasm. Aliquots (0.2-ml) were removed at 1-min time points and filtered over a membrane filter (pore size, 0.45 μm). The extracellular medium was then washed away with 5 ml of 200 mM phosphate buffer, pH 7.0, by rapid filtration, and the amount of intracellular radioactivity was determined by liquid scintillation counting. As a control, the identical strain containing the pBR322 plasmid without a *lacY* insert was also assayed for radioactive sugar uptake to obtain a value for nonspecific sugar uptake.

TABLE 2. Phenotypes on maltodextrin MacConkey plates[a]

Plasmid[b]	Amino acid substitution(s)	Phenotype on plates with the following sugars:			
		Maltose (0.4%)	Maltotriose (0.4%)	Maltotriose (0.8%)	Maltotetraose (0.8%)
pTE18	Wild type	White	White	White	ND[c]
pB15	Val-177	Red	White	White	ND
pL13	Asn-236	Pink	White	White	ND
pT-D-2	His-236	Red	White	White	ND
pTG	Thr-306	Dark pink	White	White	ND
pASN322	Asn-322	White	White	White	ND
pTL	Pro-389	Red	ND	White	ND
pBN	Val-177 and Asn-236	Red	White	Red	White
pB-8-D	Val-177 and His-236	Red	Pink	Red	White
pPHE303A	Val-177 and Phe-303	Red	ND	White	ND
pBA	Val-177 and Thr-306	Red	White	Pink	White
pBB	Val-177 and Leu-306	Red	ND	White	ND
pBI	Val-177 and Asn-319	Red	ND	White	ND
pB-5-A	Val-177 and Asn-322	Red	White	Red	White
pTG-4-A	His-236 and Thr-306	Red	Pink	Red	White
pBA-1-A	Val-177, Asn-236, and Thr-306	Red	Pink	Red	White

[a] MacConkey plates were rich plates which contained the indicated amounts of fermentable sugars. IPTG (an inducer of the *lacY* gene) was added to a final concentration of 0.1 mM. Cells unable to transport and metabolize the indicated sugars remained white, while cells capable of transporting and utilizing the indicated sugars formed red (or pink) colonies.

[b] The strain used in these experiments was HS2019/F'$I^QZ^+Y^-$ containing the indicated plasmids.

[c] ND, not determined.

RESULTS

Identification of mutants with enhanced maltotriose transport. Previous sugar specificity studies have led to the isolation of a number of lactose permease mutants which possess enhanced abilities to transport the disaccharide maltose (5, 6, 9, 16, 34). In an attempt to determine whether any of these mutations also conferred the ability to transport higher-molecular-weight maltodextrins, the strain HS2019/F'$I^QZ^+Y^-$ was transformed with plasmids carrying the wild-type or mutant lactose permease genes. It should be pointed out that HS2019/F'$I^QZ^+Y^-$ is missing the normal transport system which is required for the uptake of maltose and maltodextrins across the cytoplasmic membrane (Table 1). However, it still possesses a functional *lamB* (required for the uptake of maltodextrins into the periplasm) and a functional α-glucosidase (required for the metabolism of maltose and maltodextrins). To evaluate the ability of the wild-type and mutant permeases to facilitate transport across the cytoplasmic membrane, the transformed strains were streaked on maltose and maltodextrin MacConkey plates supplemented with 0.1 mM IPTG (an inducer of the *lacY* gene). Cells capable of efficiently transporting and metabolizing an added fermentable sugar (i.e., maltotriose) would be expected to form red colonies on these plates, while strains incapable of efficient sugar transport should remain white. The results of these experiments are shown in Table 2. As expected, the strain harboring the wild-type plasmid (pTE18) formed white colonies on these plates, since the wild-type permease exhibits a very poor recognition for maltose (and maltodextrins). Interestingly, six of the mutants capable of forming red or pink colonies on MacConkey plates containing 0.4% maltose were also seen to exhibit a similar phenotype on MacConkey plates containing 0.8% maltotriose. These six mutants all contained at least two amino acid substitutions within the lactose permease. The mutants pB-8-D (Ala-177→Val and Tyr-236→His), pBN, (Ala-177→Val and Tyr-236→Asn), pB-5-A (Ala-177→Val and His-322→Asn), pTG-4-A (Tyr-236→His and Ser-306→Thr), and pBA-1-A (Ala-177→Val, Tyr-236→Asn, and Ser-306→Thr) were seen to form red colonies on MacConkey plates containing 0.8% maltotriose, while pBA formed pink colonies. When the concentration of maltotriose was lowered to 0.4%, the mutants pB-8-D, pTG-4-A, and pBA-1-A formed pink colonies, while pBN, pBA, and pB-5-A formed white colonies. None of the six mutants listed above appeared able to form fermentation-positive colonies on MacConkey plates containing 0.8% maltotetraose. In contrast, the other double mutants and all of the single mutants which exhibited enhanced maltose transport failed to form red or pink colonies on MacConkey plates containing 0.8% maltotriose.

The multiple mutants which were able to ferment maltotriose on MacConkey plates were also tested for their abilities to grow on minimal plates in which maltose or maltotriose was provided as the only carbon source for growth. In Table 3, these results are compared with those for the corresponding single mutants. It can be seen that the single and double mutants were all able to grow well on maltose minimal plates. However, as expected from the results given in Table 2, only the multiple mutants which were red on maltotriose MacConkey plates were able to grow on maltotriose minimal plates.

Transport of αNPGs. To determine whether any of the mutants were able to transport maltodextrins longer than maltotriose, a chromogenic assay was employed in which cells were incubated with αNPGs containing two, three, four, or five glucose residues. The level of αNPG transport can be determined by the amount of *p*-nitrophenol released because of the action of the cytoplasmic enzyme α-maltosidase (29). In the experiment of Table 4, whole-cell assays were conducted to measure the rate of *p*-nitrophenol production in the wild-type and mutant strains. As expected, the wild-type lactose permease was seen to have a poor ability to transport the αNPGs. The single mutants were able to transport the αNPG_2 compound to a moderate degree, but only the Asn-236 mutant showed a significant amount of transport of the higher-molecular-weight αNPGs. The mutant pT-D-2 (Tyr-236→His) is interesting in that it has the

TABLE 3. Growth on minimal plates[a]

Plasmid[b]	Substitution	Colony size[c] on plates containing:		
		Maltose (0.4%)[d]	Maltotriose (0.8%)[e]	Maltotetraose (0.8%)[e]
pTE18	Wild type	+	−	−
pB15	Val-177	+++	−	ND
pL13	Asn-236	++	−	ND
pT-D-2	His-236	+++	−	ND
pTG	Thr-306	++	−	ND
pASN322	Asn-322	++	−	ND
pB-8-D	Val-177 and His-236	+++	+	−
pBN	Val-177 and Asn-236	+++	+	−
pBA	Val-177 and Thr-306	+++	+	−
pB-5-A	Val-177 and Asn-322	+++	+	−
pTG-4-A	His-236 and Thr-306	+++	+	−
pBA-1-A	Val-177, Asn-236, and Thr-306	+++	+	−

[a] Minimal plates contained medium 63 supplemented with 1.6% agar, 0.1 mM IPTG, and 0.1 mg of ampicillin, 0.01 mg of tetracycline, and 50 μg of vitamin B_1 per ml, along with the indicated amounts of sugars.
[b] The strain used was HS2019/F'$I^QZ^+Y^-$ containing wild-type or mutant plasmids.
[c] The relative individual colony sizes were as follows: −, no visible colonies; +, 0.2 to 0.5 mm; ++, 0.5 to 1.0 mm; and +++, 1.0 to 2.0 mm. ND, not determined.
[d] The plates were incubated at 37°C for 48 h.
[e] The plates were incubated at 37°C for 96 h.

highest rate of αNPG_2 transport among the single mutants; however, like pB15 (Ala-177→Val), pTG (Ser-306→Thr), and pTE18 (wild type), it was unable to efficiently transport αNPGs containing more than two glucose residues.

Consistent with the results obtained on maltotriose MacConkey plates (Table 2), all of the multiple mutants were observed to transport αNPG_2 faster than any of the corresponding single mutants. In some (but not all) cases, the multiple mutants were also able to transport longer αNPGs as well. For example, the mutants pBA-1-A (Ala-177→Val, Tyr-236→Asn, and Ser-306→Thr) and pBN (Ala-177→Val and Tyr-236→Asn) were able to transport αNPGs containing up to four glucose residues to the highest levels of all of the strains tested. The mutant pB-8-D (Ala-177→Val and Tyr-236→His) also appeared capable of transporting the longer αNPGs to a low level. The remaining multiple mutants seemed incapable of transporting αNPGs larger than αNPG_3. Interestingly, the highest level of αNPG_2 transport was exhibited by the mutant pTG-4-A (Tyr-236→His and Ser-306→Thr), although its ability to transport αNPG_3 was the lowest of all of the mutants with multiple amino acid substitutions.

Inhibition of single mutants by glucosides. The ability of double and triple mutants to transport maltodextrins could be due to an enhanced affinity of the lactose permease for these types of sugars and/or to an enhanced rate of transport. To gain some insight into the individual effects of substitutions at each of the four locations, corresponding single mutants were analyzed with regard to the abilities of various glucosides to competitively inhibit [^{14}C]lactose uptake. As seen in Table 5, a striking dichotomy among the four positions was observed. Compared with the wild-type strain, lactose uptake in the mutants with substitutions at positions 177 and 306 was more sensitive to inhibition by glucose, maltose, and maltotriose. These results are consistent with the idea that substitutions at positions 177 and 306 enhance the affinity for glucoside binding at the sugar recognition site. In contrast, mutations at positions 236 and 322 do not exhibit significant inhibition by the three glucosides. In previous work, the wild-type permease has been shown to have a significant affinity for a variety of monosaccharides, including glucose (29). Interestingly, the wild-type strain appears to have a markedly higher affinity for glucose than single mutants with substitutions at positions 236 and 322. Since

TABLE 4. Rate of *p*-nitrophenol release by whole cells[a]

Plasmid	Substitution	nmol of *p*-nitrophenol/(min · mg of protein)			
		αNPG_2	αNPG_3	αNPG_4	αNPG_5
pTE18	Wild type	0.947	<0.001	<0.001	<0.001
pB15	Val-177	4.85	<0.001	<0.001	<0.001
pL13	Asn-236	5.42	2.46	1.91	0.765
pT-D-2	His-236	8.11	<0.001	<0.001	<0.001
pTG	Thr-306	2.93	<0.001	<0.001	<0.001
pASN322	Asn-322	2.86	0.711	0.273	0.149
pB-8-D	Val-177 and His-236	23.2	2.06	1.61	1.26
pBN	Val-177 and Asn-236	22.7	5.79	2.84	<0.001
pBA	Val-177 and Thr-306	18.2	0.816	<0.001	<0.001
pB-5-A	Val-177 and Asn-322	12.9	2.86	<0.001	<0.001
pTG-4-A	His-236 and Thr-306	31.0	0.330	<0.001	<0.001
pBA-1-A	Val-177, Asn-236, and Thr-306	25.5	10.4	5.13	1.54

[a] See Materials and Methods.

TABLE 5. Inhibition of lactose uptake by glucosides[a]

Plasmid	Substitution	% Inhibition		
		Glucose	Maltose	Maltotriose
pTE18	Wild type	20.9	7.8	3.3
pB15	Val-177	49.2	35.9	14.8
pL13	Asn-236	2.6	<1.0	<1.0
pTG	Thr-306	42.1	16.0	15.8
pASN322	Asn-322	2.8	<1.0	<1.0

[a] See Materials and Methods.

mutations at these sites are correlated with an enhanced uptake of glucosides, it would appear that the effects of these substitutions are on the rate of transport rather than affinity changes at the sugar-binding site.

DISCUSSION

The results of this study describe the identification of mutants of the lactose permease which are capable of transporting maltotriose and αNPGs. These mutants were originally isolated in sugar specificity studies by virtue of their enhanced recognition of maltose coupled with a decreased recognition of the β-glucoside cellobiose (9) and the β-galactoside thiodigalactoside (16). In the present study, these mutants were screened for their abilities to form fermentation-positive colonies on maltotriose MacConkey plates (Table 2). Five double mutants and one triple mutant were identified as fermentation-positive colonies on these plates and were also able to grow on maltotriose minimal plates. Furthermore, when they were tested for their abilities to transport αNPGs, it seemed remarkable that some of these maltotriose-recognizing mutants were able to transport glucosides which contained three or four glucose residues in addition to the rather bulky nitrophenyl group (Table 4). Overall, a common feature of these mutants is that they contain two or three amino acid substitutions at position 177, 236, 306, or 322 within the lactose permease. In contrast, none of the corresponding single mutants at these locations were seen to form fermentation-positive colonies on maltotriose MacConkey, even though they did form positive colonies on maltose MacConkey plates. Likewise, the single mutants were defective in the ability to transport long αNPGs. Thus, it would seem that although a single mutation can lead to enhanced maltose transport, a minimum of two mutations must be present to allow significant levels of transport of maltotriose and longer maltodextrins.

It is interesting to compare the relative effects of amino acid substitutions at positions 177, 236, 306, and 322 with regard to their effects on the transport of oligosaccharides. The five mutants which were red on MacConkey plates containing 0.8% maltotriose and all three of the mutants which were pink on MacConkey plates containing 0.4% maltotriose contain a substitution at position 236. This, taken together with the observation that the only single mutant capable of significant high-molecular-weight αNPG transport was an Asn-236 mutant, seems to indicate that a mutation at position 236 is of prime importance in conferring the ability to transport longer maltodextrins. It is also noteworthy that the three mutants which exhibited the highest levels of high-molecular-weight αNPG transport (pB-8-D, pBN, and pBA-1-A) all contain the Ala-177→Val mutation in addition to a mutation at position 236. In other studies, the Val-177 mutation has been shown to cause an enhanced recognition of maltose (5, 6), sucrose (23), and arabinose (18) and has also been postulated to be involved with sugar-specific changes in turnover number (23).

It is worthwhile to estimate the sizes of the αNPGs in relation to the dimensions of the lactose permease and the width of the phospholipid bilayer. The longest glucoside which was shown to have substantial transport was the αNPG_4 derivative (Table 4). According to modelling data, each glucose residue in the αNPG_4 molecule would be 0.44 nm long (17) and the planar nitrophenyl substituent would be approximately 0.6 nm. If it is assumed that the transported form of the glucoside is in a linear, extended conformation, the total length of the molecule would be about 2.36 nm. If the transmembrane regions of the lactose permease do indeed traverse the membrane in an α-helical fashion with each amino acid residue advancing 0.15 nm along the helix, then a molecule of αNPG_4 would be able to span up to 15 or 16 amino acids of a helix. Furthermore, since it is commonly thought that an α-helix containing about 20 residues is sufficient to traverse a lipid bilayer, a molecule of αNPG_4 oriented perpendicularly to the plane of the membrane would nearly span the bilayer.

Since the lactose permease does not behave as a passively open channel, it is interesting to consider how a gated symporter would accomplish the transport of a long αNPG molecule. It may be that the αNPG_4 derivative is at the limit of the size which can be accommodated by the permease. Along these lines, the defective transport of the longer αNPG_5 derivative may be related to an interference with gate opening and/or closing. This possibility will require more-detailed structural information concerning the locations of gates within the protein. Nevertheless, the ability of the lactose permease to transport sugars which are nearly the length of the phospholipid bilayer raises important questions concerning the relationship between the binding of the sugar to the permease and the gating mechanism for transport.

Finally, it is interesting to point out that the results of this and previous studies (5, 6, 34) demonstrate that mutations within the lactose permease will enable it to transport many of the sugars which are normally transported by the *E. coli* maltose transport system. In spite of such a potential functional overlap, the transporters themselves do not appear to be evolutionarily related. Indeed, the two systems exhibit very different properties (for a review, see reference 19). At the biochemical level, the lactose permease is composed of a single subunit with 12 transmembrane segments (7, 14). In contrast, the maltose transport system has four different subunits: MalE, a periplasmic binding protein (MBP); MalG and MalF, two integral membrane components; and MalK, an ATP-binding domain (32). The membrane complex is composed of one copy each of the MalF and MalG proteins and two copies of the MalK protein. Upon the binding of maltose, the MBP is able to associate with this complex; in the absence of bound substrate, MBP is free to diffuse within the periplasm. It is noteworthy that the concentration of MBP is quite high, achieving a level of 0.3% of the total cellular protein (21). Physiologically, the two transport systems are also very different. The maltose transport system recognizes sugars with very high affinity (i.e., in the low micromolar range), whereas the affinity of the lactose permease for sugars is in the millimolar range (19). Another important difference is energy coupling. Periplasmic binding systems couple ATP hydrolysis to transport and can achieve extremely high levels of intracellular accumulation (i.e., 10^5 [36]). In contrast, the lactose transport system is coupled to

the H^+ electrochemical gradient, and the levels of accumulation are more typically in the range of 10- to 100-fold. Overall, it would appear that three features of the maltose transport system (e.g., high levels of MBP in the periplasm, high affinity for sugar substrate, and the ability to catalyze extraordinarily high levels of accumulation) make this system much better suited to scavenging sugars which would be found in very low concentrations outside the cell (see reference 19). Therefore, perhaps differences in the necessity to scavenge solutes versus the uptake of solutes which are found in relatively high abundance could explain why bacteria have evolved these two very different types of transport systems.

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