A Revised Model for the Structure and Function of the Lactose Permease

EVIDENCE THAT A FACE ON TRANSMEMBRANE SEGMENT 2 IS IMPORTANT FOR CONFORMATIONAL CHANGES*

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The lactose permease is an integral membrane protein that cotransports H⁺ and lactose into the bacterial cytoplasm. Previous work has shown that bulky substitutions at glycine 64, which is found on the cytoplasmic edge of transmembrane segment 2 (TMS-2), cause a substantial decrease in the maximal velocity of lactose uptake without significantly affecting the Kᵣ values (Jessem-Marshall, A. E., Parker, N. J., and Brooker, R. J. (1997) J. Bacteriol. 179, 2616–2622). In the current study, mutagenesis was conducted along the face of TMS-2 that contains glycine-64. Single amino acid substitutions that substantially changed side-chain volume at codons 52, 57, 59, 63, and 66 had little or no effect on transport activity, whereas substitutions at codons 49, 53, 56, and 60 were markedly defective and/or had lower levels of expression. According to helical wheel plots, Phe-49, Ser-53, Ser-56, Gln-60, and Gly-64 form a continuous stripe along one face of TMS-2. Several of the TMS-2 mutants (S56Y, S56L, S56Q, Q60A, and Q60V) were used as parental strains to isolate mutants that restore transport activity. These mutations were either first-site mutations or second-site suppressors in TMS-1, TMS-2, TMS-7 or TMS-11. A kinetic analysis showed that the suppressors had a higher rate of lactose transport compared with the corresponding parental strains. Overall, the results of this study are consistent with the notion that a face on TMS-2, containing Phe-49, Ser-53, Ser-56, Gln-60, and Gly-64, plays a critical role in conformational changes associated with lactose transport. We hypothesize that TMS-2 slides across TMS-7 and TMS-11 when the lactose permease interconverts between the C1 and C2 conformations. This idea is discussed within the context of a revised model for the structure of the lactose permease.

The lactose permease is found within the cytoplasmic membrane of *Escherichia coli* and cotransports lactose and H⁺ into the bacterial cytoplasm with a stoichiometry of 1:1 (1, 2). The inwardly directed H⁺ electrochemical gradient provides the driving force for the active accumulation of lactose (3, 4). The gene encoding the lactose permease, *lacY*, has been cloned on multicopy vectors and sequenced, revealing an open reading frame encoding a protein of 417 amino acid residues (5, 6). Several topological studies are consistent with a secondary structural model in which the lactose permease contains 12 transmembrane segments in an α-helical conformation (7–9).

The lactose permease is a member of a large superfamily of transporters called the major facilitator superfamily (MFS)¹ (10–13). This superfamily includes symporters, antiporters, and uniporters. Structurally, most members of the MFS are predicted to contain 12 membrane-spanning segments by hydropathy analysis (14). In the lactose permease, this topological arrangement has been confirmed by gene fusions with alkaline phosphatase and β-galactosidase (8, 15). The N- and C-terminal segments are cytoplasmic as shown by antibody binding studies (16–18). The general homology of the two halves of the proteins are evidence for an early evolutionary gene duplication, which led to the current superfamily of proteins (19). Analysis of the MFS for hydropobicity, amphipathicity, loop length, and potential salt bridges between helices of the lactose permease provided enough information for us to propose a tertiary structure model (14). This model depicts identical folding patterns for each half of the lactose permease, and other MFS members. The two halves are proposed to interact in a rotationally symmetrical manner. We later hypothesized that the two halves of the permease move relative to each other to facilitate H⁺/lactose cotransport (20).

Several previous studies have centered on the role of transmembrane segment 2 (TMS-2) and the connecting loop 2/3 motif in the structure and function of the lactose permease (21–23). Cysteine scanning mutagenesis of TMS-2 showed that most residues in TMS-2 tolerated cysteine replacements reasonably well (i.e. greater than 25% activity), except at Gly-64 (23). However, when lactose permease strains harboring single cysteine mutations along TMS-2 were reacted with N-ethylmaleimide (NEM), single cysteine substitutions at positions 49, 53, 56, and 65 were strongly inhibited (i.e. less than 25% activity) (23). The NEM-inhibited strains were not subjected to a kinetic analysis, so it is not known if NEM modification blocks sugar binding and/or prevents conformational changes associated with lactose transport. While investigating the role of the conserved loop 2/3 motif, our laboratory found that several different mutations at Gly-64 were very inhibitory for the velocity of lactose transport without affecting the affinity for lactose. In secondary models of the lactose permease, Gly-64 is predicted to lie along the cytoplasmic edge of TMS-2. This observation is consistent with the idea that a face on TMS-2 is important for conformational changes, which alternate the H⁺

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¹ The abbreviations used are: MFS, major facilitator superfamily; TMS-2, transmembrane segment 2; NEM, N-ethylmaleimide.
and lactose binding sites from a periplasmically accessible conformation to a cytoplasmically accessible conformation. In the current study, mutagenesis was conducted along the face of TMS-2 that contains glycine 64 to see if such mutations have an effect on lactose transport.

MATERIALS AND METHODS

Reagents—Lactose (O-β-D-galactopyranosyl-(1,4)-α-D-glucopyranose) and melibiose (O-α-D-galactopyranosyl-(1,6)-α-D-glucopyranose) were purchased from Sigma. [14C]Lactose and Sequenase (version 2.0) were purchased from Amersham Pharmacia Biotech. Restriction enzymes and ligases were purchased from New England BioLabs, Inc. (Beverly, MA). All remaining reagents were of analytical grade.

Bacterial Strains and Methods—The relevant genotypes of the bacterial strains and plasmids are described below in Table I. Plasmid DNA was purified using the PERFECT-prep Plasmid DNA kit obtained from 5 Prime → 3 Prime, Inc., Boulder, CO. Restriction digestions and ligations were performed according to the manufacturers' recommendations. Cell cultures were grown in YT media (24) supplemented with tetracycline (0.01 mg/ml).

In Vitro Galactoside Transport—Cells were grown at 37 °C with shaking to mid-log phase in YT media supplemented with 5 μg/ml tetracycline and 0.25 mM isopropyl-1-thio-β-D-galactopyranoside. The cells were pelleted by centrifugation at 5000 × g for 5 min and the resulting pellet was washed in phosphate buffer, pH 7.0, containing 40 mM K2HPO4 and 40 mM KH2PO4, then resuspended in the same buffer at a concentration of about 0.5 mg of protein/ml. The cells were equilibrated at 30 °C for 5–10 min before [14C]Lactose (2.5 μCi/ml) was added to a final concentration of 0.1 mM. Aliquots of 200 μl were removed at the appropriate time points, and the cells were captured on 0.45-μm Metricel membranes (Gelman Sciences, Inc., Ann Arbor, MI). The cells were then washed with 5–10 ml of ice-cold phosphate buffer by rapid filtration. The filter with the cells was then placed in liquid scintillation fluid and counted using a Beckman LS1801 liquid scintillation counter. Uphill and downhill transport assays were similar except that a lacZ minus strain was used in the uphill assays.

Calculations—The Km and Vmax values for lactose transport were determined by plotting [S]/v versus [S], which is a Hanes-Woolf plot (25). Straight lines were observed in all cases. The slope is 1/Vmax and the y intercept is Km/Vmax.

Membrane Isolation and Western Blot Analysis—10 ml of mid-log cells grown as for transport assays were harvested by centrifugation (5000 × g, 10 min). The pellet was quickly frozen in liquid nitrogen and resuspended in 500 μl of MTPBS (150 mM NaCl, 16 mM Na2HPO4, 4 mM KH2PO4) plus phenylmethylsulfonyl fluoride (0.1 mg/ml) and pepstatin A (1 μg/ml). The suspension was quickly frozen two more times in liquid nitrogen. The cell suspension was then sonicated three times for 20 s each. Triton X-100 was added to a final concentration of 1%, and the suspension was quickly frozen two more times in liquid nitrogen. The cell suspension was then sonicated three times for 20 s each. Triton X-100 was added to a final concentration of 1%, and the membrane fraction was harvested by centrifugation. The pellet was resuspended in 100 μl of MTPBS and subjected to a modified Lowry protein assay (Sigma). A sample of 100 μg of protein was subjected to SDS-polyacrylamide gel electrophoresis using a 12% acrylamide gel. The proteins were electroblotted onto nitrocellulose, and Western blot analysis was performed according to Sambrook et al. (26). The primary polyclonal antibody recognizes the lactose permease C-terminal 10 amino acids. The secondary antibody, goat-anti-rabbit, conjugated to peroxidase, was purchased from Sigma. The Western blot was developed using the nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate colorimetric reaction. The Western blot was then scanned using a Molecular Dynamics laser densitometer and analyzed by comparison to wild-type values for the same preparation and Western blot. As shown below in Table I, the values are reported as a percentage of wild-type for three separate preparations.

Site-directed Mutagenesis—Site-directed mutagenesis was performed using mutagenic polymerase chain reaction primers, which spanned an AvaI site located at codons 70 and 71 within the lacY gene. A mutagenic primer was used with a second primer, which spanned a SacII site within the vector to generate a 1.6-kilobase pair fragment. This fragment was ligated to pGEM-T-vector, and clones containing the polymerase chain reaction-generated fragment were identified as white colonies on plates containing 100 μM isopropyl-1-thio-β-D-galactopyranoside and 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside. The insert was removed from the T-vector by digestion with AvaI and SacII, purified on an agarose gel, and then ligated into a vector carrying the wild-type lacY gene in which the 1.6-kilobase pair fragment had been removed. The ligated DNA was then transformed into E. coli strain T84. DNA from colonies was isolated, and the mutation was verified by double stranded DNA sequencing. At least two independent clones were kept for further study.

DNA Sequencing—Mutations were confirmed by sequencing the appropriate regions of the lactose permease. Sequencing was performed on double stranded plasmid according to Kraft, et al. (27).

RESULTS

Mutagenesis of TMS-2—As already mentioned, previous work has shown that position 64 along TMS-2 appears to be important for conformational changes associated with H+/lactose cotransport. Although an alanine substitution was tolerated at codon-64, all other substitutions resulted in transport activities that were less than 4% of wild-type levels (21). In the current study, we have positioned 23 site-directed mutants at locations that are found on the side of TMS-2, which includes Gly-64. Fig. 1 presents a helical wheel plot of TMS-2. Our rationale for making substitutions along TMS-2 is that Gly-64 may be contained within a face of TMS-2 that is important for conformational changes, and that alterations in side-chain volume along such a face may disrupt the ability of the lactose permease to make these conformational changes. To test this hypothesis, the following single substitutions have been made: Phe-49 (Ala/Trp); Ile-52 (Ala/Trp); Ser-53 (Thr/Leu/Phe); Ser-56 (Thr/Tyr/Leu/Glu); Leu-57 (Ala/Phe); Phe-59 (Ala/Trp); Gin-60 (Ala/Asn/Val/Leu); Phe-63 (Ala/Trp); and Leu-66 (Ala/Trp).

Table I shows the expression levels of the strains containing the wild-type or mutant permeases. As seen here, the majority of mutants are expressed at levels that are moderate or similar to the wild-type strain. However, one position 56 mutation (S56Q) and three position 60 mutations (Q60A, Q60L, and Q60V) were very defective, as shown by their red phenotype on MacConkey plates.

Sugar transport in Wild-type and Mutant Strains—To initially explore the transport characteristics of the parental and mutant strains, their phenotype on MacConkey plates was used as a crude measure of transport activity. In general, to exhibit a white or pink phenotype on MacConkey plates, a mutation must render the lactose permease very defective (i.e. less than 10% activity). Strains with 10% or more activity usually form red colonies on sugar MacConkey plates. As shown in Table II, most of the mutant strains were able to transport lactose (a β-galactoside) and melibiose (an α-galactoside) as indicated by their red phenotype on MacConkey plates containing 0.4% or 1% concentrations of these sugars. However, certain mutations at codon-56 (i.e. S56Q, S56L, and S56Y) and codon-60 (Q60A) were very defective, as shown by their white or pink phenotypes. The Q60V mutation also produced a pink phenotype on melibiose MacConkey plates when this plasmid was transformed into other E. coli strains.
noted substitutions within the lacY gene are in the opposite transcriptional direction. The site of pACYC184. The lacY lacY so that the external lactose concentration is always higher than positive. When lactose enters the cell, it is rapidly metabolized in vivo, even though the transport activity in whole Western analysis, even though the transport activity in whole bacterial membrane, and the S56L strain had a normal level of permease, but the S56Y strain had a moderate level of permease in the membrane.

With regard to the codon-60 mutations, low levels of transport tended to correlate with low levels of expression, except that the Q60V mutation had higher levels of transport than expected. As described in other studies, some mutations cause the lactose permease to be unstable so that the protein is degraded during the membrane isolation procedure required for the Western analysis, even though the transport activity in whole cells is relatively high (29).

Taken together, the results of Tables I–III indicate that mutations at codons 56, 49, 60, and 53 often have a detrimental effect on lactose transport and/or protein expression and stability. These four sites are found adjacent to each other in a helical wheel plot of TMS-2 (see Fig. 1). Furthermore, they are found adjacent to Gly-64, which was shown in previous studies to be important for lactose transport (21).

Similar results were also obtained in uphill transport assays. In the experiment of Table III (right column), the active accumulation of [14C]lactose was measured in wild-type and mutant strains. Again, mutations at codons 66, 59, 52, 63, and 57 usually had substantial accumulation; the most defective in this region was the I52W substitution, which showed approximately 30% accumulation levels. By comparison, substitutions at codons 56, 49, 60, and 53 were generally defective in uphill accumulation, and some mutants were very defective (i.e. S56Y, S56L, S56Q, and Q60A). Overall, the results of Tables I–III, along with previous studies at position 64, suggest that a stripe of TMS-2, including Ser-56, Phe-49, Glu-60, Ser-53, and Gly-64, is critical for lactose permease function and stability.

To determine if the mutations at codons 56, 49, 60, and 53 exert their effects by inhibiting sugar binding and/or inhibiting the velocity for lactose transport, a kinetic analysis was conducted in which the Km and Vmax values for transport were measured in the wild-type and a few selected mutant strains. As shown in Table IV, the mutations at these codons primarily affected the velocity of lactose transport rather than the affinity of the sugar as judged by the apparent Km. The wild-type strain exhibited an apparent Km for lactose of 1.0 mM with a Vmax of 399.5 nmol of lactose/min/mg of protein. The F49A, S53F, S56L, Q60A, and Q60V strains showed Km values of 1.1, 1.1, 1.1, and 0.4 mM, respectively, and Vmax values of 95.9, 29.8, 1.7, 51.6, and 104.9 nmol lactose/min/mg of protein, respectively. Although some small variation is seen in the Km values, these data indicate that the primary defect in lactose transport experiments were conducted. Table III (middle column) shows the results of a “downhill” lactose transport assay that was carried out on the wild-type and mutant strains. To conduct this experiment, plasmids containing the wild-type or mutant lacY genes were transformed into an E. coli strain, HS4006/F. lacY gene and the tetracycline resistance gene (\( \text{lacO}/\text{lac-pro/lacZ}/\text{TetR} \)) were cloned into the E. coli vector pACYC184. The lacY gene and the tetracycline resistance gene are in the opposite transcriptional direction. The following plasmids are identical with pLac184 except for the noted substitutions within the lacY gene.

To obtain a quantitative description of the transport process, in vitro transport experiments were conducted. Table III (middle column) shows the results of a “downhill” lactose transport assay that was carried out on the wild-type and mutant strains. To conduct this experiment, plasmids containing the wild-type or mutant lacY genes were transformed into an E. coli strain, HS4006/F. lacY gene and the tetracycline resistance gene (\( \text{lacO}/\text{lac-pro/lacZ}/\text{TetR} \)) were cloned into the E. coli vector pACYC184. The lacY gene and the tetracycline resistance gene are in the opposite transcriptional direction. The following plasmids are identical with pLac184 except for the noted substitutions within the lacY gene.

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As described later under "Discussion," we hypothesize that the suppressor could alter the tertiary structure in a way that the suppressor could affect the topology of a helix that interacts with the sugar. Similarly, mutations that result in low levels of permease expression will have a white or pink phenotype. Finally, a third reason is related to gene induction. Melibiose is not a very good inducer of the lac operon. Mutations in the lactose permease that prevent the uphill accumulation of sugar may exhibit a pink phenotype due to poor levels of induction, even though the downhill transport rate of the sugar is moderate. The Q60V mutation appears to be pink for this reason. As shown earlier, this mutation has a moderate rate of downhill transport, but is unable to accumulate sugar against a concentration gradient.

When the S56Y, S56L, S56Q, Q60A, and Q60V strains were streaked on melibiose MacConkey plates, spontaneous mutations that restore sugar transport activity were identified as red flecks. This red phenotype could be due to an increase in the transport rate, an increase in protein expression, and/or a recovery in the ability to accumulate sugar against a concentration gradient. These suppressors were restreaked and subjected to DNA sequencing. As shown in Table V, three strains, Y56C, L56P, and Q56P, were the result of mutations involving a codon change at the original site. Codon 56 was changed to a cysteine or proline to restore transport activity. In addition to the wild-type codon (Ser-56), these results indicate that a cysteine or proline are reasonably well tolerated at position 56. In addition, several strains were intragenic suppressors in which a second mutation in the lacY gene was able to restore transport activity. Remarkably, four of these second-site suppressors (S53F, S56L, Q60P, and Q60L) involved substitutions along the same face of TMS-2 (which includes Ser-56, Phe-49, Gln-60, and Ser-53, see Fig. 1) that was shown in the experiments of Tables I–III to be important for lactose transport and/or expression.

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Transmembrane Segment 2 of the Lactose Permease

To understand how this face of TMS-2 plays an important role in permease function, it is necessary to consider its location within the tertiary structure of the protein. Several tertiary models for the lactose permease have been proposed (14, 30–32). Fig. 2 shows our revised model for the tertiary structure of the lactose permease. This arrangement of helices is only slightly different from our previous model that was proposed in 1995 (14). In our newer model, TMS-2 is shifted more toward TMS-7. In addition, TMS-1 and TMS-7 are shifted more toward the channel opening, whereas TMS-4 and TMS-10 are shifted slightly away from the channel opening. However, the basic arrangement of transmembrane segments is identical to our previous model. Our model still hypothesizes that the two halves of the lactose permease are folded in a similar manner, and interact with each other at a rotationally symmetrical interface.

Our model shown in Fig. 2 is not based on the results of the current study, but instead, is derived from bioinformatic considerations (see Ref. 14), ion pair data (shown with red bars (9, 33–40)), and cross-linking studies using bifunctional reagents (shown with black lines (30–32, 39–44)). Based on our model, it appears that cross-linking studies often times involve cross-links across the putative hydrophilic channel region. In other

| Strain        | Downhill lactose transport | Uphill lactose accumulation (in/out) |
|---------------|----------------------------|-------------------------------------|
| Wild-type     | 38.1 ± 1.6                 | 52.7 ± 3.8                          |
| Y56C         | 26.8 ± 3.4                 | 57.9 ± 2.8                          |
| L56P         | 28.2 ± 0.9                 | 33.0 ± 5.1                          |
| S56L/Q60L   | 2.1 ± 0.4                  | 12.3 ± 1.7                          |
| S56L/Y229G  | 3.9 ± 0.5                  | 13.5 ± 1.7                          |
| S56L/Q60L   | 9.0 ± 1.0                  | 19.0 ± 4.3                          |
| S56L/Y229G  | 20.9 ± 1.5                 | 34.3 ± 2.3                          |
| S56L/V229A  | 10.3 ± 1.4                 | 26.2 ± 2.1                          |
| Q60VY229H  | 17.6 ± 3.0                 | 13.3 ± 0.1                          |
| Q60VS53F   | 36.9 ± 1.5                 | 14.5 ± 1.9                          |
| Q60AS53Y   | 44.0 ± 5.6                 | 27.4 ± 4.8                          |
| Q60AS56L   | 28.0 ± 2.0                 | 45.1 ± 7.3                          |
| Q60AQ59L   | 37.5 ± 1.3                 | 20.1 ± 6.5                          |

**TABLE VI**

Downhill and uphill transport in revertant and suppressor strains.

| Strain       | Apparent $K_m$ and $V_{max}$ values$^a$ of suppressor strains |
|--------------|---------------------------------------------------------------|
|              | $K_m$ ± S.E. | $V_{max}$ ± S.E. | Expression level |
| S56L/Q60L   | 0.5 ± 0.2  | 30.7 ± 6.9       | 72 ± 2.5         |
| S56L/V229G  | 0.3 ± 0.1  | 37.5 ± 3.1       | 51 ± 2.0         |
| Q60VY229H  | 0.8 ± 0.3  | 137.8 ± 43.9     | 86 ± 2.5         |
| Q60VS53F   | 1.7 ± 0.1  | 639.7 ± 53.2     | 26 ± 10.5        |
| Q60AS53Y   | 0.4 ± 0.1  | 238.2 ± 88.5     | 71 ± 5.5         |
| Q60AS56L   | 0.7 ± 0.1  | 167.8 ± 20.5     | 89 ± 4.0         |
| Q60AQ59L   | 1.1 ± 0.1  | 447.2 ± 10.6     | 124 ± 21.0       |

$^a$ $K_m$ and $V_{max}$ values were determined in downhill lactose transport assays as described under “Materials and Methods.”

V$_{max}$ value of 29.8 nmol lactose/min/mg of protein, which was also significantly below the wild-type value (see Table IV). However, when the Q60V and S53F mutations are coupled in the same protein, the V$_{max}$ value is actually higher than the wild-type value. Taken together, these data indicate that the defect in lactose transport velocity seen in the parental strains is partially or completely restored by the second-site mutation.

**Discussion**

The results of the current study have identified a critical face on TMS-2 in the lactose permease that includes Ser-56, Phe-49, Gln-60, and Ser-53 (in the clockwise direction shown in Fig. 1). Mutations at these sites, which involve significant changes in side-chain volume, have detrimental effects on transport velocity, protein expression, and/or the uphill accumulation of sugar. Furthermore, several suppressor mutations involved changes in TMS-2, consistent with the notion that the topology of TMS-2 is critical for lactose permease function. In contrast, cysteine substitutions at these four sites were not highly inhibitory, and we found that Ser-56T, Gln-60N, and Gln-60V substitutions were also not very inhibitory. The results of the current study and the cysteine-scanning mutagenesis study indicate that substantial changes in side-chain volume are usually required at these sites to have a major impact on permease structure and function. Other studies have also shown that Gly-64 is important for protein conformational changes associated with lactose transport (21–23). At this site, an alanine substitution was tolerated, but any residue larger than alanine was very inhibitory. Taken together, the results of the current study and other studies indicate that a face of TMS-2 is indispensable for the function and stability of the lactose permease (21–23).

It is interesting to note that the S53F mutation alone had a max value of 137.8 and 639.7 nmol/min/mg of protein, respectively. Similarly, the Q60V and S53F strains had $V_{max}$ values of 104.9 nmol/min/mg of protein, whereas the Q60V/Y226H and Q60V/S53F strains had $V_{max}$ values of 137.8 and 639.7 nmol/min/mg of protein, respectively. It is interesting to note that the S53F mutation alone had a
words, the bifunctional cross-linkers covalently connect residues that are found on transmembrane faces that project into the passageway for the transport of H\(^+\) and lactose. This observation is also consistent with the relative sizes of the bifunctional reagents and the size of lactose. In the cross-linking studies, the bifunctional reagents are in the size range of 6–16 Å. Similarly, the size of the lactose, in its most stable form (i.e. the extended chair conformation), is approximately 9 Å long. Many studies have indicated that the lactose binding site is located on transmembrane segments (45–47), although it is not known if the lactose binding site is vertical or horizontal with regard to the plane of the lipid bilayer. We hypothesize that an opening of 6–16 Å should accommodate the entry of lactose into its binding site. If so, cross-linkers of the 6–16 Å size range would be able to span the binding pocket and cross-link residues on transmembrane segments that are facing the channel lumen. Such cross-links would not necessarily have to involve residues that are on transmembrane segments that are physically adjacent to one another in the tertiary structure.

The model shown in Fig. 2 has the face of TMS-2 that is...
important for conformational changes (i.e., the face containing Ser-56, Phe-49, Gln-60, Ser-53, and Gly-64) projecting toward TMS-11 and TMS-7. Studies using bifunctional cross-linking reagents have shown interactions between these transmembrane segments (30, 42). The cytoplasmic side of TMS-2 cross-links with the periplasmic side of TMS-11, whereas the periplasmic side of TMS-2 cross-links with the periplasmic side of TMS-7 (30). These results suggest that TMS-2 lies obliquely across TMS-7 and TMS-11. These cross-linking results were obtained in the absence of sugar, and would likely cause the cross-linking of the most stable conformation of the unloaded permease. It was also shown that the addition of the lactose analogue, thiodigalactoside, altered the efficiency of cross-linking, indicating that this region of the permease undergoes a sugar-induced rigid body movement (30). Because most of the residues along TMS-2, TMS-7, and TMS-11 are not thought to be near the sugar binding site, an interpretation of these data is that thiodigalactoside induces a conformational change that alters the relative arrangements of TMS-2, TMS-7, and TMS-11, and thereby has an effect on cross-linking. However, the cross-linking results cannot determine if any particular faces along these transmembrane segments are critical for such conformational changes.

A putative conformational change involving TMS-2 and TMS-11 (and perhaps TMS-7) is consistent with our previous proposal that the interconversion between the C1 and C2 conformations involves a motion at the interface between the two halves of the permease. We previously suggested that this motion would involve a change in TMS-2 relative to TMS-11, and TMS-8 relative to TMS-5, on the other side of the permease (see Ref. 20). Based on the cross-linking data described in Reference 41, as well as our own suppressor analyses, the newer evidence suggests that the interconversion may involve a scissoring motion of TMS-2 relative to both TMS-7 and TMS-11, and TMS-8 relative to both TMS-1 and TMS-5. For example, in the C1 conformation, TMS-2 may be relatively parallel to TMS-5, whereas the C2 conformation would result in TMS-8 moving to an oblique arrangement across TMS-1 and TMS-5. According to such a model, lactose could enter its binding site from the periplasm in the C1 conformation, but be prevented from entry into the cytoplasm by the presence of the large hydrophilic loop G7 (see Fig. 3). In the C2 conformation, the scissoring motion across the interface between the two halves of the protein would close the passageway from the periplasm and shift the position of loop G7 so that lactose could enter the cytoplasm.

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