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Minireview

The lactose permease of *Escherichia coli*: overall structure, the sugar-binding site and the alternating access model for transport

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Abstract Membrane transport proteins transduce free energy stored in electrochemical ion gradients into a concentration gradient and are a major class of membrane proteins, many of which play important roles in human health and disease. Recently, the X-ray structure of the *Escherichia coli* lactose permease (LacY), an intensively studied member of a large group of related membrane transport proteins, was solved at 3.5 Å. LacY is composed of N- and C-terminal domains, each with six transmembrane helices, symmetrically positioned within the molecule. The structure represents the inward-facing conformation, as evidenced by a large internal hydrophilic cavity open to the cytoplasmic side. The structure with a bound lactose homolog reveals the sugar-binding site in the cavity, and a mechanism for translocation across the membrane is proposed in which the sugar-binding site has alternating accessibility to either side of the membrane.

Key words: Transport; Bioenergetics; Membrane protein structure; Sugar-binding site; Alternating accessibility

1. Introduction

LacY is encoded by the *lacY* gene, the second structural gene in the *lac* operon [1], and is solely responsible for all the translocation reactions that typify the galactoside transport system in *Escherichia coli* (reviewed in [2]). LacY is a particularly well-studied representative of the major facilitator superfamily (MFS) [3] which presently contains more than 1000 members [4]. Like many MFS members, LacY transduces the free energy released from the downhill translocation of H^+ in response to an electrochemical H^+ gradient (interior negative and/or alkaline) to drive the uphill stoichiometric accumulation of galactosides against a concentration gradient. Conversely, LacY can also transduce the free energy released from the downhill translocation of galactosides to drive the uphill stoichiometric translocation of H^+ with the generation of a H^+ electrochemical gradient, the polarity of which depends upon the direction of the substrate concentration gradient.

The use of molecular biological approaches to engineer LacY for site-directed biochemical and biophysical studies has provided insight into both structure and mechanism (reviewed in [2,5]). In addition to other site-directed mutants, functional LacY devoid of eight native Cys residues (C-less LacY) has been constructed and used for Cys-scanning mutagenesis [5]. Analysis of the mutants has yielded the following information: (i) Almost all of the mutants are expressed normally in the membrane and have significant activity. (ii) Remarkably, only six side chains are irreplaceable with respect to active transport: Glu126 (helix IV) and Arg144 (helix V), which are crucial for substrate binding; Glu269 (helix VIII), which may be involved in both substrate binding and H^+ translocation; and Arg302 (helix IX), His322, and Glu325 (helix X), which play essential roles in H^+ translocation. (iii) Positions accessible to solvent have been identified. (iv) Positions where the reactivity of the Cys replacement is increased or decreased by ligand have been identified. (v) LacY is highly flexible conformationally. (vi) A low-resolution 3D structural model has been proposed based on the results of thiol cross-linking experiments and engineered Mn(II)-binding sites [6]. (vii) A model for the transport mechanism has been formulated on the basis of structural data and the properties of mutants in the irreplaceable residues [2].

This discussion includes the overall X-ray structure of LacY, the sugar-binding site and an alternating access model for sugar translocation across the membrane. Discussion of residues involved in H^+ translocation and coupling and description of a more detailed mechanism for galactoside/H^+ symport are omitted here for brevity. For these aspects of the work, the reader is referred to references [2] and [7].

A mutant of LacY with Gly in place of Cys154 (Cys154→Gly; C154G) is arrested in one conformation [8–10], and crystals of this mutant that diffract well have been grown successfully, leading to an X-ray structure of an inward-facing conformation of LacY with bound substrate [7] (Fig. 1). The structure clearly shows the overall fold of LacY, which is composed of unusually distorted helices and

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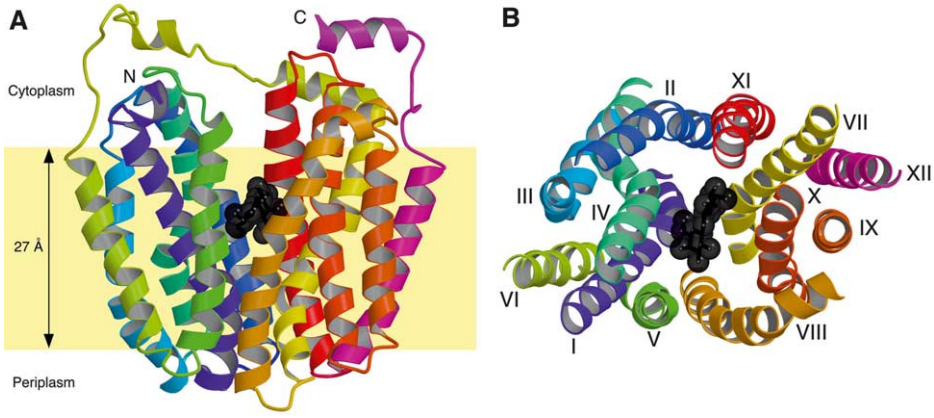

Fig. 1. Overall structure of LacY. The figures are based on the C154G mutant structure with a bound substrate homolog, TDG. A: Ribbon representation of LacY viewed parallel to the membrane. The 12 transmembrane helices are colored from the N-terminus in purple to the C-terminus in dark pink; TDG is represented by black spheres, and the membrane is in pale yellow. B: LacY in a ribbon representation, viewed along the membrane normal from the cytoplasmic side. For clarity, the loop regions have been omitted. The color scheme is the same as in A; the 12 transmembrane helices are labeled with Roman numerals.

a large water-filled internal cavity, as well as the details of the sugar-binding site. The structure is highly consistent with the proposed inward-facing conformation of LacY (see [2]).

With regard to the phenotype of the C154G mutant, helices I and V cross in the approximate middle of the membrane where Cys154 is in close proximity to Glu24 (Fig. 1). Thus, replacement of Cys154 with Gly may cause the two helices to come into closer approximation, as suggested for glycophorin A dimerization [11], thereby causing the phenotype. Evidence supporting this interpretation is provided by the demonstration that introduction of mutant G24C into the C154G mutation rescues active transport to a highly significant extent (N. Ermolova, I. Smirnova and H.R. Kaback, unpublished observations).

2. Overall structure

Structures were solved with and without the high-affinity lactose homolog β-D-galactopyranosyl-1-thio-β-D-galactopyranoside (TDG). Unexpectedly, an unidentified disaccharide of unknown origin was found in the sugar-binding site at >50% occupancy when the protein was crystallized without TDG. The two structures show little difference except for some minor alterations in the sugar-binding site.

The asymmetric unit of the LacY crystal is composed of an artificial dimer, with two molecules oriented in opposite directions, confirming the monomer as the functional unit of LacY (see [12–14]). The structures of the two monomers are almost identical. Viewed parallel to the membrane (Fig. 1A), the monomer is heart-shaped with an internal cavity open on the cytoplasmic side and largest dimensions of 60 Å (along the membrane) by 60 Å (along the membrane normal). Normal to the membrane (Fig. 1B), the molecule is oval-shaped with dimensions of 30 Å by 60 Å. Biochemical studies [13–15] estimate that the thickness of the bilayer is ca. 27 Å (Fig. 1A). A large interior hydrophilic cavity open only at the cytoplasmic side is observed with dimensions of 25 Å by 15 Å, indicating that the structure represents the inward-facing conformation (Fig. 2). Within the cavity, TDG binds at a site that is a similar distance from both sides of the membrane, and the periplasmic side is closed tightly. This is consistent with the notion that LacY has only one binding site that is alternately accessible to each side of the membrane. In this context, it is noteworthy that the P_i/glycerol-3-P antiporter (GlpT), another member of the MFS, has a similar structure, and the substrate-binding site is postulated to be in a similar location [16].

3. Transmembrane helix packing and domain structure

The monomer contains 12 transmembrane helices, as predicted [17,18]. The N- and C-terminal six helices form two distinct helical bundles connected by a long loop between helices VI and VII (Fig. 1). Although loop VI/VII has two

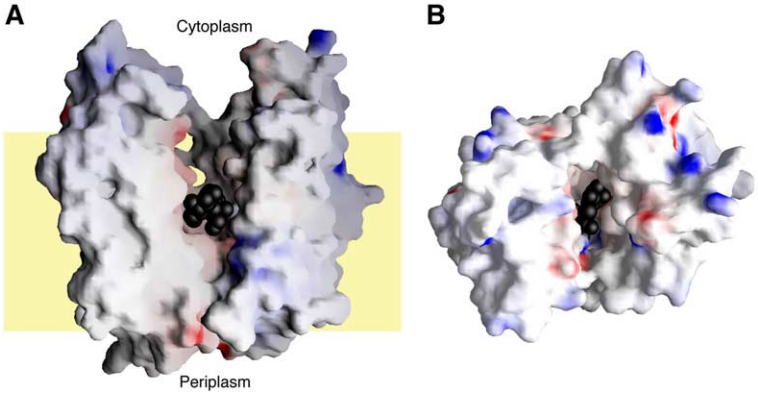

Fig. 2. The internal hydrophilic cavity of LacY. The surface model and electrostatic potential were calculated with the program GRASP [37]. The polar surfaces are colored blue (positively charged) and red (negatively charged). The black spheres denote TDG. A: View parallel to the membrane. For clarity, helices V and VIII have been removed. The membrane is in pale yellow. B: View along the membrane normal from the cytoplasmic side.

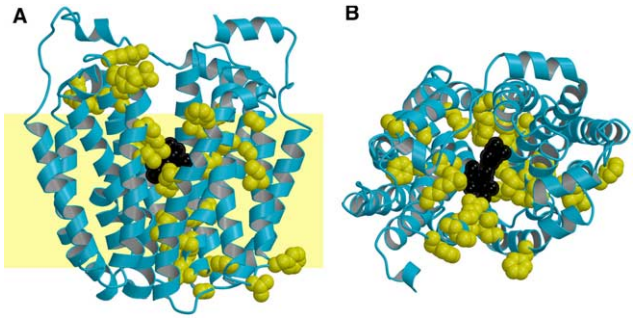

Fig. 3. Accessibility of Cys-replacement mutants to water. Individual Cys-replacement mutants were labeled with [^{14}C]NEM before and after treatment with MTSES, an impermeant thiol reagent, as described in [22,38–41]. In A, the membrane is in pale yellow.

short helical segments at the N- and C-terminal, it is extended and flexible. The N- and C-terminal six-helix domains have the same topology and are related by an approximate two-fold symmetry (Fig. 1B), as shown also with GlpT [16] and OxlT and proposed for other MFS transporters [19]. The high degree of symmetry between the N- and C-terminal domains suggests that they have the same genetic origin, although there is low sequence homology between the two domains [20].

A hydrophilic cavity is formed between helices I, II, IV, and V of the N-terminal domain and helices VII, VIII, X, and XI of the C-terminal domain. Helices III, VI, IX, and XII are largely embedded in the bilayer and not exposed to the solvent. Site-directed alkylation of single-Cys residues with *N*-ethylmaleimide (NEM) are entirely consistent with the presence of a large hydrophilic cavity in LacY. Accessibility of single-Cys mutants to the highly impermeant thiol reagent methanethiosulfonate ethylsulfonate (MTSES) [21,22] occurs mainly at positions lining the hydrophilic cavity (Fig. 3A,B). MTSES accessibility to Cys replacements at positions leading from the hydrophilic cavity to the periplasmic side may represent the pathway by which sugar gains access to the binding site.

4. The substrate-binding site

The substrate-binding site is found in the hydrophilic cavity at a similar distance from either side of the membrane and near the molecular two-fold axis of LacY (Figs. 1 and 2). The residues involved in substrate binding are shown in Fig. 4A,B. The sugar-binding site in the N-terminal domain is composed of residues from helices I, IV, and V. In the C-terminal domain, helices VII and XI – which are symmetrically related to helices I and V, respectively – form the other half of the binding site for TDG.

The binding site in the N-terminal domain bears a striking resemblance to the sites of many other sugar-binding proteins [23]. The primary hydrophobic interaction of the galactopyranosyl ring with the indole ring of Trp151 (helix V) is a common feature of galactoside-binding proteins. The C6 atom of the galactopyranosyl ring also appears in a van der Waals contact with the S atom of Met23 (helix I). The irreplaceable residue Arg144 (helix V) forms bidentate H-bonds with the O3 and O4 atoms of the galactopyranosyl ring. Another irreplaceable residue, Glu126 (helix IV), is in close proximity to Arg144 and may interact with the O4, O5, or O6 atoms of TDG via water molecules. However, individual water molecules cannot be modeled at this resolution. The results are consistent with findings that even the most conservative replacement for Arg144 (Lys; R144K) abolishes binding, and replacement of Glu126 with aspartate results in substantially reduced affinity (see [2]). Biochemical evidence [24–26] suggests that these residues form a salt bridge during turnover, although this interaction is not observed in the current structure. Instead, another irreplaceable residue, Glu269 (helix VIII), appears to form a salt bridge with Arg144 as well as a possible H-bond with Trp151. All replacements for Glu269, with the sole exception of aspartic acid, are defective with respect to substrate binding and all translocation reactions [27,28]. Glu269 in helix VIII in the C-terminal domain is the only residue that crosses domains and seems to be key in providing the important energetic link between the N- and C-terminal helix domains [29].

Recent studies (J. Vazquez-Ibar, L. Guan, M. Svrakic and H.R. Kaback, in preparation) utilizing a mutant with a single-Trp residue at position 151 are highly consistent with the conclusions drawn from the structure. Purified single-Trp151 LacY exhibits an emission maximum at 340 nm, consistent with an aqueous local environment, and addition of LacY

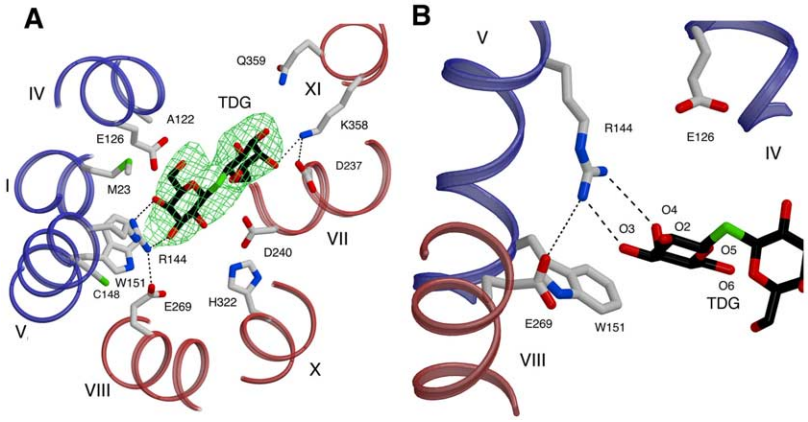

Fig. 4. Substrate-binding site of LacY. Possible H-bonds and salt bridges are represented by dashed black lines. Transmembrane helices in the N- and C-terminal domains are colored blue and red, respectively. Color code for atoms: white, carbon in side chains; black, carbon in TDG; blue, nitrogen; red, oxygen; green, sulfur. A: Residues involved in TDG binding viewed along the membrane normal from the cytoplasmic side; a $2|F_{obs}|-|F_{calc}|$ electron density map (contoured at 1.5) for TDG is also shown (green). B: Closer view of the TDG-binding site in the N-terminal domain.

substrates induces a 3–4 nm blue shift with an increase in fluorescence. Moreover, quenching by cesium, iodide or acrylamide is inhibited in the presence of TDG, and Trp phosphorescence is red-shifted by 3–4 nm in the presence of LacY substrates. In addition, when Glu269 is replaced with Asp, the fluorescence emission maximum is red-shifted by 9–10 nm, indicating that Trp151 is even more accessible to water.

Alkylation of Cys148 inactivates LacY by blocking sugar binding, and Cys148 is protected from alkylation with NEM by LacY substrates (see [2]). In similar fashion, alkylation of mutant A122C (helix IV) with NEM or replacement with Phe or Tyr abolishes binding and transport of disaccharide substrates specifically with hardly any effect on galactose binding or transport [30]. Cys148 and Ala122 are found in the vicinity of the N-terminal domain of the substrate-binding site, and the effects described are clearly explained by steric hindrance.

With regard to the C-terminal domain and TDG binding, fewer interactions are observed. Only a single H-bond is observed between Lys358 (helix XI) and the O4′ atom of TDG. Asp237 (helix VII), which forms a H-bond with Lys358, is also in the vicinity of the O4′ atom of TDG; however, interaction may occur via a water molecule. Other polar residues like Gln359 (helix XI) may also be involved in binding through water molecules, as this is a common motif for sugar-binding proteins.

It is essential to realize that galactose itself is the most specific substrate for LacY but has very low affinity, which is increased markedly by various adducts at the anomeric carbon [31]. Furthermore, the C4 OH is the most important determinant for specificity [32], although the C2, C3, and C6 OH groups on the galactopyranosyl ring also play roles in H-bonding. The hydrophobic interaction between the galactopyranosyl ring and Trp151 is likely to orient the galactopyranosyl ring so that important H-bonds can be formed [33]. The important portion of the substrate-binding site with regard to specificity is in the N-terminal domain, and the residues in the C-terminal domain that interact with the second galactopyranosyl ring in TDG (or other anomeric substituents) increase affinity for disaccharide substrates but have little or nothing to do with specificity.

5. An alternating accessibility model for translocation across the membrane

The crystal structure clearly represents the protonated, inward-facing conformation of LacY with bound substrate [2,7]. In this structure, the central hydrophilic cavity containing the sugar-binding site is open toward the cytoplasmic side only. However, an alternative, outward-facing conformation open to the periplasm must exist for substrate translocation across the membrane. Because the hydrophilic cavity exists between the N- and C-terminal domains, which are connected by a flexible loop, it seems likely that the structural change between inward- and outward-facing conformations involves rotation between the N- and C-terminal domains around the sugar-binding site, thereby allowing the binding site alternating accessibility to each side of the membrane.

There is experimental support for this conclusion. The NEM reactivity of Cys replacement mutants at the periplasmic side of LacY at the N- and C-terminal domain interface increases in the presence of ligand, suggesting that this region of LacY undergoes conformational changes that may allow access to ligand in the outward-facing conformation (Fig. 5A). As discussed above, the crystal structure is derived from the mutant C154G. Gly154 (helix V) is at the domain interface, and mutation from Cys to Gly allows tighter packing at the interface, stabilizing the inward-facing conformation.

A hint for 3D modeling of the outward-facing conformation is derived from extensive thiol cross-linking studies in which many distances were measured between positions in the N- and C-terminal domains [6]. Many observed distances are reasonably consistent with the X-ray structure; however, there is a tendency for thiol cross-linking to underestimate distances because cross-linking often traps Cys residues when they are closest to each other [34]. At the periplasmic side of LacY, distances monitored by cross-linking are reasonably consistent with those observed in the structure. However, on the cytoplasmic side, among pairs of residues that exhibit cross-linking, a group is observed where the distances between residues measured by cross-linking are consistently

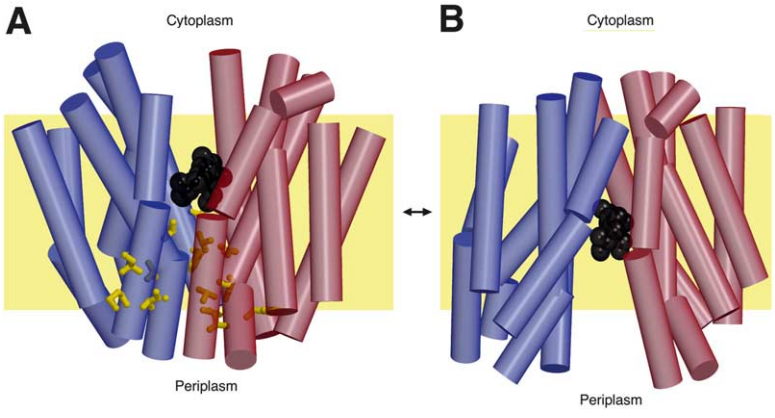

Fig. 5. Structural changes between inward- and outward-facing conformations. Transmembrane helices in the N- and C-terminal domains are shown as blue and maroon cylinders, respectively. A: Inward-facing conformation (i.e. the crystal structure) viewed parallel to the membrane. Cys replacements of the residues in yellow show an increased reactivity in NEM labeling upon substrate binding. B: A possible model for the outward-facing conformation, based on chemical modification and cross-linking experiments (see the main text), viewed parallel to the membrane. The model was obtained by applying a relative rigid-body rotation of 60° (around the axis passing near the TDG parallel to the membrane) to the N- and C-terminal domains. The membrane is in pale yellow.

underestimated to the extent of 10–15 Å relative to the crystal structure. For example, on the cytoplasmic side, the distances of residues between helix V (Phe140, Gly141, Ala143) and helix VIII (Ile275, Phe278, Ala279) or helix X (Cys333), between helix IV (Val125, Ile129, Arg134) and helix X (Lys335, Ser339), and between helix XI (Tyr350, Cys353, Phe354) and helix II (Phe63, Ser67) or helix IV (Ile129, Arg134) are all estimated to be only 9–10 Å by thiol cross-linking. Because the conformation of LacY was not arrested in the cross-linking experiments, discrepancy between distances may reflect fluctuations between the inward- and outward-facing conformations of the molecule (Fig. 5). To fulfill these distances observed in the thiol cross-linking experiments, helices II-IV-XI, V-VIII-X, and IV-X must be closely packed together, which is not observed in the current inward-facing conformation.

By applying a relative rotation of 60° between the N- and C-terminal domains, a model for the putative outward-facing conformation can be obtained that satisfies the helix packing derived from thiol cross-linking (Fig. 5B). The flexible loop connecting N- and C-terminal domains is compatible with this conformational change. In the model of the outward-facing conformation, the cytoplasmic halves of helices II, IV, and V in the N-terminal domain and helices VIII, X, and XI in the C-terminal domain form an interface that closes the cytoplasmic end of the hydrophobic cavity. Interestingly, kinks at Pro123 (helix IV) and Pro327 (helix X), which are at the domain interface in the outward-facing conformation, are in almost equivalent positions to the kinks at Pro28 (helix I) and Ala244 (helix VII) at the domain interface in the inward-facing conformation. It is likely that these kinks allow tight closure of the hydrophilic cavity ends by conferring flexibility to these helices. Each of the eight helices that form the surface of the hydrophilic cavity is heavily distorted by kinks and bends, and each also contains many Pro and Gly residues commonly found in irregular helices; in contrast, helices VI, IX, and XII, which are not part of the cavity, are unperturbed (Fig. 1). This finding implies that the irregular helices provide structural flexibility, thereby allowing the molecule to assume different conformations. The irregular shapes of many of the transmembrane helices and the dynamic conformational nature of the molecule, as well as the large hydrophilic cavity, may explain the very high rates and extent of H/D exchange observed with LacY [35,36].

Although the alternating access model depicted implies a symmetrical conformational change in LacY with the binding site becoming accessible to either side via hydrophilic cavities of approximately equal volume, the MTSES accessibility results shown in Fig. 3 suggest that the changes may not be symmetrical. From the results shown, it appears that the periplasmic side of LacY may not open to the same extent as the cytoplasmic side. In any case, it is apparent that further biochemical and computational studies based on the crystal structure and structure determinations of the other states, particularly the outward-facing conformation(s), will facilitate our understanding of the transport mechanism.

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