Gating in the LacY Symporter: Normal Mode Analysis

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Abstract

Lactose permease (LacY) is a typical facilitator, using energy stored in a transmembrane electrochemical proton gradient to drive cytosolic accumulation of galactosides against their concentration gradient. While the 3.5 Å crystal structure of LacY from *E. coli* [1] suggests a physical realization of the alternating access model [2] for the structural differences between its inward- and outward-facing conformations, the molecular details of this gating transition remain elusive. We probe large-scale conformational changes in this symporter by all-atom Normal Mode Analysis (NMA). We find that perturbing this system along the 7th all-atom normal mode initiates opening and closing via global counter-rotation of the intracellular and extracellular domains around the pore axis. The stationary plane relative to which counter-rotation occurs passes through the center of LacY parallel to the membrane. Counter-rotation of the two halves is highly cooperative and concerted. The long TM6-TM7 polypeptide loop, the small helices and the N- and C-termini on the intercellular side undergo large scale rotational motion. Pore size is essentially unaffected by these motions; it remains open toward the cytosol and occluded toward the periplasm. Perturbation along the 8th normal mode leads to large scale expansion and constriction of the intracellular mouth. The N- and C-termini of LacY alternately approach and separate, strongly affecting the size of the intracellular pore mouth.

Introduction

LacY executes coupled sugar/proton symport, accumulating galactosides against a concentration gradient by utilizing the free energy released from downhill translocation of protons [3]. In the crystal structure, the central cavity containing the sugar-binding site is open to the cytoplasmic side but closed to the periplasmic side. It has been suggested [1] that structural rearrangement from inward- to outward-facing conformation involves rotation between the N- and C-terminal domains around the sugar-binding site, thus permitting the binding site alternate access to each side of the membrane. Lactose/proton influx is viewed as a six step process: 1) protonating LacY in the outward-facing conformation; 2) lactose binding; 3) transition to the inward-facing conformation; 4) lactose release; 5) proton release; 6) transition to the outward-facing conformation. Steps 3 and 6 involve large-scale conformational changes in LacY. Moreover, they are not equivalent, representing the loaded and unloaded transition states. What are possible movements of the protein backbone for these states? Arguing from the architecture of LacY trapped in state 3, the following structural changes have been suggested [1]:

1. "*... the structural change between inward- and outward-facing conformations involves rotation between the N- and C-terminal domains around the axis parallel to the membrane.*"
2. "*By applying a relative rotation of 60° between the N- and C-terminal domains, we can obtain a model for the putative outward-facing conformation that satisfies the helix packing derived from thiol cross-linking.*"
3. "*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.*"

Computational Model

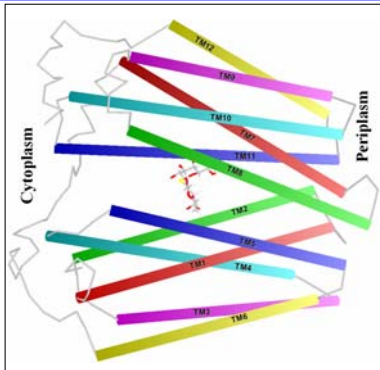
➢ We use the 1PV6 crystal structure of LacY at 3.5 Å resolution [1], a molecular system of 6,625 protein atoms, TDG (45 atoms), 277 water molecules (831 atoms), totaling 7,501 atoms. Due to low resolution, water molecules were not resolved and they were added in the intracellular pore. E325 was protonated. It is located in a hydrophobic environment close to the center of LacY and directly involved in proton translocation as suggested [1]. The crystal structure of LacY was resolved for the C154G mutant, which was trapped a conformation with an open intracellular pore. Therefore, we performed the reverse mutation, G154C, to obtain the native structure.

➢ The three minimizations were done with this E325p-G154C-LacY system, to be denoted Nat-LacY. One minimization was done with the crystal Cry-LacY. The molecular system with E325 protonated (E325p-Cry-LacY) was also minimized. All the systems were loaded with TDG. To remove steric clashes and to relax the molecular systems, ~2,000 minimization steps were done using steepest descent with a random step length; finally, the molecular systems were well tuned via conjugate gradient with guaranteed descent [4]. All degrees of freedom (bond lengths, bond angles, torsion and improper torsion angles) in the proteins, TDG and the waters were variable.

➢ The geometry of all five molecular systems reached an absolute largest gradient component of $<5\cdot10^{-10}$ kcal mol^{-1} Å^{-1}. The RMSD for 417 C_α is 1.42 Å between the crystal and minimized LacY structures; the RMSD for all 6,625 protein atoms is 1.75 Å; and, the RMSD for total 7,501 atoms (including TDG and added waters) is 1.94 Å.

➢ All-atom NMA was carried out using the LAPACK library and BLAS routines; global translational and rotational modes were removed using the Eckart conditions.

➢ All systems were described with the all-hydrogen CHARMM22 topology and parameter set, with NMA calculations carried out in vacuum.



Minimized Nat-LacY structure with a sugar at its binding site

Cylinder representation of Nat-LacY, side view. For clarity, added water molecules are not displayed. The lactose homolog, TDG, is sited at the sugar-binding site, the protein center. TM helices are shown as cylinders, with extra- and intracellular ends visualized in light and dark colors, respectively. TM helices are grouped as follows: **1)** TM1 ÷ TM6 form the N-terminal domain. TM1, TM2, TM4 and TM5 line the pore. TM3 and TM6 are peripheral helices. **2)** TM7 ÷ TM12 form the C-terminal domain. TM7, TM8, TM10 and TM11 line the pore. TM9 and TM12 are peripheral helices. Although there is low sequence homology between the two six-helix domains, they are approximately similar structurally and could be related by a two-fold symmetry. Therefore, corresponding TM helices in each domain are colored the same. A long polypeptide loop connects TM6 with TM7 on the intracellular side. The figure was generated using our MCICP code.

Eigenvalues of the various minimized LacY systems

Nat-LacY	
n	ν, cm^{-1}
1	-2.65E-05
2	-1.71E-05
3	9.48E-06
4	1.93E-05
5	2.59E-05
6	3.40E-05
7	3.54558
8	4.13860
9	4.35997
10	4.86685
11	5.25077
.....	
22501	3689.51
22502	3691.51
22503	3692.54

The first six eigenvalues are near zero and other eigenvalues are positive indicating a minimum.

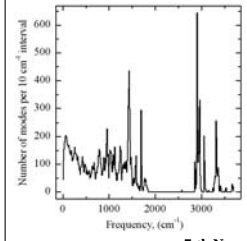
The lowest-frequency (7th) eigenvalue is nearly the same in all the three systems: 3.5, 3.6 and 3.5 cm^{-1}. The lowest-frequency 7th eigenvalue was 3.5 and 3.3 cm^{-1} for the E325p-Cry-LacY and Cry-LacY systems.

The lowest-frequency eigendirections are nearly identical for all three Nat-LacY, E325p-Cry-LacY and Cry-LacY systems; all overlaps are ~1.

Main observation: mutation of E325 or E325&G154 has no effect on the lowest-frequency (7th) eigenvector.

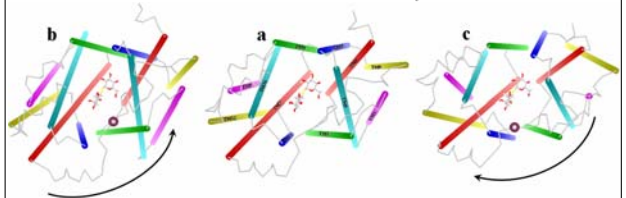
E325p-Cry-LacY	
n	ν, cm^{-1}
1	-2.30E-05
2	1.33E-05
3	2.10E-05
4	3.01E-05
5	3.78E-05
6	4.78E-05
7	3.49544
8	3.71565
9	4.31629
10	4.65913
11	5.01570
.....	
22489	3690.08
22490	3692.56
22491	3692.96

Frequency Spectrum of the Nat-LacY System



- the frequency spectrum is the same in all five minimization runs
- the region < 500 cm^{-1} describes concerted motions of secondary structure elements (α-helices and large groups of amino acids)
- the region from 500 to 1860 cm^{-1} corresponds to internal vibrations of single amino acids
- the region > 2840 cm^{-1} corresponds to hydrogen-heavy atom vibrations

7-th Normal Mode of the Nat-LacY system

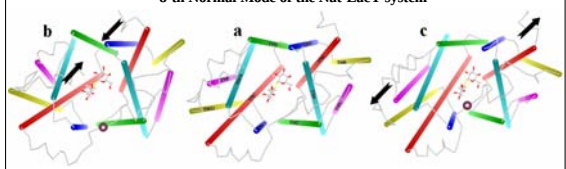


View from the intracellular side in a cylinder representation: (**a**) the minimized system; (**b**) and (**c**) displacement along the 7th all-atom NM in the "positive" and "negative" directions.

Main observations:

- Global counter-torsions of the cytoplasmic and periplasmic halves (black curved arrows) around the pore axis. The periplasmic half rotates clockwise and the cytoplasmic half anticlockwise, and vice versa (see movies [5]). The stationary plane relative to which the rotation occurs passes through the center of LacY parallel to the membrane. The coupled counter-rotation of the intra- and extracellular halves of the TM helices is highly concerted and cooperative.
- As the cytoplasmic pore is open, the intracellular halves of the TM helices are loosely coupled as compared to the extracellular ones. However, rotation of the intracellular TM half is also cooperative and concerted. The long polypeptide loop, located on the pore perimeter and connecting inter-domain TM6 (yellow) and TM7 (red), rotates (brown donut mark). On the side opposite the pore, the N-terminus (connected to TM1 (red)) and the C-terminus (small helix connected to TM12 (yellow)) rotate around the pore axis (see movies [5]). In counter-rotation pore size is nearly unaffected.

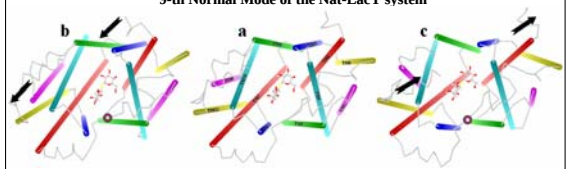
8-th Normal Mode of the Nat-LacY system



Main observations:

- Large-scale expansion and contraction of the intracellular mouth. N- and C-termini attached to TM1 (red) and TM12 (yellow) and located on the perimeter of the cytoplasmic pore alternately approach and separate (black arrows) affecting intracellular mouth size (see movies [5]). The side diagonally opposed to the pore is far less mobile. The long polypeptide loop is space-fixed and doesn't rotate (brown donut mark).
- The extracellular mouth changes little. TM helices of the N- and C-terminal domains alternately approach and separate slightly.

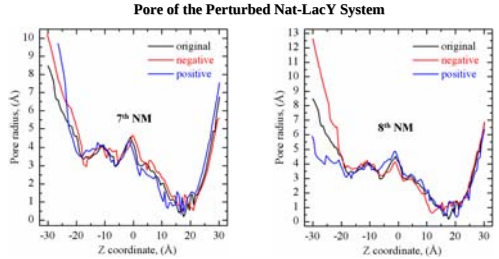
9-th Normal Mode of the Nat-LacY system



Main observations:

- N- and C-termini located on the perimeter of the cytoplasmic pore bend/twist in unison and concertedly relative to the side diagonally opposed to the pore (see movies [5]). This motion opposes that along NM 8, where the N- and C-termini alternately expand and contract the intracellular mouth. Like NM 8 but unlike NM 7, the long polypeptide loop remains space-fixed (brown donut mark) and doesn't rotate.
- The extracellular domain undergoes a small global bending/twisting motion. Pore size on both intra- and extracellular sides is nearly invariant.

Pore of the Perturbed Nat-LacY System



Main observations:

➢ In perturbing to an RMSD of 3.5 Å along low-frequency NMs in either direction, the extracellular pore stays closed. In perturbing along NM 8 in the negative direction, there is insignificant (~1.0 Å) opening.

➢ In perturbing along NM 8, the protein's intracellular mouth opens (negative direction) and closes (positive direction) widely. The region between -20 and 0 Å is nearly unaffected.

Tracking the low-frequency NMs using RTB MC-NMF

The low-frequency eigenvectors and/or their combinations were tracked using the RTB MC-NMF technique [6,7]. A number of RTB MC-NMF runs were executed for the G154C (native) mutant, always TDG-loaded, with protonated/deprotonated E325 and/or D36 and in the presence/absence of water molecules.

Main observations:

➢ In all runs, we saw neither complete closure of the inward-facing cavity nor opening of the outward-facing cavity. Presumably, TDG in the binding site and/or the protonated E325 prevents cytoplasmic mouth closure. Thus, the back transition (Introduction, step 3) from an inward-facing conformation to the outward-facing state doesn't occur in TDG-loaded systems.

➢ During positive tracking along the 7th and/or 8th NMs, the periplasmic ends of TM3&TM4 and TM9&TM10 (pink & cyan) move radially away from the pore axis, but TM1&TM7 (red) remain intact despite the protonation of D36 (TM1) that forms an inter-domain link with N245 (TM7).

Conclusions

- NMA reveals that, but for the first six NMs, describing overall translational and rotational motions of the entire system, the 7th and higher low-frequency NMs describe global torsion, bending or twisting conformational changes of the entire system. The 7th NM perfectly captures the overall counter-rotations (global counter-torsions) of the protein's intracellular and extracellular halves. The 8th NM shows large-scale alternating expansion and contraction of the intracellular mouth. These NMs identify the intrinsic directionality of conformational changes for initiating gating.
- NMA results reveal that conformational changes of the protein backbone are more complex than a simple rigid-body relative rotation of ~60^0 between the N- and C-terminal domains. However, the displacement along the 7th NM in the positive direction agrees well with the direction of a proposed rigid-body rotation. In a water-free system, we observe that the relative inter-domain movement (relative sliding of TM5 and TM11 (blue)) along the 7th NM becomes more pronounced compared to an overall counter-rotations of the water-filled protein. Displacement along the 8th NM in the positive direction leads to movement of the N- and C-terminal domains toward each other, closing the cytoplasmic mouth.
- It was suggested [1] that release of lactose and deprotonation of E325 triggers a conformational change that switches the protein's inward-facing conformation to a state with the empty binding site accessible from the periplasm. The removal of substrate from the binding site could be key for closing the intracellular mouth as observed in recent MD simulations [8]. However, many other factors, the presence/absence of TDG, the charge state of multiple ionizable residues and breakage/formation of contact networks (salt bridges) between N- and C-terminal domains, could influence conformational changes on the gating pathway. Multiple minimization and RTB MC-NMF runs are required to test a combination of these possibilities. This work is in progress. The RTB MC-NMF runs on TDG-unloaded LacY (Introduction, state 6) and TDG-loaded LacY with protonated E269 (Introduction, state preceding state 3) are also underway.

References

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