Biochimica et Biophysica Acta 1858 (2016) 1541–1552

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Modeling structural transitions from the periplasmic-open state of lactose permease and interpretations of spin label experiments

Xiaohong Zhuang [a], Jeffery B. Klauda [a,b,*]

[a] Department of Chemical and Biomolecular Engineering, College Park, MD 20742, USA
[b] Biophysics Program, University of Maryland, College Park, MD 20742, USA

ARTICLE INFO

Article history:
Received 3 December 2015
Received in revised form 25 March 2016
Accepted 19 April 2016
Available online 20 April 2016

Keywords:
Molecular simulation
Transmembrane protein
DEER
MDDS

ABSTRACT

Lactose permease of *E. coli* (LacY) is a secondary active transporter (SAT) that belongs to the major facilitator superfamily (MFS). Experimental structures of the cytoplasmic-open and more recently occluded-like structure have been determined, however, the crystal structure of LacY in the periplasmic-open state is still not available. The periplasmic-open LacY structure is important for understanding complete proton/sugar transport process of LacY as well as other similar SAT proteins. Previously, a structural model of periplasmic-open LacY has been obtained through a two-step hybrid implicit–explicit (IM–EX) simulation method (*JMB* **404**: 506). Molecular dynamics simulations are performed to further test the IM-EX model for the periplasmic-open LacY with ββ-$(Galp)_2$ in a lipid membrane. The comparison of the calculated pore radii to the data of the crystal structure indicates that the IM-EX model of LacY remains periplasmic-open in E269-protonated states. The neighbor residue distance change based on Cα are very similar in simulation results, but they are significantly different in double electron–electron resonance (DEER) experimental data, which motivates us to perform the molecular dynamics dummy spin-label (MDDS) simulations to test the effect of spin labels (size and internal flexibility) on DEER spin label distance measurements. The MDDS simulation results show that the orientation and movement of the spin labels significantly affect the residue pair distance measurement. DEER data alone may not provide an accurate guide for predicting protein structures. MDDS simulations can be applied to analyze the distance distribution due to spin labels and also aid in proper interpretation of DEER experimental data.

1. Introduction

1.1. Lactose permease

Membranes are the boundaries that protect cells and internal organelles from external environments, and they also regulate the molecular traffic across the membrane boundaries [1]. Normal functioning of cells requires energy, and disaccharides (e.g. lactose) are one example of energy source. In *Escherichia coli* (*E. coli*), lactose is transported into the cell via a transmembrane protein (e.g. lactose permease, LacY) that catalyzes the coupled translocation of a β-galactosides and a proton [2]. LacY is classified as a secondary active transporter (SAT) that utilizes the free energy from transporting of proton along the gradient to drive the accumulation of β-galactosides against their concentration gradient [3] via a symport mechanism. It belongs to the major facilitator superfamily (MFS) which is the largest and most diverse transporter that shuttles many types of substrates, such as ions, peptides and disaccharides [4] as in LacY. Moreover, the MFS can be found in membranes of many living organisms [5,6].

Based on the complete sugar/proton transport cycle in LacY proposed by Guan and Kaback [7], a similar schematic model of process with the known occluded transition states added by Madej, et al. [8] is shown in Figure S1, which involved eight transition steps: (1) Sugar induces LacY to change from *apo*-occluded to outward-facing state; (2) proton binds to LacY on periplasmic side and protonates E269 (or the proton may be shared between E269 and H322); (3) sugar binds to LacY on the periplasmic side; (4) the outward-facing LacY becomes occluded during which the proton is transferred to E325 through H322; (5) the occluded LacY changes to the inward-facing state; (6) sugar is released on the cytoplasmic side; (7) proton is released on the cytoplasmic side; and (8) LacY changes back to the *apo*-occluded state [7–9].

Structurally, LacY consists of 417 residues, among which twelve transmembrane helices are connected by hydrophilic loops, short N-terminus and C-terminal tail [12]. It is pseudo-symmetric with six helices in N-domain and C-domain respectively, and the interior hydrophilic cavity [13], like other MFS members [4]. As shown in Fig. 1, the earlier obtained LacY crystal structures are in the cytoplasmic-open (inward-facing), such as 2V8N [10], or 1PV7 [14], in which the

* Corresponding author at: Department of Chemical and Biomolecular Engineering, College Park, MD 20742, USA.
E-mail address: jbklauda@umd.edu (J.B. Klauda).

http://dx.doi.org/10.1016/j.bbamem.2016.04.008
0005-2736/

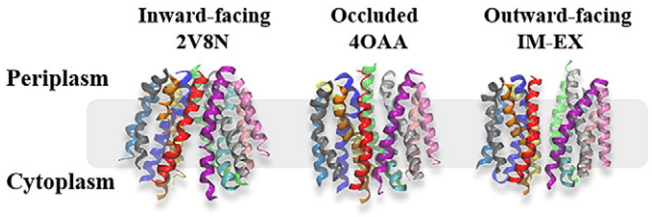

Fig. 1. Side view snapshots of the cytoplasmic-open 2V8N LacY crystal [10], the occluded-like 4OAA LacY crystal [11], and the periplasmic-open IM-EX LacY model [9], with the following color scheme for each helix: blue = H1, red = H2, gray = H3, orange = H4, yellow = H5, tan = H6, silver = H7, green = H8, pink = H9, cyan = H10, purple = H11, mauve = H12.

cytoplasmic side is open, and the periplasmic side of LacY is tightly packed. More recently an occluded-like structure (4OAA [11]) has been determined, in which both the cytoplasmic and periplasmic sides are closed based on pore radii. The crystal structure of periplasmic-open (outward-facing) LacY is still not available. The residues involved in proton translocation are near the center of the LacY hydrophilic cavity [15]. According to the alternating access mechanism [16], the periplasmic side of LacY should be able to open and close during the translocation of sugar and proton across the membrane [17–19]. The existence of periplasmic-open LacY is experimentally supported by double electron–electron resonance (DEER) [20], fluorescence resonance energy transfer (FRET) [21], site directed alkylation [22–24], and thiol crosslinking [18].

1.2. Outward-facing LacY models

The exact structure of periplasmic-open LacY is important for understanding the complete proton/sugar transport cycle of LacY and also the mechanism of other substrate transporters. Molecular simulation methods have been applied to determine the periplasmic-open LacY structure by Pendse et al. [9]. We name this model here as the IM-EX LacY model (Fig. 1), since it is obtained through a two-step hybrid implicit–explicit (IM-EX) simulation method, in which self-guided Langevin dynamics (SGLD) simulations [25,26] are performed with LacY in an implicit membrane, from which the partial periplasmic-open structures obtained are used in molecular dynamics (MD) simulations in an explicit lipid bilayer to obtain the LacY structure that is fully open on the periplasmic side. There are more bioinformatics periplasmic-open LacY models available using a structural repeats model and a FucP template [13]. MD simulations are also performed to test the accuracy of those models, the results of which will be reported in a future publication. The IM-EX LacY is the periplasmic-open model presented in this paper.

1.3. Experimental methods for LacY study

A few experimental techniques, such as DEER [20], FRET [21], site directed alkylation [22,23], and thiol crosslinking [18], have been used to understand the conformations of LacY. In single-molecule fluorescence (Förster) resonance energy transfer (sm-FRET) [27,28], the burst of fluorescence due to the energy transfer of coupling fluorophores is detected by alternating laser excitation spectroscopy [29]. FRET can be used to measure the distance changes on the cytoplasmic and periplasmic sides of LacY due to sugar binding, in which LacY is labeled with fluorophores at residues R73C/S401C (helices III/XII) at the cytoplasmic ends and residue I164C/R375C (helices V/XI) at the periplasmic ends. Then the distance change of the fluorophores can be calculated based on the shift of the energy transfer efficiency [21]. The results show that upon binding of a galactopyranoside, the distance of labeled residues decreases on the cytoplasmic side and increase on the periplasmic side [21]. Site-directed alkylation measures changes in reactivity for single-Cys replacement mutants for most residues in LacY, and the results show that with the presence of sugar, the reactivity increases on the periplasmic side [22,23]. Thiol crosslinking measures LacY transport activity with varied size of cross-linking agents, and the results demonstrate that after cross-linking with homobifunctional reagents less than 15 Å in length, LacY loses sugar transport activity [18].

In DEER, there are pairwise couplings between electron spins of two unpaired electrons of spin labels, and the coupling interactions are observed in the varied time [30]. The four-pulse DEER [31] experiment has been commonly applied to measure the distances between the electron spins. For LacY, the distance of nine residue pairs have been measured [20]. The same conformational change in FRET is observed with DEER. i.e., the galactopyranoside binding induces a closing on the cytoplasmic side and opening on the periplasmic side. The Cα distance change of most residue pairs of the outward-facing IM-EX LacY model has a reasonable agreement with DEER. The major disagreement is that the cytoplasmic side does not close as much as DEER data suggests. However, the cavity on the cytoplasmic side for the IM-EX model is closed enough to prevent sugar from transporting to the cytoplasm [9]. In this study, MD simulations are performed to test the accuracy of the IM-EX LacY model. Another key disagreement between IM-EX LacY model and DEER is that distance change between the spin-labeled neighboring residues (S136/Q340 and N137/Q340, S136/S401 and N137/S401) are very similar in simulated Cα distance [9], but are significantly different in DEER experimental data [20], which motivated us to perform MDDS simulations to test the effect of spin labels (size and internal flexibility) on the LacY residue distance measurement. DEER is very important for the study of transport of LacY and other membrane proteins [7,14,32]. Though all the experimental techniques are able to qualitatively prove that sugar transport of LacY involves opening and closing of periplasmic cavity, DEER is the only successful experimental method to date that provides detail of the outward-facing LacY conformation by measuring the distance of spin labels attached to residues. DEER analyses without considering the spin label effect is acceptable for the study of the broad structural features of a protein, such as the position of the subunits of a membrane protein with respect to one another or to the membrane, however, in order to understand the detail structure and dynamic behavior of a protein, it is necessary to consider the dynamics of the spin label [33]. Moreover, as the only available estimate for residue distance changes from the known inward crystal structure to the unknown outward-facing LacY structure, the accurate interpretation of DEER data is necessary for the structural determination of LacY and any other dynamic membrane transporters.

Schiemann et al. [34] performed MD simulations to search the possible conformations of a rigid nitroxide spin label in 2004, based on which Sale K. et al. [86] proposed to study the conformations of flexible spin labels through study torsion angles. The necessity of including the analyses of internal flexibility of spin label on DEER data interpretation was emphasized by DEER experts Borovykh et al. [35] and Jeschke et al. [36]. Since then, MD simulations have been often used to model spin label conformations in T4 lysozyme (T4L) together with their protein surroundings. The conformation of the MTSSL is characterized by the five free torsion angles in the spin label, and has been studied by performing the MD simulations of site-directed spin labeling (SDSL) with electron paramagnetic resonance (EPR) experiments [33,37–39]. Jeschke et al. developed three generations of rotamer libraries through varied methods. The first (of the three) generation rotamer library [40] was developed based on the results of the MD simulations of SDSL EPR, and it helped to obtain the structure of Na^+/H^+ antiporter of *E. coli* [41]. The second generation was developed by DFT computations and it helped in the determination of the backbone structure of Na^+/proline symporter PutP of *E. coli* [42]. The third generation was obtained by projecting conformations of spin label with long MD trajectories onto a set of canonical rotamers and it was tested by Na^+/H^+ antiporter NhaA [43]. The third generation rotamer library is implemented in an open source package Multiscale Modeling of Macromolecules (MMM). The accuracy of rotamer library is improved generation