Conformational Transition Pathways in Major Facilitator Superfamily Transporters

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Abstract

Major facilitator superfamily (MFS) of transporters consist of three subclasses: symporters, uniporters, and antiporters. We have performed a variety of equilibrium and non-equilibrium all-atom molecular dynamics (MD) simulations on bacterial proton-coupled oligopeptide transporter GkPOT, glucose transporter 1 (GluT1), and glycerol-3-phosphate transporter (GlpT) to compare the similarities and differences of the conformational dynamics of three different classes of the MFS superfamily in an explicit membrane environment. Our results of GkPOT reveal, with the aid of non-equilibrium pulling simulations and string method with swarms of trajectories, that the transition from the inward-facing (IF) to the outward-facing (OF) conformation whilst leaving the key inter-bundle salt-bridge of E310-R43 intact. Admittedly in comparison to previous GlpT simulations, the key salt-bridge must break to allow for the conformation to be explored. In spite of this, GluT1, transitions from the IF to the OF state spontaneously and then transitions to an OF-occluded conformation. All simulations are performed in the apo state of protein and reveal that despite their similarities, they use very different mechanisms to transition between the IF and OF states and they do so on very different timescales.

1 Introduction

The major facilitator superfamily (MFS) is the largest family of secondary membrane transporters.[1,2] MFS transporters posses one of three distinct mechanisms, 1. symport: the transport of two more substrates in the same direction, 2. uniport: tranport of one substrate across its concentration gradient and 3. antiport: which is the transport of substrates in opposite directions.[3] MFS transporters are composed of two transmembrane helix bundles, the N and C. Transmembrane helices 1-6 compose the N-bundle and 7-12 compose C. The MFS transporters are known to adapt a specific variation of the famous alternating access mechanism, known as the "rocker switch", in which the N and C bundles will open either inward facing (IF) or outward facing (OF), a substrate or substrates will move into the binding site of the protein. It will then transition into the occluded conformation and then move towards either inward or outward facing to release the substrate or substrates.[2–5] Three proteins whose function derives from the three different classes of MFS transporters, will be highlighted as the focus for understanding how each posses very similar and very different conformational dynamics: 1. Proton-coupled oligopeptide transporter (POT) 2. Glucose transporter 1 (GluT1) 3. Glycerol-3-phosphate transporter (GlpT).

Proton-coupled oligopeptide transporters (POTs) are of the symporter class of the transporters.[6] POTs translocate peptides, oligopeptide referring to having very few peptides i.e. 2 - 20 amino acids, and peptide like molecules across the cell membrane using an inward directed proton electrochemical gradient.[7] GluT1, a uniporter belonging to the sugar porter subfamily of MFS transporters, mediates the uptake of glucose by passively transporting it along its concentration gradient. GlpT is an antiporter member of the MFS and exchanges organic and inorganic phosphates across the membrane.

2 Theoretical Methods

Characterizing structural transitions of membrane transporters without compromising the detailed chemical description of these systems and their environments is computationally challenging mainly due to the prohibitively long timescales involved in such processes. Here we have employed brute-force all-atom MD, as well as nonequilibrium pulling and string method with swarms of trajectories to study the conformational transitions of GkPOT, GlpT, and GluT1, all in their apo states.

First, we prepared a membrane-embedded model of the wild-type *apo* GkPOT in the IF state from its crystal structure (PDB: 4IKV)[8] using Molecular Operating Environment (MOE)[9] for preparing the protein and CHARMM-GUI[10,11] for building the simulation system. All the crystal structure waters were removed, appropriate protonation states for the residues were assigned using protonate3D facility in MOE and hydrogens and other missing atoms were added using MOE. Protein was placed in the 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE) lipids, solvated in a box of TIP3P[12] waters and 0.15 M NaCl using CHARMM-GUI.[11] This simulation system consists of one protein, 113/107 POPE lipids in the upper/lower leaflet, 16,172 TIP3P waters, and 43/56 sodium/chloride ions with a total of 83,767 atoms in a box of size $\approx 106\times105\times104$ Å^3 simulated with periodic boundary conditions. Given the similarity of all crystal structures we used the same model with minor modifications to (i) protonate residue E310 and/or (ii) dock a substrate to the binding site. Three substrates were used including AA, FF, and EE, while the alafosfalin-bound GkPOT crystal structure (PDB: 4IKZ)[8] was used as a template to dock the dipeptides. In total, we modeled 8 different systems of GkPOT; four systems with the unprotonated-E310 and four with the protonated-E310. In each case one system is in the *apo* form and the other three are substrate-bound. We use CHARMM36 all-atom additive force field to describe all molecules.[13,14]

All MD simulations were performed with NAMD 2.10–11.[15] Prior to equilibration runs

we energy minimized each system for 10,000 steps using conjugate gradient algorithm[16] and relaxed using a multi-step restraining procedure of $\sim$1 ns.[10] This initial relaxation was performed in an NVT ensemble while all production runs were performed in an NPT ensemble. Simulations were carried out using a 2 fs timestep at 310 K using a Langevin integrator with a damping coefficient of γ =0.5 ps^{-1}. The pressure was maintained at 1 atm using the Nosé-Hoover Langevin piston method.[17,18] The smoothed cutoff distance for non-bonded interactions was set to 10$-$12 Å, and long-range electrostatic interactions were computed with the particle mesh Ewald (PME) method.[19] All production runs were performed starting from the relaxed models. Each system was equilibrated for 400 ns (Set 1) and repeated with a different initial velocity for another 400 ns (Set 2). The trajectories were collected every 0.5 ns for the analysis, which was conduced using VMD.[20] $2 \times 8 \times 400\, ns/5\, ns = 12,800$ data points were thus used for the statistical analysis presented in this work.

3 Results and Discussion

3.1 Monitoring the Conformational Dynamics

As GkPOT transitions from the IF to OF conformation, transmembrane helices 1,7 rotate closer together indicating the closing of the cytoplasmic gate. This is also indicated by the plotting of the Interloop distance between 4,5-10,11. As the loop distance becomes closer, it stabilizes at an approximate distance of 13-15 Angstroms . Admittedly Figure(1D) depicts a slight destabilization of the key salt-bridge of E310-R43, despite the appearance, our post SMD equilibrium simulation indicates that in the OF conformation the key salt-bridge is indeed still intact. Which would indicate that as the protein transitions into the OF state, the key salt-bridge may stay left in tact when GkPOT is unprotonated.

(Fig. 1A), N-C Bundle Domain Angle (Fig. 1B), $L_{4,5}$-$L_{10,11}$ interloop distance (Fig. 1C), H1,H7 interhelical angle (Fig. 1D), and R43-E310 salt-bridge minimum donor-acceptor distance (Fig. 1D).

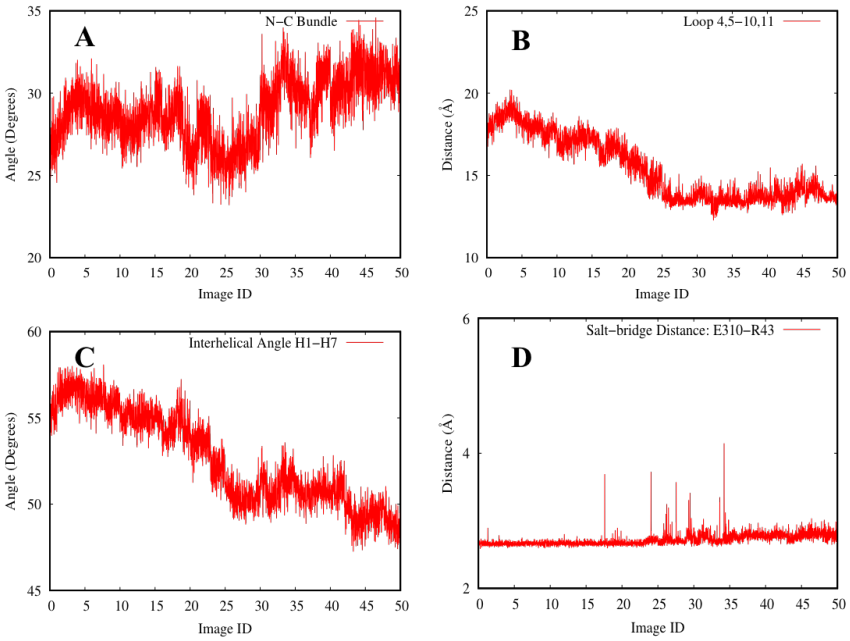

Figure 1: GkPOT transporter conformational transition from IF to OF. The definitions of some of the measures quantifying important global (A–C) and local (D) conformationl features are illustrated.

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Supporting Information Available

Figures S1–S7 and Tables S1-S3 in Supporting Information provide additional analysis based on our MD trajectories as discussed in the manuscript. This material is available free of charge via the Internet at `http://pubs.acs.org/`.

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