Gating in the LeuT<sub>Aa</sub> Symporter: A Normal Mode Analysis Study

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Abstract

Opening and closing mechanisms of the extracellular and intracellular gates of the Na<sup>+</sup>/Cl<sup>−</sup>-dependent neurotransmitter transporters that catalyze the symport of small molecules and ions across membranes remains an area of active research [1]. Large-scale conformational changes in a bacterial homologue, the LeuT<sub>Aa</sub> symporter [2], are studied using all-atom Normal Mode Analysis (NMA). Gating occurs in parallel by global cooperative motions of the extracellular and intracellular domains of LeuT<sub>Aa</sub> around the pore axis. As one half rotates clockwise, the other rotates counterclockwise, and vice versa. The stationary phase relative to which conformational motion occurs passes through the center of LeuT<sub>Aa</sub> parallel to the membrane. The consequences of the two halves are highly correlated and cooperative. On the extracellular side, the overall rotation of the peripheral TM helices affects the conformation of five of the peripheral TM helices, which are alternately strongly and weakly bent near their midpoints. Straining exchages and bindings expand the extracellular pore, with small helices on the extracellular surface of Leu<sub>T</sub> and large-scale motions. The intracellular domain of LeuT<sub>Aa</sub> rotates around the pore axis essentially as a single unit. Relative to the pore axis, the radial location of the intracellular ends of TM6b and TM3 and TM8 is not affected. Therefore, the total extracellular conformational changes of LeuT<sub>Aa</sub> and Water-LeuT<sub>Aa</sub> systems. The overlap is near ~1.

Introduction

Transporters are believed to possess two gates that provide alternating access to the binding site from opposite sides of the membrane bilayer [3]. In the crystal structure [4], the leucine molecule (Leu) at the binding site cannot access either the extracellular or the cytoplasm. The side chains of P252 and Y108 generally obstruct the pathway to the periplasm. Further towards the extracellular domain, the R30&404 charged pair forms a water mediated salt bridge which stabilizes the extracellular gate in a closed conformation. It has been suggested [5] that when the extracellular gate opens, R30 and D404 separate, impeding their extracellular side, there is ~1.3 Å gap of ordered protein structure and no pathway is readily identifiable. An angstrom from the LeuT<sub>Aa</sub> the following possible function backbone movements have been suggested [2]:

1. "The extracellular and cytoplasmic helical segments, that is TM1-TM10-TM12-TM14, are likely to move more relative to TM3, TM5 and TM7 and TM9, respectively, might shift their positions or rotate slightly. In addition, the extracellular and cytoplasmic helices could expand upon movement of water-ordered elements, including the N terminus, IL1 and EL2 and EF1.
2. Finally, the surrounding transmembrane segments, TM2, TM7, TM9, TM12 and TM14, may accommodate movement of TM3, TM5, TM7, TM9 and TM12 because they have interactions in between their helical structures. If GLY523 and PRO523 are removed, they have similar structures to GLY545 and PRO545 in TM7, GLY561 and PRO561 in TM12.

Computational Model

We use the high-resolution X-ray structure of LeuT<sub>Aa</sub> [2], a bacterial model system of 2,206 protein atoms, 210 water molecules, Leu (22 atoms), two sodium ions (+15.83 eV), one chloride, totaling 8,859 atoms. E419 was protonated; in the crystal structure its side chain was unprotonated.

Minimized LeuT<sub>Aa</sub> structure with two Na<sup>+</sup> ions (yellow spheres) and Cl<sup>−</sup> ion (green sphere) at their binding site.

For clarity, crashin diagrams are suppressed. Leu is shown as its binding site in the center of Leu<sub>T</sub>-Leu<sub>T</sub> and TM3 and TM8 are shown as the green (cytoplasmic) and grey (extracellular) sides. When R30 and D404 (from the Leu<sub>T</sub>-Leu<sub>T</sub> complex) are shown on the left (cytoplasmic) side. These residues form a bridge across the Leu<sub>T</sub>-Leu<sub>T</sub> pathway, are intracellular ends of TM10 and TM12 and TM14, respectively. All atoms in TM6 are green and red, respectively. They are broken at the Leu binding site and oriented relatively antiparallel. TM6<sub>Aa</sub> is in grey. Those atoms in the inner part of TM6<sub>Aa</sub> TM3<sub>Aa</sub> TM5<sub>Aa</sub> TM7<sub>Aa</sub> and TM10<sub>Aa</sub> form the protein for Leu and sodium ions, and comprise the inner TM helices. TM3<sub>Aa</sub> TM5<sub>Aa</sub> TM7<sub>Aa</sub> and TM10<sub>Aa</sub> are cyan and form another group of protein TM helices surrounding the inner helices, TM3<sub>Aa</sub> TM5<sub>Aa</sub> TM7<sub>Aa</sub>, and TM10<sub>Aa</sub>. The figure was generated using our MBF-3D code.

Eigenvectors of the minimized LeuT<sub>Aa</sub> systems

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

Main observations: Replacing Leu at its binding site in the LeuT<sub>Aa</sub> system of 8,204 protein atoms, 210 water molecules, Leu (22 atoms), two sodium ions, two chloride, totaling 8,859 atoms. E419 was protonated; the crystal structure its side chain was unprotonated.

<table>
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<tr>
<th>Water-LeuT&lt;sub&gt;Aa&lt;/sub&gt;</th>
<th>v, cm&lt;sup&gt;-1&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>675</td>
<td>1771.14</td>
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Frequency Spectrum of the LeuT<sub>Aa</sub>-Leu<sub>T</sub> system

The frequency spectrum is the same in all four runs (see movies [5]).

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<tr>
<th>Frequency, cm&lt;sup&gt;-1&lt;/sup&gt;</th>
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<tbody>
<tr>
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Conclusions

NMA identifies the intrinsic dynamicity of conformational changes in LeuT<sub>Aa</sub> for initiating a gating transition. The large-scale motions of the protein backbone along the low-frequency normal modes dictated by the LeuT<sub>Aa</sub> architecture are unique:

1. Highly correlated and cooperative overall conformational motion of the intracellular and extracellular LeuT<sub>Aa</sub> domains around the pore axis.
2. Large-scale expansion and contraction of the extracellular mouth.
3. In the periplasmic domain, overall rotation of the TM helices (at the inner part of TM6<sub>Aa</sub> TM3<sub>Aa</sub> TM5<sub>Aa</sub> TM7<sub>Aa</sub> and TM10<sub>Aa</sub>), TM3<sub>Aa</sub> TM5<sub>Aa</sub> TM7<sub>Aa</sub> and TM10<sub>Aa</sub> helices in agreement with suggestion it brings the extracellular gate to the extracellular permeate (suggestion 3). Leucine and small helices on the extracellular side undergo large-scale rotational motion.
4. The extracellular domain of LeuT<sub>Aa</sub> rotates around the pore axis nearly as a whole. The radial location of the extracellular ends of TM3<sub>Aa</sub> TM5<sub>Aa</sub> TM7<sub>Aa</sub> and TM10<sub>Aa</sub> relative to the pore is not affected.

8. Normal Mode of the LeuT<sub>Aa</sub> System

The pore is blocked on both the extracellular and intracellular sides. By perturbing the KMDG at 3.5 Å along low-frequency NM6<sub>Aa</sub> "open" (green arrows). TM6<sub>Aa</sub> is less affected. In the periplasmic domain, overall rotation of the TM helices (at the inner part of TM6<sub>Aa</sub> TM3<sub>Aa</sub> TM5<sub>Aa</sub> TM7<sub>Aa</sub> and TM10<sub>Aa</sub>), TM3<sub>Aa</sub> TM5<sub>Aa</sub> TM7<sub>Aa</sub> and TM10<sub>Aa</sub> helices in agreement with suggestion it brings the extracellular gate to the extracellular permeate (suggestion 3). Leucine and small helices on the extracellular side undergo large-scale rotational motion.

References

4. W. W. Hager & B. Zhang, STJ.Acknowledgement

Acknowledgement

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