Slow Gating in ClC Channels: Normal Mode Analysis

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Abstract

All-atom Normal Mode Analysis (NMA) was used to explore possible mechanisms for slow gating in CIC ClC channels. As the ‘‘potato-hat’’ architecture is well established throughout the CIC family, both channels and transporters (1), we use the high-resolution (2.5 Å) X-ray structures of E. coli ClC transporter (pdb entry 1OT5,1) and ClC-ec1 (pdb entry 2Z47,2) to describe it with the CHARMM2222 force field and carry out standard all-atom NMA. The monovalent cation concentration and the channel was resolved by dipole forces. Perturbing the system in either direction along the 7th all-atom normal mode (NM) leads to slow relative motion of the subunits, perpendicular to the membrane plane. The intracellularly oriented axis lies flat against the interface at the subunit interfaces. The position of the middle of the protein oscillates, separating and then nearly reaching. The B and A helices execute large-scale swaying, abruptly increasing and decreasing their cytoplasmic ends’ separations, motion in agreement with FREET experiments (4). The X-occupied intracellular pores behave as almost rigid units. As the subunits separate, the intracellular pores tilt relative to the membrane plane changes. By contrast, the extracellular portion of the subunit interface is less significantly altered, although small structural changes are clearly observable. These extracellular regions structurally affected by the subunits’ slow motion are localized at the extracellular C1-C2 pathways. As the subunits separate, these regions compress, shifting the extracellular pores. As they approach, the extracellular regions near the C1 pathway constricts, opening them.

Introduction

The channels and transporters of the CIC family are crucial in regulating the resting membrane potential, cell volume and excitability of muscle cells. The crystal structure of the bacterial ClC-ec1 channel was solved as a dimer with two pores, each of which is entirely contained within a single CIC-ClC subunit. The slow gate closes both CIC proteins simultaneously, with an average time of ~20 seconds in C2-ec1, modeling a long-lived inactivated state. It is difficult to obtain clear slow gating from the static picture provided by the crystal structures of CIC. The molecular mechanisms of the slow gate is largely unknown. The complex conformational changes are thought to be associated with the slow-gating transition involving the interface between the CIC subunits.

We report here a hypothesis that the X-structure of an E. coli ClC-ec1 transporter (2,7) can be used as a template for understanding eukaryotic CIC channels. We use all-atom NMA to identify the large-scale motions of the ClC-ec1 domains. We find that the most significant collective NMA of the bacterial system for the ClC-ec1 slow gating is the lowest-frequency mode, perpendicular to a membrane plane. The slow gating in physiological conditions could involve a change from the intrinsic large-scale motions of the ClC biomolecule. We find that the most relevant conformational transition for the bacterial system to the dimer is denoted by the 7th all-atom NMA.

Computational Model

We use the high-resolution (2.5 Å) X-ray structure of E. coli ClC-ec1 transporter (pdb entry 2Z47) (1) for ClC-ec1 slow gating as a template for understanding eukaryotic ClC channels. We used all-atom NMA to identify the large-scale motions of the ClC-ec1 domains. We find that the most significant collective NMA of the bacterial system for the ClC-ec1 slow gating is the lowest-frequency mode, perpendicular to a membrane plane. The extracellular part of the interface between subunits A and B is highlighted by a pore that increases and decreases between these extracellular units.

The geometry of the extracellular pore is nearly unaltered. The configuration of the R, D and other helices surrounding the pore undergoes minor changes. These pores with their constant size are nearly rigid units. As the ClC subunits swing away, they fill with respect to the membrane plane changes substantially. Consequently the intracellular extracellular interface near the protein might be trapped protein might be trapped in the extracellular space.

The molecular system was described with the all-hydrogen CHARMM22 topology and parameter set, with NMA calculations carried out in vacuo.

To remove static charges and to rule the molecular system, a 2,000-minimization step was done using standard mass-distributed sphere radii. Finally, the molecular system was then used to generate charged conformations using a conjugate gradient and highly optimized BLAS routines for performing the standard normal mode analysis.

In contrast, the extracellular portion of the interface between subunits A and B is highlighted by a pore that increases and decreases between these extracellular units.

All-atom NMA only provides the initial direction of the large-scale slow transitions in ClC-ec1. All transitions were variable. When the intracellular parts swing away, the extracellular pore regions compress, denoting the extracellular pores. In the FREET experiments (4) the closure of the slow gate was also accompanied by a physical separation between the C terminus of the two subunits. When the intracellular pores approach each other, the extracellular regions near the ClC conduction pathways relax, opening the extracellular channels. This is also in agreement with the FREET experiments (4) opening of the slow gate was accompanied by movements decreasing the distance between the C termini.

All-atom NMA only provides the initial direction of the large-scale slow transitions in ClC-ec1. It cannot elucidate any other direction, stable conformations in which the CIC pore might be trapped for seconds. Such long-lived stable states can be identified by all-atom Monte Carlo Normal Mode Following (MC- NMF) (5) along the low-frequency NMA. It can be interrogated by: 1) which nallostructural rearrangements take place on the gating pathway; 2) how and why both pores can be locked in the closed state for seconds; and 3) how specific pore mutations affect the slow gating [4].

References