MOLECULAR DETAILS OF THE LUMINAL EXIT OF CALCIUM IONS IN THE CALCIUM PUMP OF THE SARCO/ENDOPLASMIC RETICULUM

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PI: Benoît Roux[1]
Co-PI: Avisek Das[1]

[1]University of Chicago

EXECUTIVE SUMMARY:

Sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) is an integral membrane protein that uses ATP hydrolysis as a source of free energy to pump two calcium ions per ATP molecule from the calcium-poor cytoplasm of the muscle cell to the calcium-rich lumen of the sarcoplasmic reticulum, thereby maintaining a ten-thousand-fold concentration gradient. Our goal is to understand the important dynamical motions of the pump via high-resolution crystal structures of several functionally relevant states along the pumping cycle. We employed an all-atom molecular-dynamics-based rare event method called string method with swarms of trajectories to study the large-scale conformational transitions of the pump. The method involves running a large number of short trajectories that communicate with each other at a regular interval. Our recent simulations on Blue Waters revealed unprecedented molecular details of an important step of the pumping cycle.

INTRODUCTION

Membrane proteins form an important class of biomolecules that are associated with the membrane dividing the inside of a cell (or a cellular compartment) and its environment. Our project is aimed at understanding the function of an integral membrane protein called sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) [1–3] that uses ATP hydrolysis as a source of free energy to pump two calcium ions per ATP molecule from calcium-poor cytoplasm of the muscle cell to the calcium-rich lumen of the sarcoplasmic reticulum. This process is important for relaxation of skeletal muscle, which is regulated by calcium ions. Over the past few years a number of structural studies [1–6] have provided atomic-resolution models for several important states along the pumping cycle. Two major outstanding issues are the pathways of ions from either side of the membrane to the transmembrane binding sites and a detailed description of the conformational changes that will elucidate how various parts of the protein communicate over fairly large distances in order to achieve coupled ATP hydrolysis and calcium transport.

We believe our project will have a significant impact in the field of membrane protein biophysics for several reasons. First, SERCA plays an important role in the relaxation of skeletal muscle and a close analogue, which is present in the cardiac muscle, is a therapeutic target. Therefore, understanding the molecular mechanism of the active transport process is of fundamental and biomedical interest. Second, SERCA is a member of P-type ATPase family of membrane proteins that have very similar topologies and reaction cycles. A very important member of this family is Na^+-K^+-ATPase which shares high sequence similarity with SERCA. It is conceivable that the detailed knowledge acquired by studying SERCA can be applied to understand the mechanism of Na^+-K^+-ATPase which has very few high resolution structures of relevant functional states. Third, this study will serve as a test case for evaluating success and limitations of advanced methods for studying complex conformational transitions in a biologically relevant system and will greatly benefit the entire bimolecular simulation community.

METHODS & RESULTS

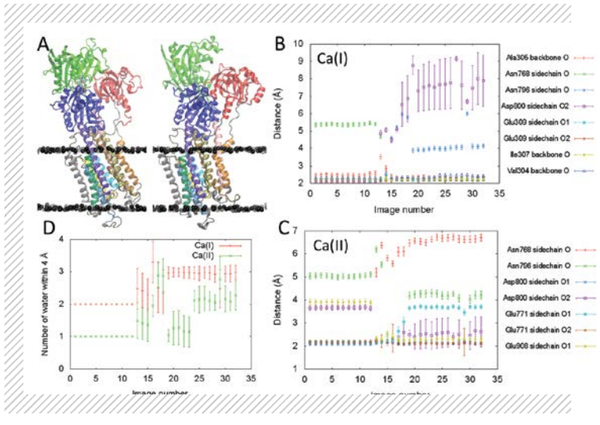
Over the past three years we have been involved in simulating large-scale conformational transitions of SERCA between experimentally resolved states. The size of the system (~290,000 atoms) and the time scale involved in the large scale motions prohibited the use of brute-force unbiased molecular dynamics (MD) [7] simulations to obtain statistically meaningful information. To circumvent this challenge, the string method with swarms-of-trajectories was used to discover the optimal minimum free-energy path between the two end states [8,9]. The path, which was represented as a chain of states or images in the space of relevant collective variables, was optimized by iterating two steps: moving each image along the drift calculated from an ensemble (swarm) of short unbiased MD trajectories initiated from the image and a re-parametrization procedure that keeps all the images equidistant.

We determined a transition pathway between the calcium-bound occluded state (PDB ID: 3BA6) and a calcium-free state with an opening toward the luminal side (fig. 1a). The transition in question is responsible for release of calcium ions into the luminal solution from the transmembrane binding sites. The string was represented by 32 images and for each image we used 32 trajectories for the estimation of the drifts (a total of 1,024 copies of the system). Each iteration of the method involved 20 picoseconds of simulation and 360 iterations were performed for production calculation. Simulations were carried out using a modified version of NAMD 2.9 [10]. The last twenty iterations were used for analysis. We have monitored the coordination environments of the calcium ions along the pathway (fig. 1b-c). The large-scale movements of the cytoplasmic domains induced important motions in several transmembrane helices that disrupted the binding sites, simultaneously forming an opening toward the luminal side. As a result of that, the water from the luminal side entered the interior of the protein and interacted with the calcium ions (fig. 1d, fig. 2). The final release of the ions presumably involved protonation of several binding site residues. The water access channel from the luminal side shed some light on the putative luminal exit channel of the calcium ions (fig. 2). These results complement the findings from structural studies and provide a comprehensive picture of a crucial step in the pumping cycle.

WHY BLUE WATERS?

The string method with swarms-of-trajectories is essential for simulating large-scale conformational changes in SERCA. In order to implement this method, one needs to simulate many copies of the system that communicate with each other at a regular interval. For meaningful results, more than a thousand copies of the system and hundreds of iterations are required. To perform this calculation on SERCA (~290,000 atoms), a single job requires more than 6,000 nodes, which is more than the total node count of an entire machine for many small- to medium-sized supercomputers. Therefore, the massively parallel architecture of Blue Waters played a crucial role in the success of our project.

FIGURE 1



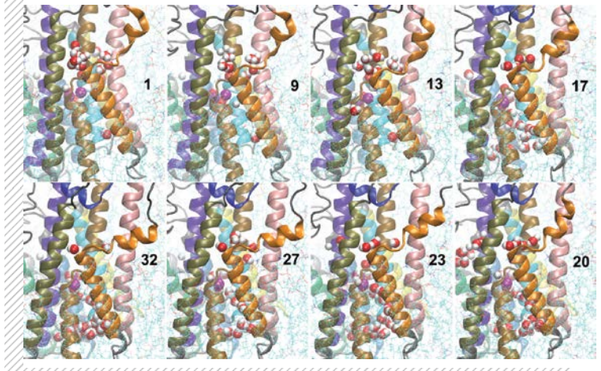


FIGURE 2: Water access pathway from the luminal side. This indicates the path ions take from the transmembrane region to the luminal side. The integers on the panels indicate the image indices of representative snapshots of the final pathway. The first and the last indices correspond to the occluded calcium-bound state and the non-occluded state with an opening toward the luminal side. The transmembrane helices are color coded as follows: M1 (orange), M2 (pink), M3 (tan), M4 (ochre), M5 (violet), M6 (cyan), M7 (dark green), M8 (sky blue), M9 (yellow), M10 (silver). Calcium ions are shown as purple spheres.

FIGURE 1: (a) Crystal structures of calcium-bound occluded state (left, PDB ID: 3BA6) and calcium-free state with an opening toward the luminal side (right, 2ZBE). (b) Distances between the coordinating residues and the first calcium ion along the pathway. (c) Distances between the coordinating residues and the second calcium ion along the pathway. (d) Number of water molecules around the calcium ions along the pathway.