Published in final edited form as:

J Comput Electron. 2007 September ; 6(1-3): 381–385.

Temporal Analysis of Valence & Electrostatics in Ion-Motive Sodium Pump

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Abstract

The present work establishes a unique framework for the simulation study of ion-motive pumps in general and the Na^+/K^+ -ATPase, or sodium pump, in particular. We shall discuss the implications of electrostatic analysis, valence calculations, and protein cavity data, each carried over data extracted from molecular dynamics simulations, on the structure-function relationship of Na^+/K^+ -ATPase. These diverse set of tools will be used to investigate atomic-level characteristics that remain undetermined such as ion binding and accessibility.

Keywords

Na^+/K^+ -ATPase; homology modeling; molecular dynamics

I. Introduction

The recent confluence of bio- and nano-engineering has drawn interest on ion transport through trans-membrane proteins. Ion transport takes place either through *passive* (ion channels) or *active* (ion pumps) proteins, which have innate properties such as selectivity and gating that allow them to be classified as bioelectric devices. Contrary to ion channels that have attracted attention from the device community recently [1,2], ion-motive pumps are still largely unexplored by the engineering community in general and device modellers in particular [3]. In this paper, we establish and use a unique methodology to study ion-motive sodium pump.

The Na^+/K^+ -ATPase, or sodium pump, is a voltage-gated membrane transport protein found in most higher-order eukaryotic cells and is essential for life. It is vital to maintaining a transmembrane voltage and regulating cellular volume. Even though electrophysiological studies have contributed the vast majority of information on the sodium pump's function in the last century, it has been only in recent years that molecular-level understanding has been forthcoming [4,5]. Still, many structure-function aspects, such as ion binding loci, extent of conformational changes, ion permeation pathway, and location of gating processes, crucial to the full understanding of this pump, remain elusive.

The sodium pump functions by using the Gibbs free energy from the hydrolysis of adenosine triphosphate (ATP) to exchange three intracellular sodium ions for two extracellular potassium ions (Fig.1). The cytoplasm consists of a sodium concentration an order of magnitude lower than the extracellular fluid, while the reverse is true for potassium. With the energy freed by ATP hydrolysis, sodium and potassium ions are moved against a strong electrochemical

gradient. This electrogenic functionality is believed to incorporate a dual-gating action that first allows ions to bind to the protein on only one side of the membrane, following with an occluded state where the inner and outer gates are both closed, and continuing with a gated release of the ions to the opposite side of the membrane. Movement of ions against a strong electro-chemical gradient, the hallmark of ion pumps, is also thought to accompany large changes in protein structure (Fig. 2), allowing this ping-pong exchange to occur. We seek to use a variety of modeling and simulation tools to investigate the effects of electrostatic and steric changes on the properties of ion binding sites as well as ion and water pathways [3]. To this end, we have developed, for the first time to our best knowledge, a comprehensive methodology, outlined in Fig. 3, beginning with homology modelling, followed by a protein-membrane system creation, leading to time-dependent analysis of molecular dynamics (MD) trajectories [6].

II. Modelling and Simulation

Recent successes in crystallography of the calcium pump by Toyoshima and others have given structures [7] of different conformations of the sarco(endo)plasmic reticulum Ca^{2+} ATP-ase, SERCA. All P-type ATPases are thought to share the same overall fold and the Na^+/K^+ -ATPase has a relatively high degree of similarity with SERCA. Although the overall identity of the two proteins is technically less than 30%, much of this difference is present in the cytoplasmic domains, which is distant from our focus on the ion binding cavity and extracellular ion pathway. Meanwhile, there is a high level of similarity in the transmembrane helices, especially in residues that are believed to coordinate ion binding [4]. Due to this similarity between the genomic sequences of these proteins, alignment of Na^+/K^+ -ATPase with SERCA gives reliable homology models [8]. Indeed, residues known to coordinate ion binding align well with their SERCA counterparts. The software Modeller [9] implements the method of spatial restraints that incorporates the secondary and tertiary structure of SERCA to improve this alignment and determine reliable models of the sodium pump.

After inclusion of the model in a lipid bilayer system, molecular dynamics simulators, such as GROMACS [11], can be used to explore water and ion accessibility and permeation pathways through simulations of the >300,000 atom protein-lipid-water-ions system. This is the first attempt to perform molecular dynamics simulations of a homology-modelled sodium pump and bilayer system, a task that has been considered previously only for the H^+/K^+ -ATPase [12]. A Beowulf cluster allows for simulations on the order nanoseconds to be analyzed for various conformational models and environmental states. Na^+/K^+ -ATPase models built from both of the primary conformational states of SERCA, E1 and E2, show different atomic interactions with Na^+ and K^+ ions that will be analyzed further to determine ion binding locations. Future work includes creation of SERCA systems that will serve as controls to determine the stability and accuracy of the sodium pump model systems. Additionally, our methodology may be used to provide estimates of physiological parameters of SERCA, since it cannot be studied easily via electrophysiological experiments.

III. Cavity Analysis

The underlying approach to our analysis is to include data from many time-steps of unrestrained MD trajectories of the protein-lipid system. Homology models provide merely an estimate at the actual structure of an unresolved protein. Even if a particular alignment is deemed highly accurate by structural data later determined, model aspects such as side-chain orientation can only be determined energetically. Our approach allows for the determination of an accurate system based on energetic considerations, coupled with analysis of system fluctuations over ns time scales to determine the most favorable protein configuration for a given environment.

Cavity analysis of the ion binding pockets of several time-steps of both SERCA and Na⁺/K⁺-ATPase systems shows no direct pathway from these regions to the extracellular lumen. This implies that this pathway of the P-type ATPases is not an unimpeded expressway as seen with some ion channels but is a fluctuating energy or steric barrier to ion release. This viewpoint may be supported by electrophysiological measurements of the relatively slow Na⁺ release rates from the E2 conformation of the sodium pump [13]. Using MD trajectories, this pathway may become distinguishable as data from many time-steps is gathered that allows fleeting sites and small cavities to be combined to create a putative pathway. This method of cavity analysis will also be used to conjecture the pathway leading from the ion binding sites to the intracellular solution, which is experimentally difficult and relatively unexplored.

IV. Valance

Site valence data are also incorporated into our analysis. The software program, VALE, has been shown to yield accurate predictions of metal ion binding site locations in a variety of protein structures [14]. Atomic structures are analyzed by VALE, which uses an empirically-based algorithm to determine favorable locations for specific ion binding due to carboxyl and side chain oxygens, as well as water. The data are post-processed with our software, CLOUD, to determine clusters that may indicate potential binding sites for a specific ion, such as Na⁺ or K⁺. Fig. 4 shows a control case using SERCA and a test case of Na⁺ site determination in a sodium pump homology model. The SERCA crystal structure displays residues in the ion-binding cavity that neatly coordinate bound ions and valence analysis pinpoints these sites. However, the valence analysis of a Na⁺/K⁺-ATPase model in Fig. 4b shows sites are not readily apparent, with only one site found that corresponds well with the literature. This situation is similar to the case of ion pathway determination via cavity analysis. The amino acid side-chains are in continual change every femtosecond during the simulation. Therefore, it is unlikely that any given frame of a MD simulation of a homology-modeled protein will give an accurate overall depiction of the state of the protein. In [15], amino acid side chains and water molecules are set into predisposed orientations in order to show putative binding sites of the Na⁺/K⁺-ATPase. We believe that results such as this can be determined through full-scale *ab initio* simulations without *a priori* knowledge of side-chain orientation. For example, data extracted at regular intervals (>500ns) of a 1 ns MD trajectory are clustered to yield sites that have a high degree of longevity, as shown in Fig. 5. These long-living sites are consistently within the transmembrane region of the protein. The grouping of spheres corresponding to SERCA site II (lower right) is due to fluctuations in the MD trajectory. Also, some sites near the extracellular lumen (upper left), also appearing in electrostatic analysis, require more investigation.

V. Electrostatics

The electropotential maps generated by APBS equipped with a multi-grid solver [10] can be augmented with protein surface/cavity calculations to show possible binding locations as well as ion/water permeation pathways between these sites and the exterior of the protein (Fig. 6). While full-scale electrostatics studies are ubiquitous in the device community, the biophysical community widely uses steric analysis of protein structures. However, only in the minority of cases does the protein analysis incorporate full electrostatics, which often consider only surface-mapped potentials without exploring the interior regions of the protein. This results from biochemical focus on the external interactions of biological structures, such as a ligand docking to a protein. However, electrostatic cross-sections in the protein's cavities yield information about the ions' environment, and will be instrumental for the accurate calculation of ion binding affinities. Fig. 7 provides a sample corner-section of electrostatic distribution in the center of sodium pump as found at the end of a 1ns MD simulation. The deep negative potential pocket found in the lower half of the transmembrane section, suitable for cation

binding, agrees with the predictions of valence analysis. However, other sites, majority of them fleeting in nature, may also be captured in such cross-sections, as can be seen in Fig.8. Thus we find that the analysis of single frames of a simulation gives little in the way of supporting experimental data (ion release rates) or providing new insights (intracellular ion pathway). This points out to the importance of our new methodology incorporating and analyzing multi-time-step data.

VI. Conclusion

The Na^+/K^+ -ATPase has been introduced and the structural and functional characteristics that remain undetermined have been outlined. The essential properties that still elude experimentation include conformational changes, gating processes, ion binding and water accessibility. To help resolve some of these problems, we have created homology models of the sodium pump based on different conformations of SERCA and incorporated these models in equilibrated bilayer systems. Using molecular dynamics simulations, we have begun investigations using several tools such as valence calculations, molecular surface determination, and electrostatic isopotential profiles to address some of these outstanding issues.

Due to inherent uncertainties created by homology modeling and thermodynamic fluctuations, time-averaged analysis seems to be required to accurately depict characteristics such as ion binding and the ion pathway. In the present work, we provide preliminary example for such an analysis of valence data from a sequence of frames from molecular dynamic trajectories in order to cull fleeting, false-positive, putative sites from those that appear on a consistent basis. This information, along with electrostatic calculations and molecular surface data will be used to further ascertain their effectiveness in verifying basic atomic-level characteristics of the Na^+/K^+ -ATPase. Thus efficient 3D solvers originally developed for electrical devices may facilitate modeling and analysis of biomolecules and biodevices not yet explored.

References

1. van der Straaten TA, Kathawala G, Trellakis A, Eisenberg RS, Ravaioli U. BioMOCA--a Boltzmann transport Monte Carlo model for ion channel simulation. *Mol Sim* 2005;31:151–171.
2. Millar C, Asenov A, Roy S. Brownian Ionic Channel Simulation. *J Comp Elec* December 2003;2:257–262.
3. Rakowski RF, Kaya S, Fonseca J. Electro-Chemical Modeling Challenges of Biological Ion Pumps. *J Comp Elec* April 2005;4:189–193.
4. Rakowski RF, Sagar S. Found: Na^+ and K^+ Binding Sites of the Sodium Pump. *News Physiol Sci* August 2003;18:164–168. [PubMed: 12869617]
5. Artigas P, Gadsby DC. Na^+/K^+ -pump ligands modulate gating of palytoxin-induced ion channels. *Proc Natl Acad Sci USA* January 21 2003;100(2):501–505. [PubMed: 12518045]
6. Fonseca, J.; Kaya, S.; Rakowski, RF. “Models, Electrostatics and Molecular Dynamics of the Na^+/K^+ -ATPase, Proceedings of the Ohio Collaborative Conference on Bioinformatics, 2006. www.occbio.org
7. Toyoshima C, Nomura H, Tsuda T. Lumenal gating mechanism revealed in calcium pump crystal structures with phosphate analogues. *Nature* November 18 2004;432:361–368. [PubMed: 15448704]
8. Sweadner KJ, Donnet C. Structural Similarities of Na,K ATPase and SERCA, the Ca^{2+} -ATPase of the sarcoplasmic reiticulum. *Biochem J* 2001;356:685–704. [PubMed: 11389677]
9. Martí-Renom MA, Stuart AC, Fiser A, Sánchez R, Melo F, Sali A. Comparative Protein Structure Modeling of Genes and Genomes. *Annu Rev Biophys Biomol Struct* 2000;29:291–325. [PubMed: 10940251]

10. Baker NA, Sept D, Joseph S, Holst MJ, McCammon JA. Electrostatics of nanosystems: Application to microtubules and the ribosome. *Proc Natl Acad Sci USA* 2001;98:10037–10041. [PubMed: 11517324]
11. Berendsen HJC, van der Spoel D, van Drunen R. Gromacs: A message-passing parallel molecular dynamics implementation. *Comp Phys Comm* 1995;91:43–63.
12. Munson K, Garcia R, Sachs G. Inhibitor and Ion Binding Sites on the Gastric H,K-ATPase. *Biochemistry* 2005;44:5267–5284. [PubMed: 15807521]
13. Sagar A, Rakowski RF. Access channel model for the voltage dependence of the forward-running Na^+/K^+ pump. *Journal of General Physiology* 1994;103:869–894. [PubMed: 8035166]
14. Nayal M, Di Cera E. Predicting Ca^{2+} -Binding Sites in Proteins. *Proc Natl Acad Sci USA* January 18 1994;91:817–821. [PubMed: 8290605]
15. Ogawa H, Toyoshima C. Homology modeling of the cation binding sites of Na+K+-ATPase. *Proc Natl Acad Sci (USA)* 2002;99:15977. [PubMed: 12461183]

Figure 1.

The Post-Albers cycle above indicates the main stages of conformational changes (E1–E2), ion binding, release and occlusion, and ATP hydrolysis. On the intracellular side, potassium is released and sodium is bound, while this process is reversed on the extracellular side. The large arrow indicates the forward pump cycle.

Figure 2.

Depiction of the large movement of the three intracellular domains and reorientation of the transmembrane helices between conformation E1, which allows ion access to the intracellular side of the membrane and E2, which allows ion access to the extracellular side. The transmembrane helices are shown in light blue.

Figure 3.

An overview of the methodology used in this paper. Homology modeling is followed by membrane and protein system preparation. After system equilibration, unrestrained molecular dynamics trajectories are analyzed with valence, electrostatic and molecular surface tools to investigate protein and ion characteristics.

Figure 4.

Cytoplasmic-side view of transmembrane helices in a) SERCA · 2Ca²⁺ and b) corresponding homology model of Na,K-ATPase, with residues involved in calcium binding shown in stick representation. Transparent blue spheres represent calcium ions from SERCA E1·2Ca²⁺ (PDB code 1SU4) with Cloud output indicating putative binding sites (I and II) in green. Valence for site I is 2.28 and 2.19 for site II. In the case of Na,K pump, four putative sites (shown with magenta spheres) were found in the cavity created by the transmembrane segments. Residues that are predicted to bind Na⁺ are shown in stick representation. The putative site where TM4 is unwound corresponds to site II in SERCA. Site I is believed to lie between TM5 and TM6, similar to that for SERCA on the left.

Figure 5.

Valance analysis of the sodium pump in the E2 state. Approximate membrane location shown by lines, with TM4 in red, TM5 in orange, TM6 in yellow, and TM8 in green. Putative K^+ sites extracted from time- and spatially-clustered valence data, over the latter half of a 1ns MD simulation, are shown as blue spheres. The agglomeration on the bottom right corresponds to Ca^{2+} site II in SERCA. Note that only the sites within 5nm radius of the agglomerate site are shown here for clarity.

Figure 6.

Transmembrane regions of the E2 state of a SERCA (PDB code: 1WPG) structure (left) and sodium pump homology model, each with a negative isopotential surface (green) indicating a high-affinity region for cations. Two bound Ca^{2+} ions, present in the crystallized SERCA structure, can be seen inside the isopotential surface at left.

Figure 7.

Electrostatic corner-section of the E2 sodium pump from one timestep of a MD simulation. Regions of high negative potential are shown in blue, with large positive potentials shown in orange. Contour lines corresponding to -5 , -70 and -140 kT/e are also shown. Note that A deep K^+ binding pocket appear near the corner and another one half-way up on the $y=0$ plane (upper-left of the central binding site) and that no continuous passage-way between the two sides of the membrane exists.

Figure 8.

Temporal analysis of electrostatic cross-section (blue: high negative potential, orange small negative potentials) of the E2 sodium pump using four MD snapshots. Contourlines corresponding to -162.1 kT/e are also shown. In all cases, the main ion binding site in the lower right is clearly visible, together with fleeting sites that fluctuate.