

Electrostatic Modeling of Ion Motive Sodium Pump

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INTRODUCTION

An ongoing convergence of bio and nano engineering has focused 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 [3].

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. The sodium pump is vital to maintaining a transmembrane voltage and regulating cellular volume. Electrophysiological studies have yielded a wealth of information on the sodium pump's function in recent years [4,5]. Yet, many aspects regarding the structure-function nature of the pump such as ion binding, permeation and gating 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). Sodium ions are moved against a strong electrochemical gradient and the pump can also be operated in the reverse mode. This functionality is believed to incorporate a dual-gating action that allows ions to bind to the protein on only one side of the membrane, followed by an occluded state where both gates are closed, and continuing with a gated release of the ions to the opposite side of the membrane. Movement of ions against a strong electrochemical gradient, the hallmark of ion pumps, is thought to accompany large changes in protein structure, 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].

HOMOLOGY MODELING

Recent successes in crystallography by Toyoshima and others have given structures [6] of different conformations of the calcium pump, SERCA, which has a relatively degree of similarity with the sodium pump. Due to this similarity between the genomic sequences of these proteins, aligning the genomic sequence of Na⁺/K⁺-ATPase with SERCA gives reliable homology models. The software Modeller [7] improves this alignment with the secondary and tertiary structure of SERCA to determine reliable models of the sodium pump (Fig.2). After inclusion of the model in an lipid

bilayer, calculated electropotential maps and pathways determined from the molecular surface can be combined with molecular dynamics simulations to investigate regions in which ion binding and permeation is believed to occur.

ELECTROSTATICS

The electropotential maps generated by APBS [8] can be augmented with protein surface/cavity calculations to show possible binding locations as well ion/water permeation pathways between these sites and the exterior of the protein (Fig.3&4). Traditionally, protein electrostatic investigations have mostly considered surface-mapped potentials. However, isopotential profiles in the protein's cavities yield information about the ions' environment, and allows the calculation of ion binding affinities. In the present work, we explore how electrostatic analysis can be coupled with molecular cavity data to refine ion and water pathways. We also show how the deployment of efficient 3D solvers originally developed for electrical devices may facilitate modeling and analysis of biomolecules and biodevices not yet explored.

WATER ACCESSIBILITY

The molecular dynamics (MD) packages, such as GROMACS [9], can be used to explore water and ion accessibility and permeation pathways through simulations of the >500,000 atom protein-lipid-water-ions system. This is the first attempt to perform molecular dynamics simulations of a sodium pump and bilayer system, a task which has been considered only for H⁺-K⁺ ATPase previously [10]. MD trajectories can be evaluated to test water permeation pathways, and to test the relationship between the binding sites and water accessibility.

The present work establishes a unique framework for the simulation study of ion-motive pumps in general and Na⁺/K⁺ pump in particular. We shall discuss the implications of electrostatic analysis and MD simulations for the structure-function relationship of Na⁺/K⁺ ATPase.

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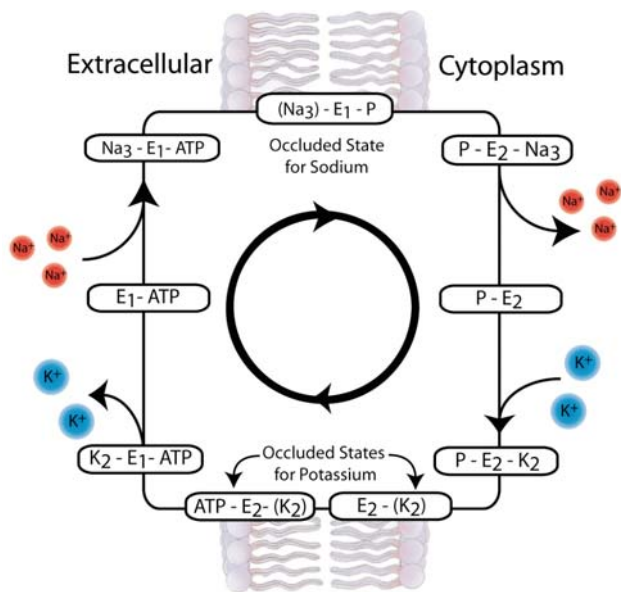


Fig.1: The Post-Albers cycle above indicates the main stages of conformational changes (E_1 - E_2), ion binding, release and occlusion, and ATP hydrolysis. Potassium is released and sodium bound on the intracellular side and this process is reversed on the extracellular side. The arrows indicate the forward pump cycle.

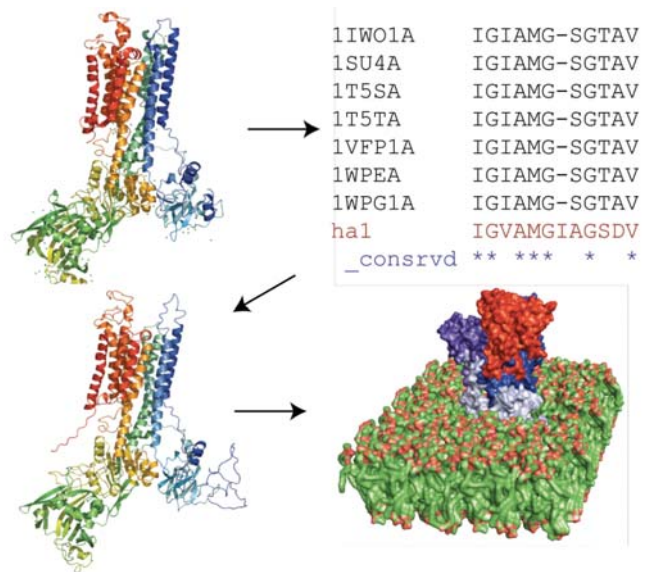


Fig.2: An overview of the homology process. From the seven available SERCA structures (top left), an alignment of the genomic sequences is made with the human α -1 isoform (hal) of the sodium pump. The alignment and coordinates of the structure file are combined to provide the homology model (lower left). At the lower right is a frame from a MD simulation that shows the model embedded in a lipid bilayer.

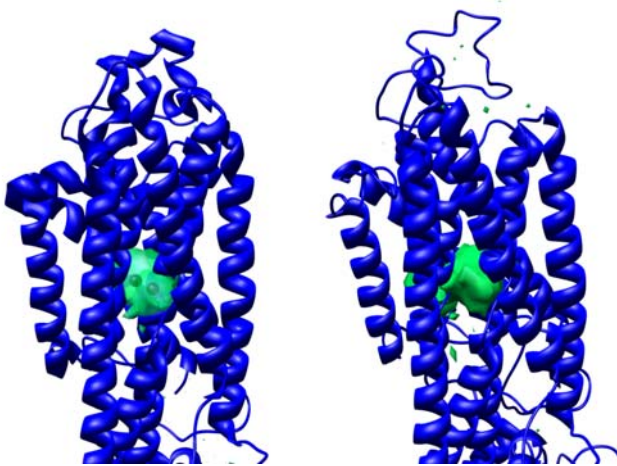


Fig.3: Transmembrane regions of the E_2 state of a SERCA (PDB: 1WPG) structure (left) and homology based model, each with a negative isopotential surface (green) indicating a high-affinity region for cations. Two bound Ca^{2+} ions can be seen inside the isopotential surface in the SERCA structure. Electrostatic investigations based on homologues can yield information on putative binding sites in the Na/K pump.

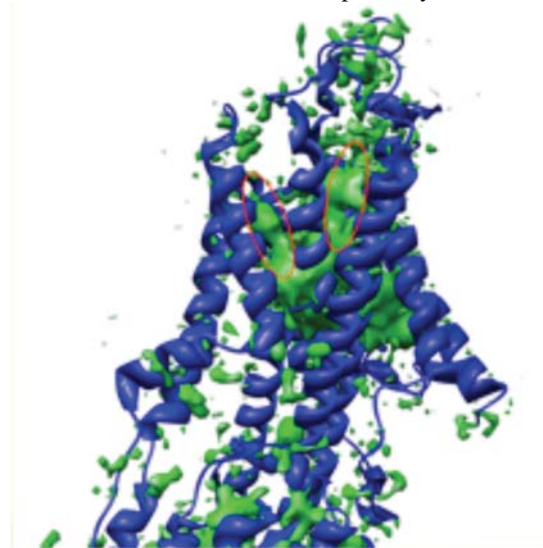


Fig.4: At smaller negative potentials, the isosurface expands towards the extracellular side (at left) of the model. The orange ovals indicate regions of negative potential that may provide a pathway conducive to ion movement between the lumen and the binding sites. Created with Chimera and electrostatic data from APBS.