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Electro-Chemical Modeling Challenges of Biological Ion Pumps

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Abstract

We outline the basic operational, structural and functional features of ion motive ATPases: transmembrane proteins central to biological functions of all animal cells. As an example we discuss the modeling problems associated with the operation of the surface membrane Na^+, K^+ -ATPase and skeletal muscle sarcoplasmic reticulum Ca^{2+} -ATPase and focus on the frameworks required for their solution. There are three basic problems: identification of the pathway for ion permeation, prediction of ion binding rate coefficients and affinities based on the structure of the protein, and prediction of conformational changes of protein structure and the associated movement of charges within the membrane dielectric. A solution strategy useful in approaching the first two problems and preliminary results obtained using molecular dynamics simulations are also presented.

Keywords

Na/K pump; P-type ATPase; molecular dynamic simulations; ion channels

1. Introduction

There are two major classes of proteins that control the movement of ions across biological membranes: channels and transporters. Ion channels allow the passive movement of ions down their electrochemical gradients whereas transporters power uphill movements. The class of transporters known as ion-motive ATPases have a number of features similar to ion channels [1], but they are more complex proteins coupling the free energy of ATP hydrolysis with the uphill movement of the transported ion species. Rather than simply acting as selective channels for passive ion movements, ion-motive ATPases are molecular batteries that maintains specific electrochemical gradients, the absence of which may lead to several common diseases such as migraine headaches and cardiac arrhythmia. A major research goal is the detailed description of structural changes that occur during the transport cycle of ATPases and the association of those changes with the mechanism of ion transport. Detailed knowledge of the cycle of conformational changes of ion motive ATPases may allow the construction of nano-electro-mechanical engineering devices [2]. Additional biophysical experiments and biomolecular simulation tools are needed to advance our knowledge of the structural changes of this important class of proteins. In this paper, we outline the fundamental features of ion motive ATPases, discuss modeling problems that remain largely unexplored and describe conceptual frameworks that may be used to tackle these challenges. To this end, we primarily focus on the Na^+ , K^+ -ATPase and the Ca^{2+} -ATPase of the sarcoplasmic reticulum of skeletal muscle. [3].

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2. Ion Motive ATPases

2.1. Structure

There are three important classes of ion motive ATPases (P-type, F_0F_1 , and vacuolar) that are different from one another both in terms of structure and the source of chemical energy required to sustain ion exchange. The P-type ATPase is the most widely distributed and occurs in the surface membrane of nearly all animal cells. P-type ATPases hydrolyze intracellular ATP, become phosphorylated (hence the name P-type) and change their structure at various stages of the transport cycle. Recently atomic resolution structures of several conformations of a P-type ATPase (SERCA, the Ca^{2+} pump of skeletal muscle), have been published [4,5]. This information has been used to postulate the structure of the closely related Na^+ , K^+ ATPase (sodium pump) as shown in top of Fig. 1 [6,7]. The transmembrane section of the sodium pump (see Fig. 1) constitutes roughly one third of the molecule. The ion transport pathway and ion binding sites are located in this transmembrane domain. The cytoplasmic two-thirds of the molecule contains sites for ATP binding and hydrolysis.

2.2. Function and Operation

In order to discuss the mechanism of ion translocation by P-type ATPases, we will consider the Na^+ , K^+ -ATPase on which a large collection of biophysical experiments has been done. The following reaction summarizes the overall function of the sodium pump that is powered by the Gibbs free energy (ΔE_G) of ATP hydrolysis:

Note that this process of exchanging 3 intracellular Na^+ with 2 extracellular K^+ is reversible. In biophysical experiments, ion concentrations and the ATP/ADP ratio can be controlled to study the rate of the reaction which takes place on a millisecond time-scale, almost 3 orders of magnitude slower than permeation through ion channels. The most-likely mechanism of active transport is an “alternating access” model in which the transported ions are first bound at one face of the membrane, then “occluded” in a state in which the ions are not directly accessible to either side, and finally released to the opposite side of the membrane as a consequence of changes in protein conformation and changes in the ion binding affinity of the transport sites [8]. A cartoon of the major structural changes associated with the Na^+ bound and K^+ bound states, and accompanying biochemical reaction cycle (Post-Albers model) are shown in Fig. 2.

3. Modeling Challenges

Comprehensive time-energy-structure resolved molecular dynamic simulations of ion-motive ATPases cannot be done at present, due to large CPU time and memory requirements. Instead, we discuss the following subset of problems that can provide insights for the design of experiments to improve our understanding of the mechanism of operation of ion motive ATPases.

3.1. Identification of the Pathway for Ion Permeation

With the recent availability of high-resolution structural data comes the possibility of identifying and studying the putative ion pathway through an ion pump using approaches similar to those used for the study of permeation through ion channels [1]. The objective of simulation studies on the sodium pump is to predict the potential landscape of the pathway that the transported ions follow. This is most easily done for the extracellular ion access pathway to Na^+ binding sites. The permeation characteristics of the ion pathway are expected to be greatly different in the two major (E_1 and E_2) configurations of the pump. Since water interacts

with the permeating ionic species and forms hydrogen bonds with atoms along the permeation pathway, such studies require the movement of the associated water molecules to be considered, thereby increasing the complexity of the simulation. However, all of the simulation tools required for this class of problem have already been developed for ion and molecular transport through channels, including Brownian motion and molecular dynamics simulation techniques. The main difference of this problem from permeation through ion channels is the absence of an 'open' channel. The access channels ("ion wells") of transporters terminate at an ion-binding site.

3.2. Ion Binding Energy Calculations

Despite the overall structural similarities among various P-type ATPases, they vary in their selectivity among cations. The ion binding sites are thought to be buried between adjacent membrane-spanning α -helical segments of the protein. The selectivity is determined by the coordination of the bound ions by carbonyl oxygens of the amino acids that form the ion-binding pocket. The ability to predict the binding site location and its affinity for a given ion species for a given conformational state of the protein is profoundly important. Once the structure of a binding site has been postulated one can test the validity of the geometry of the binding site by investigating the consequences of mutations of the nearby amino acids.

To calculate electron affinity of the binding sites, *ab-initio* electronic structure calculations with quantum mechanical corrections will be necessary. Density Functional Theory or simpler localized orbital approximations would be obvious methods of choice in dealing with this problem. Energetic modeling methods would benefit tremendously from a hybrid approach where classical approximations may be used in parts of the molecular structure, such as the protein back-bone or regions which have a weak influence on the binding affinities. Since several of the ion binding sites of the sodium pump are thought to be associated with coordination of the ion by water, the presence of water must also be accounted for. However, it appears that binding-affinity calculations are possible within the present theoretical framework.

3.3. Conformational Changes and Dielectric Relaxation Problem

Another poorly understood problem is the mechanics and kinetics of the $E_1 \leftrightarrow E_2$ transition of the protein. The mechanics of the problem are of interest to nano-engineering science. It is important to establish the relationship of the kinetics of the conformational transitions and the underlying changes in energy. It is also not known how the binding energies of various sites are affected by the conformational transition. This information would provide a deeper insight of how ion capture, occlusion and release proceed. To study this protein folding problem full-scale molecular dynamic (MD) simulations are required, but are not yet possible, mainly because of prohibitive difference between MD (fs) and mechanical (ms) time-scales. Important information concerning the changes in accessibility of the binding-sites and their ion affinity may be obtained by looking at the detailed structure of the two stable conformations of the pump that can be inferred by homology modeling of E_1 and E_2 configurations based on the known structure of the SERCA pump.

It is experimentally observed that the sodium pump exhibits a dielectric relaxation as it undergoes the $E_1 \leftrightarrow E_2$ transition [9]. In other words, the redistribution of electronic charges during the conformational change is observed as an exponentially decaying current before the pump current reaches a new steady-state. Measurement of changes in the rate of these current relaxations in various mutated forms of the pump should enhance our understanding of the structural changes that occur during this major conformational change. To simulate such dielectric relaxations, different levels of complexity, ranging from simplified non-linear electrical network approximations to a more complete MD study of charge relaxation (both

ionic and electronic) may be applied. While the former approximations may not be quantitatively precise, nonetheless, they may provide good initial guesses as to which part of the molecule dominates the charge redistribution during the conformational change.

4. Case Study: Hydration of Ca²⁺ Ion Pump

The results from an MD simulation of SERCA in the E₂ 'occluded' state are shown in Figs. 3 and 4. We employ the GROMACS [10] simulator and the SERCA structure 1SU4 from the Protein Database [4,11]. We include 100,000 water molecules in the present simulations that are run on a 24-node parallel cluster. To simplify the problem, the water-protein system undergoes an energy minimization and then a position-restrained (protein backbone fixed, ions and side-chains free) MD run for 20 ps, at which point the water distribution is obtained, as shown in Fig. 3.

Figure 4(a) provides a 3D view of the water distribution looking down the transmembrane helices from the extracellular side. There appears to be no specific accumulation or exclusion of water within the protein. However, the position history of the bound ions shown in Fig. 4 (b) reveals that, on average, they do not move during the entire 20 ps and have a very small deviation in their average position. This is contrary to H₂O molecules away from binding sites, which move at least an order of magnitude more both in their average position and standard deviation. Clearly, in this state (E₂) of the molecule the Ca²⁺ ions are bound to the protein firmly, as expected for an "occluded" state. To the best of our knowledge this is the first direct simulation of the occlusion process in any transport ATPase. Further studies of other known conformations (e.g. 1VFP) may be useful in elucidating ion accessibility and release from their binding sites.

5. Conclusions

We outline the basic structural and operational features of ion-motive ATPases, (ion pumps) that so far have been little explored by computer simulations. We identify three basic problems associated with ion pumps, which can be addressed by methods currently available. These are (i) identification of the ion permeation pathway, (ii) calculation of ion-binding energy, and (iii) modeling of the conformational changes and resulting dielectric relaxation rates. We discuss general requirements and strategies for these problems which can provide unique predictive capabilities for research for both medical and nano-engineering. Hydration of an ion pump (SERCA, the Ca²⁺ pump of skeletal muscle) illustrates the use of MD simulations to study the 'occlusion' process and the permeation pathway in ion motive ATPases.

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Figure 1.

(Top) The atomic structure of SERCA [5] used as a basis for homology modeling of its close relative Na⁺, K⁺-ATPase. Backbone amino acids and putative Na⁺ binding sites are shown. (Bottom) ribbon representation of the transmembrane part (M) of the homology model of the Na⁺, K⁺-ATPase in Na⁺- (A) and K⁺-bound (B) forms [3].

Figure 2.

(a) Alternating-gate model for the sodium pump cycle in cartoon form. From [8]. (b) Post-Albers biochemical reaction cycle, where E_1 and E_2 represent the two principal conformations of the molecule, parentheses enclose occluded ions, and P the phosphate group released by ATP used to fuel the conformational changes.

Figure 3. MD (GROMACS) simulation of hydration of SERCA (Ca pump) for 20 ps with 2 fs time steps and 100,000 water molecules. SERCA is in its ‘occluded E₂’ state, in which Ca²⁺ ions are not accessible from either side of the membrane. Only 10,000 closest H₂O atoms and protein backbones are shown for clarity.

Figure 4.

(a) Water distribution, after a 20ps MD simulation run, around the α -helices of the transmembrane section of SERCA (backbones only view). (b) Position history of twin Ca^{2+} ions in the occluded E_2 state of the protein indicates that ions remain bound during the entire simulation, consistent with ‘occlusion’ of Ca^{2+} in this state.