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ATP hydrolysis at one of the two sites in ABC transporters initiates transport related conformational transitions

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ABSTRACT

ABC transporters play important roles in all types of organisms by participating in physiological and pathological processes. In order to modulate the function of ABC transporters, detailed knowledge regarding their structure and dynamics is necessary. Available structures of ABC proteins indicate three major conformations, a nucleotide-bound "bottom-closed" state with the two nucleotide binding domains (NBDs) tightly closed, and two nucleotide-free conformations, the "bottom-closed" and the "bottom-open", which differ in the extent of separation of the NBDs. However, it remains a question how the widely open conformation should be interpreted, and whether hydrolysis at one of the sites can drive conformational transitions while the NBDs remain in contact. To extend our knowledge, we have investigated the dynamic properties of the Sav1866 transporter using molecular dynamics (MD) simulations. We demonstrate that the replacement of one ATP by ADP alters the correlated motion patterns of the NBDs and the transmembrane domains (TMD). The results suggest that the hydrolysis of a single nucleotide could lead to extracellular closure, driving the transport cycle. Essential dynamics analysis of simulations suggests that single nucleotide hydrolysis can drive the system toward a "bottom-closed" apo conformation similar to that observed in the structure of the MsbA transporter. We also found significant structural instability of the "bottom-open" form of the transporters in simulations. Our results suggest that ATP hydrolysis at one of the sites promotes transport related conformational changes leading to the "bottom-closed" apo conformation, which could thus be physiologically more relevant for describing the structure of the apo state.

1. Introduction

ABC (ATP Binding Cassette) transmembrane proteins play important roles in translocating a broad class of substrates across biological membranes in all types of organisms [1]. The failure of ABC proteins to translocate their physiological substrates results in different kinds of human diseases, including cystic fibrosis, Dubin-Johnson syndrome, diabetes mellitus type II, and adrenoleukodystrophy (http://nutrigene.4t.com/humanabc.htm). A very important phenomenon caused by some ABC transporters is multi-drug resistance of cancers against chemotherapeutic treatment [2–4]. Since some ABC proteins (e.g. MDR1, Multi-Drug Resistance protein 1; MRP1, Multi-drug Resistance associated Protein 1; ABCG2) have low substrate specificity and can remove compounds with various chemical properties from the cell, efflux of drugs through these transporters results in a decrease of drug concentration inside the cell below the effective level. The export process is driven by ATP binding and/or hydrolysis at the cytoplasmic nucleotide binding domains (NBD). The Walker A and B motifs are part of the core subdomain of NBD, while the α-helical region of the protein contains the ABC signature sequence [5,6]. Biochemical and structural studies have demonstrated that the binding of ATP to the Walker motifs in each NBD and to the signature sequence in the opposite NBD allows a tight association ("dimerization") of two NBDs [7,8]. In this ATP-bound holo state, the cytoplasmic parts of the transmembrane domains (TMD) are also close to each other, in a so called "bottom-closed" outward-facing conformation [9,10]. In the absence of nucleotides, X-ray structures indicate a "bottom-open" inward-facing conformation, where the NBDs are far from each other (Fig. 1 and S6) [10–12]. A region crucial for the transitions between the holo and apo conformations is the interface connecting the NBDs and TMDs. This interface contains short distal segments of intracellular loops,

Abbreviations: ABC, ATP Binding Cassette; NBD, nucleotide binding domain; TMD, transmembrane domain; ED, essential dynamics; ASA, accessible surface area

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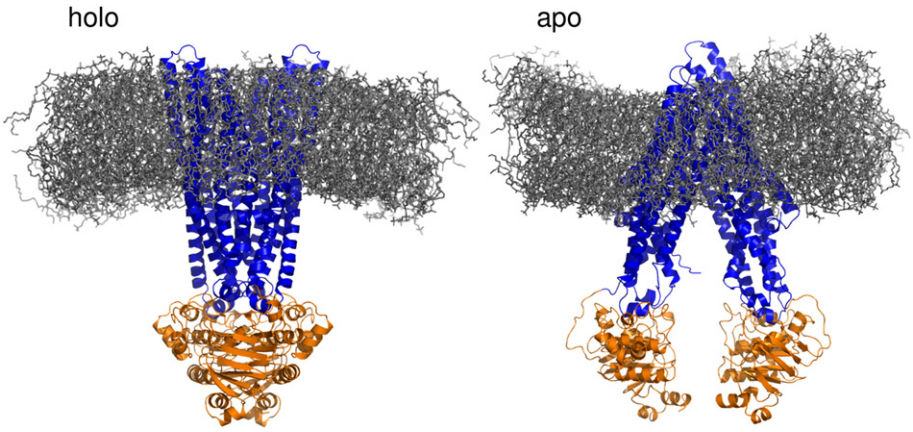

Fig. 1. The two key conformations of ABC transporters used in molecular dynamics simulations. The left panel shows the "bottom-closed" holo conformation of the Sav1866 bacterial transporter (PDB ID: 2HYD), which was used to study the effect of ATP to ADP replacement. On the right, the "bottom-open" apo conformation of the mouse MDR3 transporter (PDB ID: 3G5U) is shown, which was used for simulating the apo state. The structures in the figure are the initial conformations for the Sav1866 ATP/ATP and mouse MDR3 simulations, respectively, embedded in a POPC bilayer. Dark gray/blue: transmembrane domains, light gray/orange: nucleotide binding domains.

called coupling helices [9]. A coupling helix connects two anti-parallel helices, which are extensions of transmembrane helices, forming an intracellular "loop". The contact surface on the NBDs includes two main regions. One of them is the conserved X-loop, located C-terminal to the Walker A motif and N-terminal to the signature sequence [9]. The other is the Q-loop [13], which connects the α-helical subdomain containing the signature motif with the core subdomain and also interacts with the bound Mg^{2+} ion of ATP [7,14]. In the nucleotide-bound state, an important structural feature, which forms a significant part of the interface between the TMDs, is a tetra-helix bundle composed of the cytoplasmic parts of the TM3 and TM4 transmembrane helices of both transmembrane domains [15].

Understanding the coupling of ATP binding and/or hydrolysis to changes in the TMD conformation and substrate translocation is important for designing drugs to modulate transporter activity. The variety of available X-ray structures and drug binding studies have shown that ABC transporters are highly flexible [16], so a single static conformation, such as an X-ray crystal structure, might not be adequate to describe protein conformation and identify drug targets. Therefore, the dynamic properties of ABC proteins are being explored by both experimental and computational methods. Recently, electron paramagnetic resonance (EPR) spectroscopy has become a popular method to study the dynamics of this class of proteins [17–22]. EPR experiments with different conformations of the target protein are performed to measure distances between two spin labels attached to specifically selected amino acid pairs. Measuring distances between two specific residues by chemical cross-linking can be also used to derive information on protein conformation and dynamics [23–25]. However, both of these methods have limitations and do not provide atomic level information on the motions in the protein. In most cases these experimental methods provide only information on distances between two residues in the protein, and no directional information. In addition, while some experimental results reconcile with the wide opening of NBDs seen in some "bottom-open" X-ray structures [17], cross-linking studies with human MDR1 protein suggest that a large opening is not necessary for function [25].

To overcome this limitation of experimental methods, and to help interpret experimental data, molecular dynamics (MD) simulations have been used extensively to understand motions in ABC proteins at high resolution. Many computational studies have investigated the role of ATP in stabilizing the interactions between nucleotide binding domains in systems containing only these two cytoplasmic domains [26–31]. Simulations with NBD dimers or monomers with either ATP or ADP have shed light on the possible conformational changes induced by hydrolysis. Although there is no clear indication whether hydrolysis at one nucleotide binding site enhances or disrupts hydrolysis at the other site [30,31], most simulation results as well as half-open X-ray structures indicate that the helical domain of the *trans* NBD rotates outwards after ATP hydrolysis, facilitating nucleotide exchange [26,27]. This rotation would imply the concerted motion of the Q-loops and X-loops of the *trans* NBD, which have been shown experimentally to have an important role in signal transduction towards the TMD [32,33].

Few studies report simulations of full-length ABC proteins in a lipid environment, since sampling at biologically relevant time-scales in the case of such large proteins with lipids requires high computational power. The bacterial vitamin B12 importer (BtuCD) system has been extensively investigated using MD simulations. Using 15 ns long simulations with BtuCD in a lipid bilayer, ATP binding was shown to induce conformational changes in both NBDs and TMDs, indicating that the binding itself may be the power stroke in the catalytic cycle [34]. Elastic network normal mode analysis and biased molecular dynamics simulations have also been employed to predict the possible mechanism of transport of vitamin B12 [35]. BtuCD has been investigated in detail also by Ivetac et al. [36], whose molecular dynamics simulations combined with principal component analysis exhibited asymmetry in the ATP binding domain supporting the alternating hydrolysis mechanism [37] for ABC transporters. A recent simulation of the whole Sav1866 transporter has revealed a number of residues present at the NBD-TMD interface within the Q-loops and X-loops providing information at the atomic level how these regions could transmit conformational changes [14]. Until now, however, no systematic study has been carried out on the full pathway of conformational changes originating in the NBDs and transmitted to the TMDs. Possible pathways of transition from holo to apo conformation have been explored at atomic resolution using targeted MD simulations [15]. MD simulations also have been used to assess the global stability and structural integrity of ABC proteins [38], and have demonstrated that the now revoked structure of MsbA, whose X-ray structure had the correct transmembrane helices but an incorrect handedness and topology, suffered from large distortions even in short MD simulations.

In this study, we aim to characterize the dynamics of ABC proteins in different states, embedded in a lipid bilayer. First, long molecular dynamics simulations are performed with the holo Sav1866 X-ray structure in the presence of two ATP molecules and also one ATP and one ADP in the nucleotide binding domains, to describe the effect of ATP hydrolysis on the long range dynamics of the protein. Second, we also employ these simulations to characterize the transition of the