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How do transporters couple solute movements?

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Abstract

The availability of high-resolution atomic structures for transport proteins provides unprecedented opportunities for understanding their mechanism of action. The details of conformational change can be deduced from these structures, especially when multiple conformations are available. However, the singular ability of transporters to couple the movement of one solute to that of another requires even more information than what is supplied by a crystal structure. This short commentary discusses how recent biochemical and biophysical studies are beginning to reveal how solute coupling is achieved.

Keywords

Transport; mechanism; structure

Coupling of transport to ion gradients and potentials

The ability of solute transporters to concentrate their substrates inside a cell or organelle depends on coupling the energetically favorable movement of other solutes, usually ions, to the energetically unfavorable flux of substrate. When the two solutes move in the same direction, such as for the Na⁺-driven uptake of an amino acid or sugar across the plasma membrane, we call it symport or co-transport. If the two solutes are moving in opposite directions, we call it antiport, counter-transport or exchange. The transport process involves conformational changes that open and close pathways between the solute binding sites and the two sides of the membrane. New structures of transporter proteins are showing us the likely mechanisms for these conformational changes, an important step in understanding how transport works. However, these conformational mechanisms have not revealed how a transporter couples the flux of two solutes. Understanding solute coupling requires knowledge of how binding events control conformational change. Fortunately, such information is beginning to emerge from functional studies.

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Alternating access models and rules for coupling

The alternating access mechanism (Patlak 1957), has been used as a physical model to explain coupled transport (both symport and antiport). This model proposes two transporter conformations, each of which exposes a central binding site to one side or the other of the membrane (Figure 1). Substrate transport across the membrane requires interconversion of the two forms with substrate bound. However, there must be rules to restrict conditions for conformational change. To couple substrate and Na^+ transport (for example), this step should occur only when both substrate and Na^+ (solutes A and B, Figure 1, top) are bound. The transporter would then revert to an outward-open conformation when the binding sites were empty after release of Na^+ and substrate to the cytoplasm. For Na^+ -substrate coupling to be strictly stoichiometric, this mechanism requires that the only other conversion between outward- and inward-open conformations should occur when the binding site is empty. Antiport is also accommodated by the same kinds of conformational changes, although with different rules. For example, if efflux of K^+ (solute A, Figure 1 bottom) is coupled to substrate (solute B) influx, the two solutes must be transported in different steps, and their binding should be mutually exclusive. K^+ would be bound during the transition from inward- to outward-open and substrate bound in the opposite direction. The alternating access mechanism, in its strictest form, presumes that symported ions are bound and transported together with substrate but antiported ions are transported in the step when substrate is not bound (Figure 1).

Structures and models for transporter conformations

A clear understanding of the molecular details of structure and conformational change is essential for deducing how transport is coupled to ion gradients. Two examples of transporter families for which we have abundant structural and increasing functional information are the Neurotransmitter-Sodium Symporter (NSS) and the Dicarboxylate/Amino Acid-Cation Symporter (DAACS) families. These two families are represented in the human genome by the SLC6 and SLC1 families, respectively. In both families, Na^+ influx provides the driving force for the uptake of aminoacid substrates, and structural data comes from a prokaryotic homologues: The NSS transporter LeuT_{Aa} (Yamashita et al. 2005) and the DAACS transporter Glt_{ph} (Yernool et al. 2004).

LeuT_{Aa}

Structures have been published for LeuT_{Aa} in three distinct conformations (Yamashita et al. 2005, Krishnamurthy and Gouaux 2012) and with several substrates and inhibitors (Singh et al. 2007, 2008, Zhou et al. 2009). The three conformations include an outward-occluded conformation that seems to be the most stable form with substrate bound (Yamashita et al. 2005). An outward-open form was crystallized in the presence of tryptophan, a competitive inhibitor, or with Fab fragments that stabilized the conformation (Krishnamurthy and Gouaux 2012). An inward-open conformation was also crystallized using a Fab fragment in addition to several mutations to destabilize the outward-open conformation (Krishnamurthy and Gouaux 2012). An interesting and important aspect of the outward-occluded conformation is that it provides a way for LeuT_{Aa} to transform from outward- to inward-open forms without being simultaneously open to both sides of the membrane. Uncoupled

flux of substrate and ions could occur if the transporter opened a pathway from the binding site to the cytoplasm before it closed the pathway to the cell exterior. This uncoupled flux is prevented by the presence of an intermediate state in which the binding site is effectively sealed off from both sides.

X-ray structures of LeuT_{Aa} reveal an unexpected structural property that has since become a common feature in transporter structures. The structure of TMs 1–5 in this 12-transmembrane (TM) helix protein is repeated in the structure of TMs 6–10 except that the topological orientation of the two similar structures is inverted. The extracellular substrate permeation pathway in the occluded structure was partially open but the cytoplasmic pathway was totally closed. This asymmetry resulted from the fact that the two repeats had minor differences in their conformation, a finding that proved to be a key to understanding conformational change (Forrest et al. 2008). If the two repeats had identical conformations, the overall structure of TMs 1–10 would have been symmetrical.

The two repeats of LeuT_{Aa} each contribute equally to two domains, a 4-helix bundle (TMs 1, 2, 6 and 7) and a scaffold (TMs 3–5 and 8–10) (Forrest et al. 2008). Most LeuT_{Aa} structures show the 4-helix bundle at an angle to the scaffold, packing closely against the scaffold on the cytoplasmic side of the binding site and separating from the scaffold on the extracellular side to create the extracellular pathway (cartoon version in Figure 2). The tilt axis of the bundle is reversed in models of the inward-open structure and in structurally related transporters, suggesting a ‘rocking bundle’ mechanism that closes the extracellular pathway and opens a pathway between the binding sites and the cytoplasm (Forrest et al. 2008, Weyand et al. 2008).

Glt_{Ph}

Although the structure of Glt_{Ph} is quite different from that of LeuT_{Aa}, several interesting parallels can be drawn between them (Boudker and Verdon 2010). Like LeuT_{Aa}, Glt_{Ph} also contains an inverted structural repeat although it is discontinuous, the first repeat consisting of TMs 1–3, 7 and helical hairpin (HP) 1 and repeat 2 containing TMs 4–6, 8 and HP2 (Crisman et al. 2009). The hairpins represent helical segments that do not completely cross the membrane (Yernool et al. 2004). HP2 was shown to control access to the substrate binding site from the extracellular side of the membrane (Boudker et al. 2007).

Subsequently, modeling studies and biochemical experiments, in addition to new structures, demonstrated that a transport domain consisting of HP1–2 and TMs 3 and 6–8 moves through the rest of the protein so that HP1 can open on the cytoplasmic side to release substrates (Crisman et al. 2009, Reyes et al. 2009).

Ligand-induced conformational changes

Our understanding of conformational change in each transporter family is becoming clearer as more transporter structures are solved. However, an appreciation of how ligand binding controls conformational change is consequently becoming more important as the next step in understanding the molecular mechanism of transport. The conditions under which a transporter changes conformation from inward- to outward-open depend on rules that ensure strict coupling between ion and substrate transport. A specific set of conditions must be

satisfied for these conformational changes to occur. For both LeuT_{Aa} and Glt_{Ph}, Na⁺-amino acid symport requires that the conformational changes occur either when the transporter has bound both Na⁺ and substrate or when those binding sites are empty (Figure 1). Transporter proteins must use binding site occupancy to control conformational transitions in order to prevent conformational change when only Na⁺ or only substrate is bound.

For NSS transporters, the best structural model at present is LeuT_{Aa}, and studies with LeuT_{Aa} and other transporters already hint as to how NSS proteins accomplish the coupling of binding to conformational change. Movement of the 4-helix bundle relative to the scaffold domain is thought to control conformational changes according to the rocking bundle hypothesis. A nexus of binding sites for two Na⁺ ions and one substrate molecule near the center of the protein is the point at which these two domains have the most consistent contact (Figure 2). This interaction is optimized to directly couple binding site occupancy to conformational change.

Evidence suggesting that Na⁺ influences conformational change in the NSS family comes from studies with LeuT_{Aa} and the aromatic amino acid transporter Tyt1. Na⁺ decreased accessibility of cysteine residues in the cytoplasmic pathway of Tyt1, indicating a shift to an inward-closed (outward-open) conformation (Quick et al. 2006). Single molecule FRET experiments with LeuT_{Aa} similarly indicated that the cytoplasmic pathway was closed by Na⁺ (Zhao et al. 2011). Spin labeled probes in the LeuT_{Aa} extracellular pathway also show changes in accessibility and distance with Na⁺ using EPR measurements, but because the EPR probes were in the extracellular pathway, Na⁺ was found to increase accessibility and distances between positions, consistent with the extracellular pathway opening as the cytoplasmic pathway closed (Claxton et al. 2010). In both LeuT_{Aa} and Tyt1, substrate caused the extracellular pathway to close and the cytoplasmic pathway to open, but only in the presence of Na⁺.

NSS transporters could couple Na⁺ and substrate transport using a mechanism revealed by these basic observations. Conformational change should be prevented when only Na⁺ is bound, according to the rules for symport, and the ability of Na⁺ to stabilize one conformation could lock the transporter in that outward-open state until substrate binds. Indeed, apo-LeuT_{Aa} is distributed between inward- and outward-open states in the apo-state from smFRET and EPR experiments, and Na⁺ strongly biased Leu-T_{Aa} toward outward-open conformations with transitions between states largely eliminated (Claxton et al. 2010, Zhao et al. 2011).

Substrate binding by transporters in this family seems to depend on Na⁺, and this provides a mechanism that would prevent substrate-induced conformational change in the absence of Na⁺. The strong dependence of substrate binding on Na⁺ by LeuT_{Aa} (Shi et al. 2008) means that the substrate is unlikely to bind to the apo-transporter. Although these observations provide a way for SLC6 transporters to prevent transport of Na⁺ or substrate alone, they do not indicate how these rules ensuring strict symport are encoded in the structure.

Two densities, interpreted as Na⁺ ions were found in LeuT_{Aa} structures. One of these (Na1) is directly coordinated by the substrate carboxyl group (Yamashita et al. 2005). Therefore,

Na1 forms an essential part of the substrate binding site through this strong ionic interaction (Figure 3A) that is missing in the absence of Na⁺. TM1 (in the 4-helix bundle) and TM8 (in the scaffold) form a binding site for the other bound Na⁺ ion (Na2) (Yamashita et al. 2005) (Figure 3B). Occupation of the Na2 site could foster interaction between the scaffold and the cytoplasmic half of the bundle, thereby holding the two domains together, which would close the cytoplasmic pathway (Krishnamurthy and Gouaux 2012). Thus, Na2 binding at this interface could provide a mechanism by which Na⁺ stabilizes the outward-open form of LeuT_{Aa}. In contrast, when the Na2 site is not occupied in inward-open structures and models, the two domains separate from each other (Forrest et al. 2008, Krishnamurthy and Gouaux 2012) (Figure 3C). Thus, the key to coupling between ion and substrate transport may lie in specialization of the two Na⁺ sites, with Na2 serving to stabilize the outward-open conformation and Na1 required for substrate binding.

Despite their widely different structures and conformational mechanisms, there are several functional parallels between LeuT_{Aa} and Glt_{Ph} with respect to the effect of Na⁺ on conformation. EPR experiments examining the opening of HP2, which provides access to the binding site from outside the cell, showed that Na⁺ stabilized HP2 in an open state. In the absence of Na⁺ or in the presence of both Na⁺ and substrate, the hairpin closes over the binding site, allowing the binding site domain to move toward the cytoplasmic surface of the protein where it can release Na⁺ and aspartate (Focke et al. 2011). These conformational effects of Na and substrate are similar to those observed with LeuT_{Aa} and Tyt1 (Quick et al. 2006, Claxton et al. 2010, Zhao et al. 2011). Subsequently, single molecule FRET studies with Glt_{Ph} indicated dramatic changes in conformational stability as a function of Na⁺ and substrate, although the effect of Na⁺ alone was not tested (Akyuz et al. 2013).

For both LeuT_{Aa} and Glt_{Ph}, the mechanism out-lined above provides a conceptual framework for part of the reaction cycle. However, solid experimental validation is still lacking. We infer that Na⁺ binding initiates the process, and the strong bias toward an outward-open conformation prevents transport of the bound Na⁺ ions before substrate binds. Amino acid substrates cannot bind in the absence of Na⁺. In LeuT_{Aa} this is likely due to the participation of Na1 in forming the substrate binding site, and for Glt_{Ph}, it may be due to Na⁺ opening HP2. However, there are amine neurotransmitter substrates for some NSS transporters, and these substrates do not have carboxyl groups and do not require Na⁺ for binding. It is still not clear how, in the absence of Na⁺, binding of an amine substrate would prevent conformational change. In all cases, however, substrate binding must trigger conformational change by overcoming the conformational effect of Na⁺ binding. A proposal that substrate binding in the extracellular pathway initiates this conformational change in LeuT_{Aa} (Shi et al. 2008) has encountered resistance, in part because substrate binding has never been observed at the proposed site. At this point in time, the effect of substrate on conformational change remains unresolved.

Acknowledgments

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Figure 1.

Alternating access mechanisms. Similar conformational changes could account for symport (above) and antiport (below) of solutes A and B using different rules. Prohibited conformational changes are indicated by a red bar. This Figure is reproduced in color in the online version of *Molecular Membrane Biology*.

Figure 2.

The reaction cycle of LeuT. Transport is initiated by binding of two Na⁺ ions (upper left) followed by substrate binding (upper right). Conformational changes to the occluded state and the inward-open state follow (right). Dissociation of Na⁺ and substrate leads to the apo-state (lower left) which can re-orient to the outward open form (left). The 4-helix bundle is represented in blue and the scaffold in brown/tan. This Figure is reproduced in color in the online version of *Molecular Membrane Biology*.

Figure 3. Na⁺ binding sites in LeuT_{Aa}. Binding sites for Na1 and substrate (A) and Na2 in outward-open (B) and inward-open (C) structures (pdb 3TT1 and 3TT3, respectively). Na1 binding site residues and transmembrane helices are numbered. This Figure is reproduced in color in the online version of *Molecular Membrane Biology*.