New and Notable

How does Water Pass through a Sugar Transporter?

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Certain membrane symporters, such as the sodium-glucose transporter (SGLT), have long been known to transport water along with their substrates. Although the main job of SGLT is to uptake sugar molecules by utilizing the Na^+ gradient as the energy source, experiments on oocytes revealed substantial water fluxes into the cell when SGLTs are transporting sugar and Na^+ (1). Thermodynamically, this is not surprising. The cell was originally in osmotic equilibrium with its surrounding, with equal osmolarities (roughly determined by the total solute concentration) in the intra- and extracellular solutions. The uptake of sugar and Na^+ increases the intracellular osmolarity, and water thus enters the cell until reaching a new equilibrium. Under the experimental conditions, it was estimated that for each solute molecule taken into the cell, ~175 water molecules (1) must be added to restore the original concentration. How these water molecules pass through the transporter, however, is less obvious.

Two complementary mechanisms (Fig. 1) have been proposed for such water transport. In the coupled (active) transport mechanism (Fig. 1 *A*), some water molecules take a free ride and enter the cell in the same trip as the solutes (1). In the passive permeation mechanism (Fig. 1 *B*), in contrast, solute transport gives rise to an accumulation of the solute molecules near the intracellular side of the membrane, which in turn induces a water flux in response to this local osmotic gradient (2). Whereas the coupled transport mechanism readily explains the rapid establishment of the water flux immediately after the solute transport is started, the passive permeation mechanism is supported by the fact that the water flux does not immediately vanish after the sudden stop of the solute transport by inhibitors. The two mechanisms are by no means mutually exclusive; in fact, they could both be at work and contribute to the total water flux. Indeed, although the significance of coupled water transport has been under debate for over a decade, it is widely agreed that at least a substantial portion of the observed water flux arises from passive permeation. Recently, two independent molecular dynamics (MD) studies (3,4) revealed the behaviors of a bacterial homolog of SGLT on the multi-microsecond timescale, thanks to the power of Anton, the fastest computer in the world (created by D. E. Shaw Research, New York, NY) for MD simulations, and provided valuable insight into passive water permeation through this sugar transporter.

Both MD studies focused on the inward-facing structure of SGLT (5), in which the internal cavity appears to be closed to the extracellular solution. During the simulations (3,4), however, continuous chains of H-bonded water molecules through the protein interior are frequently formed, thus transiently connecting the bulk water on the two sides. In the inward-facing conformation (5), the bottleneck of this aqueous pathway is located, not surprisingly, at the extracellular entrance. Consequently, local motions of the protein residues (especially some bulky side chains) in that region play a major role in the forming and breaking of the water chains, with many transitions between such conducting and nonconducting states observed in the simulations (3,4). Interestingly, the sugar-binding site is also on the path of water permeation. Nonetheless, although the extracellular constriction could at times allow water molecules to pass, it is still too narrow for the sugar. Indeed, the sugar and Na^+ were spontaneously released only to the intracellular solution during the simulations (3,4). The calculated osmotic permeability for this transporter is in similar orders of magnitude to aquaporins, indicating that the bacterial SGLT can conduct water (albeit not selectively) as fast as those dedicated water channels. It is also noteworthy that the motion of the sugar molecule was not found to be significantly correlated with the water movement. In light of the better statistics obtained in these long simulations (3,4), the directional water transport concomitant with a single sugar-release event observed in a previous simulation (6) thus appears to be a coincidence rather than the norm.

Despite the atomic pictures of water dynamics revealed in these simulations, a complete understanding of water conduction in the sugar transporters still requires continued efforts from both the experimental and the theoretical fronts. Experimentally, most measurements of SGLT so far were performed on oocytes. Although ideal for the study of water transport, oocytes also involve factors (such as the presence of other water channels) that may complicate the interpretation of the observations. If SGLT could be reconstituted into simpler systems such as liposomes or planar lipid bilayers, the measurement could shed valuable new light. Computationally, whereas the correlation between water and sugar movements was closely examined in the simulations, it may be worthwhile to also look at the coupling between the Na^+ ions and water. After all, it was the concomitant Na^+ current and water flux that were directly detected in experiments. Furthermore, the hydration state and the ion affinity in protein pores are

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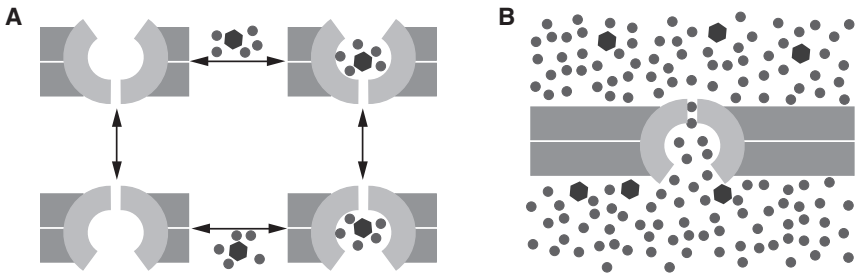

FIGURE 1 Two proposed mechanisms for water transport in SGLT. For simplicity, only one solute is shown here, whereas in reality two types of solute (sugar and Na^+) are involved. (*A*) A hypothetical molecular mechanism for the coupled transport (1). Each step in the transport cycle involves either a conformational transition (between the outward- and inward-facing states) or a collective binding / release of the solute and water molecules. A clockwise cycle in this diagram will result in a cotransport of solute and water into the cell. (*B*) The passive permeation mechanism (2), enabled by a water-conducting pore through the protein and driven by an osmotic gradient due to the directional transport of solute molecules.

closely related (7) and may be important factors in the stability of transporter conformations (8–10). But most importantly, the final definitive conclusion on the controversy of water cotransport will probably not come into sight until the transport cycle of SGLT is fully elucidated in atomic details. Mapping the major conformational transitions in MD simulations is currently a challenging task for complex proteins such as membrane transporters. Notably, these research groups (11–13) have also made promising recent progress in this aspect.

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