

Mechanistic insights from conformational ensembles of the mammalian fructose transporter GLUT5

Simple carbohydrates, such as glucose and fructose, are a major source of energy for the body. Incorporation of these molecules into mammalian cells is mediated mainly by GLUT and SGLT transporters. Humans and mammals have 14 subtypes of GLUT facilitative transporters, each of which shows a distinct pattern of substrate preference and tissue distribution [1]. Of these, GLUT5 is a fructose-specific facilitative transporter, expressed mainly on the apical surface of the enterocytes and responsible for the absorption of dietary fructose in the small intestine (Fig. 1). The current excessive consumption of fructose in the Western diet is associated with an increased incidence of metabolic disorders such as obesity and non-alcoholic steatohepatitis [2]. Growing interest has therefore been focused on the structure and mechanism of GLUT5. In this study, we used brilliant synchrotron X-rays at SPring-8 BL41XU, Diamond Light Source, and European Synchrotron Radiation Facility to analyze GLUT5 conformational ensembles by crystallography to provide mechanistic insights into the structural dynamics of this facilitative transporter during the transport process [3].

It is still challenging to determine the crystal structure of a human or mammalian membrane protein. This is reflected in the difficulties encountered with the production, stability, and crystallization of a membrane protein. An antibody-aided crystallographic approach was taken to determine the structure of rat GLUT5 in the outward-facing conformation (Figs. 2(a) and 2(c)). We generated an Fv antibody fragment that specifically bound to a conformational epitope on the intracellular hydrophilic surface of rat GLUT5 and acted as a “crystallization chaperone”. The GLUT5 structure

consists of two symmetrical six transmembrane (TM) helix bundles, plus five additional helices on the intracellular side. A central cavity between the two TM bundles allows the passage of substrate molecules. In this crystal structure, the central cavity is open to the extracellular side. A different approach was taken to shift the conformation equilibrium into an inward-facing state. A previous study has shown that C-terminal truncated mammalian GLUT1 is locked to an inward-facing form and does not have transport activity [4]. On the basis of this knowledge, we produced a 28-residue C-terminal truncation mutant of bovine GLUT5 to successfully determine the crystal structure (Figs. 2(b) and 2(d)). The C-terminal-deleted GLUT5 adopts an inward-facing conformation, in which the central cavity is open to the cytoplasmic side. Combining these structural data with biochemical studies, we identified the essential amino acid residues for substrate binding located in the central cavity. Most essential residues are located on the TM7, TM10, and TM11 in the C-terminal half, suggesting that GLUT5 has an asymmetrical substrate-binding mode. Furthermore, we have shown that a single point mutation in the substrate-binding site (Gln166 → Glu) is sufficient to switch the substrate-binding preference of GLUT5 from fructose to glucose.

A comparison between the outward-facing and inward-facing structures shows that the two major TM bundles undergo a rigid-body-like rotation of approximately 15°. The key characteristic of the global rotation is the formation and breakage of salt bridges between the two TM bundles on the cytoplasmic side. The two TM bundles are linked by a couple of salt bridges in the outward-facing state, whereas these salt bridges are broken in the inward-facing state.

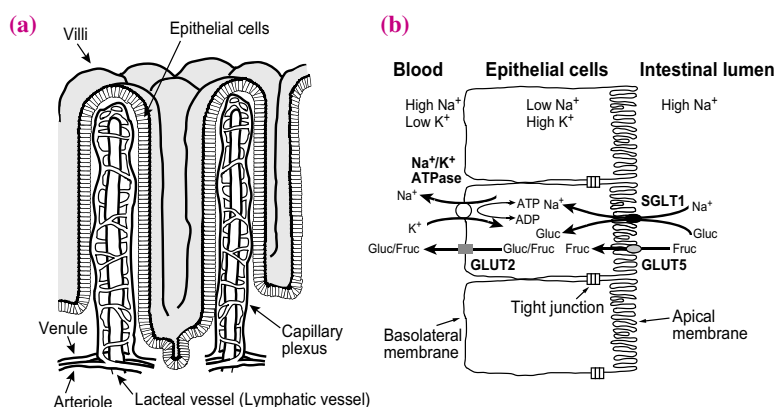


Fig. 1. Transepithelial hexose absorption in the human small intestine. (a) Villi of the small intestine. (b) Transport of monosaccharides across the epithelial cell layers. Glu: glucose, Fru: fructose.

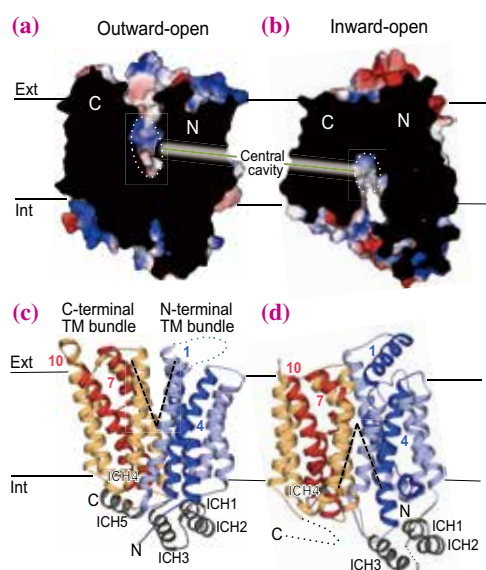


Fig. 2. Overall structure of GLUT5. Cross section through the surface electrostatic potential of the outward-facing rat GLUT5 (a) and inward-facing bovine GLUT5 (b) structures, as viewed within the plane of the membrane. The central cavity is shown as a dotted ellipse. Ribbon representations of outward-facing (c) and inward-facing (d) structures.

Interestingly, mutagenesis experiments confirmed that substrate binding is coupled with salt bridge formation in the outward-facing state. A more detailed superposition of the structures in the two different conformations revealed how the central cavity closes via a conformational change. On the extracellular side, TM1 and TM7 move to close the central cavity. In contrast, on the intracellular side, TM4 and TM10 contribute to the closing of the cavity. The movement of TM7 and TM10 is beyond the rigid-body rotation. They undergo a local conformational change to occlude the substrate-binding site. During the transition from the outward-facing state to the inward-facing state, the upper half of TM7 is markedly distorted to form an extracellular gate. On the other hand, the lower half of TM10 moves a large distance to open the intracellular gate.

There are two canonical models for transporters. In the rocker-switch mechanism, the transporter simply undergoes a rigid-body rotation of the two major TM bundles. In the gated-pore mechanism, the transporter forms an extracellular gate and an intracellular gate to control the substrate transport, but it does not undergo a rigid-body rotation of the two domains. For GLUT5, the transport mechanism is a fusion of the two canonical models. Therefore, we proposed a new model, in which the transport occurs by a combination of a global “rocker-switch”-like movement of the TM bundles and a local asymmetric “gated-pore”-like rearrangement. This scenario is depicted in Fig. 3. Initially, the transporter is waiting for the substrate in the outward-facing state

(Fig. 3(a)). The substrate molecule enters the substrate-binding site in the central cavity. TM7 moves towards TM1 and forms the extracellular gate. The substrate binding is the driving force to break the inter-domain salt bridges (Fig. 3(b)). The transporter undergoes rigid-body rotation. The TM10 moves away from the TM4 and opens the intracellular gate before the substrate molecule is released into the cytoplasm (Fig. 3(c)). The transporter undergoes a transition from the inward-facing state (Fig. 3(d)) to the outward-facing state (Fig. 3(a)), again by forming inter-domain salt bridges.

Further effort will be required to find the missing link and understand the transport cycle completely, for example, a structural determination of the GLUT5-substrate analog complexes in the occluded state as well as molecular dynamics simulations. A deeper understanding of the GLUT5 structure and mechanism, as described here, should facilitate the structure-based design of novel inhibitors with therapeutic potential.

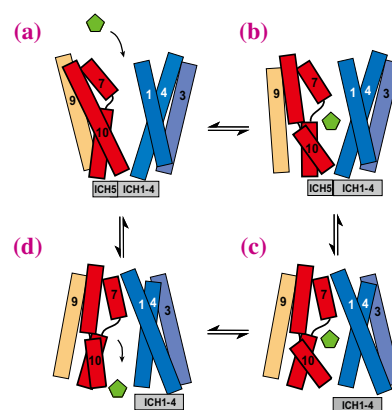


Fig. 3. Alternating-access transport mechanism in GLUT5. Green pentagons indicate fructose molecules.

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