Structural insights into a bacterial homologue of glucose transporters GLUT1–4

Glucose, one of the essential simple sugars, plays a central role in energy transfer in most living organisms. Mammalian cells take in glucose principally by two subfamilies of glucose transporters, one named GLUT, the other, SGLT.

GLUTs belong to the major facilitator superfamily (MFS), an ancient and the largest secondary transporter superfamily [1]. Previous studies indicate that they facilitate glucose uptake and prevent unwanted glucose analogues from passing through the plasma membrane. GLUTs are highly related to metabolic diseases, such as diabetes mellitus, yet no crystal structure of GLUTs has been obtained up to now. Structures of other MFS members have been obtained. Unfortunately, none of the available structures could provide enough information for further understanding of GLUTs' mechanism at a molecular level, owing to the lack of primary sequence similarity.

Important yet poorly understood, GLUTs are ideal protein targets for X-ray crystallography research. In the case of difficulties in handling eukaryotic membrane protein, we screened prokaryotic homologues and chose XylE originating from *E. coli* as a reasonable substitution. XylE is a H^+: D-xylose symporter with relatively high sequence homology to human GLUTs (especially GLUT1–4) and also has similar biochemical properties. The molecule of D-xylose consists of the same elements of a six-member ring as that of D-glucose.

After data set collection at beamline **BL41XU**, the structure of XylE bound to D-xylose was determined and refined, with the resolution of 2.8 Å (as shown in Fig. 1(a, b)) [2]. It is the first structure binding to its natural substrate in MFS proteins with solved structures. The structure shows that 12 transmembrane α-helices (TMs) are arranged in two domains, each with 6 TMs forming a bundle around the substrate binding center, which is a common feature of the MFS fold. Its amino and carboxy termini are both located in the cytoplasm. In a total view, the structure represents a new conformation of the MFS, namely, an outward facing, partially occluded and ligand-bound structure (Fig. 1(c)). It satisfies a clear step of the putative transport process theoretically

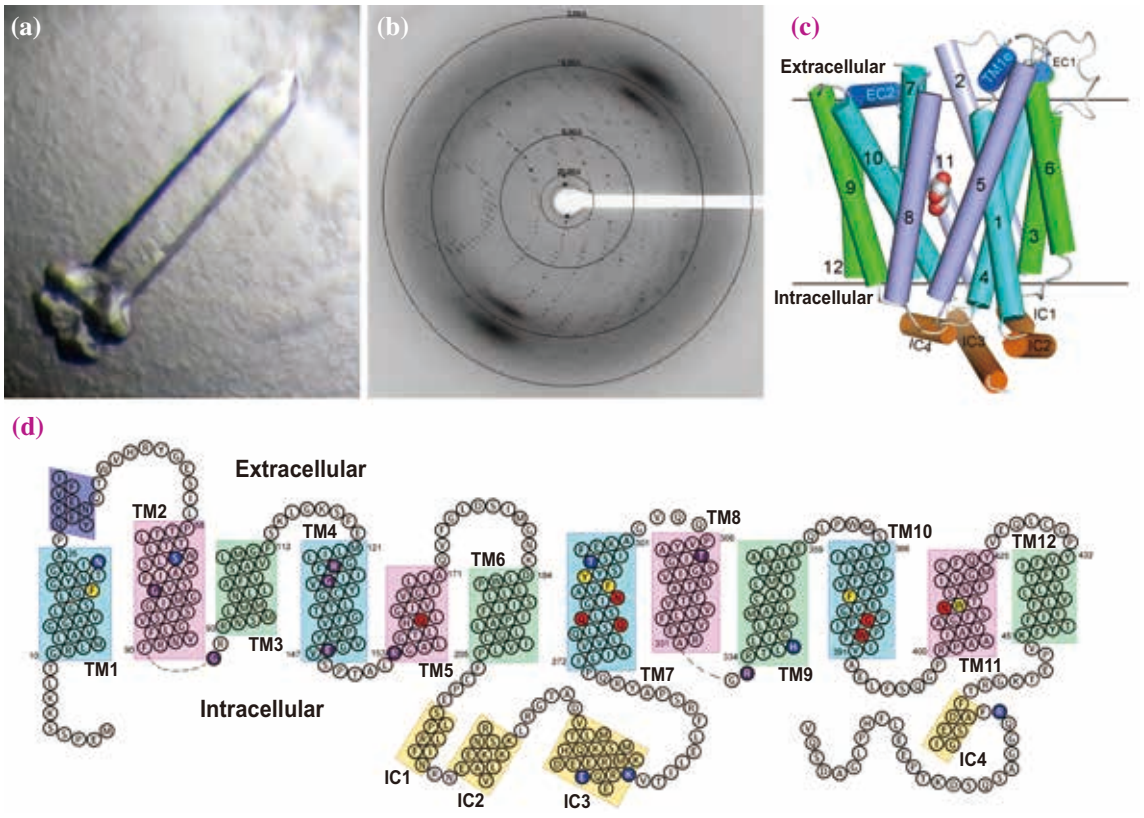

Fig. 1. **(a)** crystal of XylE protein viewed by microscopy, **(b)** diffraction data collected at BL41XU beamline, **(c)** 3D structure of XylE, and **(d)** proposed secondary structure model of GLUT1.

proposed a long time ago.

Distinct from other known MFS structures, XylE contains four more intracellular α-helices in which key residues are conserved in GLUT1–4. Furthermore, XylE is also able to bind D-glucose but not any other natural monosaccharide studied. The binding affinity between XylE and D-xylose or D-glucose is within the same order of magnitude as estimated by ITC. We then successfully captured the structures of XylE bound to D-glucose at 2.9 Å and XylE bound to 6-bromo-6-deoxy-D-glucose (6-BrGlc; a synthesized D-glucose derivative) at 2.6 Å. Br anomalous signals from 6-BrGlc help to locate the precise position and orientation of D-glucose inside XylE. The conformations of the three structures are almost identical.

It is observed that no charged residue is involved in ligand binding in XylE, which differs from other proton symporters. The hydroxyl groups of D-xylose are stabilized through direct hydrogen bonds by polar and aromatic residues including Gln 168, Gln 288, Gln 289, Asn 294, Trp 392 and Gln 415. Some more water-mediated hydrogen bonds are contributed by Tyr 298 and Gln 415. On the basis of the high-resolution data, both *in vitro* and *in vivo* biochemical assays were designed to prove the validity of substrate spacing in XylE. Generally, proteoliposome-based counterflow and cell-based uptake assays are used to measure transport activity. Transport missense mutations of the mentioned residues (to be more specific, single residue mutated to alanine) show loss of function in the transport assays.

Substrate specificity is also examined. Sugar selectivity is in agreement with former reports. With such a delicate hydrogen-bond network, it is not hard to imagine that only one possible form of a sugar ring can be tolerated. The comparisons with structures containing different ligands reveal that D-xylose and D-glucose interact with XylE similarly. Both six-member rings of the sugar molecules are coordinated by hydrogen bonds in a conformed manner. Moreover, residues Gln 168, Gln 175, and Gly 388 contribute additional hydrogen bonds towards the hydroxyl groups of D-glucose. The 6-hydroxyl group of D-glucose is also coordinated through van der Waals contacts by Ile 171 and Phe 383. The extra interactions may explain why XylE can bind to both D-xylose and D-glucose separately but transport only D-xylose.

Most residues participating in substrate recognition of XylE are invariant in GLUT1–4, so that the coordination network is presumably more or less the same between GLUT1–4 and D-glucose. Some reported disease-related mutations in GLUT1–4 could also affect the function of XylE. For instance, single missense mutation of Arg 133 or Arg 341 in XylE (corresponding to Arg 126 or Arg 333 in GLUT1) results in complete loss of transport activities in the assays.

To put the above findings together, we find that XylE is more practical and accurate for functional simulations of GLUTs than any previous models. In the newly built GLUT1–4 structure model based on XylE, the boundaries of transmembrane helices are revised, and four more intracellular α-helices are added, as Fig. 1(d) shows. The signature-sequence conserved, so-called “sugar porter motifs,” are highlighted as well. These motifs are usually far from the sugar binding site. However, they may interact with some charged or polar residues on intracellular α-helices, which implies that the transport mechanism is somehow different from those at other branches of MFS. Mutations of these motifs or of the presumed residues involved in interactions still lead to the impairment of transport activity. A more profound mechanism of how proton motivates sugar transport may lie under the sophisticated hydrogen-bond network among transmembrane α-helices and the intracellular domain. Most significantly, the common natural substrate of GLUT1–4, D-glucose, is included in the picture in a detailed pattern that has never been worked out before.

Furthermore, small-molecule inhibitors of GLUTs can now be designed and modified on the basis of the new model. Specific inhibition or activation of one certain GLUT might be achieved as well. Since the MFS-type sugar transporter is widely spread besides mammalian cells, homologue models for other species such as plants and yeasts may also be built by the same approach. These structures and models will shed more light for further deciphering the sugar transporter mechanism and will promote related medical and agricultural research. The above results were published in Ref. [2]. A “News and Views” [3] was published in the same issue to highlight our study.

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