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Review

Pathogenic mutations causing glucose transport defects in GLUT1 transporter: The role of intermolecular forces in protein structure-function

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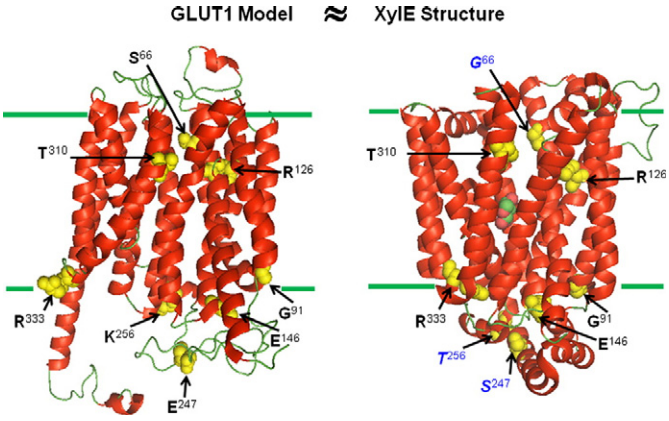
HIGHLIGHTS

- The mechanism of GLUT1 loss-of-function (LOF) mutations is not well understood.
- The GLUT1 homology model and bacterial XylE structure share notable similarities.
- LOF mutations may destabilize intermolecular interactions causing transport defects.
- The structure/function of sugar transporters is stabilized via similar mechanism.

GRAPHICAL ABSTRACT



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ABSTRACT

Two families of glucose transporter – the Na^+-dependent glucose cotransporter-1 (SGLT family) and the facilitated diffusion glucose transporter family (GLUT family) – play a crucial role in the translocation of glucose across the epithelial cell membrane. How genetic mutations cause life-threatening diseases like GLUT1-deficiency syndrome (GLUT1-DS) is not well understood. In this review, we have combined previous functional data with our *in silico* analyses of the bacterial homologue of GLUT members, XylE (an outward-facing, partly occluded conformation) and previously proposed GLUT1 homology model (an inward-facing conformation). A variety of native and mutant side chain interactions were modeled to highlight the potential roles of mutations in destabilizing protein–protein interaction hence triggering structural and functional defects. This study sets the stage for future studies of the structural properties that mediate GLUT1 dysfunction and further suggests that both SGLT and GLUT families share conserved domains that stabilize the transporter structure/function via a similar mechanism.

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Contents

1. Introduction

Glucose uptake into mammalian cells is mediated mostly by members of the sodium-independent glucose transporter (GLUT) and sodium-dependent glucose cotransporter (SGLT) [1,2]. GLUTs, as major facilitator superfamily (MFS) type passive transporters, are absolutely crucial for sugar metabolism in almost every cell type [3]. GLUT1 is highly expressed in the endothelial cells of erythrocytes and the blood-brain barrier where it is mainly involved in glucose transport into the brain [4,5]. GLUT1-deficiency syndrome (GLUT1-DS), an autosomal dominant haploinsufficiency disorder characterized by a low glucose concentration in the cerebrospinal fluid [6,7], is caused by genetic mutations in GLUT1 (*SLC2A1* gene). The classical effects of GLUT1-DS include infantile drug-resistant seizures, mild to severe developmental delay and an acquired microcephaly. A wide spectrum of mutations in the GLUT1 has been illustrated in more than 250 patients since the disease was identified in 1991 [8]. Multiple deletions, missense and frame shift mutations lead to either loss or disappearance of GLUT1 protein function [9,10].

The structure and function of the GLUT1 protein has been extensively investigated [11].The cloning of cDNA encoding the red cell plasma membrane GLUT1 was carried out in 1985 [12]. Later, 13 other members of the *SLC2A* were subsequently identified in the human [3]. *SLC2A1* is a relatively small gene which consists of 10 exons, spanning 2842 base pairs encoding 493 amino acids [13] and 12 transmembrane (TM) alpha-helices linked by extra and intracellular loops with both N- and C-termini located in the cytosol [11]. Furthermore, there exists a high (97–98%) sequence identity among the human, rat, rabbit and mouse GLUT1 indicating that all domains of GLUT1 are functionally important in these species [14].

GLUT1-DS can be confirmed by mutation analysis of the *SLC2A1* gene. Hence, several hot spots for persistent mutations have been identified including N34, G91, S113, R126, R153, R264 or R333 in GLUT1 [10]. However, the mechanism by which these mutations cause transport defects remain indefinable. In this study, the GLUT1 homology model [15] exhibiting an inward-facing conformation, which was derived from the crystal structures of *E. coli* glycerol phosphate transporter and *lac* permease, and XylE transporter crystal structure in outward-facing, partly occluded conformation [16] were compared to define inter-molecular interactions that stabilize the structure of the human GLUT1 transporter. *In silico* mutagenesis and modeling analyses were carried out to get insights into molecular mechanism of pathogenic mutations. Our studies indicate that LOF mutations may alter protein-protein and protein-membrane interactions thereby causing defects in glucose transport or mistrafficking of GLUT1 protein to the plasma membrane.

2. Comparison of GLUT1 homology model and XylE structures

Despite several extensive efforts in solving the structure, the homology model of GLUT1 structure was proposed [15]. According to this model, the central channel is composed of TM helices 2, 4, 5, 7, 8, and 10. The internal segment of the transport pathway is lined by both hydrophilic and hydrophobic amino acids that provide perfect interaction for the glucose molecule. OH groups render it hydrophilic and the pyranose ring offers hydrophobic interaction sites. The very well conserved QLS motif in TM7 starting at Q279 determines the transport selectivity of GLUTs. The residues crucial for transport activity include Q161, R126, Q279, Q282, N317, T321, W65, W388, W412, and V165 [15,17,18].

It was a major breakthrough when a crystal structure of XylE, an *Escherichia coli* homologue of GLUT1–4 and an archetypal member of the sugar porter subfamily, was solved for the first time [16]. The main structure consists of a characteristic MFS fold of 12 TM segments and a unique intracellular four-helix domain. The overall structure has an outward-facing conformation with D-xylose bound in the centre of the TM domain. The sequence comparison of XylE and GLUT1 is shown in Fig. 1. Similar to D-glucose interaction with GLUT1, the hydroxyl groups of D-glucose/xylose are also recognized through several hydrogen bonds in XylE as determined by mutagenesis and transport activity assays. Such residues include Q168 (Q161-GLUT1) on TM5, Q288/Q 289/N294 (Q282/Q283/N288-GLUT1) on TM7, and Q415 (replaced by N411 in GLUT1) on TM11. Also, several aromatic residues are located in the vicinity of D-xylose including F24 (F26-GLUT1) on TM1, Y298 (Y292-GLUT1) on TM7, W392 (W388-GLUT1) on TM10, and W416 (W412-GLUT1) on TM11. These residues are highlighted by red asterisks in Fig. 1. In comparison with D-xylose coordination, an extra hydrogen bond is formed between the carbonyl oxygen of G388 (G384-GLUT1) on TM10 and the 1-OH of D-glucose. Q175, which is replaced by I168 in GLUT1, also forms an H-bond to the 6-OH of D-glucose. There is also an interaction between hydroxyl methyl group of D-glucose and Ile 171 (I164-GLUT1) on TM5 and F383 (F379-GLUT1) on TM10 (highlighted by blue asterisks) through van der Waals contacts. Hence, a combination of different amino acids (Q175/Q415 in XylE and I168/N411 in GLUT1) might suggest a difference in binding affinities of D-glucose among both proteins.

Previously proposed GLUT1 homology model [15] fits quite nicely with an existing knowledge of the XylE transporter crystal structure which defines the precise binding pockets for glucose and several inhibitors and also elaborate the dynamic and flexibility. According to XylE structure, the intracellular helices (ICα1, 2 and 3) interact with the cytosolic parts of the transmembrane domains via extensive polar interactions. Interestingly, the residues that constitute the intracellular helices are highly conserved in GLUT family and the amino acids mediating the inter-domain interactions are quite similar among GLUT and XylE proteins.

Despite functional and mechanistic conservations of residues crucial for sugar (D-xylose or D-glucose) binding and transport there is considerable lack of sequence identity among XylE and GLUT. XylE shares only 29% sequence identity and 49% sequence similarity with GLUT1, as shown in Fig. 1. Also, the amino acid sequences forming extracellular helices

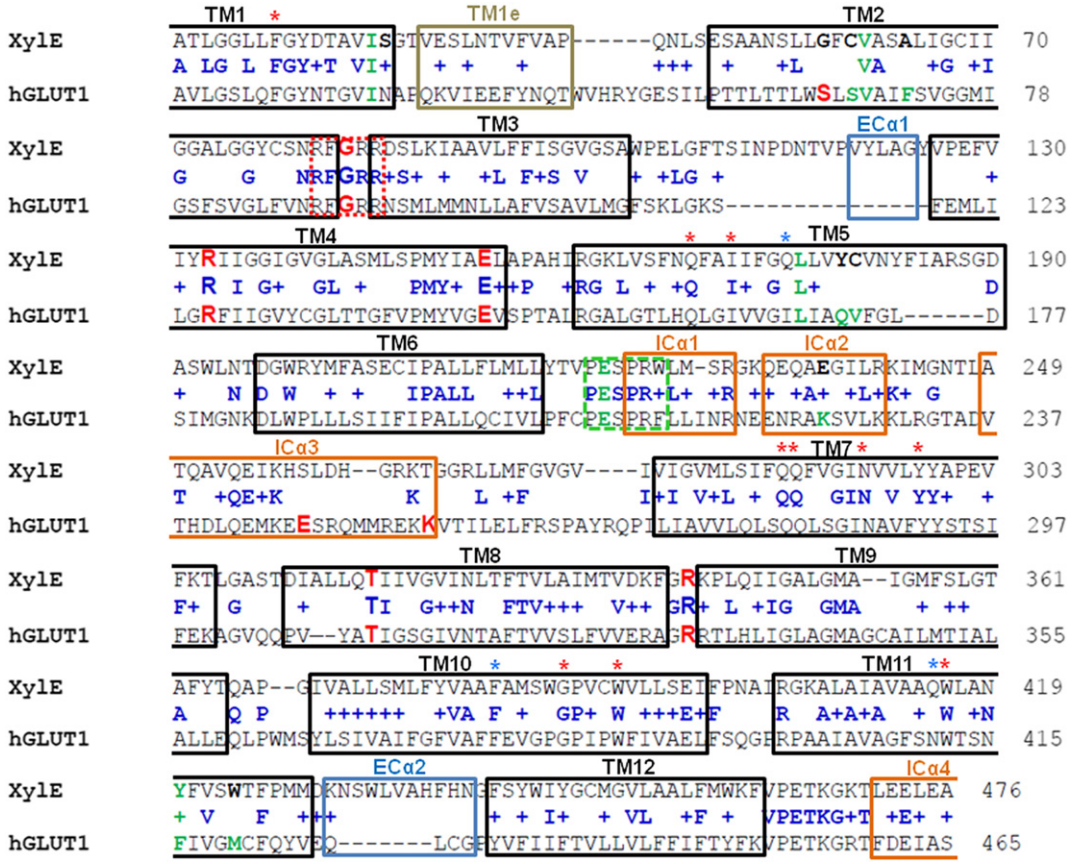

Fig. 1. Amino acid sequence comparison of XylE with human GLUT1 (hGLUT1). The sequences of both transporters were aligned using a sequence alignment program Clustal W (www.ebi.ac.uk) with manual adjustments. According to the crystal structure of XylE [16], the secondary structures in TM helices, intracellular (IC) and extracellular (EC) loops are highlighted in black, orange and blue boxes, respectively. Sequence identified or common residues among both proteins are indicated in blue. The residues that cause LOF/GLUT-DS and potentially involved in inter-molecular interactions are highlighted in red and green respectively. The descriptions to red and blue asterisks, green and red dotted boxes are given in the text.

(ECα1 and 2) in XylE structure (highlighted by blue boxes in Fig. 1) are absent in GLUT1. Such differences might suggest that the GLUT1 and XylE transporters undergo a dissimilar set of conformational changes as a part of their transport cycle.

3. Molecular mechanism of GLUT1-DS causing mutations

Fig. 1 depicts the amino acid sequence comparison of XylE and GLUT1 proteins. The positions of 8 genetic mutations are highlighted in red. For comparison, the GLUT1homology model and XylE structures are shown in Fig. 2a and b, respectively. The positions of genetic mutations S66, G91, R126, E146, E247, K256, T310 and R333 are also highlighted. Both protein models allocate similar positioning of genetic mutations. Five residues (G91, E146, E247, K256 and R333) that are known to be crucial for pathogenicity are clustered on the intracellular side of the transporter. S66 and T310 are positioned on the extracellular side of the presumed transport pathway whereas R126 is located away from the transport path in both structures. Out of 8 positions, there are 5 residues (G91, R126, E146, T310 and R333) that are found to be similar in both XylE and GLUT1 (compare Fig. 2a and b) whereas S66, E247 and K256 are replaced by Gly, Ser and Thr residues, respectively, in XylE (highlighted in blue in Fig. 2b). Considering these differences, we therefore sought to analyze and compare both GLUT1 homology model and XylE crystal structure and to investigate whether both model systems provide similar mechanism by which LOF mutations affect the GLUT1 transporter activity. In particular, the predictions of the side chain interactions that might stabilize the transporter structure, folding and stability are a centerpiece of this review.

3.1. S66F genetic variation

One of the autosomal dominant LOF mutations causing GLUT-DS is due to substitution of serine with phenylalanine at position 66. S66F mutation, present in exon 3, is caused by mutation of a single nucleotide (TCC➔TTC) at nucleotide position 197 [19]. According to GLUT1 homology modeling studies [15], S66 on helix 2 is able to form a hydrogen bond with a M420 (replaced by W424 in XylE) in helix 11 via serine's OH and methionine's ample Hs group (Fig. 3a). In addition, S66 also interacts with F416 (Y420 in XylE) in helix 11 [15] most probably via mechanism similar to intermolecular Phe/Ser protonated dimer complex formation [20]. Such interactions between pair of residues govern helix-helix interaction in GLUT1 [15].

In silico analysis indicates that S66F mutation can cause structure perturbation via destabilization of S66-M420 as well as S66-F416 interaction pairs [15]. Interestingly, S66F substitution seems to position the F66 ring closer to F416 (with measured distance of 1.9 Å) suggesting the close coupling of both phenylalanine and possible formation of Phe-Phe ring stacking non-covalent interactions (Fig. 3b). Such type of interactions are known to be crucial in stabilization of protein structures [21]. It has been shown that S66F mutant protein can be expressed and targeted to the cell membrane. However, a significant reduced membrane association and an impaired glucose transport function [19] supports our analysis suggesting that S66F mutation leads to the formation of new yet redundant interaction between S66F and F416, rather than the native interaction between S66-F416. Such alteration would also be expected to modify the transmembrane helix-helix interaction and energetic parameters of packing of helices 2 and 11 thereby impairing the transport function.

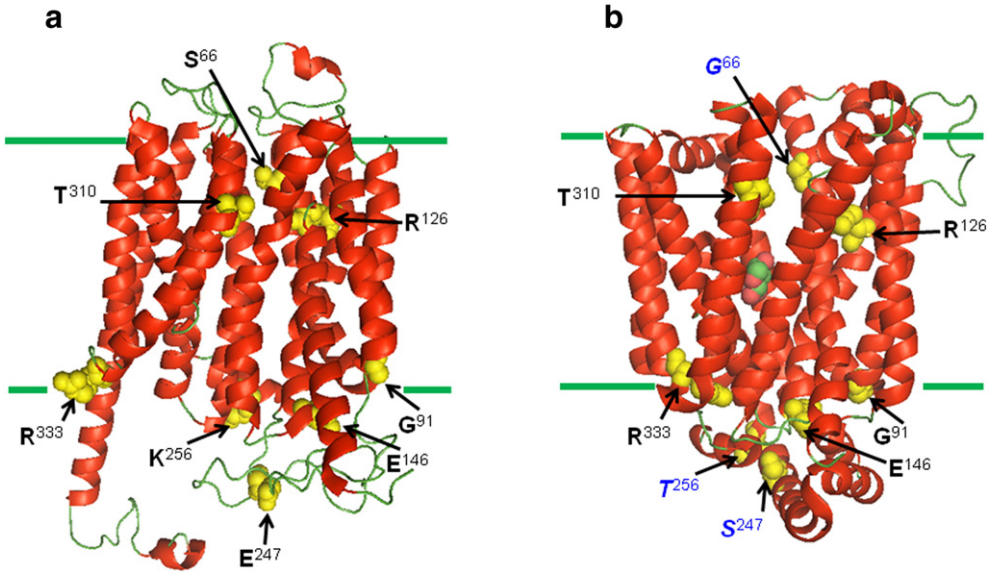

Fig. 2. Comparison of (a) previously proposed GLUT1 homology model (PDB ID: 1SUK) and (b) XylE crystal structure (PDB ID: 4GBY) in the membrane plane depicting the positions of pathogenic mutations causing GLUT-DS. In both figures, the residues are numbered according to GLUT1 amino acid sequence. Dissimilar residues in XylE compared to GLUT1 are highlighted in blue. All structures were analyzed in a PyMol computer modeling program (http://www.pymol.org/).

3.2. Genetic mutations at R126 position

Several LOF mutations at position Arg-126 (Arg126➔His/Leu/Cys) cause GLUT-DS. R126H (CGC➔CAC), R126L (CGC➔CTC) and R126C (CGC➔CGT) mutations are due to a single nucleotide polymorphisms in exon 4 [22–24]. R126 is located away from the transport path and buried within the N- and C-domains (Fig. 2A and B). According to GLUT1 homology model [15] and our *in silico* analysis, R126 (R133 in XylE) interacts with F72, V69 and S68 (A64, V61 and G58 in XylE, respectively) in the adjacent H2 helix (Fig. 4A).

The interaction of R126 with F72 may be caused by cation–π interaction. Such an interaction between aromatic amino acids (Trp/Tyr/Phe) and the positively charged residues (Lys/Arg) has been shown to play an important role in stabilizing protein structures [25,26]. However, replacement of R126 by charged histidine could result in the formation of 'redundant' H126-F72 pair (Fig. 4B) via formation of an intra-helical bridge between the aromatic F72 and H126 residues at physiological pH [27]. In case of R126L mutation, the substituted hydrophobic leucine seems to be positioned in the vicinity of another hydrophobic residue (isoleucine 33 in helix 1) as depicted in Fig. 4C. This may promote a hydrophobic interaction/cluster formation among R126L, I33 and V69 thereby altering the helix-helix interaction and ultimate protein conformation. Substitution of R126 with cysteine in R126C variation could also cause destabilization of interactions among R126C, F72, V69 and S68 (not shown). It has been shown that R126 variants carrying His, Leu or Cys substitutions exhibit reduced targeting of GLUT1 to the plasma membrane as determined by Western blot of plasma membrane rich fractions and confocal microscopy [22–24]. In all cases, the amounts of mutant GLUT1 were found to be within 20% of native GLUT1 except for R126C, which was reduced by ~75%. Furthermore, all variants exhibited glucose transport defects. Site-directed mutagenesis studies in *Xenopus* oocytes demonstrated only 3.2% uptake of 3-O-methyl-D-glucose (3-OMG) by the R126L carrying GLUT1 [28]. It was also documented that charge, but not the side-chain size, facilitates substrate translocation, as determined by the transport defective R126L mutant [28].

Interestingly, the XylE variants carrying R133H, R133L and R133C (R126 in GLUT1) mutations also resulted in a significant decrease in substrate transport [28]. According to *in silico* analysis, such pairs of interaction are absent in XylE structure. Instead, the side-chain of R133 (R126 in GLUT1) is located quite close to the negatively charged D27 (replaced by N35 in GLUT1) at a distance of 3.1 Å. Such situation may

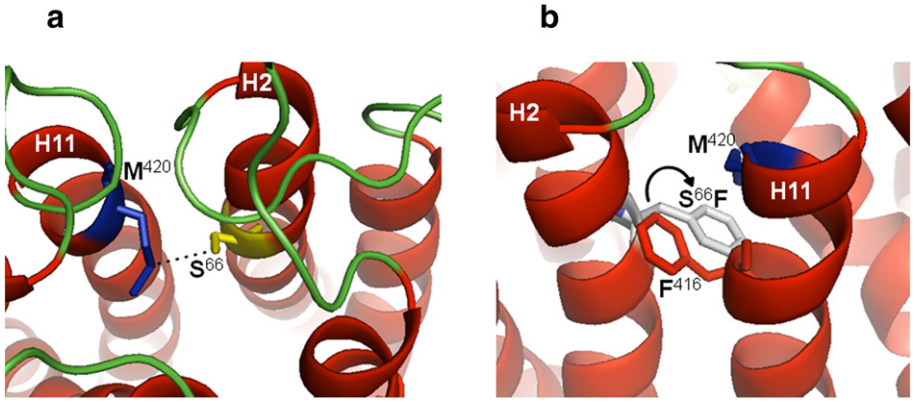

Fig. 3. (a) Modeling of S66 in helix H2 depicting the interaction with M420 in helix H11. The dotted line indicates the bond length distance (3.5 Å) between the side chains. (b) The possible phe-phe ring formation via non-covalent interaction between S66F with F416. All structures were analyzed in a PyMol computer modeling program. The best rotamer configurations were selected from two possible orientations to depict the closest inter-residue interactions.

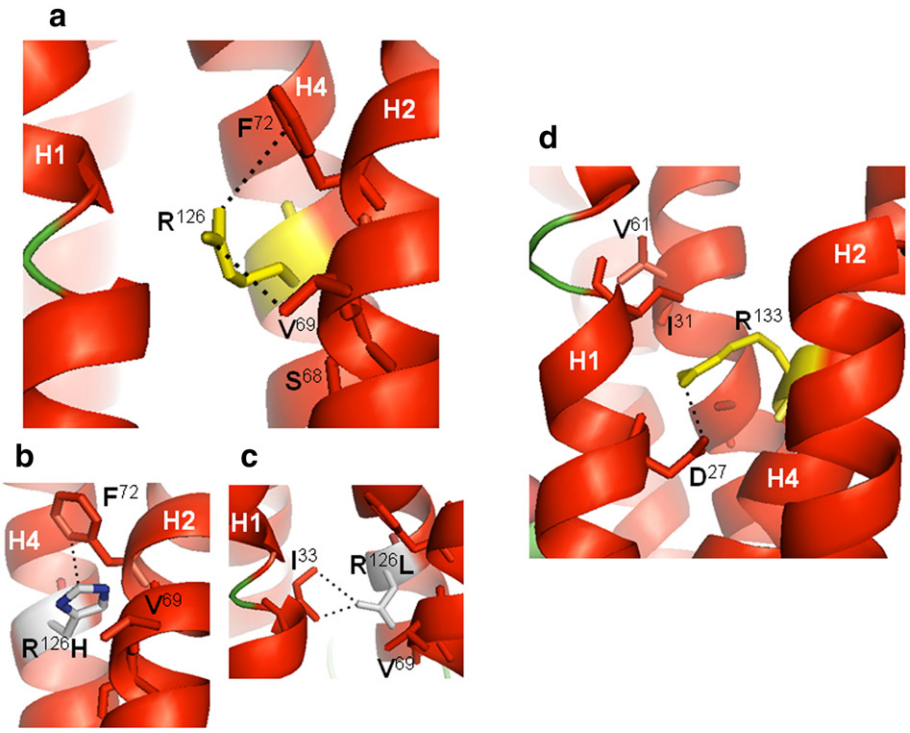

Fig. 4. (a) Interaction of R126 in H4 with V69 and F72 in H2. (b) R126H mutation can cause R126H-F72 via formation of intra-helical bridge thereby altering the conformational stability of H4 and H2 helices. (c) For R126L mutation, the substituted hydrophobic leucine may interact with another hydrophobic isoleucine (I33) in H1 via hydrophobic interaction or may involve cluster formation of R126L, I33 and V69. (d) Modeling of R133-D27 interaction in XylE structure. R133 mutations can destabilize electrostatic and helix-helix interactions between H4 and H1. The dotted lines represent the interactions between side chains.

induce a salt bridge formation among Arg-Asp (Fig. 4D). Similar to GLUT1, yet via different mechanism, destabilizing this electrostatic interaction (R133-D27) in XylE may destabilize the helix-helix interaction thereby causing drastic changes in the protein conformation and function. These analyses agree well with the observations on mistrafficking or defective function of both GLUT1 and XylE proteins.

3.3. G91D genetic variation

G91 residue is located on the intracellular side on loop 2-3 of both XylE and GLUT1 [29]. The genetic mutation G91D in exon 4 is caused by single nucleotide polymorphism at nucleotide position 272 (GGC➔GAC). G91D mutation has been shown to affect an R-X-G-R-R motif (Fig. 1, highlighted in red dotted box) between helices 2 and 3 that corresponds to a cytoplasmic anchor point and is highly conserved among transporters of the major facilitator superfamily (MFS). Furthermore, the substitution of the positively charged arginine with glycine in R-X-G-R-R motif completely abolished the transport activity indicating that amino acid charges are important determinants for membrane topology. The functional importance of G91 to aspartate mutation within a highly conserved sequence of GLUT1 was determined in which the glucose uptake into erythrocytes was significantly reduced, suggesting a quantitatively normal, but functionally impaired, GLUT1 translocation to the cell membrane [29].

Substitution of glycine (a particularly conserved small, hydrophobic and neutral amino acid) with aspartate (a long, hydrophilic and negatively charged amino acid) is expected to perturb the membrane topology of the GLUT1 protein. In this regard, several interesting phenomena can point to the possible effects of G91D mutation. First, *in silico* analysis suggests that G91D mutation localizes the substituted aspartate side chain quite close to E209 and may cause strong electrostatic repulsion due to negative charges on both side chains (Fig. 5A). Similar situation was observed for XylE. The positioning of G83D in the vicinity of E222 (corresponding to G91D and E209 in GLUT1, respectively) may also cause electrostatic repulsion (Fig. 5B). Second, the positioning of G91D may cause alteration in the packing of helices 2 and 3 with regard to the membrane as denoted by black arrows. The introduction of negatively charged (G91D) in a highly conserved and positively charged R-X-G-R-R motif may disrupt protein-lipid interaction(s) that might stabilize the helices H2/H3 via negatively charged phospholipids in the membrane [30,31]. Third, the presence of similar negative charges (E209 on loop 6–7 and G91D) may trigger an aberrant translocation of the large cytoplasmic loop 6–7 into the exoplasm similar to the effect described for arginine to glycine substitution in R-X-G-R-R motif [32].

3.4. E146K and E247D genetic variations

E146 and E247 residues are located on the intracellular side on helix 4 and loop 6–7, respectively. Mutations E146K (GAA➔AAA) in exon 4 [23], and E247D (GAA➔CAA) in exon 6 [24] are also caused by a single nucleotide polymorphism. Mutation of a negatively charged E146 to lysine results in impaired glucose transport [23]. According to GLUT1 and XylE model, E146 is located quite close to the long intracellular loop 6–7 (Fig. 2). *In silico* analyses indicate that E146 interacts with K225 on loop 6–7 via electrostatic interactions with a bond length of ~3.6 Å between the functional groups (Fig. 6A). Similarly, in XylE structure E153 (corresponding to E146 in GLUT1) in helix 4 is localized quite close to the positively charged R225 (K225 in GLUT1) in ICα1 of loop 6–7 (Fig. 6B) which can stabilize the same segment of loop 6–7 via electrostatic interactions. Removal of this interaction could cause aberrant conformation of loop 6–7 hence sugar transport defect.

E247D position, which is located on loop 6–7, is a part of the segment of the transport pathway. Substitution of negatively charged glutamate with similarly charged aspartate decreases the side chain length by 1.5 Å. Modeling indicates that E247, which is replaced by S259 in XylE (not shown), might not be involved in stabilizing the intermolecular interactions. However, its side chain sticks out of the unstructured loop (Fig. 6C) and might interact with glucose substrate during translocation.

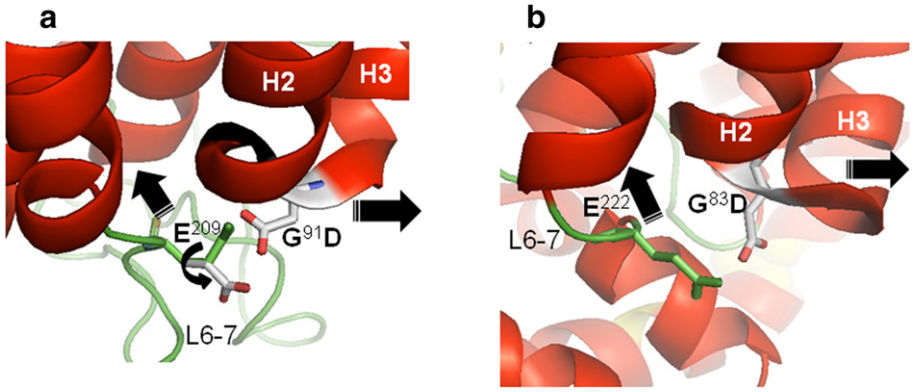

Fig. 5. (a) Possible electrostatic repulsion between G91D and E209 located on H2-H3 and loop 6–7, respectively. (b) In XylE, G83D mutation can also cause electrostatic repulsion due to the presence of E222. The dark blue arrow depicts the hypothetical movement of glutamate side chain as a result of electrostatic repulsion and the black arrows indicate alteration in the conformation of loop 6–7 and H2-H3 helices.

Decreasing the side chain length upon E247D mutation may obstruct the proper interaction of E247D with the glucose thereby triggering LOF or defective transport function.

3.5. Missense mutation T310I

Genetic mutation of threonine to isoleucine, located on helix 8, at position 310 (T310I) is due to a single nucleotide mutation (ACC➔ATC) at 929 nucleotide position in exon 7 [6]. It has been shown that the amount of immunoreactive GLUT1 protein is not decreased in erythrocyte membranes of a GLUT-DS patient carrying a T310I missense mutation [19,33] indicating that T310I does not cause any trafficking defect.

T310 is located on the extracellular side of the putative transport path (Fig. 2). According to GLUT1 homology modeling [15], T310 is involved in formation of three interaction pairs with the residues located on helix 6. T310-L169, T310-Q172 and T310-V173 interaction pairs, that are formed via hydroxyl group of threonine, might stabilize helix-helix interactions (Fig. 7A). Due to the presence of dissimilar residues in TM6 of XylE such pairs are unlikely to exist. It has been shown that mutation of threonine/serine residues to non-polar residues abolishes oligomerization suggesting that the interaction between these positions is specific and requires an extended motif of serine/threonine hydroxyl groups [34]. Similarly, mutation of T310 to a non-polar isoleucine may destabilize the interaction pairs (Fig. 7B) as well as the helix–helix interaction between H6 and H8. In addition, an introduction of bulky

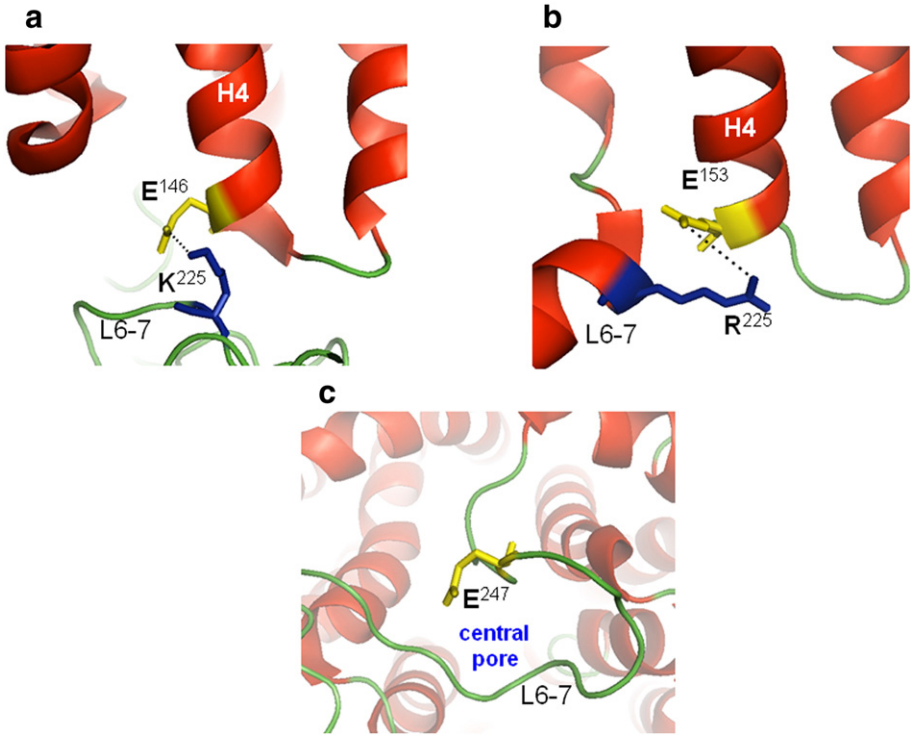

Fig. 6. (a) Stabilization of H4 and loop 6–7 via ionic interaction between E146 and K225. The dotted line represents the interaction between the side chains with measured bond distance of about 3.6 Å. (b) In XylE, similar electrostatic interaction between negatively charged E153 and positively charged R225 (K225 in GLUT1) stabilizes the protein structure at the inner mouth. (c) Localization of E247 in loop 6–7 along the transport pathway. Decreasing the side chain length upon E247D mutation may interfere with the proper interaction between the glucose and the negatively charged residue at 247 position hence causing transport defects.

isoleucine would also affect the packing of residues around the TM6 and TM8 thereby causing transport defect.

3.6. K256V and R333W GLUT1 mutations

Non-sense mutation K256V (AAG➔GTG) is located in exon 6 [24]. K256 being conserved in human GLUT1–2, is positioned near the segment of the long intracellular loop 6–7. The K256V mutant GLUT1 protein retains significant residual catalytic activity (12.7%) as demonstrated by site-directed mutagenesis and 3-OMG uptake studies in *Xenopus* oocytes. However, the maternally inherited K256V mutation causes a mild decrease in GLUT1 protein expression (~85%) with no clinical symptoms [35].

According to GLUT1 homology modeling and XylE structure, K256 is localized in the ICα3 of loop 6–7 (Figs. 1 and 2) and quite close to the membrane surface. K256 in GLUT1 is either replaced by T266 or one position C-terminal (K265) in XylE (Fig. 1). The most common feature of several transmembrane domains is that they are frequently flanked by lysine residues that well known for their snorkeling effect. The aliphatic part of lysine is buried in the hydrophobic region of the lipid bilayer, while the charged amino group is positioned in the more polar interface [36]. Interestingly, ICα3 and the late part of loop 6–7 are rich in positively charged residues (R253, K255 or R264) where they might interact with the negatively charged lipids on the cytoplasmic side (Fig. 8A) thereby stabilizing transmembrane helices [37,38]. Mutation of K256 to a hydrophobic valine may modify the protein-lipid interaction most likely by causing a shift in the positioning of ICα3 short helix with regard to the membranes thereby changing the protein conformation and altering the function of GLUT1 protein.

A basic R333 (R341 in XylE) is found highly conserved from *Archaebacteria* to humans throughout members of the GLUT family and the MFS membrane transport proteins. Missense mutation of R333 to a non-polar tryptophan (R333W) is caused by a single nucleotide polymorphism (CGG➔TGG) in exon 8 [23]. R333 in loop 8–9 (Fig. 8B) has been shown to be involved in determining the membrane topology of human GLUT1. R333W mutation leads to severe defects in GLUT1 function, as determined by 2-deoxy D-glucose (DOG) uptake in *Xenopus* oocytes. Interestingly, a corresponding R380W mutation in GLUT9 causes renal hypouricemia type-2 [39]. According to GLUT1 and XylE modeling, R333-side chain might stick out of the membrane and may contribute to stabilize the segment between helix 8 and 9. However, R333W mutation may position the aromatic side chain of substituted tryptophan at the membrane-water interfacial region (Fig. 8B). The preferential positioning of tryptophan residues at the membrane/water interface has been shown to be associated with the structure and function of many membrane proteins [40,41]. The substitution of R333 to tryptophan may therefore 'push' the short loop 8–9 into the membrane thereby altering the conformation of the sugar transport pathway.

4. Discussion

The lack of sequence similarity among resolved structures of several MFS proteins and GLUT family members has made it an intimidating task to generate precise models of GLUT members, particularly GLUT1. The availability of the proton-coupled *E. coli* D-xylose transporter XylE, as the first member of the MFS, which has been structurally characterized in multiple transporting conformations, including both the outward [16] and inward-facing states [45] can help predict the GLUT1 transporter structure and transport mechanism. The sequence similarities among XylE and GLUT1 to a certain extent and presumably similar meshwork of interaction pairs stabilizing the transporter structure provide a rationale that how LOF mutations might change the stability, structure and function of the GLUT1 proteins, as investigated in the present study. Currently, when the GLUT1 structure is not available, the *in silico* analyses carried out for both XylE crystal structure and homology model of GLUT1 support the available experimental results discussed above.

Combining previous transport studies and our *in silico* analyses it is interesting to consider how LOF genetic mutations may destabilize protein-protein and/or protein-membrane interactions thereby causing mistrafficking, misfolding and defective transport function of GLUT1 protein. It is intriguing to note that the mechanism of LOF mutations is quite similar to what has been proposed for SGLT1-GGM mutations that also seems to destabilize hydrogen and ionic bonds among pairs of residues and therefore change protein transport function [42].

The XylE structure has been crystallized in the presence of the competitive inhibitor 6-Br-deoxy-D-glucose [16], while the GLUT1 model was derived by an evolutionary molecular modeling strategy, using the crystal structure of the glycerol-3-phosphate translocase as template [15]. The crystal structures of the MFS members, like YajR transporter [43] as well as SGLT1 [44] reveal three different conformations: outward-facing, inward-facing and intermediate partially occluded conformations. The crystal structure for XylE corresponds to an outwardly facing partially occluded conformation, whilst the GLUT1 homology model represents an inward-facing conformation, since it was derived using the inwardly facing conformation of the GlpT crystal. However, upon comparison of both structures in two distinct conformations, it is intriguing to note that the amino acid positions, particularly where genetic mutations occur, remain unaltered while the carrier undergoes

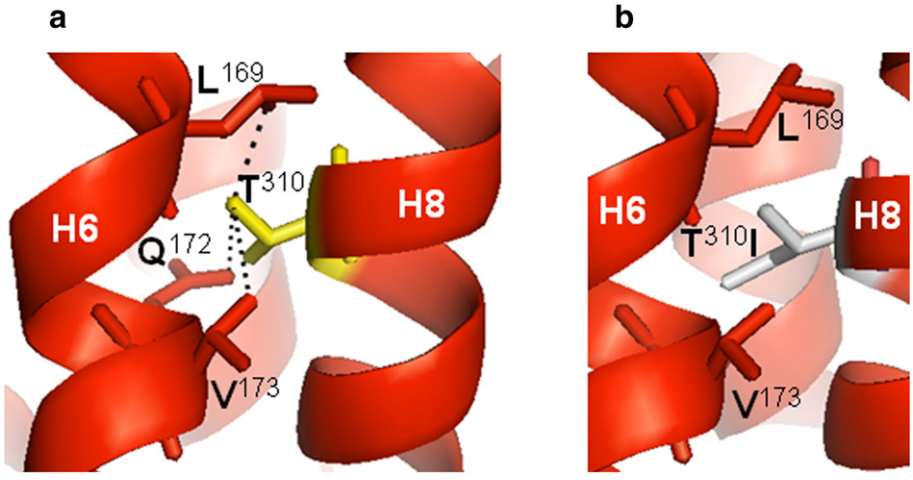

Fig. 7. (a) Interaction of T310 in H8 with L169, Q172 and V173 in H6. (b) LOF mutation of T310 to a non-polar isoleucine (T310I) destabilizes the interaction pairs and increases the bulk in the vicinity of H8/H6 thereby causing GLUT-DS.

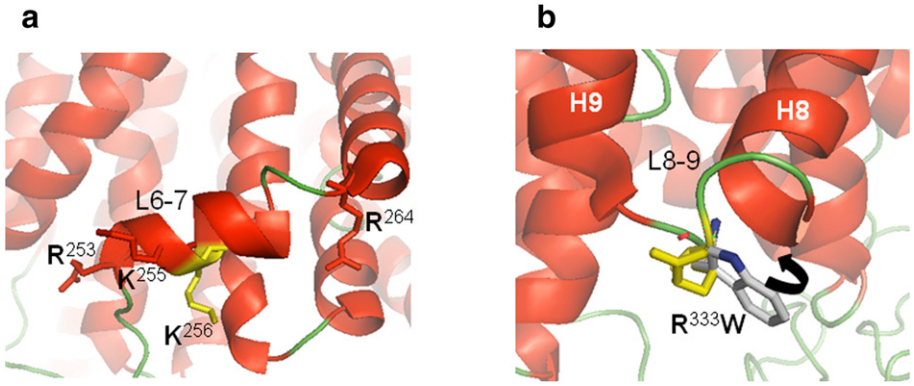

Fig. 8. (a) Localization of K256 in loop 6–7. Other positively charged residues R253, K255 and R264 are also shown that might interact with negatively charged lipids on the cytoplasmic side and stabilize TM helices. (b) Substitution of R333 to tryptophan (R333W) may affect the packing of helices, hence the sugar translocation.

various conformational changes during substrate translocation. In other words, the XylE protein or GLUT1 homology model do not seem to exhibit any considerable movements within the transmembrane/extramembranous regions in either conformation. It is however difficult to predict any possible motion within the protein structure while transitioning from an outward to an inward facing conformation or *vice versa*.

The MFS transporter lactose permease (LacY) has been shown to alternate between cytoplasmic and periplasmic open conformations to co-transport a sugar molecule across the plasma membrane. Such occluded state of LacY transporter is quite similar to the occluded crystal structures of several other MFS transporters, including XylE [46,47]. Interestingly, a salt bridge formation between E325 and R302 has been proposed to be involved in driving the conformational transition in this transporter. Our modeling also indicate a salt bridge formation between R133 and D27 in the TM region (which is at almost similar position with regard to the salt bridge formation in LacY) or between E153 and R225 (E146-K225 in GLUT1) on the intracellular side, suggesting a similar mechanism by which XylE/GLUT1 may exhibit a complicated coupling between two flexible gates [46,47].

Another important aspect of GLUT1 structure/function is that it forms dimers and tetramers in cell membranes [48]. It has been shown that TM2, -5, -8, and -11 contribute to GLUT1 dimerization, whereas TM9 acts as a major determinant of transporter tetramerization. Interestingly, the tetrameric or dimeric GLUTs exhibit cooperative sugar binding behavior where tetramers are at least 4-fold more catalytically active than GLUT dimers. The allosteric transport behavior may therefore reflect subunit interactions [48]. In addition to the destabilization of inter-molecular interactions upon LOF mutations we hypothesize that such mutations (e.g., S66F on helix 2 or T310I on helix 8) may also lead to dissociation of subunit-subunit interaction between dimeric/tetrameric GLUT1 monomers causing inefficient substrate translocation.

Most mutations are found in structurally defined parts of the protein and the rest are localized in unordered loops. In particular, mutations in the cytoplasmic domains may affect the structural content of short helices that might be crucial in determining the proper interaction of transport inhibitors. It is known that the binding of a competitive inhibitor cytochalasin B with the cytoplasmic domains is followed by a conformational change at the cytoplasmic face of the GLUT1 protein [49] which presumably contains small helices, like ICα1, 2, 3 and 4 in loop 6–7, as predicted in XylE. In support of this notion, the predicted small helices in the C-terminal loop 13 of SGLT1 also seem to undergo conformational changes upon interaction of the glucose cotransport inhibitors [50,51] suggesting that both GLUT and SGLT proteins share similar binding domains that undergo mechanistically similar conformational changes upon inhibitor binding.

Despite the fact that the GLUT1 homology model and bacterial XylE crystal structure share remarkable identities, as explored in this review, a word of caution is still necessary. Generally, a sequence identity more than 50% leads to reliable modeling, with only limited errors in side chain and loops positioning. From sequence analysis shown in Fig. 1 it is clear that multiple regions in XylE and GLUT1 share little sequence identity (~29% total identity). Yet, GLUT1 homology model/XylE structure exhibit several limitations, i.e., (1) they fail to mimic the structure/function of post-translationally modified GLUT1; (2) based on the sequence analysis (Fig. 1) and possibility of lack of several structural elements in GLUT1, as compared to XylE, these models cannot predict the roles of several regions of GLUT1, i.e., the extramembranous unstructured loops that might also be involved in substrate translocation; (3) they are not able to predict any particular movement(s) within the GLUT1 protein which might change the side-chain interaction and therefore the stability of protein, and (4) they are not able to predict the conformational/structural changes in dimeric or tetrameric forms of functionally active GLUT1 [48] during substrate translocation and/or upon LOF mutations.

Although, the precision and validation of homology models by comparing our analyses with the GLUT1 structure is required, if structural biology succeeds to resolve the human GLUT1 structure, our approach of combining functional studies with structural perspectives in this review provides a step forward in understanding the mechanism of sugar transport defects. The long term goals may allow to seek drugs or corrector molecules as discovered for other transport proteins, like cystic fibrosis transmembrane conductance regulator (CFTR) [52], that might improve cell surface expression, stability or folding of misfolded GLUT1proteins.

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