Transmembrane IV of the high-affinity sodium-glucose cotransporter participates in sugar binding

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Liu T, Lo B, Speight P, Silverman M. Transmembrane IV of the high-affinity sodium-glucose cotransporter participates in sugar binding. Am J Physiol Cell Physiol 295: C64–C72, 2008.—Investigation of the structure/function relationships of the sodium-glucose transporter (SGLT1) is crucial to understanding the cotransporter mechanism. In the present study, we used cysteine-scanning mutagenesis and chemical modification by methanethiosulfonate (MTS) derivatists to test whether predicted transmembrane IV participates in sugar binding. Five charged and polar residues (K139, Q142, T156, K157, and D161) and two glucose/galactose malabsorption missense mutations (I147 and S159) were replaced with cysteine. Mutants I147C, T156C, and K157C exhibited sufficient expression to be studied in detail using the two-electrode voltage-clamp method in Xenopus laevis oocytes and COS-7 cells. I147C was similar in function to wild-type and was not studied further. Mutation of lysine-157 to cysteine (K157C) causes loss of phloridzin and α-methyl-D-glucopyranoside (αMG) binding. These functions are restored by chemical modification with positively charged (2-aminoethyl) methanethiosulfonate hydrobromide (MTSEA). Mutation of threonine-156 to cysteine (T156C) reduces the affinity of αMG and phloridzin for T156C by ~5-fold and ~20-fold, respectively. In addition, phloridzin protects cysteine-156 in T156C from alkylation by MTSEA. Therefore, the presence of a positive charge or a polar residue at 157 and 156, respectively, affects sugar binding and sugar-induced Na⁺ currents.

The high-affinity sodium-glucose cotransporter (SGLT1) belongs to the homologous family of Na⁺/solute symporters, SLC5 (22). It is a secondary active transporter that uses the sodium electrochemical gradient to transport sugar substrates uphill against a concentration gradient (2, 9, 22). SGLT1 is expressed most abundantly at the mucosal surface of the small intestine and serves as the principal uptake pathway for glucose derived from dietary sources. Dysfunctional mutations in SGLT1 cause intestinal glucose/galactose malabsorption (22). For many years, SGLT1 has served as a model system for studying the molecular basis of ion-coupled cotransporters. In pursuit of this objective, structure/function studies have formed an important experimental strategy to identify key residues participating in cotransporter function.

Application of the substituted cysteine accessibility method (SCAM) has shown that the Na⁺ interaction domain is located in the NH₂-terminal half of SGLT1 and involves residues 163, 166, 170, and 173 in the putative external loop joining transmembranes (TM) IV-V (7, 8, 10, 11). The polar residues at position 176 hydrogen bond to the hydroxyl group on the β-phenyl ring of phloridzin (18). There is also evidence that D454 in the putative external loop joining TM X-XI is involved in the coupling of Na⁺ and sugar in the transport process (4). The sugar binding domain, on the other hand, has been localized to the COOH-terminal half of SGLT1 (16). Q457, located in the putative external loop joining TM X-XI, appears to be particularly important because sugar transport is abolished by reaction of methanethiosulfonate (MTS) reagents and maleimides with Q457C (13). However, under these conditions, the transporter still binds Na⁺ and sugar (13). These results suggest that although residue 457 is at or near the sugar-translocation site, other unidentified residue(s) must be involved in the interaction with sugar ligand. Interestingly, there is evidence that A166 is important for the interaction between the Na⁺ and sugar pathways and that helices TM IV-V are close to TM X-XI (14). Therefore, the aim of the present investigation was to explore whether residues within predicted TM IV participate in sugar interaction.

In the 34 different missense mutations that were identified for glucose/galactose malabsorption (GGM) (22), four mutations are located in TM IV and the putative loop joining TM IV-V (R135W, L147R, S159P, and A166T). In the region of TM IV, 19 of 24 residues (138-161) are conserved in all members of SGLT1 and SGLT2. Previous studies have shown that several residues important for substrate and cation recognition are located in the putative external loop joining TM IV-V in the NH₂-terminal half of the transporter (7, 8, 10, 11, 14). Therefore, it is possible that TM IV may be important for SGLT1 function.

The present study describes the results from detailed investigation of two single cysteine mutants, K157C and T156C, which are conserved across species in SGLT1 and SGLT2. Our results suggest that manipulation of positive charge and polarity at positions 157 and 156, respectively, significantly affects sugar binding and sugar-induced Na⁺ currents.

MATERIALS AND METHODS

Molecular Biology

The cysteine mutations were generated via the megaprimer protocol of polymerase chain reaction mutagenesis as described previously and confirmed by sequencing (21).

Oocyte Preparation and Injection

Xenopus laevis were prepared as described previously (10). The oocytes were injected with 60 ng cDNA [empty vector pMT4, wild-type (WT) rSGLT1 or mutant rSGLT1]. The injected oocytes for

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the electrophysiology were stored at 16–18°C for 4 days or more before study. The present study was reviewed and approved by the Committee on Animal Research at the University of Toronto, Toronto, and was conducted in accordance with the committee’s guidelines.

**Electrophysiology**

Voltage clamping and recordings were performed using a GeneClamp 500 amplifier, Digidata 1200B interface, and pClamp 9.0 data acquisition software (Axon Instruments, Union City, CA) as described previously (10). The oocytes were constantly superfused with a voltage-clamping solution consisting of 100 mM NaCl, 2 mM KCl, 1 mM MgCl2, 1 mM CaCl2, and 10 mM HEPES-Tris base (pH 7.4) and held at a holding potential, Vh, of −50 mV, then was subjected to a series of voltage test pulses, Vt. The current responses were recorded with a sampling interval of 20 μs for pre-steady-state and steady-state experiments. The sampling frequency was 50 KHz. Results were filtered via a 1-kHz, 5-point Gaussian filter. Additional curve fitting was performed in ORIGIN 7.0 with the Levenberg-Marquardt algorithm; n is the number of observations.

**Transient Current Measurements**

The rSGLT1 pre-steady-state currents were determined as described previously (10). The pre-steady-state currents for each Vt were integrated over the entire course of the trace to calculate the total charge transferred by the cotransporter. The charge, Q, was plotted as a function of the test pulses, and these Q (Vt) curves were fitted to the two-state Boltzmann relation

\[
Q = -N \cdot e \cdot z \cdot \frac{1}{1 + e^{(z \cdot u \cdot (V_t - V_{th})} + Q_{dep}}
\]  

(1)

where Q is the total charge transferred, Q_{dep} is the charge due to depolarizing pulses, e is the elementary charge, z is the apparent valence of the movable charge, V_{th} is the potential at which half of the total charge transfer is complete, and N is the number of cotransporters expressed at the surface. The term n = FRT; F is Faraday’s constant, R is the gas constant, and T is absolute temperature.

Steady-state parameters were determined with the difference in the steady-state currents obtained before and after exposure to the substrate as described previously (10). Steady-state currents were acquired with test pulses of 300-ms duration. The final 100 ms of a test pulse was selected and the average current value of this range was acquired. The average current values were plotted versus [substrate] and the following equation was fit to the curve

\[
I = I_{max} \cdot [S]^n / (K_o^n + [S]^n)
\]  

(2)

where S is the substrate of investigation (Na+, αMG), I_{max} is the maximal current induced at saturating substrate concentration ([S]), n is the Hill coefficient, and K_o is the Michaelis constant, which is the S at which the I = I_{max}/2, which serves as an approximation of substrate affinity. The calculation of substrate affinity values used the I_{max} values of −150 mV test pulses.

**Protocols for Chemical Modification**

Cysteine-specific reagents (1 mM) (2-aminoethyl) methanethiosulfonate hydrobromide (MTSEA), amidonic sodium (2-sulfonatoethyl)methanethiosulfonate (MTSES), or [2-(trimethylammonium)ethyl] methanethiosulfonate bromide (MTSET) (Toronto Research Chemicals, Toronto, ON, Canada) were dissolved in a voltage-clamping solution consisting of 100 mM NaCl, 2 mM KCl, 1 mM MgCl2, 1 mM CaCl2, and 10 mM HEPES-Tris base (pH 7.4) immediately before use. The oocytes expressing mutant were labeled with the bath solution including cysteine-specific reagents for 10 min, with membrane clamped at −50 mV.

**Cell Transfection and Western Blot Detection**

NH2-terminal myc-tagged WT and mutants used for COS-7 transfections were prepared as described previously (21). Nontransfected cells and COS-7 cells transfected with vector alone served as controls. Proteins samples were resolved on 10% SDS-PAGE and transferred to nitrocellulose. The myc-epitope was detected with mouse monoclonal 9E10 (anti-myc, 1:1,000) antibody (Berkeley Antibody), followed by peroxidase-conjugated anti-mouse IgG (1:200,000) (Sigma). Immunoblots were developed by chemiluminescence, and area analysis was performed using the public domain NIH Image program (developed at National Institutes of Health). Western blot analysis for β-actin was performed to check equal loading.

**Labeling of Surface-Expressed T156C in COS-7 Cells With Biotine-MTSEA**

MTS compounds (Toronto Research Chemicals) were prepared fresh in (DMSO) and diluted to 1 mM concentrations in PBS (pH 7.4) immediately before use. Various protocols were used as described in RESULTS. Typically, cells in each well (1 × 10^5 cells/well, 12-well plate) were first preincubated for varying times at room temperature with either 500 μl PBS (control), 1.35 mM phloridzin, 1 mM MTSEA-alone, or in different combinations. The cells in each well were then washed (over 10–20 s) and immediately exposed to 500 μl 1 mM biotin-MTSEA for varying times up to 10 min at room temperature. Cells in each well were then washed with 3 times with 2 ml cold PBS and individual wells were scraped into 0.5 ml lysis buffer, (50 mM Tris·HCl, 150 mM NaCl, 1% Triton, 1% SDS, 1 mM EDTA, and protease inhibitor cocktail). Samples were rocked at 4°C for 30 min, and the insoluble protein was removed by centrifugation at 14,000 rpm for 15 min. Biotin-labeled proteins were isolated from the cell lysates with immobilized streptavidin-agarose (Sigma) (10% total volume, ~50 μl) by incubating overnight at 4°C with gentle agitation. The beads were washed, and the biotinylated protein was eluted from the beads by the addition of 50 μl SDS-PAGE sample buffer (4% SDS) at 100°C for 3 min.

**Statistical Comparisons of Means**

Data are presented with means ± SE. A one-way analysis of variance followed by Tukey’s honestly significant difference post hoc test was applied to the entire data set using SPSS software (SPSS, Chicago, IL) to determine whether significant differences existed between mean values. Statistical significance was accepted at an α-level of P < 0.05.

**RESULTS**

In the present study, we used SCAM and chemical modification by MTS derivatives to study the role of residues of K139-D161 that lie within predicted TM IV (Fig. 1). Five charged and polar residues (K139, Q142, T156, K157, and D161) and two GGM missense mutations (I147 and S159) (22) were replaced with cysteine. Several of these (I147C, T156C, and K157C) exhibited sufficient expression to be studied in detail using the two-electrode voltage-clamp method in Xenopus laevis oocytes. I147C was similar in function to WT and was not studied further (unpublished observations).

**Characterization of K157C**

Pre-steady-state behavior of K157C compared with WT. Figure 2 shows representative pre-steady-state currents from oocytes expressing WT or mutant K157C in the presence of 100 mM Na+. Saturating concentrations of the competitive inhibitor phloridzin (200 μM) (Fig. 2, A and B) or sugar substrate, αMG (10 mM) (Fig. 2, A and C), eliminate WT
transient currents, indicating that binding of phloridzin and αMG are intact. Transfection with empty vector (pMT4) does not give rise to pre-steady-state currents (Fig. 2D). The specific phloridzin-sensitive transient currents for WT are shown in Fig. 2E. In contrast to WT, the transient current of mutant K157C remains essentially unaffected by exposure to either phloridzin (Fig. 2, H and I) or αMG (Fig. 2, H and J). The phloridzin-sensitive transient currents for the mutant K157C are shown in Fig. 2F and are significantly lower than WT (Fig. 2E). The phloridzin binding of mutant K157C could be studied by the effect of the external phloridzin on the pre-steady-state charge movement. The pre-steady-state charge movement of mutant K157C, which shows the loss of phloridzin binding for mutant K157C, was obtained by integrating the phloridzin-sensitive pre-steady-state currents (Fig. 2G).

Site-directed alkylation of cysteine-157 (K157C) rescued activity of SGLT1. Since mutation of positively charged lysine-157 to neutral cysteine abolishes both phloridzin and αMG binding, we developed a rescue strategy designed to recover phloridzin and αMG binding by restoring a positive charge at position 157. Accordingly, we exposed oocytes expressing K157C to the positively charged MTS derivative, MTSEA, which mimics the lysine side chain at position 157 (Fig. 3). Previous work has established that exposure of oocytes expressing WT to various MTS derivatives does not alter SGLT1 function (9).
Boltzmann relation. The porter. The charge, trace to calculate the total charge transferred by the cotransporter. The net phloridzin-sensitive K157C transient currents are restored, demonstrating that exposure of K157C to MTSEA rescues phloridzin and αMG binding. The net phloridzin-sensitive K157C transient currents (obtained by subtracting the currents in Fig. 4A from Fig. 4B) are shown in Fig. 4E. We attempted to study phloridzin and αMG binding in mutant K157R. We found that there was insufficient expression of mutant K157R in oocytes to permit further investigation (confirmed by Western blot analyses of COS-7 cells; unpublished observations).

The pre-steady-state currents of mutant K157C-MTSEA for each $V_t$ (Fig. 4E) were integrated over the entire course of the trace to calculate the total charge transferred by the cotransporter. The charge, $Q$, was then plotted as a function of the test pulses, and these $Q (V_t)$ curves were fitted to the two-state Boltzmann relation. The $V_{0.5}$ value of K157C-MTSEA is $-9.2 \pm 1.4 \text{ mV (} n = 5 \text{)},$ whereas the $V_{0.5}$ value of WT is $-1.5 \pm 5.1 \text{ mV (} n = 5 \text{).}$ The $z$-values of the two are also comparable ($0.88$ for K157C-MTSEA vs. $1.01$ for WT). The pre-steady-state Boltzmann parameters in $100 \text{ mM Na}^+$ for both WT and rescued K157C-MTSEA are very similar, suggesting equivalent voltage sensitivity.

To determine the extent of the rescue achieved by restoring a positive charge at position 157, we estimated mutant K157C expression relative to WT. SGLT1 pre-steady-state currents yields three time constants $\tau$ ($\tau_{fast}$, $\tau_{medium}$, and $\tau_{slow}$) (9, 12), and the oocyte capacitive membrane current, which is not relevant to transporter activity, is $<1 \text{ ms}$ (2, 9). Loo et al. (12) estimated WT hSGLT1 expression by adding the charge transferred associated with the medium and slow decays ($Q_{med}$ and $Q_{slow}$). We used the same approach to calculate the charge transferred in nC from the medium and slow decays for K157C and rWT (K157C $3.8 \pm 0.7 \text{ nC, } n = 8$ vs. WT $12.8 \pm 0.4 \text{ nC, } n = 3$). From this ratio, the estimated expression of mutant K157C is $\sim 30\%$ of WT. Thus we estimate the rescue of K157C sugar binding activity following chemical modification by MTSEA to be $>85\%$ (Fig. 5).

Figure 5 also shows the results following chemical modification of cysteine-157 in K157C by two membrane-impermeant MTS derivatives (positively charged MTSET and negatively charged MTSES). In both cases, rescue of phloridzin and αMG-dependent charge transfer is much less than that achieved following treatment with MTSEA (Fig. 5; lines 7 and 11 versus line 5; lines 9 and 13 versus line 6), but pretreatment with MTSET or MTSES blocks the ability of MTSEA to restore K157C phloridzin and αMG binding (Fig. 5; lines 8 and 12 versus line 5; lines 10 and 14 versus line 6). Since the MTS reagents did not affect WT hSGLT1 (10), the changes observed in Fig. 5 must arise from alkylation of cysteine-157 after exposure to MTSEA. Therefore, the inability of MTSET or MTSES to rescue K157C sugar binding is not due to inaccessibility to cysteine-157 because MTSEA rescued sugar binding. Since MTSET and MTSES are both membrane-impermeant and since chemical modification of cysteine-157 in K157C by either derivative blocks reactivity to MTSEA, this indicates that cysteine-157 is located exofacially.

Figure 6, A and B, shows that the charge transfer following reaction of K157C with MTSEA (i.e., of K157C-MTSEA)
depends on the sodium concentration in the bath solution. αMG-induced Na\(^{+}\) currents are also Na\(^{+}\) dependent (Fig. 6C). Na\(^{+}\) dependence of K157C-MTSEA thus mirrors Na\(^{+}\) dependence of WT rSGLT1 (8) and provides further evidence that MTSEA exposure restores sugar-induced Na\(^{+}\) currents and phloridzin binding.

To determine whether reactivity of K157C with MTSEA depends on sodium, oocytes were exposed to MTSEA in either 100 mM Na\(^{+}\) or 0 mM Na\(^{+}\) (choline buffer). As shown in Fig. 7, A and B, reactivity of 1 mM MTSEA with cysteine-157 in K157C is not influenced by the presence of Na\(^{+}\). K157C-MTSEA, labeled in the absence of Na\(^{+}\), restores both phloridzin and αMG binding. Furthermore, when the same oocyte is pretreated with MTSEA in the absence of Na\(^{+}\) and is then treated with MTSEA in the presence of 100 mM Na\(^{+}\), there is no additive effect on rescue of phloridzin and αMG binding.

Steady-state αMG-induced Na\(^{+}\) currents of K157C and K157C-MTSEA. Figure 8A shows the maximal current induced by 10 mM αMG for K157C and for K157C reacted with various MTS reagents. Neither K157C-MTSET nor K157C-MTSES rescues sugar binding and sugar-induced Na\(^{+}\) currents (Fig. 8A, lines 6 and 8 versus line 4). This is in contrast to the effect of chemical modification by MTSEA. As shown in Fig. 6C, reaction with MTSEA (K157C-MTSEA) restores Na\(^{+}\)-dependent sugar-induced Na\(^{+}\) currents, and the Na\(^{+}\) leak is very small (unpublished observations). However, when the same oocyte that was pretreated with MTSET or MTSES is then reacted with MTSEA in the presence of
To determine the affinity of rescued K157C-MTSEA for sugar substrate αMG, we measured αMG-induced steady-state currents at various sugar concentrations over a range of holding potentials, and the resulting curves were fitted to the Michaelis-Menten relation. As shown in Fig. 8B, the $K_{0.5}$ of restored mutant K157C-MTSEA activity exhibited voltage dependence from $-150 \text{ mV}$ to $-50 \text{ mV}$, similar to WT rSGLT1 ($K_{0.5}$ is $0.15 \pm 0.02 \text{ mM at } -150 \text{ mV}$) (10). The αMG apparent $K_{0.5}$ of K157C-MTSEA was $2.65 \pm 0.93 \text{ mM (} V = -150 \text{ mV) and}$ $5.81 \pm 2.46 \text{ mM (} V = -50 \text{ mV) (significantly different, } P \approx 0.05)$. K157C-MTSEA $I_{\text{max}}$ was $28.99 \pm 5.99 \text{ nA (} V = -150 \text{ mV) and}$ $6.48 \pm 1.68 \text{ nA (} V = -50 \text{ mV), (significantly different, } P < 0.05)$. Therefore the αMG affinity of K157C-MTSEA is decreased about 18-fold compared with WT.

In summary, mutation of the positively charged lysine residue at position 157 abolishes sugar and phloridzin binding. Replacement of the positive charge by MTSEA, however, restores activity.

**Characterization of T156C**

Given the substantial effects on sugar binding and sugar-induced Na⁺ currents that arise following mutation of lysine-157 to neutral cysteine, we investigated whether mutation of the neighboring residue, threonine-156, would affect SGLT1-sugar interaction.

**Steady-state kinetics of T156C.** A plot of the apparent αMG affinity as functions of $V$ is shown in Fig. 8B. The affinity of T156C for αMG (at $V = -50 \text{ mV, } K_{0.5} = 0.95 \pm 0.06 \text{ mM}$) was decreased about 5-fold compared with WT. When Na⁺ dependence of T156C sugar-induced Na⁺ currents was ana-

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**Fig. 7.** Typical results demonstrating the effects of pretreatment with 1 mM MTSEA on mutant K157C charge transfer in the absence of Na⁺ (choline replacement) or in the presence of 100 mM Na⁺. A: effects on charge transfer. B: effects on αMG binding. C: effects on αMG-induced Na⁺ currents.

Na⁺, sugar binding and sugar-induced Na⁺ currents were not restored (Fig. 8A, lines 7 and 9 versus line 5). Although MTSET or MTSES does not affect sugar binding and sugar-induced Na⁺ currents, pretreatment with these agents is able to block reactivity of MTSEA, thus indicating that MTSET and MTSES are able to alkylate cysteine-157 in K157C.
lized by fitting the Hill equation, $K_{0.5}$ ranged from 28.9 ± 1.4 mM at −30 mV to 22.9 ± 3.7 mM at −150 mV. The corresponding values for $n$, the Hill coefficient, ranged from 1.0 to 2.0. The Na$^+$ affinities of T156C were decreased about 6-fold at −150 mV and same at −30 mV compared with WT (11, 17).

**Apparent affinity of T156C for phloridzin.** Binding of phloridzin to WT SGLT1 inhibited αMG-induced currents by competing for the sugar-binding site, reduced the leak current by locking the transporter in a nontransporting state, and eliminated the charge movements that give rise to the transient currents. By measuring the amount of transient current eliminated as a function of phloridzin concentration, an estimate of the apparent affinity of the transporter for phloridzin could be made.

We have determined that the phloridzin binding affinity of T156C was decreased ~20-fold compared with WT (Krofchick D and Silverman M, unpublished observations). These values for phloridzin binding are consistent with the apparent reduced affinity for αMG demonstrated in Fig. 8B. Therefore, a threonine-to-cysteine mutation at position 156 causes a significant reduction in binding affinity for both sugar and phloridzin.

**Chemical modification of T156C by MTS reagents.** The T156C I-V curves in the absence or presence of 2 mM MTSEA are shown in Fig. 9A. After reacting mutant T156C with MTSEA, the Na$^+$ leak current is increased ~10-fold, and the αMG-induced Na$^+$ current is almost abolished (Fig. 9A). These results are similar to those reported for mutant Q457C (13). This effect was observed in the absence of Na$^+$ (choline+ replacing Na$^+$) as well as in its presence (unpublished observations). The fluorophore TMR6M, which blocks sugar-induced Na$^+$ currents in Q457C (11), also abolishes αMG-induced Na$^+$ currents in T156C (Fig. 9B), but the Na$^+$ leak currents of T156C are very small (unpublished observations). A similar exposure (10 min) to 2 mM MTSEA was previously shown to have no effect on the function of WT SGLT1 (10). When phloridzin was infused at the same time as MTSEA, phloridzin conferred partial protection against MTSEA modification of T156C. Phloridzin protection was only observed when inhibitor was present together with MTSEA in the infusing solution. It has not been possible to definitively test whether saturating concentrations of αMG could provide similar protection since prolonged exposure to high concentrations of αMG leads to paradoxical inactivation of the transporter.

We then studied the apparent competition between phloridzin and MTSEA with respect to MTSEA reactivity with cysteine-156 (21). COS-7 cells expressing WT SGLT1 and T156C were each reacted with biotin-MTSEA. Fig. 10A demonstrates that biotin-MTSEA specifically labels cells expressing T156C, but not WT SGLT1. These results demonstrate that MTSEA has reacted with cysteine-156 in T156C. We then developed an assay system based on competition of MTSEA and biotin-MTSEA for cysteine-156. Fig. 10B shows that MTSEA prevents biotin-MTSEA from reacting with T156C, but it takes ~6–7 min for maximal MTSEA effects to be observed. Phloridzin was able to protect the mutant T156C from reacting with MTSEA because after removal of phloridzin/MTSEA, then cells could still react with biotin-MTSEA.

In summary, mutation of the polar amino acid to a neutral residue (T156C) reduces the affinity for both αMG and phloridzin. Conversely, phloridzin protects cysteine-156 from alkylation by MTSEA.
DISCUSSION

We identify two residues (threonine-156 and lysine-157) in predicted TM IV that are important for sugar binding. Wright and coworkers (16) have previously proposed the sugar domain to be established by TM X-XIII in the COOH-terminal half of SGLT1. Their laboratory also demonstrated that reacting Q457C with MTSEA inhibited sugar transport, but the mutant could still bind sugar (11). These results suggest that other residues may be important for binding of sugar. Recently, Punthiranarak et al. (19) expressed rSGLT1 in COS-7 and G6D3 cells and found that mutants C255 and C511 form a disulfide bridge, suggesting the putative loop TM VI-VII is close to TM XI-XIII. Despite the discrepancy between these two sets of observations (which may be due to the different experimental systems used), both studies suggest that the putative loop TM VI-VII also participates as part of the extracellular binding pocket for sugar.

Lysine-157 is conserved in all members of both SGLT1 and SGLT2. The finding that mutation of lysine-157 to alanine in hSGLT1 apparently results in “impaired transport” (20) supports the notion that lysine-157 is an important residue in sugar binding. In the present study, we also found that lysine-157 is important for sugar binding. Mutation of positively charged lysine to cysteine abolishes sugar and phloridzin binding. Restoration of the positive charge, however, by chemical modification of cysteine-157 with MTSEA rescued sugar binding by 10.220.33.6 on June 23, 2017 http://ajpcell.physiology.org/ Downloaded from

also independent of Na⁺. However, phloridzin binding and sugar-induced Na⁺ currents of mutants T156C, K157C-MTSEA, and Q457C are all dependent on sodium. Binding of Na⁺ appears to produce a conformational change in the TM XI region of mutant Q457 compared with TM IV and/or the putative external loop between TM IV and V region of mutants T156, K157, and A166. A large conformational change in the region of TM XI (Q457) may be required to allow access to extracellular ligand in the late stages of the transport cycle.

Finally, there are two alternative interpretations of the collective data presented in the present study. One possibility is that both lysine-157 and threonine-156 directly participate in binding of sugar substrate. This is plausible in view of the evidence derived from the crystallographic structure of lactose permease, that positively charged residues can participate in hydrogen bonding with sugar ligands (1). Another possibility is that the mutation of the charge and polarity at positions 157 and 156 causes a conformational change in SGLT1, which alters sugar interaction with its binding pocket in the protein. Whichever interpretation is correct, there seems to be reciprocal in that phloridzin protects against alkylation of MTSEA at position 156 due to either the glycoside moiety of phloridzin competing directly for binding to position 156 or phloridzin occupancy of the sugar binding site at some other location which induces a conformational change that prevents MTSEA accessibility to position 156. Taken together, these results suggest that TM IV participates in sugar interaction with SGLT1.

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GRANTS

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