The Endogenous CXXC Motif Governs the Cadmium Sensitivity of the Renal Na\(^+\)/Glucose Co-Transporter

Xiaobing Xia,*† Gang Wang,* Yanchun Peng,† Ming-Gene Tu,‡§ Jimmy Jen,‡ and Hongqing Fang‡

*Beijing 302 Hospital, Beijing, China; †Medical Sciences Division, University of Oxford, Headington, Oxford, United Kingdom; ‡School of Dentistry, China Medical University, §Department of Dentistry, China Medical University Hospital, and ‖Graduate Institute of Dental Sciences, Kaohsiung Medical University, Taiwan; and §Beijing Institute of Biotechnology, Beijing, China

Cadmium (Cd\(^{2+}\)) poisoning causes severe renal disorders manifested by defects in reabsorptive transport of various compounds. It is reported here that the renal brush-border membrane Na\(^+\)/glucose co-transporter-1 (SGLT1) is a molecular target for Cd\(^{2+}\) toxicity. In micromolar concentrations, Cd\(^{2+}\) acted as a noncompetitive, partial inhibitor of methyl-d-glucopyranoside uptake in vesicles from COS-7 cells transiently expressing SGLT1. In contrast, only a modest effect in the closely related Na\(^+\)/myo-inositol co-transporter-1 (SMIT1) was observed. The factor responsible for this difference was the CXXC motif (X can be any residue) at the cytoplasmic end of the eighth transmembrane segment (TM8) of SGLT1. Thus, a mutational transfer of this motif conveyed Cd\(^{2+}\) sensitivity to SMIT1. Moreover, mimicking the inhibitory effect of Cd\(^{2+}\), the biarsenical molecule FlAsH-EDT\(_2\) strongly inhibited the SGLT1 that had an engineered tetracysteine motif at the cytoplasmic end of TM8. The experiments also showed that covalent binding of the sulfhydryl reactive biotin-PEO-maleimide to the SGLT1 wild type but not to the mutant lacking the CXXC motif was suppressed by Cd\(^{2+}\). Taken together, these results suggest that in SGLT1, Cd\(^{2+}\) binding to the CXXC motif induces conformational changes that cause a partial inhibition of d-glucose transport.

C

hronic exposure to cadmium (Cd\(^{2+}\)) by ingestion of contaminated food and water or by inhalation of metal dust leads to accumulation of this metal in the kidney (1). Nephropathy in humans and experimental animals as a result of chronic exposure to Cd\(^{2+}\) is manifested by reabsorptive and secretory dysfunction of renal tubules. The principal symptoms of tubular damage (glucosuria, aminoaciduria, phosphaturia, hypercalciuria, and proteinuria) indicate that Cd\(^{2+}\) preferentially targets various transporters in the proximal tubular brush-border membrane (BBM) (2,3). Some Na\(^+\)-dependent BBM transporters, such as those for phosphate, glucose, and amino acids, were inhibited by inorganic Cd\(^{2+}\) in experimental animals that were treated with CdCl\(_2\) for 2 to 3 wk (4–6). Several studies have shown that the adverse effect of Cd\(^{2+}\) on BBM transporters is due both to the indirect action of Cd\(^{2+}\) on the cell-to-extracellular sodium gradient and to the reactive radicals that are able to selectively diminish the abundance of various transporters in epithelial cells (7–9). However, some of the effects of Cd\(^{2+}\) exposure on the renal proximal tubule, such as decreased uptake of nutrients by the BBM, closely resemble those associated with the direct inhibition of the activity of the secondary transporters (10,11), suggesting that the primary target for Cd\(^{2+}\) includes the renal transporters.

One of these transporters is the glucose transporter Na\(^+\)/glucose co-transporter-1 (SGLT1), a highly conserved, approximately 662-amino acid residue protein that couples sugar transport to the inward movement of Na\(^+\) across the plasma membrane (12). In the proximal tubule, SGLT1 is located at the apical membrane, where it catalyzes transport of glucose and thus is important for the maintenance of a constant blood glucose level (13). The SGLT1 belongs to a distinct gene family that at least includes Na\(^+\)-dependent plasma membrane carriers for glucose (SGLT1, SGLT2, and SGLT3), myo-inositol (SMIT1 and SMIT2), iodide, and multivitamin (14). It contains 14 transmembrane segments, and the N-terminal and C-terminal regions are involved in binding Na\(^+\), d-glucose and the nontransporter inhibitor phlorizin, respectively (15–18). A series of missense mutations in the SGLT1 coding sequence is responsible for the autosomal recessive glucose-galactose malabsorption, demonstrating the relevance of this protein to human pathophysiology (19). Despite this knowledge, it is yet unknown whether SGLT1 is a molecular target of Cd\(^{2+}\) and the site at which Cd\(^{2+}\) binds.

In this study, we compared the uptake of the glucose analog methyl-d-glucopyranoside (AMG) by SGLT1-containing vesicles with the uptake of myo-inositol (MI) by SMIT1-containing vesicles in the presence of various concentrations of Cd\(^{2+}\). For enabling Cd\(^{2+}\) to move across the membrane, vesicles from COS-7 cells expressing the two proteins were pretreated with
ETH-129, a lipophilic divalent metal ion carrier that can embed within the bilayer lipid membrane (20). We thereby demonstrated that by modulation of the pair of cysteine residues at the cytoplasmic end of the eighth transmembrane segment (TM8), Cd\(^{2+}\) noncompetitively inhibits the SGLT1.

**Materials and Methods**

**Chemicals**

Methyl-\(\alpha\)-d-[\(^{14}\)C]glucopyranoside (approximately 300 \(\mu\)Ci/\(\mu\)mol) was purchased from Amersham Biosciences (Uppsala, Sweden), and \(^{3}\)H]M1 (185 GBq/mmol) was obtained from Perkin Elmer Life Sciences (Norwalk, CT). FLASH-EDT\(_{2}\), biotin-PEO-maleimide, and avidin-peroxidase were the products of Pierce Chemical (Rockford, IL). All chemicals used were of analytical grade and purchased from commercial sources.

**DNA Constructs**

cDNA fragments encoding the full-length SGLT1 and SMIT1 were obtained by amplification of the rat kidney QUICK-Clone cDNA library (Clontech, Palo Alto, CA). The generated PCR fragments were digested with appropriate enzymes, cleaned with DNA cleaning kit (Qiagen, Studio City, CA), and subcloned in-frame into pCMV-Tag1 (Stratagene, La Jolla, CA) tagged with a FLAG epitope (DYKDDDDK) at the N-termini. The point mutations were generated by PCR-based mutagenesis using wild-type cDNA as template. The presence of each point mutation was verified by sequence analysis.

**Expression of Co-Transports in COS-7 Cells**

COS-7 cells were grown in DMEM (Invitrogen, San Diego, CA) that contained 0.5 units/ml penicillin and 100 \(\mu\)g/ml streptomycin and was supplemented with 10% FCS. On the second day of culture, the cells were transiently transfected with the empty vector or constructs encoding SGLT1 or SMIT1 proteins using the calcium phosphate method. Experiments were performed on the fourth day of culture.

**Membrane Preparation**

The cells were washed with an osmotically balanced medium KRH-choline (Krebs-Ringer-HEPES: 120 mM choline\(^{+}\), 5.6 mM KCl, 1.2 mM Mg\(_{2}\)Cl\(_{2}\), 1.2 mM CaCl\(_{2}\), and 20 mM HEPES-Tris [pH 7.5]). For membrane preparation, the cells were lysed and pressurized for 15 min at 900 psi of nitrogen in a Parr pressure homogenizer, and the undisrupted cells and nuclear debris were removed by centrifugation at 9000 \(\times\)g for 10 min at 22°C. The membrane preparations were highly reproducible.

**Sulfhydryl Modification**

Biotin-PEO-maleimide (BPM; 0.1 mM) was added to vesicle suspension (approximately 200 \(\mu\)g of protein) and incubated for 10 min at 25°C. The reaction was stopped by incubation with 10 mM cysteinine-HCl. After labeling, vesicles were washed three times and then solubilized with TBS buffer (25 mM Tris-HCl and 100 mM NaCl [pH 7.5]) that contained 1% Triton X-100. Triton solubilized proteins were added to ANTI-FLAG M2–coated 96-well plates (Sigma, St. Louis, MO) and incubated at room temperature for 90 min. The plates then were washed three times with TBS and blocked with in TBS 500 (100 mM Tris, 500 mM NaCl, and 0.1% Tween 20 [pH 7.5]) that contained 2.5% BSA. After 1 h at 37°C, the wells were washed four times with TBS 500 and once with CBST 500 (20 mM sodium citrate, 500 mM NaCl, and 0.1% Tween 20 [pH 5.5]), then blocked with in CBST 500 2.5% BSA for 1 h at room temperature. Wells then were incubated with avidin-peroxidase (1:4000 diluted with 2% BSA) at room temperature. After 30 min, plates were washed four times with CBST 500 and once with TBS 200 (100 mM Tris-HCl, 200 mM NaCl, and 0.1% Tween 20 [pH 7.5]). Freshly prepared SIGMAFAST OPD (Sigma) was used to develop the plate, with color being read at 492 nm. The results given were representative of experiments that were repeated at least three times, with triplicate data obtained from each individual experiment. Each plate was set to zero on wells that contained vesicle proteins that had not been incubated with avidin-peroxidase. Several control wells that con-
tained all reagents but biotinylated proteins were used for detecting nonspecific binding. Nonspecific binding of the anti-FLAG to cellular proteins was subtracted from each value within an experiment.

**Fluorescence Measurement**

Fluorescence was measured in a volume of 300 μl that contained 30 μg of protein using a Hitachi 500 spectrophotometer. Emission spectra were recorded over the range 500 to 600 nm with a fixed excitation wavelength of 500 nm, averaging five scans. The spectral band pass of excitation and emission was 5 nm. Spectra were fully corrected for the wavelength response of the detection system.

**Results**

**Membrane Sidedness and Other Considerations**

Studies of factors that alter transmembrane transport in intact cells are always complicated by the fact that more than one of the proteins involved in the chain of events that compose the transport might be altered. We therefore chose to functionally study vesicles from COS-7 cells that respectively expressed the proteins SGLT1 and SMIT1. The presence of the recombinant transporters in the membrane vesicles was confirmed by Western blotting (data not shown). The ouabain-sensitive (Na<sup>+</sup>, K<sup>+</sup>)-ATPase activity, measured in the absence of Triton X-100 A (0.2% vol/vol), was approximately 20% of that in the presence of this detergent, suggesting that a large fraction of the isolated vesicles were sealed (approximately 80%). The determination of vesicle sidedness was performed by analysis of surface markers in the membrane preparations. The addition of Triton X-100 (0.2% vol/vol) to the vesicles increased activity of the ectomarker, alkaline phosphatase, 1.5 ± 0.2-fold above the level detectable in the absence of detergent. The amounts of sialic acid liberated by the neuraminidase treatment in the absence and presence of 0.2% (vol/vol) Triton X-100 were 35 ± 5 and 120 ± 16 nmol/mg protein, respectively. From these values, we estimate that 60 to 70% of the vesicles were RSO. Thus, the first task of this study was to minimize any potential complications arising from the fact that 30 to 40% of the vesicles remained oriented ISO.

In a recent study, Quick et al. (25) showed that the affinity of SGLT1 for AMG was approximately 350-fold higher in RSO than in ISO vesicles, and AMG uptake by RSO but not ISO vesicles was strongly inhibited by micromolar concentrations of the transport inhibitor phlorizin. With regard to the SMIT1, it was reported previously that phlorizin can completely block MI uptake by RSO vesicles (K<sub>i</sub> = 64 μM) (e.g., 26). Thus, we admitted simultaneously the radioactive MI tracer with phlorizin to the vesicles to characterize the reverse transport mode of SMIT1. Striking, the accumulation of [3H]MI into the isolated vesicles was not completely blocked by 0.5 M phlorizin (data not shown). In addition, we found that in the presence of phlorizin, vesicles expressing the SMIT1 exhibited >100-fold lower MI apparent binding affinity than that measured in the phlorizin-free buffer (37 ± 8 versus 0.15 ± 0.06 mM). Furthermore, the above phenomena were observed in the mutant SGLT1 and SMIT1 proteins. These results, together with the previous report that SGLT1 is a functional asymmetrical molecule (25), suggest that the reverse transport reactions of the two transporters are insensitive to inhibition by phlorizin and that the contribution of AMG or MI uptake by the ISO fraction to the total uptake is small. Hence, in the subsequent experiments, in vitro transport assays were performed in both the phlorizin-containing (0.5 M) and the phlorizin-free media to separate sugar uptake by RSO and ISO vesicles. As the sugar transport proteins studied here are kinetically asymmetrical, we calculated the amount of AMG and MI influxes into RSO vesicles by subtracting the phlorizin-uninhibitable fraction from the total uptake.

**Cd<sup>2+</sup> Inhibits SGLT1-Mediated AMG Uptake**

Application of Cd<sup>2+</sup> (100 μM) for 10 min to vesicles that expressed SGLT1 slightly inhibited AMG influx in RSO vesicles (approximately 15% inhibition; Figure 1A). However, inspection of the published data revealed that 100 μM Cd<sup>2+</sup> could inhibit AMG uptake in BBM vesicles by approximately 60% (27). One possible reason for the discrepancy may lie in the incapability of vesicles derived from COS-7 cells to take up Cd<sup>2+</sup> efficiently. We therefore treated the vesicles with 5 μM ETH-129, a lipophilic metal ion transporter that allows Cd<sup>2+</sup> to move across the membrane (20), for 10 min before initiating transport assays. Figure 1A shows that in the presence of ETH-129, Cd<sup>2+</sup> suppressed SGLT1 in a concentration-dependent manner, and a 10-min preincubation with 100 μM CdCl<sub>2</sub> gave 40% further inhibition of AMG influx into RSO vesicles from COS-7 cells expressing the SGLT1. This could be due to binding of Cd<sup>2+</sup> to either a cytoplasmic site of SGLT1 or the inner membrane. To address this issue, we compared the ability of Cd<sup>2+</sup> to inhibit MI influx in the presence and absence of ETH-129. If the inhibitory effect of Cd<sup>2+</sup> relies on its interaction with the inner membrane, then Cd<sup>2+</sup> should decrease the activity of SMIT1 by approximately 50% when vesicles are treated with ETH-129. However, whether ETH-129 was present or not, MI influx into RSO vesicles expressing SMIT1 was not much affected by 1 to 100 μM Cd<sup>2+</sup> (Figure 1B). It seems that the cytoplasmic part of SGLT1 possesses an endogenous site that is sensitive to Cd<sup>2+</sup> inhibition.

The mechanism by which Cd<sup>2+</sup> inhibits SGLT1 was explored by measuring the dependence of AMG influx into ETH-129–embedded vesicles on the concentration of AMG in the presence and absence of CdCl<sub>2</sub> (Figure 2A). Analysis of the transport data revealed that Cd<sup>2+</sup> caused a dramatic decrease in V<sub>max</sub> with no significant change in the K<sub>m</sub> value. In the presence of 100 μM CdCl<sub>2</sub>, the V<sub>max</sub> value was approximately 52% of the control level (Figure 2B). These results suggest that Cd<sup>2+</sup> inhibits AMG transport by specifically inhibiting the translocation process, without affecting AMG binding. Thus, Cd<sup>2+</sup> may be thought of as a noncompetitive, partial inhibitor of SGLT1.

**Identification of the Molecular Target for Cd<sup>2+</sup> Inhibition in SGLT1**

In the following experiments, we used site-directed mutagenesis to identify amino acids that may form the Cd<sup>2+</sup> binding site in SGLT1 and also investigated the differential Cd<sup>2+</sup> sensitivity of SGLT1 compared with SMIT1. Chemically, Cd<sup>2+</sup> reacts with sulfhydryl groups and exhibits high affinity to vicinal thiols.
Thus, the adverse effects of Cd\(^{2+}\) on biologic systems may be caused by its reaction with closely spaced cysteine residues on critical cellular proteins (e.g., 28,29). Most Na\(^{+}\)/glucose co-transporters have vicinal cysteines located at the cytoplasmic end of TM8 (Figure 3A). In rat SGLT1, this sequence is...; however, the CXXC motif is absent in rat SMIT1.

To investigate whether this pair of cysteine residues served as a Cd\(^{2+}\) sensor, we replaced the CXXC motif in SGLT1 with VXXA (Cys → Val at position 314 and Cys → Ala at position 317). Wild-type and C314V/C317A SGLT1 in isolated vesicles had a similar apparent binding affinity for AMG (data not shown), suggesting that Cys-314 and Cys-317 do not play any structurally important role through disulfide bonding. Strikingly, co-application of 100 μM CdCl\(_2\) and 5 μM ETH-129 (10-min incubation) only slightly inhibited AMG influx across RSO vesicles expressing SGLT1-C314V/C317A (Figure 4), indicating a predominant role of the CXXC motif in Cd\(^{2+}\) sensitivity. To gain some further insight into the role of the CXXC motif in Cd\(^{2+}\) inhibition, we replaced two vicinal residues in SMIT1,
present at the cytoplasmic end of TM8, with cysteine (Thr → Cys at position 299 and Ala → Cys at position 302). The RSO vesicles expressing the T299C/A302C SMIT1 displayed far less MI uptake compared with the wild-type transporter when both Cd2⁺/H11001 and ETH-129 were present (Figure 5). This suggests that introducing the CXXC motif in SGLT1 to the corresponding position in SMIT1 (SMIT1-T299C/A302C) results in transfer to SMIT1 of the Cd2⁺/H11001 sensitivity with respect to inhibition of MI uptake. Thus, it seems likely that we had generated a Cd2⁺-binding site in SMIT1.

To investigate whether other intracellular SH groups of SGLT1 are involved in Cd2⁺ binding, we further examined the effect of dithiolthreitol (DTT) on Cd2⁺ inhibition (Figure 6). In these experiments, three 5-min incubations in MHT buffer were used (first, with or without DTT; second, with Cd2⁺; third, without or with DTT). The Cd2⁺ effect was almost completely blocked when DTT was added to the wild-type SGLT1-containing vesicles either before or after Cd2⁺ preincubation. However, in RSO vesicles that expressed the C314V/C317A mutant, these treatments did not affect Na⁺-stimulated AMG influx at all (data not shown). In control experiments, we observed that three incubations in the transport buffer with DTT (with DTT, with Na⁺, and then with DTT) had no effect on the wild-type transporter-mediated AMG uptake. The data suggest that the thiol compound DTT inhibits the
formation of $\text{Cd}^{2+}$-SH bonds by directly interacting with SH radicals and that cysteines other than the pair within TM8 are not the main target for $\text{Cd}^{2+}$ toxicity.

In addition, we investigated the mechanism underlying $\text{Cd}^{2+}$ inhibition of SMIT1-T299C/A302C by performing MI transport assay in the presence and absence of $\text{Cd}^{2+}$. MI influx into SMIT1-containing vesicles responds to $\text{Cd}^{2+}$ in a similar way to that observed in SGLT1-containing vesicles: A significant reduction of $V_{\text{max}}$ and an unchanged $K_{m}$ value for MI (data not shown).

**Engineering a Diarsenical Ligand Binding Site in SGLT1**

To investigate further the sensitivity of SGLT1 to heavy metals, we next engineered an artificial diarsenical ligand binding site at the cytoplasmic end of TM8. Proteins that contain four cysteines at the $i, i+1, i+4,$ and $i+5$ positions of an $\alpha$-helix can specifically interact with the diarsenical reagent FIAsh-EDT$_2$, in which fluorescence is quenched until its arsenic substitute binds vicinal thiols in a polypeptide target (30). Assuming that the CXXC motif in SGLT1 is situated in an $\alpha$-helix environment, a FIAsh-EDT$_2$ binding site would be expected to be generated by mutating Gly313 ($i+1$) and Gly318 ($i+5$) to cysteine (Figure 3B). As illustrated in Figure 7A, the addition of membrane proteins from COS-7 cells expressing SGLT1-G313C/G318C but not SGLT1-C314V/C317A to a FIAsh-EDT$_2$ solution caused a dramatic increase in fluorescence emission, indicating that a specific interaction between the diarsenical ligand and the tetracysteine motif in G313C/G318C occurred. Figure 7B shows that, when the membrane-permeable FIAsh-EDT$_2$ (40 $\mu$M) was included in the uptake buffer, AMG influx into RSO vesicles expressing the G313C/G318C SGLT1 was dramatically decreased (approximately 35% reduction), whereas SGLT1-C314V/C317A-mediated AMG uptake was not so much affected. These data provide indirect evidence that $\text{Cd}^{2+}$ exerts its inhibitory effect on SGLT1 through binding to the CXXC motif.

$\text{Cd}^{2+}$ Induces a Conformational Change in SGLT1

The reactivity of a target cysteine depends on its location with respect to the face of the protein on which the hydrophilic
cysteine-reactive reagent is presented and on steric hindrance in the activated complex between the cysteine thiolate and the reagent. Here we used a cysteine-accessibility method to investigate the effect Cd\(^{2+}\) binding on the conformation of SGLT1. Biotin-avidin ELISA evidenced that in vesicles that were pretreated with ETH-129, Cd\(^{2+}\) (100 μM) decreased the reactivity of the sulfhydryl groups of the SGLT1 wild type but not the C314V/C317A mutant to BPM by approximately 50% (Figure 8). Application of the vesicles with ETH-129 alone had no significant effect on the sulfhydryl modification. In addition, without an incubation of the vesicles with ETH-129, such a decrease was not observed. Together, they suggest SGLT1 undergoes a conformational change upon Cd\(^{2+}\) binding.

**Discussion**

Because the kidney is one of the primary organs involved in excretion of metals such as Cd\(^{2+}\), mercury, and lead, it is also a site for heavy metal intoxication. However, to date, few studies have examined the interaction between heavy metals and specific renal transporters. This is the first study that has combined biochemical and molecular biology approaches to examine the interaction of Cd\(^{2+}\) with the proximal tubular brush border Na\(^{+}\)/glucose co-transporter SGLT1.

In this article, we provide evidence that SGLT1 is a primary target of Cd\(^{2+}\). First, we show that in RSO vesicles from transfected COS-7 cells, SGLT1 was more sensitive than SMIT1 to inhibition by Cd\(^{2+}\). The differential Cd\(^{2+}\) sensitivity of SGLT1 and SMIT1 seems to be attributed, at least in part, to the direct inhibitory effect of SGLT1. The second argument supporting a direct SGLT1–Cd\(^{2+}\) interaction is based on the finding that SGLT1-C314V/C317A is less sensitive to Cd\(^{2+}\). Finally, we demonstrate that the inhibitory effect of Cd\(^{2+}\) on SGLT1 can be reversed by the presence of DTT, a thiol compound that sequesters metal ions from the sulfhydryls, indicative of an existence of Cd\(^{2+}\)-SH bonds in the transporter. Inspection of the data presented in Figures 1 through 3 also revealed that at a concentration of 100 μM, “extravesical” Cd\(^{2+}\) has a similar inhibitory effect (10 to 15% inhibition) on the transport capacity of both the RSO-orientated, SGLT1- and SMIT1-containing vesicles. The binding of Cd\(^{2+}\) to the membrane might account for the slight decrease in the V\(_{\text{max}}\) of the two Na\(^{+}\)-dependent transporters. However, we also found that allowing Cd\(^{2+}\) to enter the intracellular compartment did not double its inhibitory effect (Figures 1 through 3). It seems likely that under our experimental conditions, the amount of Cd\(^{2+}\) accumulated in the interior of ETH-129–treated vesicles is too low to disrupt the membrane.

To attain a better understanding of the structural basis for the mechanism of action of Cd\(^{2+}\) on Na\(^{+}\)/glucose co-transporters, we studied the Cd\(^{2+}\) binding site in SGLT1 using a site-directed mutagenesis strategy. Taking advantage of the differential Cd\(^{2+}\) sensitivity of SMIT1 and SGLT1, we identified a pair of cysteines on the intracellular end of TM8, which, when mutated, greatly reduced Cd\(^{2+}\) affinity without affecting AMG binding, suggesting that these residues represent the molecular determinant for Cd\(^{2+}\) inhibition. In this study, we also mimicked the inhibitory effect of Cd\(^{2+}\) on SGLT1 by showing that the activity of the G314C/G319C mutant could be strongly inhibited by FlAsH-EDT\(_2\), a biaxial ligand that specifically binds to the tetracysteine motif. Surprising, even Cd\(^{2+}\) concentrations (100 μM) that would be expected to saturate this CXXC motif cause only 40 to 50% inhibition of AMG uptake. One explanation could be that the transporter can still transport substrates even when Cd\(^{2+}\) is bound, but only at reduced efficacy. Alternatively, it may reflect the existence of two conformational states of the transporter with distinct Cd\(^{2+}\) sensitivity. We cannot distinguish between the two hypotheses; however, they both are consistent with the major implication from this study that Cd\(^{2+}\) bound to the CXXC motif acts as a noncompetitive inhibitor of AMG translocation.

It is assumed that transporters operate by alternating their access mechanism, where the transporter interchanges between an “outward facing” conformation, in which the substrate binding is accessible to extracellular medium, and an “inward facing” conformation, in which the binding is accessible to the intracellular environment (31). Thus, it is interesting to consider the possibility that CXXC motif is located in an intracellular “gating” domain and that Cd\(^{2+}\) binding probably stabilizes the transporter in the outward configuration, allowing AMG binding but inhibiting its translocation. In this scenario, it can be envisaged that removing the two cysteines restores the conformational equilibrium of the transport cycle that is restrained by Cd\(^{2+}\).
binding. Similarly, introducing a CXXC motif transfers the ability of Cd\(^{2+}\) to break down the conformational equilibrium of the transport cycle of SMIT1. A changed conformational equilibrium of SGLT1 is strongly supported by the substantial decrease in the accessibility of BPM to the sulfhydril groups of SGLT1 in the presence of Cd\(^{2+}\). However, in this study, we were unable to distinguish whether Cd\(^{2+}\) affects the covalent binding of BPM to the extracellular side or the cytoplasmic side of SGLT1, as vesicles isolated from COS-7 cells have a mixture of orientations. We were also unable to determine whether the Cd\(^{2+}\) binding introduces a positive charge into a crucial site that may provide an electrostatic repulsion for the SGLT1-mediated transport process, because replacement of the two vicinal cysteine residues with lysine abolished the expression of the protein in COS-7 cells (data not shown). Thus far, more work is required to clarify the precise mechanism involved in the inhibition of SGLT1 induced by Cd\(^{2+}\) binding.

In summary, we have proved for the first time that the renal SGLT1 can be noncompetitively inhibited by Cd\(^{2+}\) and that the CXXC motif at the cytoplasmic end of TM8 determines the sensitivity of SGLT1 to Cd\(^{2+}\). Thus, some of the effects of Cd\(^{2+}\) exposure on reabsorption of nutrients from the renal proximal tubule are partially due to the dysfunction of co-transporters.

Acknowledgments
We express sincere thanks to Prof. R.H.K. Kinne for granting X.X. access to the Max Planck Institute for Molecular Physiology and providing him opportunities to gain an insight into the complex field of routing and regulation of renal transporters.

References


Correction


In Results, found on page 1259, the correct amounts of sialic acid liberation in the absence and presence of Triton X-100 was 85 ± 5 nmol/mg protein. The authors regret this error.

Correction to October Highlights


GLomerulosclerosis—Can Proteomics Help Us Understand It Better?

We currently identify sclerotic glomeruli only by light and electron microscopy, but clearly there must be more subtle changes that precede these. Others have searched for such changes with molecular techniques such as DNA arrays with limited success. Xu *et al.* have employed a newer proteomic methodology to compare sclerotic to nonsclerotic and normal glomeruli in the remnant kidney model of secondary focal sclerosis. Not surprisingly, the sclerotic glomeruli differed substantially from normal ones. But of more interest, the nonsclerotic glomeruli in remnant kidneys were more like sclerotic than normal glomeruli, thus identifying early changes that likely predict, and may even cause, the development of sclerosis. A clue is provided by further studies of thymosin β4, one of the upregulated proteins, that itself exerts a profibrotic effect by mediating angiotensin induction of PAI-1, which impairs breakdown of fibrin and extracellular matrix. These studies are important because they not only show the feasibility of applying proteomics to the detection and study of glomerular scarring, but also yield a new insight into what the mechanisms involved may be. *See Wu et al., pages 2967–2873.*