Regulated transport of sulfate and oxalate by SLC26A2/DTDST

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Henehan JF, Akhavein A, Salas MJ, Shmukler BE, Karniski LP, Vandorpe DH, Alper SL. Regulated transport of sulfate and oxalate by SLC26A2/DTDST. Am J Physiol Cell Physiol 298: C1363–C1375, 2010. First Published March 10, 2010; doi:10.1152/ajpcell.00004.2010.—Nephrolithiasis in the Slc26a6−/− mouse is accompanied by 50–75% reduction in intestinal oxalate secretion with unchanged intestinal oxalate absorption. The molecular identities of enterocyte pathways for oxalate absorption and for Slc26a6-independent oxalate secretion remain undefined. The reported intestinal expression of SO42−/cell was at wild-type levels. Surface abundance was at wild-type levels.

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We found that SLC26A2 exchanges oxalate with trans-
$SO_4^{2-}$, Cl$^-$, or oxalate itself, in addition to mediating $SO_4^{2-}$ / 
Cl$^-$ and Cl$^-$/Ca$^{2+}$ exchange. The two species orthologs exhibit
similar anion transport affinities, share nonsaturating extracel-
lar Cl$^-$ dependence, and exhibit low rates of $^{36}$Cl$^-$ efflux
except as Cl$^-$/Ca$^{2+}$ self-exchange. hSLC26A2 is differentially regul-
ated by extracellular pH (pHs) and intracellular pH (pHi).
hSLC26A2 is inhibited by protein kinase C (PKC), likely
through regulation of surface abundance. Two diastrophic
dysplasia mutants of hSLC26A2 exhibit parallel reductions in
transport of sulfate and oxalate but differ in impairment of
surface expression.

**METHODS**

Materials. Na$^{35}$Cl, Na$_2^{35}$SO$_4^{2-}$, and [1$^{4}$C]butyrate were from ICN
(Irvine, CA). [1$^{4}$C]oxalate originally from NEN-DuPont was the
generous gift of C. Scheid and T. Honeymay (Univ. of Massachusetts
Medical Center). Restriction enzymes and T4 DNA ligase were from
New England Biolabs (Beverly, MA). EXPAND High-fidelity
PCR System was from Roche Diagnostics (Indianapolis, IN). 4$^{4}$-
Diothiocyanostilbene-2,2$^-$-disulfonic acid (DIDS) was from Calbio-
chem (La Jolla, CA). 4$^{4}$-Dinitrostilbene-2,2$^-$-disulfonic acid (DNDS)
was from Pfalz and Bauer (Waterbury, CT). 4$^-$Phorbol-12-myristate-
13-acetate (PMA) and 4$^-$phorbol-didecanoate (4$^-$PDD) were from LC
Laboratories (Woburn, MA). All other chemical reagents were from
Sigma (St. Louis, MO) or Fluka (Milwaukee, WI) and were of reagent
grade.

Solutions. MBS consisted of (in mM) 85 NaCl, 1 KCl, 2.4
NaHCO$_3$, 0.82 Mg(SO$_4$)$_2$, 0.33 Ca(NO$_3$)$_2$, 0.41 CaCl$_2$, and 10 HEPEs
(pH adjusted to 7.40 with NaOH). ND-96 (pH 7.40) consisted of (in
mM) 96 NaCl, 2 KCl, 1.8 CaCl$_2$, 1 MgCl$_2$, and 5 HEPEs. In Cl$^-$/free
ND-96 or partial Cl$^-$ substitution solutions, NaCl was replaced
mole-for-mole with Na cyclamate or, in the case of two-electrode
voltage-clamp experiments, Na gluconate. In some experiments, 96
mM NaCl was replaced with 64 mM Na$_2^{35}$SO$_4^{2-}$. As needed, the Cl
salts of K$^+$, Ca$^{2+}$, and Mg$^{2+}$ were substituted on an equimolar basis
with the corresponding gluconate salts. Na oxalate-containing bath
solutions were nominally Ca$^{2+}$ free. Addition to flux media of the
weak acid salt sodium butyrate (40 mM) was in equimolux substitution
for Na cyclamate. Bath addition of NH$_4$Cl (20 mM) was in equimolux substitution
for NaCl. pH 5.0 ND/96 solutions were buffered with 5
mM MES. (Occasional use of 5 mM HEPEs during the first 4-min
exposures to pH 5.0 was without bath pH drift and without change in
results.)

Construction and mutagenesis of cDNA expression plasmids. Hu-
man SLC26A2/DTDST (hSLC26A2) cDNA in a modified pGEM
vector as described (29) was subcloned into the Xenopus oocyte
expression vector pXT7. Mouse Slc26a2 (mScl26a2) cDNA in
pCMV-SPORT6 (clone ID 4014785; BC028345) was obtained from
Open Biosystems (Huntsville, AL). The cDNA plasmid encoding a
COOH-terminal green fluorescent protein (GFP)-fusion hSLC26A2-
GFP (gift of J. Schwartzbauer, Princeton University) was subcloned
into pXT7.

hSLC26A2 mutations R297W and A386V were generated by four
primer polymerase chain reaction (PCR) as described (8) with specific
mutagenic oligonucleotides and flanking oligonucleotides hA2-F and
hA2-R (see supplemental Table 1 online at the AJP-Cell Physiology
website). All PCR products were sequenced in entirety in both strands
to ensure absence of unintended mutations.

Expression of cRNAs in Xenopus oocytes. Capped cRNA was
transcribed from linearized cDNA templates with T7 or SP6 RNA
polymerase (Ambion, Austin, TX) and purified with an RNasey
mini-kit (Qiagen). cRNA concentration ($A_{260}$) was measured by
Nanodrop spectrometer (ThermoFisher), and integrity was confirmed
by formaldehyde agarose gel electrophoresis. Mature female Xenopus
(Dept. of Systems Biology, Harvard Medical School; or NASCO,
Madison, WI) were maintained and subjected to partial ovariectomy
under hypothermic tricaine anesthesia following protocols approved
by the Institutional Animal Care and Use Committee of Beth Israel
Deaconess Medical Center. Stage V-VI oocytes were prepared by
overnight incubation of ovarian fragments in MBS with 1.3 mg/ml
collagenase B (Roche, Indianapolis, IN), followed by a 20-min rinse
in Ca$^{2+}$/free MBS with subsequent manual section and defollicula-
tion as needed. Oocytes were injected on the same day with cRNA
(0.5–50 ng) or with water in a volume of 50 nl. Injected and
uninjected oocytes were then maintained before use for 2–6 days at
19°C in MBS containing gentamicin. Injection of increasing amounts
of hSLC26A2-GFP cDNA led to corresponding increases in GFP
fluorescence intensity at or near the oocyte surface (see supplemental
Fig. 1 online at the AJP-Cell Physiology website). Injection of
increasing amounts of untagged hSLC26A2 also led to corresponding
increases in transporter polypeptide at or near the oocyte surface, as
detected by confocal immunofluorescence microscopy (see supple-
mental Fig. 2 at the AJP-Cell Physiology website).

$^{35}$SO$_4^{2-}$/H$^{110}$O influx studies were carried out for periods of 15 or 30
min in a bath containing (in mM) 1 Na$_2$SO$_4$, 94.5 Na cyclamate, 2 K glutamate, 1.8 Ca glutamate,
1 Mg glutamate, and 5 HEPEs (2 $\mu$Ci/150 $\mu$l in a microtiter plate
well) (6). $^{35}$SO$_4^{2-}$/ uptake increased as a function of the amount of
cRNA previously injected (supplemental Fig. 1). $^{36}$Cl$^-$ influx studies
were carried out for periods of 15 or 30 min in ND-96 as previously
described (7). Total bath [Cl$^-$] was 103.6 mM (0.5 $\mu$Ci/mM).

$^{35}$Cl$^-$oxalate influx studies were carried out for 30-min periods in
nominally Ca$^{2+}$/ and Mg$^{2+}$/free influx medium containing (in
mM) 96 mM Na cyclamate, 2 K glutamate, 5 HEPEs, pH 7.40, with added
1.0 mM sodium oxalate (0.375 $\mu$Ci/mM; 150 $\mu$l), or the indicated
oxalate concentrations with balancing cyclamate.

Influx experiments were terminated with four washes in Cl$^-$/free
ND96, followed by oocyte lysis in 150 $\mu$l of 2% dodecyl
sulfate (SDS). Duplicate 10-$\mu$l aliquots of influx solution were used
to calculate specific activity of radiolabeled substrate anions. Oocyte
anion uptake was calculated from oocyte-associated counts per
minute (cpm) and bath specific activity.

For unidirectional $^{36}$Cl$^-$ efflux studies individual oocytes in Cl$^-$/free
ND-96 were injected with 50 nl of 260
mM Na$^{35}$Cl (20,000–24,000 cpm). After a 5- to 10-min recovery
period in Cl$^-$/free ND-96, the efflux assay was initiated by transfer of
individual oocytes to 6-mL borosilicate glass tubes, each containing 1
ml efflux solution. At intervals of 1 or 3 min, 0.95 ml of this efflux
solution was removed for scintillation counting and replaced with an
equal volume of fresh efflux solution. After completion of the assay
with a final efflux period either in Cl$^-$/free cyclamate solution or in
the presence of the inhibitor DIDS (100 or 200 $\mu$m), each oocyte was
lysed in 150 $\mu$l of 2% SDS. Samples were counted for 3–5 min such
that the magnitude of 2SD was $<5%$ of the sample mean.

For $^{[14]}$Coxalate efflux assays, oocytes were injected with 50 nl of
50 mM Na$^{[14]}$Coxalate (6,000–8,000 cpm, with final estimated
intracellular concentration 5 mM). After a recovery period of at least
20 min, efflux was measured in baths containing up to 103.6 mM
NaCl or 64 mM Na$_2$SO$_4$, or as indicated. In baths of reduced [Cl$^-$] or
[SO$_4^{2-}$], the respective anions were replaced with equimolar cycla-
mate. Bath anion concentration-response curves were generated from
individual oocytes subjected sequentially to increasing concentrations
of the bath anion under study. Single oocytes were exposed to a
maximum of six conditions.

To vary pHi, oocytes were preexposed to 40 mM Na butyrate
(substituting for Na cyclamate) for 30 min before initiation of an
efflux experiment to produce intracellular acidification to pH 6.8.
Upon removal of bath butyrate (with substitution by Na cyclamate)
during the efflux experiment, pH rapidly alkalized back toward
initial pH$_i$ while pH$_e$ remained constant. Variation of pH$_e$ was
achieved at near-constant pH$_i$ (39). Some oocyte groups were

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exposed to 20 mM NH4Cl during the efflux experiments, acidifying pH, to 6.9 (26). Drugs were added to the bath or were injected into oocytes either before or together with an isolate as indicated.

Efflux data were plotted as the natural logarithm (ln) of the quantity (%cpm remaining in the oocyte) versus time. Efflux rate constants for 35SO4<sup>2-</sup>, 36Cl<sup>-</sup>, and [14C]oxalate were measured from linear fits to data from the last three time points sampled within each experimental period. For each experiment, water-injected or un.injected oocytes from the same frog were subjected to parallel measurements with cRNA-injected oocytes. Each experimental condition was tested in oocytes from at least two frogs. Two exclusion criteria were defined to categorize efflux experiments as reflecting "nonspecific" efflux or "leaky" oocytes. One criterion was ≤50% reduction in efflux rate constant upon final addition of the inhibitor DIDS or upon final bath substitution of exchangeable substrate anion for the impermeant anion, cyclamate. The second criterion was loss of >85% of injected isolate before completion of the efflux assay.

Application of these a priori criteria resulted in exclusion from analysis of 11% of 17636Cl<sup>-</sup>, 36Cl<sup>-</sup>, and [14C]oxalate were measured from linear fits to data from the last three time points sampled within each experimental period. For each experiment, water-injected or uninjected oocytes were blocked in PBS with 1% bovine serum albumin (PBS-BSA). Oocytes were incubated overnight at 4°C with a substitution of exchangeable substrate anion for the impermeant "leaky" oocytes. One criterion was 50% reduction in efflux rate constant upon final addition of the inhibitor DIDS or upon final bath substitution of exchangeable substrate anion for the impermeant anion, cyclamate.

**Data acquisition and analysis** utilized pCLAMP 8.0 software (Axon). The voltage pulse protocol generated with the Clampex subroutine consisted of 20-mV steps between −100 mV and +40 mV, with durations of 738 ms separated by 30 ms at the holding potential of −30 mV. Bath resistance was minimized by the use of agar bridges filled with 3 M KCl, and a virtual ground circuit clamped bath potential to zero during voltage-clamp experiments.

Confocal immunofluorescence microscopy. Two days after injection with H2O or with cRNA encoding COOH terminally GFP-tagged hSLC26A2, 10–12 oocytes were fixed in 3% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) for 30 min at room temperature, washed three times in PBS supplemented with 0.002% Na azide (PBS-azide), and saved at 4°C for direct imaging of GFP. Oocytes were aligned in uniform orientation along a Plexiglas groove and sequentially imaged through the ×10 objective of a Zeiss LSM510 laser scanning confocal microscope using the 488-nm laser line at 512 × 512 resolution at a uniform setting of 80% intensity, pinhole 61 μm (1.0 Airy units), detector gain 650, Amp gain 1, 0 amp offset.

Two or three days after injection with H2O or with cRNA encoding wild-type or mutant untagged hSLC26A2, groups of 10–12 oocytes were similarly fixed with 3% PFA and washed with PBS-azide. Oocytes were then placed in PBS containing 1% SDS to permeabilize the surface membranes and unmask epitope. Fixed, permeabilized oocytes were blocked in PBS with 1% bovine serum albumin (PBS-azide), and saved at 4°C for direct imaging of GFP. Oocytes were incubated overnight at 4°C with mouse monoclonal anti-hSLC26A2 antibody 1-G2 at 1:400 dilution in PBS-BSA (28) and then washed three times in cold PBS-BSA.

Fig. 1. *Cis*-inhibition of SLC26A2-mediated SO4<sup>2-</sup> uptake by extracellular anions. A: extracellular [Cl<sup>-</sup>] dependence for *cis*-inhibition of 35SO4<sup>2-</sup> uptake by hSLC26A2. K<sub>i,cl</sub> = 24.1 ± 3.8 mM Cl<sup>-</sup> (R<sup>2</sup> = 0.90, n = 12–14). B: extracellular [oxalate] dependence for *cis*-inhibition of 35SO4<sup>2-</sup> uptake by hSLC26A2. K<sub>i,ox</sub> = 5.1 ± 1.7 mM oxalate (R<sup>2</sup> = 0.86, n = 7–9). C: extracellular [Cl<sup>-</sup>] dependence for *cis*-inhibition of 35SO4<sup>2-</sup> uptake by mSlc26a2. K<sub>i,cl</sub> = 18.4 ± 3.4 mM Cl<sup>-</sup> (R<sup>2</sup> = 0.93, n = 6–7). D: *cis*-inhibition of mSlc26a2-mediated 35SO4<sup>2-</sup> uptake by oxalate or HCO3<sup>-</sup> at the indicated concentrations for (n) oocytes compared with complete inhibition by DIDS (200 μM). Oocytes previously injected with 50 ng cRNA or water were subjected to 30 min uptake in the presence of 1 mM extracellular SO4<sup>2-</sup>. Uptake data were normalized to same-day experiments carried out in cyclamate. Solid bar indicates water-injected oocytes. Normalized uninhibited SO4<sup>2-</sup> uptake was (in nmol/oocyte·h<sup>-1</sup>) 1.42 ± 0.05 (A), 1.48 ± 0.07 (B), 1.13 ± 0.05 (C), and 1.03 ± 0.19 (D). Values are means ± SE. **P < 0.01.
Antibody-labeled oocytes were then incubated 2 h with Cy3-conjugated secondary goat anti-mouse Ig (dilution 1:500, Jackson Immunochemicals), again thoroughly washed in PBS-BSA, and stored at 4°C until imaging.

Cy3-labeled oocytes were aligned in uniform orientation along a Plexiglas groove and sequentially imaged through the /H11003\ 10 objective of a Zeiss LSM510 laser scanning confocal microscope by using the 543-nm laser line at 512 \times 512 resolution at a uniform setting of 80% intensity, pinhole 54 (1.0 Airy units), detector gain 650, Amp gain 1, 0 amp offset.

Polypeptide abundance at or near each oocyte surface was estimated by quantitation of specific fluorescence intensity (FI) at the circumference of one quadrant of an equatorial focal plane (Image J v. 1.38, National Institutes of Health). Mean background-corrected FI

Fig. 2. hSLC26A2 mediates bidirectional oxalate/SO\(_4^{2-}\) exchange. A: bath [oxalate] dependence of 30 min oxalate uptake by oocytes expressing hSLC26A2. \(K_{1/2} = 0.65 \pm 0.08\) mM oxalate, Hill coefficient = 2.01 \pm 0.41 (\(R^2 = 0.66, n = 19–20\)). Normalized oxalate uptake = 1.0 represented 0.15 nmol/h. B: bath [SO\(_4^{2-}\)] dependence for cis-inhibition of 1 mM bath oxalate uptake by oocytes expressing hSLC26A2. \(K_{1/2} = 3.07 \pm 0.74\) mM sulfate (\(R^2 = 0.72, n = 7–17\)). C: traces of \(^{35}\)SO\(_4^{2-}\) efflux from individual oocytes previously injected with water (open squares) or hSLC26A2 (filled circles) during sequential exposure to Cl- free cyclamate baths in the initial presence and subsequent absence of 25 mM oxalate. D: traces of \(^{14}\)Coxalate efflux from individual oocytes previously injected with water (open squares) or hSLC26A2 (filled circles) during sequential exposure to Cl- free baths in the initial presence and subsequent absence of 64 mM SO\(_4^{2-}\). Oocytes previously injected with 50 ng cRNA or water were assayed 4 days postinjection. Values are means \(\pm\) SE.

Fig. 3. [Substrate] dependence of SLC26A2-mediated oxalate/SO\(_4^{2-}\) exchange. A: bath [oxalate] dependence of hSLC26A2-mediated \(^{35}\)SO\(_4^{2-}\) efflux. \(K_{1/2} = 1.58 \pm 0.44\) mM oxalate (n = 4–5). B: bath [SO\(_4^{2-}\)] dependence of hSLC26A2-mediated oxalate efflux. \(K_{1/2} = 0.14 \pm 0.05\) mM SO\(_4^{2-}\) (n = 5–22). C: bath [oxalate] dependence of mSlc26a2-mediated \(^{35}\)SO\(_4^{2-}\) efflux. \(K_{1/2} = 3.74 \pm 0.73\) mM oxalate (n = 3–15). D: bath [SO\(_4^{2-}\)] dependence of mSlc26a2-mediated oxalate efflux. \(K_{1/2} = 0.20 \pm 0.08\) mM SO\(_4^{2-}\) (n = 4–20). Oocytes previously injected with 50 ng cRNA were assayed 3–4 days postinjection. Values are means \(\pm\) SE. Curve fits in B and D were unchanged by inclusion of additional \(^{14}\)Coxalate efflux rate constants measured at higher bath [SO\(_4^{2-}\)] values of 8 and 9 mM (n = 5–6, data not shown). There was no sulfate self-inhibition at still higher bath [SO\(_4^{2-}\)] values of 16, 32, or 64 mM (not shown).
for quadrants of oocytes previously injected with water was subtracted from the background-corrected FI for quadrants of individual cRNA-injected oocytes to yield intensity values for surface-associated specific FI for each oocyte.

Statistics. Data are reported as means ± SE. Flux data were compared by Student’s paired or unpaired two-tailed t tests (Microsoft Excel), or by ANOVA with Bonferroni post hoc analysis (SigmaPlot). Concentration-dependence data were fit to a four-parameter Hill equation in SigmaPlot 8.0. Two-electrode voltage-clamp data were analyzed by ANOVA followed by Bonferroni’s T-test for multiple samples (SigmaPlot 8.0). Image intensity data were compared by ANOVA. P < 0.05 was interpreted as significant.

RESULTS

SO$_4^{2-}$ uptake by SLC26A2 is cis-inhibited by Cl$^-$ and oxalate. Previous isotopic flux studies of recombinant rat and human SLC26A2 have been limited to 35SO$_4^{2-}$. hSLC26A2-mediated 35SO$_4^{2-}$ influx exhibited a $K_{1/2}$ of 67 µM in Xenopus oocytes (29), was cis-inhibited by 100–150 mM Cl$^-$ or HCO$_3^-$, and by 5 mM oxalate, SO$_4^{2-}$, or thiosulfate (37). We confirmed that 35SO$_4^{2-}$ influx mediated by hSLC26A2 was cis-inhibited by extracellular Cl$^-$ with $K_{1/2}$ of 24.1 ± 3.8 mM (Fig. 1A). 35SO$_4^{2-}$ influx mediated by mSlc26a2 was inhibited by extracellular Cl$^-$ with a similar $K_{1/2}$ of 18.4 ± 3.4 mM (Fig. 1C). hSLC26A2-mediated 35SO$_4^{2-}$ influx was cis-inhibited by extracellular oxalate with $K_{1/2}$ of 5.1 ± 1.7 mM (Fig. 1B), and mSlc26a2 showed similar results (Fig. 1D). cis-Inhibition by 24 mM HCO$_3^-$ was 20% for hSLC26A2 and 45% by mSlc26a2. 35SO$_4^{2-}$ influx by both orthologs was completely inhibited in the presence of 200 µM DIDS (Fig. 1D and data not shown), consistent with previous reports (29, 37). Inhibition by DIDS (12 min exposure) was only partially reversible, whereas the lower affinity inhibition by DNDS was completely reversible (supplemental Fig. 3).

SCL26A2 mediates bidirectional oxalate/SO$_4^{2-}$ exchange. Although oxalate had been shown to cis-inhibit uptake of SO$_4^{2-}$ by SLC26A2, SLC26A2-mediated transport of oxalate had not been reported. We found that hSLC26A2 transported oxalate with a $K_{1/2}$ for [14C]oxalate uptake of 0.65 ± 0.08 mM (Fig. 2A). [14C]oxalate uptake was cis-inhibited by extracellular SO$_4^{2-}$ with $K_{1/2}$ of 3.1 ± 0.7 mM. Bidirectional oxalate/SO$_4^{2-}$ exchange by hSLC26A2 was demonstrated by 35SO$_4^{2-}$ efflux dependent on extracellular oxalate (Fig. 2C, SO$_4^{2-}$/oxalate exchange) and by [14C]oxalate efflux dependent on extracellular SO$_4^{2-}$ (Fig. 2D, oxalate/SO$_4^{2-}$ exchange). hSLC26A2-

Fig. 4. SLC26A2-mediated exchange of intracellular 35SO$_4^{2-}$ for bath Cl$^-$ exhibits very low Cl$^-$ affinity. A: traces of 35SO$_4^{2-}$ efflux from individual oocytes previously injected with water (open squares, n = 2) or hSLC26A2 cRNA (filled circles, n = 6) during sequential increases in bath [Cl$^-$], followed by exposure to 200 µM DIDS. B: extracellular Cl$^-$ concentration ([Cl$^-$]) dependence of hSLC26A2-mediated [35SO$_4^{2-}$]/[Cl$^-$] exchange determined from A. C: traces of 35SO$_4^{2-}$ efflux from individual oocytes previously injected with water (open squares, n = 2) or mSlc26a2 cRNA (filled circles, n = 6) during sequential increases in bath [Cl$^-$], followed by addition of 200 µM DIDS. D: [Cl$^-$]$_i$ dependence of mSlc26a2-mediated [35SO$_4^{2-}$]/[Cl$^-$]$_o$ exchange determined from C. Values in B and D are means ± SE measured 3 days after oocyte injection with cRNA encoding hSLC26A2 (1 ng) or mSlc26a2 (5 ng). Data are representative of 2–3 similar experiments.

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mediated oxalate/$\text{SO}_4^{2-}$ exchange measured as $35\text{SO}_4^{2-}$ efflux was characterized by $K_{1/2}$ for extracellular oxalate of $1.6 \pm 0.4$ mM, and a $K_{1/2}$ for extracellular $\text{SO}_4^{2-}$ of $0.14 \pm 0.05$ mM measured as $[^{14}\text{C}]\text{oxalate}$ efflux. The corresponding extracellular $K_{1/2}$ values for mSlc26a2 were $3.7 \pm 0.7$ mM for oxalate (Fig. 3C) and $0.20 \pm 0.08$ mM for $\text{SO}_4^{2-}$ (Fig. 3D). These $K_{1/2}$ values for extracellular $\text{SO}_4^{2-}$ in $[^{14}\text{C}]\text{oxalate}/\text{SO}_4^{2-}_i$ exchange were within two- to threefold of the $K_{1/2}$ for $\text{SO}_4^{2-}$ influx of 0.067 mM reported by Karniski for hSLC26A2 (29).

SLC26A2-mediated exchange of intracellular $\text{SO}_4^{2-}$, $[^{14}\text{C}]\text{oxalate}$, and $^{36}\text{Cl}^-$ for extracellular $\text{Cl}^-$ is nonsaturable with respect to extracellular $\text{Cl}^-$. Efflux of $35\text{SO}_4^{2-}$ from oocytes expressing hSLC26A2 or mSlc26a2 into bath $\text{Cl}^-$ ($\text{SO}_4^{2-}/\text{Cl}^-$ exchange) exhibited a quasilinear, nonsaturable extracellular $[\text{Cl}^-]$ dependence of low affinity (Fig. 4). hSLC26A2-mediated $[^{14}\text{C}]\text{oxalate}$ efflux into bath $\text{Cl}^-$ ($\text{oxalate}/\text{Cl}^-$ exchange, supplemental Fig. 3) exhibited similar quasi-linear $[\text{Cl}^-]$ dependence of low affinity (Fig. 5, A and B). The low affinity for extracellular $\text{Cl}^-$ was also evident in measurements of hSLC26A2-mediated $^{36}\text{Cl}^-$ efflux into the $\text{Cl}^-$ bath ($\text{Cl}^-/\text{Cl}^-$ exchange, Fig. 5, C and D). In oocytes expressing mSlc26a2 or hSLC26A2, $^{36}\text{Cl}^-$ influx in exchange for intracellular anion (nominal $\text{Cl}^-$) was also linear and nonsaturable out to 104 mM (supplemental Fig. 4, A and C). Thus inward translocation of extracellular $\text{Cl}^-$ was of low affinity in exchange for each of the three intracellular anions tested. This property of SLC26A2 differed from its higher-affinity, saturable transport of extracellular $\text{SO}_4^{2-}$ and oxalate. The nonsaturable extracellular $[\text{Cl}^-]$ dependence for SLC26A2-mediated anion exchange also contrasted markedly with the saturable cis-inhibition of $\text{SO}_4^{2-}$ uptake by extracellular $\text{Cl}^-$ (Fig. 1).

SLC26A2 exhibits very low rates of exchange of intracellular $^{36}\text{Cl}^-$ for extracellular $\text{SO}_4^{2-}$ or oxalate. The unusual interaction of extracellular $\text{Cl}^-$ with SLC26A2 led us to examine exchange of intracellular $\text{Cl}^-$. The substantial uptake rates of $\text{SO}_4^{2-}$ and $\text{Cl}^-$, and the moderate uptake of oxalate, appeared likely to reflect exchange with intracellular $\text{Cl}^-$. However, rates of SLC26A2-mediated exchange of intracellular $^{36}\text{Cl}^-$ into saturating extracellular $[\text{SO}_4^{2-}]$ (supplemental Fig. 4, B and D) or saturating extracellular [oxalate] (data not shown) were 10- to 30-fold lower (supplemental Fig. 4, C–F) than that of $^{36}\text{Cl}^-$ efflux into 104 mM extracellular $\text{Cl}^-$ (Fig. 5, C and D). Nonetheless, the $K_{1/2}$ values for extracellular $\text{SO}_4^{2-}$ and oxalate at these low exchange rates were similar to those measured at the higher transport rates of the respective isotopically labeled anions. Thus the intracellular $\text{Cl}^-$ binding

![Graphs](http://ajpcell.physiology.org/)
site of SLC26A2 exhibits highly specific trans-anion dependence. Occupancy of the intracellular anion binding site of SLC26A2 by $^{35}\text{SO}_4^{2-}$ allows robust $^{35}\text{Cl}^-/\text{Cl}^-$ exchange. Rates of $\text{Cl}^-/\text{SO}_4^{2-}$ and $\text{Cl}^-/\text{oxalate}$ exchange are much lower, despite the preservation of $K_{1/2}$ values for these extracellular anion substrates.

SLC26A2-mediated exchange of extracellular oxalate or sulfate with intracellular anion is not detectably electrogenic. The above data shows that SLC26A2/DTDST is capable of sulfate with intracellular anion is not detectably electrogenic. However, SLC26A2 with mSlc26a6 and with nonmammalian Slc26a5/prestin (8, 38). Inhibition of SLC26A2 by acidic pH also applied to efflux of $^{35}\text{SO}_4^{2-}$ into ND96 (oxalate/Cl$^-$ exchange). The strong inhibition of SLC26A2/DTDST by acidic pH is paralogous. It contrasts with the modest inhibition by extracellular protons of hSLC26A6-mediated $[^{14}]\text{oxalate}/\text{Cl}^-$ exchange (supplemental Fig. 5) and $[^{14}]\text{oxalate}$ uptake (8), and with pH$_i$-insensitive $^{35}\text{Cl}^-/\text{Cl}^-$ exchange by hSLC26A3 (7).

Acidic pH$_i$ activates SLC26A2-mediated exchange of bath Cl$^-$ for intracellular $^{35}\text{SO}_4^{2-}$ but not with intracellular $^{35}\text{Cl}^-$ or $[^{14}]\text{oxalate}$. hSLC26A2 pH$_i$ sensitivity was tested by addition and removal of 40 mM butyrate, which reversibly decreases oocyte pH$_i$, 0.5 pH units (39). hSLC26A2-mediated $^{35}\text{SO}_4^{2-}/\text{Cl}^-$ exchange was more active at acidic pH$_i$ (in the presence of butyrate) than at resting pH$_i$ (in the absence of butyrate), as shown in Fig. 8, A and B. However, hSLC26A2 was nearly or completely pH$_i$ insensitive when it mediated efflux of $^{35}\text{Cl}^-$ (Fig. 8C) or of $[^{14}]\text{oxalate}$ (Fig. 8D) in exchange for extracellular Cl$^-$ (Cl$^-$/Cl$_i^-$ exchange or oxalate/Cl$_i^-$ exchange).

The apparent pH$_i$ sensitivity of SO$_4^{2-}$ efflux might also be explained by SCL26A2-mediated butyrate/SO$_4^{2-}$ exchange.
However, Fig. 9 demonstrates that extracellular butyrate did not itself serve as a substrate for hSLC26A2-mediated exchange with $^{35}$SO$_4$$^2$-, whereas subsequent introduction of bath Cl$^-$/H$^+$ elicited robust $^{35}$SO$_4$$^2$-/H$^+$ efflux activity sensitive to DIDS.

These data include those of A: C$^{36}$Cl$^-$ efflux rate constants of oocytes expressing hSLC26A2 (10 ng cRNA) during sequential exposure to ND96 baths of pH 5.0, pH 8.0, and after addition of DIDS (open bars, n = 12). Solid bars show water-injected oocytes (n = 6). D: $[^{14}$C]oxalate efflux rate constants of oocytes expressing hSLC26A2 (50 ng cRNA) during sequential exposure to ND96 baths of pH 5.0, pH 8.0, and after addition of DIDS (open bars, n = 11). Solid bars show water-injected oocytes (n = 4). Values in B–D are means ± SE measured 3–4 days post-cRNA injection.

Exposure of oocytes to 20 mM NH$_4$Cl also acidifies pH_i (−0.4 pH units) (4, 26, 36), likely reflecting a native ratio of NH$_4^+$/NH$_3$ permeabilities higher than that of mammalian tissue culture cells. This route of intracellular acidification, in con-

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REGULATED OXALATE TRANSPORT BY SLC26A2

Fig. 9. hSLC26A2 does not mediate \( {^{35}}\text{SO}_4^{2-}/\text{butyrate} \) exchange. A. \( {^{35}}\text{SO}_4^{2-} \) efflux traces from individual un.injected oocytes (open squares, \( n = 2 \)) or from oocytes previously injected with 0.5 ng hSLC26A2 cRNA (closed circles, \( n = 7 \)) during sequential exposure to baths containing Cl\(^-\)-free cyclamate, cyclamate with 40 mM Na butyrate, butyrate-free ND96, and ND96 containing 200 \( \mu \text{M} \) DIDS. B. \( {^{35}}\text{SO}_4^{2-} \) efflux rate constants (means \( \pm \text{SE} \)) of oocytes shown in A. Values are measured 3 days post-cRNA injection.

PKC inhibits hSLC26A2 through reduction of surface expression. Phorbol ester-sensitive PKC inhibits mSlc26a6 activity, largely through reduction in oocyte surface expression, whereas hSLC26A4/pendrin lacks this response to PKC (21). We reproduced this pattern of hSLC26A6 regulation by PKC (data not shown) and then tested the response of hSLC26A2 to PKC. As shown in Fig. 10, A and B, exposure to the classical PKC activator PMA in the absence of Cl\(^-\) dramatically reduced subsequent \( {^{35}}\text{SO}_4^{2-} \) efflux after reintroduction of bath Cl\(^-\), whereas exposure to the inactive PMA analog 4\( \alpha \)-PDD was without effect. PKC activation was associated with reduced abundance of hSLC26A2-GFP at or near the oocyte surface, as shown in Fig. 10, C and D. Again, 4\( \alpha \)-PDD was without effect. Thus PKC inhibits hSLC26A2/DTDST largely by reducing its abundance at the cell surface, similar to its regulation of mSlc26a6.

DTDST mutations inhibit hSLC26A2 activity at the cell surface or by regulating surface abundance. Mutations in hSLC26A2/DTDST cause several related clinical phenotypes of chondrodysplasia (22, 35). A related murine phenotype was generated by genetic knock-in of a mutation homologous to a human mutation associated with disease of moderate severity (10, 14). The several hSLC26A2 disease mutations that have been subjected to functional study exhibited decreased \( {^{35}}\text{SO}_4^{2-} \) uptake into oocytes (29) or HEK-293 cells (28). We therefore compared the effects of chondrodysplasia mutations on oxalate and \( {^{35}}\text{SO}_4^{2-} \) transport. The severe hSLC26A2 mutation R279W impaired uptake of \( {^{35}}\text{SO}_4^{2-} \) (Fig. 11A), as described earlier (28, 29).

DISCUSSION

hSLC26A2 was the first human member of the SulP gene superfamily linked to human genetic disease. Its homology with the previously identified \( {^{35}}\text{SO}_4^{2-} \) uptake transporters hSAT-1 and sulfate permease II of *Neurospora crassa* led to the demonstration of loss of \( {^{35}}\text{SO}_4^{2-} \) uptake by primary fibroblasts from a patient with diastrophic dysplasia (DTD) (22). Subsequent studies of recombinant SLC26A2 documented \( {^{35}}\text{SO}_4^{2-} \) transport, defined cis-inhibitor anions (37) and \( \text{K}_{1/2} \) for extracellular \( {^{35}}\text{SO}_4^{2-} \) in *Xenopus* oocytes, and documented reduced \( {^{35}}\text{SO}_4^{2-} \) uptake by most SLC26A2 mutants of DTD patients in oocytes and HEK-293 cells (28, 29).

In the current paper, we have extended the functional characterization of SLC26A2. We establish that hSLC26A2 and mSlc26a2 mediate bidirectional anion exchange with similar characteristics. We find that SLC26A2 transports oxalate and Cl\(^-\) (in addition to \( {^{35}}\text{SO}_4^{2-} \)) by an apparently electroneutral mechanism. We show that SLC26A2 is regulated independently by \( \text{pH}_4 \), \( \text{pH}_o \), \( \text{NH}_4^+ \), and PKC, and that disease mutations alter oxalate transport in parallel with \( {^{35}}\text{SO}_4^{2-} \) transport. We also report anion substrate-selective asymmetries of anion exchange.

Lack of species differences. The 78% amino acid sequence identity between mSlc26a6 and hSLC26A6 is associated with substantial differences in anion transport selectivity and affinity (6, 8). Thus the 80% amino acid sequence identity between mSlc26a2 and hSLC26A2/DTDST similarly prompted us to compare functional properties of these two species orthologs. In contrast to anion transport by SLC26A6, we found that anion transport by mouse and human SLC26A2 did not differ in their tested transport properties. This functional similarity strengthens the possibility that study of mSlc26a2 and the
hypo- and mutant knock-in mouse (14) will continue to provide data applicable to hSLC26A2 and its deficiency diseases.

Oxalate transport by wild-type and mutant SLC26A6. The expression of mSlc26a2 polypeptide in the apical pole of colonocytes (20) prompted us to test it as an oxalate transporter possibly contributing to oxalate absorption or to the 25–50% of transepithelial oxalate secretion that is mSlc26a6 independent (15, 27). We demonstrated that both mouse and human SLC26A2 orthologs mediate oxalate uptake cis-inhibited by SO$_4^{2-}$, as well as bidirectional, saturable oxalate/SO$_4^{2-}$ exchange. $[^{14}C]$Oxalate/Cl$^-$ exchange by SLC26A2 was more rapid than $[^{14}C]$oxalate/SO$_4^{2-}$ exchange. However, Cl$^-$/oxalate, exchange efflux rate constants were ~10-fold lower than for oppositely directed anion exchange. We confirmed the earlier observation (37) that 5 mM oxalate cis-inhibits SO$_4^{2-}$ uptake but less potently (Fig. 1B) than previously noted.

SLC26A2 is abundant in chondrocytes, suggesting the possibility of chondrocyte involvement in primary hyperoxaluria. Oxalosis can indeed involve bones, joints (32), and skin (40), but oxalate transport by chondrocytes and fibroblasts has not yet been reported. Urinary oxalate excretion has not been measured in the Slc26a2 hypomorph mutant mice, in which nephrocalcinosis has not been radiologically evident (A. Rossi, University of Pavia, personal communication).

SLC26A2 chondrodyplasia mutant polypeptides have been shown previously to exhibit varying degrees of decreased SO$_4^{2-}$ transport. We studied two human disease mutations, one of which (A386V) corresponds to the mouse knock-in hypomorph mutation but had not been studied as an anion transporter. We found that transport of SO$_4^{2-}$ and oxalate was reduced in parallel for both mutants, but that only A386V exhibited parallel reduction in abundance at or near the oocyte surface. The reduced function of the R279W mutant in the setting of apparently wild-type surface abundance suggests that the mutation reduces the rate of the conformational cycle that mediates anion transport. The R279W mutant should thus prove a useful point of entry for future structure-function studies of SLC26A2.

SLC26A2-mediated transport of SO$_4^{2-}$ and Cl$^-$. Our results extend previous measurements of SO$_4^{2-}$ transport by SLC26A2 with demonstrations of exchange with oxalate and with Cl$^-$. Whereas the $K_{1/2}$ of SO$_4^{2-}$ for cis-inhibition of $[^{14}C]$oxalate was 3 mM, the $K_{1/2}$ of SO$_4^{2-}$ for exchange with intracellular $[^{14}C]$oxalate exhibited a 15- to 20-fold higher affinity. This difference is due in part to the presence or absence of cis-competitor, but with these additional contributions. The oxalate injection required to measure $[^{14}C]$oxalate efflux is expected to saturate the internal binding site and promote oxalate translocation and exposure of the outward-facing substrate binding site. A large increase in the population of outward-facing sites available to extracellular substrate should increase apparent affinity. In addition, the required oxalate injection likely reduces intracellular [Ca$^{2+}$], and may trigger stress responses or other regulatory pathways potentially altering apparent extracellular substrate affinity.

In contrast to the saturable, bidirectional transport of SO$_4^{2-}$ and oxalate, Cl$^-$ exhibited nonsaturable uptake. In addition, Cl$^-$ was nonsaturable with respect to exchange of $^{36}$Cl$^-$/oxalate, $^{34}$Cl$^-$/oxalate, and $^{36}$Cl$^-$, suggesting that the extracellular anion binding site of SLC26A2 has very low affinity for Cl$^-$. SLC26A2-mediated $^{36}$Cl$^-$ efflux exhibited trans-anion selectivity, in that exchange with Cl$^-$ was approximately 10-fold...
more rapidly than exchange with SO\textsuperscript{2−}_o or with [\textsuperscript{14}C]oxalate\textsubscript{i}. Thus, the free energy required for the conformational transition associated with Cl\textsuperscript{−} translocation may vary with the extracellular anion, suggesting possible simultaneous anion binding on both sides of the membrane, an external regulatory anion binding site, or anion-specific influence on substrate off-rate after translocation.

Of note, the low-affinity [Cl\textsuperscript{−}]o dependence of mSlc26a2 for \textsuperscript{35}SO\textsuperscript{2−} efflux exhibited a sigmoid pattern suggestive of positive cooperativity (Fig. 4D), as was also observed for hSLC26A2-mediated \textsuperscript{35}Cl\textsuperscript{−}/Cl\textsuperscript{−} exchange. However, the [Cl\textsuperscript{−}]o dependence of hSLC26A2-mediated [\textsuperscript{14}C]oxalate efflux did not show this sigmoid pattern. Thus, in some conditions, extracellular Cl\textsuperscript{−} may exert a regulatory function beyond that of substrate. Complex, condition-dependent substrate cooperativity for electroneutral cation exchange has been observed previously for NHE Na\textsuperscript{+}/H\textsuperscript{+} exchangers (17) and NCX Na\textsuperscript{+}/Ca\textsuperscript{2+} exchangers (3).

\textit{SLC26A2-mediated anion exchange is not detectably electrogenic.} SO\textsuperscript{2−}_o/Cl\textsuperscript{−} exchange and oxalate/Cl\textsuperscript{−} exchange were unaccompanied by measurable currents in two-electrode voltage-clamped oocytes (Fig. 6). An electrogenic SLC26A2-mediated SO\textsuperscript{2−}_o influx of magnitude equivalent to that measured in Fig. 1 should be reflected in a DIDS-sensitive outward current of 30–40 nA at the resting potential of approximately −30 to −40 mV. This level of current is detectable by two-electrode voltage clamp at that potential but was not evident (Fig. 6). We therefore conclude that SLC26A2-mediated SO\textsuperscript{2−}_o/Cl\textsuperscript{−} exchange is very likely electro-neutral. This electroneutral exchange could be SO\textsuperscript{2−}_o/2Cl\textsuperscript{−} exchange or H\textsuperscript{+}/SO\textsuperscript{2−}_o/Cl\textsuperscript{−} exchange.

An electrogenic SLC26A2-mediated oxalate influx of magnitude equivalent to that measured in Fig. 2 should be reflected in an outward current of only 4 nA at resting potential. This level of current is below the detection threshold (Fig. 6). Thus our present results do not allow a definitive conclusion that SLC26A2-mediated oxalate/Cl\textsuperscript{−} exchange is electroneutral. Nonetheless, SLC26A2-mediated oxalate/Cl\textsuperscript{−} exchange and SO\textsuperscript{2−}_o/Cl\textsuperscript{−} exchange likely employ the same mechanism. In contrast, electrogenic oxalate/Cl\textsuperscript{−} exchange has been observed in oocytes expressing mSlc26a6 (8), nonmammalian orthologs of Slc26a5/prestins (38), and rat Slc26a1/Sat-1 (30).

\textit{Regulation of SLC26A2 by pH and PKC.} Acidic pH\textsubscript{o} strongly suppresses SLC26A2-mediated exchange of Cl\textsubscript{o} for intracellular \textsuperscript{35}SO\textsuperscript{2−}_o, \textsuperscript{36}Cl\textsuperscript{−}, and [\textsuperscript{14}C]oxalate (Fig. 7). In contrast, acidic pH\textsubscript{o} activated \textsuperscript{35}SO\textsuperscript{2−}_o/Cl\textsuperscript{−} exchange but had no effect on \textsuperscript{36}Cl\textsuperscript{−}/Cl\textsubscript{o} exchange and [\textsuperscript{14}C]oxalate/Cl\textsubscript{o} exchange (Fig. 8). These data are compatible with mechanisms of SO\textsuperscript{2−}_o/OH\textsuperscript{−} exchange (or the formally equivalent SO\textsuperscript{2−}_o/H\textsuperscript{+} cotransport) but not with oxalate\textsuperscript{−}/OH\textsuperscript{−} exchange. However, the great reduction in SO\textsuperscript{2−}_o transport rate by Cl\textsuperscript{−} removal suggests that pH\textsubscript{o}-sensitive SO\textsuperscript{2−}_o/Cl\textsuperscript{−} exchange is predominant. Regulation of SLC26A2-mediated anion exchange by NH\textsubscript{4} also

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\textbf{Fig. 11.} Two diastrophic dysplasia sulfate transporter (DTDST) mutants of hSLC26A2 exhibit impaired efflux of \textsuperscript{35}SO\textsuperscript{2−}_o and of oxalate but differ in surface expression. A: normalized \textsuperscript{35}SO\textsuperscript{2−}_o uptake by (n) un.injected oocytes or oocytes expressing wild-type (WT) hSLC26A2 or the indicated hSLC26A2 mutants (0.5 ng cRNA). WT uptake 0.17 ± 0.02 nmmol/h. B: normalized \textsuperscript{14}C]oxalate uptake by (n) un injected oocytes or oocytes expressing WT or mutant hSLC26A2 (50 ng cRNA). WT uptake was 0.20 ± 0.02 nmol/h. C: confocal immunofluorescence micrographs of representative oocytes expressing WT hSLC26A2 or the indicated mutants. D: normalized mean fluorescence intensity (FI) measured at the periphery of (n) oocytes expressing WT or mutant SLC26A2. WT hSLC26A2 intensity was assigned a mean FI of 1.0. ***p < 0.001.
exhibits substrate selectivity, but not of the same type as regulation by pH (supplemental Fig. 6).

Inhibition by acidic pH$_r$ is of pathophysiological importance in the hypoxic and elevated lactate conditions of inflamed and septic joints. Septic or inflammatory joint synovial fluid pH can reach pH 6.6 or below (42). The cells of the hypoxic nucleus pulposus of vertebral bodies lack carbonic anhydrase activity and exhibit resting pH$_r$ of 6.7, with pH$_r$ as low as 6.4 (34). In this acidic environment, reduced SLC26A2-mediated SO$_4^{2-}$ transport by fibroblasts and chondrocytes in these acidic environments could thus impair local glycosaminoglycan biosynthesis. However, potential activation of SO$_4^{2-}$ uptake by acidic pH$_r$ could counter this impairment, suggesting a balance of two counter-regulatory effects.

PKC activation markedly inhibits SLC26A2, in part by reducing its abundance at the oocyte surface. This mode of regulation resembles that previously shown for the apical anion exchanger SLC26A6 but is unlike the insensitivity to PKC shown by the apical anion exchanger SLC26A4/pendrin (21). Thus activation of chondrocyte SLC26A2 through administration of PKC inhibitors might be envisioned as a useful component of treatment for degenerative joint diseases. Future experiments could determine the specific PKC type involved in regulation of SLC26A2.

**Possible roles of SLC26A2 in intestine and kidney.** Although SLC26A2 can exchange oxalate, it is likely a minor contributor to intestinal transepithelial transport of oxalate under normal conditions. The strong DIDS sensitivity of all SLC26A2 transport modes does not support its contribution to DIDS-insensitive intestinal oxalate absorption (23). In oocytes, SLC26A2-mediated oxalate influx and efflux were both at considerably lower rates than SLC26A6-mediated oxalate influx and efflux (6, 8). Moreover, small interfering RNA (siRNA) knockdown of hSLC26A6 by $\sim$60% in Caco2 cells decreased unidirectional oxalate fluxes in both directions by 50% (16), suggesting that most oxalate is carried by SLC26A6 in this cell model. The direction of colonic oxalate/Cl$^{-}$ exchange will depend on transmembrane membrane gradients of oxalate and Cl$.^{-}$ The Cl$^{-}$ gradient varies along the length of the intestine and with the Cl$^{-}$ secretory state of the enterocyte. The oxalate gradient is a function of dietary oxalate intake, net oxalate transport by upstream epithelia, enterocyte oxalate production, and (in normal colon) bacterial consumption. In the presence of a stable oxalate gradient, SLC26A2-mediated electroneutral oxalate/Cl$^{-}$ exchange might switch from oxalate absorption to secretion upon reduction in intracellular [Cl$^{-}$] triggered by Cl$^{-}$-secretagogue stimulation. SLC26A2-mediated oxalate transport should be unaffected by elevated colonic luminal [NH$_{2}^{+}$] (supplemental Fig. 6D) but reduced by mildly acidic intestinal luminal pH (Fig. 7).

However, hSLC26A2 could mediate a significant portion of that oxalate secretion dependent on exchange with luminal SO$_4^{2-}$, and this exchange mode might be sensitive to inhibition by both the mildly acidic luminal pH and the elevated [NH$_{2}^{+}$] of the colon. Although median [SO$_4^{2-}$] in municipal water supplies was 0.28 mM, [SO$_4^{2-}$] in private well water can exceed 20 mM (11, 19), and common dietary sources rich in SO$_4^{2-}$ include preserved fruits, Brassica vegetables, and beer. SLC26A2 might thus, also serve, in states of positive SO$_4^{2-}$ balance, to secrete SO$_4^{2-}$ in exchange for luminal Cl$^{-}$, complementing renal SO$_4^{2-}$ excretion.

hSLC26A2 mRNA levels have been suggested as a biomarker for the differential diagnosis of Crohn’s disease and ulcerative colitis. SLC26A2 mRNA levels are increased severalfold in Crohn’s disease and unchanged or strongly decreased in ulcerative colitis (9, 43). SLC26A2 and SLC6A14 mRNA levels used as part of a seven gene panel yielded rates of correct prediction, sensitivity, and specificity higher than with previously available diagnostic indices (43). In addition, SLC26A2 is downregulated in colon cancer biopsies compared with surrounding normal tissue (18).

SLC26A2 mRNA is expressed in the kidney, and SLC26A2 polypeptide has been immunolocalized in the brush border of the rat proximal tubule (5). In that location, oxalate/SO$_4^{2-}$ exchange by SLC26A2 might contribute to tertiary active Cl$^{-}$ reabsorption across the proximal tubular epithelium, coupled to sodium-sulfate cotransport (1).