Solute Carrier family 26 member a2 (Slc26a2) functions as an electroneutral SO$_4^{\circ}/$OH$^-$/Cl$^-$ exchanger regulated by extracellular Cl$^-$

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Running Title: Slc26a2 transport properties and regulation

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Keywords: Slc26a2; SO$_4^{\circ}$/OH$^-$/Cl$^-$ exchange; regulation; Cl$^-$ out.

Background: Slc26a2 is SO$_4^{\circ}$ transporter, mutations in which cause diastrophic dysplasia. How Slc26a2 transports SO$_4^{\circ}$ is unknown.

Results: We found that Slc26a2 transport 1SO$_4^{\circ}$ in exchange for 2OH$^-$ or 2Cl$^-$ and is regulated by a promiscuous extracellular anion site.

Conclusion: Slc26a2 functions as SO$_4^{\circ}$/2OH$^-$ or SO$_4^{\circ}$/2Cl$^-$ exchanger regulated by extracellular Cl$^-$ out.

Significance: The findings should help in understanding aberrant SLC26A2 function in diastrophic dysplasia.

Abstract

Slc26a2 is a ubiquitously expressed SO$_4^{\circ}$ transporter with high expression levels in cartilage and several epithelia. Mutations in SLC26A2 are associated with diastrophic dysplasia. The mechanism by which Slc26a2 transports SO$_4^{\circ}$ and the ion gradients that mediate SO$_4^{\circ}$ uptake are poorly understood. We report here that Slc26a2 functions as a SO$_4^{\circ}$/2OH$^-$, SO$_4^{\circ}$/2Cl$^-$ and SO$_4^{\circ}$/OH$^-$/Cl$^-$ exchanger, depending on the Cl$^-$ and OH$^-$ gradients. At inward Cl$^-$ and outward pH gradients (high Cl$^-$ out, low pH$_{out}$), Slc26a2 functions primarily as SO$_4^{\circ}$/2OH$^-$ in exchanger. At low Cl$^-$ out, high pH$_{out}$, Slc26a2 functions increasingly as SO$_4^{\circ}$/2Cl$^-$ in exchanger. The reverse is observed for SO$_4^{\circ}$/2OH$^-$ out and SO$_4^{\circ}$/2Cl$^-$ in exchange. Slc26a2 also exchanges Cl$^-$ for I$^-$, Br$^-$ and NO$_3^-$ and Cl$^-$ competes with SO$_4^{\circ}$ on the transport site. Interestingly, Slc26a2 is regulated by an extracellular anion site, required to activate SO$_4^{\circ}$/2OH$^-$ out exchange. Slc26a2 can transport oxalate in exchange for OH$^-$ and/or Cl$^-$ with properties similar to SO$_4^{\circ}$ transport. Modeling of the Slc26a2 transmembrane domain (TMD) structure identified a conserved extracellular sequence 367GFXXP371 between TMD7 and TMD8 close to the conserved E417 in the permeation pathway. Mutation of E417 eliminated transport by Slc26a2, whereas mutation of F368 increased the affinity for SO$_4^{\circ}$ out 8 fold while reducing the affinity for Cl$^-$ out 2 fold, but without affecting regulation by Cl$^-$ out. These findings clarify the mechanism of net SO$_4^{\circ}$ transport and describe a novel regulation of Slc26a2 by an extracellular anion binding site and should help in further understanding aberrant SLC26A2 function in diastrophic dysplasia.

Introduction

Protein sulfation, and thus SO$_4^{\circ}$, is essential for cellular and tissue survival. Many proteins undergo posttranslational modification by sulfation. Tyrosine sulfation of signaling molecules, like the G proteins coupled receptors chemokine receptors (1), modifies signaling pathways. Protein sulfation contributes to detoxification of endogenous compounds (2). A critical role of protein sulfation is sulfation of proteoglycans (3). Proteoglycans are constituents of the extracellular matrix that mediate the cell response to growth factors (4). Several disorders are caused by mutations in genes that affect proteoglycans synthesis or sulfation. The sulfate groups in proteoglycans are critical in formation of active domains and the high polyanionic charge density of the proteoglycans is neutralized by SO$_4^{\circ}$ (5). Sulfation of secretory proteins, like digestive enzymes and mucins, is essential for their synthesis, processing through the biosynthetic pathway and packaging in secretory granules (6). Hence, understanding SO$_4^{\circ}$ homeostasis is essential for understanding cell development and function.

Cells have two sources of SO$_4^{\circ}$, a minor source from degradation of cysteine and methionine and active uptake of SO$_4^{\circ}$ mediated largely by the SO$_4^{\circ}$ transporters Slc26a1 and Slc26a2 (7,8). Slc26a1 and Slc26a2 belong to the family of the SLC26 transporters, which includes 11 genes with Slc26a10 being pseudogene (9). Members of the family transport remarkably diverse substrates, including Cl$^-$, HCO$_3^-$, I$^-$, SO$_4^{\circ}$, formant and oxalate and can function as coupled electroneutral or electrogenic transporters or as ion channels (9,10). Mutations in several members of the
family are associated with human diseases, including autosomal recessively inherited chondrodysplasias (SLC26A2) (11,12) congenital chloride diarrhea (SLC26A3) (13), Pendred’s syndrome (SLC26A4) (14), deafness (SLC26A5) (15), and perhaps reduced fertility (SLC26A8) (16). In addition, deletion of Slc26a6 in mice resulted in nephrolithiasis due to aberrant oxalate transport (17) and in aberrant pancreatic and parotid ducts HCO3- transport (18,19).

While Slc26a1 has limited tissue distribution, Slc26a2 is ubiquitously expressed with particularly high levels in developing and mature cartilage as well as in epithelial tissues like pancreas, salivary glands, colon, bronchial glands, tracheal epithelium, and eccrine sweat glands (20,21). The central role of Slc26a2 in supplying the bulk of cellular SO4- is evident from the lethality of deletion of the SLC26A2 gene in humans and mice (20,22), mainly due to under-sulfation of proteoglycans leading to aberrant development (23). Indeed, measurement of SO4- uptake in fibroblast from patients with a severe form of the disease showed reduced or lack of SO4- uptake (20,24). Most mutations causing diastrophic dysplasia are missense mutations that affect either trafficking to the plasma membrane or showed reduced SO4- transport (25,26).

The phenotype of chondrodysplasias is highly variable, ranging from mild (27) to lethal before or shortly after birth (11). To better understand the disease and cellular SO4- homeostasis it is necessary to understand transport and regulation of Slc26a2. To date, characterization of transport by Slc26a2 was based on measurement of isotopic fluxes (24,25,28) that are the sum of both net and exchange fluxes, with the exchange dominating the fluxes. These studies revealed that Slc26a2 can transport SO4-, Cl- and oxalate (24,25,28) and a recent detailed characterization of the fluxes suggested that Slc26a2 functions as an electroneutral transporter when mediating isotopic fluxes. SO4- fluxes appeared to be sensitive to intracellular and extracellular pH (24). An unusual finding was that inhibition of SO4- and oxalate isotopic uptake by external Cl- exhibited simple saturation, while Slc26a2-mediated exchange of intracellular SO4-, oxalate or Cl- for external Cl- was nonsaturable (24), suggesting that the measured fluxes, at least isotopic efflux, is mostly exchange rather than net fluxes.

The available information is not sufficient to determine the mode of SO4- and other ions transport by Slc26a2 and the cellular ionic gradients that drive net transport. We used Xenopus oocytes expressing Slc26a2 to report that Slc26a2 functions as SO4-/2OH-, SO4-/2Cl- and SO4-/OH-/Cl- exchanger, depending on the cellular Cl- and OH-(H+) gradients. Slc26a2 can also mediate ONa+OH-/Cl- exchange and transport I-, Br- and NO3-. Slc26a2 activity is regulated by an extracellular anion binding site which is not involved in ion transport. Modeling of the Slc26a2 transmembrane sector identified an extracellular loop which contains the conserved sequence 367GFXXP371 in the vicinity of the gating E417 as a potential part of the permeation pathway. These findings should help in further understanding ion transport by the SLC26 transporters and aberrant SLC26A2 function in diastrophic dysplasia.

**Experimental Procedures**

**Solutions and reagents:** For experiments in oocytes, the standard HEPES-buffered ND96 solution contained (in mM): 96 NaCl, 2 KCl, 1.8 CaCl2, 1 MgCl2, and 5 HEPES, pH 7.5. Cl- free solutions were prepared by replacing chloride with gluconate in the presence of Ca-ethylendiaminetetraacetic acid substituted for CaCl2. A 100 mM solution of Dисothiocyanoestilbene-2,2'-disulfonic acid (DIDS) (Invitrogen, Carlsbad, CA) dissolved in DMSO was prepared freshly and diluted to a final concentrations of 10 or 50 µM in the relevant solutions. All other chemicals and reagents were purchased from Sigma (St. Louis, MO).

**cRNA preparation:** The pCMV-Sport6-Slc26a2 (GenBank/EMBL/DDBJ, accession no. BC028345) was purchased from Open Biosystems and was used as template for cRNA preparation. The plasmid was linearized with NotI and used to transcribe cRNA with mMessage mMachine Sp6 kit (Life technologies, Applied Biosystems), respectively. Mutation in Slc26a2 were generated by a site-directed mutagenesis kit (Agilent Technologies) and verified by sequencing.

**Biotinylation and Western Blot Analysis:** To monitor surface expression of Slc26a2 WT, E417A and E417K, HEK cells transfected with vector alone or myc-tagged Slc26a2 constructs were incubated with EZ link Sulfo-NHS-LC-Biotin (0.5 mg/ml; Thermo Fisher Scientific) for 30 min at room temperature. Subsequent parameters as previously described (29) with the following modifications: 50 µl of 1:1 slurry of immobilized avidin beads (Thermo Fisher Scientific) was added to 300 µg of protein in 300 µl of cell extract and incubated overnight. To monitor protein expression the PVDF membranes were incubated overnight with anti-myc antibodies diluted 1:1000 (cell signaling) and for 1 hr with HRP conjugated goat anti-mouse (Invitrogen) diluted 1:2000. For β-actin detection membranes were incubated for 1 hr with monoclonal anti-β-actin peroxidase (Sigma-Aldrich) diluted 1:20,000.
Xenopus laevis oocytes preparation: All experiments in this study were conducted under the National Institutes of Health guidelines for research on animals, and experimental protocols were approved by the Institutional Animal Care and Use Committee. Oocytes were isolated by partial ovariectomy of anaesthetized female Xenopus laevis (Xenopus Express, Brooksville, FL) and treated by collagenase B (Roche, Indianapolis, IN), as described previously (30). Stage V–VI oocytes were injected with 10 ng cRNA using glass micropipettes and a microinjection device (Nanoliter 2000; World Precision Instruments) in a final volume of 27.6 nL. Control oocytes were injected with equal volumes of H2O. Oocytes were incubated at 18°C in ND96 supplemented with 2.5 mM pyruvate and antibiotics, and were studied 72–144 h after injection.

Voltage, pH, and Cl− measurement in oocytes: Voltage recordings were performed at room temperature with two-electrode voltage clamp, exactly as described previously (29,30). Voltage, pH_in and Cl_in concentrations were measured as detailed previously (31,32). In the present study, the Cl− sensitive electrode was also used to record intracellular Br−, I− and NO3− with the resin used to measure Cl− with the procedure used to measure Cl− (see results section).

Measurement of buffer capacity: To determine OH− (H+) fluxes by Slc26a2 we determine the buffer capacity of oocytes bathed in HEPES-buffered medium. Since we can measure both Cl− and pH determined the buffer capacity directly rather than rely on pH_in changes induced by weak acids. Supplement Fig. 1A shows that two consecutive injections of the oocytes with 13.8 nL of 100 mM HCl reduced pH_in and increased Cl_in. Similar determination in 5 experiments and using the pH_in and Cl_in changes of the first injection resulted in a buffer capacity of 17.1±2.2/pH unit, which is similar to that reported by others (33).

Modeling and prediction of the Slc26a2 transmembrane domains structure: The transmembrane sector of the mouse Slc26a2 model was generated using the deepview swiss-PDB viewer by Raw sequence fit of the Slc26a2 sequence (NCBI accession no. NP_031911) onto the putative Slc26a6 model previously generated by us based on structural similarity to the bacterial ClC-ec protein (29). The predicted binding site of DIDS on the Slc26a2 model was performed with the Autodock Vina software (34), according to software tutorial instructions. Briefly, the box grid determining the Slc26a2 region of binding was set using the AutoDockTools software with the following coordinates (center x=0.472, y=1.222, z=0.472) (size x=30, y=26, z=24). Exhaustiveness level was set to default. AutoDockTools was further used to select all rotatable bonds of the DIDS molecule. The AutoDockVina software generated 9 different models and here we present the best model as ranked by the software with a predicted affinity of -8.9 Kcal/mol for the binding of Slc26a2 and DIDS. The final model (cartoon and surface representations) was generated using PyMol software (Schrödinger, LLC).

Results and Discussion

Slc26a2 functions as SO4^2-/OH-, SO4^2-/Cl and possibly SO4^2-/OH/Cl exchanger: Slc26a2-mediated net fluxes were assayed in Xenopus oocytes by measuring intracellular pH (pH_in) and Cl− (Cl_in) and the membrane potential in the same oocytes. Fig. 1A shows that removal of extracellular Cl− (Cl_out) had no effect on pH_in and the membrane potential with a slow rate of reduction in Cl_in. Exposing Slc26a2-expressing oocytes bathed in Cl−-free solution to 0.2 mM SO4− resulted in a precipitous reduction in pH_in and Cl_in. Removal of SO4− out with the concomitant addition of Cl− out resulted in increased pH_in and Cl_in. Fig. 1C shows almost no change in Cl_in and pH_in in water-injected oocytes under the same conditions.

Reduction in pH_in can be due to H+ influx or OH− efflux. Since some of the SO4− transport is coupled to Cl−, and the Cl− coupling is affected by pH_out (see below), we will refer to the transported ion as OH−, although we cannot distinguish between the transport of OH− and H+. The average Slc26a2-mediated SO4−-coupled net Cl− and OH− transport are shown in Fig. 1B and indicate that under the conditions of Fig. 1A about 40% of SO4− is transported in exchange for Cl− and about 60% in exchange with OH−. SO4− transport is electroneutral since it is not associated with a change in membrane potential (Fig. 1) and SO4−-coupled OH− (Fig. 1D) and Cl− (not shown) fluxes are the same at membrane potential of +40 and -100 mV. This indicates that the coupling stoichiometry of SO4− exchange with Cl− and OH− is likely 1:2 with Slc26a2 functioning as SO4^2-/2OH−, SO4^2-/2Cl− and possible SO4^2-/OH-/Cl− exchanger.

To further determine the relationship between SO4^2− and Cl− we measured the effect of Cl_out on the apparent affinity for SO4^2− out. Fig. 2A shows an example of the protocol used for these experiments. Oocytes expressing Slc26a2 were exposed to solutions containing the desired Cl− out (0, 5, 20 or 50 mM) and SO4^2− concentration for 5 min to obtain the rate of OH− efflux. Then the oocytes were incubated in Cl−-containing solution without SO4^2− to extrude the SO4^2− and recover pH_in before exposure to the subsequent SO4^2− concentration. The plots in Fig. 2B obtained from...
dependence of Cl\- intracellular oxalate (24). This may reflect the different opposite effect. On the other hand, during SO\(_4\) and the columns show the associated OH\-(H\+) and Cl\- removal of SO\(_4\) traces) and 8.2 (gray traces). The rates of OH\- and Cl\- can accommodate I\-, Br\- and NO\(_3\) Slc26a2 permeation pathway is not very selective and as a result of SO\(_4\) turnover cycle (net) of transport by Slc26a2. Many of the SLC26 transporters can transport HCO\(_3\) in exchange for Cl\- (9). However, Fig. 2D shows that Slc26a2 does not function as a Cl\-/HCO\(_3\) exchanger. The capacity of Slc26a2 to transport other anions, such as I\-, Br\- and NO\(_3\), in addition to SO\(_4\), OH\- and Cl\- was further tested by measuring their intracellular concentration. Supplementary Fig. 1B shows that the resin used to detect Cl\- can also detect Br\- and NO\(_3\) about 10 times better than Cl\- and I\- about 100 times better than Cl\- (see also (29)). The left panel of Fig. 2D shows that exposing Slc26a2-expressing oocytes to Cl\-free solution containing 2 mM I\- resulted in a rapid influx of I\-. Removal of I\- in the absence of Cl\- out stopped the influx. To initiate I\- efflux it was necessary to add Cl\- out with as little as 1 mM Cl\- out resulting is nearly maximal rate of I\- efflux. Similar behavior was observed with Br\- and NO\(_3\) (Fig. 2D, right panel) and no I\- (Fig. 2D, gray trace, left panel), Br\- or NO\(_3\) (not shown) fluxes were observed in water-injected oocytes. These findings indicate that the Slc26a2 permeation pathway is not very selective and can accommodate I\-, Br\- and NO\(_3\) to mediate I\-/Cl\-, Br\-/Cl\- and NO\(_3\)/Cl\- exchange.

The ratio of SO\(_4\)/2OH and SO\(_4\)/2Cl exchange is determined by pH\(_{out}\). Coupling of SO\(_4\) transport to OH\- and Cl\- raised the question of how the availability of substrate would affect the coupling. We addressed this question by examining the effect of pH\(_{in}\) and pH\(_{out}\) on SO\(_4\) transport. Fig. 3A shows example traces of the changes in pH\(_{in}\) (left panel) and of the Cl\- (right panel) as a result of SO\(_4\) transport at pH\(_{out}\) of 6.5 (black traces) and 8.2 (gray traces). The rates of OH\- and Cl\- influx and efflux under both conditions are summarized in Fig. 3B. The models in Fig. 3B show the direction of ion fluxes during SO\(_4\) influx (left) and SO\(_4\) efflux (right) and the columns show the associated OH\-(H\+) and Cl\- fluxes at pH\(_{out}\) of 6.5 and 8.2. SO\(_4\) influx is coupled to Cl\- and OH\- efflux, while SO\(_4\) efflux initiated by removal of SO\(_4\) and addition of Cl\- is coupled to Cl\- and OH\- influx. During SO\(_4\) influx acidic pH\(_{out}\) increases OH\- efflux with low Cl\- efflux while alkaline pH\(_{out}\) has the opposite effect. On the other hand, during SO\(_4\) efflux acidic pH\(_{out}\) inhibits OH\- efflux and increases Cl\- efflux while alkaline pH\(_{out}\) has the opposite effect.

Fig. 3C further illustrates the reciprocal effect of pH\(_{out}\) on OH\- and Cl\- fluxes. Exposing Slc26a2-expressing oocytes to a solution buffered to pH 7.5 and containing 110 mM Cl\- and 2 mM SO\(_4\) resulted in a reduction in pH\(_{in}\) at a rate of about 0.18±0.03 mM/min (n=8), with no change in Cl\-in. Removal of SO\(_4\) resulted in recovery of pH\(_{in}\). H\(_2\)O injected oocytes showed no response to SO\(_4\). Hence, at high Cl\-out and pH\(_{out}\) of 7.4 all the Slc26a2-mediated SO\(_4\) influx is mediated by SO\(_4\)/2OH\-(2H\+) exchange (or SO\(_4\)/2H\+ cotransport). When the same oocytes were exposed to the same solution containing 110 Cl\- and 2 mM SO\(_4\), but now buffered to pH of 6.5, SO\(_4\) uptake resulted in a large reduction in pH\(_{in}\) with no change in Cl\-in, while SO\(_4\) efflux initiated by removal of SO\(_4\) resulted in a small increase in pH\(_{in}\) and a large Cl\- influx (Fig. 3C). Thus at low pH\(_{out}\) SO\(_4\) uptake is predominantly mediated by SO\(_4\)/2OH\-(2H\+) exchange, while SO\(_4\) efflux is dominated by SO\(_4\)/in/Cl\- out exchange.

The Sulfate transported species can be SO\(_4\) or HSO\(_3\). Although we did not examine this in great detail, the results in Figs. 1-3 favor SO\(_4\). Thus, if the transported species is HSO\(_3\) then acidic pH\(_{out}\) should markedly enhance Sulfate influx. Fig. 3B indicates that is not the case. Second, SO\(_4\) efflux after removal of SO\(_4\) should be independent of pH\(_{out}\) since pH\(_{out}\) should have no effect of the transported SO\(_4\) species. Again, this is not the case. Third, changes on pH\(_{out}\) have the same effect on SO\(_4\) and Ox\- transport (see below), suggesting that the transport rate follows the pH gradient rather than substrate species.

Coupling of SO\(_4\) transport to both Cl\- and OH\- may function to ensure SO\(_4\) uptake under acidic and alkaline conditions. Slc26a2 is expressed in the luminal membrane of polarized cells (31,35) that can be exposed to acidic and alkaline pH. In the stomach and synovial fluid pH is acidic (36,37) and SO\(_4\)/2OH\- exchange mediates most SO\(_4\) uptake. On the other hand, in secretory glands, like the pancreas (38) and salivary glands (39), luminal pH is alkaline, which inhibits SO\(_4\)/in/Cl\- exchange (Fig. 3B) and most SO\(_4\) uptake is by SO\(_4\)/out/2Cl\- exchange.

Regulation of Slc26a2 by Cl\-out: While measuring net SO\(_4\) efflux we noticed that removal of SO\(_4\) in the continuous absence of Cl\-out never resulted in SO\(_4\) efflux, as would be expected from SO\(_4\)/in/2OH\- out exchange. This is illustrated in the period bordered by the dashed box of Fig. 4A. However, addition of as little as 1 mM Cl\-out triggered a robust SO\(_4\)/in/2OH\- out exchange and a small Cl\- influx (Fig. 4A, period marked by gray box). The dependence of the SO\(_4\)/in/2OH\- out exchange rate of Cl\-out followed simple saturation curve.
with apparent $K_m$ of 3.7±0.9 mM (Fig. 4B). Activation of the exchange was not specific for Cl', Fig. 4C shows that 1 mM external Cl', Br', I', NO$_3^-$ and SCN$^-$ similarly activated SO$_4^{2-}$/2OH$^-$/2Cl$^-$ exchange. Only 1 mM F$^-$ did not activate the exchange (Fig. 4C), but actually inhibited the exchange initiated by the other anions (not shown).

The findings in Fig. 4 suggest that SO$_4^{2-}$ transport by Slc26a2 is regulated by interaction of an anion with a regulatory site. The regulatory site is not selective for Cl', but because Cl' is the major extracellular anion, Slc26a2 is likely regulated by Cl'inter action with the regulatory site. The Cl'out regulatory site is likely different from the transport site since increased Cl'out should increase SO$_4^{2-}$/2Cl'out exchange while reducing SO$_4^{2-}$/2OH$^-$/out exchange. However, the opposite is observed. Activation of Slc26a2-mediated SO$_4^{2-}$/2OH$^-$/out by Cl'out may be by stabilization of an active Slc26a2 conformation. However, the exact mechanism remains to be elucidated. The physiological significance of regulation of Slc26a2 activity by Cl'out is not known at present. The Cl' content in the GI tract is high in the intestine (42) and the vas deferens (43). One possibility such as the pancreatic (38) and salivary (32) ducts, the concentration of SO$_4^{2-}$/2Cl' when luminal Cl' becomes very low due to Cl' absorption. Perhaps in Cl' absorbing epithelia completion of Cl' absorption may be used to signal termination of the SO$_4^{2-}$/Cl' absorptive activity. Additionally, regulation by Cl'out may be used to stop reverse Slc26a2 transport to prevent SO$_4^{2-}$/Cl' loss by SO$_4^{2-}$/Cl' efflux across the luminal membrane due to SO$_4^{2-}$/2OH$^-$/out when luminal Cl' becomes very low due to Cl' absorption.

A Potential Slc26a2 permeation pathway: In a previous study we developed a model of the Slc26a6 transmembrane sector to search for motifs that determine the function of the electrogenic Slc26 transporters as coupled and uncoupled transporters (29). The modeling identified a glutamate (Glu') conserved in all Slc26 transporters that has the same orientation as Glu' E148 in the Cl' permeation pathway of the ClCs transporters (44-47). Interestingly, a recent study utilized our predicted Slc26a6 model and the crystal structure of the Slc26a5 STAS domain to assemble a detailed putative structure of Slc26 transporters (48). This structure showed a surprising similarity to the low resolution structure of a bacterial Slc26 homologue obtained using SANS (small angle neutron scattering) method in terms of symmetry and size. Notably, this study suggested that Slc26 functions as a dimer, as was previously suggested based on the predicted similarity of Slc26a6 to the bacterial ClC-ec dimeric crystal structure. Therefore, assuming a similar overall architecture for Slc26 transporters, we thread the Slc26a2 transmembrane sector on the Slc26a6 model to determine the localization of the conserved Glu' E417 (Fig. 5). Another purpose of the modeling was to identify additional determinants of the Slc26a2 ion permeation pathway and perhaps the extracellular Cl' regulatory site. The motif GSGIP was identified as a potential anion (Cl') binding site that is conserved in the CLC transporters (44,49). Mutations of residues within this motif altered ionic selectivity and coupling in the yeast and mammalian ClCs (50,51). We searched for a similar motif in the Slc26 transporters. Although identical motif is not present in the Slc26 transporters, Supplementary Fig. 2 shows the presence of the well conserved sequence GFXXP. The structural model in Fig. 5 shows the predicted localization of the Slc26a2 conserved Glu' E417 and phenylalanine F368 and of a potential DIDS binding site.

To test the prediction in Fig. 5 we first determined the sensitivity of Slc26a2 to DIDS. Fig. 6A shows that 50 µM DIDS completely inhibited SO$_4^{2-}$/OH$^-$/H$^+$ efflux and most of the SO$_4^{2-}$/Cl' efflux. The residual DIDS-insensitive Cl' efflux is likely not mediated by Slc26a2 but by a DIDS-insensitive transporter native to the oocytes. Fig. 6B shows that DIDS inhibited SO$_4^{2-}$/Cl' efflux when added after SO$_4^{2-}$/Cl' uptake. Also in this case DIDS completely inhibited OH$^-$/H$^+$ influx, but with a residual Cl' influx. Similar results were obtained with 10 and 50 µM DIDS, indicating that the DIDS sensitivity of Slc26a2 is in the same range of that reported for Slc26a6 (52). Fig. 6C summarizes the rates of OH$^-$ and Cl' fluxes in the absence and presence of SO$_4^{2-}$/Cl' and DIDS, indicating that at pH$_{out}$ of 7.5 and the absence of Cl'out about 60% of SO$_4^{2-}$/Cl' uptake in coupled to OH$^-$/Cl' efflux and 40% to Cl' efflux. Fig. 6D test another prediction of the model in Fig. 5 by neutralizing (Slc26a2(E417A)) or reversing (Slc26a2(E417K)) the charge of the conserved E417. Both mutations eliminated SO$_4^{2-}$/Cl' uptake and transport activity. Inhibition of transport was not due to altered trafficking of the mutants to the plasma membrane (Fig. 6E).

The sequence GFXXP is predicted to be in the extracellular loop between transmembrane domains (TMD) 7 and 8, with F368 predicted to be in the entrance of the permeation pathway (Fig. 5). The mutations G367A and P371A had no effect on SO$_4^{2-}$/Cl' transport or its coupling to Cl' and OH$^-$ (not shown). However the F368A mutation had multiple effects. Fig. 7A shows that Slc26a2(F368A) is about 50% less active
than wild-type Slc26a2 in exchanging $\text{SO}_4^{\text{in}}$ for $\text{OH}^-$ (left traces) and $\text{Cl}^-$ (right traces). Most notably, the F368A mutation increased the apparent affinity of Slc26a2 for $\text{SO}_4^{\text{in}}$ by about 8 fold to reduce the apparent $K_m$ for $\text{SO}_4^{\text{out}}$ from 79±7 to 9.7±0.7 µM. Unexpectedly from competition between $\text{SO}_4^{\text{out}}$ and $\text{Cl}^-$ (Fig. 2), the F368A mutation increased the apparent $K_m$ for inhibition of $\text{SO}_4^{\text{in}}$ uptake by $\text{Cl}^-$ from 26 to 50 mM (Fig. 7C). Hence, F368 appears to control the access of $\text{SO}_4^{\text{in}}$ and $\text{Cl}^-$ to the permeation pathway. Interestingly, Fig. 7D shows that the F368A mutation had no effect of the apparent affinity for the $\text{Cl}^-$ regulatory site that activates $\text{SO}_4^{\text{in}}/\text{OH}^-$ exchange. This finding provides the strongest evidence that inhibition of $\text{SO}_4^{\text{in}}$ uptake by $\text{Cl}^-$ (Figs. 4B and 7C) and activation of $\text{SO}_4^{\text{in}}/\text{OH}^-$ exchange by $\text{Cl}^-$ out probably involves interaction of $\text{Cl}^-$ in with two separate sites.

The findings in Fig. 7A-C provide additional evidence for the importance of the GSGIP or the GFXXP motifs in the function of the $\text{Cl}^-$ transporters, in addition to the two additional GXXXP motifs that participate in $\text{Cl}^-$ transport in the bacterial CLCs (44). The bacterial CLC-ec1 crystal structure shows that the permeation pathway has three $\text{Cl}^-$ interacting sites (44-46,49). Ser107 and Gly108 in the GSGIP motif coordinate the $\text{Cl}^-$ ion in the internal substrate site and the side chain of S107 participates in binding of the middle $\text{Cl}^-$ (45,49). In Slc26a2 F368 appears to control the affinity for the substrate ($\text{SO}_4^{\text{in}}$), suggesting that F368 may participate in the access of $\text{SO}_4^{\text{in}}$ to the permeation pathway or in shaping the external $\text{SO}_4^{\text{in}}$ binding site. The increased apparent affinity for $\text{SO}_4^{\text{in}}$ and reduced apparent affinity for $\text{Cl}^-$ by the F368A mutation suggests that F368 may hinder access of $\text{SO}_4^{\text{in}}$ and facilitate access of $\text{Cl}^-$ to the permeation pathway or reduces the time $\text{SO}_4^{\text{in}}$ spends in the external binding site on its way across the plasma membrane. Perhaps this is necessary to allow $\text{SO}_4^{\text{in}}/\text{Cl}^-$ exchange at high $\text{SO}_4^{\text{in}}$ when $\text{SO}_4^{\text{out}}$ efflux is required. Irrespective of the exact role of F368, the present findings further support the notion of similarities between the CLC and SLC26 transporters permeation pathways and that the opening of the permeation pathway is situated in the region of TMDs 7 and 8.

**Properties of Slc26a2-mediated Oxalate transport:** Slc26a2 was reported to transport Oxalate ($\text{Ox}^-$) by mediate $\text{Ox}^-/\text{SO}_4^{\text{in}}$ exchange (8,24-26,28) and that $\text{Ox}^-/\text{SO}_4^{\text{in}}$ exchange is 10 times slower than $\text{SO}_4^{\text{in}}/\text{Cl}^-$ exchange (24). However, the properties and mode of $\text{Ox}^-$ transport and the capacity of net $\text{Ox}^-$ transport by Slc26a2 are not known. We set to estimate net $\text{Ox}^-$ transport by measuring $\text{Ox}^-$-mediated $\text{OH}^-$ and $\text{Cl}^-$ fluxes. Fig. 8A shows that Slc26a2 mediates net $\text{Ox}^-_{\text{out}}/\text{OH}^-_{\text{in}}$ and $\text{Ox}^-_{\text{out}}/\text{Cl}^-_{\text{in}}$ exchange in oocytes bathed in $\text{Cl}^-$-free solution containing 1 mM $\text{Ox}^-$, pH 7.5. Removal of $\text{Ox}^-$ was not followed by $\text{Ox}^-$ efflux until the addition of 1 mM $\text{Cl}^-_{\text{out}}$ to activate the efflux. Importantly, addition of 1 mM $\text{Cl}^-_{\text{out}}$ resulted in minimal $\text{Ox}^-_{\text{in}}/\text{Cl}^-_{\text{out}}$ exchange but near maximal $\text{Ox}^-_{\text{out}}/\text{OH}^-_{\text{in}}$ exchange. Increasing $\text{Cl}^-_{\text{out}}$ to 110 mM caused a small additional increase in $\text{Ox}^-_{\text{in}}/\text{OH}^-_{\text{in}}$ exchange and modest $\text{Ox}^-_{\text{in}}/\text{Cl}^-_{\text{out}}$ exchange. As expected, Figure 8B shows that reducing pH$_{\text{out}}$ to 6.5 increased the rate of $\text{Ox}^-_{\text{in}}/\text{OH}^-_{\text{in}}$ exchange and increasing pH$_{\text{out}}$ to 8.5 inhibited the $\text{Ox}^-_{\text{out}}/\text{OH}^-_{\text{in}}$ exchange. Fig. 8C shows the opposite effect of pH$_{\text{out}}$ on the $\text{Ox}^-_{\text{in}}/\text{OH}^-_{\text{in}}$ exchange. Finally, the F368A mutation increased the apparent affinity for $\text{Ox}^-$ and reduced the apparent $K_m$ for $\text{Ox}^-$ from 90±12 to 50±8 µM. Although this was not as prominent as the increased apparent affinity for $\text{SO}_4^{\text{in}}$ (Fig. 7), it was in the same direction. The results in Figs. 8A-D indicate that the properties of $\text{Ox}^-$ transport closely resemble those of $\text{SO}_4^{\text{in}}$ transport, although at the same conditions $\text{Ox}^-$ transport rate is about 50% slower than $\text{SO}_4^{\text{in}}$ transport rate.

In summary, the present study reports the mechanism of $\text{SO}_4^{\text{in}}$ and $\text{Ox}^-$ transport by Slc26a2. Both anions are transported in exchange for $\text{Cl}^-$ and $\text{OH}^-$ or by cotransport with $\text{H}^+$. Based on the rate of the coupled $\text{OH}^-(\text{H}^+)$ fluxes in the absence of $\text{Cl}^-_{\text{out}}$ and at substrate concentration of 1 mM, net $\text{SO}_4^{\text{in}}$ transport by Slc26a2 is about twice faster than net $\text{Ox}^-$ transport. Under normal conditions plasma oxalate is the µM range and even in patients with primary hyperoxaluria plasma oxalate is around 40 µM (53). Moreover, although Slc26a2 is expressed at high level in the luminal membrane of colonic crypts (54), $\text{SO}_4^{\text{in}}$ in the colon can be in the mM range both in human (55) and animals (56) that will favor $\text{SO}_4^{\text{in}}$ uptake by Slc26a2. Indeed, the colon is a major site of $\text{SO}_4^{\text{out}}$ absorption (55,57) that is likely mediated by Slc26a2. Similarly, although Slc26a2 is expressed in the proximal tubule luminal membrane (31), $\text{SO}_4^{\text{in}}$ concentration in the proximal tubule is in the mM range and although the role of Slc26a2 in the kidney is not known, if any it is likely to function mainly as a $\text{SO}_4^{\text{in}}$ transporter (8). The only possible scenario where Slc26a2 can affect $\text{Ox}^-$ homeostasis is by mediating $\text{Ox}^-$ secretion in exchange for external $\text{SO}_4^{\text{in}}$ when external $\text{Cl}^-$ is low and pH is high. Even then, this process will be inhibited by the high cytoplasmic $\text{Cl}^-$ typical of epithelia and by intracellular $\text{SO}_4^{\text{in}}$. Thus Slc26a2 is not likely to play a major role in oxalate metabolism in the colon or the kidney.

The permeation pathway includes the conserved SLC26 transporters Glu and may lay between TMD7 and TMD8, where a phenylalanine conserved in the loop predicted to connect the TMDs may control $\text{SO}_4^{\text{in}}$ and $\text{Cl}^-$ access to the permeation pathway. As yet
mutations of these residues, or even in the vicinity of these residues, have not been found in patients with diastrophic dysplasia (58). This is most likely because Slc26a2 is an essential gene and the mutations markedly affect Slc26a2 activity, and thus may not be compatible with life. Indeed, analysis of several disease causing Slc26a2 mutations showed that retention of some SO_4^{2-} transport capacity by the mutants and a good correlation between loss of SO_4^{2-} transport and disease severity (25,26). The coupling of SO_4^{2-} transport to both OH^- and Cl^- likely serves to ensure transport at both acidic pH when most SO_4^{2-} uptake is mediated by SO_4^{2-}/2OH^- exchange and alkaline pH when most SO_4^{2-} uptake is mediated by SO_4^{2-}/2Cl^- exchange. Slc26a2 is also regulated by an extracellular anion binding site different from the transport site, the physiological function of which remains to be determined, although it may control SO_4^{2-} uptake when Cl^- out is very low.

References


Abbreviations: Slc26a2, Solute Carrier family 26 isoform a2; DTDST, diastrophic dysplasia; pH(SO4,Cl-)in, intracellular pH(SO4,Cl-); pH(SO4,Cl-)out, extracellular pH(SO4,Cl-); DIDS, Diisothiocyanostilbene-2,2′-disulfonic acid; TMD, transmembrane domain.

Acknowledgement
This research was supported by the Intramural Research Program of the NIH, NIDCR grant Z1A-DE000735. E.O was supported in part by the Machiah Fellowship grant 2008-0702 awarded through the Machiah Foundation, a supporting foundation of the Jewish Community Federation of San Francisco, the Peninsula, Marin and Sonoma Counties.

Figure Legends
Fig. 1: Slc26a2 functions as an electroneutral SO4+/OH-(H+)/Cl- exchanger. pHin (dark traces) and Cl- in (dark gray traces) and membrane potential (light gray traces) were simultaneously recorded in Slc26a2 (A) or H2O injected (C) oocytes. Note that the membrane potential did not change following addition of SO4 2-. (B) Averaged (mean±S.E.M of the indicated number of experiments) transport rates of Cl- and OH(H+) in the presence and absence of 0.2 mM SO4 2- were used to determine net transport (right most columns). (D) pHin was recorded while clamping membrane potential at -100 , +40 or -30 mV, as indicated.

Fig. 2: Effect of Cl out on Slc26a2-mediated SO4 2-/OH- exchange. (A) Slc26a2 expressing oocytes were alternately perfused with a solution containing 110 mM Cl- and Cl-free solutions containing the indicated SO4 2- concentrations for about 5 min. The plots in (B) are the relative average rates of OH fluxes as a function of SO4 2- at Cl- out of 0 (.), 5 (m), 20 (A) and 50 (b) mM. The rates were normalized to the rate at 30 mM SO4 2- that was taken as 100% and the plots were fitted to the Hill equation. The averages are the mean±S.E.M of 3-5 experiments at each Cl out concentration. The resulting apparent Km values for SO4 2- are plotted as a function of Cl out (C). (D) Control (, H2O injected, black trace) and Slc26a2-expressing oocytes (gray trace) bathed in HCO3- buffered solution were...
exposed to Cl⁻-free solution at the indicated time to assay Cl⁻/HCO₃⁻ exchange activity. In (E) the exchange of I⁻, Br⁻ and NO₃⁻ with Cl⁻ was measured by incubating oocytes in Cl⁻-free solution containing 2 mM of I⁻ (left traces), Br⁻ or NO₃⁻ (right traces). The influx was terminated by removal of the anions from the bath and anion efflux was initiated by addition of Cl⁻ to the bath. The lack or minimal anion efflux in the absence of Cl⁻ out indicates low net and exchange rate of the anions with OH⁻ and the rapid efflux of the anions upon addition of Cl⁻ out indicates fast exchange rate of the anions with Cl⁻. The control in the left panel is representative of oocytes injected with H₂O.

**Fig. 3: Effect of pH out on SO₄²⁻ out influx and SO₄²⁻ in efflux.** (A) Example traces for measurement of pH in and Cl⁻ in solutions buffered to pH out of 6.5 (black traces) or 8.2 (gray traces). The models in (B) shows the direction of the fluxes and the average influx and efflux rates of OH⁻ (H⁺) (dark columns) and Cl⁻ (gray columns) fluxes are summarized in (B). The results are the mean±S.E.M of the number of experiments listed in the columns. In (C) pH in and Cl⁻ in were measured at 110 mM Cl⁻ out and first at pH out of pH7.5 and then at pH out of 6.5. The columns on the right show the average rates (mean±S.E.M) of Cl⁻ and OH⁻ influx at pH in of 6.5 upon removal of SO₄²⁻ and the lower traces are example traces obtained in H₂O-injected oocytes.

**Fig. 4: Activation of Slc26a2-mediated SO₄²⁻ out/OH⁻ out exchange by Cl⁻ out.** (A) Example traces of Cl⁻ in (black) and pH in (gray) in oocytes expressing Slc26a2. SO₄²⁻ influx was terminated by removal of SO₄²⁻ out (period bordered by dashed line). SO₄²⁻ efflux did not start until the addition of 1 mM Cl⁻ out (period bordered by gray square), which triggered robust SO₄²⁻ out/OH⁻ out exchange with minimal SO₄²⁻ in/Cl⁻ out exchange. (B) The relative rate of SO₄²⁻ in/OH⁻ out exchange is plotted as a function of the activating Cl⁻ out. The rates at each Cl⁻ out were normalized to the rate measured at 110 mM Cl⁻ out which was taken as 100%. The plot is the average of 3-5 experiments and fitted to the Hill equation. (C) The protocol in (A) was used to measure activation of SO₄²⁻ in/OH⁻ out exchange by 1mM of the indicated anions (period marked by gray square). The results are mean±S.E.M. of the number of experiments indicated in the columns.

**Fig. 5: In Silico model of the putative structure of the Slc26a2 TMDs.** The model was derived by threading the TMD sector of Slc26a2 on the TMDs of Slc26a6 reported before (29) (see Methods). (A) Shows the predicted position of the 12 transmembrane helices of Slc26a2. (B) shows the space filling of the GFMPG sequence. Highlighted in (C) are the position of the extracellular loop located between TMDs 7 and 8 (light blue), the conserved phenylalanine F368 (blue), the conserved Glu E417 (green) and the putative position of DIDS binding site (red). (D) Illustrates a cross-section (~20Å) through the surface representation of the putative Slc26a2 revealing a potential binding cavity. Interestingly, Glu 417 (green) and the EC loop (orange) are constituents of the binding pocket (outlined in cyan) in which the inhibitor DIDS (red) is also bound.

**Fig. 6: Inhibition of Slc26a2 by DIDS and by mutations of E417.** Example traces depicting inhibition of SO₄²⁻ influx (A) and SO₄²⁻ efflux (B) by 50 µM DIDS. Note the complete inhibition of the coupled OH⁻ but not of Cl⁻ fluxes. (C) The average rates (mean±S.E.M of the indicated number of experiments) of OH⁻ and Cl⁻ fluxes. In (D) shown are examples of oocytes expressing either wild-type Slc26a2, Slc26a2(E417A) or Slc26a2(E417K) that were used to measure the SO₄²⁻-associated OH⁻ and Cl⁻ fluxes. (E) shows the surface expression of Slc26a2 and mutants with actin used as a control for the biotinylation.

**Fig. 7: F368 in Slc26a2 permeation pathway.** (A) Example traces for pH in and Cl⁻ in measurement in oocytes expressing either wild-type (gray traces) or Slc26a2(F368A) (black traces). This protocol was used to monitor Slc26a2-mediated the SO₄²⁻ dependence of SO₄²⁻ out/OH⁻ in exchange (B) and inhibition of the exchange by Cl⁻ out (C). SO₄²⁻ in/OH⁻ out exchange was used to monitor activation of the reverse exchange by Cl⁻ out (D). All plots (B-D) were fitted to the Hill equation and Km values are given as mean±S.E.M.

**Fig. 8: Properties of Slc26a2-mediated oxalate (Ox⁻) transport.** (A) Example traces of the Slc26a2-mediated OH⁻ (black trace) and Cl⁻ (gray trace) efflux in response to addition of 1mM Ox⁻ out to Cl⁻-free solution at pH 7.5. Removal of Ox⁻ out terminated the influx (dashed box) and addition of 1 mM Cl⁻ out was required to initiate Ox⁻ in/OH⁻ out exchange (gray box) with minimal Ox⁻ in/Cl⁻ out exchange. (B, C) Effect of pH out of Ox⁻ fluxes was as in (A), except that the Ox⁻ efflux was initiated by the addition of 110 mM Cl⁻ out and pH out was set at 6.5 (dark columns), 7.5 (gray columns) or 8.2 (empty columns). The results are the mean±S.E.M of the number of experiments indicated in the columns. (D) The dependence of Ox⁻ out/OH in exchange on Ox⁻ out concentrations was measured with wild-type Slc26a2 (○) and Slc26a2(F368A) (●). The apparent Kms are given as the mean±S.E.M of 4-5 experiments.
Figure 1, Ohana et al

A

B

C

D

Ion Flux rate (mM/min)

0.0 0.5 1.0 1.5

OH⁻(H⁺) Total Net

0 Cl⁻ 0 SO₄²⁻ 0 Cl⁻ 0 SO₄²⁻

0.2 mM SO₄²⁻ 0.2 mM SO₄²⁻

Cl⁻ 10 min

+40 +40 -30 mV

10 min

+40 +40 -30 mV

Cl⁻ 10 min

0 Cl⁻ 0 SO₄²⁻ 0 Cl⁻ 0 SO₄²⁻

0.2 mM SO₄²⁻ 0.2 mM SO₄²⁻

pHi Cl-i mV

0 Cl⁻ 0 SO₄²⁻ 0 Cl⁻ 0 SO₄²⁻

0.2 mM SO₄²⁻ 0.2 mM SO₄²⁻
Figure 2, Ohana et al

A) pH

B) Extracellular Cl\(^-\) (mM) vs. V (% of Vmax)

C) Apparent Km for SO\(_4^{2-}\)

D) pH

E) I- influx (Arbitrary Units)

V (% of Vmax) vs. mM external SO\(_4^{2-}\)

- Control
- Slc26a2

<table>
<thead>
<tr>
<th>Cl(^-) out (mM)</th>
<th>AppKm (mM)</th>
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<tbody>
<tr>
<td>0</td>
<td>0.08±0.01</td>
</tr>
<tr>
<td>5</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>20</td>
<td>3.0±0.5</td>
</tr>
<tr>
<td>50</td>
<td>5.5±1.3</td>
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</table>

10 min

<table>
<thead>
<tr>
<th>Cl(^-)</th>
<th>I- influx (Arbitrary Units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Cl(^-)</td>
<td>0</td>
</tr>
<tr>
<td>2 mM Br(^-), NO(_3^-)</td>
<td>0</td>
</tr>
</tbody>
</table>

110 mM Cl\(^-\)
Figure 3, Ohana et al

**A**

- pH\textsubscript{in} vs. time
- Cl\textsuperscript{-} and SO\textsubscript{4}\textsuperscript{2-} concentrations

**B**

- SO\textsubscript{4}\textsuperscript{2-}-dependent OH\textsuperscript{-} and Cl\textsuperscript{-} efflux
- SO\textsubscript{4}\textsuperscript{2-}-dependent OH\textsuperscript{-} and Cl\textsuperscript{-} influx

**C**

- pH\textsubscript{in} vs. time
- Cl\textsuperscript{-} concentrations
- Cl\textsuperscript{-} fluxes in mM/min

**Graphs**

- H\textsuperscript{+} (OH\textsuperscript{-}) Fluxes
- Cl\textsuperscript{-} fluxes
Figure 4, Ohana et al

A

Flux rate (% of Vmax)

Extracellular Cl\textsuperscript{-} (mM)

18

20

22

24

0 Cl\textsuperscript{-}

0.2 SO\textsubscript{4}\textsuperscript{2-}

1 Cl\textsuperscript{-}

110 Cl\textsuperscript{-}

10 min

pH\textsubscript{in}

7.3

7.0

6.7

B

Km=3.7±0.9 mM

50

100

Flux rate (% of Vmax)

0

Extracellular Cl\textsuperscript{-} (mM)

C

Transport Rate (∆pH/min)

0.00

0.02

0.04

Cl\textsuperscript{-}

Br\textsuperscript{-}

I\textsuperscript{-}

NO\textsubscript{3}\textsuperscript{-}

SCN\textsuperscript{-}

F\textsuperscript{-}

5

4

4

3

3
Figure 5, Ohana et al

A

EC loop between TMD7 and TMD8

Phe 368

Glu 417

DIDS

B

Gly367
Phe368
Met369
Pro370,371
Glu417

Pocket contour

EC loop

Phe368

Met369

DIDS

14
Figure 6, Ohana et al

A  

pH<sub>in</sub>  

10 min  

6.8  

7.0  

7.2  

7.4  

22  

24  

26  

28  

50 μM DIDS  

0 Cl<sup>-</sup> 0.2 SO<sub>4</sub><sup>2-</sup>  

B  

pH<sub>in</sub>  

10 min  

6.8  

7.0  

7.2  

7.4  

110 Cl<sup>-</sup>  

50 μM DIDS  

C  

H<sup>+</sup> Fluxes  

Cl<sup>-</sup> Fluxes  

Cl<sup>-</sup>/OH<sup>-</sup> Flux in mM  

0 Cl<sup>-</sup> 0.2 SO<sub>4</sub><sup>2-</sup> DIDS  

D  

pH<sub>in</sub>  

E417K  

E417A  

Wild-type  

5 min  

0 Cl<sup>-</sup> 

1 mM SO<sub>4</sub><sup>2-</sup>  

E  

Vector SLC26a2 SLC26a2(E417A) SLC26a2(E417K)  

Surface Input  

Surface Input  

Actin SLC26a2  

Wild-type
Figure 7, Ohana et al

A

\[ \text{pH}_{\text{in}} \]

F368A

Wild-type

\[ 0 \text{ Cl}^- \]

\[ 1 \text{ mM SO}_4^= \]

\[ 10 \text{ min} \]

B

\[ \text{OH}-\text{influx rate (v/Vmax)} \]

\[ \text{SO}_4^= \text{ concentration (mM)} \]

\[ \text{Km}=9.7\pm0.7 \mu\text{M} \]

\[ \text{Km}=79\pm7 \mu\text{M} \]

\[ \text{Wild-type} \]

\[ \text{F386A} \]

C

\[ \text{OH}-\text{influx rate (% inhibition)} \]

\[ \text{Cl}^- \text{ concentration (mM)} \]

\[ \text{Km}=26\pm4 \text{ mM} \]

\[ \text{Km}=50\pm7 \text{ mM} \]

\[ \text{Wild-type} \]

\[ \text{F368A} \]

D

\[ \text{OH}-\text{efflux rate (v/Vmax)} \]

\[ \text{Cl}^- \text{ concentration (mM)} \]

\[ \text{Wild-type, Km}=8.4\pm4.0 \text{ mM} \]

\[ \text{F368A, Km}=9.7\pm4.3 \text{ mM} \]
S1c26a2 functions as an electroneutral SO4=/OH-/Cl- exchanger regulated by extracellular Cl-
Ehud Ohana, Nikolay Shcheynikov, Meeyoung Park and Shmuel Muallem

J. Biol. Chem. published online December 21, 2011

Access the most updated version of this article at doi: 10.1074/jbc.M111.297192

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