SLC26A7 Is a Cl⁻ Channel Regulated by Intracellular pH*

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Members of the SLC26 transporter family play an essential role in several epithelial functions, as revealed by diseases associated with mutations in members of the family. Several members were shown to function as Cl⁻ and HCO₃⁻ transporters that likely play an important role in epithelial Cl⁻ absorption and HCO₃⁻ secretion. However, the mechanism of most transporters is not well understood. SLC26A7 is a member of the SLC26 transporter family reported to be expressed in the basolateral membrane of the cortical collecting duct and parietal cells and functions as a coupled Cl⁻/HCO₃⁻ exchanger. In the present work we examined the transport properties of SLC26A7 to determine its transport characteristics and electrogenicity. We found that when expressed in Xenopus oocytes or HEK293 cells SLC26A7 functions as a pH₁-regulated Cl⁻ channel with minimal OH⁻/HCO₃⁻ permeability. Expression of SLC26A7 in oocytes or HEK293 cells generated a Cl⁻ current with linear IV and an instantaneous current that was voltage- and time-independent. Based on measurement of reversal potential the selectivity of SLC26A7 is NO₃⁻ >> Cl⁻ = Br⁻ = I⁻ > SO₄²⁻ = Glu⁻, although I⁻ partially inhibited the current. Incubating the cells with HCO₃⁻ or butyrate acidified the cytosol and increased the selectivity of SLC26A7 for Cl⁻. Measurement of membrane potential and pH₁ showed minimal OH⁻ and HCO₃⁻ transport by SLC26A7 when the cells were incubated in Cl⁻-containing or Cl⁻-free media. The activity of SLC26A7 was inhibited by all inhibitors of anion transporters tested, 4,4'-disothiocyanostilbene-2,2'-disulfonic acid, diphenylamine-2-carboxylic acid, and glybenclamide. These findings reveal that SLC26A7 functions as a unique Cl⁻ channel that is regulated by intracellular H⁺.

The SLC26A7 is a relatively new family of Cl⁻ and HCO₃⁻ transporters that is coded by at least 10 genes, most of which have several splice variants (1). The founding member of the family is SLC26A3, mutations of which are associated with congenital Cl⁻ diarrhea (2). Other members of the family include SLC26A2, mutations of which are associated with diastrophic dysplasia (3), pendrin (SLC26A4), mutations of which cause Pendred syndrome (4) and Prestin (SLC26A5), which is involved in hearing (5). SLC26A3 and SLC26A6 appear to play important roles in epithelial Cl⁻ absorption and HCO₃⁻ secretion (1). These SLC26 transporters are expressed in the luminal membrane of ductal systems that also express the cystic fibrosis transmembrane conductance regulator (CFTR) (6). All SLC26 transporters examined are activated by CFTR and in turn activate CFTR by increasing its open probability (7).

All members of the family tested so far were shown to function as exchangers with defined substrate specificity. SLC26A1 (8) and SLC26A2 (9) are SO₄²⁻ transporters but can also transport Cl⁻. SLC26A3 is a Buna feed coupled Cl⁻/HCO₃⁻ exchanger (6, 10), SLC26A4 is an I⁻ transporter that can also function as a Cl⁻/HCO₃⁻ exchanger (4, 6, 11), and SLC26A6 functions as a Cl⁻/oxalate (12) and Cl⁻/HCO₃⁻ exchanger (6, 13). The exact transport mechanism of most SLC26 transporters is not well understood. However, an important recent finding was that the SLC26 transporters are electrogenic (6, 13) with isoform-specific stoichiometry (6).

Another SLC26 transporter that was characterized recently is SLC26A7 (14–16). SLC26A7 shows very restricted distribution, and so far it has been found only in gastric parietal cells (15) and the intercalated cells of the outer medullary collecting duct (16). Unlike other members of the family that are expressed at the luminal membrane of epithelial cells (1), SLC26A7 was found at the basolateral membrane of both parietal cells and intercalated cells (15, 16). It was reported that SLC26A7 functions as a coupled Cl⁻/HCO₃⁻ exchanger (15, 16) and was thus suggested to play an important role in clearing HCO₃⁻ from the parietal cells during acid secretion (15). Furthermore, SLC26A7 was reported to be markedly activated by cell shrinkage and thus to mediate HCO₃⁻ efflux into the hypertonic fluid of the collecting duct (16).

As part of our effort to understand the role of the SLC26 transporters in epithelial Cl⁻ absorption and HCO₃⁻ secretion and to determine whether SLC26A7 is an electrogenic transporter, we characterized the transport properties of SLC26A7. Surprisingly, we were unable to show that SLC26A7 functions as a Cl⁻/HCO₃⁻ exchanger or even that SLC26A7 transports much of HCO₃⁻. In fact, of all the SLC26 transporters examined to date in our laboratory (SLC26A3, -4, -6, and -7), SLC26A7 is the least permeable to HCO₃⁻. Rather, when expressed in Xenopus oocytes or HEK293 cells SLC26A7 behaves as a Cl⁻ channel. The selectivity of SLC26A7 was NO₃⁻ >> Cl⁻ = Br⁻ = I⁻ inhibited the current. Interestingly, SLC26A7 is regulated by pH₁, where H⁺ does not increase the current but rather increases the selectivity of SLC26A7 for Cl⁻.

**EXPERIMENTAL PROCEDURES**

*Materials—2',7'-Bis(2-carboxyethyl)-5,6-carboxyfluorescein ace-toxymethyl ester was from Telf Laboratories, DIDS (4,4'-disothiocyanostilbene-2,2'-disulfonic acid) was from Molecular Probes, diphenylamine-2-carboxylic acid (DPC) was from Alexis Corp., and glybenclamide was from Sigma. All other chemicals and salts were from Sigma. The expressed sequence tag clone of mouse SLC26A7 was ob-

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1 The abbreviations used are: CFTR, cystic fibrosis transmembrane conductance regulator; MQA, N-ethoxycarbonylmethyl-6-methoxyquinolinium bromide; RP, reversal potential; DIDS, 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid; DPC, diphenylamine-2-carboxylic acid; Glyb, glybenclamide.
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FIG. 1. Cl⁻ current in Xenopus oocytes expressing SLC26A7. A, control oocytes (▲, ▼) and oocytes expressing SLC26A7 (●, ○) were used to measure the I/V in the presence (●, ▲) and absence (○, ▼) of Cl⁻. B shows the instantaneous current. C shows the current in the presence of Cl⁻ (●, NO₃⁻ (○) or I⁻ (▲)). D summarizes the reversal potential as measured with the indicated ions. The boxed numbers indicate the number of experiments under each condition.

Measurement of pH, and [Cl⁻], in HEK293 Cells—The procedure for pH measurement in HEK293 cells was identical to that described previously (17). HEK293 cells were loaded by incubation with 5 mM MQAE for 1 h at room temperature. MQAE fluorescence was recorded at an excitation wavelength of 360 nm while the cells were perfused with solutions containing 150 mM Cl⁻ or 150 mM NO₃⁻.

Electrophysiology—The whole cell configuration of the patch clamp technique was used to measure the Cl⁻ current in control and SLC26A7-transfected HEK293 cells as detailed before (19). The pipette solution contained (in mM) 140 N-methyl-D-glucamine-Cl, 1 MgCl₂, 2 EGTA, 5 ATP, and 10 HEPES (pH 7.3 with Tris), and the bath solution was Na⁺-free solution A. The current was recorded using an Axopatch 200A patch clamp amplifier and digitized at 2 kHz. The membrane conductance was probed by stepping the membrane potential from a holding potential of 0 mV to membrane potentials between −100 and +60 mV at 10-mV steps for 200 ms with 500-ms intervals between steps. Pipettes had resistance between 5 and 7 megohms when filled with an intracellular solution, and seal resistance was always more than 8 gigohms. Current recording and analysis were performed with the pClamp 6.0.3 software.

For current recording in oocytes the oocytes were obtained by partial ovariectomy of anesthetized female Xenopus laevis. Follicles were reovulated by incubation of 10–25 ng of cRNA in a final volume of 50 nl. Injected oocytes were incubated at 18 °C in an ND-96 solution composed of (in mM) 96 NaCl, 2 KCl, 1.8 CaCl₂, 1 MgCl₂, 2.5 pyruvate, 5 HEPES-Na, pH 7.5, and oocytes were used 48–96 h post-injection. Na⁺-free media were prepared by replacing Na⁺ with N-methyl-D-glucamine, and Cl⁻-free media were prepared by replacing Cl⁻ with gluconate. To
prepare solutions containing Br\textsuperscript{−}, I\textsuperscript{−}, or NO\textsubscript{3}\textsuperscript{−}, NaCl and KCl were replaced with the relevant salt that was supplemented with 1.8 mM Ca\textsuperscript{2+}-cycamate, 1 M\textsubscript{g}SO\textsubscript{4}, 2.5 pyruvic acid, 5 HEPES, pH 7.5, with Tris-Acetate voltage clamp setup was used to record the current as detailed before (6). Current and voltage were digitized with a Digitex 1222A A/D converter and analyzed by the Clampex 8.1 system.

The pH of oocytes was measured with single-barreled borosilicate-silanized microelectrodes as described before (20). In brief, ~0.5 µl of proton exchanger resin was introduced into the tip of the microelectrode, and the electrodes were backfilled with an ND-90 solution and calibrated against standard solutions of pH 6, 7, and 8 before and after each experiment. The electrodes were fitted with a holder with an Ag-AgCl wire attached to a high impedance probe of a two-channel FD-223 electrometer. A second channel was used for measurement of membrane potential using a standard microelectrode. The bath was grounded via a 3 M KCl agar bridge connected to an Ag-AgCl wire. The signal from the voltage microelectrode was subtracted from the voltage of the pH electrode to obtain the pH changes. Initial rates of change in pH were determined from the slope of the line obtained by fitting pH versus time to a linear regression line. The slope of the pH electrodes was between 56 and 57 mV (pH unit\textsuperscript{-1}). For measurement of pH under voltage clamp conditions, a three-electrode setup was used. Two standard microelectrodes connected to an OC-725C amplifier were used for voltage and current clamps, and one electrode containing the pH resin was attached to a FD-223 electrometer. A common reference electrode was used for both amplifiers. Data were acquired as described above for pH measurement.

**Fig. 1.** The effect of HCO\textsubscript{3}/H\textsubscript{2}CO\textsubscript{3} on SLC26A7 Cl\textsuperscript{−} Channel Activity. A, the IV curves were measured in oocytes expressing SLC26A7 and incubated in HEPES- or HCO\textsubscript{3}-buffered media containing 75 or 0 mM Cl\textsuperscript{−} as indicated on the figure. The IV relations were measured 5–7 min after exposure to HCO\textsubscript{3}-buffered media to allow completion of acidification and stabilization of pH. B, oocytes incubated in HEPES-buffered media containing 0 or 60 mM Cl\textsuperscript{−} as indicated and without (●) or with (○, ▲) 40 mM butyrate (Buty). The IV relations were measured 10 min after the exposure to butyrate when pH was stable. C shows summary of the reversal potential recorded under each condition from the number of experiments indicated next to the columns. Note that HCO\textsubscript{3}/H\textsubscript{2}CO\textsubscript{3} and butyrate had minimal effect on the current and reversal potential in the presence of Cl\textsuperscript{−} but markedly shifted the reversal potential in the absence of Cl\textsuperscript{−}. D, effect of pH on the RP. The oocytes were incubated in solutions containing 40 mM butyrate, and after completion of the acidification and recording of the IV relation at the most acidic pH the oocytes were successively incubated in solutions containing 30, 20, 10, 5, and 0 mM butyrate. About 5 min after each change in butyrate concentration pH was stable, and the IV relation was recorded. The mean ± S.E. of the RP are plotted as a function of internal H\textsuperscript{+} concentration that was calculated from the average pH in three separate experiments.

**RESULTS AND DISCUSSION**

**SLC26A7 Is an Electrogenic Transporter**—Two recent studies expressed SLC26A7 in Xenopus oocytes and measured pH to conclude that SLC26A7 is a cell shrinkage-activated Cl\textsuperscript{−}/HCO\textsubscript{3} exchanger (15, 16). To test whether SLC26A7 is an electrogenic transporter the protein was expressed in Xenopus oocytes, and the resulting current was measured in Cl\textsuperscript{−}-containing and Cl\textsuperscript{−}-free media. Fig. 1A displays the nearly linear IV curves recorded from control oocytes and oocytes expressing SLC26A7. The expression of SLC26A7 generated a current that at +60 mV averaged 2.7 ± 0.3 µA (n = 18). Removal of external Cl\textsuperscript{−} reduced the outward current at +60 mV to 1.1 ± 0.2 µA. To further characterize the current mediated by SLC26A7, Fig. 1B shows that the instantaneous inward and outward currents are time- and voltage-independent, similar to the properties of the current mediated by CFTR (21).

The ion selectivity of SLC26A7 was measured by ion substitution. Replacing external Na\textsuperscript{+} or K\textsuperscript{+} with N-methyl-d-glucamine had no effect on the current, indicating that SLC26A7 does not conduct these ions (data not shown). Measurement of reversal potentials (RP) as in Fig. 1C showed that SLC26A7 has a similar low permeability to glutamate and SLC26A7, identical permeability to Cl\textsuperscript{−} and Br\textsuperscript{−} and very high permeability to NO\textsubscript{3}\textsuperscript{−} (Fig. 1D). Interestingly, I\textsuperscript{-} showed anomalous behavior. Replacing external Cl\textsuperscript{−} with I\textsuperscript{−} had no effect on the reversal potential, but reduced both the inward and outward currents. Because the inward current is mediated by Cl\textsuperscript{−} efflux, I\textsuperscript{−} is an inhibitor of Cl\textsuperscript{−} transport by SLC26A7. Therefore, the anionic selectivity of SLC26A7 is NO\textsubscript{3}\textsuperscript{−} > Cl\textsuperscript{−} = Br\textsuperscript{−} > Glu, and is similar to that reported for CFTR (21), including the anomonic effect of I\textsuperscript{−} (22).

**Regulation of SLC26A7 Selectivity by pH**—As a first test of HCO\textsubscript{3} transport by SLC26A7 we recorded the effect of HCO\textsubscript{3} on the Cl\textsuperscript{−} current. The current was recorded 5–7 min after the exposure to CO\textsubscript{2}/HCO\textsubscript{3} to allow completion of cellular acidification. The CO\textsubscript{2}/HCO\textsubscript{3} acidifies the oocytes because of the rapid diffusion of CO\textsubscript{2} into the cell and its hydration in the cytosol. In all experiments (n = 14) HCO\textsubscript{3} had no effect on the current recorded in the presence of Cl\textsuperscript{−} (Fig. 2A). However, HCO\textsubscript{3} noticeably shifted the reversal potential in Cl\textsuperscript{−}-free media from +2.75 ± 1.1 mV (n = 32) in HEPES-buffered media to +19.5 ± 1.4 mV (n = 14) in HCO\textsubscript{3}-buffered media (Fig. 2C). The effect of HCO\textsubscript{3} can be mediated by HCO\textsubscript{3} itself or by the reduction in pH caused by exposing the oocytes to a HCO\textsubscript{3}-buffered media equilibrated with CO\textsubscript{2}. To distinguish between the two possibilities we measured the effect of butyrate on pH (see Fig. 3) and the IV in Cl\textsuperscript{−}-containing and Cl\textsuperscript{−}-free media. Butyrate acidified pH similar to HCO\textsubscript{3} (Fig. 2, B and C), showing that butyrate had the same effect as HCO\textsubscript{3} by having no effect on the current in Cl\textsuperscript{−}-containing media and shifting the RP in Cl\textsuperscript{−}-free media to +21.3 ± 3.4 mV (n = 8). The pH dependence of the shift in RP is shown in Fig. 2D. In preliminary experiments we found that the most reliable data could be obtained by acidifying the oocytes with 40 mM butyrate and then reducing the butyrate concentration stepwise from 40 to 30, 20, 10 and 5 mM. Reducing pH from a resting level of 7.35 ± 0.02 (n = 3, H\textsuperscript{+} concentration of 44.7 nM) to 6.93 ± 0.02 (n = 3, H\textsuperscript{+} concentration of 117 nM) was sufficient to cause the maximum shift in RP. Therefore, the findings in Fig. 2 indicate that pH regulates SLC26A7 channel selectivity. Acidic pH (increased intracellular H\textsuperscript{+} or reduced OH\textsuperscript{−} ions) increases the selectivity of SLC26A7 for Cl\textsuperscript{−}. To our knowledge SLC26A7 is the first Cl\textsuperscript{−} channel the selectivity of which is regulated by intracellular acidification.

**SLC26A7 Is a Poor OH\textsuperscript{−}/HCO\textsubscript{3} Transporter**—The previous reports of HCO\textsubscript{3} transport by SLC26A7 (15, 16) and the effect

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The previous reports of HCO\textsubscript{3} transport by SLC26A7 (15, 16) and the effect
of pH on SLC26A7 Cl− selectivity (Fig. 2) prompted us to measure HCO₃⁻ transport by SLC26A7. Oocytes were used to measure the membrane potential and pH. A, control oocytes (gray traces) and oocytes expressing SLC26A7 (black traces) in HEPES-buffered media were exposed to Cl⁻-free media that hyperpolarized control and depolarized oocytes expressing SLC26A7. Then the oocytes were incubated in HCO₃⁻-buffered media, and where indicated the oocytes were incubated in Cl⁻-free, HCO₃⁻-buffered media. The shaded area marks the period of incubation in Cl⁻-free medium. Finally, Cl⁻ was added back to the perfusate, and then HCO₃⁻ was removed by perfusing the oocytes in HEPES-buffered media. A similar protocol was used in B except that the oocytes were incubated in media containing 40 mM butyrate (Buty) instead of HCO₃⁻. C shows the averaged rates of pH increase in 7 control (Cont) and 14 SLC26A7-expressing (A7) oocytes before and after removal of external Cl⁻. * indicates difference from the respective control at p < 0.05. D summarizes the change in membrane potential measured at the indicated number of experiments.

FIG. 3. Lack of HCO₃⁻ transport by SLC26A7. Oocytes were used to measure the membrane potential and pH. A, control oocytes (gray traces) and oocytes expressing SLC26A7 (black traces) in HEPES-buffered media were exposed to Cl⁻-free media that hyperpolarized control and depolarized oocytes expressing SLC26A7. Then the oocytes were incubated in HCO₃⁻-buffered media, and where indicated the oocytes were incubated in Cl⁻-free, HCO₃⁻-buffered media. The shaded area marks the period of incubation in Cl⁻-free medium. Finally, Cl⁻ was added back to the perfusate, and then HCO₃⁻ was removed by perfusing the oocytes in HEPES-buffered media. A similar protocol was used in B except that the oocytes were incubated in media containing 40 mM butyrate (Buty) instead of HCO₃⁻. C shows the averaged rates of pH increase in 7 control (Cont) and 14 SLC26A7-expressing (A7) oocytes before and after removal of external Cl⁻. * indicates difference from the respective control at p < 0.05. D summarizes the change in membrane potential measured at the indicated number of experiments.

FIG. 4. Effect of membrane potential and external pH on pHi. A, pHi was measured in control oocytes (upper gray trace) and oocytes expressing SLC26A7 (lower black trace). pH was alternated between 7.5, 8.5, and 9.0 as indicated by the bars, first in Cl⁻-containing and then in Cl⁻-free, HEPES-buffered media. B, pHi was measured in oocytes expressing SLC26A7 while clamping the membrane potential at −80 or +40 mV as indicated. Note that at constant external Cl⁻ and pH, hyperpolarization acidified and depolarization alkalinized the cells.

of pH on SLC26A7 Cl⁻ selectivity (Fig. 2) prompted us to measure HCO₃⁻ transport by SLC26A7. In Fig. 3A the effect of removal and addition of Cl⁻ on membrane potential and pH was measured in the presence and absence of HCO₃⁻. Removal of Cl⁻ in both HEPES- and HCO₃⁻-buffered media depolarized the cells. However, in the absence of HCO₃⁻ removal of Cl⁻ depolarized the cells by 17 ± 1 mV, whereas in the presence of HCO₃⁻ removal of Cl⁻ depolarized the cells by 50 ± 2.5 mV (n = 14) (Fig. 3D), consistent with the increased selectivity to Cl⁻ in HCO₃⁻-buffered media shown in Fig. 2. The same pattern of depolarization was observed in oocytes incubated with butyrate (Fig. 3B) when removal of Cl⁻ in the absence of butyrate
depolarized the oocytes by 16 ± 1.3 and in the presence of butyrate by 52 ± 1.3 mV (n = 8) (Fig. 3D).

Surprisingly, SLC26A7 showed minimal OH⁻ or HCO₃⁻ transport. Thus, removal of external Cl⁻ in the presence or absence of HCO₃⁻ or butyrate resulted in a very slow rate of alkalinization (Fig. 3, A and B). In fact, in the presence of HCO₃⁻ the rate of alkalinization before and after removal of Cl⁻ in oocytes expressing SLC26A7 was the same and was less than 0.01 ΔpH/min (Fig. 3C). A small difference was found when the rate of alkalinization was compared between control oocytes and oocytes expressing SLC26A7 (control, 0.0029 ± 0.0005, n = 7; SLC26A7 0.0074 ± 0.0008 ΔpH/min, n = 14, p < 0.05). Previous work used medium buffered with 33 mM HCO₃⁻. We repeated the experiments in Fig. 3 in media buffered with 33 mM Cl⁻ and then at increasing blockers concentrations. After each increase in blocker concentration the IV curves were recorded to verify no change in reversal potential and similar inhibition in all voltages. 100% was taken as the Cl⁻-dependent current, and inhibition was calculated as percent of this current. A, C, and D show representative IV curves, and B and E show the summary of multiple experiments. A and B show inhibition by DIDS, C and E show inhibition by DPC, and D and E show inhibition by Glyb.

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The results in Fig. 3 suggest that SLC26A7 may have a finite permeability to HCO₃⁻ and perhaps OH⁻. To verify this possibility and explore the role of Cl⁻ in OH⁻ transport we measured the effect of increasing pHᵢ on pHₑ in the presence and absence of external Cl⁻. The upper trace in Fig. 4A is the control. The lower trace in Fig. 4A shows that in oocytes expressing SLC26A7 increasing pHᵢ from 7.5 to 8.5 and 9.0 increased pHₑ accordingly. Importantly, restoring pHᵢ to 7.5 resulted in recovery of pHₑ, and the increase and recovery of pHᵢ in response to changes in pHₑ were minimally affected by removal of external Cl⁻. To test whether SLC26A7 OH⁻ permeability is conductive we measured the effect of the membrane potential on pHᵢ. The experiments were done in HEPES-buffered media because at the acidic pHᵢ in HCO₃⁻-buffered media changes in membrane potential had minimal effect on pHᵢ (data not shown). The darkly shaded areas in Fig. 4B show that at constant Cl⁻ and pHᵢ, clamping the membrane potential at −80 mV acidified the cytosol by 0.07 ± 0.01 pH units, whereas clamping the membrane potential at +40 mV alkalinized the cytosol by 0.22 ± 0.03 pH units (n = 4).

Sensitivity to Blockers—Common blockers used to probe anion transporters are DIDS, DPC, and glybenclamide (Glyb). The effect of these blockers on the SLC26A7 current is depicted in Fig. 5. Fig. 5, A and B, shows the complex inhibition by DIDS. In HEPES-buffered media the inhibition saturated at 100 μM DIDS but was partial. Increasing DIDS to 500 μM did not change the extent of the inhibition, which plateaued at 56 ± 8% (n = 5). HCO₃⁻ increased the apparent affinity to DIDS from about 17 ± 4 to 8.1 ± 2.3 μM and the maximal inhibition to 74 ± 13% (n = 5) (Fig. 5B). This finding further indicates that pHᵢ and HCO₃⁻ modify the properties of SLC26A7 as was found in Fig. 2 for the selectivity to Cl⁻. DPC also inhibited the current mediated by SLC26A7 (Fig. 5C) in a dose-dependent manner (Fig. 5E), with 78 ± 14% (n = 3) inhibition at 0.5 mM DPC. Finally, SLC26A7 current was inhibited by Glyb (Fig. 5D) with 65 ± 9% (n = 3) inhibition by 0.5 mM Glyb. The inhibitor
profile found for the current mediated by SLC26A7 emphasizes the need to interpret such studies with much caution because these blockers were shown to inhibit other Cl\(^{-}\) transporters. Of particular interest is the inhibition by Glyb. Often, inhibition of a Cl\(^{-}\) current by Glyb is taken as evidence that the current is chloride independent (Fig. 7, A and C). Interestingly, at 150 mM intracellular Cl\(^{-}\) the current in HEK293 cells maintained in HEPES-buffered media was highly Cl\(^{-}\) selective, as evident from the shift in the reversal potential to 58 ± 5 mV (n = 5) and the lack of outward current in Cl\(^{-}\)-free medium (Fig. 7A). The lack of outward current made it difficult to demonstrate the effect of pH\(_i\) on SLC26A7 selectivity in HEK293 cells. Therefore, we measured the SLC26A7-mediated current in cells infused with the more physiological Cl\(^{-}\) concentration of 30 mM. Fig. 7, D and E, show that the RP of the SLC26A7 current in HEK293 cells infused with a pipette solution containing 30 mM Cl\(^{-}\), pH of 7.5, and incubated in Cl\(^{-}\)-free media is about 43 ± 3 mV. Exposing these cells to HCO\(_3\)\(^{-}\)-buffered media for 1 min, a time sufficient for completion of the acidification and before substantial recovery of pH\(_i\) (Fig. 6), shifted the RP to 57 ± 2 mV. When the cells were infused with a pipette solution buffered with HEPES to a pH of 6.5, the RP was 65 ± 3.5 mV, and exposing these cells to a HCO\(_3\)\(^{-}\)-buffered media had no further effect on the RP, which averaged 53 ± 4.4 mV. These findings indicate that in HEK293 cells, SLC26A7 Cl\(^{-}\) channel activity is also regulated by pH\(_i\) and that HCO\(_3\)\(^{-}\) likely does not regulate the channel independent of pH\(_i\) because it did not affect the SLC26A7 current in cells maintained at a pH\(_i\) of 6.5.

In conclusion, measurement of membrane current and voltage and pH\(_i\) in Xenopus oocytes and HEK293 cells expressing SLC26A7 reveal that SLC26A7 functions as a Cl\(^{-}\) channel that is regulated by pH\(_i\). Several protocols were used to evaluate OH\(^{-}\)/HCO\(_3\)\(^{-}\) transport by SLC26A7, and all reported very low permeability. In fact, OH\(^{-}\)/HCO\(_3\)\(^{-}\) transport was found only when pH\(_i\) was above 7 in either oocytes (Fig. 4) or HEK293 cells (Fig. 6). Even under these conditions the OH\(^{-}\)/HCO\(_3\)\(^{-}\) permeability was conductive as evident from the effect of the membrane potential on pH\(_i\) and inhibition of the pH\(_i\) changes by membrane depolarization (Figs. 4 and 6). We, therefore, have to conclude that SLC26A7 does not function as a Cl\(^{-}\)/HCO\(_3\)\(^{-}\) exchanger as suggested before (15, 16) but rather as a pH\(_i\)-regulated Cl\(^{-}\) channel. We have no immediate explanation for the difference between our findings and those reported before, although expression in Xenopus oocytes sometimes leads to activation of native transporters. Previous work did not measure membrane current or the behavior of SLC26A7 in HEK293 cells. Such measurements may clarify the disparity between the findings.

The finding that SLC26A7 functions as a channel highlights the remarkable diverse functions and substrate selectivity of the members of the SLC26 transporters family. For example, SLC26A3 (DRA), SLC26A4 (Pendrin), and SLC26A6 function as Cl\(^{-}\)/HCO\(_3\)\(^{-}\) exchangers (6, 7, 10–13, 23, 24) that are activated in the conditions of Fig. 6 were inhibited by about 60–75% by 0.25 mM DIDS (data not shown).

Two assays were used to show that SLC26A7 expressed in HEK293 cells functions as a Cl\(^{-}\) channel. First, we measured [Cl\(^{-}\)]\(_i\), with MQAE and followed the rate of Cl\(^{-}\)/NO\(_3\)\(^{-}\) exchange as was used before to assay CFTR channel activity (6, 18). Fig. 6E is the control, and Fig. 6F shows the high rate of Cl\(^{-}\)/NO\(_3\)\(^{-}\) exchange mediated by SLC26A7. Importantly, the SLC26A7-mediated Cl\(^{-}\)/NO\(_3\)\(^{-}\) exchange was inhibited 78 ± 6% (n = 3) by depolarizing the cells with 40 mM external K\(^{+}\). The SLC26A7-mediated Cl\(^{-}\)/NO\(_3\)\(^{-}\) exchange was inhibited by preincubation of the cells with 0.25 mM DIDS (Fig. 6G).

In the second assay we characterized the current generated by expressing SLC26A7 in HEK293 cells. Fig. 7A shows that expression of SLC26A7 in HEK293 cells resulted in a large Cl\(^{-}\)-current that averaged 673 ± 114 pA (n = 5). In symmetrical Cl\(^{-}\) concentrations the current followed linear IV with reversal potential of 2 ± 3 mV. As was found in oocytes (Fig. 1), the instantaneous current in HEK293 cells was time- and voltage-independent (Fig. 7, B and C). Interestingly, at 150 mM intracellular Cl\(^{-}\) the current in HEK293 cells maintained in HEPES-buffered media was highly Cl\(^{-}\) selective, as evident from the shift in the reversal potential to 58 ± 5 mV (n = 5) and the lack of outward current in Cl\(^{-}\)-free medium (Fig. 7A). The lack of outward current made it difficult to demonstrate the effect of pH\(_i\) on SLC26A7 selectivity in HEK293 cells. Therefore, we measured the SLC26A7-mediated current in cells infused with the more physiological Cl\(^{-}\) concentration of 30 mM. Fig. 7, D and E, show that the RP of the SLC26A7 current in HEK293 cells infused with a pipette solution containing 30 mM Cl\(^{-}\), pH of 7.5, and incubated in Cl\(^{-}\)-free media is about 43 ± 3 mV. Exposing these cells to HCO\(_3\)\(^{-}\)-buffered media for 1 min, a time sufficient for completion of the acidification and before substantial recovery of pH\(_i\) (Fig. 6), shifted the RP to 57 ± 2 mV. When the cells were infused with a pipette solution buffered with HEPES to a pH of 6.5, the RP was 65 ± 3.5 mV, and exposing these cells to a HCO\(_3\)\(^{-}\)-buffered media had no further effect on the RP, which averaged 53 ± 4.4 mV. These findings indicate that in HEK293 cells, SLC26A7 Cl\(^{-}\) channel activity is also regulated by pH\(_i\) and that HCO\(_3\)\(^{-}\) likely does not regulate the channel independent of pH\(_i\) because it did not affect the SLC26A7 current in cells maintained at a pH\(_i\) of 6.5.
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by CFTR (6). However, although SLC26A3 functions only as a Cl⁻/HCO₃⁻ exchanger (10), SLC26A4 also transports I⁻ (4) and SLC26A6 also transports oxalate (12). Although all transporters examined so far function as electrotransporters, SLC26A3 may function as a 2Cl⁻/HCO₃⁻ exchanger, SLC26A6 as a 2HCO₃⁻/Cl⁻ exchanger, and SLC26A7 as a Cl⁻ channel with limited HCO₃⁻ permeability that is modulated by pH₁. A comparative structure-function study of representative members of the family with diverse transport modes should be very informative.

SLC26A7 is expressed in the basolateral membrane of the acid-secreting cells in the cortical collecting duct and the gastric parietal cells. How might a pH₁-modulated Cl⁻ channel function in such cells? A clue may be suggested by the behavior of SLC26A7 as a selective Cl⁻ channel at acidic pH₁ (Fig. 2) and the reduced Cl⁻ selectivity and increased OH⁻/HCO₃⁻ permeability at a pH₁ above 7 (Figs. 4 and 6). It is possible that at rest SLC26A7 functions as a selective Cl⁻ channel. Stimulation of acid secretion leads to an increase in pH₁ of the parietal cell (25). This will result in an increased OH⁻/HCO₃⁻ permeability of SLC26A7 that may provide a pathway to clear the excess base equivalents while allowing Cl⁻ entry into the cells that is needed for acid secretion. The same mechanism may function at the cortical collecting duct. Although the net effect is Cl⁻/HCO₃⁻ exchange, the regulation of SLC26A7 by pH₁ may function as a sensor to activate the exchange only at the time of acid secretion. It remains to be discovered why a channel is preferable to an exchanger in fulfilling this function. However, it is worth noting that the Cl⁻ exit pathway at the apical membrane is a Cl⁻ channel (26). A Cl⁻ entry pathway at the basolateral membrane will allow for electrical coupling between the Cl⁻ exit and Cl⁻ entry pathways during stimulated acid secretion.

REFERENCES

1. Mount, D. B., and Romero, M. F. (2004) Pfleugers Arch. Eur. J. Physiol. 447, 710–721
2. Hologa, P., Haila, S., Socha, J., Tomaszewski, L., Saarialho-Kere, U., Karpalainen-Lindeberg, M. L., Airola, K., Holmberg, C., de la Chapelle, A., and Kere, J. (1996) Nat. Genet. 14, 316–319
3. Hastbacka, J., de la Chapelle, A., Mahtani, M. M., Clines, G., Reever-Daly, M. P., Daly, M., Hamilton, B. A., Kusumi, K., Trivedi, B., Weaver, A., Coloma, A., Lovett, M., Buckler, A., Kaitila, I., and Lander, E. S. (1994) Cell 77, 1073–1087
4. Everett, L. A., Glaser, B., Beck, J. C., Idol, J. R., Buxes, A., Heyman, M., Adawi, F., Hazani, E., Nassir, E., Baexevanis, A., Sheffeld, V. C., and Green, E. D. (1997) Nat. Genet. 17, 411–422
5. Zheng, J., Shen, W., He, D. Z., Long, K. B., Madison, L. D., and Dallas, P. (2000) Nature 405, 149–155
6. Ko, S. B. H., Shcheynikov, N., Chi, J. Y., Luo, G., Ishiihishi, K., Thomas, P. J., Kim, J. Y., Lee, M. G., Naruse, S. and Mualliem, S. (2002) EMBO J. 21, 5562–5572
7. Ko, S. B. H., Zeng, W., Darvart, M. R., Luo, G., Kim, K. H., Millen, L., Get, H., Naruse, S., Suyombe, A., Thomas, P. J., and Mualliem, S. (2004) Nat. Cell Biol. 6, 343–350
8. Lee, A., Beck, L., and Markovitch, D. (2003) DNA Cell Biol. 22, 19–31
9. Satoh, H., Susuki, M., Shukunami, C., Iyama, K., Negoro, T., and Hiraki, Y. (1998) J. Biol. Chem. 273, 12307–12315
10. Melvin, J. B., Park, K., Richardson, L., Schulthei, P. J., and Shull, G. E. (1999) J. Biol. Chem. 274, 22855–22861
11. Royaux, I. E., Wall, S. M., Karniski, L. P., Everett, L. A., Suzuki, K., Knepper, M. A., and Green, E. D. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 4221–4226
12. Knauf, F., Yang, C. L., Thomson, R. B., Montone, S. A., Giebisch, G., and Arras, P. S. (2003) Proc. Natl. Acad. Sci. U. S. A. 98, 9425–9430
13. Xie, Q., Welch, R., Mercado, A., Romero, M. F., and Mount, D. B. (2002) Am. J. Physiol. 283, F826–F838
14. Koh, H., Kajala, M., Makela, S., Lehtonen, E., Kestila, M., Saarialho-Kere, U., Markovich, D., and Kere, J. (2002) J. Biol. Chem. 277, 14246–14254
15. Petrovic, S., Xu, J., Barone, S., Seidler, U., Alper, S. L., Lohi, H., Kere, J., and Soleimani, M. (2003) DNA Cell Biol. 22, 19–31
16. Petrovic, S., Barone, S., Xu, J., Conforti, L., Ma, L., Kajula, M., Kere, J., and Soleimani, M. (2004) Am. J. Physiol. 286, F161–F169
17. Lee, M. G., Choi, J. Y., Luo, X., Strickland, E., Thomas, P. J., and Mualliem, S. (1999) J. Biol. Chem. 274, 14670–14677
18. Choi, J. Y., Muallieem, D., Kisekay, K., Lee, M. G., Thomas, P. J., and Mualliem, S.
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S. (2001) Nature 410, 94–97

19. Zeng, W., Lee, M. G., and Muallem, S. (1997) J. Biol. Chem. 272, 32956–32965
20. Shcheynikov, N., Kim, K. H., Kim, K.-M., Derwart, M. R., Ko, S. B. H., Goto, H., Naruse, S., Thomas, P. J., and Muallem, S. (2004) J. Biol. Chem. 279, 21857–21865
21. Dawson, D. C., Smith, S. S., and Mansoura, M. K. (1999) Physiol. Rev. 79, Suppl 1, 47–75
22. Tabcharani, J. A., Lindsell, P., and Hanrahan, J. W. (1997) J. Gen Physiol. 110, 341–354

23. Soleimani, M., Greeley, T., Petrovic, S., Wang, Z., Amlal, H., Kopp, P., and Burnham, C. E. (2001) Am. J. Physiol. 280, F356–F364
24. Wang, Z., Petrovic, S., Mann, E., and Soleimani, M. (2002) Am. J. Physiol. 282, G575–G579
25. Muallem, S., Blissard, D., Cragoe, E. J., Jr., and Sachs, G. (1988) J. Biol. Chem. 263, 14703–14711
26. Hersey, S. J., and Sachs, G. (1995) Physiol. Rev. 75, 155–189