Specificity of Anion Exchange Mediated by Mouse Slc26a6*

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Recently, CFEX, the mouse orthologue of human SLC26A6, was localized to the brush border membrane of proximal tubule cells and was demonstrated to mediate Cl⁻-formate exchange when expressed in Xenopus oocytes. The purpose of the present study was to examine whether mouse Slc26a6 can mediate one or more of the additional anion exchange processes observed to take place across the apical membrane of proximal tubule cells. Influx of [¹⁴C]formate into Slc26a6-expressing oocytes was inhibited by sulfate, oxalate, and p-aminohippurate (PAH), indicating affinity for these anions. Measurements of uptake of [¹⁴C]oxalate, [¹⁴C]PAH, and [³⁵S]sulfate indicated that Slc26a6 can mediate transport of oxalate and sulfate but not PAH. Studies of the effect of external anions on [¹⁴C]oxalate efflux demonstrated Slc26a6-mediated Cl⁻-oxalate, oxalate-formate, oxalate-sulfate exchange. Two-electrode voltage clamp measurements indicated that Slc26a6-mediated Cl⁻-oxalate exchange is electronegative. Intracellular pH recordings demonstrated that Slc26a6 can mediate Cl⁻-HCO₃⁻ exchange, but Cl⁻-OH⁻ exchange was not detected. The presence of 100 μM oxalate inhibited the rate of Cl⁻-HCO₃⁻ exchange by 60%. We conclude that mouse Slc26a6 has affinity for oxalate, sulfate, and HCO₃⁻ in addition to Cl⁻ and formate and can function in multiple exchange modes involving pairs of these anions. In the presence of high oxalate concentrations as found in renal tubular fluid and urine, Slc26a6 may largely function as a Cl⁻-oxalate exchanger.

The majority of Na⁺-, Cl⁻-, and HCO₃⁻ filtered by the kidney is reabsorbed in the proximal tubule. Studies using isolated brush border vesicles and perfused tubules are consistent with the concept that a major fraction of Cl⁻ entry across the apical membrane of proximal tubule cells occurs via Cl⁻-formate exchange and Cl⁻-oxalate exchange (1). The molecular identification of the transporter(s) responsible for these anion exchange activities has yet to be established with certainty. Recently, CFEX, the mouse orthologue of human SLC26A6 (2, 3), was demonstrated to be capable of mediating Cl⁻-formate exchange when expressed in Xenopus oocytes, and it was localized to the brush border membrane of proximal tubule cells by immunocytochemistry (4). Thus, Slc26a6 was proposed as a candidate to mediate Cl⁻-formate exchange in the proximal tubule (4).

The purpose of the present study was to characterize the anion specificity of Slc26a6 in more detail and to test specifically the ability of Slc26a6 to mediate additional anion exchange processes known to take place across the apical membrane of proximal tubule cells, such as Cl⁻-oxalate exchange. We find that mouse Slc26a6 can mediate transport of oxalate, sulfate, and HCO₃⁻ in addition to Cl⁻ and formate and can function in multiple exchange modes involving pairs of these anions, including Cl⁻-oxalate exchange.

MATERIALS AND METHODS

Measurements of Radiolabeled Solute Fluxes—Mouse Slc26a6 (CFEX) cDNA was subcloned into the Xenopus expression plasmid pGH19 (5) and heterologously expressed in Xenopus oocytes as described previously (4). For the experiments in this study, oocytes were injected with 50 nl of water (control) or Slc26a6 cRNA (25 ng), and transport was assayed 2 days later. For measurements of solute influx, oocytes were washed twice in 1 ml chloride-free buffer (98 mM potassium-gluconate/1.8 mM hemi-calcium-gluconate/1 mM hemi-magnesium-glucuronate/5 mM Tris-Hepes, pH 7.5) and then incubated in 500 μl of uptake solution (100 mM potassium-glucuronate/5 mM Tris, pH adjusted to 7.5 with Hepes) containing the radiolabeled solutes to be tested (³⁵Cl⁻, [¹⁴C]formate, [¹⁴C]oxalate, [³⁵S]sulfate, [¹⁴C]PAH). After 30-min incubation at room temperature, external isotope was removed by washing the oocytes three times with 1 ml of ice-cold chloride-free buffer. For efflux measurements, oocytes were first preloaded with radioisotope by incubating for 60 min in chloride-free buffer containing [¹⁴C]oxalate. After three washes in ice-cold chloride-free buffer, the radioisotope content of oocytes was measured both initially and 15 min after suspension in solution containing test anions. Net efflux was calculated as the difference between the initial oocyte radioisotope content and that remaining after 15-min reincubation. For experiments in which anions were tested for effects on radiolabeled solute influx or efflux, glutamate was isosmotically replaced by each test anion, and media were buffered with 5 mM Tris titrated with Hepes to pH 7.5. Radiosotope content of each individual oocyte was measured by scintillation spectrophotometry after solubilization in 0.2 ml of 10% SDS and addition of 3 ml scintillation fluid (Opti-fluor, Packard). Results shown in the bar graphs represent means ± S.E. for 9–12 oocytes in each group. In some cases the S.E. values are too small to be visible in the figures.

Measurements of Membrane Currents, Voltage, and Intracellular pH—Membrane currents were measured using a two-microelectrode voltage clamp (model OC-725B Oocyte Clamp, Warner Instruments Corp., Hamden, CT). As described previously (6), cells were impaled with microelectrodes filled with 3 M KCl (resistance = 0.3–1.0 megohms) the holding potential (Vhold) was −60 mV, and currents were filtered at 20 Hz using a 4-pole Bessel filter. In separate experiments, microelectrodes were used to monitor intracellular pH and membrane voltage (7). The voltage- and pH-sensitive microelectrodes were prepared as described previously (8). The pH electrode tip was filled with proton ionophore 1 mixture B (Fluka Chemical Corp., Ronkonkoma, NY), and back-filled with a pH 7 phosphate buffer (9). Electrodes were connected to high impedance electrometers (model FD-223, WPI, Inc., Sarasota, FL), the signal from the voltage microelectrode was digitized for digitization and analysis by computer. Oocytes were held in a chamber that was...

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‡ The abbreviations used are: PAH, p-aminohippurate; DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid.
10 mM acetate (4). Taken together, the findings in Fig. 1 sug-
lactate. We had previously found the absence of inhibition by
cant inhibition, while little or no interaction was detected with
alate, PAH, and sulfate. Succinate caused modest but signifi-
cmate uptake with potency greater than formate, including ox-
formate also confirmed previous findings (4), consistent with
have previously observed the absence of inhibition by 10 mM NaHCO3 in the HCO3-
Gluconate replaced Cl– in the Cl–-free medium. NaCl was isosmotically
medium, pH 7.5, which was gassed with 1.5% CO2. In some solutions, 16 mM NaCl was replaced
and sulfate. Acetate, which was previously found to
results confirmed that Slc26a6 is
capable of transporting sulfate and oxalate in addition to for-
cute involvement of these additional anions.
Effects of Anions on \( [14C] \)Formate Influx—As an initial ap-
port of an outside-acid pH gradient, consistent with a
Effects of Anions on \( [14C] \)Oxalate Influx—Having demo-
Effects of Anions on \( [36Cl] \) Influx—To evaluate in more detail
The oocytes were then washed and resuspended in media con-
load with \([14C]\)oxalate by preincubation for 60 min under the
cxulated by external anions in the order Cl– > oxalate > formate > sulfate. Acetate, which was previously found to

**RESULTS**

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![Fig. 1. Effects of 5 mM test anions added to the external medium on 30 min uptake of 20 \( \mu \text{M} \) \([14C]\)formate.](image)

![Fig. 2. Uptake of 20 \( \mu \text{M} \) \([14C]\)formate, \([14C]\)oxalate, \([14C]\)PAH, and \([35S]\)sulfate in the absence and presence of 100 \( \mu \text{M} \) DIDS.](image)

![Fig. 3. Effects of 1 mM test anions added to the external medium on 30 min uptake of 3 mM \( ^{36}\text{Cl} \).](image)
found that oxalate transport via Cl−-oxalate exchange in renal brush border membrane vesicles is electrogenic, consistent with 1:1 exchange of divalent oxalate for monovalent Cl− (11). We therefore performed voltage clamp experiments in Slc26a6-expressing Xenopus oocytes to test whether oxalate transport mediated by Slc26a6 is electrogenic. In the experiment illustrated in Fig. 7, Cl− was removed from the external medium to impose an outward Cl− gradient. Under these conditions, addition of 5 mM oxalate to the external medium caused a large outward current, indicating net inward movement of negative charges associated with uptake of oxalate. The mean outward current induced by addition of 5 mM oxalate in three experiments was 810 ± 38 nA. This outward current gradually decayed and then returned to base line when external oxalate was removed. Readdition of external Cl− then caused a marked inward current, reflecting net outward movement of negative charge. The large inward current resulting from Cl− addition after cellular oxalate loading most likely represents electrogenic exchange of intracellular oxalate for external Cl−. The presence of 100 μM DIDS almost completely abolished the oxalate induced currents. Shown in the inset, oxalate failed to induce currents in water-injected oocytes. Thus, the results shown in Fig. 7 indicated that Cl−-oxalate exchange mediated by Slc26a6 is electrogenic.

Relationship between Cl−-Oxalate Exchange and Cl−-HCO3− Exchange—Recent experiments showed that removal of extracellular Cl− in the presence of CO3/HCO3− causes a rise in pH in Slc26a6-expressing oocytes, indicating that Slc26a6 (PAT1) can mediate Cl−-OH− and/or Cl−-HCO3− exchange (12). We conducted a final series of experiments to evaluate the relative affinities of HCO3− and oxalate as exchange partners for Cl−. Intracellular pH was directly monitored with a pH-sensitive microelectrode in oocytes injected with Slc26a6 cRNA. As shown in Fig. 8A, isohydric, pH 7.5, replacement of Heps-buffered external medium with a medium containing 10 mM HCO3−, 1.5% CO2 resulted in intracellular acidification due to entry of CO2. In the presence of HCO3−, removal of external Cl− caused an intracellular alkalinization that was blocked by DIDS. In contrast, as shown in Fig. 8B, when intracellular pH was acidified in the absence of HCO3−/CO2 by addition of the weak acid anion butyrate to the external medium, Cl− removal failed to cause intracellular alkalinization. As shown in Fig. 8C, in the presence of HCO3−, Cl− removal failed to induce intracellular alkalinization in control water-injected oocytes. Taken together, these findings indicated that Slc26a6 does indeed mediate Cl−-HCO3− exchange but does not mediate detectable Cl−-OH− exchange under similar conditions.

To evaluate the relative affinities of HCO3− and oxalate as substrates to exchange for Cl−, the protocol for assaying Cl−-HCO3− exchange was repeated in the presence and absence of 100 μM oxalate. As illustrated in Fig. 9, 100 μM external oxalate greatly reduced the rate of exchange of intracellular Cl− for 10 mM external HCO3−, indicating that Slc26a6 has much greater affinity for oxalate than for HCO3−. In three experiments, 100 μM oxalate reduced the mean rate of pH recovery by 60% from 4.27 ± 0.10 to 1.67 ± 0.11 × 10−4 pH units/s. This inhibition of the rate of pH recovery was not due to the difference in starting pH because oxalate caused similar inhibition of pH recovery (4.8 versus 1.5 × 10−4 pH units/s) when the order of ion substitution was reversed (not shown).

Fig. 9 also demonstrates the relatively large changes in membrane potential resulting from electrogenic oxalate transport. In three experiments, the mean hyperpolarization resulting from addition of 100 μM oxalate to the external solution in the absence of Cl− was 29.3 ± 0.4 mV, reflecting electrogenic oxalate influx. Removal of external oxalate and re-addition of

Electrogenicity of Cl−-Oxalate Exchange—Previous studies

either inhibit formate influx nor stimulate formate efflux (4), failed to stimulate [14C]oxalate efflux. These findings indicated that Slc26a6 is capable of functioning in Cl−-oxalate, oxalate-oxalate, oxalate-formate, and oxalate-sulfate exchange modes, with greatest activity as a Cl−-oxalate exchanger under the tested conditions.

Effects of 10 mM test anions on efflux of [14C]oxalate. Content of [14C]oxalate was measured initially and after 15-min reincubation in the presence of the indicated test anions.

Anion Specificity of Slc26a6

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Cl\(^{-}\) caused a mean depolarization of 114.0 ± 3.6 mV in the three experiments, with a shift in the membrane potential to a peak of +58.7 ± 5.1 mV. This marked depolarization is consistent with electrogenic efflux of intracellular oxalate in exchange for external Cl\(^{-}\).

DISCUSSION

In the present study we have shown that mouse Slc26a6 can mediate transport of oxalate, sulfate, and HCO\(_3\)\(^{-}\), in addition to Cl\(^{-}\) and formate, and can operate in multiple exchange modes involving pairs of these anions. These exchange modes include Cl\(^{-}\)-oxalate and oxalate-sulfate exchange in addition to the previously described Cl\(^{-}\)-formate exchange (4).

Immunolocalization studies have demonstrated the presence of Slc26a6 (CFEX) on the apical or brush border membrane of renal proximal tubule cells (4). Microperfusion experiments have indicated that physiologic concentrations of formate and oxalate markedly stimulate Cl\(^{-}\) and fluid absorption in the rat proximal convoluted tubule (13–16) and that different mechanisms of anion recycling underlie the effects of formate and oxalate (17). Whereas evidence suggests that formate-stimulated NaCl uptake across the apical membrane arises from Cl\(^{-}\)-formate exchange in parallel with Na\(^{+}\)-H\(^{+}\) exchange and H\(^{+}\)-coupled formate entry (17, 18), oxalate-induced transport appears to involve oxalate-Cl\(^{-}\) exchange in parallel with sodium-sulfate cotransport and sulfate-oxalate exchange (17). The present findings clearly raise the possibility that the Cl\(^{-}\)-formate, Cl\(^{-}\)-oxalate, and oxalate-sulfate exchange activities nec-

FIG. 7. Oxalate-induced currents measured by two-electrode voltage clamp. Results in water-injected oocytes are shown in inset.

FIG. 8. Effects of removal of extracellular Cl\(^{-}\) on intracellular pH in the presence (A) and absence (B) of CO\(_2\)/HCO\(_3\)\(^{-}\), and in water-injected oocytes (C).

FIG. 9. Effect of oxalate on rate of alkalinization caused by removal of extracellular Cl\(^{-}\) in the presence of CO\(_2\)/HCO\(_3\).

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essary for proximal tubule NaCl absorption may all be mediated by Slc26a6.

However, previous studies (10, 11, 19) of anion transport in brush border vesicles strongly suggested the presence of at least three separate transporters mediating Cl−-formate, Cl−-oxalate, and oxalate-sulfate exchange. For example, although formate was found to be a substrate for the transporter mediating Cl−-oxalate exchange (11), the major fraction of Cl−-formate exchange was insensitive to competition by oxalate (11), suggesting that most Cl−-formate exchange is mediated by a pathway separate from that mediating Cl−-oxalate exchange. In addition, the major fraction of Cl−-formate exchange was only partially inhibited by concentrations of disulfonic stilbenes that abolished Cl−-oxalate exchange (11, 20), further suggesting that most of the Cl−-formate exchange is mediated by a pathway separate from that mediating Cl−-oxalate exchange. Similarly, although a small activity of Cl−-sulfate exchange was observed (10, 11), the major fraction of oxalate-sulfate exchange was insensitive to competition by Cl− (10), suggesting that most of the oxalate-sulfate exchange is mediated by a pathway separate from that mediating Cl−-oxalate exchange. The existence of separate apical membrane pathways mediating oxalate-sulfate exchange and Cl−-oxalate exchange is also supported by reconstitution experiments showing that these activities purified in different protein fractions from solubilized renal brush border membranes (21).

The present study indicates that mouse Slc26a6 has relatively high affinity for oxalate, Cl−, and disulfonic stilbenes and that Slc26a6 can mediate electroneutral Cl−-oxalate exchange. These features closely resemble the properties of the pathway found to mediate Cl−-oxalate exchange in renal brush border membrane vesicles (24). One possibility is that additional transporter(s) yet to be identified rather than Slc26a6 mediate most of the Cl−-formate exchange and oxalate-sulfate exchange in renal brush border membrane vesicles. Another possibility is that Slc26a6 associates with additional subunits in native kidney membranes so as to form the three different anion exchange pathways characterized in previous kinetic studies. It should be noted that the previous studies of anion exchange in brush border membranes were conducted using rabbit kidneys (10, 11, 20, 21). Thus, a third possibility is that Slc26a6 accounts for all three anion exchange activities in mouse kidney, whereas additional transporters are involved in rabbit.

Finally, we confirmed the previous observation that removal of external Cl− alkalinizes Slc26a6-expressing oocytes (12). In addition, we found that this effect of Cl− removal was HCO3−-dependent, indicating that Slc26a6 can mediate Cl−-HCO3− exchange but not significant Cl−-OH− exchange. The inability of Slc26a6 to mediate significant Cl−-OH− exchange is also supported by the previous finding that Cl− uptake into Slc26a6-expressing oocytes was not stimulated by acidification of the external medium in the absence of HCO3− (4). Data concerning the presence of Cl−-OH− exchange activity in renal brush border membrane vesicles has been conflicting (22, 23), but if appreciable Cl−-OH− exchange activity does occur physiologically, the present study suggests it must be mediated by a pathway other than Slc26a6. Similarly, the inability of Slc26a6 to facilitate PAH transport indicates that the anion exchange process that transports PAH and urate in renal brush border vesicles (24–26) must also be mediated by a pathway other than Slc26a6.

In our initial characterization of Cl− transport mediated by Slc26a6, we found that Cl− influx was inhibited by external HCO3−, as expected for Cl−-HCO3− exchange, but Cl− efflux was only modestly and not significantly stimulated by external HCO3− (4). There are at least two possible explanations for the failure to detect Cl−-HCO3− exchange in the earlier study. First, the use of a relatively late time point at which almost 50% of the intracellular Cl− content had already been lost may have reduced the sensitivity for detecting stimulation of Cl− efflux. Second, as shown in Fig. 8 of the present study, exposure to CO2 causes significant intracellular acidification. Oocytes were not gassed with CO2 in the earlier study. If intracellular acidification stimulates the rate of Cl−-HCO3− exchange mediated by Slc26a6, then the conditions of the prior study may not have been optimal for detection of this transport activity.

Measurements of intracellular pH have indicated that Cl−-HCO3− exchange activity is present on the apical membrane of proximal tubule cells (16, 27), although there is nephron heterogeneity in this regard (16). The present and previous (12) findings suggest that Slc26a6 may account for this transport activity. However, we observed that a concentration of oxalate in the range found in renal tubular fluid and urine (100 μM) can significantly convert the function of Slc26a6 from Cl−-HCO3− exchange to Cl−-oxalate exchange. Accordingly, it is possible that in cells physiologically exposed to appreciable oxalate concentrations, as in the kidney, Slc26a6 may predominantly function as a Cl−-oxalate exchanger, whereas in the absence of such high oxalate concentrations, Slc26a6 may principally mediate Cl−-HCO3− exchange. It should be noted that Slc26a6 is expressed in a wide range of epithelial and non-epithelial tissues (2–4, 12) in most of which it is therefore likely to function as a Cl−-HCO3− exchanger.

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