Role of Anion Transporter SLC26A6 (CFEX) in Prevention of Hyperoxaluria and Urolithiasis

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Abstract. In the proximal tubule of the kidney, a significant fraction of Cl⁻ is transported by apical membrane Cl⁻-formate exchange and Cl⁻-oxalate exchange. Studies to identify the transporter(s) mediating apical membrane Cl⁻-anion exchange in the proximal tubule led to the characterization of CFEX (SLC26A6). Functional expression of CFEX in Xenopus oocytes demonstrated that the transporter is capable of mediating multiple modes of anion exchange including Cl⁻-oxalate exchange. Comparison of wild-type and CFEX null mice with respect to transport in renal brush border vesicles and microperfused tubules demonstrated that CFEX primarily mediates Cl⁻-oxalate exchange rather than Cl⁻-formate exchange in the proximal tubule in vivo. CFEX null mice were observed to have a high incidence of calcium oxalate urolithiasis that was attributable to hyperoxaluria. Hyperoxaluria in CFEX null mice was found to result from a defect in oxalate secretion in the intestine, thereby causing increased net absorption of ingested oxalate and elevated plasma oxalate concentration. Thus, by mediating intestinal oxalate secretion, CFEX plays an essential role in preventing hyperoxaluria and calcium oxalate nephrolithiasis.

Keywords: Kidney stones, mouse, membrane transport, brush border membrane vesicles

PACS: 87.16.Uv

INTRODUCTION

The majority of the Na⁺ and Cl⁻ filtered by the kidney is reabsorbed in the proximal tubule. We used brush border membrane vesicles as an experimental system to identify apical membrane transport activities that might mediate Cl⁻ absorption in this nephron segment. Although we could not detect significant Na⁺-Cl⁻ or Na⁺-K⁺-2Cl⁻ cotransport [1], we did demonstrate Cl⁻-formate exchange and Cl⁻-oxalate exchange [2,3]. In addition, others found evidence for Cl⁻-OH⁻ exchange activity in renal brush border vesicles [4], and for Cl⁻-OH⁻ and Cl⁻-HCO₃⁻ exchange activities on the apical membrane of intact proximal tubule cells [5].

Cl⁻-OH⁻ or Cl⁻-HCO₃⁻ exchange in parallel with Na⁺-H⁺ exchange is a potential mechanism for NaCl entry across the apical membrane of proximal tubule cells, as shown in Fig. 1. But Cl⁻-formate exchange and Cl⁻-oxalate exchange can mediate substantial quantities of Cl⁻ absorption only if there are mechanisms to recycle these organic anions from the lumen back into the cell. Studies using renal brush border vesicles failed to demonstrate direct Na⁺-formate cotransport, but instead found that an inward H⁺ gradient markedly stimulated formate uptake [2,6]. These findings supported a model by which Cl⁻-formate exchange could mediate NaCl entry across...
the apical membrane of proximal tubule cells [2], as illustrated in Fig. 1. The inward 
\( H^+ \) gradient generated by \( \text{Na}^+\)-\( H^+ \) exchange would drive filtered formate into the cell 
by \( H^+ \)-coupled transport, and then the outward formate gradient would drive \( \text{Cl}^- \) entry 
by \( \text{Cl}^-\)-formate exchange.

![Diagram of NaCl transport mechanisms](image)

FIGURE 1. Models of apical membrane NaCl entry mediated by different modes of \( \text{Cl}^-\)-base exchange.

Possible mechanisms to recycle oxalate from lumen to cell were similarly evaluated 
in renal brush border vesicles. Although there was no significant \( \text{Na}^+\)-oxalate 
cotransport activity, oxalate-sulfate exchange was found [7], consistent with 
previously described sulfate-anion exchange activity [8]. These findings supported a 
model by which \( \text{Cl}^-\)-oxalate exchange could mediate NaCl entry across the apical 
membrane of proximal tubule cells [7], as also illustrated in Fig. 1. Sulfate absorption 
by \( \text{Na}^+\)-sulfate cotransport would generate an outward sulfate gradient that could then 
drive oxalate uptake by sulfate-oxalate exchange. The resulting cell to lumen oxalate 
gradient would then drive \( \text{Cl}^- \) entry by \( \text{Cl}^-\)-oxalate exchange.

The models of NaCl transport shown in Fig. 1 were tested in tubule microperfusion 
studies. These studies showed that formate and oxalate strongly stimulated the rate of 
transepithelial NaCl absorption [9]. Moreover, the tubule perfusion experiments 
supported the proposed mechanisms of formate and oxalate recycling illustrated in 
Fig. 1 [10]. For example, luminal application of the \( \text{Na}^+\)-\( H^+ \) exchange inhibitor EIPA 
abolished stimulation of \( \text{Cl}^- \) absorption by formate, but did not affect stimulation by 
oxalate. Conversely, deletion of sulfate from the perfusion solutions abolished oxalate 
stimulation of \( \text{Cl}^- \) absorption, but did not affect stimulation by formate. These findings 
were entirely consistent with the models for NaCl transport in Fig. 1, in which formate 
recycling is dependent on \( \text{Na}^+\)-\( H^+ \) exchange, and oxalate recycling is sulfate-
dependent as it occurs by \( \text{Na}^+\)-sulfate cotransport in parallel with sulfate-oxalate 
exchange. These models were also supported by the observations that formate-
stimulated \( \text{Cl}^- \) absorption was abolished in NHE3 null mice, whereas oxalate-
stimulated transport was not affected [11].
STUDIES ON THE ANION EXCHANGER SLC26

An important advance toward the molecular identification of the apical membrane anion exchanger(s) mediating proximal tubule Cl− reabsorption was the discovery that pendrin (SLC26A4), a member of the SLC26 “sulfate transporter” family, can actually function as a monovalent anion exchanger and mediate Cl−-formate exchange [12]. We therefore evaluated pendrin as a candidate transporter to mediate apical membrane Cl−-formate exchange in the proximal tubule. Using immunocytochemical methods, we were unable to detect pendrin expression in proximal tubule cells, but instead observed pendrin expression on the apical membrane of some cells in the collecting tubule [13]. Studies by others identified this pendrin-expressing cell population as non-alpha intercalated cells and demonstrated that pendrin mediates HCO3− secretion in the cortical collecting tubule [14]. Additional evidence against a role for pendrin in the proximal tubule was that there was no reduction in either brush border membrane Cl−-formate exchange or transepithelial NaCl absorption in pendrin null mice [15].

However, based on the finding that pendrin could mediate Cl−-base exchange, we screened the mouse EST database to try to identify one or more novel homologues of pendrin that might be expressed in the proximal tubule [13]. We successfully identified a cDNA that encoded a pendrin homologue expressed on the brush border membrane in mouse kidney [13]. It proved to be the mouse orthologue of human SLC26A6, which had been independently identified by other groups searching for novel SLC26 gene family members [16,17]. Expression of SLC26A6 was detected widely in both epithelial and non-epithelial tissues including heart, placenta, liver, skeletal muscle, kidney, pancreas, stomach and small intestine [13,16-18]. Immunolocalization studies revealed that SLC26A6 has polarized expression on the apical membrane of cells in many epithelia in addition to the proximal tubule [13,19] including pancreatic duct [16], stomach [20], and small intestine [18].

We performed functional expression studies of mouse SLC26A6 in Xenopus oocytes and demonstrated Cl−-formate exchange activity [13]. We therefore called the transporter CFEX [13]. But subsequent functional expression studies by our group and others demonstrated that SLC26A6 has broad anion specificity and can mediate multiple anion exchange reactions involving Cl−, formate, oxalate, sulfate, HCO3− and OH− as substrates (e.g. Cl−-oxalate exchange, Cl−-HCO3− exchange, oxalate-sulfate exchange) [18,21-23]. We found that Cl−-oxalate exchange mediated by CFEX is electrogenic, consistent with exchange of divalent oxalate for monovalent Cl− [21], similar to the electrogenic Cl−-oxalate exchange activity we had observed in renal brush border vesicles many years earlier [3]. Competition studies indicated that affinity of CFEX for oxalate is appreciably greater than for Cl−, HCO3−, sulfate or formate [21,22]. Moreover, when tested at equal substrate concentrations, transport of oxalate was twice as high as that of sulfate or formate [21], as shown in Fig. 2. These findings underscored the potential role of CFEX as an oxalate transporter.

Studies in CFEX null mice have provided insight into the actual physiological roles of CFEX in vivo. For example, as shown in Fig. 3a, the ability of an outward Cl− gradient to stimulate oxalate influx into renal brush border membrane vesicles was completely abolished in CFEX null mice, demonstrating that CFEX mediates all of the apical membrane Cl−-oxalate exchange activity in proximal tubule cells [24]. In

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contrast to the complete loss of Cl\(^{-}\)-oxalate exchange activity, there was only a very modest partial defect in Cl\(^{-}\)-formate exchange in renal brush border membrane vesicles from CFEX null mice [24], as shown in Fig. 3b. The results of tubule microperfusion studies are illustrated in Fig. 3c. Consistent with the predominant role of CFEX as a Cl\(^{-}\)-oxalate exchanger rather than a Cl\(^{-}\)-formate exchanger, the increment in transepithelial NaCl absorption induced by the addition of oxalate to the luminal perfusate was completely abolished in CFEX null mice, whereas the increment in NaCl absorption induced by formate was only partially inhibited [25].

![FIGURE 2. Functional expression studies of mouse SLC26A6 in Xenopus oocytes [21].](image)

![FIGURE 3. Proximal tubule transport defects in CFEX null mice. (a) Cl\(^{-}\)-oxalate exchange, and (b) Cl\(^{-}\)-formate exchange assayed in renal brush border vesicles from wild-type and CFEX null mice [24]. (c) Effects of formate and oxalate on volume absorption (J\(_v\)), a measure of transepithelial NaCl transport, by proximal tubules microperfused in situ in wild-type and CFEX null mice [25].](image)

In view of this evidence for the role of CFEX as an oxalate transporter, a striking phenotype observed in CFEX null mice was a high frequency of calcium oxalate urolithiasis [24]. We found calcium oxalate stones in the bladders of many null mice, as shown in Fig. 4a, and detected calcium oxalate deposits in the kidneys of CFEX null mice, as illustrated in Fig. 4b.
The propensity toward calcium oxalate urolithiasis was attributable to severe hyperoxaluria in CFEX null mice [24], as shown in Fig. 5a. Moreover, as illustrated in Fig. 5b, plasma oxalate was greatly elevated in CFEX null mice [24], thereby explaining hyperoxaluria on the basis of enhanced filtration of oxalate rather than a primary renal leak of oxalate due to impaired tubular reabsorption. The high levels of plasma and urine oxalate in CFEX null mice were greatly reduced when mice were fed an oxalate-free diet [24], as also shown in Figs. 5a and 5b. These observations demonstrated that dietary oxalate must be the source of most of the excess oxalate appearing in the urine and plasma of CFEX null mice. Thus, CFEX null mice appeared to represent a genetic model of enteric hyperoxaluria in which there is enhanced net absorption of dietary oxalate.

**FIGURE 4.** Calcium oxalate urolithiasis in CFEX null mice [24]. (a) Stones found in the bladder of a CFEX null mouse. (b) Calcium oxalate mass in the urinary space of a CFEX null mouse.

**FIGURE 5.** Hyperoxaluria and hyperoxalemia in CFEX null mice [24]. (a) urine, and (b) plasma oxalate concentrations in wild-type and CFEX null mice fed a control oxalate-containing diet or an oxalate-free diet.

As described earlier, CFEX is expressed on the apical membrane in many gastrointestinal tissues including the pancreas and small intestine [16,18]. Moreover, many years ago we had observed Cl⁻-oxalate exchange activity in brush border membrane vesicles isolated from small intestine [26]. Given the relatively high concentration of Cl⁻ in the lumen of the small intestine, it would be expected that Cl⁻-oxalate exchange would function in the direction of mediating Cl⁻ absorption and
oxalate secretion, as in the proximal tubule. To directly assess if CFEX mediates oxalate secretion in the small intestine, we measured unidirectional absorptive and secretory fluxes of radiolabeled oxalate across strips of duodenal epithelium mounted in Ussing chambers *in vitro* [24]. We found that duodenal tissue from CFEX null mice has a major defect in the serosa to mucosa secretory flux of oxalate, resulting in conversion of net oxalate transport from secretion to absorption [24]. As additional evidence for the concept that CFEX plays a major role in intestinal oxalate secretion, we observed a significant reduction in fecal oxalate concentration in CFEX null mice *in vivo* [24].

Hatch and colleagues similarly reported hyperoxaluria in an independent line of CFEX null mice generated by Soleimani [27]. They measured unidirectional oxalate fluxes across ileal epithelium *in vitro*, and also demonstrated conversion of net oxalate transport from secretion to absorption in intestinal tissue from CFEX null mice [27]. Moreover, they demonstrated a specific defect in Cl-dependent oxalate secretion in CFEX null mice, consistent with the role of CFEX as a Cl−-oxalate exchanger [27].

**FIGURE 6.** Oxalate homeostasis in normal and CFEX knockout mice [28]. The primary defect in CFEX null mice is a greatly reduced secretory flux of oxalate in the intestine, leading to increased net absorption of ingested oxalate, elevated plasma oxalate concentration, and increased renal oxalate excretion.

Oxalate homeostasis in wild-type and CFEX knockout mice are contrasted schematically in Fig. 6 [28]. Under normal conditions there is net absorption of a small fraction of ingested oxalate [29]. As indicated by the results of feeding an oxalate-free diet in Fig. 5, the absorption of ingested oxalate is the source of most of the plasma and urine oxalate even in wild-type mice. The components of plasma and urine oxalate that remain in mice fed an oxalate-free diet reflect endogenous hepatic production. We propose that net absorption of ingested oxalate in wild-type mice is maintained at a low level due to the large secretory backflux of oxalate mediated by CFEX in the intestine, as illustrated in Fig. 6. In CFEX null mice, this secretory flux is defective, leads to greatly augmented net absorption of oxalate, and results in hyperoxalemia and hyperoxaluria, as also shown in Fig. 6. Thus, according to this model, CFEX has a major constitutive role in limiting net intestinal absorption of oxalate, thereby preventing hyperoxaluria and calcium oxalate urolithiasis.
CONCLUSIONS

These studies suggest that inherited or acquired defects in function or regulation of CFEX are potential molecular mechanisms causing hyperoxaluria and calcium oxalate urolithiasis. For example, the increased incidence of hyperoxaluria and nephrolithiasis in patients with small bowel disease or resection [30] has been attributed to the direct and indirect effects of increased luminal levels of bile salts and fatty acids to stimulate enteric oxalate absorption [31]. The studies of oxalate homeostasis in CFEX null mice raise the possibility that loss of CFEX-mediated oxalate secretion in the small intestine may also contribute to enteric hyperoxaluria.

ACKNOWLEDGMENTS

Work in the author’s laboratory has been supported by National Institutes of Health grants R01-DK37933 and P01-DK17433.

REFERENCES