

## Physiological Characterization of Epithelial Oxalate Exchangers

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It has recently been appreciated that the intestinal absorption and secretion of oxalate may be a major determinant of both renal oxalate excretion and the natural history of kidney stone disease. We and others have shown that the apical anion exchanger Slc26a6, known to be expressed in the murine proximal tubule and intestine, is capable of mediating Cl<sup>-</sup>-oxalate and SO<sub>4</sub><sup>2-</sup>-oxalate exchange in *Xenopus* oocytes; data from knockout mice suggest a prominent role for this paralog in intestinal oxalate secretion. Data from red cells have suggested that Band 3/Anion Exchanger-1 (AE1), the cardinal member of the SLC4 bicarbonate transporter family, is capable of transporting oxalate; this AE1-activity has not been verified by heterologous expression of the AE1 cDNA. To formally evaluate the functional, kinetic, pharmacological, and electrophysiological characteristics of epithelial oxalate exchangers, we have assembled cDNAs encoding the entire 10-member SLC26 gene family, along with the three AE (SLC4A1-3) cDNAs. Transport of oxalate, SO<sub>4</sub><sup>2-</sup>, Cl<sup>-</sup>, and other substrates has been measured in *Xenopus laevis* oocytes injected with cRNA for each of these exchangers; electrophysiology of Cl<sup>-</sup>-base, oxalate, and sulphate exchange has also been assessed, using contemporaneous measurement of V<sub>m</sub>, pH<sub>i</sub>, and intracellular ion activities. The human and mouse SLC26A2 and SLC26A6 orthologs transport oxalate, sulphate, and chloride, but differ in electrophysiology and apparent stoichiometry. Oxalate and sulphate transport by Slc26a1 is uniquely activated by extracellular Cl<sup>-</sup>, unlike the cis-inhibition of paralogs that also transport Cl<sup>-</sup>. Unlike published data for human SLC26A7, -A8, and -A9, the respective murine orthologs do not transport oxalate. SLC26A3 (DRA) does not in our experiments transport SO<sub>4</sub><sup>2-</sup>, contrary to some published data, but appears to function as a low-affinity, high capacity oxalate transporter. Finally, oocytes expressing AE1 and AE2 mediate significant uptake of oxalate that is highly significant at extracellular pH 7.4 but markedly increased at pH 5.5; whereas SLC26 exchangers appear to be electrogenic, oxalate transport by AEs likely involves electroneutral exchange of co-transported H<sup>+</sup>-oxalate with intracellular anions. Whereas Cl<sup>-</sup> transport by AE1 and AE2 is completely inhibited by the appropriate concentrations of DIDS, NPPB, and niflumic acid, the SLC26 exchangers exhibit a heterogeneous response, with Slc26a9 being the least sensitive. Chimeric cDNAs generated between Slc26a6, Slc26a9, and SLC26A3 indicate that the transport characteristics of these paralogs are conferred by the central core of transmembrane domains.

To develop a cell culture model, we have turned to the human Caco-2 cell line, a widely utilized model of intestinal ion and solute transport. Thus far, we have demonstrated by RT-PCR that post-confluent Caco-2 cells express SLC26A2, -A3, -A6, and -A7, along with SLC4A2 and SLC4A3 ("brain"-AE3 only), but not SLC4A1. Apical and basolateral uptake of oxalate in these cells is DIDS-sensitive, consistent with involvement of SLC26 and SLC4 transporters. Based on *Xenopus* oocyte data and the published distribution of SLC26 and SLC4 proteins in the intestine, we tentatively propose that apical oxalate transport in Caco-2 cells is primarily mediated by SLC26A2, SLC26A6, ± SLC26A3, whereas basolateral transport is mediated by SLC4A2 and SLC4A3.

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