Recent evidence suggests that apical membrane Cl\(^-\)-oxalate exchange plays a major role in mediating Cl\(^-\) absorption in the renal proximal tubule. To sustain steady-state Cl\(^-\) absorption by a mechanism of exchange for intracellular oxalate requires the presence of one or more pathways for recycling oxalate from lumen to cell. Accordingly, we evaluated the mechanisms of oxalate transport in luminal membrane vesicles isolated from the rabbit renal cortex. We found that transport of oxalate by Na\(^+\) cotransport is negligible compared to the transport of sulfate. In contrast, we demonstrated that oxalate shares the electroneutral pathway mediating Na\(^+\)-independent sulfate-carbonate exchange. We also demonstrated the presence of OH\(^-\)-oxalate exchange (indistinguishable from H\(^+\)-oxalate cotransport). The process of OH\(^-\)-oxalate exchange was electrogenic and partially inhibited by Cl\(^-\), indicating that it occurs, at least in part, as a mode of the Cl\(^-\)-oxalate exchanger described previously. An additional component of OH\(^-\)-oxalate exchange was insensitive to inhibition by either Cl\(^-\) or sulfate, suggesting that it takes place by neither the Cl\(^-\)-oxalate exchanger nor the sulfate-carbonate exchanger. We conclude that multiple anion exchange mechanisms exist by which oxalate can recycle from lumen to cell to sustain Cl\(^-\) absorption occurring via apical membrane Cl\(^-\)-oxalate exchange in the renal proximal tubule.

Studies using isolated renal microvillus membrane vesicles have identified a transport pathway mediating Cl\(^-\)-oxalate exchange across the apical membrane of proximal tubule cells (1). The addition of physiological concentrations of oxalate to microperfused proximal and distal tubules markedly stimulates NaCl absorption via a pathway sensitive to inhibition by the disulfonic stilbene DIDS,\(^1\) consistent with a major role for oxalate-Cl\(^-\) exchange in mediating transtubular Cl\(^-\) transport (2–4). To sustain steady-state Cl\(^-\) absorption by a mechanism of exchange for intracellular oxalate requires the presence of one or more pathways for recycling oxalate from lumen to cell.

The purpose of the present study was, therefore, to use microvillus membrane vesicles isolated from rabbit renal cortex as a model system to evaluate possible mechanisms for mediating uphill uptake of oxalate across the luminal membrane of renal tubular cells. We report that multiple anion exchange mechanisms exist by which oxalate can recycle from lumen to cell to sustain Cl\(^-\) absorption occurring via apical membrane Cl\(^-\)-oxalate exchange in the renal proximal tubule. A preliminary account of this study has been published previously as an abstract (5).

EXPERIMENTAL PROCEDURES

Preparation of Microvillus Membrane Vesicles—Male New Zealand White rabbits (2–3 kg) were sacrificed by intravenous sodium pentobarbital. Microvillus membrane vesicles were prepared from the renal cortices by magnesium aggregation and differential centrifugation as described previously (6), except that hemimagnesium gluconate salt was used rather than MgSO\(_4\). The homogenization medium contained 200 mM mannitol, 41 mM K\(^+\), and 80 mM HEPES at pH 7.5. The final membrane pellets were suspended in this medium to a protein concentration of 30–50 mg/ml, as determined by the Peterson modification of the Lowry assay (7), using bovine serum albumin as the standard. Membranes were stored at –70 °C and used within 1 month of preparation. The vesicle preparations were enriched 10–15-fold in specific activity of the luminal membrane marker enzyme, γ-glutamyl transpeptidase.

Uptake of \(^{[35S]}\)Sulfate and \(^{[14C]}\)Oxalate—Freshly thawed microvillus membrane vesicles were suspended and washed twice in a medium consisting of 100 mM potassium gluconate and buffers to achieve experimental pH values of 7.5 (50 mM HEPES, 25 mM TMA-OH), 8.0 (60 mM Tris, 57 mM HEPES), or 6.0 (50 mM Mes, 10 mM HEPES, and 15 mM TMA-OH). Appropriate concentrations of mannitol were added to maintain a calculated osmolarity of 345 mosm. During the washing procedure, membranes were pelleted by centrifugation at 20,800 \(\times g\) for 15 min. The final pellets were resuspended in iso-osmotic solutions of desired composition (as detailed in the figure legends) to a protein concentration of 10–20 mg/ml and then were prequillibrated for 2 h at room temperature (20–23 °C). The membranes were continuously gassed with humidified 100% N\(_2\) or desired N\(_2\)/CO\(_2\) mixtures.

The timed uptakes of \(^{[35S]}\)Sulfate and \(^{[14C]}\)Oxalate into the membrane vesicles were measured at 30 °C by a rapid filtration method described previously (8). The specific conditions for each experiment are described in the figure legends. In general, uptake measurements were performed in triplicate as follows. Forty to 790 \(\mu\)l of radioactive medium containing 1–4 \(\times 10^3\) cpm/\(\mu\)l \(^{[35S]}\)Sulfate or \(^{[14C]}\)Oxalate were placed in the bottom of a 10-ml test tube; the tube was briefly gassed with 100% N\(_2\) or N\(_2\)/CO\(_2\) mixtures, and then the tube was capped and preincubated for 5 min in a 30 °C water bath. Ten \(\mu\)l of membrane suspension containing 100–200 \(\mu\)g protein that had been gassed for 2 h were placed on the side of the tube just above the radioactive solution. The uptake reaction was started by vortex mixing the two droplets together. The uptake period was stopped by rapid addition of 3 ml of an ice-cold isosmotic solution (100 mM potassium gluconate, 89 mM mannitol, 40 mM Hepes, and 1.6 mM TMA-OH, pH 7.5). The resulting mixture was poured over a 0.65-μm Millipore filter (DAWP) and washed with an additional 9 ml of the same ice-cold solution. Filters were placed in vials containing 3 ml of Opti-Fluor (Packard Instrument Co.) and counted by scintillation spectroscopy. Values of solute uptake were corrected for retention of isotope by the Millipore filters in the absence of membrane protein. A metronome was used for the timing of uptake periods shorter than 15 s.
Materials—Solid [U-14C]oxalic acid (112 mCi/mmole) was purchased from Amersham Corp. and dissolved at a concentration of 0.40 mCi/ml in a medium consisting of 125 mM TMA-OH, 100 mM glutamate, 60 mM mannitol, and 60 mM Hepes, pH 7.5. Aqueous [35S]sulfuric acid (1 mCi/ml, carrier-free) was purchased from DuPont NEN. Valinomycin, DIDS, and furosemide were bought from Sigma.

RESULTS

Studies using microvillus membrane vesicles have indicated the presence of a Na\(^+\)-sulfate cotransport system at the apical membrane of proximal tubule cells (9, 10). Oxalate inhibits the transport of sulfate via this system, suggesting that it is an alternative substrate for the Na\(^+\)-cotransporter (11). We, therefore, tested whether imposing an inward Na\(^+\) gradient would stimulate the uptake of sulfate and oxalate under similar conditions. As shown in the upper panel of Fig. 1, an inward Na\(^+\) gradient markedly stimulated the initial rate of sulfate uptake and induced its transient accumulation 8-fold over the equilibrium value. In contrast, imposition of the same inward Na\(^+\) gradient failed to appreciably stimulate the uptake of oxalate (Fig. 1, lower panel). These findings indicate that oxalate is a poor substrate for transport via the Na\(^+\)-sulfate cotransporter in renal microvillus membrane vesicles.

In renal basolateral membrane vesicles, oxalate is a substrate for transport via the sulfate-carbonate exchanger (8). Because a similar Na\(^+\)-independent sulfate-carbonate exchanger has been described in renal microvillus membrane vesicles (12), we next tested whether oxalate can be transported via this apical membrane pathway. The effects on oxalate and sulfate uptake of outward gradients of OH\(^-\) and HCO\(_3\)\(^-\)/CO\(_3\)\(^-\) were examined. These experiments were conducted in the presence of the K\(^+\) ionophore valinomycin with K\(^+\) \(\text{in} = \text{K}^+\) \(\text{out}\) to prevent the generation of OH\(^-\) or HCO\(_3\)\(^-\) diffusion potentials.

In the absence of a pH gradient (pH, 6.0/pH, 6.0), uptake of
Sulfate-carbonate exchange is a prominent transport activity in renal basolateral membrane vesicles (8, 13, 14). Pritchard fractionated rat renal cortical membrane vesicles on Percoll gradients and demonstrated that there were two peaks of sulfate-carbonate exchange activity, corresponding to the peaks in activity of basolateral and apical membrane markers (12). Similarly, we fractionated rabbit renal cortical membrane vesicles on a 30–42% continuous sucrose gradient and confirmed that oxalate-carbonate exchange activity was also present in two peaks, corresponding to the peaks for basolateral (Na-K-ATPase) and apical (maltase) membrane markers (data not shown). Thus, sulfate-oxalate-carbonate exchange activity in renal microvillus membrane vesicles reflects an apical localization and cannot be attributed to basolateral membrane contamination.

Whereas our current findings indicate that sulfate and oxalate share the sulfate-oxalate-carbonate exchanger in renal microvillus membrane vesicles, previous studies indicated that sulfate is not transported appreciably via the Cl⁻-oxalate exchanger (1). However, these studies were conducted at pH 7.5. Transport of sulfate via red cell band 3 is known to be markedly pH dependent, consistent with H⁺-sulfate cotransport (15). We, therefore, tested the effects of an outward Cl⁻ gradient on oxalate uptake in renal microvillus membrane vesicles and induced its uphill accumulation (Fig. 4, top), whereas the same Cl⁻ gradient minimally affected sulfate uptake (Fig. 4, bottom). The results in Fig. 4 confirm that sulfate is a poor substrate for transport via the Cl⁻-oxalate exchanger.

In the preceding experiments, the membrane potential was short circuited by use of valinomycin with K⁺ in = K⁺ out to avoid effects of the potential on any component of sulfate or oxalate uptake that was conductive. We next tested whether conductive pathways contribute at all to oxalate or sulfate uptake in renal microvillus membrane vesicles. As shown in the top panel of Fig. 5, an inside-positive membrane potential generated by an inward K⁺ gradient in the presence of valinomycin markedly stimulated the uptake of oxalate and induced its transient uphill accumulation (Fig. 5, top). This voltage-stimulated oxalate uptake was almost completely inhibited by 1 mM DIDS (Fig. 5, top). The same inward K⁺ gradient only minimally stimulated the uptake of sulfate (Fig. 5, bottom). These findings indicate that oxalate but not sulfate is transported via a DIDS-sensitive conductive pathway in renal microvillus membrane vesicles.

One example of a DIDS-sensitive conductive pathway for oxalate but not sulfate is the Cl⁻-oxalate exchanger (1). It is possible that the voltage-stimulated oxalate uptake observed in the absence of Cl⁻ in Fig. 5 results from the fact that the electronegative Cl⁻-oxalate exchanger can also operate as a OH⁻-oxalate exchanger, even in the absence of an imposed pH gradient. To test this hypothesis, we examined whether the increment in oxalate uptake resulting from imposition of an outward OH⁻ gradient, which we operationally define as due to OH⁻-oxalate exchange, is stimulated by an inside-positive potential difference. To confirm that oxalate-carbonate exchange is electroneutral, as in basolateral membrane vesicles (8), we also examined the voltage dependence of the increment in oxalate uptake due to imposition of an outward HCO₃⁻/CO₂⁻ gradient.

In the experiment illustrated in the left panel of Fig. 6, the effect of an inward K⁺ gradient in the presence of valinomycin was measured with and without imposition of an inside-alkaline pH gradient alone (OH⁻ gradient only) or an inside-alkaline pH gradient in the presence of a CO₂/HCO₃⁻ buffer system (OH⁻ gradient and HCO₃⁻/CO₂⁻ gradient). In the upper right panel of Fig. 6, the increment in oxalate uptake due to an
outward OH\(^{-}\) gradient in the absence of CO\(_2\)/HCO\(_3\), operationally defined as OH\(^{-}\)-oxalate exchange, is replotted as a function of voltage. This panel demonstrates that OH\(^{-}\)-oxalate exchange is markedly stimulated by an inside-positive potential generated by an inward K\(^{+}\) gradient. In the lower right panel of Fig. 6, the increment in oxalate uptake due to adding an outward HCO\(_3\)/CO\(_3\)\(^{-}\) gradient to an outward OH\(^{-}\) gradient, operationally defined as oxalate-carbonate exchange, is replotted as a function of voltage. It can be seen that oxalate-carbonate exchange is completely insensitive to the imposition of an inside-positive potential. Thus, the findings in Fig. 6 are consistent with the idea that at least a component of OH\(^{-}\)-oxalate exchange takes place via the electrogenic Cl\(^{-}\)-oxalate exchanger, whereas oxalate-carbonate exchange is electroneutral.

To test further the hypothesis that OH\(^{-}\)-oxalate exchange occurs, at least in part, via the Cl\(^{-}\)-oxalate exchanger, we compared the sensitivity of the three modes of oxalate transport (i.e. OH\(^{-}\)-oxalate exchange, oxalate-carbonate exchange, and Cl\(^{-}\)-oxalate exchange) to inhibition by the anion transport inhibitors DIDS and furosemide. Illustrated in Fig. 7, oxalate-carbonate exchange, OH\(^{-}\)-oxalate exchange, and Cl\(^{-}\)-oxalate exchange were each sensitive to inhibition by both agents. However, OH\(^{-}\)-oxalate exchange was significantly less inhibited by 0.01 mM DIDS and 1 mM furosemide than were Cl\(^{-}\)-oxalate exchange and oxalate-carbonate exchange. Thus, although these findings are consistent with the concept that OH\(^{-}\)-oxalate exchange takes place in part via the Cl\(^{-}\)-oxalate exchanger, they suggest that an additional component of OH\(^{-}\)-oxalate exchange occurs by a pathway with less sensitivity to DIDS and furosemide than those mediating Cl\(^{-}\)-oxalate exchange and oxalate-carbonate exchange.

Finally, we compared the sensitivity of each of the three modes of oxalate transport (i.e. OH\(^{-}\)-oxalate exchange, oxalate-carbonate exchange, and Cl\(^{-}\)-oxalate exchange) to inhibition by unlabeled oxalate. At the two concentrations tested, external Cl\(^{-}\) markedly inhibited Cl\(^{-}\)-oxalate exchange.
but minimally affected oxalate-carbonate exchange. In contrast to its lack of sensitivity to Cl⁻, oxalate-carbonate exchange was inhibited by a concentration of sulfate (0.1 mM) that did not affect Cl⁻-oxalate exchange. These results are consistent with the idea that oxalate-carbonate exchange takes place via the sulfate-carbonate exchanger, which is separate from the Cl⁻-oxalate exchanger. OH⁻-oxalate exchange was sensitive to inhibition by Cl⁻, supporting the hypothesis that a component of OH⁻-oxalate exchange takes place via the Cl⁻-oxalate exchanger. However, the inhibition of OH⁻-oxalate exchange by external Cl⁻ was less than that observed for Cl⁻-oxalate exchange, again suggesting that a portion of OH⁻-oxalate exchange occurs by a pathway other than the Cl⁻-oxalate exchanger. OH⁻-oxalate exchange was unaffected by 0.1 mM sulfate, indicating that it does not occur via the sulfate-carbonate exchanger. However, at a higher concentration (1.0 mM), sulfate inhibited both Cl⁻-oxalate exchange and OH⁻-oxalate exchange, although this inhibition was less than that observed for oxalate-carbonate exchange.

DISCUSSION

We have evaluated possible pathways capable of mediating the uphill transport of oxalate across the apical membrane of proximal tubule cells. Despite previous findings that oxalate can inhibit Na⁺-sulfate cotransport (11), we found that actual transport of oxalate by Na⁺-cotransport is negligible compared to the transport of sulfate. In contrast, oxalate was a well transported substrate via the electroneutral pathway mediating Na⁺-independent sulfate-carbonate exchange that had been identified previously in renal microvillus membrane vesicles (12). This pathway is separate from the electrogenic Cl⁻-oxalate exchanger (1), for which sulfate is a poor substrate.

We also demonstrated the presence of OH⁻-oxalate exchange (indistinguishable from H⁻-oxalate cotransport). The process of OH⁻-oxalate exchange was electrogenic and partially inhibited by Cl⁻, indicating that it occurs, at least in part, as a mode of the Cl⁻-oxalate exchanger. An additional component of OH⁻-oxalate exchange was less sensitive to inhibition by either sulfate or Cl⁻, suggesting that it takes place by neither the sulfate-carbonate exchanger nor the predominant pathway mediating Cl⁻-oxalate exchange. It remains possible that this additional pathway mediating OH⁻-oxalate exchange can also mediate Cl⁻-oxalate exchange, albeit with lower affinity for Cl⁻, DIDS, and furosemide than the predominant pathway mediating Cl⁻-oxalate exchange in these membranes. OH⁻-
oxalate exchange by a pathway separate from the Cl\(^{-}\)-oxalate exchanger has been described previously in rat renal microvillus membrane vesicles (16), although it was found to be insensitive to the membrane potential, in contrast to our results using rabbit microvillus membrane vesicles.

Physiological oxalate concentrations markedly stimulate NaCl absorption in proximal and distal tubule segments (2–4). Oxalate-stimulated NaCl absorption is inhibited by luminal...
DIDS, consistent with the proposal that Cl⁻ entry across the apical membrane occurs by Cl⁻-oxalate exchange (1-4). To sustain steady-state Cl⁻ absorption by a mechanism of exchange for intracellular oxalate requires the presence of one or more pathways for recycling oxalate from lumen to cell. Transport of oxalate via the apical membrane sulfate-carbonate exchanger provides two possible mechanisms for recycling oxalate from lumen to cell, as illustrated in Fig. 9, A and B. 1) Oxalate-carbonate exchange may take place driven by the outward HCO₃⁻/CO₃²⁻ gradient resulting from operation of the apical membrane Na⁺-H⁺ exchanger (Fig. 9A); and 2) oxalate-sulfate exchange may take place driven by the outward sulfate gradient resulting from operation of the apical membrane Na⁺-sulfate cotransporter (Fig. 9B). In addition, the process of OH⁻-oxalate exchange, occurring either as a mode of the Cl⁻-oxalate exchanger or as a separate exchanger, provides another mechanism by which oxalate uptake can be coupled to the apical membrane Na⁺-H⁺ exchanger (Fig. 9C). Similarly, since formate-oxalate exchange has been demonstrated as a mode of the Cl⁻-oxalate exchanger (1), oxalate uptake may take place driven by the outward formate gradient resulting from operation of the Na⁺-H⁺ exchanger in parallel with H⁺-coupled formate transport (Fig. 9D).

The four models in Fig. 9 each result in a net process of Na⁺-coupled Cl⁻ transport dependent on the presence of oxalate. To the extent that recycling of oxalate from lumen to cell can keep pace with the rate of Cl⁻-oxalate exchange, steady-state NaCl absorption by these mechanisms can be sustained. Future studies will be necessary to establish the relative contribution of these oxalate recycling mechanisms under physiologic conditions in the intact renal tubule.

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REFERENCES

Fig. 9. Pathways for oxalate transport across the luminal membrane of the renal proximal tubule cell. See text for details.
Pathways for Oxalate Transport in Rabbit Renal Microvillus Membrane Vesicles
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