Oxalate transport by anion exchange across rabbit ileal brush border.

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Research Article

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Oxalate Transport by Anion Exchange Across Rabbit Ileal Brush Border

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Abstract

This study demonstrates the presence of oxalate transporters on the brush border membrane of rabbit ileum. We found that an inside alkaline (pH = 8.5 inside, 6.5 outside) pH gradient stimulated [^{14}C]oxalate uptake 10-fold at 1 min with a fourfold accumulation above equilibrated uptake at 5 min. 1 mM 4,4'-disothiocyanostilbene-2,2'-disulfonate (disodium salt; DIDS) profoundly inhibited the pH-gradient stimulated oxalate uptake. Using an inwardly directed K^+ gradient and valinomycin, we found no evidence for potential sensitive oxalate uptake. In contrast to Cl:HCO_3 exchange, HCO_3 did not stimulate oxalate uptake more than was seen with a pH gradient in the absence of HCO_3. An outwardly directed Cl gradient (50 mM inside, 5 mM outside) stimulated oxalate uptake 10-fold at 1 min with a fivefold accumulation above equilibrated uptake. Cl-stimulated oxalate uptake was largely inhibited by DIDS. Addition of K^+ and nigericin only slightly decreased the Cl gradient-stimulated oxalate uptake, which indicates that this stimulation was not primarily due to the Cl gradient generating an inside alkaline pH gradient via Cl:OH exchange. Further, an outwardly directed oxalate gradient stimulated ^{36}Cl uptake. These results suggested that both oxalate:OH and oxalate:Cl exchange occur on the brush border membrane. To determine if one or both of these exchanges were on contaminating basolateral membrane, the vesicle preparation was further fractionated into a brush border and basolateral component using sucrose density gradient centrifugation. Both exchangers localized to the brush border component. A number of organic anions were examined (outwardly directed gradient) to determine if they could stimulate oxalate and Cl uptake. Only formate and oxaloacetate were found to stimulate oxalate and Cl uptake. An inwardly directed Na gradient only slightly stimulated oxalate uptake, which was inhibited by DIDS.

Introduction

Increased dietary oxalate absorption has been implicated in the etiology of calcium oxalate nephrolithiasis both in the presence and apparent absence of intestinal disease (1–6). Until recently most investigators had concluded that oxalate was absorbed by a passive, noncarrier-mediated pathway and that increased absorption resulted from increased passive permeability and/or increased solubility of oxalate (2–4, 7–14). Hatch et al. (15), however, have recently reported that net oxalate absorption occurs in the distal rabbit colon under short-circuited conditions, which suggests a carrier-mediated, active process (15). Further, net absorption was inhibited by the mucosal addition of 4-acetamido-4'-isothiocyanostilbene 2,2'-disulfonate (disodium salt; SITS),[1] which suggests an anion exchange process on the brush border. In this report, we present evidence that oxalate (Ox):OH and Ox:Cl exchange occur across rabbit ileal brush border membrane.

Methods

Rabbit ileal brush border vesicles prepared as previously described (16), were used on the day of preparation. Briefly, ileum is excised, washed with saline, and the mucosa is scraped to remove epithelial tissue. The mucosa is homogenized in a mannitol-Tris-Hepes buffer, and 10 mM $CaCl_2$ is added followed by three sets of low and high speed centrifugation steps to purify the brush border membrane fraction. The membrane vesicles were preloaded by incubating with appropriate solution for 2 h at room temperature; then a 10-μl aliquot was added to 40 or 90 μl reaction medium unless stated otherwise in the figure legend. Uptake of [^{14}C]oxalate or ^{36}Cl was determined at 30°C and was terminated at the desired time with 3 ml ice-cold stopping solution immediately followed by filtration on a 0.45-μm filter (HAWP; Millipore Corp., Bedford, MA). The filter was washed twice with 3 ml ice-cold stopping solution (10 mM Tris, 16 mM Hepes, pH 7.5, and a K gluconate concentration resulting in an osmolality equal to both the preincubation and reaction mixtures). Radioactivity was determined with a beta scintillation counter.

When basolateral and brush border membranes were studied simultaneously, they were prepared as previously described (17) utilizing a sucrose density gradient. Briefly, membranes were initially prepared in the same manner as for the brush border preparation, except that only two sets of low and high centrifugation steps were performed; then the membranes were suspended in 50% sucrose. A 50, 40, 20% discontinuous sucrose gradient was made, and the membranes were centrifuged for 90 min at 190,000 *g*. The 20–40% interface was the basolateral membrane fraction and the 40–50% interface was the brush border membrane fraction.

Statistical analysis. Each experimental datum was determined by performing triplicate analysis on three separate membrane preparations unless stated otherwise. Error bars are not shown when inclusive within the symbol.

Results

Fig. 1 illustrates that a pH gradient (inside alkaline) will stimulate oxalate uptake compared with no pH gradient. The stimulation by a 2-U pH-gradient was ~10-fold at 1 min and a fourfold overshoot was observed at 5 min. This pH gradient-stimulated oxalate uptake was largely inhibited by 4,4'-disothiocyanostilbene-2,2'-disulfonate (disodium salt; DIDS) which suggests that it occurred by anion exchange. DIDS inhibited oxalate uptake,

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1. *Abbreviations used in this paper:* DIDS, 4,4'-disothiocyanostilbene-2,2'-disulfonate (disodium salt); MES, 2-(*N*-morpholino) ethanesulfonate; Ox, oxalate; SITS, 4-acetamido-4'-isothiocyanostilbene 2,2'-disulfonate (disodium salt); TMA, tetramethylammonium.

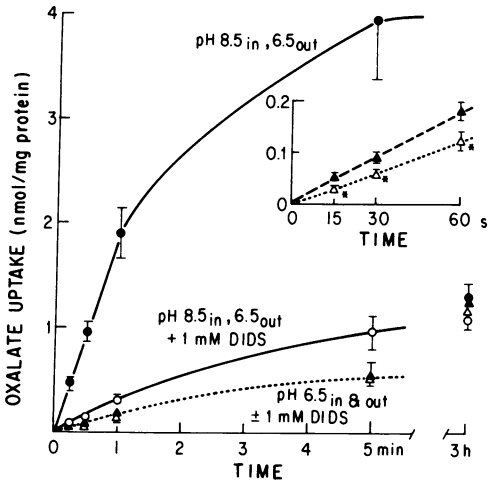

Figure 1. The effect of a pH gradient on oxalate uptake. Vesicles were preincubated with 150 mM mannitol and 100 mM TMA gluconate at pH 8.5 (103 mM Tris, 37 mM Hepes) or pH 6.5 (31 mM Tris, 74 mM Hepes, 37 mM 2-(*N*-morpholino)ethanesulfonate (MES). [^{14}C]Oxalate uptake was determined by adding 10 μl preincubated vesicles to 40 μl of reaction medium, which resulted in an extravesicular concentration of 500 μM oxalate, 150 mM mannitol, 100 mM TMA gluconate, 31 mM Tris, 74 mM Hepes, 37 mM MES, pH = 6.5, with (open symbols) or without (solid symbols) 1 mM DIDS. The asterisks in the insert indicate a P value of <0.05.

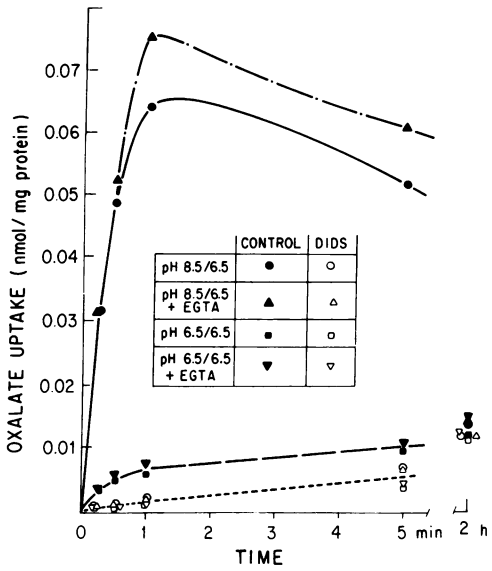

Figure 2. The effect of EGTA on oxalate uptake. Vesicles were preincubated with 150 mM mannitol and 100 mM TMA gluconate at pH 8.5 (103 mM Tris, 37 mM Hepes) or pH 6.5 (31 mM Tris, 74 mM Hepes, 37 mM MES). [^{14}C]Oxalate uptake was determined by adding 10 μl preincubated vesicles to 40 μl of reaction medium resulting in an extravesicular concentration of 5 μM oxalate, 150 mM mannitol, 100 mM TMA gluconate, 31 mM Tris, 74 mM Hepes, 37 mM MES, pH = 6.5, with and without 1 mM DIDS and/or 0.1 mM EGTA (see *inset* for key to symbols). No error bars are shown in this figure because n = 2.

even in the absence of a pH gradient (inset, Fig. 1), which suggests that some Ox:OH exchange occurred in the absence of a pH gradient.

Since calcium oxalate is very insoluble, there was some concern that the pH gradient-stimulated oxalate uptake might be due to the precipitation of oxalate by calcium associated with the brush border membranes. Therefore, the effect of a pH gradient on oxalate uptake was also determined at a much lower oxalate concentration (5 μM presumably below the ion activity product of calcium oxalate) and in the presence of 100 μM EGTA (Fig. 2). As can be seen in Fig. 2, an inside alkaline pH gradient still stimulated oxalate uptake and EGTA did not diminish this stimulation.

Further proof that oxalate uptake is not due to precipitation by calcium is shown in Fig. 3. If oxalate uptake is due to crystallization with calcium, rather than Ox:OH exchange, then Ox:Ox exchange should not occur. Fig. 3 illustrates that an outwardly directed oxalate gradient will stimulate oxalate uptake, consistent with Ox:Ox exchange. Further, preloading the vesicles with 10 mM oxalate, as done in Fig. 3, should have precipitated any available calcium.

Since an inside alkaline pH gradient can generate an inside positive diffusion potential, oxalate uptake could be stimulated indirectly by electrical coupling. To test this possibility, we generated an inside-positive membrane potential utilizing an inwardly directed K^+ gradient and the K^+ ionophore, valinomycin (Fig. 4). Oxalate uptake was slow with the inwardly directed K gradient and not increased by valinomycin, which suggests that the pH gradient-stimulated oxalate uptake was due to Ox:OH exchange rather than electrical coupling. We have previously shown that the K gradient and valinomycin concentration used in these experiments will generate an inside positive diffusion potential (16).

A HCO_3 gradient can stimulate Cl uptake more than the same OH gradient in the absence of HCO_3 (17). Fig. 5 illustrates, however, that HCO_3 does not have a similar stimulatory effect on oxalate uptake. The initial rate of oxalate uptake was unaffected by the addition of HCO_3. The stimulation by HCO_3 at 5 min is probably due to a longer maintenance of the pH gradient because of increased buffering capacity when HCO_3 is added.

Fig. 6 illustrates that an outwardly directed Cl gradient can also stimulate oxalate uptake. Indeed, compared with gluconate, Cl will stimulate oxalate uptake even when no gradient is present (Fig. 6). Most of the Cl-gradient stimulated oxalate uptake is

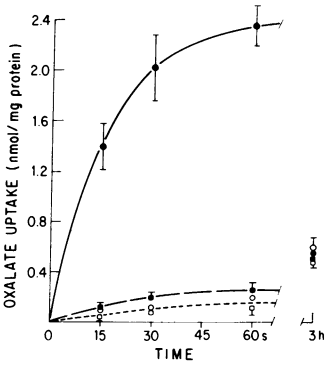

Figure 3. The effect of an outwardly directed oxalate gradient on oxalate uptake. Vesicles were preincubated with 150 mM mannitol, 31 mM Tris, 74 mM Hepes, 37 mM MES (pH 6.5), and either 10 mM TMA_2 oxalate plus 85 mM TMA gluconate or 0.25 TMA_2 oxalate plus 99.6 mM TMA gluconate. The uptake of ^{14}C-oxalate was determined after either a 50-fold or fivefold dilution into a reaction medium consisting of 150 mM mannitol, 31 mM Tris, 74 mM Hepes, 37 mM MES (pH 6.5), and 99.6 mM TMA gluconate, resulting in either 10 mM oxalate in, 0.25 mM out (●, ○), or 0.25 mM oxalate in and out (■, □). Uptake was determined in the presence (open symbols) or absence (solid symbols) of 1 mM DIDS.

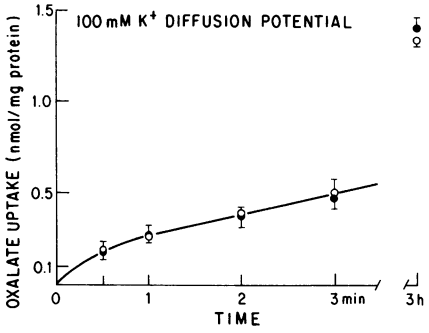

Figure 4. Effect of a transmembrane potential on oxalate uptake. Vesicles were preincubated with 250 mM mannitol, 50 mM Tris, and 96 mM Hepes (pH 7.5). Preincubated vesicles with (○) or without (●) 10 μg valinomycin per mg protein were added to reaction medium resulting in a final concentration of 50 mM Tris, 96 mM Hepes, 50 mM mannitol, 100 mM K gluconate, and 1 mM [^{14}C]oxalate (pH 7.5).

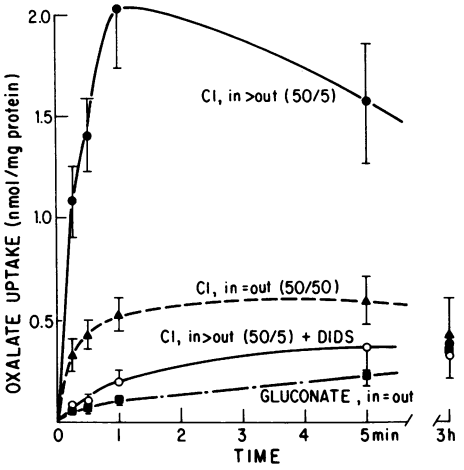

Figure 6. The effect of a chloride gradient on oxalate uptake. Vesicles were preincubated with 50 mM Tris, 96 mM Hepes (pH 7.5), 150 mM mannitol, and either 100 mM TMA gluconate, or 50 mM TMA gluconate and 50 mM TMACl. The vesicles were diluted 10-fold into a reaction medium containing 500 μM [^{14}C]oxalate, 50 mM Tris, 96 mM Hepes, 149 mM mannitol, and 100 mM TMA gluconate (■, no Cl in or out), 50 mM TMA gluconate, and 50 mM TMACl (▲, 50 mM Cl inside and outside), or 95 mM TMA gluconate, 5 mM TMACl (50 mM Cl inside, 5 mM Cl outside) with (○) and without (●) 2 mM DIDS.

inhibited by 2 mM DIDS, which suggests an anion exchange process.

An outwardly directed Cl gradient could stimulate oxalate uptake by generating a pH gradient, resulting in Ox:OH exchange. A Cl gradient could generate a pH gradient either electrically (inside positive diffusion potential) or by Cl:OH exchange (17). To test this possibility, we determined Cl gradient-stimulated oxalate uptake in the presence and absence of nigericin and K^+. Nigericin, a K:H exchanger, will collapse any pH gradient generated by the Cl gradient (16). Fig. 7 shows that Cl-gradient stimulated oxalate uptake was inhibited only 10% by nigericin at 1 min, which indicates that most of the oxalate uptake stimulated by Cl is independent of any effect on pH. The stimulation of oxalate uptake by Cl in the absence of a Cl gradient (Fig. 6) also argues for the direct exchange of oxalate and Cl. Finally, Fig. 8 shows that an oxalate gradient will stimulate Cl uptake, which is inhibited by DIDS, further suggesting that Cl:Ox exchange occurs.

Our data indicate that both Ox:OH and Ox:Cl exchange occur on the brush border. We have made similar observations

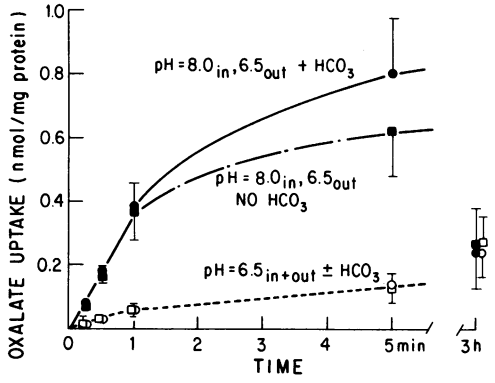

Figure 5. The effect of bicarbonate on pH-gradient stimulated oxalate uptake. Vesicles were preincubated with 100 mM TMA gluconate and pH 8.0 buffer either in the presence of bicarbonate (26.8 mM Tris, 23.2 mM Hepes, 90.6 mM choline bicarbonate, and 150 mM mannitol with 5% CO_2, 95% N_2 gassing) or the absence of bicarbonate (75 mM Tris, 65 mM Hepes, and 240 mM mannitol with 100% N_2 gassing). Uptake of 100 μM ^{14}C-oxalate was determined after 10-fold dilution into a reaction medium at pH 6.5 containing either 5% CO_2 and HCO_3 (●) (21.8 mM Tris, 26 mM MES, 52.2 mM Hepes, 2.9 mM choline HCO_3, 262 mM mannitol, 100 mM TMA gluconate) or no CO_2 and bicarbonate (■) (31 mM Tris, 74 mM Hepes, 37 mM MES, 240 mM mannitol, and 100 mM TMA gluconate). Oxalate uptake in the absence of a pH or bicarbonate gradient was determined in vesicles preincubated in solution identical to the pH 6.5 reaction medium either in the presence (○) or absence (□) of CO_2 and HCO_3.

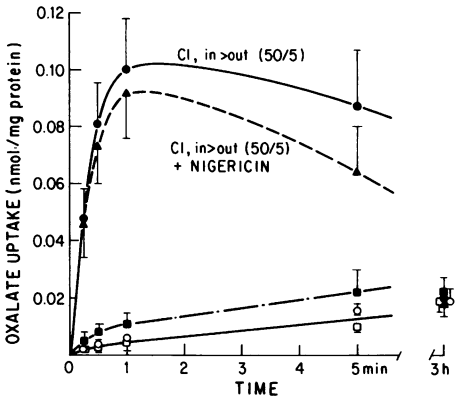

Figure 7. The effect of pH clamping on chloride-stimulated oxalate uptake. 25 μM [^{14}C]oxalate uptake was determined in vesicles preincubated with 50 mM Tris, 96 mM Hepes (pH 7.5), 150 mM mannitol, 50 mM K gluconate, and either 50 mM TMACl (chloride gradient: ●, ○, ▲) or 50 mM TMA gluconate (no chloride: ■, □). The vesicles were diluted 10-fold into a reaction mixture with a final concentration of 50 mM Tris, 95 mM Hepes (pH 7.5), 150 mM mannitol, 50 mM K gluconate, 25 μM [^{14}C]oxalic acid, and either 5 mM TMACl, 45 mM TMA gluconate (chloride gradient), or 50 mM TMA gluconate (no chloride). Uptake was determined in the presence of nigericin, 10 μg/mg protein (▲), or in the presence (○, □) or absence (●, ■) of 1 mM DIDS.

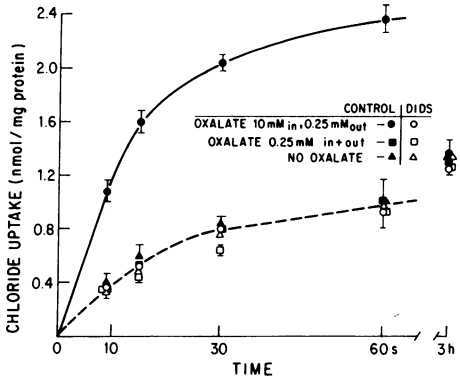

Figure 8. The effect of an oxalate gradient on chloride uptake. The vesicles were preincubated with 140 mM buffer at pH 6.5 (see Fig. 1 for composition), 150 mM mannitol, and 100 mM TMA gluconate (▲, △) or 99.625 mM TMA gluconate and 0.25 mM TMA_2 oxalate (■, □) or 85 mM TMA gluconate 10 mM TMA_2 oxalate (●, ○). Uptake of 3 mM TMA ^{36}Cl was determined by diluting the vesicles 40-fold with reaction medium containing 140 mM buffer (pH 6.5), 144 mM mannitol, and 100 mM TMA gluconate or 99.625 mM TMA gluconate and 0.25 mM TMA_2 oxalate. The experiment was carried out in the presence (open symbols) or absence (solid symbols) of 3 mM DIDS.

with SO_4, i.e., SO_4:OH and SO_4:Cl exchange (18). When our "brush border membranes" were further purified on a sucrose density gradient, however, and separated into brush border and basolateral membrane components, SO_4:OH exchange was localized to the brush border and SO_4:Cl exchange was isolated to the basolateral membrane (18). When similar studies were performed with oxalate (Fig. 9), both pH gradient and Cl gradient-stimulated oxalate uptake were greater in the purified brush border subfraction, compared with the crude mixed preparation. Since the purified brush border fraction was significantly less purified in basolateral marker than was the mixed membrane preparation (see fig. 9 legend and reference 17), these data suggest that Ox:OH and Ox:Cl exchange activities reside on the brush border itself and are unlikely to be due to basolateral contamination of the brush border preparation. The small stimulation of oxalate uptake by the pH and Cl gradient in the basolateral membrane preparation could be due to contaminating brush border membrane.

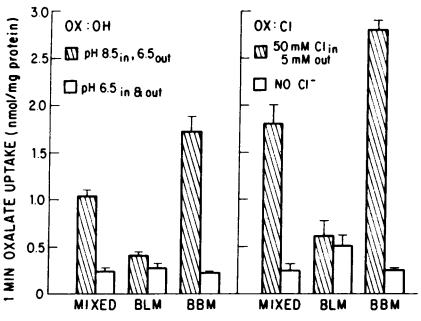

Figure 9. Membrane localization of Ox:OH and Ox:Cl exchangers. A "mixed" population of plasma membrane vesicles was separated into basolateral (BLM) and brush border (BBM) enriched membranes by sucrose density gradient centrifugation (17). 1-min oxalate uptake was determined for each membrane population in the presence (hatched bar) or absence (clear bar) of either; a pH gradient (see Fig. 1 or media composition) or chloride gradient (see Fig. 4 for media composition). The enrichment of the "mixed" membranes, BBM and BLM, in sucrase activity was 15.6, 24.1, and 0.6-fold, respectively, and in Na-K ATPase activity was 2.9, 1.0, and 7.2-fold, respectively (17).

The effect of various transport inhibitors on pH gradient-stimulated oxalate uptake and Cl gradient-stimulated oxalate uptake is shown in Table I. Amiloride and harmaline, Na:H exchange inhibitors, had no effect. Acetazolamide, a carbonic anydrase inhibitor, also had no effect. Bumetanide and furosemide, Na:Cl and Na:K:Cl cotransport inhibitors, caused ~50% inhibition. These two inhibitors will inhibit anion exchange at the concentrations used in this experiment (17). Probenecid, an organic anion transport inhibitor, inhibited pH gradient-stimulated oxalate uptake about twice as much as Cl gradient-stimulated oxalate uptake, whereas SITS, an anion exchange inhibitor, resulted in 70–80% inhibition in both exchanges.

We looked for evidence that other organic acids could be transported by determining the ability of outwardly directed organic acid gradients to stimulate oxalate and Cl uptake (trans-stimulation). Since organic acid exit from the vesicle by passive nonionic diffusion would result in an inside alkaline pH gradient, stimulating Ox:OH and Cl:OH exchange, the vesicles were pH clamped with K^+ and nigericin (Table II). Formate caused a sevenfold stimulation of oxalate uptake and a ninefold stimulation of Cl uptake, which suggests formate:Ox and formate:Cl exchange. Oxalacetate produced a fourfold stimulation of Cl uptake and a twofold stimulation of oxalate uptake, which suggests oxaloacetate:Cl and oxaloacetate:Ox exchange.

Finally, we wanted to determine if Na would stimulate oxalate uptake. Fig. 10 illustrates that an inwardly directed Na gradient produced only a slight stimulation of Na uptake compared with tetramethylammonium (TMA) and K. This Na-stimulated oxalate uptake was inhibited by DIDS.

Discussion

In this study we found that oxalate can be transported across the brush border membrane in exchange for either OH or Cl ions. This conclusion is based on the observations that: (*a*) an outwardly directed oxalate, OH or Cl gradient will stimulate oxalate uptake and an outwardly directed oxalate gradient will

Table I. Effect of Transport Inhibitors on Oxalate Uptake

	Uptake	
	Ox:OH	Ox:Cl
	Percent of control	*Percent of control*
Amiloride	105.2±10.7	94.7±5.5
Harmaline	107.7±7.8	98.6±8.9
Acetazolamide	87.8±6.6	98.0±5.0
Bumetanide	53.1±1.5	38.4±3.6
Furosemide	53.7±3.3	44.1±2.5
Probenecid	27.5±3.7	66.1±2.4
SITS	31.8±0.6	22.2±0.6
DIDS	3.7±0.6	2.7±0.6
Oxalate	10.0±0.8	44.3±6.2

Uptake of 50 μM oxalate was determined at 6 s either in the presence of a pH gradient (pH 8.5 in, 6.5 out) or a chloride gradient (50 mM in, 5 mM out). See Figs. 1 and 4 for composition of media. 1 mM inhibitor was added to the vesicles 10 min before the initiation of uptake (no preincubation when 1 mM oxalate was added).

Table II. Organic Acid Stimulation of Cl and Oxalate Uptake

Organic acid	Percent of control	
	Oxalate uptake	Chloride uptake
Formic acid	762±24	962±65
Propionic acid	110±4	163±28
Butyric acid	103±7	165±19
Valeric acid	80±8	162±19
Oxalacetic acid	201±27	421±90
Malonic acid	157±25	78±9
α-Ketoglutaric acid	97±12	82±7
p-aminohippuric acid	58±8	100±8
B-Hydroxybutyric acid	—	113±9
Lactic acid	—	139±11

Vesicles were preincubated for 2 h at 20°C with 50 mM TMA salt of the organic acid (50 mM TMA gluconate for control), 50 mM Tris, 96 mM Hepes (pH 7.5), 150 mM mannitol, 50 mM K gluconate, and enough TMA gluconate for a final osmolality of 546 mosm. The vesicles were diluted 20-fold into a reaction medium containing 50 mM Tris, 96 mM Hepes, 50 mM K gluconate, either 50 μM [^{14}C]oxalate and 150 mM mannitol or 5 mM TMA ^{36}Cl and 140 mM mannitol, and enough TMA gluconate for a final osmolarity of 546 mosm. Nigericin, 10 μg/mg protein was added to pH clamp the vesicles. Oxalate uptake was determined at 15 s and Cl uptake, at 9 s. n = 4 for oxalate uptake and n = 6 for Cl uptake.

stimulate Cl uptake; (*b*) pH-gradient and Cl-gradient stimulated oxalate uptake is inhibited by anion exchange inhibitors; (*c*) an inside-positive membrane potential will not stimulate oxalate uptake, which rules out indirect electrical coupling; (*d*) pH clamping with nigericin and K excludes the possibility that Cl gradient-stimulated oxalate uptake is secondary to Cl:OH exchange; and (*e*) further separation of our membranes into brush border-enriched and basolateral-enriched fractions indicate that Ox:OH and Ox:Cl exchange observed is not due to contaminating basolateral membrane. We have found that a pH or Cl gradient will stimulate oxalate uptake over a wide range of oxalate concentrations (5–500 μM, Figs. 1, 2, 6, 7), likely to encompass

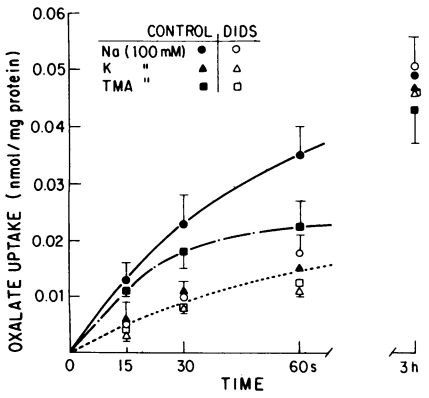

Figure 10. The effect of Na on oxalate uptake. The vesicles were incubated for 2 h at 20°C with 50 mM Tris, 96 mM Hepes (pH 7.5), 150 mM mannitol, and 200 mM TMA gluconate. Uptake of 25 μM ^{14}C-oxalate was determined by adding the vesicles to a reaction medium containing 100 mM TMA gluconate and 100 mM Na gluconate (●), 100 mM TMA gluconate, and 100 mM K gluconate (▲) or 200 mM TMA gluconate (■). Uptake was determined in the presence (open symbols) and absence (closed symbols) of 0.5 mM DIDS.

the range of oxalate concentrations found in vivo, suggesting a physiologic function. These studies do not resolve the question of how oxalate exits across the basolateral membrane.

Hatch et al. (15), studying intact, distal colonic mucosa of rabbit in vitro, found net oxalate absorption under short-circuit conditions, which was abolished by adding the anion exchange inhibitor, SITS, to the mucosal solution. Their results suggest that anion exchange is responsible for oxalate transport across the brush border membrane, which agrees with our findings. They also found that net oxalate absorption was partially inhibited by acetazolamide, which suggests that Ox:HCO_3 exchange may be preferred over Ox:OH exchange in the colon. In our studies, HCO_3 did not provide additional stimulation of oxalate uptake (Fig. 5). This observation is in contrast to our findings with pH-stimulated Cl uptake, in which HCO_3 caused further stimulation of Cl uptake (17).

A previous study in intact rabbit ileum in vitro (7) reported that oxalate uptake by ileal mucosa was nonsaturable, and thus not suggestive of a carrier-mediated process. This study, however, employed a relatively long uptake time (45 min); thus, equilibration may have occurred via noncarrier-mediated pathways, making saturability undetectable. Further, no pH gradient was utilized (7), and oxalate transport in the absence of a pH gradient is small (Fig. 1). Saturability may be difficult to detect in the absence of a favorable pH gradient across the brush border membrane. Finally, in intact tissue experiments as used above, the inwardly directed Cl gradient would tend to prevent the mucosal accumulation of oxalate by Ox:OH exchange.

Oxalate transport has been examined previously, using brush border membrane vesicles prepared from rat small intestine (19). These investigators found stimulation of oxalate uptake by an outwardly directed OH gradient but concluded that it was not due to the pH gradient since the stimulated uptake was not abolished by the proton ionophore carbonyl cyanide *p*-trifluoromethoxyphenylhydrazine (FCCP) (19). These investigators used relatively low buffer capacity (1–2 mM) and began the uptake studies 5 min after diluting their vesicles in reaction media to generate the pH gradient. These conditions may have led to the collapse of the pH gradient before the determination of [^{14}C]oxalate uptake even in the absence of FCCP. Of interest, these investigators found trans-stimulation of [^{14}C]oxalate uptake by unlabeled oxalate, indicating Ox:Ox exchange (19), an observation we have also made (Fig. 3).

The importance of these "transmembrane" carrier-mediated pathways of oxalate transport in net oxalate absorption in vivo and in the pathogenesis of calcium oxalate nephrolithiasis remains to be determined. There may even be species variation in the presence of these transporters, since three studies in the rat colon found oxalate transport to be nonsaturable (8–10) (see above for potential pitfalls in these studies), although a fourth study claimed active transport (20). Further, present evidence indicates that bile acids and fatty acids, which increase oxalate absorption in the colon, probably do so by causing a nonspecific increase in permeability (10, 11, 20–22), and probably at the tight junction (23) rather than affecting a specific transport process. Bile acids and long-chain fatty acids do block NaCl absorption and induce electrogenic Cl secretion, however, which are transcellular, carrier-mediated processes (24–26). Thus, it is possible that bile acids and long-chain fatty acids may have some direct or indirect effect on the oxalate transporters in the ileum and colon. Finally, net oxalate secretion rather than absorption occurs in the rat small intestine in vitro (unpublished observation

and reference 27) and these exchangers may be involved in this secretory process.

To determine what other organic acids might also be transported on the Ox:OH and Ox:Cl exchange, we performed trans-stimulation experiments (Table II). Formic acid caused the greatest degree of trans-stimulation of oxalate uptake. These results strongly suggest that formate can also be transported on the same exchangers as oxalate. Formic acid is a product of the bacterial fermentation of carbohydrates and it has been measured in human feces at a concentration of 1–2 mM (28). The concentration may be much higher in the proximal colon or distal ileum because it is further converted by bacteria to hydrogen and CO_2. The amount in feces will also be reduced by the amount that is absorbed. In most studies of short-chain fatty acid metabolism in the intestine, formic acid has not been measured. Therefore, we will not know the full biologic importance of formate transport until these studies have been done. Note that exchange of formate and oxalate with Cl has been described recently in brush border vesicles isolated from the proximal tubule (29, 30). Thus, affinity for formate may be a general feature of Cl:Ox exchangers.

Oxaloacetate, a dicarboxylic acid like oxalate, stimulated Cl uptake and to a lesser extent, oxalate uptake. The other organic acids only slightly stimulated the uptake of either oxalate or Cl but not both. Thus, it would appear that only a limited number of organic acids are readily transported by the oxalate exchangers.

Finally, we found little evidence that oxalate could be absorbed by cotransport with Na. The slight stimulation of oxalate transport by Na could be due to indirect coupling via dual exchange; Na:H and Ox:OH exchange, since it was inhibited by DIDS.

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