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Substrate and Inhibitor Specificity of Anion Exchangers on the Brush Border Membrane of Rabbit Ileum

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Summary. In previous studies we have found that several anions can be transported by an exchange process in rabbit ileal brush border membranes. We demonstrated exchanges of Cl for OH or HCO_3, SO_4 for OH, oxalate for OH, and oxalate for Cl. The purpose of these studies was to determine the number of distinct carriers mediating these exchanges. We utilized substrate and inhibitor specificity studies to distinguish between different anion exchange transporters. We conclude that SO_4 : OH and oxalate : OH exchange occur on the same carrier because: (i) pH-gradient stimulated transport of both ^{14}C-oxalate and $^{35}SO_4$ were equally sensitive to *cis*-inhibition by unlabeled SO_4 or oxalate; and (ii) both were inhibited equally by K. We conclude that oxalate : OH and oxalate : Cl exchanges occur on different carriers because: (i) Cl or SO_4 caused unequal *cis*-inhibition of these two exchanges; and (ii) as compared to oxalate : Cl exchange, oxalate : OH exchange was more sensitive to inhibition by probenecid and K and less sensitive to inhibition by bumetanide. Finally, we conclude that oxalate : Cl exchange and Cl : HCO_3 exchange occur on different carriers because: (i) Cl : HCO_3 exchange was almost completely insensitive to *cis*-inhibition by oxalate; and (ii) oxalate : Cl exchange was more sensitive to inhibition by DIDS and bumetanide than Cl : HCO_3 exchange. Thus we have found that there are at least three separate anion exchangers on rabbit ileal brush border: (i) a Cl : HCO_3 exchanger; (ii) a SO_4 : OH exchanger, which also transports oxalate; and (iii) an oxalate : Cl exchanger.

Key Words anion exchange · Cl : HCO_3 exchange · SO_4 : OH exchange · Ox : Cl exchange · brush border membrane · ileum

Introduction

We have previously demonstrated that Cl : HCO_3(OH) and SO_4 : OH exchange occurs across the brush border membrane of rabbit ileum [5, 12]. SO_4 did not compete with Cl for transport [12], suggesting that Cl : HCO_3 and SO_4 : OH exchange occurred on separate carriers. More recently we have found that oxalate : Cl exchange and oxalate : OH exchange occurs across the same membrane [6]. In these experiments we provide evidence that Ox : OH[1] and SO_4 : OH exchange occur on the same carrier, but that Ox : Cl exchange occurs on a separate carrier from either the SO_4 : OH or the Cl : HCO_3 carrier.

Materials and Methods

Rabbit ileal brush border vesicles were prepared as previously described [4] and used on the day of preparation. Following a 2-hr preincubation at room temperature, a 10-μl aliquot of vesicles (150–200 μg protein) was added to a reaction solution containing $^{35}SO_4$, ^{14}C-oxalate, or ^{36}Cl and uptake allowed to proceed at 30°C (*see* figure legends). When HCO_3 was used, all media were gassed with 95% N_2, 5% CO_2 or 99% N_2, 1% CO_2. At varying time intervals, isotope uptake was stopped with 3 ml ice-cold stop solution consisting of 10 mM Tris, 16 mM HEPES, pH 7.5 plus the desired K gluconate concentration to maintain isosmolarity (preincubation medium, reaction medium and stopping solution were always kept isosmotic). The stopped solution was immediately filtered on 0.45 μm Millipore filter (HAMP) and washed twice with 3 ml of ice-cold stopping solution. Radioactivity was determined using a beta scintillation counter.

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.

[1] *Abbreviations used*: Ox, oxalate; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid; MES, 2-(N-morpholino) ethanesulfonate; Tris, tris(hydroxymethyl) aminomethane; TMA, tetramethylammonium; SITS, 4-acetamido-4′-isothiocyanostilbene 2,2′-disulfonate (disodium salt); DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonate (disodium salt).

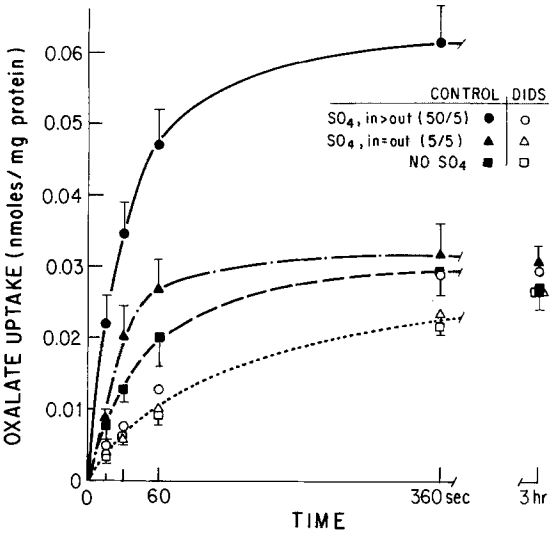

Fig. 1. The effect of an outwardly directed SO_4 gradient on oxalate uptake. Vesicles were preincubated with 50 mM Tris, 96 mM HEPES (pH 7.5), 150 mM mannitol and either 100 mM TMA gluconate (■,□), 92.5 mM TMA gluconate and 5 mM TMA_2SO_4 (▲,△), or 25 mM TMA gluconate and 50 mM TMA_2SO_4 (●,○). ^{14}C-oxalate (25 μM) uptake was determined following dilution into a reaction medium consisting of 50 mM Tris, 96 mM HEPES, 150 mM mannitol, and 100 mM TMA gluconate (■,□, no SO_4) or 92.5 mM TMA gluconate, 5 mM TMA_2SO_4 resulting in either 50 mM SO_4 inside, 5 mM SO_4 outside (●,○) or 5 mM SO_4 inside and outside (▲,△). The inhibitory effect of 1 mM DIDS (open symbols) was also determined

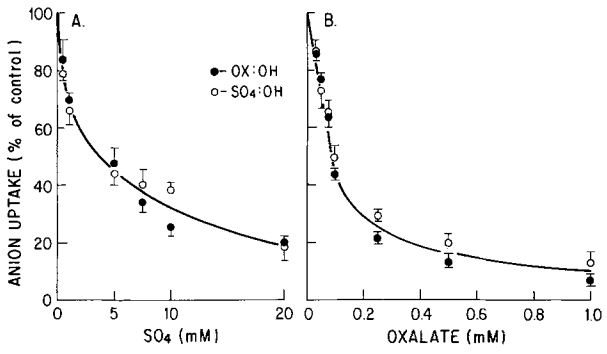

Fig. 2. *Cis*-inhibition of SO_4 : OH and Ox : OH exchange. The uptake of $^{35}SO_4$ or the uptake of ^{14}C-oxalate was determined under pH gradient conditions (pH 7.7 inside, pH 6.5 outside) at 3 sec. The vesicles were preincubated in 70 mM Tris, 70 mM HEPES, 150 mM mannitol and 100 mM TMA gluconate and diluted into a reaction media containing 31 mM Tris, 74 mM HEPES, 37 mM MES, 150 mM mannitol and 100 mM TMA gluconate (control). Uptake was corrected for the DIDS-insensitive component as determined in the control with 1 mM DIDS. (*A*) The inhibitory effect of SO_4 was determined by increasing [TMA_2SO_4] from 25 μM (control) to 20 mM. Simultaneously, the TMA gluconate concentration was decreased to keep total osmolarity constant. The TMA_2 oxalate concentration was 25 μM. (*B*) The inhibitory effect of oxalate was determined by increasing TMA_2 oxalate from 25 μM (control) to 1 mM. The TMA_2SO_4 concentration was 25 μM

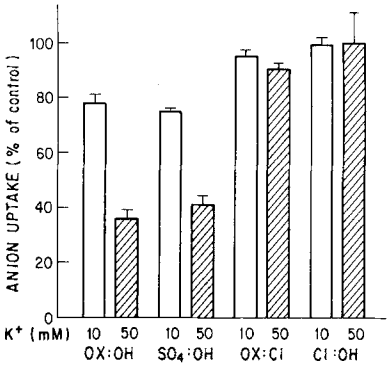

Fig. 3. The effect of potassium on anion exchange. The uptake of 25 μM ^{14}C-oxalate, 25 μM $^{35}SO_4$ or 3 mM ^{36}Cl stimulated by an outwardly directed OH gradient, or of oxalate stimulated by an outwardly directed chloride gradient was determined at 3 sec in the presence of 10 or 50 mM K gluconate, or the absence of K (control). In all cases, the reaction medium contained 31 mM Tris, 74 mM HEPES, 37 mM MES (pH 6.5), 150 mM mannitol, and 50–100 mM TMA gluconate. Oxalate : OH, SO_4 : OH, and Cl : OH was determined in the presence of a pH 7.7 inside, pH 6.5 outside gradient by preincubating the vesicles with 70 mM Tris, 70 mM HEPES, 150 mM mannitol, and 100 mM TMA gluconate. Oxalate : Cl exchange was determined in the presence of a 50 mM inside, 5 mM outside chloride gradient by preincubating the vesicles with 31 mM Tris, 74 mM HEPES, 37 mM MES, 150 mM mannitol, 50 mM TMA gluconate, and 50 mM TMACl. Uptake was corrected for the DIDS-insensitive component as determined in the control with 1 mM DIDS for Ox : OH, SO_4 : OH and Ox : Cl exchange and 3 mM DIDS for Cl : OH exchange

Results

An outwardly directed OH gradient can stimulate both oxalate uptake and SO_4 uptake [6, 12], raising the possibility that these processes occur on the same carrier. If this were the case, then SO_4 should stimulate oxalate uptake and vice versa. Figure 1 illustrates that an outwardly directed SO_4 gradient stimulated oxalate uptake. Even the presence of SO_4 without a gradient (5 mM in and out) stimulated oxalate uptake, indicating that Ox : SO_4 exchange is preferred over Ox : OH exchange at the pH and SO_4 concentrations used in these experiments. SO_4-stimulated oxalate uptake was inhibited by 1 mM DIDS, consistent with an anion exchange process.

If SO_4 : OH and Ox : OH exchange occur on the same carrier, then each anion should cause similar *cis*-inhibition of SO_4 and Ox uptake by competition for transport. To test this, pH-stimulated SO_4 and oxalate transport were determined using reaction media of identical composition (except for the labeled substrate). In Fig. 2*A*, increasing concentrations of SO_4 produced equal inhibition of pH gradient-stimulated SO_4 and oxalate uptake, and in Fig. 2*B* the same is seen with increasing concentrations of oxalate.

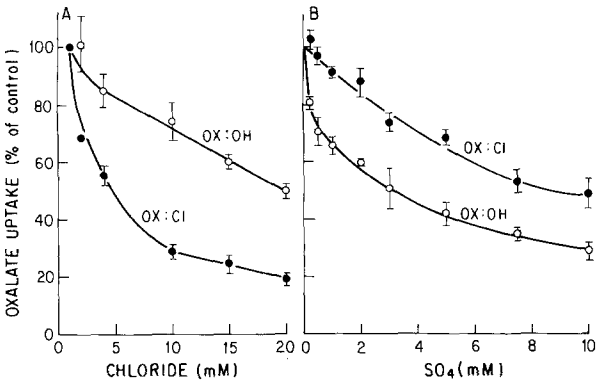

Fig. 4. (*A*) *Cis*-inhibition of Ox : OH and Ox : Cl exchange by Cl. The uptake of 50 μM ^{14}C-oxalate stimulated by a 40-fold pH (pH 8.1 inside, 6.5 outside) or Cl gradient (40 mM in, 1 mM out) was determined at 3 sec with vesicles preincubated in pH 8.1 buffer (84 mM Tris, 56 mM HEPES, 150 mM mannitol, 100 mM TMA gluconate) or in pH 6.5, 40 mM Cl buffer (31 mM Tris, 74 mM HEPES, 37 mM MES, 150 mM mannitol, 60 mM TMA gluconate, 40 mM TMACl). The vesicles were diluted 40-fold (Ox : Cl) or fivefold (Ox : OH) to a reaction medium containing 31 mM Tris, 74 mM HEPES, 37 mM MES (pH 6.5), 150 mM mannitol, 50 μM ^{14}C-oxalate, 99 mM TMA gluconate (control), and TMACl. The TMACl concentration was varied from 1 mM (control) to 20 mM in the reaction media with a corresponding decrease in TMA gluconate to keep ionic strength constant. Uptake was corrected for the DIDS-insensitive component as determined in the control with 1 mM DIDS. (*B*) *Cis*-inhibition of Ox : OH and Ox : Cl exchange by SO_4. The uptake of 50 μM ^{14}C-oxalate stimulated by an outwardly directed 20-fold OH (pH 7.8 in, 6.5 out) or chloride gradient (20 mM in, 1 mM out) was determined at 3 sec using vesicles preincubated in pH 7.8 buffer (71 mM Tris, 69 mM HEPES, 150 mM mannitol, 100 mM TMA gluconate) or pH 6.5, 20 mM Cl buffer (31 mM Tris, 74 mM HEPES, 37 mM MES, 150 mM mannitol, 20 mM TMACl, 80 mM TMA gluconate). The vesicles were diluted 20-fold into a reaction medium containing 31 mM Tris, 74 mM HEPES, 37 mM MES (pH 6.5), 150 mM mannitol, 50 μM ^{14}C-oxalate, 99 mM TMA gluconate, and 1 mM TMACl (control). The TMA_2SO_4 concentration was varied from 250 μM to 10 mM, with a corresponding decrease in TMA gluconate to keep osmolarity constant. Uptake was corrected for the DIDS-insensitive component as determined in the control with 1 mM DIDS

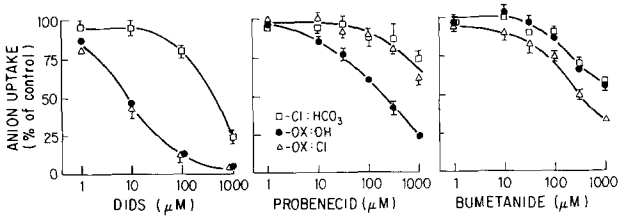

Fig. 5. The effect of DIDS, probenecid, and bumetanide on anion exchange. The uptake of 50 μM ^{14}C-oxalate was determined with an outwardly directed pH (8.1 inside, 6.5 outside) gradient (Ox : OH) using vesicles preincubated with 84 mM Tris, 56 mM HEPES (pH 8.1), 150 mM mannitol and 150 mM TMA gluconate (●). The 3-sec uptake of 50 μM ^{14}C-oxalate was also determined with an outwardly directed Cl gradient (40 mM_{in}, 2 mM_{out}) using vesicles preincubated as described in Fig. 4*A* (Δ). Two mM ^{36}Cl uptake was determined with an outwardly directed pH and HCO_3 (7.7 in, 6.5 out) gradient using vesicles preincubated with 35 mM Tris, 35 mM HEPES, 50 mM choline HCO_3, 100 mM TMA gluconate, 222 mM mannitol and gassed with 5% CO_2, 95% N_2 (□). Uptake was initiated by diluting vesicles fivefold (Ox : OH), 20-fold (Ox : Cl) or 50-fold (Cl : HCO_3) into a reaction medium gassed with 1% CO_2, 99% N_2 and with a final composition of 31 mM Tris, 74 mM HEPES, 37 mM MES (pH 6.5), 150 mM mannitol, 147 mM TMA gluconate, 50 μM TMA_2 oxalate and 2 mM TMACl. The effect of 1 to 1000 μM transport inhibitor on each of the exchangers was determined. Probenecid and bumetanide were preincubated with the vesicles for 10 min. All uptakes were determined at 3 sec

We have previously shown that external K^+ inhibits SO_4 : OH exchange [13]. If Ox : OH exchange occurs on the same carrier, then one would expect to see similar inhibition by external K^+, and this was the case (Fig. 3). 10 and 50 mM K^+ caused similar inhibition of pH-stimulated SO_4 (SO_4 : OH exchange) and Ox (Ox : OH exchange) uptake. In contrast K^+ had little effect on pH-stimulated Cl uptake (Cl : OH(HCO_3) exchange) or Cl-stimulated oxalate uptake (Ox : Cl exchange) (Fig. 3).

The next question was to determine whether Ox : Cl exchange occurs on the same carrier as Ox : OH (SO_4 : OH) exchange. In Fig. 3, K caused little inhibition of Ox : Cl exchange, whereas 50 mM K caused greater than 50% inhibition of Ox : OH exchange, suggesting these events occur on different carriers.

If Ox : OH exchange and Ox : Cl exchange occur on the same carrier, then one might see a similar degree of *cis*-inhibition of both processes by external Cl. Figure 4*A* illustrates that increasing concentrations of Cl cause greater inhibition of Cl gradient-stimulated oxalate uptake (Ox : Cl exchange) than pH-gradient stimulated oxalate uptake (Ox : OH exchange). Continuing with the same reasoning, if Ox : Cl exchange and Ox : OH exchange occur on the same carrier, then external SO_4 should cause similar *cis*-inhibition, since our data indicate that Ox : OH exchange and SO_4 : OH exchange occur on the same carrier. Figure 4*B* illustrates that SO_4 causes greater inhibition of Ox : OH exchange than of Ox : Cl exchange, further suggesting that these two processes occur on separate carriers.

Figure 5 illustrates the effect of three different anion exchange inhibitors on Ox : OH exchange and Ox : Cl exchange. Although both exchanges were similarly sensitive to DIDS, Ox : OH exchange was more sensitive to inhibition by probenecid and Ox : Cl exchange was more sensitive to inhibition by bumetanide. In the aggregate, therefore, the data from Figs. 3, 4 and 5 strongly suggest that Ox : Cl

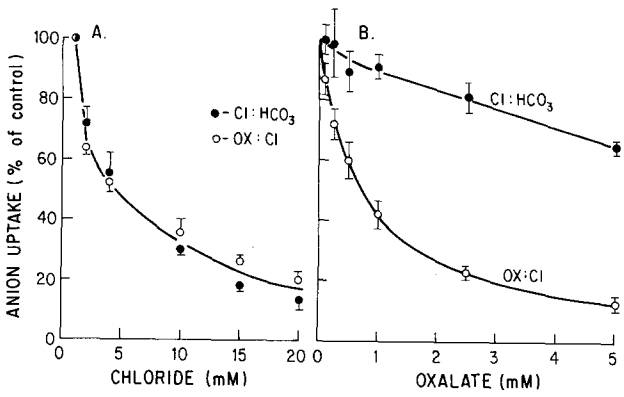

Fig. 6. *Cis*-inhibition of Ox : Cl and Cl : HCO_3 exchange. (*A*) The uptake of 50 μM ^{14}C-oxalate was determined at 3 sec with an outwardly directed chloride gradient (40 mM inside, 1 mM outside) using vesicles preincubated with 31 mM Tris, 74 mM HEPES, 37 mM MES (pH 6.5), 150 mM mannitol, 40 mM KCl and 110 mM K gluconate. The uptake of ^{36}Cl was determined with an outwardly directed pH (7.7 inside, 6.5 outside) and HCO_3 gradient using vesicles preincubated with 35 mM Tris, 35 mM HEPES, 50 mM $KHCO_3$, 100 mM K gluconate, and 222 mM mannitol while gassing with 5% CO_2, 95% N_2. Uptake was determined by diluting the vesicles 40-fold (Ox : Cl) or 50-fold (Cl : HCO_3) into a reaction medium gassed with 1% CO_2, 99% N_2 and containing 31 mM Tris, 74 mM HEPES, 37 mM MES (pH 6.5), 150 mM mannitol, 25 μM K_2 oxalate, 0.27 mM $KHCO_3$, and 149 mM K gluconate (control). KCl was varied from 1 mM (control) to 20 mM with a corresponding change in K gluconate to maintain a constant osmolarity. Uptake was corrected for the DIDS-insensitive component as determined in the control with 1 mM (Ox : Cl exchange) or 3 mM (Cl : HCO_3 exchange) DIDS. (*B*) The uptake of ^{14}C-oxalate was determined at 3 sec with an outwardly directed chloride gradient (60 mM inside, 3 mM outside) using vesicles preincubated with 31 mM Tris, 74 mM HEPES, 37 mM MES (pH 6.5), 150 mM mannitol, 60 mM KCl and 90 mM K gluconate. The 3-sec uptake of 3 mM TMA^{36}Cl was determined with an outwardly directed pH and HCO_3 gradient (pH 7.7 inside, 6.5 outside) using vesicles preincubated with 35 mM Tris, 35 mM HEPES, 50 mM $KHCO_3$, 100 mM K gluconate, 222 mM mannitol and gassed with 5% CO_2, 95% N_2. The vesicles were diluted 20-fold (Ox : Cl) or 50-fold (Cl : HCO_3) into a reaction medium with a final concentration of 31 mM Tris, 74 mM HEPES, 37 mM MES (pH = 6.5), 3 mM TMACl, 25 μM K_2 oxalate, 144 mM mannitol and 150 mM K gluconate (control) and gassed with 1% CO_2, 99% N_2. Oxalate in the reaction medium was varied from 25 μM (control) to 5 mM with K_2 oxalate with a concomitant change in K gluconate to maintain a constant osmolarity. Uptake was corrected for the DIDS-insensitive component as determined in the control with 1 mM (Ox : Cl exchange) or 3 mM (Cl : HCO_3 exchange) DIDS

exchange and Ox : OH (SO_4 : OH) exchange occur on separate carriers.

Finally, we wished to determine whether Ox : Cl exchange and Cl : HCO_3(OH) exchange occurred on the same or different carriers. Figure 5 illustrates that DIDS caused much greater inhibition

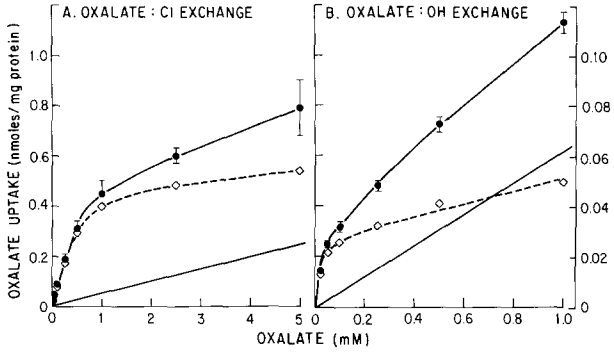

Fig. 7. Kinetics of Ox : Cl and Ox : OH exchange. (*A*) Chloride-stimulated (60 mM inside, 3 mM outside) oxalate uptake was determined with increasing extravesicular TMA_2 oxalate concentrations ranging from 25 μM to 5 mM (Fig. 6*B*). The uptakes (top line) were computer analyzed using a nonlinear least squares program [9]. The best fit resulted in a linear component (bottom line) and a saturable component with a K_m of 0.566 ± 0.034 mM and V_{max} of 12.04 ± 0.92 nmol min^{-1} mg protein^{-1}. Cl gradient-stimulated oxalate uptake was linear for 3 sec with 2.5 mM oxalate and only slightly less than linear (86% of expected) at 3 sec with 5 mM oxalate (N = 2). (*B*) pH gradient-stimulated oxalate uptake was determined with increasing extravesicular oxalate concentrations ranging from 25 μM to 1 mM (Fig. 2*B*). The oxalate uptakes (upper line) were computer analyzed and the best fit resulted in a linear component (bottom line) and a saturable component with a K_m of 0.086 ± 0.009 mM and V_{max} of 0.994 ± 0.064 nmol min^{-1} mg protein^{-1}. Under the pH gradient conditions used in these experiments, 1 mM oxalate uptake is linear for 15 sec (N = 2)

of Ox : Cl exchange than Cl : HCO_3 exchange, as did bumetanide to a lesser extent, suggesting these events occur on different carriers. In Fig. 6, although external Cl caused a similar degree of *cis*-inhibition of Cl : HCO_3 exchange and Ox : Cl exchange (Fig. 6*A*), there was a marked difference in degree of *cis*-inhibition produced by external oxalate (Fig. 6*B*). Oxalate only caused 20–30% inhibition of HCO_3-stimulated Cl uptake, while causing 80% inhibition of Cl gradient-stimulated oxalate uptake. Thus, all data considered, Cl : HCO_3 exchange and Ox : Cl exchange appear to occur on separate carriers.

The data from Figs. 2 and 6 can be used to determine the apparent kinetic parameters of the Ox : OH and Ox : Cl exchangers and has been replotted for this purpose in Fig. 7. Utilizing a nonlinear least squares curve-fitting program [9], oxalate uptake can be resolved into a linear and saturable component (dashed line). For Ox : OH exchange, the apparent K_m is 86 ± 9 μM and J_{max} is 1.0 ± 0.06 nmol mg^{-1} protein min^{-1}. For Ox : Cl exchange, the apparent K_m is 566 ± 34 μM and J_{max} is 12.0 ± 0.9 nmol mg^{-1} protein min^{-1}.

Table. Apparent K_i values for various substrates and inhibitors on anion exchange processes

	$Cl:HCO_3$	$SO_4:OH$	Ox:OH	Ox:Cl
Chloride	3.5 ± 0.1 mM[a]	—	19.1 ± 5.5 mM	4.0 ± 0.3 mM
Sulfate	>50 mM[b]	4.6 ± 0.3 mM	4.6 ± 0.3 mM	9.0 ± 0.8 mM
Oxalate	>5 mM	96 ± 10 μM	83 ± 5 μM	774 ± 74 μM
DIDS	314 ± 31 μM	—	9.3 ± 0.5 μM	8.5 ± 2.0 μM
Probenecid	>1 mM	—	271 ± 61 μM	>1 mM
Bumetanide	>1 mM	—	>1 mM	358 ± 80 μM
Potassium	−	+	+	−

The results shown are the apparent K_i values (determined by Dixon plot) of the substrates and inhibitors used in this study, utilizing the data in Figs. 2–6. For potassium, only presence (+) or absence (−) of inhibition is shown.

[a] The K_m for Cl, taken from ref. 5.

[b] This K_i was calculated from data in ref. 12.

Discussion

In the past few years, we have found that a number of anions can be transported across the rabbit ileal brush border membrane by an exchange process. As the number of "anion exchangers" increased, it became important for us to determine whether several carriers were involved, or whether a single carrier could mediate the exchange of all of the transported anions. On the basis of the experiments in this paper and our previous findings, we can conclude that there are at least three separate anion exchangers on the brush border membrane in the rabbit ileum: (i) a $Cl:HCO_3$ exchanger, (ii) a $SO_4:OH$ exchanger, and (iii) and Ox:Cl exchanger. The Table summarizes our studies to date which distinguish between the three anion exchangers.

In the *cis*-inhibition experiments (Figs. 2, 3, 4 and 6), uptake was corrected for the DIDS-insensitive component. Since DIDS inhibits pH gradient-stimulated SO_4 [13] and oxalate uptake (Fig. 5) and Cl-stimulated oxalate uptake (Fig. 5) by 95% and there are no detectable conductive pathways for SO_4 and oxalate [6, 12], it can be assumed that the inhibition of SO_4 and oxalate uptake observed was due to inhibition of anion exchange. DIDS only inhibits 80% of pH and HCO_3 gradient-stimulated Cl uptake (reference 5, Fig. 5), however; thus it is possible that oxalate inhibited Cl uptake (Fig. 6) via the DIDS-insensitive conductive pathway [5]. This possibility, however, would only further establish that Ox:Cl exchange and $Cl:HCO_3$ exchange occur on separate carriers since even less inhibition of $Cl:HCO_3$ exchange (DIDS-sensitive) by oxalate would occur than currently indicated in Fig. 6.

The $Cl:HCO_3$ exchanger also functions as a Cl:OH exchanger, but prefers HCO_3 at physiological OH concentrations [5]. It will probably also transport other halides (fluoride, bromide, and iodide) and NO_3, but not PO_4, SO_4, lactate, PAH [5] or oxalate (Fig. 6). The $Cl:HCO_3$ exchanger is probably involved in the coupled absorption of Na and Cl via dual exchange (Na:H and $Cl:HCO_3$) [5]. In *Necturus* renal microvillus membranes there is a $Cl:HCO_3$ exchanger which is not stimulated by OH [14]. NO_3, bromide, iodide and fluoride can substitute for Cl on this exchanger, but lactate and PAH do not. These properties differ from the $Cl:HCO_3$ exchanger on ileal membrane only in that OH will stimulate Cl uptake in the ileum, though not as well as HCO_3 [5].

The $SO_4(Ox):OH$ exchanger, unlike the $Cl:HCO_3$ exchanger, is not stimulated by HCO_3 compared to OH [5, 12]. The $SO_4(Ox):OH$ exchanger also differs from the $Cl:HCO_3$ exchanger in its sensitivity to DIDS, probenecid and potassium (Figs. 3 and 5). Since it has not been demonstrated that SO_4 can be actively absorbed in the intact intestine in the absence of Na [15], the physiologic role of the $SO_4:OH$ exchanger remains undefined. Of interest in this regard, however, Langridge-Smith et al. found that a 0.6 unit pH gradient across rabbit ileal brush border (pH = 7.4 intracellularly, pH = 6.8 lumen) resulted in a fivefold stimulation of SO_4 uptake compared to no pH gradient, in the presence of only 3 mM Na [7]. This observation raises the possibility that $SO_4:OH$ occurs in intact tissue; however, the effect of a pH gradient in the total absence of luminal Na needs to be determined. Further, the dependence on luminal Na for SO_4 transport in intact tissue does not exclude participation of $SO_4:OH$ exchange in the transport process, since $Na:SO_4$ cotransport could occur indirectly via dual exchange (Na:H and $SO_4:OH$) rather than direct cotransport. Clearly, further studies are

needed to determine the role of SO_4 : OH exchange in intestinal SO_4 transport. SO_4 : OH exchange has been found on rat renal basolateral membrane [8], and on flounder renal basolateral membrane [10]. SO_4 : HCO_3 (but not SO_4 : OH) exchange has been found on flounder renal brush border membrane [11].

Oxalate is also transported on the SO_4 : OH exchanger and probably has a higher affinity for the exchanger than SO_4 ([12] and Fig. 7). The SO_4 : OH exchanger on rat renal basolateral membrane also transports oxalate [8]. Oxalate has no known physiologic role in humans, however; thus there is no need to absorb it from the diet. Oxalate is excreted in the urine and this carrier may be involved in that process in the kidney. Obviously, other anions, which we have not tested, may utilize this exchanger.

The Ox : Cl exchanger may play a role in organic acid transport since formate and oxaloacetate will *trans*-stimulate Cl and oxalate uptake [6] and thus probably utilize the same carrier. Recently an Ox : Cl exchanger which will transport formate has been described in the dog renal brush border membrane and therefore is similar to the Ox : Cl exchanger in our membranes [3]. The Ox : Cl exchanger differs from the Cl : HCO_3 exchanger in its sensitivity to DIDS and bumetanide (Fig. 5) and its degree of *cis*-inhibition by oxalate (Fig. 6) and sulfate (Fig. 4 and ref. 12). The Ox : Cl exchanger differs from the Ox : OH exchanger in its sensitivity to probenecid, bumetanide and K (Figs. 3 and 5), its degree of *cis*-inhibition by Cl and SO_4 (Figs. 4 and 6) and in its apparent affinity for oxalate (Fig. 7).

The urate exchanger in dog proximal tubule microvillus membranes will transport a number of organic acids, including lactate, oxaloacetate, α-ketoglutarate, but not oxalate [2]. It is therefore likely that the urate exchanger is different from the Ox (SO_4) : OH and Ox : Cl exchangers. Whether there is yet another exchanger in the ileum that is comparable to the urate exchanger in the kidney and will transport organic acids such as lactate, but not oxalate, needs to be evaluated. Of interest in this regard, there is also a lactate : OH exchanger on red cell membranes which is separate from the Ox (SO_4) : OH exchanger [1].

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References

1. Deuticke, D., Beyer, E., Forst, B. 1982. Discrimation of three parallel pathways of lactate transport in the human erythrocyte membrane by inhibitors and kinetic properties. *Biochim. Biophys. Acta* **684:**96–110
2. Guggino, S.E., Martin, G.J., Aronson, P.S. 1983. Specificity and modes of the anion exchanger in dog renal microvillus membranes. *Am. J. Physiol.* **244:***F*612–*F*621
3. Karniski, L.P., Aronson, P.S. 1985. Chloride/formate exchange with formic acid recycling: A mechanism of active chloride transport across epithelial membranes. *Proc. Natl. Acad. Sci. USA* **82:**6362–6365
4. Knickelbein, R., Aronson, P.S., Atherton, W., Dobbins, J.W. 1983. Sodium and chloride transport across rabbit ileal brush border: I. Evidence for Na-H exchange. *Am. J. Physiol.* **245:***G*504–*G*510
5. Knickelbein, R.G., Aronson, P.S., Schron, C.M., Dobbins, J.W. 1985. Na and Cl transport across rabbit ileal brush border: II. Evidence for Cl : HCO_3 exchange and mechanism of coupling. *Am. J. Physiol.* **249:***G*236–*G*245
6. Knickelbein, R.G., Schron, C.M., Aronson, P.S., Dobbins, J.W. 1985. Anion exchange across rabbit ileal brush border membrane. *Gastroenterology* **88:**1449 (*abstr.*)
7. Langridge-Smith, J.E., Sellin, J.H., Field, M. 1983. Sulfate influx across the rabbit ileal brush border membrane: Sodium and proton dependence, and substrate specificities. *J. Membrane Biol.* **72:**131–139
8. Low, I., Friedrich, T., Burckhardt, G. 1984. Properties of an anion exchanger in rat renal basolateral membrane vesicles. *Am. J. Physiol.* **246:***F*334–*F*342
9. Marquardt, D.W. 1963. An algorithm for least squares estimation of non-linear parameters. *J. Soc. Ind. Appl. Math.* **11:**431–441
10. Renfro, J.L., Pritchard, J.B. 1982. H^+-dependent sulfate secretion in the marine teolost renal tubule. *Am. J. Physiol.* **243:***F*510–*F*519
11. Renfro, J.L., Pritchard, J.B. 1983. Sulfate transport by flounder renal tubule brush border: Presence of anion exchange. *Am. J. Physiol.* **244:***F*488–*F*496
12. Schron, C.M., Knickelbein, R.G., Aronson, P.S., Della Puca, J., Dobbins, J.W. 1985. pH gradient-stimulated sulfate transport by rabbit ileal brush border membrane vesicles: Evidence for SO_4 : OH exchange. *Am. J. Physiol.* (*in press*)
13. Schron, C.M., Knickelbein, R.G., Aronson, P.S., Della Puca, J., Dobbins, J.W. 1985. Effects of cations on pH gradient-stimulated sulfate transport in rabbit ileal brush border membrane vesicles. *Am. J. Physiol.* (*in press*)
14. Seifter, J.L., Aronson, P.S. 1984. Cl transport via anion exchange in *Necturus* renal microvillus membranes. *Am. J. Physiol.* **247:***F*888–*F*895
15. Smith, P.L., Orellana, S.A., Field, M. 1981. Active sulfate absorption in rabbit ileum: Dependence on sodium and chloride and effects of agents that alter chloride transport. *J. Membrane Biol.* **63:**199–206

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