Evidence for the Existence of a Distinct SO_4^{--}-OH^- Exchange Mechanism in the Human Proximal Colonic Apical Membrane Vesicles and Its Possible Role in Chloride Transport

SANGERTA TYAGI,* REENA J. KAVILAVEETTIL,† WADDAH A. ALREFAI,* SHADWAN ALSAFWAH,† KRISHNAMURTHY RAMASWAMY,*,† AND PRADEEP K. DUDEJA*,†,[1]

Department of Medicine, University of Illinois, Chicago, Illinois 60612; and †Westside Veterans Affairs Medical Center, Chicago, Illinois 60612

Recent studies have demonstrated that mutations in human downregulated in adenoma gene (DRA) result in congenital chloride diarrhea (CLD), and that DRA may be involved in chloride transport across the intestinal apical domains. DRA is highly homologous to sulfate transporters, but not to any member of the anion exchanger gene family (AEs). Our previous studies have characterized the existence of a distinct Cl^--OH^- (HCO_3^-) exchanger, with minimal affinity for sulfate in the human colonic apical membrane vesicles (AMV). However, the mechanism(s) of sulfate movement across the colonocyte plasma membranes in the human colon is not well understood. Current studies were undertaken to elucidate sulfate transport pathways in AMVs of human proximal colon. Purified AMV and rapid filtration $^{35}SO_4^{--}$ uptake techniques were used. Our results demonstrate the presence of a pH gradient-driven carrier-mediated SO_4^{--}-OH^- exchange process in the human proximal colonic luminal membranes based on the following: a marked increase in the SO_4^{--} uptake in the presence of an outwardly directed OH^- gradient; a significant inhibition of SO_4^{--} uptake by the membrane anion transport inhibitor, DIDS; demonstration of saturation kinetics (K_m for SO_4^{--}: 0.80 ± 0.17 mM and V_{max} 649 ± 74 pmol/mg protein/10 sec); competitive inhibition of SO_4^{--}-OH^- exchange by oxalate; SO_4^{--} uptake was insensitive to alterations in the membrane potential; and inwardly directed Na^+ gradient under non-pH gradient conditions did not stimulate SO_4^{--} uptake. SO_4^{--} uptake was significantly inhibited by increasing concentrations of chloride (1–10 mM) in the incubation media with a K_i for Cl^- of 9.3 ± 1.4 mM. In contrast, OH^-/HCO_3^- gradient-driven $^{36}Cl^-$ uptake into these vesicles was unaffected by increasing concentrations of sulfate (10–50 mM). The above data indicate that two distinct transporters may be involved in SO_4^{--} and Cl^- transport in the human intestinal apical membranes: an anion exchanger with high affinity for SO_4^{--} and oxalate but low affinity for Cl^-, and a distinct Cl^--OH^- (HCO_3^-) exchanger with low affinity for SO_4^{--}.

[Exp Biol Med Vol. 226(10):912–918, 2001]

Key words: human intestine; congenital chloride diarrhea; downregulated in adenoma; sulfate transport

The product of the downregulated in adenoma (DRA) gene, which is highly expressed in normal colonic epithelia, has been shown to be involved in SO_4^{--}, oxalate (1), as well as chloride transport (2, 3). Previous studies demonstrated that the DRA gene is mutated in congenital chloride diarrhea (CLD) patients (4), where the basic physiological defect is an impaired ileal and colonic Cl^-/HCO_3^- exchange process. These studies suggested that DRA may be the mammalian intestinal apical membrane anion exchanger (Cl^-/HCO_3^-) involved in the electroneutral sodium chloride absorption (4, 5). In this regard, previous studies from our laboratory have demonstrated the presence of a Cl^--HCO_3^- exchange process in the human intestinal ileal and colonic apical membrane, which exhibited little affinity for the luminal sulfate (6–8).

With respect to SO_4^{--} transport in the mammalian intestine, previous studies had demonstrated a SO_4^{--}-OH^- exchange activity in the brush-border membranes of rabbit intestine that was shown to be distinct from the previously reported Cl^--HCO_3^- (OH^-) exchange process (9, 10). In addition to a SO_4^{--}-OH^- exchange activity, a number of earlier studies also demonstrated the existence of a distinct Na^+-SO_4^{--} co-transport process in brush-border membrane vesicles from rabbit and rat ileum that is energized by the transmembrane Na^+ gradient maintained by the activity of a Na^+-K^+ ATPase pump on the basolateral membrane (10–12). Other distinct mechanisms for SO_4^{--} transport have also

This work was supported by the Department of Veterans Affairs and by National Institute of Diabetes, Digestive & Kidney Diseases (grants DK 54016 to P.K.D., DK 33349 to K.R., and DK 09930 to W.A.A.).

[1] To whom requests for reprints should be addressed at University of Illinois, Medical Research Service (600/151), V.A. Medical Center, 820 South Damen Avenue, Chicago, IL 60612. E-mail: PKDUDEJA@UIC.EDU

Received March 15, 2001.
Accepted July 12, 2001.

1535-3702/01/22610-0912$15.00

been described on the basolateral membrane domains of the intestinal epithelial cells (10, 11, 13, 14). For example, in rabbit ileum, studies showed the presence of distinct Cl^--SO_4^{--} and SO_4^{--}-HCO_3^- exchange mechanisms in the basolateral membrane vesicles (13–15). Additionally, Cl^--SO_4^{--}, SO_4^{--}-OH^-, and SO_4^{--}-HCO_3^- exchange mechanisms have also been described in the basolateral membranes of rat intestine (16, 17). An SO_4^{--}-HCO_3^- exchanger has also been reported in brush-border membrane vesicles prepared from marine teleosts (18) and human placenta (19).

To date, however, detailed mechanisms of SO_4^{--} transport and possible interaction of a sulfate transporter with chloride and oxalate transport across the apical membrane domains of the human colonic epithelia have not been investigated. Current studies were, therefore, undertaken to define the characteristics of SO_4^{--} transport mechanisms in human proximal colonic apical membrane vesicles (AMVs) and to study the interactions of chloride and oxalate with this transport process. Our present studies clearly demonstrate that SO_4^{--} uptake in the AMVs of the human proximal colon occurs via a pH-dependent 4,4′-diisothiocyanatostilbene-2, 2′-disulfonic acid (DIDS)-sensitive carrier-mediated SO_4^{--}-OH^- exchange activity, but not by Na^+-SO_4^{--} co-transport process. This SO_4^{--}-OH^- exchange process in these vesicles was significantly inhibited by oxalate and chloride, but it exhibited much lower inhibition by chloride compared with SO_4^{--} and oxalate. In contrast, the Cl^-/HCO_3^- exchange process in these vesicles was not inhibited by sulfate. Based on these findings, the presence of an OH^- gradient-dependent carrier-mediated SO_4^{--}-OH^- exchange mechanism distinct from the previously described Cl^--HCO_3^- (OH^-) exchange process in the human proximal colonic apical membranes has been proposed.

Material and Methods

Materials. Valinomycin, 4-acetamido-4′-isothiocyano-2, 2′-disulfonic acid stilbene (SITS), DIDS, amiloride, bumetanide, and acetazolamide were obtained from Sigma Chemical Co. (St. Louis, MO). Stock solutions (1 M) of *N*-methyl D-glucamine gluconate (N-MGG) were made by titrating 1 M *N*-methyl D-glucamine (N-MG) with solid D-gluconic acid lactone. Radionuclide ^{35}S [sulfuric acid] was obtained from DuPont Research Products (Boston, MA). All other materials were obtained from either Fisher Scientific (Fairlawn, NJ) or Sigma Chemical Co., unless otherwise stated, and were of the highest purity available.

Isolation of Human Proximal Colonic AMV. These investigations were approved by the Institutional Review Board of the University of Illinois at Chicago. Colons from healthy adult organ donors were obtained after harvest of transplantable organs. After discarding the cecum, the remaining large intestine was divided into two equal parts, proximal and distal. The bowel was cleansed with an ice-cold 0.9% NaCl solution, and the mucosa of proximal colon was then scraped and frozen at –80°C. Purified apical membranes were prepared from thawed mucosa utilizing divalent cation (Mg^{+2}) chelation and differential centrifugation technique (20). To reduce any further cellular activity or metabolism, all the steps were carried out on ice. The purity of membrane vesicles and the degree of contamination with intracellular organelles were assessed by appropriate marker enzymes. Membrane vesicles demonstrated approximately 8- to 10-fold enrichment in cysteine-sensitive alkaline phosphatase activity (colonic apical membrane marker) compared with crude homogenate. The corresponding values for succinate dehydrogenase, NADPH cytochrome-c reductase, and sodium-potassium-dependent adenosine triphosphatase, marker enzymes for mitochondrial, microsomal, and basolateral membranes, respectively, ranged from 0.5- to 2.2-fold compared with crude membranes in all apical membrane preparations. For loading vesicles with various constituents, the desired intravesicular medium buffer was utilized in the last two centrifugations and in all the resuspension steps for the purification procedure and has been described in the figure legends.

After the final suspension, the vesicles were used for uptake studies either within 1 to 2 hr of purification or were quick frozen and stored at –80°C for use within 2 weeks of preparation without any significant loss of transport activity. The membrane protein was assessed by Bradford technique, using bovine plasma globulin as standard (21).

$^{35}SO_4^{--}$ Uptake Studies. The AMVs (20 μl) were incubated in incubation media (80 μl) with known buffer composition (described in detail in figure legends) and containing 25 μM radioactive $^{35}SO_4^{--}$ (unless stated otherwise), as previously described (10, 19). The transport was studied at 25°C in a water bath with uniform temperature. The uptake was stopped at various time points using 5 ml of ice-cold stop solution containing 50 mM Tris/MES and 150 mM N-MGG, pH 6.5. The diluted sample was immediately filtered utilizing a rapid filtration technique employing 0.65 μm nitrocellulose filters. Filters were then washed twice with 5 ml of ice-cold stop solution. The filters were then dissolved in Filtercount and the radioactivity was measured in a Packard TR1600 liquid scintillation counter (Packard Inc., Downers Grove, IL). All values were corrected for nonspecific $^{35}SO_4^{--}$ binding to filters and/or vesicles by subtracting the radioactivity present in zero-time vesicle blanks.

Statistical Analysis. All experiments were performed using at least three or four freshly isolated membrane preparations prepared from proximal colons of different organ donors. Results are expressed as mean ± SEM. Paired or unpaired student's *t* tests were used in statistical analysis as appropriate. A P value of <0.05 was considered statistically significant.

Results

Effect of pH Gradient on SO_4^{--} Uptake. To determine whether a carrier-mediated anion exchange process is involved in sulfate uptake into the human proximal colonic AMVs, the effect of an outwardly directed OH^- gra-

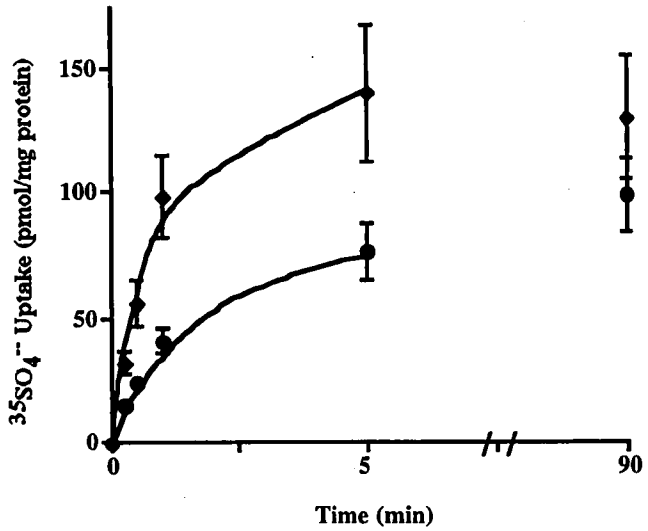

Figure 1. Time course of SO_4^{--} uptake. Sulfate uptake was determined at different time points at 25°C by diluting the AMVs (60–70 μg) preloaded with 150 mM NMGG and 50 mM Tris-Hepes (pH 8.2) in the reaction medium containing 150 mM NMGG, 25 μM $^{35}SO_4$ and either 50 mM Tris-Hepes, pH 8.2 (•), or 50 mM Tris-Mes, pH 6.5, (♦). The data shown are representative of mean ± SEM of six separate preparations.

dient on sulfate uptake was determined. The data presented in Figure 1 demonstrate that in the presence of an outwardly directed OH^- gradient (pH 8.2_{in}, pH 6.5_{out}) sulfate uptake was markedly stimulated into the colonic AMVs compared with the nongradient conditions. As shown in Figure 1, the uptake of sulfate under pH gradient conditions was significantly higher than the uptake in the absence of an OH^- gradient ($P < 0.05$) up to 5 min. These results suggest the presence of a pH gradient-dependent carrier-mediated transport process for sulfate across the colonic luminal membrane vesicles.

Effect of Transmembrane Potential on SO_4^{--} Uptake. To assess the effect of transmembrane electrical potential on sulfate influx into the human colonic AMVs, the effect of valinomycin-induced potassium diffusion potential on sulfate uptake in the presence of pH gradient conditions was determined. The sulfate uptake was measured after creating the intravesicular positive membrane potential and was compared with uptake under voltage-clamped conditions. As shown in Table I, the voltage clamping of the vesicles or making the membrane inside positive failed to significantly alter the sulfate uptake, suggesting that under pH gradient conditions, the OH^- gradient-stimulated sulfate uptake is largely mediated by an electroneutral anion exchange mechanism and is not due to any electrical coupling.

Effect of Extravesicular Na^+ on SO_4^{--} Uptake. To assess the possible existence of a Na^+-independent pathway for sulfate uptake, the effect of extravesicular Na^+ (50 mM) in the reaction medium on SO_4^{--} uptake was also investigated. In the presence of an inwardly directed Na^+ gradient under non-pH gradient conditions, the sulfate uptake was relatively slow and no stimulation was observed (Table II). The lack of stimulation of SO_4^{--} uptake in the presence of Na^+ confirms the absence of a Na^+-SO_4^{--} co-transport process for sulfate transport in these membranes.

Effect of Inhibitors on SO_4^{--} Uptake. To further characterize the carrier-mediated SO_4^{--}-OH^- exchange process in the human proximal colonic AMVs, the effect of various membrane transport inhibitors (0.5 mM) on OH^- gradient-driven sulfate uptake was determined. As shown in Figure 2a, the anion exchange inhibitors DIDS and SITS resulted in significant inhibition (>70%) of the OH^- gradient-stimulated sulfate uptake in the proximal colonic AMV. Under pH gradient conditions, sulfate transport into the vesicles was inhibited by DIDS in a dose-dependent manner (Fig. 2b). Although furosemide and bumetanide (inhibitors of Na-Cl and Na-K-Cl co-transport processes) also resulted in inhibition, amiloride (Na^+-H^+ exchange inhibitor) and acetazolamide (carbonic anhydrase inhibitor) failed to inhibit the pH gradient-stimulated sulfate uptake. These observations further support the presence of a carrier-mediated SO_4^{--}-OH^- exchange process in the human proximal colonic AMVs.

Effect of Anions on SO_4^{--} Uptake. A variety of organic and inorganic anions have been reported to be alternative substrates for sulfate and/or oxalate anion exchange processes in a number of tissues (9, 10). The anion specificity of the SO_4^{--}-OH^- exchange process in the human proximal colonic AMVs was determined by examining the effect of various anions (1 mM) on pH gradient-stimulated sulfate uptake (Fig. 3). Of all the anions, oxalate and unlabeled sulfate had a maximal inhibitory effect (60%–70%) on sulfate uptake into the vesicles. Although bromide also showed slight inhibition of sulfate transport, the uptake was relatively unaffected by chloride, nitrate, bicarbonate, acetate, and fluoride, respectively. These findings indicate that

Table I. Effect of Diffusion Potential on $^{35}SO_4^{--}$ Uptake[a]

Groups	Conditions	$^{35}SO_4^{--}$ uptake (pmol/mg protein/15 sec)
Voltage clamped	$K^+_{out} = K^+_m$ + valinomycin	19.5 ± 0.50
Inside positive	$K^+_{out} > K^+_{in}$ + valinomycin	16.5 ± 1.5

Note. The colonic membrane vesicles were preloaded with either 150 mM N-MGG (for inside positive experiments) or 100 mM N-MGG and 50 mM K. Glu (for voltage clamping experiments), and 50 mM Tris-Hepes (pH 8.2). Uptake was measured by incubating the AMVs in reaction medium consisting of 100 mM N-MGG, 50 mM K. Glu, 50 mM Tris-Mes (pH 6.5), 25 μM $^{35}SO_4^{--}$, and ± 20 μM valinomycin.
[a] Values are mean ± SEM of three membrane preparations.

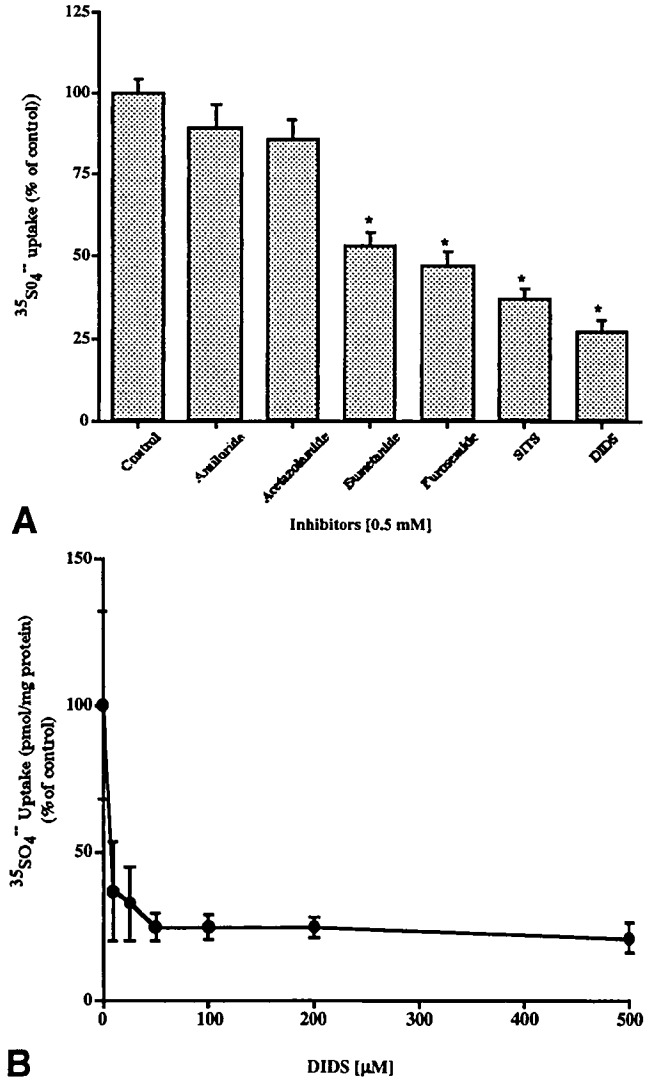

Figure 2. A, Effect of transport inhibitors on $^{35}SO_4^{--}$ uptake. The colonic AMVs preloaded with 150 mM NMGG and 50 mM Tris-Hepes (pH 8.2) were incubated with the reaction medium containing 150 mM NMGG, 25 μM $^{35}SO_4^{--}$, and 50 mM Tris-Mes (pH 6.5) ± 0.5 mM of the transport inhibitor. Results are expressed as the percentage of control, where control represents uptake in the absence of inhibitor. Values represent mean ± SEM (*$P < 0.05$) of four separate membrane preparations. B, Dose-dependent inhibition of $^{35}SO_4^{--}$ uptake by DIDS. $^{35}SO_4^{--}$ uptake was determined under pH gradient conditions at 25°C for 15 sec. The AMVs preloaded with 150 mM NMGG and 50 mM Tris-Hepes (pH 8.2) were diluted in the incubation medium consisting of 150 mM NMGG, 25 μM $^{35}SO_4^{--}$, 50 mM Tris-Mes (pH 6.5), and increasing concentrations of DIDS over the range of 0.01 to 0.5 mM. Results are expressed as the percentage of control, and values represent mean ± SEM of four separate membrane preparations.

this SO_4^{--}-OH^- antiporter is highly substrate specific for sulfate and oxalate.

Kinetics of SO_4^{--}-OH^- Exchange Process. To determine the kinetic characteristics of the transport process, uptake of sulfate as a function of increasing substrate concentrations in the incubation medium was investigated. As shown in Figure 4, the OH^- gradient-dependent SO_4^{--} uptake was saturable in the presence of increasing concentrations of sulfate over the range of 0.01 to 1.0 mM. Analysis of these results with Lineweaver-Burk plot yielded a K_m of 0.80 ± 0.17 mM and a V_{max} of 649 ± 74 pmol/mg protein/10 sec. This data again confirms the presence of a carrier-mediated transport process for sulfate in these membranes.

Table II. Effect of Extravesicular Na^+ on $^{35}SO_4^{--}$ Uptake[a]

Time (min)	-Na gluconate	+Na gluconate
0.25	7.0 ± 4.0	8.0 ± 5.0
1.00	17 ± 11.5	20 ± 10.0
5.00	38 ± 26.0	37 ± 23.5
90.0	38 ± 19.5	55 ± 21

Note. Vesicles loaded with 150 mM N-MGG and 50 mM Tris-Hepes (pH 8.2) were diluted into an incubation medium containing either 150 mM N-MGG and 50 mM Tris-Hepes (8.2) or 100 mM N-MGG, 50 mM Na^+ Glu, 25 μM $^{35}SO_4^{--}$, and 50 mM Tris-Hepes (pH 8.2). $^{35}SO_4^{--}$ uptake was determined at 25°C at different time intervals.
[a] Values are mean ± SEM of three membrane preparations.

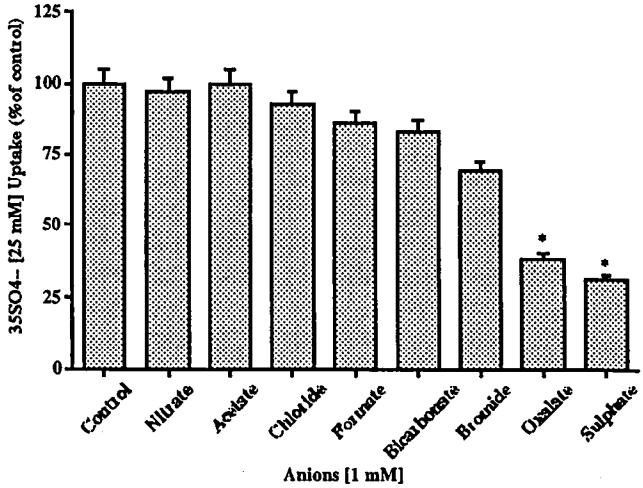

Figure 3. Effect of anions on pH gradient-stimulated $^{35}SO_4^{--}$ uptake. Vesicles preloaded with 150 mM N-MGG and 50 mM Tris-Hepes (pH 8.2) were incubated with buffers containing 150 mM N-MGG, 50 mM Tris-Mes (pH 6.5), 25 μM SO_4^{--}, and 1 mM potassium salts of various organic and inorganic anions. Sulfate uptake was determined for 15 sec at 25°C. Values are expressed as the percentage of control, and they represent mean ± SEM (*$P < 0.05$) of three separate membrane preparations.

Effect of Chloride and Oxalate on SO_4^{--} Uptake. The uptake of OH^- gradient-driven sulfate uptake was significantly inhibited in the presence of increasing concentrations of chloride in the extravesicular media (1–10 mM). Dixon plot analysis of SO_4^{--} uptake inhibition by chloride yielded a K_i of 9.3 ± 1.4 mM, indicating a lower affinity of the exchanger for chloride (Fig. 5) compared with SO_4^{--}. In contrast, the OH^- gradient-stimulated SO_4^{--}-OH^- exchange was competitively inhibited with very high affinity in the presence of varying concentrations of oxalate (0.5–1.5 mM) in the incubation media (Table III). Therefore, the inhibition of OH^- gradient-stimulated sulfate uptake with oxalate and low affinity of the exchanger for chloride strongly indicates that SO_4^{--}-OH^- and Cl^--HCO_3^- exchangers are distinct carriers.

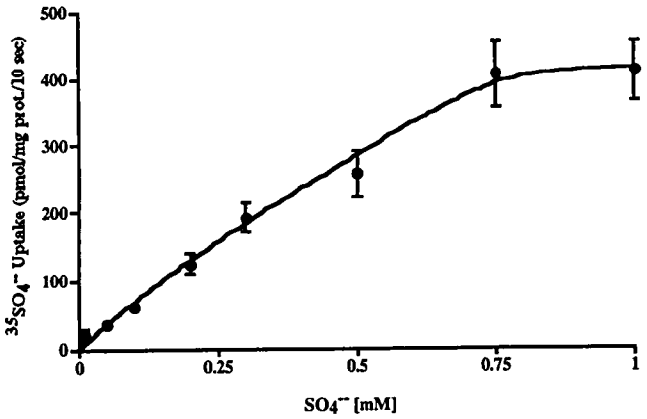

Figure 4. Kinetics of $^{35}SO_4^{--}$ uptake. $^{35}SO_4^{--}$ uptake was determined at 25°C with increasing extravesicular concentrations of sulfate (0.01–1.0 mM). The vesicles were preloaded with 150 mM N-MGG and 50 mM Tris-Hepes (pH 8.2). Osmolarity was kept constant on both sides of the vesicle membranes by altering N-MGG concentration in the incubation medium. Results shown are representative of mean ± SEM of 11 separate membrane preparations.

Effect of SO_4^{--} on Cl^--HCO_3^- Exchange Process. Previous studies from our laboratory have identified an electroneutral Cl^--HCO_3^- (OH^-) exchange process in the human proximal colonic AMVs. To examine that the observed pH gradient-driven sulfate uptake does not occur by the Cl^--HCO_3^- exchanger, pH and HCO_3^- gradient-driven $^{36}Cl^-$ uptake in the presence of increasing concentrations of SO_4^{--} were examined. Under pH gradient conditions, the OH^- and HCO_3^- gradient-stimulated $^{36}Cl^-$ uptake was not inhibited in the presence of varying concentrations of SO_4^{--} over the range of 0 to 50 mM (Fig. 6). If SO_4^{--} was being transported by the Cl^--HCO_3^- exchanger, some inhibition of $^{36}Cl^-$ uptake by sulfate would have been observed under these conditions. Therefore, the lack of inhibition of pH and HCO_3^- gradient-stimulated $^{36}Cl^-$ uptake by SO_4^{--} and low affinity of the SO_4^{--}-OH^- exchanger for chloride strongly indicate that SO_4^{--}-OH^- and Cl^--HCO_3^- exchanger processes in the human proximal colonic AMVs occur via two distinct transporters.

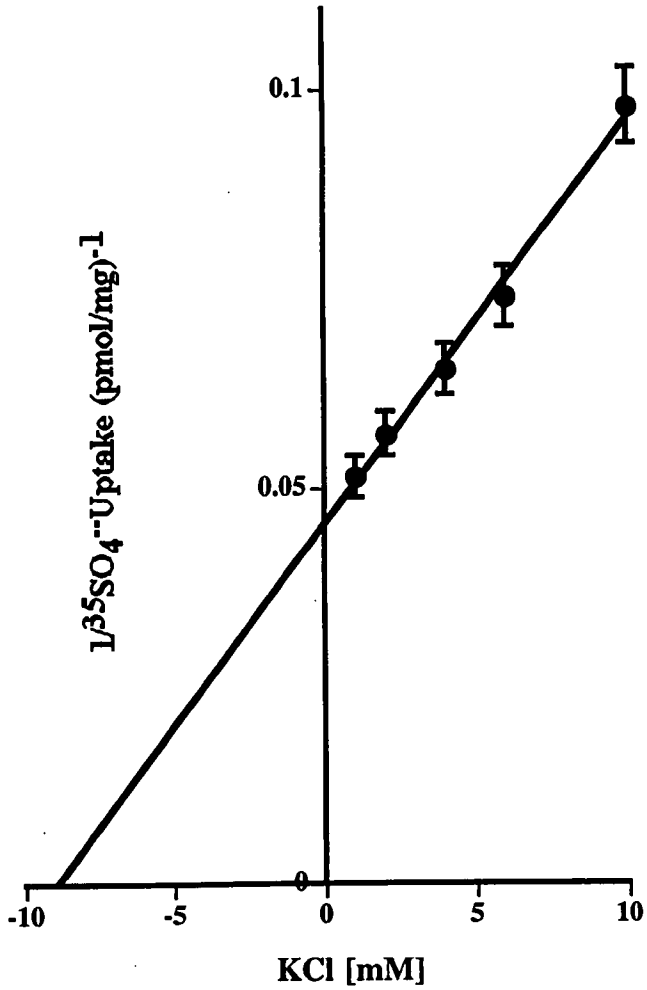

Figure 5. Effect of chloride on $^{35}SO_4^{--}$ uptake. Initial rate of $^{35}SO_4^{--}$ uptake was determined at 25°C in the presence of increasing chloride concentrations over the range of 1 to 10 mM. AMVs were preloaded with 150 mM N-MGG and 50 mM Tris-Hepes (pH 8.2). Uptake was measured by incubating the AMVs in medium containing 150 mM N-MGG, 50 mM Tris-Mes (pH 6.5), 25 μM $^{35}SO_4^{--}$, and varying concentrations of potassium chloride. The results are representative of four separate membrane preparations. Dixon plot analysis of SO_4^{--} uptake inhibition by chloride yielded a K_i of 9.3 ± 1.4 mM, indicating lower affinity of chloride for the carrier.

Discussion

In the present study, our results, for the first time, provide evidence to support the presence of a distinct SO_4^{--}-OH^- exchanger in the human proximal colonic AMVs. This exchanger involves a carrier-mediated electroneutral anion exchange activity rather than an electrogenic process. Additionally, Na^+-SO_4^{--} co-transport activity was not detected in these membranes. The characteristics of the human apical proximal colonic SO_4^{--}- OH^- exchange process are similar to previously described SO_4^{--}-OH^- exchange activity in rabbit ileum (10). The K_m for SO_4^{--} reported in the current study (0.8 ± 0.17 mM) and the anion selectivity of human colonic AMVs is comparable to those of the SO_4^{--}-OH^- exchange process in rabbit ileal brush-border membrane vesicles. Of all the anions tested, oxalate and unlabeled sulfate were found to be potential substrates for this exchange system.

In parallel studies performed with Caco-2 cells, we have also demonstrated the existence of an SO_4^{--}-OH^- exchange process that is highly sensitive to inhibition by DIDS and can transport oxalate as well as chloride, but with lower affinity for chloride compared with SO_4^{--} and oxalate (22). Similar to the studies in Caco-2 cells, in current study, the SO_4^{--}-OH^- exchanger of the apical colonic AMVs also exhibits high affinity for oxalate and DIDS and a very low affinity for Cl^-.

Recent studies from our laboratory have identified a Cl^--HCO_3^- exchange process in the human proximal colonic AMVs (6), raising the possibility that this exchanger might be involved in the OH^- gradient-dependent sulfate uptake. To further elucidate whether the SO_4^{--}-OH^- ex-

Table III. Effect of Oxalate on the Kinetics of $^{35}SO_4^{--}$ Uptake[a]

Kinetic parameters	Control	Oxalate (0.5 mM)	Oxalate (1.5 mM)
V_{max} (pmols/mg protein/10 sec)	409 ± 95.5	400 ± 126	776 ± 289
K_m (mM)	0.640 ± 0.23	1.28 ± 0.14[a]	2.77 ± 0.87[a]

Note. Values are mean ± SEM of three membrane preparations. The membrane vesicles were loaded with 150 mM N-MGG and 50 mM Tris-Hepes (pH 8.2). Sulfate uptake (0.1 mM) was determined at 25°C for 10 sec by incubating the vesicles in the incubation media consisting of 150 mM N-MGG and 50 mM Tris-Mes (pH 6.5) and increasing concentrations of oxalate over the range of 0.5–1.5 mM.
[a] $P < 0.05$ compared with control.

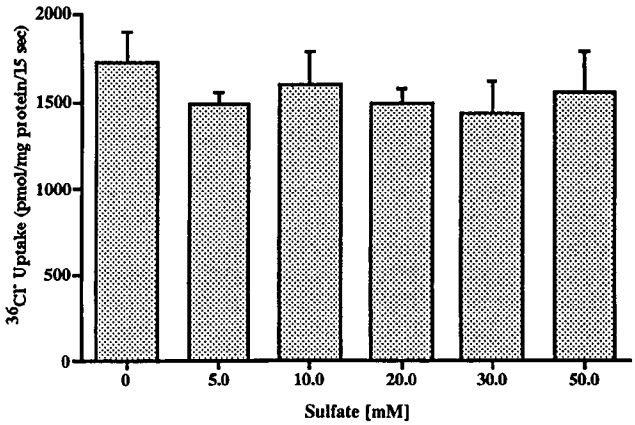

Figure 6. Effect of SO_4^{--} on $^{36}Cl^-$ uptake. $^{36}Cl^-$ uptake under pH and HCO_3^--gradient conditions was examined at 15 sec in the presence of increasing concentrations of sulfate in the incubation medium. As SO_4^{--} concentration was varied from 5 to 50 mM, changes in osmolarity were kept constant by adjusting potassium gluconate concentration in the medium. The results are expressed as the percentage of control, and they represent mean ± SEM of three separate membrane preparations.

change is mediated by the previously described Cl^--HCO_3^- exchanger, inhibition of OH^- gradient-driven SO_4^{--} uptake by Cl^-, as well as inhibition of HCO_3^- (OH^-) gradient-stimulated Cl^- uptake by SO_4^{--}, was also examined. Although, increasing concentrations of Cl^- significantly inhibited OH^- gradient-driven SO_4^{--} uptake (Fig. 5), increasing concentrations of SO_4^{--} failed to influence HCO_3^- (OH^-) gradient-driven Cl^- uptake (Fig. 6). Also, as compared with the K_m for SO_4^{--} (0.80 ± 0.17 mM) of this exchanger, Dixon plot analysis yielded a K_i for Cl^- of 9.3 ± 1.4 mM, indicating a lower affinity of the carrier for chloride. A significant competitive inhibition of OH^- gradient-dependent SO_4^{--} uptake was also seen in the presence of external oxalate. Additionally, OH^- gradient-driven sulfate uptake was highly sensitive to inhibition by DIDS, whereas HCO_3^- (OH^-) gradient-dependent Cl^- uptake demonstrated a much lower sensitivity to inhibition by DIDS. Based on the above observations, SO_4^{--}-OH^- and Cl^--HCO_3^- exchange processes appear to be mediated by distinct carriers.

To date, a family of at least three structurally and functionally related genes for anion exchangers termed as AE1, AE2, and AE3 (brain and cardiac subtypes: bAE3 and cAE3, respectively) has been identified (23). Recent studies from our laboratory have clearly shown the expression of AE2 and bAE3, but not AE1 and cAE3 along the entire length of the human intestine (24). Furthermore, we have shown that both AE2 and bAE3 polypeptides are restricted to the basolateral membranes of the polarized epithelial cells in the human intestine (24). However, the apical anion exchanger, a potential candidate for luminal chloride absorption, still needs to be identified.

In this regard, the DRA gene has been shown to be mutated in CLD patients where the basic defect has been demonstrated to be in the intestinal Cl^-/HCO_3^- exchange process (4). Moreover, recent genetic studies indicated that DRA is a candidate for CLD, and suggested that DRA may be the luminal Cl^-/HCO_3^- exchanger (25). The cDNA sequence of DRA has been shown to exhibit high homology to sulfate transporters, but not to the members of the AE family (1, 2). Additionally, earlier in vitro studies have demonstrated that DRA is sulfate and oxalate transporter (1). However, more recent studies have shown DRA to be capable of transporting chloride as well (2, 3). Along with the demonstration of two distinct SO_4^{--}/OH^- and Cl^-/OH^- exchange processes in Caco-2 cells (22) and human proximal colonic AMVs, recent studies from our laboratory also showed that thyroxine down-regulated the relative abundance of hDRA mRNA in parallel to a reduction in SO_4^{--}-OH^- but not Cl^--OH^- exchange process (22). Altogether, these data strongly suggest that DRA may be primarily responsible for SO_4^{--}-OH^- exchange process which appears to be distinct from the previously described Cl^--OH^- (HCO_3^-) exchange activity across the apical membrane of the human proximal colonocytes and Caco-2 cells. Our findings, therefore, appear to support one of the proposed models of Kere *et al* (5) for the epithelial apical chloride transport, where they also suggested that the apical Cl^-/HCO_3^- (OH^-) exchange process might require two or more transporters having functions that are tightly coupled by a common substrate. In this model, a mutation in only one of the proteins could block the functions of both transporters and cause the observed defect in the Cl^-/HCO_3^- (OH^-) exchange in CLD patients.

Therefore, based on the data of our present studies, we speculate the existence of two distinct exchangers that may be involved in chloride and sulfate absorption across the human intestinal luminal domain: an SO_4^{--}-OH^- exchanger with high affinity for sulfate and oxalate but low affinity for chloride, and a distinct Cl^--HCO_3^- exchanger with low affinity for sulfate. SO_4^{--}-OH^- exchanger with low affinity for Cl^- might be the gene product of DRA, how-

ever, the molecular nature of the apical membrane Cl^--HCO_3^- exchanger remains to be defined. Further studies are required to elucidate, in detail, the functional role of DRA in luminal Cl^- absorption and its relationship to other chloride transporters.

1. Silberg DG, Wang W, Moseley RH, Traber PG. The down-regulated in adenoma (dra) gene encodes an intestine-specific membrane sulfate transport protein. J Biol Chem **270**:11897–11902, 1995.
2. Moseley RH, Hoglund P, Wu GD, Silberg DG, Haila S, Chapelle ADL, Holmberg C, Kere J. Downregulated in adenoma gene encodes a chloride transporter defective in congenital chloride diarrhea. Am J Physiol **267**(39):G185–G192, 1999.
3. Melvin JE, Park K, Richardson L, Schultheis PJ, Shull GE. Mouse down-regulated in adenoma (DRA) is an intestinal Cl^-/HCO_3^- exchanger and is up-regulated in colon of mice lacking the NHE_3 Na^+/H^+ exchanger. J Biol Chem **274**(32):22855-22861, 1999.
4. Hoglund P, Haila S, Socha J, Tomaszewski L, Saarialho-kere U, Lindsberg MLK, Chapelle ADL, Kere J. Mutations of the down-regulated in adenoma (DRA) gene cause congenital chloride diarrhea. Nat Genet **14**:316–319, 1996.
5. Kere J, Lohi H, Hoglund P. Genetic Disorder of Membrane Transport. III. Congenital chloride diarrhea. Am J Physiol **276**(39):G7–G13, 1999.
6. Mahajan RJ, Baldwin ML, Harig JM, Ramaswamy K, Dudeja PK. Chloride transport in human proximal colonic apical membrane vesicles. Biochim Biophys Acta **1280**:12–18, 1996.
7. Ramaswamy K, Chung M, Barry JA. Chloride/bicarbonate exchange in human ileal brush-border membrane vesicles. Gastroenterology **94**:A366, 1988.
8. Ramaswamy K, Harig JM, Kleinman JG, Harris MS. Characteristics of Na^+/H^+ and Cl^-/HCO_3^- antiport systems in human ileal brush border membrane vesicles. NY Acad Sci **574**:128–130, 1989.
9. Knickelbein RG, Aronson PS, Dobbins JW. Substrate and inhibitor specificity of anion exchanger on the brush border membrane of rabbit ileum. J Membr Biol **88**:199–204, 1985.
10. Schron CM, Knickelbein RG, Aronson PS, Della Puca J, Dobbins JW. pH gradient-stimulated sulfate transport by rabbit ileal brush-border membrane vesicles: Evidence for SO_4^{--}-OH exchange. Am J Physiol **249**(12):G607–G613, 1985.
11. Langridge-Smith JE, Field M. Sulfate transport in rabbit ileum: Characterization of the serosal border anion exchange process. J Membr Biol **63**(3):207–214, 1981.
12. Lucke H, Stange G, Murer H. Sulfate-sodium cotransport by brush-border membrane vesicles isolated from rat ileum. Gastroenterology **80**(1):22–30, 1981.
13. Schron CM, Knickelbein RG, Aronson PS, Dobbins JW. Evidence for carrier-mediated Cl/SO4 exchange in rabbit ileal basolateral membrane vesicles. Am J Physiol **253**:G404–G410, 1987.
14. Knickelbein R, Dobbins JW. Sulfate and oxalate exchange for bicarbonate across the basolateral membrane of rabbit ileum. Am J Physiol **259**(22):G807–G813, 1990.
15. Kuo SM, Aronson PS. Oxalate transport via the sulfate/HCO_3 exchanger in rabbit renal basolateral membrane vesicles. J Biol Chem **263**(20):9710–9717, 1988.
16. Hagenbuch B, Stange G, Murer H. Transport of sulphate in rat jejunal and rat proximal tubular basolateral membrane vesicles. Pflugers Arch **405**(3):202–208, 1985.
17. Weinberg SL, Burckhardt G, Wilson FA. Taurocholate transport by rat intestinal basolateral membrane vesicles: Evidence for the presence of an anion exchange transport system. J Clin Invest **78**(1):44–50, 1986.
18. Renfro JL, Pritchard JB. Sulfate transport by flounder renal tubule brush border: Presence of anion exchange. Am J Physiol **244**(5): F488–F496, 1983.
19. Cole DE. Sulfate transport in brush border membrane vesicles prepared from human placental syncytiotrophoblast. Biochem Biophys Res Commun **123**(1):223–229, 1984.
20. Harig JM, Dudeja PK, Knaup SM, Shoshara J, Ramaswamy K, Brasitus TA. Apical plasma membrane vesicles formed from organ donor colon demonstrate Na^+ and H^+ conductances and Na^+/H^+ exchange. Biochem Biophys Res Commun **167**:438–443, 1990.
21. Bradford M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem **72**:248–254, 1976.
22. Alrefai WA, S. T, Mansour F, Saksena S, Syed I, Ramaswamy K, Dudeja PK. Sulfate and chloride transport in Caco-2 cells: Differential regulation by thyroxine and the possible role of DRA gene. Am J Physiol Gastrointest Liver Physiol **280**:G603–G613, 2001.
23. Alper SL. The band 3-related AE anion exchanger gene family. Cell Physiol Biochem **4**:265–281, 1994.
24. Alrefai WA, Tyagi S, Nazir TM, Barakat J, Anwar SS, Hadjiagapiou C, Bavishi J, Sahi J, Malik P, Goldstein J, Layden TJ, Ramaswamy K, Dudeja PK. Human intestinal anion exchanger isoforms: Expression, distribution and membrane localization. Biochem Biophys Acta **1511**(1):17–27, 2001.
25. Hoglund P. Positional candidate genes for congenital chloride diarrhea suggested by high-resolution physical mapping in chromosome region 7q31. Genome Res **6**:202–210, 1996.