Electrogenic $\text{H}^+$-regulated sulfate-chloride exchange in lobster hepatopancreatic brush-border membrane vesicles

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Cattley, Mark A., George A. Gerencser, and Gregory A. Ahearn. Electrogenic $\text{H}^+$-regulated sulfate-chloride exchange in lobster hepatopancreatic brush-border membrane vesicles. Am. J. Physiol. 262 (Regulatory Integrative Comp. Physiol. 31): R255-R262, 1992.—Transport of $[^{35}\text{S}]$sulfate by brush-border membrane vesicles (BBMV) of lobster ($\text{Homarus americanus}$) hepatopancreas was stimulated by an outwardly directed chloride gradient. In contrast, sulfate uptake was not enhanced by inwardly directed Na$^+$ or K$^+$ transmembrane gradients. An inside-positive membrane potential (valinomycin and K$^+$) stimulated SO$_4^{2-}$-Cl$^-$ exchange, whereas an inside-negative membrane potential was inhibitory. Sulfate-sulfate exchange was not affected by alterations of transmembrane potential. An inwardly directed proton gradient, or the presence of low bilateral pH, enhanced SO$_4^{2-}$-Cl$^-$ exchange, but the $\text{H}^+$ gradient alone did not stimulate sulfate uptake in chloride-equilibrated BBMV or in vesicles lacking internal Cl$^-$. The stilbenes 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid (SITS) and 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS) strongly inhibited SO$_4^{2-}$-Cl$^-$ exchange. Sulfate influx occurred by a combination of carrier-mediated transfer, exhibiting Michaelis-Menten kinetics, and nonsaturable "apparent diffusion." $^{35}\text{Cl}^-$ influx into sulfate-loaded BBMV was stimulated by an inside-negative transmembrane potential compared with short-circuited vesicles. These results suggest that sulfate-chloride exchange in hepatopancreatic BBMV occurred by an electrogenic carrier mechanism exhibiting a 1:1 flux ratio that was modulated by internal and external $\text{H}^+$-sensitive regulatory sites. The role of this antiport process in anion secretion is discussed.

sulfate transport; anion exchange; pH regulation; gastrointestinal physiology; $\text{Homarus americanus}$

GASTROINTESTINAL and renal transport of the divalent anion sulfate across epithelial apical membranes has been investigated in various vertebrate groups including mammals (6, 15, 20), teleost fish (23, 24), and the domestic chicken (22). A number of mechanisms for brush border carrier-mediated sulfate transport across epithelial membranes have been proposed and include sodium-sulfate cotransport (6, 15, 26), anion exchange (20, 24, 29), and pH gradient-dependent transfer (23, 27). These processes contribute to transepithelial regulation of sulfate, which maintains physiological levels of this anion, and may affect acid-base balance and cell and plasma osmolarity.

The crustacean hepatopancreas has been described as a digestive and absorptive organ based on morphological and histochemical studies of the epithelial cells that compose most of the tissue. Previous studies also suggest that the organ may in addition play a role in secretion (11, 14, 31). Recently, the use of isolated membrane vesicles provided data to suggest that nutrient absorption across the hepatopancreas of the lobster, $\text{Homarus americanus}$, occurs as a result of transport activities at the apical border via both Na-dependent and -independent mechanisms (2, 3, 5). An electrogenic sodium-proton exchanger, with physiological properties unlike those of the vertebrate antipporter, has also been shown to be present on the brush border of the hepatopancreas epithelium (4). Other than the occurrence of this Na-H exchanger there is no direct evidence as to possible functions of the hepatopancreas in inorganic ion transport.

The lobster, being a marine invertebrate, periodically ingests seawater during both eating and drinking (18). The sulfate content of seawater is relatively high (25 mM), whereas the blood concentration of this anion in many marine decapods, including the lobster, has been shown to be maintained at lower levels than the surrounding seawater (21, 25). The present study investigates the role of the brush-border membrane of the hepatopancreatic epithelium in the transport of sulfate. Results demonstrate the presence of an anion exchange mechanism in this membrane that is inhibitable by stilbenes, is modified by pH, and operates in an electrogenic fashion.

METHODS

Live Atlantic lobsters ($\text{H. americanus}$, 0.5 kg each) were purchased from commercial dealers in Hawaii and maintained unfed at 10°C for up to 1 wk in filtered seawater. All animals were in either intermolt or early premolt as assessed by the molt stage classification scheme introduced by Aiken (7). Hepatopancreatic brush-border membrane vesicles (BBMV) were prepared from fresh tissue removed from individual lobsters. Each membrane batch was produced from a single organ (15-25 g fresh wt) by a method of combined osmotic disruption, differential centrifugation, and magnesium precipitation described previously by Ahearn and co-workers (2, 5). Marker enzyme assays confirmed that vesicles prepared by these methods were highly enriched in brush-border membranes with minimal contamination from basolateral or organelle membranes (5).

Transport studies were conducted at 15°C using the rapid filtration technique developed by Hopfer et al. (13). For time course experiments, a volume of vesicles (e.g., 20 μl) was added to a volume of incubation media (e.g., 180 μl) containing 0.1 mM radiolabeled $^{35}\text{SO}_4^-$. At various incubation times a known volume (20 μl) of reaction mixture was removed and plunged into 2 ml of ice-cold stop solution (stop solution composition varied with experiment and generally consisted of incubation media without any sulfate) to stop the uptake process. The vesicle suspension was then rapidly filtered through 0.22-μm Millipore filters (presoaked in distilled water) and washed with another 5 ml of ice-cold stop solution. Filters were transferred to vials containing Beckman Ready Solv HP scintillation cocktail and counted for radioactivity in a Beckman LS-8100 scintillation counter. Transport experiments involving incubations of <10 s were conducted using a rapid-exposure uptake apparatus.
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ratus (Inovativ Labor, Adliswil, Switzerland). Uptake was initiated by mixing 5 μl of vesicles with a volume (e.g., 45 μl) of radiolabeled incubation media, and filters were washed and counted for radioactivity as above. For short-term incubations, a blank was also run for each condition by mixing stop solution, vesicles, and radiolabeled incubation media simultaneously; the resulting value was subtracted from corresponding experimental results before uptake was determined. For long-term time courses, an estimation of the nonspecific binding of radioactivity to vesicles and filters was determined. Incubation and intravesicular media varied between experiments and are indicated in the figure legends. Sulfate uptake values were usually expressed as picomoles per milligram protein (Bio-Rad protein assay) per filter using the specific activity of sulfate in the incubation media.

Unless otherwise indicated, valinomycin (50 μM) and bilaterally equal potassium concentrations across the vesicular wall were present to short-circuit the membranes. Each experiment was generally repeated three to five times using membranes prepared from different animals. Within a given experiment each point was determined from three to five replicate samples. Data are presented as means ± SE of a single representative experiment. SE values indicate variation due to assay procedure, not individual variation. Similar qualitative experimental findings were obtained in the repetition of an experiment.

33SO₄ as the Na⁺ salt was obtained from New England Nuclear. Valinomycin, carbonyl cyanide m-chlorophenylhydrazone (CCCP), 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid (SITS), 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS), bumetanide, furosemide, and other reagent grade chemicals were purchased from Sigma Chemical.

RESULTS

Driving force for sulfate uptake. Figure 1 indicates that the transport of 0.1 mM 33SO₄⁻ as the Na⁺ salt was not affected by an inwardly directed 100 mM Na⁺-gluconate gradient compared with uptake with bilateral tetramethylammonium (TMA) gluconate. This suggests that sulfate uptake by BBMV was not sodium dependent. In the same experiment, a portion of the vesicle preparation was preloaded with 25 mM HCO₃⁻. As before, there was no increased stimulation of sulfate uptake compared with the TMA gluconate control. In all conditions there was no accumulation of sulfate above that of the equilibrium value.

Sulfate uptake into BBMV was only slightly stimulated by incubation in media containing 100 mM K⁺ gluconate (no valinomycin or internal K⁺) in the presence of 50 μM CCCP (Fig. 2). However, when vesicles were preloaded with 25 mM KCl and incubated in TMA gluconate media containing equimolar K⁺ and valinomycin there was an "overshoot" accumulation twice that of the equilibrium value. Such results, and those from Fig. 1, indicate an anion exchange mechanism that can utilize a Cl⁻ gradient, but not a gradient of HCO₃⁻, as a driving force to move sulfate across the apical membrane. The slight K⁺ stimulation seen during inwardly directed K⁺ gradients may result from electrical coupling between diffusional substrate movements and a small membrane potential not completely eliminated by CCCP short-circuiting.

Effect of membrane potential on sulfate uptake. The possible membrane potential sensitivity of sulfate-chloride exchange was examined by imposing a valinomycin-induced potassium diffusion potential across the vesicular wall and measuring the time course of 0.1 mM sulfate uptake. Transport was determined under both inside-negative and inside-positive conditions and compared with uptake in short-circuited conditions (equal K⁺ across membrane). Figure 3 shows that when vesicles were incubated in media containing 100 mM K⁺ (no internal K⁺), sulfate uptake was stimulated above that with bilateral potassium. In contrast, when vesicles were preloaded with 100 mM K⁺ (no external potassium), sulfate uptake was significantly inhibited. These data suggest that sulfate-chloride exchange is enhanced by an

**Fig. 1.** Time course of 0.1 mM 33SO₄⁻ uptake by hepatopancreatic brush-border membrane vesicles (BBMV). Vesicles contained (in mM) 100 tetramethylammonium (TMA) gluconate and 50 K⁺ gluconate (●) or 100 TMA gluconate, 25 K⁺ gluconate, and 25 K⁺ gluconate (○). Incubation media contained (in mM) 100 TMA gluconate and 50 K⁺ gluconate (○) or 100 Na⁺ gluconate and 50 K⁺ gluconate (●). All media contained 40 mM HEPES-Tris and 50 μM valinomycin at pH 7.0.

**Fig. 2.** Time course of 0.1 mM 33SO₄⁻ uptake by hepatopancreatic BBMV. Vesicles were preloaded with 100 mM TMA gluconate, 25 mM K⁺ gluconate, 25 mM KCl, and 50 μM valinomycin (●), 150 mM TMA gluconate and 50 μM carbonyl cyanide m-chlorophenylhydrazone (CCCP; ○), or 100 mM TMA gluconate, 50 mM K⁺ gluconate, and 50 μM valinomycin (●). Incubation media contained (in mM) 100 mM TMA gluconate and 50 K⁺ gluconate (●) or 100 K⁺ gluconate and 50 TMA gluconate (○). All media contained 40 mM HEPES-Tris at pH 7.0.
inside-positive membrane potential and inhibited by an inside-negative vesicular interior, supporting the electrogenic nature of the exchange process.

A second series of experiments was designed to investigate the effect of membrane potential on 0.5 mM $^{35}$SO$_4^-$ transport. In these experiments, vesicles were preloaded with either 100 mM Cl$^-$ or 10 mM SO$_4^{2-}$ and subjected to valinomycin-induced potassium diffusion potentials as above. Figure 4 indicates that sulfate-chloride exchange responds to both inside-negative and inside-positive conditions, whereas sulfate-sulfate exchange appears to be an electroneutral process. At both 15-s and 1-min incubations, sulfate-chloride exchange was inhibited by a transmembrane negative potential and enhanced by an imposed positive potential. In contrast, sulfate-sulfate exchange was unaffected by potential manipulations. These data suggest that the transport protein can accept either one Cl$^-$ or one SO$_4^{2-}$ molecule during the translocation process.

Effects of pH on sulfate-chloride exchange. Sulfate uptake has been shown to be stimulated by proton (or hydroxyl) gradients in the basolateral membrane of the teleost fish renal epithelium (23). The possible proton (hydroxyl) gradient stimulation of sulfate-chloride exchange in hepatopancreatic BBMV was investigated in a series of experiments in which the pH of internal and external media was varied and 0.1 mM $^{35}$SO$_4^-$ uptake was determined.

Figure 5 shows that when the pH of the incubation media was maintained at 7.0 and the internal pH was varied from 5.0 to 8.0, there was differential transport of sulfate into BBMV preloaded with 100 mM Cl$^-$. The lowest internal pH of 5.0 corresponded to the slowest uptake of sulfate, whereas when the internal pH was greater (>8.0) than the external pH, sulfate uptake was enhanced over that of all other conditions. Such results suggest that at a constant external pH of 7.0 an increase in internal proton concentration inhibits the transport of sulfate by BBMV. Alternatively, the observed phenomena may have been attributed to competition of internal OH$^-$ with Cl$^-$. In a reciprocal experiment where the internal pH was kept constant at pH 7.0 and the pH of the incubation media was raised from 5.4 to 8.0, there was again a gradation of sulfate uptake into vesicles preloaded with 100 mM Cl$^-$ (Fig. 6). With a decrease in external pH
Fig. 6. Influence of external pH on uptake of 0.1 mM \(^{35}\)SO\(_4\)\(^-\) into hepatopancreatic BBMV. Vesicles contained 100 mM TMA Cl, 50 mM K gluconate, 50 \(\mu\)M valinomycin, and 40 mM HEPES-Tris at pH 7.0. Incubation media contained 100 mM TMA gluconate and 50 mM K gluconate, and pH was adjusted to 7.0 or 8.0 with 40 mM HEPES-Tris or to pH 5.4 or 6.0 with MES-Tris.

Fig. 7. Effect of various bilateral pH conditions on uptake of 0.1 mM \(^{35}\)SO\(_4\)\(^-\) into hepatopancreatic BBMV. Vesicles contained 100 mM TMA Cl, 50 mM K gluconate, and 50 \(\mu\)M valinomycin and were adjusted to pH 7.0 or 8.0 with 40 mM HEPES-Tris or to pH 5.4 or 6.0 with 40 mM MES-Tris. Incubation media contained 100 mM TMA gluconate and 50 mM K gluconate with pH adjusted as above.

there was a corresponding increase in maximal transient sulfate accumulation. These results indicate that an increase in external proton (or decrease in external hydroxyl) concentration can stimulate sulfate-chloride exchange.

In contrast to the previous two experiments, Fig. 7 shows the result of sulfate-chloride exchange at various bilateral pH conditions ranging from 5.4 to 8.0. The maximal uptake of sulfate into BBMV occurred at pH 5.4 and decreased as pH was raised to 8.0. Results of this experiment indicate that it was not the proton (or hydroxyl) gradient that stimulated sulfate uptake but rather the absolute proton (hydroxyl) concentration.

The observation that increased external [H\(^+\)] can enhance sulfate uptake suggests a possible regulation of the transport protein by external protons. Information on the regulatory role of protons was investigated by measurement of \(^{35}\)SO\(_4\)\(^-\) transport in vesicles equilibrated with 0.1 mM radiolabeled sulfate, 100 mM TMA Cl, 50 K gluconate, 100 mM TMA gluconate, and 50 \(\mu\)M valinomycin at a pH of either 6.0 or 8.0. Incubation media had the same constituents (same specific activity inside and outside) at pH 6.0. A control condition was included where external TMA Cl was replaced by TMA gluconate (Cl gradient) at pH 6.0. In this experiment the only driving force was either the pH gradient or the Cl gradient. Figure 8 shows that the Cl\(^-\) gradient was capable of driving sulfite against a concentration gradient, whereas the pH gradient was unable to stimulate a change in sulfate content within the vesicle. These results indicate that neither a pH gradient nor equilibrated chloride was responsible for driving sulfate uptake. The previous experiments addressed the effect of pH on sulfate-chloride exchange, while Fig. 9 compared the pH effect on vesicles prepared without Cl\(^-\) and vesicles preloaded with Cl\(^-\). It was observed that 15-s uptake of 0.1 mM \(^{35}\)SO\(_4\)\(^-\), under the influence of low vs. high pH or opposing pH gradients (in the absence of internal Cl\(^-\)), was not stimulated to the level of vesicles preloaded with Cl\(^-\). There was also an effect of bilateral pH on Cl\(^-\) preloaded vesicles, where uptake was fourfold greater at pH 6 vs. pH 9. Based on these results the conclusion was made that OH\(^-\) ions were unable to substitute for Cl\(^-\) as an exchangeable substrate and that a high absolute concentration of protons stimulates SO\(_4\)\(^-\)-Cl\(^-\) exchange.

Kinetic characteristics of sulfate influx. Preliminary investigation determined that \(^{35}\)SO\(_4\)\(^-\) uptake into hepatopancreatic BBMV was linear over the first 12 s at the concentrations utilized throughout this study (data not shown). Sulfate influx (7 s uptake) from incubation media to vesicular interior was measured in membranes preloaded with 100 mM TMA Cl, 50 mM K gluconate, and 50 \(\mu\)M valinomycin at pH 7.0 and external media of 100 mM TMA gluconate, 50 mM K gluconate, and variable sulfate (0.1–20 mM) at pH 7.0. Figure 10 shows that...
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SULFATE UPTAKE (pmol/mg protein/15 s)

Fig. 9. Effect of pH and internal Cl- on 0.1 mM $^{35}$SO$_4^{2-}$ uptake into hepatopancreatic BBMV. Vesicles were preloaded with 100 mM TMA gluconate, 100 mM K gluconate, and 50 $\mu$M valinomycin and adjusted to pH 9 with 40 mM HEPES-Tris or to pH 6 with 40 mM MES-Tris. Chloride-preloaded vesicles (hatched bars) contained 100 KCl replacing K gluconate. Incubation media contained (in mM) 0.10 $^{35}$SO$_4^{2-}$, 100 TMA gluconate, and 100 K gluconate and were adjusted to pH 9 with 40 mM HEPES-Tris or to pH 6 with 40 MES-Tris.

SULFATE CONCENTRATION (mM)

Fig. 10. Effect of external sulfate concentration on uptake of 0.1 mM $^{35}$SO$_4^{2-}$ into hepatopancreatic BBMV. Vesicles were preloaded with 100 mM TMA Cl, 50 mM K gluconate, and 50 $\mu$M valinomycin. Incubation media contained 100 mM TMA gluconate, 50 mM K gluconate, and indicated sulfate concentrations. All media contained 40 mM HEPES-Tris at pH 7.0. $P$, rate constant of the linear entry component; $J_{\text{mm}}$, apparent maximal carrier-mediated influx; $K_t$, apparent sulfate concentration resulting in half-maximal uptake.

SULFATE INFLUX WAS A CURVILINEAR FUNCTION OF EXTERNAL SULFATE CONCENTRATION. AN INFLUX RELATIONSHIP SUCH AS THIS CAN BE DESCRIBED AS THE SUM OF AT LEAST TWO INDEPENDENT PROCESSES ACTING SIMULTANEOUSLY: 1) A MICHAELIS-MENTEN CARRIER MECHANISM ILLUSTRATING SATURATION KINETICS AND 2) A LINEAR ENTRY SYSTEM WITH A RATE PROPORTIONAL TO THE EXTERNAL SULFATE CONCENTRATION. THESE TWO PROCESSES OPERATING TOGETHER CAN BE DESCRIBED BY THE EQUATION

\[
J = \frac{J_{\text{mm}} [S]}{K_t + [S]} + P[S]
\]  

WHERE $J$ IS TOTAL $^{35}$SO$_4^{2-}$ INFLUX IN NANO moles PER MILLIGRAM PROTEIN PER 7 s, $J_{\text{mm}}$ IS APPARENT MAXIMAL CARRIER-MEDIATED INFLUX, $K_t$ IS THE APPARENT SULFATE CONCENTRATION RESULTING IN HALF MAXIMAL UPTAKE, $[S]$ IS THE EXTERNAL SULFATE CONCENTRATION, AND $P$ IS THE RATE CONSTANT OF THE LINEAR ENTRY COMPONENT, WHICH CAN BE DEFINED AS APPARENT DIFFUSIONAL PERMEABILITY.

A NONLINEAR ITERATIVE BEST-FIT COMPUTER PROGRAM WAS USED TO ANALYZE THE DATA IN FIG. 10 BY Eq. 1. APPARENT TRANSPORT PARAMETERS CALCULATED IN THIS MANNER ARE AS FOLLOWS: APPARENT $K_t = 0.27$ mM, APPARENT $J_{\text{mm}} = 1.28$ nmol-mg protein$^{-1}$.7 s$^{-1}$ AND $P = 0.36$ nmol-mg protein$^{-1}$.7 s$^{-1}$.mM$^{-1}$.

IN AN ATTEMPT TO DETERMINE THE INTERNAL BINDING COEFFICIENT OF SULFATE TO HEPATOPANCREATIC BBMV DURING SULFATE SULFATE EXCHANGE, VESICLES WERE PRELOADED WITH VARIOUS CONCENTRATIONS OF $\text{SO}_4^{2-}$ (0.05-10 mM) AND INCUBATED IN MEDIA THAT CONTAINED 0.5 mM RADIOLabeled SULFATE. FIGURE 11 SHOWS THAT UNDER THESE CONDITIONS INFUX VS. INTERNAL SULFATE CONCENTRATION EXHIBITED A HYPERBOLIC RELATIONSHIP THAT CAN BE DESCRIBED BY THE MICHAELIS-MENTEN EQUATION

\[
J = \frac{J_{\text{mm}} [S]}{K_t + [S]} + P[S]
\]  

WHERE $\alpha$ IS THE BOUNDING COEFFICIENT OF SULFATE TO VESICULAR INTERIOR, $[S]$ CORRESPONDS TO THE INTERNAL SULFATE CONCENTRATION, AND OTHER SYMBOLS ARE THE SAME AS IN Eq. 1. TRANSPORT PARAMETERS WERE CALCULATED AS ABOVE AND WERE APPARENT $K_t = 3.68 \pm 1.8$ mM AND APPARENT $J_{\text{mm}} = 125.9 \pm 25.7$ pmol-mg protein$^{-1}$.7 s$^{-1}$.

FIGURE 12 INDICATES THE INFUX OF 0.1 mM $^{35}$SO$_4^{2-}$ INTO BBMV THAT WERE PRELOADED WITH VARIOUS CONCENTRATIONS OF Cl$^-$ (10-400 mM). THESE DATA APPROXIMATED A HYPERBOLIC RELATIONSHIP THAT WAS FIT TO THE MICHAELIS-MENTEN EQUATION

\[
J = \frac{J_{\text{mm}} [Cl]}{K_t + [Cl]} + P[Cl]
\]  

WHERE $\beta$ IS THE APPARENT BINDING COEFFICIENT OF Cl$^-$ TO THE VESICLE INTERIOR, [Cl] IS THE INTERNAL Cl$^-$ CONCENTRATION.
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+ = 173.1 pmol/mg protein/6 s

INTERNAL CHLORIDE CONCENTRATION (mM)

Fig. 12. Effect of internal chloride concentration on influx of 0.1 mM $^{35}$SO$_4^-$ into hepatopancreatic BBMV. Vesicles were preloaded with 100 mM K gluconate, 10–250 mM TMA Cl, 50–290 mM TMA gluconate, and 50 mM valinomycin. Incubation media contained (in mM) 300 TMA gluconate, 100 K gluconate, and 0.1 $^{35}$SO$_4^-$. All media contained 40 mM HEPES-Tris at pH 7.0. $K_c^i$, apparent binding coefficient of chloride to vesicle interior.

Fig. 13. Effect of inside-negative vesicular interior on $^{35}$Cl$^-$-chloride exchange (A) and $^{35}$Cl$^-$-sulfate exchange (B) in hepatopancreatic BBMV. A: vesicles were preloaded with (in mM) 100 KCl and 100 TMA gluconate and incubated in media containing 0.1 $^{35}$SO$_4^-$ and either 100 K gluconate and 100 TMA gluconate (open bar) or 200 TMA gluconate (hatched bar). B: vesicles were preloaded with (in mM) 50 K$_2$SO$_4$ and 75 TMA gluconate and incubated in media containing 10 $^{35}$Cl$^-$ and either 100 K gluconate and 100 TMA gluconate (open bar) or 200 TMA gluconate (hatched bar). All media contained 50 mM valinomycin and 40 mM HEPES-Tris at pH 7.5.

Effect of inhibitors and competitors on sulfate influx. Potential anion exchange transport inhibitors and competitive ions were tested in BBMV preloaded with 200 mM TMA gluconate, 100 mM KCl, and 50 mM valinomycin (Fig. 14). Incubation media consisted of 0.1 K$_2$SO$_4$, 200 TMA gluconate, 100 K gluconate, and either 1 SITS, DIDS, bumetanide, or furosemide or 10 NaCl, NaHCO$_3$, or Na$_2$SO$_3$. All media contained 40 mM HEPES-Tris at pH 7.0.

SULFATE UPTAKE (pmol/mg protein/15 s)

Fig. 14. Effect of potential inhibitors on $^{35}$SO$_4^-$-Cl$^-$ exchange in hepatopancreatic BBMV. Vesicles were preloaded with 200 mM TMA gluconate, 100 mM KCl, and 50 mM valinomycin. In incubation media contained (in mM) 0.1 K$_2$SO$_4$, 200 TMA gluconate, 100 K gluconate, and either 1 SITS, DIDS, bumetanide, or furosemide or 10 NaCl, NaHCO$_3$, or Na$_2$SO$_3$. All media contained 40 mM HEPES-Tris at pH 7.0.

SULFATE UPTAKE (pmol/mg protein/15 s)

Fig. 14. Effect of potential inhibitors on $^{35}$SO$_4^-$-Cl$^-$ exchange in hepatopancreatic BBMV. Vesicles were preloaded with 200 mM TMA gluconate, 100 mM KCl, and 50 mM valinomycin. Incubation media contained (in mM) 0.1 K$_2$SO$_4$, 200 TMA gluconate, 100 K gluconate, and either 1 SITS, DIDS, bumetanide, or furosemide or 10 NaCl, NaHCO$_3$, or Na$_2$SO$_3$. All media contained 40 mM HEPES-Tris at pH 7.0.

DISCUSSION

In the current investigation we presented evidence for the existence of a carrier-mediated sulfate-chloride exchange on BBMV isolated from lobster hepatopancreatic epithelium. Sulfate carriers have been described for the
brush border of several vertebrate tissues. Both sodium-sulfate cotransport and sulfate-hydroxyl exchange mechanisms, in the same BBMV, have been demonstrated in rabbit ileal brush border (26, 27). In avian renal BBMV, multiple pathways were shown to transport sulfate: sodium-sulfate cotransport, sulfite-bicarbonate exchange, and proton-dependent sulfate transport (22). Marine teleost renal tubule BBMV have been shown to contain a sulfate-anion exchange mechanism that is most effective with bicarbonate (24), whereas no sodium or proton stimulation of sulfate transport was observed in this brush border (23).

In the lobster hepatopancreas in vivo there is a pH gradient maintained across the epithelium with a lower pH in the lumen than in the blood (11). We therefore tested the effect of pH gradients on the uptake of sulfate into BBMV. Sulfate-chloride exchange was diminished when the internal pH was less than that of the external pH, and sulfate uptake was enhanced as the external pH was decreased (constant internal pH). This suggested that an extravesicular pH lower than internal pH could stimulate sulfate-chloride (or sulfate-hydroxyl) exchange. When pH was held constant on both sides of BBMV (Fig. 7), there was increased uptake at lower pH, an effect similar to that obtained in Fig. 6. This result suggested that it was not the pH gradient but the lower pH (increased external protons) that stimulated sulfate-chloride exchange. An equilibrium shift experiment was designed to determine whether a pH gradient (internal pH 8.0, external pH 6.0) could provide the driving force to accumulate $\text{SO}_4^{2-}$ into BBMV under equilibrated $\text{SO}_4^{2-}$ and chloride conditions (Fig. 8). A pH gradient or varying absolute pH did not stimulate $\text{SO}_4^{2-}$ uptake in the absence of internal Cl$^-$(Fig. 9).

This series of experiments suggests that a proton (or hydroxyl) gradient does not act as a driving force during sulfate-chloride exchange, nor could it stimulate uptake alone, yet there were significant effects of varying pH on the magnitude of sulfate-chloride exchange. All the experiments were short-circuited by the presence of valinomycin and equimolar potassium across the BBMV so that H$^+$-generated transmembrane diffusion would be unlikely to cause the results observed in Figs. 5 and 6. Protons have been shown to act as allosteric activators of the Na-H exchanger in rabbit renal BBMV (8) and Na-SO$_4$ cotransport in rabbit ileum BBMV (6). The results observed in Figs. 7 and 9 would support the idea of external protons having a modifier role of stimulating the sulfate-chloride exchanger. There may also be a pH-sensitive regulatory site on the internal surface of the transport protein that can inhibit sulfate-chloride exchange. One must be careful in comparing results from different vesicle preparations, but the magnitude of the external pH effect (Figs. 6 and 7) is twice that of the internal effect (Fig. 5), which suggests that the external modifier site is more important in transport regulation.

The existence of internal pH-sensitive regulatory sites for the Cl$^-$-HCO$_3^-$ exchanger has been demonstrated on the rabbit ileal brush-border membrane using membrane vesicles (17) and in isolated cell preparations of lymphocytes (16) and Vero cells (19). An external pH-sensitive regulatory site on the sulfate-chloride exchanger in the hepatopancreatic brush-border membrane would be physiologically important due to the 2 Na-H exchanger also present in the membrane (4). The Na-H exchanger operates during luminal acidification after ingestion of a meal and would provide a stimulus for enhanced sulfate-chloride exchange. When the sodium-proton exchanger is operating, there would be an increase in the [H$^+$] in the hepatopancreatic lumen and a corresponding decrease of protons in the cytoplasm. This would tend to enhance the sulfate-chloride exchanger due to modification at an external site as in Fig. 6 and regulation at an internal site as in Fig. 5 (via a decrease in internal [H$^+$]).

The effect of valinomycin-induced K$^+$ diffusion potentials on sulfate-chloride exchange was investigated to determine the effect of membrane potential on transport in BBMV. Uptake was measured under both inside-negative and inside-positive membrane potentials (100 mM K$^+$ on respective side of vesicle) and compared with short-circuited conditions with bilaterally equimolar potassium. Figure 3 indicates that sulfate-chloride exchange is stimulated by a positive vesicular interior and inhibited by a negative vesicular interior. These data suggest that there is an excess of negative charge transferred into the vesicle during the exchange process. The effect of membrane potential on sulfate-sulfate exchange is compared with sulfate-chloride exchange in Fig. 4. Vesicles were preloaded with either 100 mM Cl$^-$ or 10 mM SO$_4^{2-}$, and the uptake of $^{35}$SO$_4^{2-}$ was measured under the above membrane potentials. It was observed that alteration of transmembrane potential had no effect on sulfate-sulfate exchange while sulfate-chloride exchange was affected as above (Fig. 3). These data support the idea that the carrier can accommodate either one SO$_4^{2-}$ ion or one Cl$^-$ ion, which would result in electroneutral sulfate-sulfate exchange or electrogenic sulfate-chloride exchange. These findings are in contrast with the reports of other investigations on sulfate carriers and electrical coupling in BBMV. Sulfate-bicarbonate exchange was unaffected by valinomycin-induced potassium diffusion potentials in both flounder renal brush border (24) and rat renal cortex BBMV (20). Electrical coupling was not supported in pH gradient-stimulated SO$_4^{2-}$ uptake in rabbit ileal BBMV (27), while Na$^+$-SO$_4^{2-}$ cotransport, in the same organ, was variably affected depending on pH and Na$^+$ concentration (6). In rat and rabbit cortical BBMV Na-dependent sulfate transport was shown to be an electroneutral process (15, 26).

The influx of $^{35}$SO$_4^{2-}$ in hepatopancreatic BBMV occurred by at least one carrier-mediated mechanism exhibiting Michaelis-Menten kinetics and a second process that may be simple diffusion (Fig. 10). The apparent binding constant for sulfate association with the vesicular interior is an order of magnitude higher than that observed for the vesicular exterior. The estimated $J_{\text{max}}$ of both sulfate- and chloride-loaded vesicles was similar, while the estimated binding of sulfate to vesicle interior was considerably less than that for chloride. The cellular concentrations of these ions is unknown; hence it is not possible to determine which of these binding constants is physiologically relevant. It is possible that the high internal Cl$^-$ would have caused a cis-inhibition of sulfate-
sulfate exchange due to the experimental conditions that resulted in a spillover of unlabeled chloride. The magnitude of cis-inhibition by Cl\(^{-}\) has not yet been determined.

The sulfate-chloride exchanger was significantly inhibited by thioulsulfate and the disulfonic stilbenes DIDS and SITS (Fig. 14) as reported by various investigators (20, 22, 24). Furosemide and bumetanide were not as effective as with rabbit ileal BBMV (27) or bovine kidney tubule BBMV (29). The strong inhibition of DIDS and SITS provides further evidence for the presence of an anion exchanger in hepatopancreatic BBMV.

Under the assumption that exchange mechanisms such as this can operate in both directions, it should be possible to measure the uptake of \(^{36}\)Cl\(^{-}\) into vesicles in exchange for internal sulfate. If the exchange operated at a 1:1 ratio it would be expected to also react to a membrane potential. It was observed that \(^{36}\)Cl\(^{-}\) influx did respond to an inside-negative potential (Fig. 13B) by enhancing the uptake at a 1 min incubation. With this arrangement there would be a secretion of sulfate from the vesicular interior in exchange for luminal chloride that is further driven by the inside negative potential that is characteristic of epithelial cells (e.g., Refs. 1, 10). Given the above binding constants there is a higher apparent affinity of the vesicular interior for sulfate, which supports the idea of sulfate being transferred from the cell interior to the lumen. This phenomenon would support the work of some investigators who have postulated that the crustacean gut can provide an excretory function in the elimination of certain solutes (9, 12).

From this study it can be tentatively suggested that the hepatopancreas of the intermolt lobster may play a role in the secretion of the divalent anion sulfate. The mechanism of sulfate transport across the basolateral membrane needs to be characterized more clearly to ascertain the net flux direction of this ion across the epithelium. It is very likely that the hepatopancreas can transport sulfate in both directions depending on physiological, environmental, and even hormonal conditions.

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