Sulfate/oxalate exchange by lobster hepatopancreatic basolateral membrane vesicles

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Gerencser, George A., Mark A. Cattey, and Gregory A. Ahearn. Sulfate/oxalate exchange by lobster hepatopancreatic basolateral membrane vesicles. *Am. J. Physiol.* 269 (*Regulatory Integrative Comp. Physiol.* 38): R572–R577, 1995.—Purified basolateral membrane vesicles (BLMV) were prepared from lobster hepatopancreas by osmotic disruption and discontinuous sucrose gradient centrifugation. Radiolabeled sulfate uptake was stimulated by 10 mM intravesicular oxalate compared with gluconate-loaded vesicles. Sulfate/oxalate exchange was not affected by transmembrane valinomycin-induced potassium diffusion potentials (inside negative or inside positive), suggesting electroneutral anion transport. Sulfate uptake was not stimulated by the similar carboxylic anions formate, succinate, oxaloacetate, or ketoglutarate. Sulfate influx occurred by at least one saturable Michaelis-Menten carrier system [apparent K_m = 6.0 ± 1.7 mM; maximum flux (J_{max}) = 382.3 ± 37.0 pmol·mg protein^{-1}·7 s^{-1}]. Sulfate/oxalate exchange was significantly reduced by the anion antiport inhibitors 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid and 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid but was not affected by bumetanide or furosemide. The possible physiological role of this exchange mechanism in anion/sulfate transport across the crustacean hepatopancreas is discussed.

sulfate transport; anion exchange; gastrointestinal physiology; *Homarus americanus*

SULFATE TRANSPORT across serosal plasma membranes has been described in a wide variety of organisms and cell types, including intestinal (17, 22, 24, 25, 36), renal (30, 32, 33, 37), and liver (20, 21, 27) epithelia. The mechanisms involved in sulfate uptake differ considerably among the many systems that have been investigated. Transport can be coupled to proton (21, 33) and sodium (25) cotransport or anion exchange, i.e., bicarbonate or chloride (24, 30, 32, 36). In many of these reports it has been stated that sulfate transport can be stimulated by exchange with a number of organic anions, especially oxalate (20–22, 24, 27, 37, 38). Although carrier-mediated sulfate transport is observed in the basolateral membrane of numerous vertebrate systems, the net handling of this anion can be either absorptive or secretory in nature, depending on the specific animal and organ. The physiological properties associated with sulfate regulation in these diverse organisms include cell and plasma osmoregulation, maintenance of ion composition, and acid-base balance.

In crustaceans, the hepatopancreas is involved in both digestion and absorption of nutrients (14, 26, 40); in addition, some investigators have implicated this organ as a site of excretion as well (12, 15, 18). Recently the use of isolated membrane vesicles has led to the definition of absorptive and exchange mechanisms for a number of solutes in the hepatopancreatic brush border (1–4, 10) and a chloride/bicarbonate exchange process (5) in the basolateral membrane.

The present study uses hepatopancreatic basolateral membrane vesicles (BLMV) to characterize a sulfate/oxalate exchange mechanism that is specific with respect to organic anions, is inhibited by stilbenes, and is unaffected by pH.

METHODS

Live Atlantic lobsters (*Homarus 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 in this study were either in intermolt or early premolt as assessed by the molt stage classification scheme introduced by Aiken (6).

Hepatopancreatic BLMV were prepared from fresh tissue removed from individual lobsters. Each membrane batch was produced from 10 g fresh wt from one organ with the use of a modification of the method described by Ahearn et al. (5). In the present study, hepatopancreatic tissue was homogenized for 30 s with a Polytron (Brinkmann Instruments) in 300 ml of bomb buffer [25 mM NaCl, 10 mM tris(hydroxymethyl)aminomethane *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (Tris-HEPES), pH 8.0] containing 0.2 mM phenylmethylsulfonyl fluoride (PSMF). The homogenate was centrifuged for 15 min at 500 *g* with a Sorvall RC-5C high-speed centrifuge with an SS-34 fixed angle rotor. After suction removal of a surface lipid layer, the supernatant was centrifuged for 33 min at 100,000 *g* with a Beckman L8–55 ultracentrifuge with an SW-28 swinging bucket rotor. The resulting pellet consisted of a dark, highly condensed central region and a fluffy, light external area. The light portion was separated from the remainder of the pellet and collected by pipette, brought up in 10 ml of 50% sucrose in gradient buffer (12.5 mM NaCl, 10 mM HEPES-Tris, and 0.5 mM ethylenediaminetetraacetic acid, pH 7.4), and resuspended by passage 10 times through a small-bore Pasteur pipette. The solution was brought to a final volume of 36 ml and placed in the bottom of 4 centrifuge tubes (9 ml each) and overlayed with successive 9-ml volumes of 40, 30, and 20% sucrose in gradient buffer. The discontinuous sucrose gradient was centrifuged at 100,000 *g* for 93 min in a Beckman ultracentrifuge. Vesicles were collected from the 30–40% sucrose interface and brought up in a small volume of inside buffer (composition indicated in Figs. 1–6). Vesicles were allowed to equilibrate in internal buffer at 4°C for 2 h and the solution was centrifuged at 100,000 *g* for 33 min. Final vesicle pellets were brought up in a small volume of inside buffer and homogenized with 15 passes through a 22-gauge needle.

Spectrophotometric assays of marker enzymes were used to establish the relative purity of the membrane preparation. Alkaline phosphatase activity was determined with the use of Sigma kit no. 104, leucine aminopeptidase was measured by the technique of Haase et al. (16), and cytochrome-*c* oxidase activity was assessed by the method of Cooperstein and Lazarow (11). Na^+-K^+-stimulated ATPase was measured with

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the use of the phosphate liberation technique of Ames (7) with the modifications described by Murphy and Riley (28).

Transport studies were conducted at 15°C, using the rapid filtration technique developed by Hopfer et al. (19). For time course experiments, 20 μl of vesicles were added to 180 μl of incubation media containing 0.1 mM radiolabeled $^{35}SO_4^{2-}$. At various incubation times, a known volume (20 μl) of reaction mixture was removed and plunged into 2 ml of ice-cold stop solution (composition varied with experiment and generally consisted of incubation media without sulfate) to stop the uptake process. The vesicle suspension was then rapidly filtered through a 0.65-μm Millipore filter and washed with another 7 ml of ice-cold stop solution. Filters were transferred to vials containing Beckman Ecolume scintillation cocktail and counted for radioactivity in a Beckman LS-8100 scintillation counter. Transport experiments involving incubations of < 10 s were conducted with the use of a rapid-exposure uptake apparatus (Inovativ Labor, Adliswil, Switzerland). Uptake was initiated by mixing 5 μl of vesicles with 95 μl of radiolabeled incubation media, and filters were washed and counted for radioactivity as above. Blank values were 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. Incubation and intravesicular media varied between experiments and are indicated in Figs. 1–7. Sulfate uptake values were 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 repeated at least three to five times using membranes prepared from different animals, and there was no qualitative difference among any of the experiments. 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. Similar quantitative experimental findings were obtained in the repetition of an experiment.

$^{35}SO_4^{2-}$ as the Na^+ salt was obtained from New England Nuclear. Valinomycin, 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

Physical characteristics of hepatopancreatic BLMV. Purity of BLMV, prepared by the centrifugation methods used in this study, was assessed by comparing the activities of membrane-bound marker enzymes assayed in the initial hepatopancreas homogenate with activities of the same enzymes in the final vesicle suspension (Table 1). Putative brush-border marker enzymes alkaline phosphatase and leucine aminopeptidase did not exhibit increased activity in the BLMV suspension. Likewise, cytochrome-*c* oxidase, a mitochondrial marker enzyme, was not enriched in the vesicle fraction. In contrast, the activity of the basolateral membrane marker enzyme Na^+-K^+-ATPase was found to be increased in the vesicle suspension, with respect to the initial homogenate. These observations suggest that vesicles prepared by the above technique contained predominately basolateral membranes with minimal contamination from brush-border or organelle membrane sources.

Table 1. *Enzyme characterization of Atlantic lobster (Homarus americanus) hepatopancreatic basolateral membrane vesicles*

Enzyme	Homogenate Activity, μmol·mg⁻¹·h⁻¹	BLMV Activity, μmol·mg⁻¹·h⁻¹	Purification Factor	*n*
Alkaline phosphatase	1.21 ± 0.37	1.29 ± 0.38	1.07 ± 0.16	8
Leucine aminopeptidase	804.3 ± 113.4	488.0 ± 35.1	0.66 ± 0.12	3
Cytochrome-*c* oxidase	65.6 ± 2.6	15.5 ± 1.9	0.24 ± 0.03	3
Na^+-K^+-ATPase	3.81 ± 0.71	47.3 ± 5.7	13.9 ± 2.2	5

Values are means ± SE; *n*, no. of preparations. Purification factors are means of individual basolateral membrane vesicle (BLMV) activities/individual homogenate activities.

To confirm the closure of hepatopancreatic BLMV formed by the above preparation and to determine the movement of sulfate into an osmotically reactive space, equilibrium (180 min) uptake of 0.1 mM radiolabeled sulfate was determined at a variety of transmembrane osmotic gradients. Vesicles were loaded with 90 mM tetramethylammonium gluconate (TMA-gluconate), 100 mM K-gluconate, 10 mM K-oxalate, pH 7, and incubated in media containing 100 mM TMA-gluconate, 100 mM K-gluconate, 0.1 mM radiolabeled sulfate, and variable quantities of sucrose at the same pH. Figure 1 indicates a linear association between sulfate uptake and the reciprocal of external osmolarity, suggesting uptake into a defined vesicular space. Extrapolation of the curve to the vertical intercept shows some nonspecific isotope binding at infinite osmolarity.

Transport properties of hepatopancreatic BLMV. Figure 2 shows the stimulation of an outwardly directed

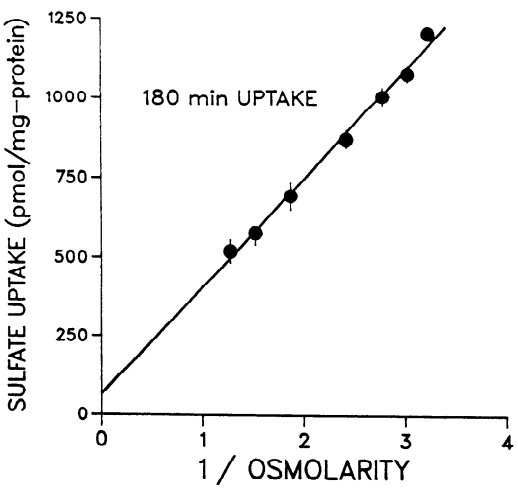

Fig. 1. Effect of transmembrane osmotic gradients on 180-min equilibrium uptake of 0.1 mM $^{35}SO_4^{2-}$ by hepatopancreatic basolateral membrane vesicles (BLMV). Vesicles were loaded with 100 mM tetramethylammonium gluconate (TMA-gluconate), 80 mM K-gluconate, and 10 mM K_2-oxalate and incubated in external media containing 100 mM TMA-gluconate and 100 mM K-gluconate and variable quantities of sucrose. All media contained 40 mM HEPES-Tris and 50 μM valinomycin, pH 7.0.

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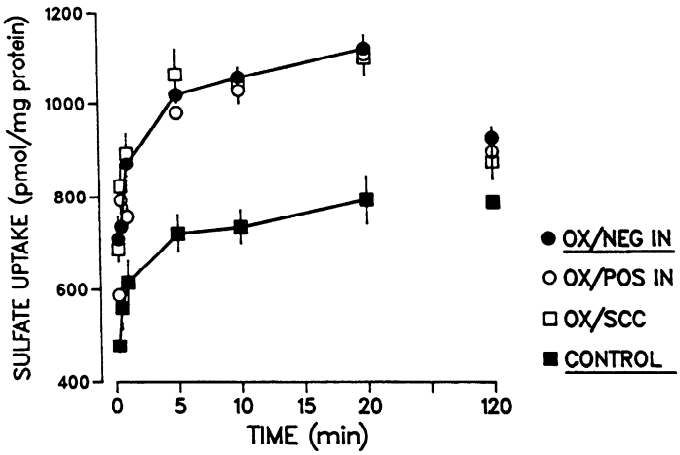

Fig. 2. Time course of 0.1 mM $^{35}SO_4^{2-}$ uptake by hepatopancreatic BLMV. Oxalate inside-positive potential (OX/POS IN) vesicles contained 180 mM TMA-gluconate and 10 mM K_2-oxalate; OX/inside-negative (OX/NEG IN) and OX/short-circuited condition (OX/SCC) vesicles contained 100 mM TMA-gluconate, 80 mM K-gluconate, and 10 mM K_2-oxalate; Control vesicles contained 100 mM TMA-gluconate and 100 mM K-gluconate. Incubation media for OX/POS IN, OX/SCC, and control contained 100 mM TMA-gluconate and 100 mM K-gluconate, or for OX/NEG IN, 200 mM TMA-gluconate. All media contained 40 mM HEPES-Tris and 50 μM valinomycin at pH 7.0.

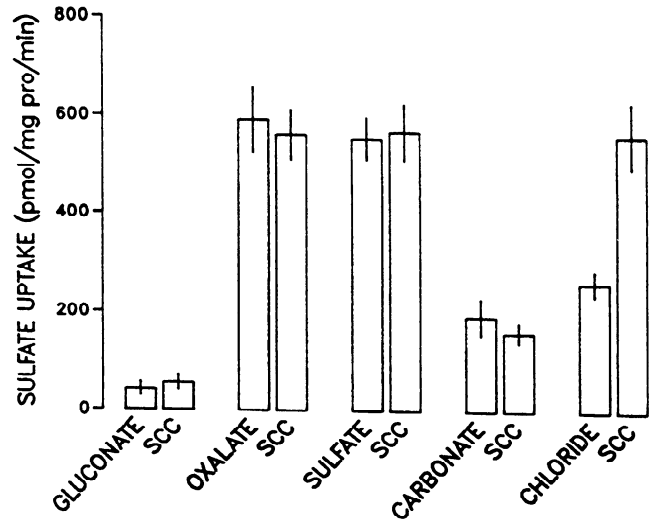

Fig. 3. Effect of membrane potential on exchange-driven uptake of 0.1 mM $^{35}SO_4^{2-}$ uptake into hepatopancreatic BLMV. Vesicles were loaded with 100 mM TMA-gluconate and 100 mM K-gluconate and 10 mM of indicated anion (substituted isosmotically for K-gluconate). Incubation media contained 100 mM TMA-gluconate and 100 mM K-gluconate). Incubation media contained 100 mM TMA-gluconate and 100 mM K-gluconate. SCC also contained 50 μM valinomycin. All media contained 40 mM HEPES-Tris at pH 7.0.

oxalate gradient on the uptake of 0.1 mM $^{35}SO_4^{2-}$ by BLMV when compared with vesicles lacking an exchangeable anion (gluconate only). Additionally, the possible effect due to alteration of membrane potential by valinomycin-induced K^+-diffusion potentials across the vesicular wall was observed. An inside-negative potential was established with the use of 100 mM intravesicular K^+ and incubation media without K^+, and an inside-positive potential was established with the use of 100 mM external K^+ and no internal K^+. The short-circuited condition contained bilaterally equal K^+. Figure 2 indicates that these manipulations of membrane potential had no effect on sulfate/oxalate exchange. This would suggest that the antiport process is electroneutral in nature.

To further ascertain the electrogenic nature of sulfate/oxalate exchange, another experimental protocol was employed. Vesicle batches were loaded with 10 mM oxalate, sulfate, carbonate, or chloride. Both internal and external media contained 100 mM K^+; a portion of each membrane suspension was short-circuited by exposure to valinomycin. The remaining vesicle suspension was not incubated in the ionophore. Figure 3 indicates that only vesicles that had been loaded with chloride exhibited differential sulfate uptake. These results suggest that, in the absence of a freely moveable charge, there is a buildup of negative potential that inhibited the movement of sulfate that exchanged for the monovalent anion. When vesicles were loaded with divalent anions there was no significant difference between the experimental conditions. These results suggest that sulfate will exchange at a 1:1 ratio with the above anions and that sulfate/oxalate exchange was electroneutral. It is uncertain whether the reduced uptake with carbonate was due to the divalent species or the monovalent bicarbonate.

Whether sulfate would exchange with a wide range of organic anions as transferable substrates was investigated by *trans*-stimulation with 5 mM oxalate, oxaloacetate, succinate, formate, ketoglutarate, and citrate (Fig. 4). Only oxalate was able to stimulate 0.1 mM $^{35}SO_4^{2-}$ uptake greater than the response obtained in the presence of the nonexchangeable anion gluconate. This experiment indicates that in BLMV the antiporter is relatively specific and will not accept similar dicarboxylic acids or the tricarboxylic acid citrate as a substrate.

Information about the effect of various bilateral pH conditions and proton (hydroxyl) gradients was obtained for sulfate/oxalate exchange. Vesicles were loaded with 10 mM oxalate, equilibrated to pH 6, 7, or 8, and incubated in media of the same respective pH. All conditions were significantly stimulated compared with

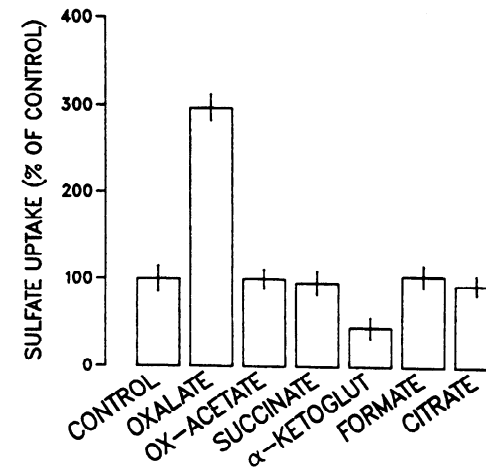

Fig. 4. Effect of internal organic anion on 0.1 mM $^{35}SO_4^{2-}$ uptake into hepatopancreatic BLMV. Vesicles were loaded with 100 mM TMA-gluconate, 100 mM K-gluconate, and 5 mM of indicated anion (substituted isosmotically for TMA-gluconate). External media contained 100 mM TMA-gluconate and 100 mM K-gluconate. All media contained 40 mM HEPES-Tris and 50 μM valinomycin at pH 7.0.

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gluconate-loaded vesicles (pH 7), but the absolute proton (hydroxyl) concentration did not differentially effect sulfate uptake (Fig. 5). Likewise, a transmembrane proton gradient established across the vesicular wall, pH 8 out and pH 6 in or pH 6 out and pH 8 in, had no effect on sulfate/oxalate exchange. These results suggest that the simultaneous imposition of transmembrane proton (hydroxyl) gradients, in addition to an outwardly directed oxalate gradient, do not enhance sulfate uptake into lobster BLMV.

Potential anion-exchange inhibitors of sulfate/oxalate antiport were tested in BLMV loaded with 10 mM oxalate (Fig. 6). Inhibitors were added to the external media at 1 mM concentration, whereas the control condition was sulfate/oxalate exchange without any inhibitor. The potent stilbene compounds DIDS and SITS significantly reduced sulfate uptake, whereas both furosemide and bumetanide had no inhibitory effect. This is further evidence that sulfate uptake into BLMV is an antiport mechanism.

The last set of experiments assessed the role of external sulfate concentration on sulfate/oxalate exchange in BLMV. Vesicles were loaded with 10 mM K-oxalate and incubated in media containing various concentrations of sulfate (0.25–30 mM). Initial 7-s uptake was measured and found to be a hyperbolic function of external sulfate concentration. Figure 7 shows this relationship, which can be described by the Michaelis-Menten equation

$$J = \frac{J_{max}\,[S]}{K_t + [S]} \qquad (1)$$

where K_t is the apparent sulfate concentration resulting in one-half maximal uptake, and [S] is the external sulfate concentration. Transport parameters were calculated and are the following: apparent K_t = 6.0 ± 1.7 mM and apparent J_{max} = 382.3 ± 37.0 pmol·mg protein^{-1}·7 s^{-1}.

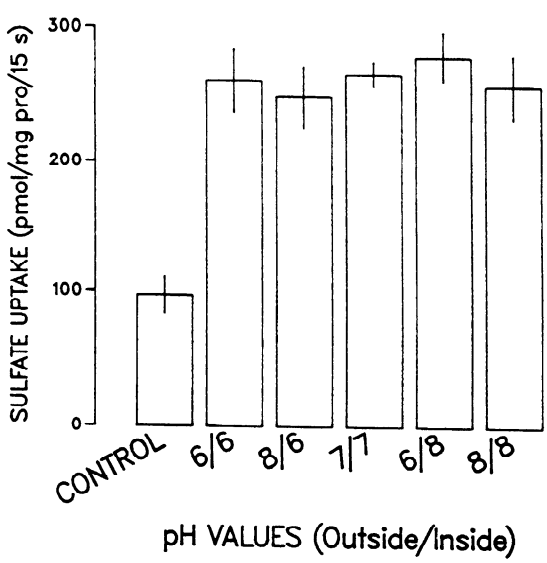

Fig. 5. Effect of various bilateral pH conditions and pH gradients on uptake of 0.1 mM $^{35}SO_4^{2-}$ into hepatopancreatic BLMV. Vesicles contained 100 mM TMA-gluconate, 80 mM K-gluconate, 10 mM K_2-oxalate adjusted to pH 7.0 or 8.0 with 40 mM HEPES-Tris, or to pH 6.0 with 40 mM 2-(*N*-morpholino)ethanesulfonic acid (MES-Tris). Incubation media contained 100 mM TMA-gluconate and 100 mM K-gluconate with pH adjusted as above. All media contained 50 μM valinomycin.

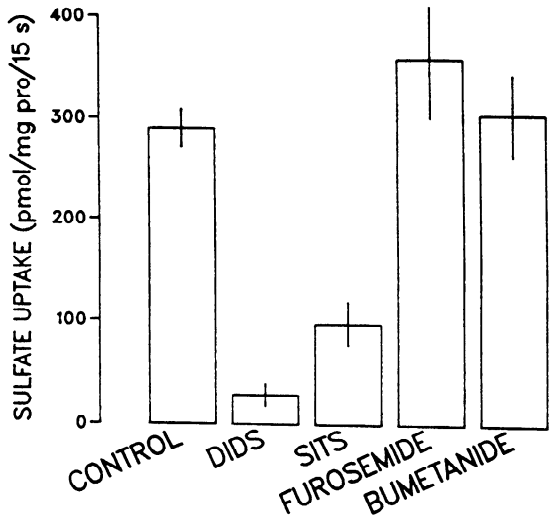

Fig. 6. Effect of potential inhibitors on $^{35}SO_4^{2-}$/oxalate exchange in hepatopancreatic BLMV. Vesicles were loaded with 100 mM TMA-gluconate, 80 mM K-gluconate, and 10 mM K_2-oxalate. Incubation media contained 0.1 mM $K_2{}^{35}SO_4$, 100 mM TMA-gluconate, 100 mM K-gluconate, and either 1 mM 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid (SITS), 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS), bumetanide, or furosemide. All media contained 50 μM valinomycin and 40 mM HEPES-Tris at pH 7.0. pro, Protein.

DISCUSSION

The present study provides evidence for the occurrence of a carrier-mediated sulfate/oxalate exchanger found in BLMV isolated from lobster hepatopancreas. A number of marine invertebrates, including the lobster, have been shown to maintain plasma levels of sulfate below that which is found in seawater (31, 35). This

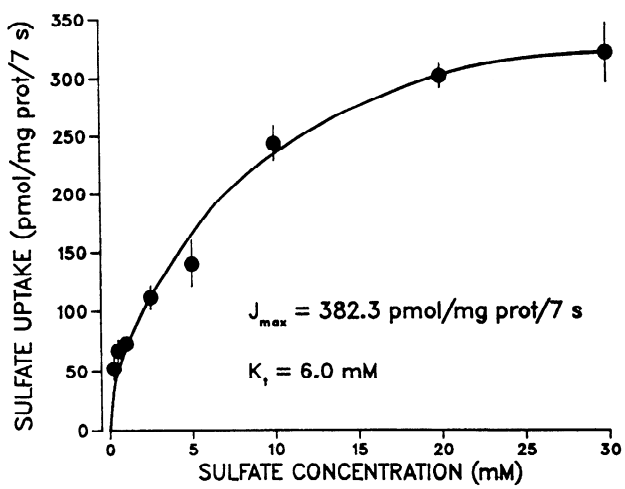

Fig. 7. Effect of external sulfate concentration on uptake of 0.1 mM $^{35}SO_4^{2-}$ into hepatopancreatic BLMV. Vesicles were loaded with 100 mM TMA-gluconate, 80 mM K-gluconate, and 10 mM K_2-oxalate. Incubation media contained 100 mM TMA-gluconate, 100 mM K-gluconate, and indicated sulfate concentrations. All media contained 50 μM valinomycin and 40 mM HEPES-Tris at pH 7.0. J_{max}, maximum flux; K_t, apparent sulfate concn resulting in one-half maximum uptake; prot, protein.

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suggests that the organism is faced with a constant need to eliminate sulfate against a concentration gradient. The mechanism elucidated here could be used as one means of removing sulfate from the blood to assist in maintaining anion homeostasis.

Sulfate/dicarboxylic acid exchange has been observed in the basolateral membrane of a variety of tissues, such as the rabbit ileum (24), rabbit renal cortex (37), and elasmobranch liver (20). As shown in Fig. 2, when BLMV are loaded with 10 mM oxalate there is stimulation of sulfate uptake over gluconate-loaded vesicles. Other sulfate/oxalate exchangers will accept similar dicarboxylic acids as *trans*-stimulators. Oxaloacetate (24) and succinate (24) were able to exchange with sulfate in the rabbit ileum and the skate liver, respectively. Lobster BLMV were found to be conservative in the acceptance of a suitable substrate and did not use oxaloacetate, succinate, formate, ketoglutarate, or citrate. It appears that in hepatopancreatic BLMV the sulfate exchanger does not extend its substrate specificity to other important physiological anions.

Recently, we have described an electrogenic sulfate/chloride exchanger in brush-border membranes of hepatopancreas (10). To determine if the BLMV antiporter was similar in nature, we employed experiments that altered membrane potential. First, sulfate/oxalate exchange was measured in the presence of both inside-negative and inside-positive conditions and compared with short-circuited vesicles. Figure 2 indicates that sulfate uptake was unaffected by these manipulations of transmembrane potential. In the second experiment we compared sulfate uptake in the presence of internal monovalent versus divalent anions under short-circuited conditions (Fig. 3). Sulfate was found to exchange for oxalate, sulfate, carbonate, and chloride. Uptake was similar when each of the divalent anions was used with or without the ionophore valinomycin present. Sulfate/chloride exchange was inhibited in vesicles that were not short circuited, suggesting a buildup of intravesicular negative charge. From this set of experiments we conclude that sulfate/oxalate exchange in BLMV is electroneutral and consists of 1 sulfate:1 oxalate antiport.

To determine if pH had an effect on sulfate/oxalate exchange, we measured uptake in the presence of various bilateral pH conditions and under both inward and outward proton gradients (Fig. 5). No effects of these pH manipulations were observed, even when oxalate was substituted with chloride (unpublished observation); this is in contrast to the proton stimulation of sulfate/chloride exchange described for the hepatopancreatic brush border (10).

Sulfate/oxalate exchange was strongly inhibited by the stilbene derivatives DIDS and SITS, which have been shown to inhibit sulfate/anion exchange in other systems (20, 24, 27, 30) but it also inhibits anion transport through channels (29), which is not relevant to the electroneutral antiporter. Furosemide and bumetanide will inhibit sulfate antiport in rat liver (26) and rabbit ileum (24) BLMV but were not effective in our lobster preparation.

Sulfate influx into BLMV occurred by at least one carrier-mediated mechanism that exhibited Michaelis-Menten kinetics (Fig. 7). Sulfate association with the vesicle exterior was shown to have an apparent K_m of 6 mM, and this is in agreement with the level of sulfate (18 mM) reported in lobster plasma (31, 35). These observations suggest the possibility that the high sulfate concentration in lobster blood could act as the driving force for sulfate/oxalate exchange across the BLMV, assuming, of course, that the chemical potential for extracellular sulfate exceeds its intracellular counterpart.

Epithelial sulfate transport in mammals has been shown to be primarily absorptive in nature, yet a brush-border sulfate/anion exchange pathway has been described for both rabbit proximal tubule (9) and rat renal cortex (29). This brush-border antiport mechanism is similar to sulfate/anion exchangers observed in nonmammalian vertebrates (32, 34) and may allow for bidirectional sulfate transport in these cells.

In contrast to mammals, the marine teleost kidney has been shown to be predominately involved in sulfate secretion. It has been proposed that sulfate crosses the basolateral membrane of the renal tubule epithelium via a pH-dependent electroneutral process (33) and exits by way of a sodium-independent sulfate/anion exchanger located at the brush-border membrane (34).

Multiple transport pathways have been described for sulfate in the avian kidney (32) and are suggested to play a part in different functional roles. On the brush-border membrane there is a strong sodium/sulfate cotransport system similar to that found in mammals that may be involved in reabsorption and a bicarbonate/sulfate exchanger that could mediate sulfate secretion. The basolateral membrane contains a bicarbonate/sulfate exchanger that could function in either direction, depending on the respective ion concentrations. Thus the chick renal tubule maintains transport processes that allow for both sulfate absorption and secretion.

The basolateral membrane sulfate/oxalate exchanger described in the present investigation cannot provide definitive evidence for absorption or secretion for either oxalate or sulfate. However, speculatively, this mechanism could provide a cellular means for ridding the cell of oxalate and/or taking up sulfate for conjugation functions. This transporter would allow for the elimination of oxalate from the lobster hepatopancreatic cells as a means of anion regulation. Oxalate binds divalent cations, such as Ca^{2+}, and precipitates as calcium oxalate, which can be deleterious for cellular survival (39). On the other hand, intracellular uptake of sulfate can be beneficial for cellular viability by its provision for cellular metabolic reactions, such as conjugation (13). Therefore, this antiport mechanism may provide both cytoprotective and cytoanabolic functions for the lobster hepatopancreas. However, a more realistic function of oxalate may be that of storing Ca^{2+} in hepatopancreatic epithelial cells as calcium oxalate concretions. It is known that many premolt crustaceans store Ca^{2+} as calcium oxalate in hepatopancreatic lysosomes and mitochondria for the use of Ca^{2+} during the molt cycle (8).

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The anion antiporter could then be used for ridding the cell of excessive oxalate as a cytoprotective mechanism after completion of the molting cycle.

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