Electrogenic proton-regulated oxalate/chloride exchange by lobster hepatopancreatic brush-border membrane vesicles

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Summary

The transport of [14C]oxalate (Ox2–) by epithelial brush-border membrane vesicles (BBMV) of lobster (Homarus americanus) hepatopancreas, formed by a magnesium precipitation technique, was stimulated by an outward Cl– gradient (in > out). By contrast, Ox2– uptake was not enhanced by an inward Na+ or K+ transmembrane gradient. Generation of an inside-positive membrane potential by K+ in the presence of valinomycin inhibited this process. Neither Ox2–/Ox2– nor Ox2–/SO42– transport exchange were affected by alterations of transmembrane potential. An inwardly directed proton gradient, or the presence of low bilateral pH, enhanced Ox2–/Cl– exchange, yet the H+ gradient alone could not stimulate Ox2– uptake in Cl–-equilibrated BBMV or in vesicles lacking internal Cl–. The stilbenes 4-acetamido-4¢-isothiocyanostilbene-2,2¢-disulfonic acid (SITS) and 4,4¢-diisothiocyanato-2,2¢-disulfonic stilbene (DIDS) strongly inhibited Ox2–/Cl– exchange. Oxalate influx occurred by a combination of carrier-mediated transfer, exhibiting Michaelis–Menten kinetics, and nonsaturable ‘apparent diffusion’. Apparent kinetic constants for Ox2–/Cl– exchange were Ki=0.20 mmol l–1 and Jmax=1.03 mmol l–1 mg–1 protein 7 s–1. 36Cl– influx into oxalate-loaded BBMV was stimulated by an inside-negative transmembrane potential compared with short-circuited vesicles. These results suggest that Ox2–/Cl– exchange in crustacean hepatopancreatic BBMV occurred by an electrogenic carrier mechanism exhibiting a 1:1 flux ratio that was modulated by an external proton-sensitive regulatory site.

Key words: brush-border membrane vesicle, BBMV, oxalate, ion transport, hepatopancreas, electrogenic carrier mechanism, lobster, Homarus americanus.

Introduction

Gastrointestinal and renal transport of the divalent anion oxalate (Ox2–) has been investigated in various vertebrate groups including mammals (Renfro et al., 1987; Shiu-Ming and Aronson, 1988; Schneider et al., 1984; Schron et al., 1985) and teleost fish (Renfro and Pritchard, 1983). A number of Ox2– transport mechanisms for epithelial membranes have been proposed and include anion exchange (Gerencser et al., 1995; Hagenbuch et al., 1985; Shiu-Ming and Aronson, 1988; Talor et al., 1987), conductive transport (Freeel et al., 1998; Hatch et al., 1994) and paracellular diffusion (Hatch et al., 1984, 1994). These processes contribute to transepithelial regulation of Ox2–, which may affect the physiology or pathophysiology of the animal (Binder, 1974; Earnest, 1974; Gerencser et al., 2000; Yendt and Cohanim, 1985).

In invertebrates, specifically crustaceans, the hepatopancreas is involved in both digestion and absorption of nutrients (Gibson and Barker, 1979); in addition, some investigators have implicated this organ as a site of excretion (Dall, 1970). The use of isolated membrane vesicles has led to the definition of absorptive transport mechanisms for a number of solutes in the hepatopancreatic brush border (Ahearn and Clay, 1987a,b; Ahearn et al., 1985; Gerencser et al., 1996) and also for a number of transport processes, including those for Ox2–, in the basolateral membrane (Gerencser et al., 1995, 2000).

The present study uses hepatopancreatic brush-border membrane vesicles (BBMV) to characterize an electrogenic Ox2–/Cl– exchange mechanism that is relatively specific with respect to organic anions, is inhibited by stilbenes and is regulated by pH. The mechanism is reversible and, therefore, it can be utilized for either absorption or secretion of Ox2– (for excretory purposes) from the lobster hepatopancreatic epithelium.

Materials and methods

Live Atlantic lobsters (Homarus americanus L.; 0.5 kg each) were purchased from commercial dealers in Florida and
Hawaii and maintained unfed at 10°C for up to 1 week in filtered seawater. All animals were either in intermolt or early premolt as assessed by the molt stage classification scheme introduced by Aiken (1973).

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 mass) using a method of combined osmotic disruption, differential centrifugation and magnesium precipitation described previously by Ahearn and co-workers (Ahearn and Clay, 1987a; Ahearn et al., 1985). Purity of BBMV prepared by these methods was assessed by comparing the activities of membrane-bound enzymes in vesicles with the activities of the same enzymes in the original homogenate. These comparisons showed final pellet enrichments of alkaline phosphatase (marker enzyme for apical membranes), Na+/K+-ATPase (marker enzyme for basolateral membranes) and cytochrome c oxidase (marker enzyme for mitochondrial membranes) of 19.3-, 0.7- and 0.2-fold, respectively, suggesting that the isolated vesicles were highly enriched with apical membranes and contained minimal contamination from basolateral or organelle membranes.

Transport studies were conducted at 15°C using the rapid filtration technique developed by Hopfer et al. (1973). 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 mmol l⁻¹ radiolabeled [¹⁴C]oxalate (specific activity 12.2 µCi mmol⁻¹). 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 oxalate to stop the uptake process). The vesicle suspension was then rapidly filtered through 0.65 µm Millipore filters (presoaked in distilled water) and washed with another 5 ml of ice-cold stop solution. Filters were transferred to vials containing Ready Solv HP scintillation cocktail (Beckman, Chicago, IL, USA) and counted for radioactivity in a Beckman LS-8100 scintillation counter. Transport experiments involving incubations of less than 10 s were conducted using a rapid-exposure uptake apparatus (Inovativ Labor AG, 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 5 µl of 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 5 µl of incubation media, and filters were washed and counted for radioactivity as above.

Results

Driving force for oxalate uptake

Fig. 1 indicates that the transport of 0.1 mmol l⁻¹ [¹⁴C]Ox²⁻ was not affected by an inward 100 mmol l⁻¹ Na⁺ gradient when compared with Ox²⁻ uptake with bilateral tetramethylammonium (TMA)-gluconate (control). This suggests that Ox²⁻ uptake by the BBMV was not sodium dependent. In the same experiment, a portion of the vesicle preparation was preloaded with 25 mmol l⁻¹ HCO₃⁻. As before, there was no accumulation of Ox²⁻ compared with the TMA-glucanate control. In all conditions, there was no accumulation of Ox²⁻ above that of the equilibrium value.

Fig. 1. Time course of 0.1 mmol l⁻¹ [¹⁴C]oxalate uptake by hepatopancreatic brush-border membrane vesicles (BBMV). Vesicles contained 100 mmol l⁻¹ TMA-gluconate and 50 mmol l⁻¹ K-glucanate ( ), 100 mmol l⁻¹ TMA-glucanate, 25 mmol l⁻¹ K-glucanate and 25 mmol l⁻¹ KHCO₃ ( ). Incubation media contained 100 mmol l⁻¹ TMA-glucanate and 50 mmol l⁻¹ K-glucanate ( ), or 100 mmol l⁻¹ Na-glucanate and 50 mmol l⁻¹ K-glucanate ( ). All media contained 40 mmol l⁻¹ Hepes-Tris and 50 µmol l⁻¹ valinomycin at pH 7.0.
There is some evidence that Ox2– transport across plasma membranes might occur via a Cl– channel (Freel et al. 1998; Hatch et al. 1994); therefore, the following experiment investigated this premise (Fig. 2). Vesicular and incubation media contained 100 mmol l⁻¹ TMA-gluconate, 100 mmol l⁻¹ K-gluconate and 50 μmol l⁻¹ valinomycin. [14C]Ox 2– vesicular uptake was monitored in the presence (test) or absence (control) of 50 μmol l⁻¹ 9-AC, which is a Cl– channel blocker (Freel et al., 1998; Fig. 2). 9-AC was placed in both the incubation and vesicular media. As can be seen, 9-AC had no effect on the uptake of [14C]Ox 2– into the vesicle preparation.

Oxalate uptake into BBMV was not stimulated by incubation in media containing 100 mmol l⁻¹ K-gluconate (no valinomycin or internal K+) nor in the presence of 50 μmol l⁻¹ CCCP (a protonophase used to short-circuit the membrane potential in the absence of K+ and valinomycin, which are the normal reagents used to short-circuit the membrane potential; Fig. 3). However, when vesicles were preloaded with 25 mmol l⁻¹ KCl and incubated in TMA-gluconate media containing equimolar K+ and valinomycin, there was an overshoot Ox2– accumulation approximately twice that of the equilibrium value. Such results, and those from Figs 1, 2, indicate an anion exchange mechanism that can utilize a Cl– gradient, but not a gradient of HCO3–, as a driving force to move Ox2– across the apical membrane.

Effect of membrane potential on oxalate uptake

The possible membrane potential sensitivity of Ox2–/Cl– exchange was examined by imposing a valinomycin-induced K+ diffusion potential across the vesicular wall and measuring the time course of 0.1 mmol l⁻¹ [14C]oxalate uptake. Transport was determined under both inside-negative and inside-positive conditions and was compared with uptake in short-circuited conditions (equal K+ across the membrane). Fig. 4 shows that when vesicles were incubated in media containing 100 mmol l⁻¹ K+ (no internal K+) and 50 μmol l⁻¹ valinomycin, Ox2– uptake was stimulated above that with bilateral K+. By contrast, when vesicles were preloaded with
100 mmol l\(^{-1}\) K\(^+\) and 50 mmol l\(^{-1}\) valinomycin (no external K\(^+\)), Ox\(^2-\) uptake was significantly inhibited. These data suggest that Ox\(^2-\)/Cl\(^-\) exchange is enhanced by an inside-positive membrane potential and inhibited by an inside-negative vesicular interior, supporting the electrogenic nature of the exchange process.

**Specificity of the oxalate-exchange process**

The questions of whether Ox\(^2-\) would exchange with a wide range of organic ions as transferable substrates was investigated by trans-stimulation with either 5 mmol l\(^{-1}\) oxalate, sulfate, oxaloacetate, succinate, formate, \(\alpha\)-ketoglutarate or citrate (Fig. 5). Only oxalate and sulfate were able to stimulate 0.1 mmol l\(^{-1}\) [\(^{14}\)C]Ox \(^2-\) uptake greater than the response obtained in the presence of the nonexchangeable anion gluconate. This experiment indicates that in the lobster hepatopancreatic BBMV the antiporter is relatively specific and will not accept similar dicarboxylic acids or the tricarboxylic acid citrate as a substrate.

**Effects of pH on oxalate–chloride exchange**

Oxalate uptake has been shown to be stimulated by proton (or hydroxyl) gradients in the basolateral membrane (BLM) of the teleost fish renal epithelium (Renfro and Pritchard, 1982). The possible proton (hydroxyl) gradient stimulation of Ox\(^2-\)/Cl\(^-\) 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 mmol l\(^{-1}\) [\(^{14}\)C]Ox\(^2-\) uptake was determined.

Fig. 6 shows that when the pH of the incubation media was maintained at 7.0 and the internal pH was varied from pH 5.0 to 8.0, there was an inequality of Ox\(^2-\) transport into the BBMV preloaded with 100 mmol l\(^{-1}\) Cl\(^-\). The lowest internal pH of 5.0 corresponded to the slowest uptake of Ox\(^2-\), whereas when the internal pH was greater (pH\(_{in}\)=8.0) than the external pH, Ox\(^2-\) uptake was enhanced over that of all other conditions. Alternatively, the observed phenomena may have been attributed to competition of internal hydroxyl concentration 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 5.0 to 8.0, there was again a gradation of Ox\(^2-\) uptake into vesicles preloaded with 100 mmol l\(^{-1}\) Cl\(^-\) (Fig. 7). With a decrease in external pH there was a corresponding increase in Ox\(^2-\) accumulation. These results indicate that an increase in external proton (or decrease in external hydroxyl) concentration can stimulate Ox\(^2-\)/Cl\(^-\) exchange.

In contrast to the previous two experiments, Fig. 8 shows the result of Ox\(^2-\)/Cl\(^-\) exchange at various bilateral pH conditions ranging from 5.0 to 8.0. The maximal uptake of Ox\(^2-\) into BBMV occurred at pH 5.0 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 Ox\(^2-\) uptake but rather the external absolute proton (hydroxyl) concentration.

**\(^{36}\)Cl\(^-\)/Ox\(^2-\) exchange in BBMV**

An experiment was designed to determine if \(^{36}\)Cl\(^-\) would exchange for internal Ox\(^2-\) and respond to a membrane potential in hepatopancreatic BBMV. A portion of the vesicle preparation was preloaded with 10 mmol l\(^{-1}\) Ox\(^2-\) (50 mmol l\(^{-1}\) valinomycin) and incubated in media containing 10 mmol l\(^{-1}\) Na\(^{36}\)Cl, with or without 100 mmol l\(^{-1}\) K-glucanate. Another portion was preloaded with 100 mmol l\(^{-1}\) KCl (valinomycin)
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and incubated in media containing \[ {^{14}C} \text{Ox}^2- \], with or without 100 mmol l\(^{-1} \) K-glucolate. These conditions provided vesicles that were either short-circuited or inside-negative. Fig. 9A indicates that \[ {^{14}C} \text{Ox}^2- \] influx was inhibited by an inside-negative vesicular membrane potential compared with short-circuited vesicles. Fig. 9B also indicates that \[ {^{36}}\text{Cl}^- \] influx was enhanced by an inside-negative vesicular membrane potential compared with the short-circuited vesicular membrane potential condition. These data suggest that the \[ \text{Ox}^2/\text{Cl}^- \] exchange occurred in a 1:1 ratio and that excess negative charge of the internal \[ \text{Ox}^2- \] was repelled by a negative intravesicular space.

**Effect of inhibitors and competitors on oxalate influx**

Potential anion exchange transport inhibitors were tested in the BBMV preparation in order to further delineate the type of mechanism by which \[ \text{Ox}^2- \] is being transported. The vesicles were preloaded with 100 mmol l\(^{-1} \) TMA-glucolate, 100 mmol l\(^{-1} \) KCl and 50 mmol l\(^{-1} \) valinomycin and incubated in media containing 10 mmol l\(^{-1} \) \[ {^{36}}\text{Cl}^- \] and either 100 mmol l\(^{-1} \) K-glucolate and 100 mmol l\(^{-1} \) TMA-glucolate (open bar) or 200 mmol l\(^{-1} \) TMA-glucolate (hatched bar). All media contained 40 mmol l\(^{-1} \) Hepes-Tris at pH 7.0.

**Kinetic characteristics of oxalate influx**

Oxalate influx (7 s uptake) from incubation media to vesicular interior was measured in BBMV preloaded with 100 mmol l\(^{-1} \) TMA-Cl, 50 mmol l\(^{-1} \) K-glucolate and...
50 μmol l⁻¹ valinomycin at pH 7.0 and external media of 100 mmol l⁻¹ TMA-glucanate, 50 mmol l⁻¹ K-glucanate and variable Ox²⁻ concentrations (0.1–20 mmol l⁻¹) at pH 7.0.

Fig. 11 shows that Ox²⁻ influx was a curvilinear function of external Ox²⁻ 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 Ox²⁻ concentration. These two processes operating together can be described by the equation:

$$J = \frac{J_{\text{max}} [S]}{K_t + [S]} + P [S],$$

where $J$ is total [14C]Ox²⁻ influx (in nmol mg⁻¹ protein 7 s⁻¹), $J_{\text{max}}$ is apparent maximal carrier-mediated influx, $K_t$ is the apparent Ox²⁻ concentration resulting in half-maximal uptake, $[S]$ is the external Ox²⁻ 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 utilized to analyze the data in Fig. 11 by equation 1. Apparent transport parameters calculated in this manner are as follows: apparent $K_t=0.20$ mmol l⁻¹; apparent $J_{\text{max}}=1.03$ nmol mg⁻¹ protein 7 s⁻¹ and $P=0.31$ nmol mg⁻¹ protein 7 s⁻¹ mmol l⁻¹.

**Discussion**

In the current investigation, we presented evidence for the existence of a secondarily active carrier-mediated Ox²⁻/Cl⁻ exchange in BBMV isolated from lobster hepatopancreatic epithelium (Fig. 3). Oxalate carriers have been described in the plasma membranes of several vertebrate tissues (Pritchard, 1987; Renfro et al., 1987; Shiu-Ming and Aronson, 1988). Marine animal epithelial BBMV have been shown to contain a sulfate/Ox²⁻ anion exchange mechanism (Hugentobler et al., 1987; Renfro and Pritchard, 1982), whereas no proton stimulation of Ox²⁻ transport was observed in this brush-border preparation (Renfro and Pritchard, 1983). Previously, we had demonstrated an Ox²⁻/sulfate antiporter present in basolateral membrane vesicles (BLMV) of lobster hepatopancreas (Gerencser et al., 1995, 2000). This Ox²⁻/sulfate exchanger was not influenced by protons or proton gradients, sodium concentration, sodium gradients or vesicular membrane potential (Gerencser et al., 1995).

In the present study, extravascular Na⁺ or K⁺ gradients caused no intravesicular accumulation of Ox²⁻ (Fig. 1), which rules out cotransport of Ox²⁻ with either of these two cations. An outward vesicular gradient of HCO₃⁻ had no accumulative effect on Ox²⁻ into the BBMV, which negates an Ox²⁻/HCO₃⁻ antiporter as a possible mechanism for Ox²⁻ transport (Fig. 1). Ruling out the possibility that Ox²⁻ transport across the brush-border membrane occurs via a Cl⁻ channel, as has been shown and speculated in other species (Freel et al., 1998; Hatch et al., 1984, 1994), we demonstrated that 9-AC, a known Cl⁻ channel blocker (Freel et al., 1998), had no effect on the downhill energetic movement of Ox²⁻ into the BBMV of lobster hepatopancreatic epithelium (Fig. 2).

The effect of valinomycin-induced K⁺ diffusion potentials on Ox²⁻/Cl⁻ exchange was investigated to determine the effect of membrane potential on Ox²⁻ transport in the BBMV of
lobster hepatopancreatic epithelium. Oxalate uptake was measured under both inside-negative and inside-positive vesicular membrane potential conditions and compared with short-circuited conditions. Fig. 4 indicates that Ox2–/Cl– exchange is stimulated by a positive vesicular interior while 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. These data also support the idea that the carrier can accommodate one oxalate ion and one chloride ion, which would result in an electrogenic Ox2–/Cl– exchange, as has been demonstrated for SO42–/Cl– exchange in the same epithelium (Gerencser et al., 1995).

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 (Gibson and Barker, 1979). We therefore tested the effect of pH gradients on the uptake of Ox2– into the BBMV. Ox2–/Cl– exchange was diminished when the internal pH was less than the external pH (Fig. 6), and Ox2– uptake was enhanced as the external pH was decreased (constant internal pH), as shown in Fig. 7. This suggested that an extravesicular pH lower than internal pH could stimulate Ox2–/Cl– (or Ox2–/OH–) exchange. When the pH was held constant on both sides of the BBMV (Fig. 8), 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 external pH (increased external protons) that stimulated Ox2–/Cl– exchange.

This series of experiments suggests that a proton (or hydroxyl) gradient does not act as a driving force during Ox2–/Cl– exchange, nor could it stimulate uptake alone, yet there were significant effects of varying pH on the magnitude of Ox2–/Cl– exchange. All of the experiments were short-circuited by the presence of valinomycin and equimolar K+ across the BBMV so that H+-generated transmembrane diffusion potentials would be unlikely to cause the results observed in Figs 6 and 7. Protons have been shown to act as allosteric activators of the Na+/H+ exchanger in rabbit renal BBMV (Aronson et al., 1982) and Na+/SO42– cotransport in rabbit ileum BBMV (Ahearn and Murer, 1984). Similarly, the results observed in Figs 6–8 would support the idea of external protons having a modifier role for stimulating the Ox2–/Cl– exchanger.

The existence of internal pH-sensitive regulatory sites for the Cl–/HCO3– exchanger has been demonstrated on the rabbit ileal brush-border membrane using membrane vesicles (Mughrabil et al., 1990), in isolated cell preparations of lymphocytes (Mason et al., 1989) and in Vero cells (Olsnes et al., 1986). An external pH-sensitive regulatory site on the Ox2–/Cl– exchanger in the hepatopancreatic brush-border membrane would be physiologically important due to the 2Na+/H+ exchanger also present in the membrane (Ahearn et al., 1990). The 2Na+/H+ exchanger operates during luminal acidification following ingestion of a meal and would provide a stimulus for enhanced Ox2–/Cl– exchange. When the 2Na+/H+ exchanger is operating there would be an increase in the proton concentration in the hepatopancreatic lumen and a corresponding decrease of protons in the cytoplasm. This would tend to enhance the Ox2–/Cl– exchanger due to modification at an external site, as demonstrated in Fig. 8.

The influx of [14C]oxalate 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. 11). The apparent affinity constant (Kt) for oxalate binding to the vesicle exterior was 0.20 mmol l−1, and the maximal transport velocity (Jmax) was 1.03 mmol mg−1 protein 7 s−1. The apparent diffusional component had a rate of 0.31 mmol mg−1 protein 7 s−1 mmol l−1. These kinetic constants are similar to those obtained for oxalate transport across the BLMV of lobster hepatopancreas (Gerencser et al., 1995, 2000).

The Ox2–/Cl– exchanger was significantly (P<0.05) inhibited by the disulfonic stilbenes DIDS and SITS, as shown in Fig. 10. Oxalate transport has been shown to be inhibited by SITS and DIDS in other epithelial preparations (Pritchard, 1987; Renfro et al., 1987; Renfro and Pritchard, 1983). Furosemide and bumetanide were not effective as inhibitors in the present study (Fig. 10). The strong inhibition by DIDS and SITS provides further evidence for the presence of an anion exchanger in hepatopancreatic BBMV since both SITS and DIDS are relatively specific inhibitors for anion exchange processes (Renfro and Pritchard, 1983; Shiu-Ming and Aronson, 1988; Talor et al., 1987).

Under the assumption that exchange mechanisms such as this can operate in both directions, it should be possible to measure the uptake of 36Cl– into vesicles in exchange for internal Ox2–. If the exchange operated at a 1:1 ratio it would be expected to react to a membrane potential. It was observed that 36Cl– influx did respond to an inside-negative potential (Fig. 9) by an increased uptake during its incubation. With this arrangement there would be a secretion of Ox2– from the vesicular interior in exchange for luminal Cl– that is further driven by the inside negative potential that is characteristic of epithelial cells (Gerencser, 1985). This secretion of Ox2– would lead to its excretion unless it were recycled by the same antiporter. 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 (Dall, 1970; Gifford, 1962).

The brush-border membrane Ox2–/Cl– exchanger described in the present investigation cannot provide definitive evidence for absorption or secretion for either Ox2– or Cl–. However, speculatively, this mechanism could provide a cellular means for ridding the cell of Ox2– and/or taking up Cl– for metabolic functions. This transport would allow for the elimination of Ox2– from the lobster hepatopancreatic cells as a means of anion regulation. Oxalate binds divalent cations, such as Ca2+, and precipitates as calcium oxalate, which could be deleterious for cellular survival (Binder, 1974; Dobbins and Binder, 1977; Earnest, 1974). However, a more realistic function of Ox2– may be that of storing Ca2+ as calcium oxalate in hepatopancreatic lysosomes and mitochondria for the use of Ca2+ during the molt cycle, as has been previously reported (Gerencser et al., 1996,
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