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^{3}H-L-histidine and $^{65}Zn^{2+}$ are cotransported by a dipeptide transport system in intestine of lobster *Homarus americanus*

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Summary

The tubular intestine of the American lobster *Homarus americanus* was isolated *in vitro* and perfused with a physiological saline whose composition was based on hemolymph ion concentrations and contained variable concentrations of ^{3}H-L-histidine, ^{3}H-glycyl-sarcosine and $^{65}Zn^{2+}$. Mucosa to serosa (M→S) flux of each radiolabelled substrate was measured by the rate of isotope appearance in the physiological saline bathing the tissue on the serosal surface. Addition of 1–50 μmol l^{-1} zinc to the luminal solution containing 1–50 μmol l^{-1} ^{3}H-L-histidine significantly (P<0.01) increased M→S flux of amino acid compared to controls lacking the metal. The kinetics of M→S ^{3}H-L-histidine flux in the absence of zinc followed Michaelis–Menten kinetics (K_m=6.2±0.8 μmol l^{-1}; J_{max}=0.09±0.004 pmol cm^{-2} min^{-1}). Addition of 20 μmol l^{-1} zinc to the luminal perfusate increased both kinetic constants (K_m=19±3 μmol l^{-1}; J_{max}=0.28±0.02 pmol cm^{-2} min^{-1}). Addition of both 20 μmol l^{-1} zinc and 100 μmol l^{-1} L-leucine abolished the stimulatory effect of the metal alone (K_m=4.5±1.7 μmol l^{-1}; J_{max}=0.08±0.008 pmol cm^{-2} min^{-1}). In the absence of L-histidine, M→S flux of $^{65}Zn^{2+}$ also followed the Michaelis–Menten relationship and addition of L-histidine to the perfusate significantly (P<0.01) increased both kinetic constants. Addition of either 50 μmol l^{-1} Cu^{+} or Cu^{2+} and 20 μmol l^{-1} L-histidine simultaneously abolished the stimulatory effect of L-histidine alone on transmural $^{65}Zn^{2+}$ transport. Zinc-stimulation of M→S ^{3}H-L-histidine flux was significantly (P<0.01) reduced by the addition of 100 μmol l^{-1} glycyl-sarcosine to the perfusate, as a result of the dipeptide significantly (P<0.01) reducing both L-histidine transport K_m and J_{max}. Transmural transport of ^{3}H-glycyl-sarcosine was unaffected by the presence of either L-histidine or L-leucine when either amino acid was added to the perfusate alone, but at least a 50% reduction in peptide transport was observed when zinc and either of the amino acids were added simultaneously. These results show that ^{3}H-L-histidine and $^{65}Zn^{2+}$ are cotransported across the lobster intestine by a dipeptide carrier protein that binds both substrates in a bis-complex (Zn-[His]$_2$) resembling the normal dipeptide substrate. In addition, the transmural transports of both substrates may also occur by uncharacterized carrier processes that are independent of one another and appear relatively specific to the solutes used in this study.

Key words: L-histidine, zinc, bis-complex, dipeptide, PEPT-1, glycyl-sarcosine, copper, heavy metal, *Homarus americanus*, intestine, transmural transport, epithelium, L-leucine, cotransport.

Introduction

In crustaceans ions and organic solutes such as amino acids and sugars, obtained in the diet, are absorbed across the epithelial lining of the hepatopancreas and intestine to the blood for organ distribution (Ahearn, 1987a,b, 1988; Ahearn and Clay, 1988a; Ahearn et al., 1992; Wright and Ahearn, 1997). Metals such as copper and zinc, associated with dietary elements, are also transported across the mucosal membrane barrier of epithelial cells in these two organs and either undergo sequestration and detoxification processes in the epithelial cells or are transferred across the basolateral epithelial cell border to the blood (Ahearn et al., 1994; Chavez-Crooker et al., 2001). In recent years studies have shown hepatopancreatic mitochondria and lysosomes to be sites of metal sequestration where complexation with divalent anions leads to precipitation formation in these organelles, thereby lowering effective concentrations of the metals and reducing their potentially toxic effects to the cells (Chavez-Crooker et al., 2002, 2003).

Dietary metals, at low concentrations, also have an important role in protein function and act as cofactors in many cellular reactions. It is important, therefore, to characterize the membrane transport mechanisms by which luminal metals are transferred into gastrointestinal absorptive cells where they can help regulate a variety of cellular processes. Zinc is a dietary metal that has a vital role in the operation of several hundred proteins and its deficiency leads to impairments in growth and development as well as in immune reactions and reproductive status of many animals (Hambridge, 2000; Bury et al., 2003).

Zinc enters cells by a variety of known transport systems belonging to the ZTL and ZIP gene families (Cragg et al., 2002; Gaither and Eide, 2001a,b), through relatively unspecific DMT-1 transporters characterized for iron (Gunshin et al., 1997), or through putative calcium channels (Bury et al., 2003). An additional zinc transport process that has received attention in recent years is the apparent coupling of the metal with specific amino acids such as L-histidine and L-cysteine (Horn et al., 1995; Horn and Thomas, 1996; Glover and Hogstrand, 2002a,b; Glover et al., 2003). These latter studies have suggested processes whereby luminal zinc complexes with two amino acids in solution in a bis-complex (Zn-[His]$_2$) and the combination is transported as a unit across the cellular membrane. Other mechanisms accounting for the transfer of both metal and amino acid across a given membrane may also be possible and to date the identity of this amino acid-dependent zinc transport system is unclear.

The present investigation is a study of transmural ^{3}H-L-histidine and $^{65}Zn^{2+}$ transport across the isolated and perfused intestine of the American lobster *Homarus americanus*. Results show that the metal and amino acid may cross this organ from lumen to blood *via* a dipeptide transporter that has recently been reported to occur in lobster hepatopancreas (Thamotharan and Ahearn, 1996) and may be similar to PEPT-1 described for vertebrates. In addition, both substrates may also cross the tissue by way of separate carrier processes that both show a high degree of specificity for their respective solutes.

Materials and methods

Live American lobsters *Homarus americanus* Milne-Edwards (0.5 kg each) were purchased from a local commercial dealer and maintained in holding tanks containing filtered seawater at 15°C until needed for the experiments. Lobsters were fed frozen mussel meat several times a week while being maintained.

A physiological saline solution was developed in conjunction with the salt composition and osmolarity of lobster hemolymph. This medium included the following salt concentrations (in mmol l^{-1}): NaCl, 415; $CaCl_2$, 25; KCl, 10.0; $NaH_2PO_4.2H_2O$, 1.0; $NaHCO_3$, 4.0; Na_2SO_4, 8.4; Hepes, 30. The osmotic pressure of this incubation medium was approximately 950 mosmol kg^{-1} and the pH of the solution was adjusted to 7.1 for experimental conditions.

In vitro transmural transports of L-histidine, Zn^{2+} and glycyl-sarcosine were examined using a simple perfusion apparatus as described in detail previously (Ahearn and Hadley, 1977a,b; Ahearn and Maginniss, 1977; Brick and Ahearn, 1978; Wyban et al., 1980; Chu, 1986). Briefly, intact intestines were flushed of contents and mounted with surgical thread on blunted 18–20 gauge stainless steel needles in a lucite chamber containing the incubation medium (10 ml), which served as the serosal medium. This solution was also perfused through the intestines as the mucosal medium using a peristaltic pump (Instech Laboratories, Inc., Plymouth Meeting, PA, USA) at a flow rate of 380 µl min^{-1} for periods of time up to 180 min. Previous studies using other crustacean species have shown intestinal viability under the conditions used in the present work for up to 5 h of continuous perfusion (Ahearn and Hadley, 1977a,b; Ahearn and Maginniss, 1977; Chu, 1986). Variable concentrations of L-histidine, Zn^{2+} or glycyl-sarcosine were added to the mucosal medium as needed. Experiments were conducted at 23°C.

An intestine was perfused with an unlabelled mucosal medium for 10–20 min for tissue stabilization before a 60 min control flux interval with radiolabelled uptake medium. This control flux period was followed by one or two additional 60 min experimental flux periods using labeled perfusate of various compositions. Control experiments showed that a steady state appearance of isotope in the serosal compartment occurred after only 10 min of perfusion. All unidirectional flux measurements reported in this paper were conducted on intestines after they had reached the steady state. Experimental mucosal solutions containing L[2,5]^{3}H-histidine (Amersham Biosciences Corp., Piscataway, NJ, USA), $^{65}ZnCl_2$ (Oak Ridge National Laboratory, Oakridge, TN, USA), or glycyl-1,2-^{3}H-sarcosine (Moravek Biochemicals, Brea, CA, USA) were next perfused through the intestine at pH 7.1. Triplicate 200 µl samples were removed from the serosal bath, added to scintillation cocktail, and counted for radioactivity in a Beckman LS6500 scintillation counter. Upon removal of the sample, an equal volume of saline solution was added back into the bath to maintain the volume of the surrounding medium. Subsequent corrections for isotope removal and bath dilution were made during transmural flux calculations. Samples of serosal media were taken every 5–10 min during a 90–180 min time-course experiment. Unidirectional transmural flux rates (mucosa to serosa) were determined over 30 min periods with bath samples taken every 5 min. The mucosal test solutions consisted of varying ^{3}H-L-histidine (1–50 µmol l^{-1}), $^{65}Zn^{2+}$ (1–1000 µmol l^{-1}), ^{3}H-glycyl-sarcosine (100 µmol l^{-1}), L-leucine (100 µmol l^{-1}), and CuCl or $CuCl_2$ (50 µmol l^{-1}) concentrations. pH experiments were conducted in a similar fashion where the first flux interval was measured with a pH 7.0 saline delivered from one perfusion tube and this was followed immediately by exchanging perfusion tubes with a pH 6.0 saline. A bubble introduced between the two salines took less than 30 s to pass through a perfused gut, suggesting that minimal time occurred between tissue exposures to the two pH treatments. Specific conditions for each experiment are outlined in the figure legends. The rate of radioactivity increase in the serosal bathing medium was used to calculate the transmural mucosal-to-serosal transport rate of the isotope under the conditions of each experiment.

^{3}H-glycyl-sarcosine was used as a representative substrate for the dipeptide transport system previously identified for this lobster species (Thamotharan and Ahearn, 1996) and for the vertebrate PEPT-1 carrier system (Adibi, 1997). L-leucine was used in the present study as a potential inhibitor of L-histidine transport since both amino acids are known to be transported by the L-lysine transport protein, and in lobster hepatopancreas

this carrier process is strongly inhibited by L-leucine (Ahearn and Clay, 1987a). Copper was used in the present investigation as a potential inhibitor of zinc transport as a result of published competitive interactions between these two metals at the hepatopancreatic brush border membrane (Chavez-Crooker et al., 2001).

Each of the experiments was subjected to statistical tests with analysis of variance (ANOVA). Both ^{3}H-L-histidine and $^{65}Zn^{2+}$ transmural transport kinetics were fitted to Michaelis–Menten functions using Sigma Plot software (Systat Software Inc., Point Richmond, CA, USA). Slopes of time-course curves were determined with linear regression analysis functions (first point of treatment to last point of treatment) using Sigma Plot software. Results are reported as representative experiments that were repeated three times producing qualitatively similar results. Data points on individual figures represent mean values from three replicates ±1 S.E.M.

Results

Time course of mucosal-to-serosal ^{3}H-L-histidine transport

Fig. 1 shows the effects of 20 μmol l^{-1} zinc or 100 μmol l^{-1} L-leucine on the time course of mucosal-to-serosal transport of 20 μmol l^{-1} ^{3}H-L-histidine. During the first 60 min of mucosal perfusion with radiolabelled amino acid, a slow transmural transport rate (0.02 pmol cm^{-2} min^{-1}) was observed. Addition of 20 μmol l^{-1} zinc to the luminal perfusate along with the radiolabelled amino acid resulted in a threefold increase in mucosal to serosal transfer of the amino acid (0.07 pmol cm^{-2} min^{-1}) during the second hour of perfusion. During the third perfusion period 20 μmol l^{-1} zinc and 100 μmol l^{-1} L-leucine were both added to the luminal perfusate along with 20 μmol l^{-1} ^{3}H-L-histidine. Under the latter experimental conditions, the mucosal-to-serosal transmural transport rate of radiolabelled amino acid was reduced almost threefold from the stimulatory condition occurring in the presence of zinc alone (0.03 pmol cm^{-2} min^{-1}) and only slightly higher than the rate observed under control conditions. These results show that an otherwise slow transfer of ^{3}H-L-histidine from the intestinal lumen to serosal medium is strongly stimulated by luminal zinc and that this metal stimulation was significantly reduced when the amino acid L-leucine was added to the perfusate along with the zinc.

To ensure that the results reported in Fig. 1, and other time-course experiments, represented substrate-induced changes in transmural transfer of radiolabelled solutes, control experiments were performed with both ^{3}H-L-histidine and $^{65}Zn^{2+}$. Both labeled substrates were perfused separately through the intestinal lumen for 180 min without the addition of other interacting luminal molecules and the appearance rate of the respective isotope in the serosal medium monitored. In both cases linear rates of isotope appearance in the serosal medium were observed over the entire incubation interval with no tendency toward isotope equilibration between the media on both intestinal surfaces (data not shown).

Kinetics of transmural ^{3}H-L-histidine transport in the presence and absence of luminal zinc and L-leucine

Because at least a portion of the transmural transport rate of ^{3}H-L-histidine was significantly affected by both zinc and the amino acid L-leucine, the involvement of a carrier-mediated transport system appeared likely in the transfer of this amino acid across intestinal tissues. Fig. 2 illustrates the effects of varying luminal ^{3}H-L-histidine concentration on the rate of mucosal-to-serosal transmural transport of the amino acid in the absence of either zinc or L-leucine. As shown in Fig. 2, the movement of this amino acid across lobster intestine was a

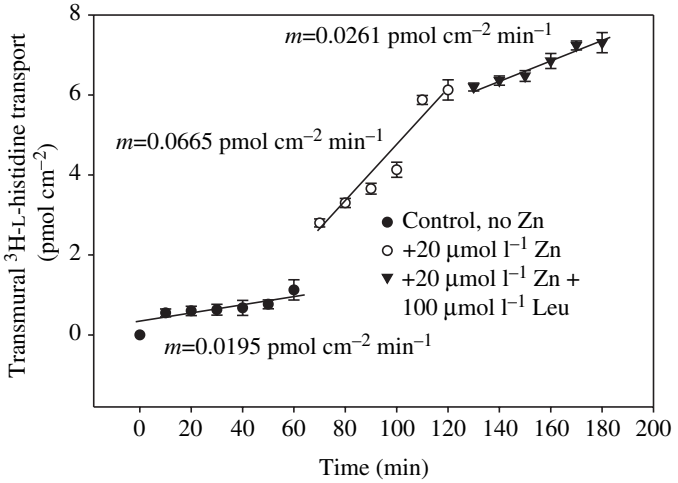

Fig. 1. Effects of 20 μmol l^{-1} zinc and 100 μmol l^{-1} L-leucine on the time course of transmural transport of 20 μmol l^{-1} ^{3}H-L-histidine. Values displayed are means ± S.E.M. of 3 replicates per time point. Slopes of the lines were calculated as regression lines using Sigma Plot software and the values on the figure (i.e. *m*-values) are the slopes obtained from these analyses at each condition used.

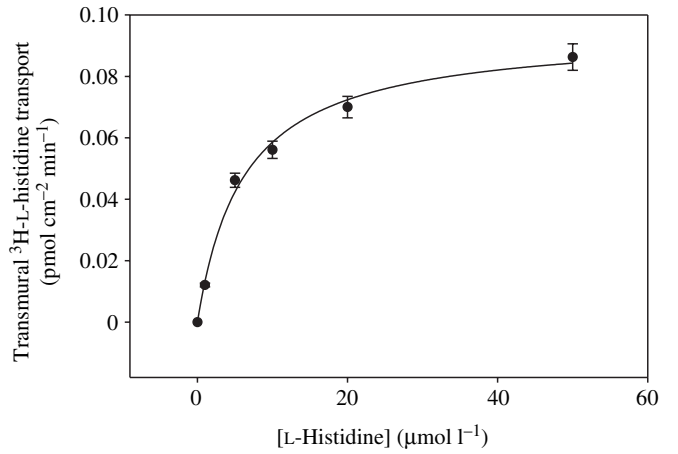

Fig. 2. Effect of luminal ^{3}H-L-histidine concentration (1–50 nmol l^{-1} μmol l^{-1}) on the rate of mucosal-to-serosal transmural transport measured in the absence of mucosal zinc over 30 min time intervals at each luminal amino acid concentration. Unidirectional fluxes were calculated from regression slopes determined over this time interval for each amino acid concentration. Values displayed are means ± S.E.M. of 3 replicates per concentration.

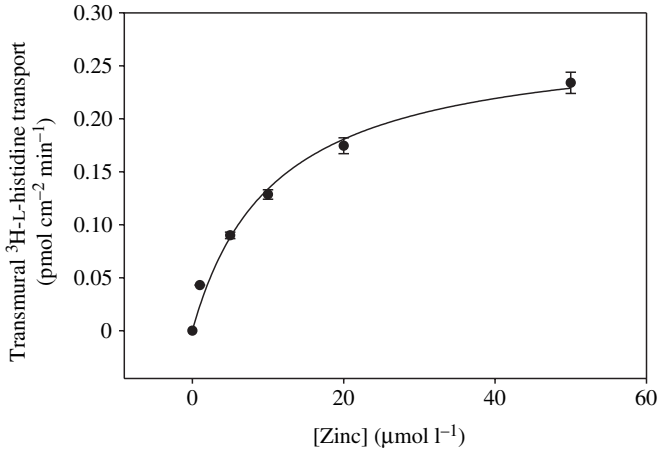

Fig. 3. Effect of varying luminal zinc concentration (1–50 μmol l^{-1}) on transmural transport rate of 20 μmol l^{-1} ^{3}H-L-histidine over 30 min time intervals at each luminal zinc concentration. Unidirectional fluxes were calculated from regression slopes determined over this time interval for each zinc concentration. Values displayed are means ± S.E.M. of 3 replicates per concentration.

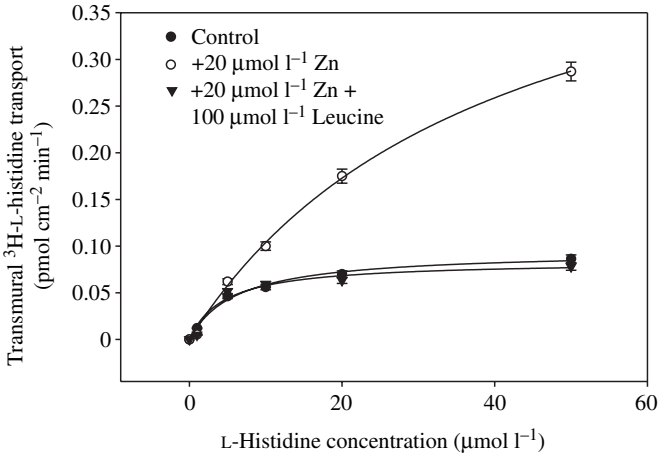

Fig. 4. Effect of luminal zinc (20 μmol l^{-1}) and L-leucine 100 μmol l^{-1}) on the kinetics of transmural transport of ^{3}H-L-histidine (1–50 μmol l^{-1}) measured over 30 min time intervals at each luminal ^{3}H-L-histidine concentration. Unidirectional fluxes were calculated from regression slopes determined over this time interval for each ^{3}H-L-histidine concentration. Values displayed are means ± S.E.M. of 3 replicates per concentration.

hyperbolic function of luminal amino acid concentration (2.5–50 μmol l^{-1}) and followed the Michaelis–Menten equation:

$$J_H = J_{max}[H] / K_H + [H] , \quad (1)$$

where J_H is mucosa-to-serosal flux of radiolabelled amino acid in the absence of either luminal zinc or L-leucine, J_{max} is apparent maximal transmural transport rate, K_H is an apparent affinity constant of the transport system for the amino acid, and [H] is luminal amino acid concentration. The apparent affinity constant for mucosa-to-serosal ^{3}H-L-histidine transport was 6.2±0.8 μmol l^{-1} L-histidine and the apparent maximal transport rate was 0.09±0.004 pmol cm^{-2} min^{-1}.

As shown in Fig. 1, there was a marked increase in transmural ^{3}H-L-histidine transport when 20 μmol l^{-1} zinc was added to the luminal perfusate. To assess the nature of this stimulatory action of the metal on amino acid transport, an experiment was conducted to determine the effects of variable luminal zinc concentrations (2.5–50 μmol l^{-1}) on the mucosal-to-serosal transmural transport rate of 20 μmol l^{-1} ^{3}H-L-histidine. Fig. 3 shows that a hyperbolic relationship occurred between the transmural amino acid transport rate and luminal zinc concentration and followed a modified Michaelis–Menten equation given below:

$$J_H = J_{max}[Zn] / K_{Zn} + [Zn] , \quad (2)$$

where J_H is rate of mucosa-to-serosa transmural ^{3}H-L-histidine transport, J_{max} is apparent maximal amino acid transport, K_{Zn} is an apparent affinity constant of the amino acid transporter for the metal, and [Zn] is luminal zinc concentration. The calculated apparent affinity of the transport system for zinc was 19±3 μmol l^{-1} zinc and the apparent maximal amino acid transport rate was 0.28±0.02 pmol cm^{-2} min^{-1}. These results, and those of Fig. 2, show that addition of zinc to the luminal perfusate increased the apparent maximal transmural amino acid transport rate by a factor of three (no Zn, J_{max}=0.09; added Zn, J_{max}=0.28 pmol cm^{-2} min^{-1}).

L-Leucine (100 μmol l^{-1}) inhibited the stimulation of transmural ^{3}H-L-histidine transport by luminal zinc (Fig. 1), suggesting that both amino acids may interact with a common membrane agency. An experiment was conducted examining the transmural transport kinetics of ^{3}H-L-histidine in the presence and absence of both 20 μmol l^{-1} zinc and 100 μmol l^{-1} L-leucine. Fig. 4 shows that a hyperbolic, Michaelis–Menten type, response occurred between ^{3}H-L-histidine transport and luminal histidine concentration in the absence of either metal or L-leucine. Addition of 20 μmol l^{-1} zinc to the mucosal perfusate increased the mucosal-to-serosal flux of the radiolabelled amino acid as before. However, addition of both 20 μmol l^{-1} zinc and 100 μmol l^{-1} L-leucine simultaneously to the perfusing mucosal medium abolished the stimulatory action of zinc on ^{3}H-L-histidine transport. These data suggest that zinc stimulates carrier-mediated ^{3}H-L-histidine transport by a system that is markedly inhibited by the presence of 100 μmol l^{-1} L-leucine. In a separate control experiment, carrier-mediated, 20 μmol l^{-1} ^{3}H-L-histidine transport in the absence of luminal zinc (control flux=0.037±0.01 pmol cm^{-2} min^{-1}; N=3) was unaffected by the presence of 50 μmol l^{-1} L-leucine (flux with inhibitor=0.044±0.003 pmol cm^{-2} min^{-1}; N=3) or 100 μmol l^{-1} L-leucine (flux with inhibitor=0.043±0.007 pmol cm^{-2} min^{-1}; N=3) (data not shown).

Data presented in Table 1 summarize the effects of both 20 μmol l^{-1} zinc and 100 μmol l^{-1} L-leucine on the mucosa-to-serosa transport kinetic constants of ^{3}H-L-histidine in lobster intestine. The data in this table indicate that the apparent binding affinity of the carrier mechanism involved in transport

Table 1. *Kinetic constants for transmural ^{3}H-L-histidine transport in the presence and absence of 20 μmol l^{-1} zinc and 100 μmol l^{-1} L-leucine*

Perfusate	K_m (μmol l^{-1})	J_{max} (pmol cm^{-2} min^{-1})
L-Histidine control	6.2±0.8	0.09±0.004
L-Histidine + Zinc	19±3*	0.28±0.02*
L-Histidine + Zinc + Leucine	4.5±1.7†	0.08±0.01†

Values are means ± S.E.M. of three replicates per point.
*Significantly different (P<0.01) than control; †significantly different (P<0.01) than L-His + Zn treatment.

of ^{3}H-L-histidine across the intestine was significantly (P<0.01) reduced by the presence of zinc (control=6.2±0.8; test=19±3 μmol l^{-1}), while the apparent affinity constant was not significantly different (P>0.05) when both zinc and L-leucine were present together in the mucosal medium (control=6.2±0.8; test=4.5±1.7 μmol l^{-1}). Similarly, the apparent maximal transport rate was significantly (P<0.01) increased (control=0.09±0.004; test=0.28± 0.02 pmol cm^{-2} min^{-1}) by a factor of three when zinc alone was present in the mucosal medium, but no significant (P>0.05) increase in maximal transport rate (control=0.09±0.004; test=0.08±0.01 pmol cm^{-2} min^{-1}) was observed when both zinc and L-leucine were added together in the perfusate.

Kinetics of transmural $^{65}Zn^{2+}$ transport in the presence and absence of luminal L-histidine and copper

To assess whether the transmural transport of $^{65}Zn^{2+}$ across lobster intestine was influenced by L-histidine or copper ions, an experiment was conducted examining the time course of mucosal-to-serosal 20 μmol l^{-1} $^{65}Zn^{2+}$ transport in the presence or absence of luminal 20 μmol l^{-1} L-histidine or 50 μmol l^{-1} Cu^+ (cuprous ions). Fig. 5 indicates that the transmural transport rate of $^{65}Zn^{2+}$ across lobster intestine in the absence of either amino acid or copper was 0.1 pmol cm^{-2} min^{-1}. This rate was increased twofold to 0.19 pmol cm^{-2} min^{-1} when 20 μmol l^{-1} L-histidine was perfused through the intestinal lumen with the radiolabelled ion. When 50 μmol l^{-1} Cu^+ was perfused through the intestine with 20 μmol l^{-1} L-histidine and 20 μmol l^{-1} $^{65}Zn^{2+}$, the transmural transport rate of the radiolabelled ion was reduced to 0.1 pmol cm^{-2} min^{-1}, a value that was half that of the stimulated condition in the presence of L-histidine alone, and equal to the transport rate of $^{65}Zn^{2+}$ in the absence of either amino acid or copper.

Fig. 6 indicates that the mucosal-to-serosal transport rate of $^{65}Zn^{2+}$ across lobster intestine was a hyperbolic function of luminal L-histidine concentration following the Michaelis-Menten relationship:

$$J_{Zn} = J_{max}[H] / K_H + [H] , \quad (3)$$

where J_{Zn} is mucosa to serosa $^{65}Zn^{2+}$ transport rate, J_{max} is apparent maximal transmural zinc transport, K_H is an apparent affinity constant of the zinc transport protein for L-histidine,

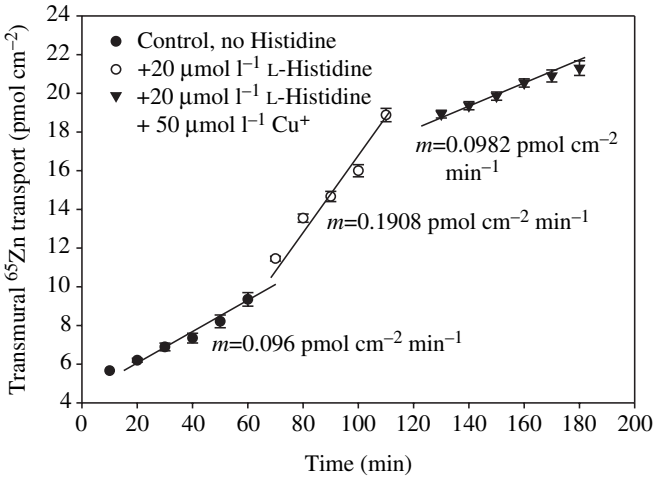

Fig. 5. Effect of 20 μmol l^{-1} L-histidine and 50 μmol l^{-1} Cu^+ on the time course of transmural transport of 20 μmol l^{-1} $^{65}Zn^{2+}$. Values displayed are means ± S.E.M. of 3 replicates per time point. Slopes of the lines were calculated as regression lines using Sigma Plot software and the values on the figure (i.e. m-values) are the slopes obtained from these analyses at each condition used.

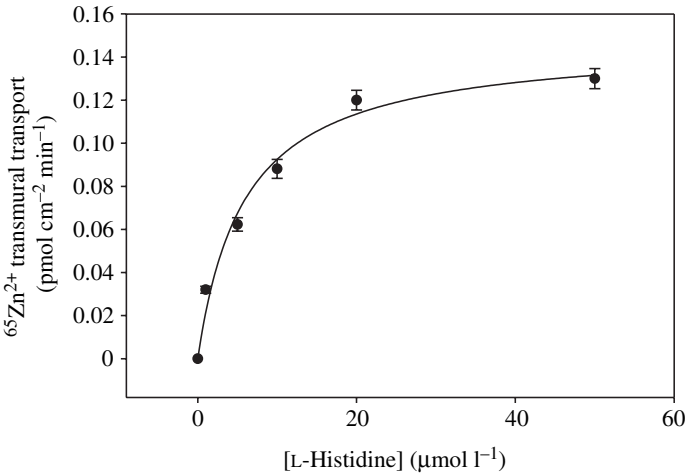

Fig. 6. Effect of varying luminal L-histidine concentration (1–50 μmol l^{-1}) on transmural transport rate of 20 μmol l^{-1} $^{65}Zn^{2+}$over 30 min time intervals at each luminal L-histidine concentration. Unidirectional fluxes were calculated from regression slopes determined over this time interval for each L-histidine concentration. Values displayed are means ± S.E.M. of 3 replicates per concentration.

and [H] is luminal L-histidine concentration. The apparent affinity constant for L-histidine stimulation of mucosal-to-serosal $^{65}Zn^{2+}$ transport was 5.9±1.3 μmol l^{-1} and the apparent maximal transport rate of zinc in the presence of L-histidine was 0.15±0.01 pmol cm^{-2} min^{-1}.

Fig. 7 shows the result of an experiment varying luminal zinc concentration on the kinetics of transmural transport of $^{65}Zn^{2+}$ across lobster intestine in the presence and absence of 20 μmol l^{-1} L-histidine, 50 μmol l^{-1} cuprous ions (Cu^+) and 50 μmol l^{-1} cupric ions (Cu^{2+}). In the absence of either L-histidine or copper ions, the transmural transport rate of $^{65}Zn^{2+}$

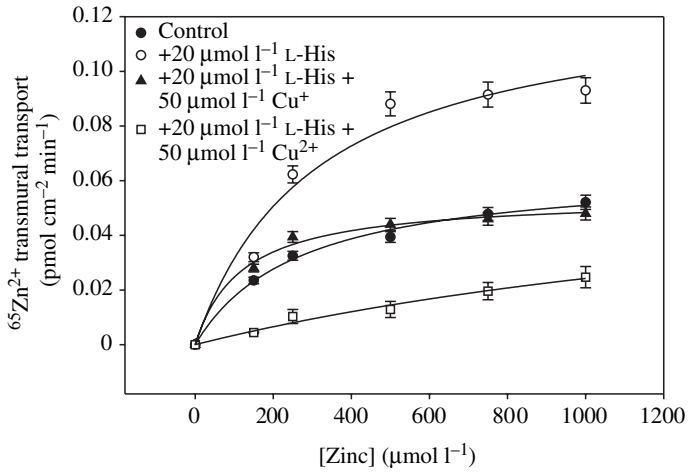

Fig. 7. Effect of luminal L-histidine (L-His; 20 μmol l^{-1}) and both Cu^{+} and Cu^{2+} (50 μmol l^{-1}) on the kinetics of transmural transport of $^{65}Zn^{2+}$ (150–1000 μmol l^{-1}) measured over 30 min time intervals at each luminal $^{65}Zn^{2+}$ concentration. Unidirectional fluxes were calculated from regression slopes determined over this time interval for each $^{65}Zn^{2+}$ concentration. Values displayed are means ± S.E.M. of 3 replicates per concentration.

followed the Michaelis–Menten equation, as given in Equation 1. Addition of 20 μmol l^{-1} L-histidine to the luminal perfusate doubled the apparent maximal transport rate of zinc and adding both 20 μmol l^{-1} L-histidine and 50 μmol l^{-1} Cu^{+} together abolished the stimulation by the amino acid alone. Addition of 20 μmol l^{-1} L-histidine and 50 μmol l^{-1} Cu^{2+} reduced $^{65}Zn^{2+}$ transport to values significantly below those observed under control conditions. While a straight line function could be fitted to these data, Sigma Plot software indicated a better fit to the results with a hyperbolic relationship. The kinetic constants for zinc transport under each of these four conditions are displayed in Table 2. These results show that addition of L-histidine to the luminal solution doubled the apparent maximal transport rate of $^{65}Zn^{2+}$ across the tissue without affecting the apparent binding affinity of the transport system for zinc itself. Addition of Cu^{+} ions to the mucosal surface of the intestine increased the apparent binding affinity of the transport system to $^{65}Zn^{2+}$ and abolished the stimulation of apparent maximal $^{65}Zn^{2+}$ transport rate in the presence of the L-histidine. Addition of Cu^{2+} ions to the perfusate blocked both L-histidine-stimulated $^{65}Zn^{2+}$ transport as well as a portion of the L-histidine-independent $^{65}Zn^{2+}$ transport.

In order to assess whether differences in $^{65}Zn^{2+}$ transport in the absence of L-histidine could be observed with cupric and cuprous ions, an experiment was conducted to see the effect of copper valence on zinc transport. In the absence of L-histidine the control transport of 20 μmol l^{-1} $^{65}Zn^{2+}$ across the intestine was 0.056±0.007 pmol cm^{-2} min^{-1} (N=3; data not shown). Addition of 100 μmol l^{-1} cuprous ions (Cu^{+}) to the perfusate had no effect on transmural 20 μmol l^{-1} $^{65}Zn^{2+}$ transport (0.064±0.007 pmol cm^{-2} min^{-1}, N=3; data not shown), but

Table 2. *Kinetic constants for transmural $^{65}Zn^{2+}$ transport in the presence and absence of 20 μmol l^{-1} L-histidine and 50 μmol l^{-1} copper*

Perfusate	K_m (μmol l^{-1})	J_{max} (pmol cm^{-2} min^{-1})
Zinc control	267±37	0.07±0.003
Zinc + L-Histidine	310±107	0.13±0.02*
Zinc + L-Histidine + Cu^{+}	119±21†	0.05±0.002†
Zinc + L-Histidine + Cu^{2+}	2079±1231†	0.08±0.03†

Values are means ± S.E.M. of three replicates per point.
*Significantly different (P<0.01) than control; †significantly different (P<0.01) than Zn + L-Histidine treatment.

100 μmol l^{-1} cupric ions (Cu^{2+}) significantly (P<0.01) reduced the transfer of 20 μmol l^{-1} $^{65}Zn^{2+}$ across the tissue (0.032±0.002 pmol cm^{-2} min^{-1}, N=3; data not shown). These results show that the cuprous ion inhibited zinc transport when the latter was cotransported with L-histidine, but only cupric ion appeared to inhibit the transfer of $^{65}Zn^{2+}$ in the absence of the amino acid.

Effects of the dipeptide, glycyl-sarcosine, on transmural ^{3}H-L-histidine transport in the presence and absence of zinc

To test the hypothesis that zinc and L-histidine were complexing in solution and being transported together across the lobster intestine as a binary complex containing 2 L-histidine/1 zinc ion, the effects of the dipeptide, glycyl-sarcosine, on the transfer of ^{3}H-L-histidine were examined in the presence and absence of luminal zinc. Time-course experiments showed that in the absence of either zinc or dipeptide the transmural transport rate of ^{3}H-L-histidine across lobster intestine was very slow (e.g. 0.02 pmol cm^{-2} min^{-1}; data not shown). Addition of 20 μmol l^{-1} zinc to the luminal solution increased the transmural transport rate of labeled amino acid by a factor of 3 (0.06 pmol cm^{-2} min^{-1}). When 20 μmol l^{-1} zinc and 100 μmol l^{-1} glycyl-sarcosine were added together to the luminal solution, the transport rate of ^{3}H-L-histidine across the intestine dropped to 0.03 pmol cm^{-2} min^{-1}), a value that was approximately half that of the stimulated rate induced by zinc alone, but still higher than the value observed in the control condition without either zinc or dipeptide.

In order to determine the specific effect that the dipeptide, glycyl-sarcosine, was having on the transmural transport rate of ^{3}H-L-histidine across lobster intestine, the kinetics of zinc-stimulated radiolabelled amino acid transport were observed in the presence and absence of the dipeptide. Fig. 8 indicates that addition of 100 μmol l^{-1} dipeptide to the luminal medium during transit of ^{3}H-L-histidine resulted in changes in K_H (control=18.9±2.9; test=7.9±2.3 μmol l^{-1}) and J_{max} (control=0.3±0.02; test=0.13±0.01 pmol cm^{-2} min^{-1}). These results suggest that the dipeptide inhibited the transmural transport of L-histidine by a mixed type of inhibitor response (Segel, 1975).

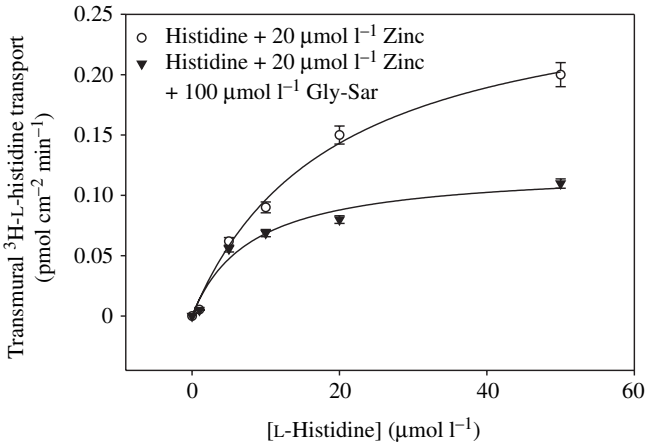

Fig. 8. Effect of zinc (20 μmol l^{-1}) and glycyl-sarcosine (Gly-Sar; 100 μmol l^{-1}) on the kinetics of transmural transport of ^{3}H-L-histidine (1–50 μmol l^{-1}) measured over 30 min time intervals at each luminal ^{3}H-L-histidine concentration. Unidirectional fluxes were calculated from regression slopes determined over this time interval for each ^{3}H-L-histidine concentration. Values displayed are means ± S.E.M. of 3 replicates per concentration.

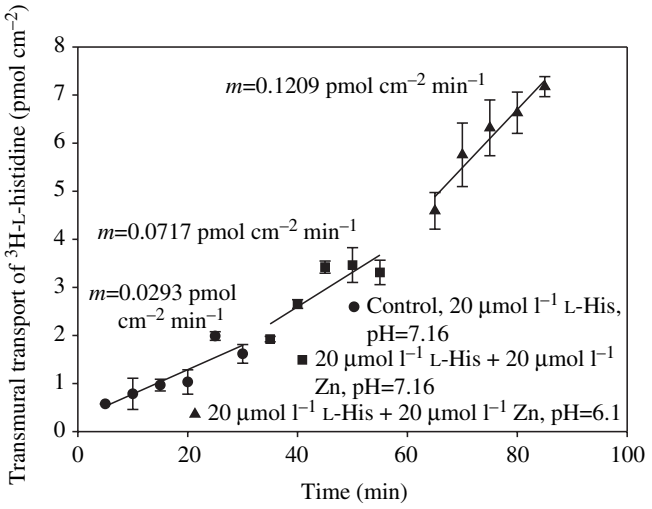

Fig. 9. Effect of luminal pH on the time course of 20 μmol l^{-1} ^{3}H-L-histidine (L-His) transport by perfused lobster intestine in the presence of 20 μmol l^{-1} zinc (control, pH 7.1; test, pH 6.1). Values displayed are means ± S.E.M. of 3 replicates per time point. Slopes of the lines were calculated as regression lines using Sigma Plot software and the values on the figure (i.e. *m*-values) are the slopes obtained from these analyses at each condition used.

Effect of luminal pH on transmural ^{3}H-L-histidine transport in the presence of zinc

In order to further characterize the transport of ^{3}H-L-histidine in the presence of zinc across perfused intestine, the transmural transport of the amino acid was measured at two luminal pH conditions: control pH (pH 7.1) and acidic pH (pH 6.1). As shown in Fig. 9, addition of luminal zinc significantly increased the transmural transport of the radiolabelled amino acid and this transfer rate was further elevated when the perfusate pH was lowered from 7.1 to 6.1. These results suggest that zinc-dependent ^{3}H-L-histidine transport was pH sensitive.

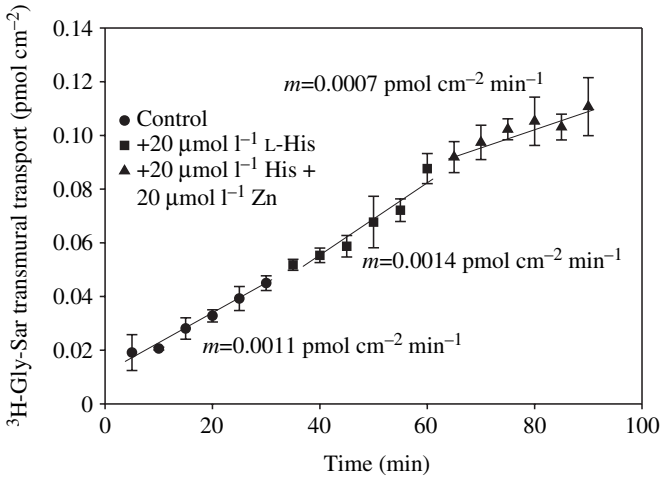

Fig. 10. Effect of 20 μmol l^{-1} L-histidine (L-His) and 20 μmol l^{-1} zinc on the time course of transmural transport of 100 μmol l^{-1} ^{3}H-glycyl-sarcosine. Values displayed are means ± S.E.M. of 3 replicates per time point. Slopes of the lines were calculated as regression lines using Sigma Plot software and the values on the figure (i.e. *m*-values) are the slopes obtained from these analyses at each condition used.

Effects of L-histidine, L-leucine and zinc on transmural transport of ^{3}H-glycyl-sarcosine

Figs 10 and 11 describe transmural transport of 100 μmol l^{-1} ^{3}H-glycyl-sarcosine in the presence and absence of two L-amino acids and zinc. Addition of either L-histidine or L-leucine to the luminal perfusate simultaneously with ^{3}H-glycyl-sarcosine had no effect on the transmural transport of the dipeptide (P>0.05). Furthermore, transmural ^{3}H-glycyl-sarcosine transport was not affected by the presence (0.006±0.0004 pmol cm^{-2} min^{-1}; data not shown) or absence (0.007±0.0006 pmol cm^{-2} min^{-1}; data not shown) of luminal zinc in the absence of amino acids. However, addition of zinc and either amino acid together to the luminal solution with the radiolabelled dipeptide, resulted in highly significant reduction in dipeptide transport across the tissue (P<0.01). These effects suggest that a bis-complex between two amino acids and the metal ion competes with ^{3}H-glycyl-sarcosine for transport by the peptide transport protein.

Discussion

The results of the present investigation suggest that the amino acid L-histidine and the ion Zn^{2+} are transported across the isolated and perfused intestine of the American lobster *Homarus americanus* by transport proteins that are specific to each of the solutes and by a shared cotransport protein that binds and transports both solutes simultaneously. Fig. 2 indicates that ^{3}H-L-histidine is able to cross the lobster intestine by a carrier-mediated transport system in the absence of metal ions. Similarly, Fig. 7 shows that $^{65}Zn^{2+}$ is transported across

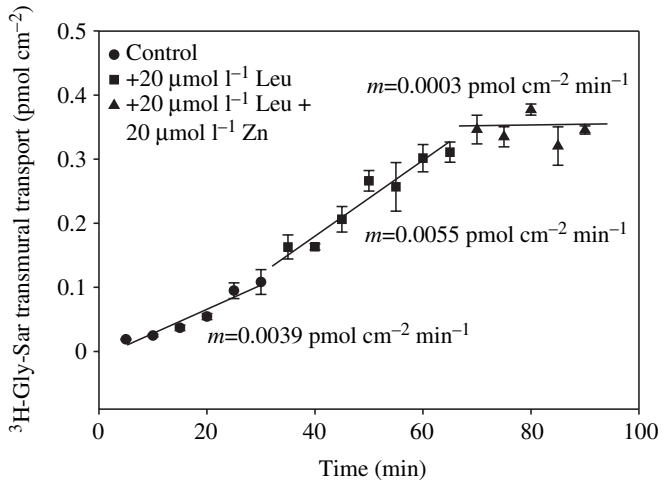

Fig. 11. Effect of 20 µmol l^{-1} L-leucine (L-Leu) and 20 µmol l^{-1} zinc on the time course of transmural transport of 100 µmol l^{-1} ^{3}H-glycyl-sarcosine. Values displayed are means ± S.E.M. of 3 replicates per time point. Slopes of the lines were calculated as regression lines using Sigma Plot software, and the values on the figure (i.e. *m*-values) are the slopes obtained from these analyses at each condition used.

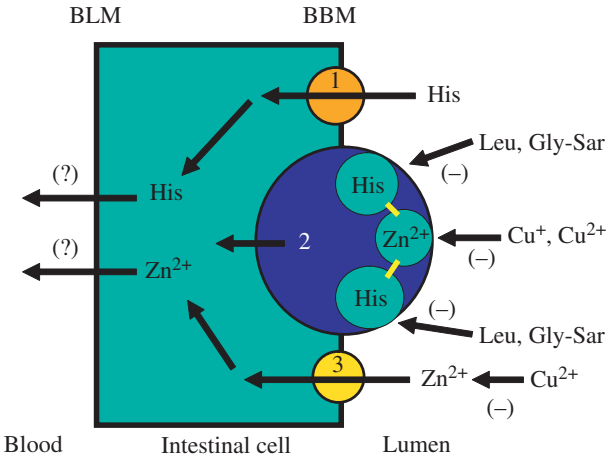

Fig. 12. Working model of transmural mucosal-to-serosal transport of ^{3}H-L-histidine (His) and ^{65}Zn^{2+} across the perfused intestine of the American lobster *Homarus americanus*. The figure shows three mucosal membrane carrier proteins involved in the movement of these two solutes across the intestine. (1) A relatively specific L-histidine carrier that is not inhibited by L-leucine; (2) a relatively non-specific dipeptide transporter that accepts two histidine molecules (His) linked to a zinc ion in an apparent bis-complex; and (3) a relatively specific zinc transporter that is inhibited by luminal cupric ions (Cu^{2+}). Luminal L-leucine (Leu) and glycyl-sarcosine (Gly-Sar) inhibit ^{3}H-L-histidine transport by interacting with the dipeptide carrier in a mixed type inhibition. Luminal copper (Cu$^+$ and Cu^{2+}) inhibits ^{65}Zn^{2+} transport by interacting with the dipeptide carrier in a mixed type inhibition. It is proposed that all interactions observed in this study occur on the brush border membrane (BBM) of intestinal epithelial cells and the mechanisms for efflux of both L-histidine and zinc from the cells to the blood across the basolateral membrane (BLM) are currently unclear.

the intestine by a hyperbolic process that occurs in the absence of the amino acid. The nature of these two carrier processes is unclear at the present time, but data in Fig. 4 and discussed in the text suggest that the transporter accommodating ^{3}H-L-histidine transport alone was not inhibited by 100 µmol l^{-1} L-leucine and therefore was unlikely to be shared by these amino acids. The data in Fig. 7 show that the carrier process responsible for transferring zinc alone across the intestine was not affected by cuprous ions, suggesting that monovalent copper may be excluded from this transporter. However, data reported in Fig. 7 and in the text suggest that divalent copper inhibited L-histidine-independent ^{65}Zn^{2+} transport and was likely shared by the zinc carrier.

In contrast to the apparent high specificity of the transport systems accommodating the amino acid or ion alone, the shared transporter that simultaneously transferred both L-histidine and zinc across the intestine was affected by both L-leucine and copper. Fig. 4 and Table 1 show that addition of 100 µmol l^{-1} L-leucine significantly (P<0.01) reduced ^{3}H-L-histidine transport in the presence of 20 µmol l^{-1} zinc by lowering both the apparent K_m and J_{max} of the carrier process. In the presence of L-leucine the kinetic constants were not significantly different (P>0.05) than those under control conditions lacking both zinc and L-leucine. Such results show that L-leucine exerted a mixed inhibitory effect (modification in both K_m and J_{max}) on zinc-stimulated ^{3}H-L-histidine transport (Segel, 1975). Data in Fig. 7 and Table 2 show a similar pattern of effect. In this example, copper significantly (P<0.01) reduced both apparent K_m and J_{max} of ^{65}Zn^{2+} transport by the cotransport carrier process by acting as a mixed inhibitor of L-histidine-stimulated ^{65}Zn^{2+} transport.

Fig. 8 provides strong evidence that the cotransport system in lobster intestine, accommodating simultaneous L-histidine and zinc transport, is the dipeptide transporter previously described for the hepatopancreas of this animal (Thamotharan and Ahearn, 1996), which may be related to the vertebrate PEPT-1 gene system. Additional support for this notion is provided in Fig. 9, showing that an acidic luminal condition stimulates transmural ^{3}H-L-histidine transport in the presence of zinc. The PEPT-1 transport system is proton-stimulated and the transport of any substrates by that carrier system would likely be enhanced by acidic conditions. The dipeptide glycyl-sarcosine is a substrate of this transport mechanism in both invertebrates (Thamotharan and Ahearn, 1996) and vertebrates (Adibi, 1997; Fei et al., 1994; Thamotharan et al., 1996a,b). Fig. 8 indicates a significant (P<0.01) and mixed inhibitory effect of the dipeptide on zinc-stimulated ^{3}H-L-histidine transport, suggesting that the dipeptide and amino acid interact with each other for the cotransport process with zinc. If this is the case, then the cotransport process transferring both L-histidine and zinc across lobster intestine likely accommodates two L-histidine amino acids linked to the zinc cation in a bis-complex, as described by Horn et al. (1995) and Horn and Thomas (1996), and in this configuration sufficiently resembles dipeptides in solution to utilize a transport system that normally would accommodate two amino acids linked by a peptide bond. The dipeptide transporter (e.g. PEPT-1) has a very broad specificity for peptides, accepting a wide

range of amino acid substrates. The role of the peptide bond between two amino acids in a peptide being transported on PEPT-1 has not been examined, but this study suggests that it may not be critical for the successful transfer of the peptide components to the *trans* side of the membrane. All that may be needed for PEPT-1 to transport two amino acids across a membrane may be that they are associated in solution with either a peptide bond or as a bis-complex with a metal cation. If this is the case, the dipeptide transport system may be a significant means by which cells are able to accumulate essential metals from their environment.

Supporting evidence for the role of PEPT-1-like transporters as the responsive agents for transmural transport of L-histidine or L-leucine across the lobster intestine in the presence of luminal zinc is shown in Figs 10 and 11. Neither L-histidine nor L-leucine added alone to the luminal perfusate were able to influence the transmural transport of ^{3}H-glycyl-sarcosine, but when the complexing ion, zinc, was included in the luminal solution, a marked reduction in the transfer of dipeptide across the gut was observed. These data suggest that when zinc was present, bis complexes between the metal and either amino acid were able to occur in solution and that, once formed, these complexes were able to compete with the dipeptide for transport by the peptide carrier system.

The model shown in Fig. 12 illustrates the results of the present investigation and suggests a mechanism that would allow the independent transport of both L-histidine and zinc on highly specific carrier proteins and allow the shared transport of both substrates on a PEPT-1-like dipeptide transport protein. The model suggests an apical location of all three carrier proteins. The vertebrate PEPT-1 dipeptide transporter has been localized to the brush border membrane in vertebrate intestine (Adibi, 1997), and physiological studies with other animals such as lobsters (Thamotharan and Ahearn, 1996) and fish (Thamotharan et al., 1996a; Verri et al., 2000) have also confirmed this location for the analogous transporter. The model shows that zinc likely occupies a separate binding site on the cotransport protein than occurs for either amino acid in the bis complex, since Cu^{+} or Cu^{2+} may inhibit zinc-stimulated L-histidine transport by competing with zinc for this site (Fig. 7). Similar inhibitory interactions between metal components have been reported for cadmium and zinc stimulation of L-histidine transport in human erythrocytes (Horn and Thomas, 1996). Alternatively, Zn^{2+} and either Cu^{+} or Cu^{2+} may interact in solution and compete with each other as bis-forming substrates with amino acids. L-Leucine and Gly-Sar are shown to inhibit L-histidine binding to the amino acid binding sites in Fig. 12, but not L-histidine transport by the high specificity amino acid transporter occurring on the same membrane, because the same amount of L-histidine transport under control conditions (e.g. no zinc, no L-leucine) occurred when both zinc and L-leucine were present together (Fig. 4). Lastly, zinc is shown to be transferred across the apical membrane by a highly specific carrier protein that was not apparently inhibited by cuprous ions, but was inhibited by cupric ions (Fig. 7; time-course data reported in the text). The model shows that once inside the intestinal epithelial cell, the exit processes to the blood for either L-histidine or zinc are unclear at the present time.

The nature of zinc-independent ^{3}H-L-histidine transport in lobster intestine is not known. As shown in Figs 2 and 4, in the absence of zinc stimulation, ^{3}H-L-histidine transport occurred by a saturable mechanism that had a high apparent binding affinity of about 6 μmol l^{-1}. Previous studies with lobster hepatopancreatic brush border membrane vesicles have characterized a number of amino acid transport proteins that occur on the luminal membrane of this absorptive organ and are responsible for the transapical transfer of L-alanine (Ahearn et al., 1986), L-lysine (Ahearn and Clay, 1987a), L-glutamate (Ahearn and Clay, 1987b), L-leucine (Ahearn and Clay, 1988b), and L-proline (Monteilh-Zoller et al., 1999). To date there has not been any specific study of a carrier-mediated transport process for L-histidine in either lobster hepatopancreas or intestine. The L-lysine transport system described for hepatopancreas (Ahearn and Clay, 1987a) would be a likely candidate for L-histidine transport, but this transporter is strongly inhibited by L-leucine, and since Fig. 4 and the results of a control experiment involving ^{3}H-L-histidine transport in the presence of 100 μmol l^{-1} L-leucine (reported in the text) both suggest a minimal effect of L-leucine on ^{3}H-L-histidine transport in the present study of lobster intestine, it is unlikely that L-histidine was using the hepatopancreatic L-leucine-inhibited L-lysine transporter. Clearly, further studies are needed to clarify the mechanism by which L-histidine is transported across lobster intestine in the absence of metals.

The nature of L-histidine-independent $^{65}Zn^{2+}$ transport suggested in Fig. 12 and experimentally described in Fig. 7 is similarly unclear at the present time. Experimental data presented in this report indicated that cuprous ions (Cu^{+}) had negligible effects on the transport of $^{65}Zn^{2+}$ in the absence of L-histidine, while cupric ions (Cu^{2+}) were effective inhibitors of zinc transport under these conditions. These results suggest that L-histidine-independent $^{65}Zn^{2+}$ transport may take place by a divalent cation transport system that does not recognize ions of other valences. Previous work supports the suggestion of a divalent cation-specific brush border antiporter in crustacean hepatopancreas (Aslamkhan and Ahearn, 2003). This study found that both calcium and cadmium acted as strong *trans*-stimulators of lobster hepatopancreatic brush border transport of both $^{55}Fe^{2+}$ and $^{59}Fe^{2+}$, implying a tight coupling between the divalent cation fluxes across this cell border. Other studies with lobster hepatopancreas have also shown a coupling between the uptakes of copper and zinc from dietary constituents and intracellular calcium activities (Chavez-Crooker et al., 2001). Interactions between the transport of zinc and calcium have also been recorded for gastrointestinal epithelial cells of asteroid echinoderms (Zhuang et al., 1995). These studies, and the present investigation, provide strong support for the occurrence of an invertebrate gastrointestinal brush border transport system that accepts a wide range of divalent cations, including both metals and calcium, but the nature of this mechanism is still unclear, as is its relationship to other metal-transporting membrane proteins from vertebrate cells.

A number of membrane-bound transport proteins that transport zinc into or out of cells have been cloned in mammalian tissues, and include members of the ZIP or ZTL families (Gaither and Eide, 2001a,b; Cragg et al., 2002) of zinc uptake proteins, the ZnT transport group for zinc efflux from cells (McMahon and Cousins, 1998), and the more generic DMT-1 heavy metal transporter that accepts a wide variety of metal ions (Gunshin et al., 1997). It therefore appears that metals such as zinc may enter cells in the absence of organic solutes by binding to either zinc-specific transporters (e.g. ZIP or ZnT proteins), or transporters that are mainly used by other cations (Zuang and Ahearn, 1996) but accept metals when they are present (e.g. DMT-1, calcium transport proteins). At present it is not known which of these mechanisms is responsible for zinc entry into lobster intestinal epithelial cells in the absence of L-histidine as defined in the present investigation. Future studies may help to clarify this situation.

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References

Adibi, S. A. (1997). The oligopeptide transporter (Pept-1) in human intestine: biology and function. *Gastroenterol*. **113**, 332-340.

Ahearn, G. A. (1987a). Nutrient transport by the crustacean gastrointestinal tract: recent advances with vesicle techniques. *Biol. Rev*. **62**, 45-63.

Ahearn, G. A. (1987b). Nutrient transport by invertebrate gastrointestinal organs and their diverticula. In *Terrestrial vs. Aquatic Life: Contrasts in Design and Function*, Vol. 9 (ed. P. Dejours, L. Bolis, C. R. Taylor and E. R. Weibel), pp. 167-179. Padova: Liviana Press.

Ahearn, G. A. (1988). Nutrient absorption by the invertebrate gut,. In *Advances in Environmental and Comparative Physiology*, Vol. 2 (ed. R. Gilles), pp. 91-129. Berlin: Springer-Verlag.

Ahearn, G. A. and Clay, L. P. (1987a). Membrane-potential-sensitive, Na-independent lysine transport by lobster hepatopancreatic brush border membrane vesicles. *J. Exp. Biol.* **127**, 373-388.

Ahearn, G. A. and Clay, L. P. (1987b). Na-Cl-glutamate cotransport by lobster hepatopancreatic brush border membrane vesicles. *J. Exp. Biol.* **130**, 175-191.

Ahearn, G. A. and Clay, L. P. (1988a). Sodium-coupled sugar and amino acid transport in an acidic microenvironment. *Comp. Biochem. Physiol*. **90A**, 627-634.

Ahearn, G. A. and Clay, L. P. (1988b). Electroneutral Na-2Cl-leucine cotransport by lobster hepatopancreatic brush border membrane vesicles. *J. Exp. Biol.* **136**, 363-381.

Ahearn, G. A., Gerencser, G. A., Thamotharan, M., Behnke, R. D. and Lemme, T. H. (1992). Invertebrate gut diverticula are nutrient absorptive organs. *Am. J. Physiol.* **263**, R472-R481.

Ahearn, G. A., Grover, M. L. and Dunn, R. E. (1986). Effects of Na, H, and Cl on alanine transport by lobster hepatopancreatic brush border membrane vesicles. *J. Comp. Physiol. B* **156**, 537-548.

Ahearn, G. A. and Hadley, N. F. (1977a). Functional role of luminal sodium and potassium in water transport across scorpion ileum. *Nature* **261**, 66-68.

Ahearn, G. A. and Hadley, N. F. (1977b). Water transport in perfused scorpion ileum. *Am. J. Physiol.* **233**, R198-R207.

Ahearn, G. A. and Maginniss, L. A. (1977). Kinetics of glucose transport by the perfused mid-gut of the freshwater prawn, *Macrobrachium rosenbegii. J. Physiol. (Lond.)*. **271**, 319-336.

Ahearn, G. A., Zhuang, Z., Duerr, J. and Pennington, V. (1994). Role of the invertebrate electrogenic $2Na^+/1H^+$ antiporter in monovalent and divalent cation transport. *J. Exp. Biol.* **196**, 319-336.

Aslamkhan, A. G. and Ahearn, G. A. (2003). Iron uptake by hepatopancreas brush border membrane vesicles (BBMV) of the lobster (*Homarus americanus*). *J. Exp. Zool.* **295A**, 145-150.

Brick, R. W. and Ahearn, G. A. (1978). Lysine transport across the mucosal border of the perfused midgut of the freshwater prawn, *Macrobrachium rosenbergii. J. Comp. Physiol.* **124**, 169-179.

Bury, N. R., Walker, P. A. and Glover, C. N. (2003). Nutritive metal uptake in teleost fish. *J. Exp. Biol.* **206**, 11-23.

Chavez-Crooker, P., Garrido, N. and Ahearn, G. A. (2001). Copper transport by lobster hepatopancreatic epithelial cells separated by centrifugal elutriation: measurements with the fluorescent dye, Phen Green. *J. Exp. Biol.* **204**, 1433-1444.

Chavez-Crooker, P., Garrido, N. and Ahearn, G. A. (2002). Copper transport by lobster (*Homarus americanus*) hepatopancreatic mitochondria. *J. Exp. Biol.* **205**, 405-413.

Chavez-Crooker, P., Garrido, Pozo, P. and Ahearn, G. A. (2003). Copper transport by lobster (*Homarus americanus*) hepatopancreatic lysosomes. *Comp. Biochem. Physiol.* **135C**, 107-118.

Chu, K. H. (1986). Glucose transport by the *in vitro* perfused midgut of the blue crab, *Callinectes sapidus*. *J. Exp. Biol.* **123**, 325-344.

Cragg, R. A., Christie, G. R., Phillips, S. R., Russi, R. M., Kury, S., Mathers, J. C., Taylor, P. M. and Ford, D. (2002). A novel zinc-regulated human zinc transporter, HzTL1, is localized to the enterocyte apical membrane. *J. Biol. Chem.* **277**, 22789-22797.

Fei, Y. J., Kanai, Y., Nussberger, S., Ganapathy, V., Leibach, F. H., Romero, M. F., Singh, S. K., Boron, W. F. and Hediger, M. A. (1994). Expression cloning of a mammalian proton-coupled oligopeptide transporter. *Nature* **368**, 563-566.

Gaither, L. A. and Eide, D. (2001a). Eukaryotic zinc transporters and their regulation. *Biometals* **14**, 251-270.

Gaither, L. A. and Eide, D. (2001b). The human ZIP1 transporter mediates zinc uptake in human K562 erythroleukaemia cells. *J. Biol. Chem.* **276**, 22258-22264.

Glover, C. N., Bury, N. R. and Hogstrand, C. (2003). Zinc uptake across the apical membrane of freshwater rainbow trout intestine is mediated by high affinity, low affinity, and histidine-facilitated pathways. *Biochim. Biophys. Acta* **1614**, 211-219.

Glover, C. N. and Hogstrand, C. (2002a). In vivo characterization of intestinal zinc uptake in freshwater rainbow trout. *J. Exp. Biol.* **205**, 141-150.

Glover, C. N. and Hogstrand, C. (2002b). Amino acid modulation of *in vivo* intestinal zinc absorption in freshwater rainbow trout. *J. Exp. Biol.* **205**, 151-158.

Gunshin, H., MacKenzie, B., Berger, U. V., Gunshin, Y., Romero, M. F., Boron, W. F., Nussberger, S., Gollan, J. L. and Hediger, M. A. (1997). Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* **388**, 482-488.

Hambridge, M. (2000). Human zinc deficiency. *J. Nutr.* **130**, 1344S-1349S.

Horn, N. M. and Thomas, A. L. (1996). Interactions between the histidine stimulation of cadmium and zinc influx into human erythrocytes. *J. Physiol.* **496**, 711-718.

Horn, N. M., Thomas, A. L. and Tompkins, J. D. (1995). The effect of histidine and cysteine on zinc influx into rat and human erythrocytes. *J. Physiol.* **489**, 73-80.

McMahon, R. J. and Cousins, R. J. (1998). Mammalian zinc transporters. *J. Nutr.* **128**, 667-670.

Monteilh-Zoller, M. K., V. Zonno, C. Storelli, and Ahearn, G. A. (1999). Effects of zinc on ^{3}H-L-proline transport in hepatopancreatic brush border membrane vesicles of the American lobster, *Homarus americanus. J. Exp. Biol.* **202**, 3003-3010.

Segel, I. H. (1975). *Enzyme Kinetics*. New York: Wiley-Interscience.

Thamotharan, M. and Ahearn, G. A. (1996). Dipeptide transport by crustacean hepatopancreatic brush border membrane vesicles. *J. Exp. Biol.* **199**, 635-641.

Thamotharan, M., Gomme, J., Zonno, V., Maffia, M., Storelli, C. and Ahearn, G. A. (1996a). Electrogenic, proton-coupled, intestinal dipeptide transport in herbivorous and carnivorous teleosts. *Am. J. Physiol.* **270**, $939-R947.

Thamotharan, M., Zonno, V., Storelli, C. and Ahearn, G. A. (1996b). Basolateral dipeptide transport by the intestine of the teleost *Oreochromis mossambicus*. *Am. J. Physiol.* **270**, R948-R954.

Verri, T., Maffia, M., Danieli, A., Herget, M., Wenzel, U., Daniel, H. and Storelli, C. (2000). Characterization of the H+/peptide cotransporter of eel intestinal brush border membranes. *J. Exp. Biol.* **203**, 2991-3001.

Wright, S. H. and Ahearn, G. A. (1997). Nutrient absorption in invertebrates. In *Handbook of Physiology (Sect. 13: Comparative Physiology),* Vol. II, Chap. 16 (ed. W. H. Dantzler), pp. 1137-1206. New York: American Physiol. Soc.

Wyban, J. A., Ahearn, G. A. and Maginniss, L. A. (1980). Effects of organic solutes on transmural PD and Na transport in freshwater prawn intestine. *Am. J. Physiol.* **239**, C11-C17.

Zhuang, Z. and Ahearn, G. A. (1996). Calcium transport process of lobster hepatopancreatic brush border membrane vesicles. *J. Exp. Biol.* **199**, 1195-1208.

Zhuang, Z., Duerr, J. and Ahearn, G. A. (1995). Ca^{2+} and Zn^{2+} are transported by the electrogenic $2Na^+/1H^+$ antiporter in echinoderm gastrointestinal epithelium. *J. Exp. Biol.* **198**, 1207-1217.