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DIPEPTIDE TRANSPORT BY CRUSTACEAN HEPATOPANCREATIC BRUSH-BORDER MEMBRANE VESICLES

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Summary

Epithelial brush-border membrane vesicles (BBMVs) of lobster (*Homarus americanus*) hepatopancreas were formed by a Mg^{2+} precipitation technique. In these BBMVs, [^{14}C]glycylsarcosine ([^{14}C]Gly-Sar) uptake was stimulated by a transmembrane proton gradient. transmembrane K^+ diffusion potential (inside negative) stimulated [^{14}C]Gly-Sar uptake above that observed with short-circuited vesicles, while an inwardly directed Na^+ gradient had no stimulatory effect on peptide uptake. [^{14}C]Gly-Sar influx (over 10 s) occurred by a low-affinity, saturable, proton-gradient-dependent carrier system (K_t=5.90±0.13 mmol l^{-1}, J_{max}=4662±487 pmol mg^{-1} protein 10 s^{-1}; mean ± S.E.M., N=3). This carrier exhibited a high-affinity proton binding site (K_H=235±25 nmol l^{-1}; pK=6.6) and an apparent 1H^+:1Gly-Sar transport stoichiometry. Influx of 0.1 mmol l^{-1} [^{14}C]Gly-Sar into lobster hepatopancreatic BBMVs was significantly (P<0.01) *cis*-inhibited by 10 mmol l^{-1} diethylpyrocarbonate and by a variety of other dipeptides (10 mmol l^{-1}), suggesting a broad transport specificity. These observations strongly suggest that transport of peptides into crustacean hepatopancreas is proton-gradient-dependent and electrogenic, qualitatively resembling the peptide transport paradigm proposed for fish and mammals.

Key words: peptide, dipeptide, glycylsarcosine, proton-coupled transport, electrogenic transport, hepatopancreas, *Homarus americanus*, crustacean, epithelium.

Introduction

In crustaceans, the hepatopancreas is a large branching gut diverticulum involved in the absorption of nutrients. A number of studies have focused on the characterization of sugar (Ahearn *et al.* 1985), amino acid (Ahearn and Clay, 1988) and vitamin (Siu and Ahearn, 1988) transport by isolated hepatopancreatic brush-border membrane vesicles (BBMVs) of the lobster *Homarus americanus*. These studies indicate that lobster hepatopancreatic BBMVs possess at least seven distinct transport proteins for nutrient transport (Ahearn *et al.* 1992). They also point out that hepatopancreatic brush-border membrane transporter properties are modified, compared with those of the vertebrate system, to accommodate the very low pH at the absorption site of the gut (Ahearn, 1987; Gibson and Barker, 1979).

A proton-gradient-energized cotransport of di- and tripeptides has been shown to be present in the intestine of mammals (Ganapathy *et al.* 1984) and teleosts (Verri *et al.* 1992; M. Thamotharan, J. Gomme, V. Zonno, M. Maffia, C. Storelli and G. A. Ahearn, in preparation). Advances continue rapidly in the field of peptide absorption and its probable importance to the animal (Gardner, 1984). Until now, the characteristics of proton-coupled dipeptide transport systems in the crustacean hepatopancreas, where a proton gradient of 3 pH units (pH 4–7) normally exists from lumen to cell, have remained unexplored.

For this reason, in the present study we used [^{14}C]glycylsarcosine ([^{14}C]Gly-Sar), which is generally very resistant to hydrolysis and appears intact in mammalian (Ganapathy and Leibach, 1983) and teleost (M. Thamotharan, J. Gomme, V. Zonno, M. Maffia, C. Storelli and G. A. Ahearn, in preparation) intestinal tissues following absorption, to determine the characteristics of hepatopancreatic dipeptide transport in the lobster *Homarus americanus*.

Materials and methods

Collection and maintenance of animals

Live Atlantic lobsters *Homarus americanus* (L.) were purchased from commerical dealers in Hawaii and maintained in sea water at 10 °C until required.

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Preparation of brush-border membrane vesicles

Hepatopancreatic brush-border membrane vesicles (BBMVs) were prepared from fresh tissue removed from lobsters using a Mg^{2+} precipitation technique described previously (Ahearn *et al.* 1985). Briefly, the tissue was homogenized in a buffer solution containing 60 mmol l^{-1} D-mannitol with 12 mmol l^{-1} Tris/HCl, 10 mmol l^{-1} EGTA and 0.1 mmol l^{-1} *p*-methylsulphonyl fluoride (PMSF). The suspension was centrifuged at 27 000 ***g*** for 30 min. The resulting pellet was resuspended in 250 ml of the above mannitol buffer solution using a Potter-Elvehjem tissue homogenizer, and magnesium chloride was added and mixed with the homogenate to a final concentration of 10 mmol l^{-1}. The resulting solution was allowed to stand on ice for 15 min (step 1). The suspension was centrifuged at 3000 ***g*** for 15 min and the resulting supernatant was centrifuged at 27 000 ***g*** for 30 min (step 2). The pellet from this high-speed spin was resuspended in 35 ml of the mannitol buffer solution using a tissue homogenizer. Steps 1 and 2 were repeated on this homogenate and the resulting pellet was resuspended with the tissue homogenizer in 10 ml of ice-cold transport buffer of appropriate composition (details are given in the figure legends). The final suspension was centrifuged at 27 000 ***g*** for 30 min. The purified membrane pellet was resuspended in sufficient transport buffer to provide a final protein content of 6–8 mg ml^{-1}. The protein content of the preparation was quantified using the Bio-Rad protein assay.

Transport measurements

Transport studies using hepatopancreas BBMVs were conducted at 15 °C using the Millipore filtration technique (Hopfer *et al.* 1973). [^{14}C]Gly-Sar was provided by Dr F. H. Leibach, Department of Cell and Molecular Biology, Medical College of Georgia, USA. Long-term [^{14}C]Gly-Sar uptake experiments were initiated by mixing 20 μl of membrane suspension with 180 μl of radiolabelled incubation medium. The composition of the incubation medium varied with the nature of the experiments (see Results). Uptake of [^{14}C]Gly-Sar was terminated by injecting 20 μl of reaction mixture into 2 ml of ice-cold stop solution (same composition as the incubation medium without radiolabelled solute). This was then filtered using a Millipore filter (0.65 μm pore diameter) and washed with another 5 ml of ice-cold stop solution. Filters containing the washed vesicles were placed in Beckman Ecolume scintillation cocktail and counted using a Beckman LS-8100 scintillation spectrometer.

Short-term influx experiments were initiated by mixing 5 μl of membrane suspension with 45 μl of radiolabelled incubation medium containing variable concentrations of unlabelled solute (see Results). Solute influx was terminated by injecting 20 μl of the appropriate reaction mixture into 2 ml of ice-cold stop solution. Carrier-mediated [^{14}C]Gly-Sar influx kinetics were determined by a curve-fitting procedure in which the individual influx values for all replicates were fitted, using an iterative nonlinear method, to the Michaelis–Menten kinetic equation:

$$J_{oi} = (J_{max}\,[S])/(K_t + [S]), \quad (1)$$

where J_{oi} is [^{14}C]Gly-Sar influx (in pmol mg^{-1} protein 10 s^{-1}), [*S*] is the external [^{14}C]Gly-Sar concentration (in mmol l^{-1}), J_{max} is the maximal rate of [^{14}C]Gly-Sar influx, and K_t is the [^{14}C]Gly-Sar concentration that yielded 0.5J_{max}. All isotope transport values were corrected for a 'vesicle blank' obtained by adding the incubation medium and vesicles directly to the respective stop solution, filtering onto Millipore filters, and performing the counting procedure as above. Each experiment was repeated using membrane vesicles prepared from different lobsters to confirm consistent experimental findings. Within a given experiment, each value represents a mean of three replicates ± S.E.M. Significant differences between values were determined using Student's *t*-tests.

Specificity of the [^{14}C]Gly-Sar carrier was assessed by measurement of the influx of 0.1 mmol l^{-1} [^{14}C]Gly-Sar in the presence of 10 mmol l^{-1} of several external unlabelled dipeptides (*cis*-inhibition). All experiments employed an inwardly directed H^+ gradient.

Results

Osmotic reactivity of BBMVs

To confirm that dipeptide transport by these vesicles was into an osmotically reactive space, 60 min equilibrium uptake of 0.1 mmol l^{-1} [^{14}C]Gly-Sar was assessed at a series of transmembrane osmotic gradients. Vesicles were loaded during their preparation with 200 mmol l^{-1} mannitol, 100 mmol l^{-1} KCl, 20 mmol l^{-1} Hepes/Tris at pH 8.5 and 50 μmol l^{-1} valinomycin and were incubated for 60 min in external media containing 0.1 mmol l^{-1} [^{14}C]Gly-Sar, 200 mmol l^{-1} mannitol, 100 mmol l^{-1} KCl, 20 mmol l^{-1} Mes/Tris at pH 5.5 and 0–600 mmol l^{-1} sucrose. Fig. 1 shows that uptake increased as a linear function of the reciprocal of medium osmolarity, indicating that these vesicles were sealed and exhibited osmotic reactivity. Extrapolation of this relationship to the vertical axis shows no equilibrium binding at infinite osmolarity because the intercept is not significantly different from zero (P>0.05).

Time course of [^{14}C]Gly-Sar uptake in lobster hepatopancreas BBMVs

Effect of a pH gradient

In order to assess whether the transport of [^{14}C]Gly-Sar was specifically activated by a proton gradient, uptake of 0.1 mmol l^{-1} [^{14}C]Gly-Sar was measured with vesicles preloaded with 100 mmol l^{-1} KCl, 200 mmol l^{-1} mannitol, 20 mmol l^{-1} Hepes/Tris at pH 7.5 and 50 μmol l^{-1} valinomycin. Fig. 2 illustrates the effects of a pH gradient on the time course of 0.1 mmol l^{-1} [^{14}C]Gly-Sar uptake by hepatopancreatic BBMVs. [^{14}C]Gly-Sar uptake was characterized by a slow, hyperbolic time course and was higher when pHi (7.5) was greater than pHe (5.5) than when both pHi and pHe were 7.5.

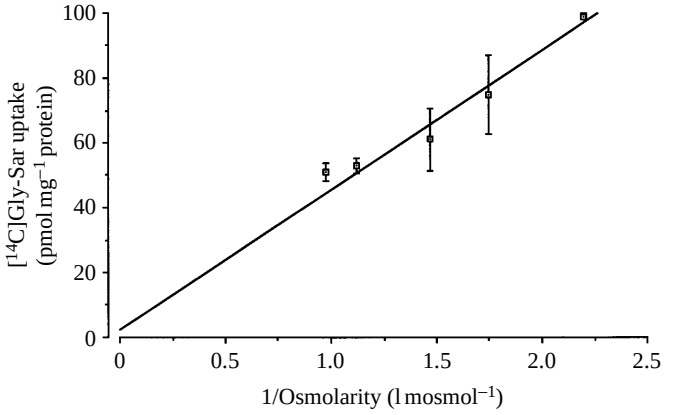

Fig. 1. Osmotic reactivity of hepatopancreatic brush-border membrane vesicles (BBMVs). Vesicles were preloaded with 200 mmol l^{-1} mannitol, 100 mmol l^{-1} KCl, 20 mmol l^{-1} Hepes/Tris at pH 8.5 and 50 μmol l^{-1} valinomycin and were subsequently incubated at 15 °C for 60 min in external media containing 0.1 mmol l^{-1} [^{14}C]Gly-Sar, 200 mmol l^{-1} mannitol, 100 mmol l^{-1} KCl, 20 mmol l^{-1} Mes/Tris at pH 5.5 and 0–600 mmol l^{-1} sucrose. In all figures, values are means ± S.E.M., *N*=3.

These data show that external pH had a marked effect on the transport of this dipeptide and that greater peptide transport was attained with an inwardly directed H^+ gradient.

Effect of a membrane potential

Fig. 2 also shows the effect of a K^+-generated diffusion potential (inside negative) on 0.1 mmol l^{-1} [^{14}C]Gly-Sar transport in the presence of an inwardly directed H^+ gradient. Vesicles were preloaded with 100 mmol l^{-1} KCl, 200 mmol l^{-1} mannitol, 20 mmol l^{-1} Hepes/Tris at pH 7.5 and 50 μmol l^{-1} valinomycin. Transport was initiated by exposing 20 μl of preloaded vesicle suspension to 180 μl of transport medium containing 0.1 mmol l^{-1} [^{14}C]Gly-Sar and 100 mmol l^{-1} choline chloride, 200 mmol l^{-1} mannitol and 20 mmol l^{-1} Mes/Tris at pH 5.5. Imposing a membrane potential resulted in increased uptake of [^{14}C]Gly-Sar into hepatopancreatic BBMVs compared with the uptake under short-circuited conditions (Fig. 2).

Effect of a Na^+ gradient

The effect of a Na^+ gradient on the uptake of 0.1 mmol l^{-1} [^{14}C]Gly-Sar by hepatopancreatic BBMVs was studied in the presence of an inwardly directed H^+ gradient together with a K^+-generated diffusion potential (inside negative). In these experiments, vesicles were preloaded with 100 mmol l^{-1} KCl, 200 mmol l^{-1} mannitol, 20 mmol l^{-1} Hepes/Tris at pH 8.5 and 50 μmol l^{-1} valinomycin. Transport was initiated by exposing 20 μl of vesicle suspension to 180 μl of transport medium containing 0.1 mmol l^{-1} [^{14}C]Gly-Sar and (1) 100 mmol l^{-1} KCl, 200 mmol l^{-1} mannitol, (2) 100 mmol l^{-1} choline chloride, 200 mmol l^{-1} mannitol, or (3) 100 mmol l^{-1} sodium chloride, 200 mmol l^{-1} mannitol. Each medium also contained 20 mmol l^{-1} Mes/Tris at pH 5.5. Addition of a 100 mmol l^{-1} inwardly directed Na^+ gradient together with an outwardly directed K^+-generated diffusion potential (inside negative) inhibited membrane-potential-dependent [^{14}C]Gly-Sar uptake (Fig. 3). This reduced H^+/peptide uptake in the presence of a

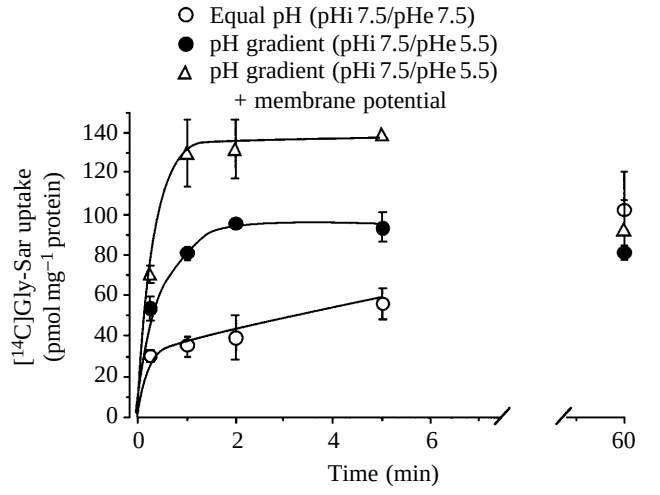

Fig. 2. Effects of a pH gradient and membrane potential on the time course of [^{14}C]Gly-Sar uptake by hepatopancreatic BBMVs. Vesicles were preloaded with 100 mmol l^{-1} KCl, 200 mmol l^{-1} mannitol, 20 mmol l^{-1} Hepes/Tris at pH 7.5 and 50 μmol l^{-1} valinomycin. Outside media containing 0.1 mmol l^{-1} [^{14}C]Gly-Sar were as follows: 100 mmol l^{-1} KCl, 200 mmol l^{-1} mannitol and 20 mmol l^{-1} Hepes/Tris at pH 7.5 (open circles); 100 mmol l^{-1} KCl, 200 mmol l^{-1} mannitol and 20 mmol l^{-1} Mes/Tris at pH 5.5 (filled circles); or 100 mmol l^{-1} choline chloride, 200 mmol l^{-1} mannitol and 20 mmol l^{-1} Mes/Tris at pH 5.5 (triangles).

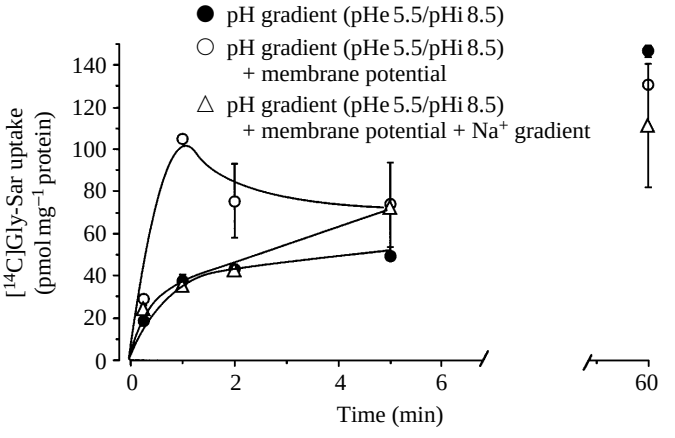

Fig. 3. Effect of a transmembrane Na^+ gradient on the time course of [^{14}C]Gly-Sar uptake by hepatopancreatic BBMVs. Vesicles were preloaded as in Fig. 2 (except that a pHi of 8.5 was used in this case) and the resulting membrane vesicles were incubated in one of the following external media containing 0.1 mmol l^{-1} [^{14}C]Gly-Sar: 100 mmol l^{-1} KCl, 200 mmol l^{-1} mannitol and 20 mmol l^{-1} Mes/Tris at pH 5.5 (filled circles); 100 mmol l^{-1} choline chloride, 200 mmol l^{-1} mannitol and 20 mmol l^{-1} Mes/Tris at pH 5.5 (open circles); or 200 mmol l^{-1} mannitol, 100 mmol l^{-1} NaCl and 20 mmol l^{-1} Mes/Tris at pH 5.5 (triangles).

transmembrane Na^+ gradient was presumably due either to the shorting out of the K^+ diffusion potential by Na^+ entry or to *cis*-inhibition of H^+ binding to its external symport site on the transporter. Future experiments will be designed to clarify the potential role of Na^+ in H^+/peptide cotransport in this animal.

Kinetics of [^{14}C]Gly-Sar influx into hepatopancreatic BBMVs

Influx of [^{14}C]Gly-Sar into hepatopancreatic BBMVs was measured over a concentration range of 0.05–20 mmol l^{-1} [^{14}C]Gly-Sar in the presence of an inwardly directed H^+ gradient (pHi 8.5/pHe 5.5) for 10 s at 15 °C. Membrane vesicles were preloaded with 200 mmol l^{-1} mannitol, 100 mmol l^{-1} KCl, 20 mmol l^{-1} Hepes/Tris at pH 8.5 and 50 μmol l^{-1} valinomycin. Data for total dipeptide influx were used in equation 1 to generate transport kinetic constants for a single Gly-Sar carrier system assuming minimal contribution from diffusional influx or from a second peptide carrier process. Fig. 4A indicates that peptide influx into BBMVs appeared to follow a single Michaelis–Menten relationship for carrier transport with negligible diffusional entry over the concentration range used. Transport kinetic constants (±S.E.M., N=3) for the apparent carrier influx of Gly-Sar obtained using equation 1 were as follows: K_t=5.99±0.42 mmol l^{-1} and J_{max}=5006±148.7 pmol mg^{-1} protein 10 s^{-1}. Influx data in Fig. 4 were linearized using an Eadie–Hofstee relationship and the resulting regression line is shown (Fig. 4B). Transport kinetic constants obtained from this treatment of the data were as follows: K_t=5.8±1.3 mmol l^{-1} and J_{max}=4317±713 pmol mg^{-1} protein 10 s^{-1}. The mean values for the constants from these two estimations were K_t=5.90±0.13 mmol l^{-1} and J_{max}= 4662±487 pmol mg^{-1} protein 10 s^{-1}.

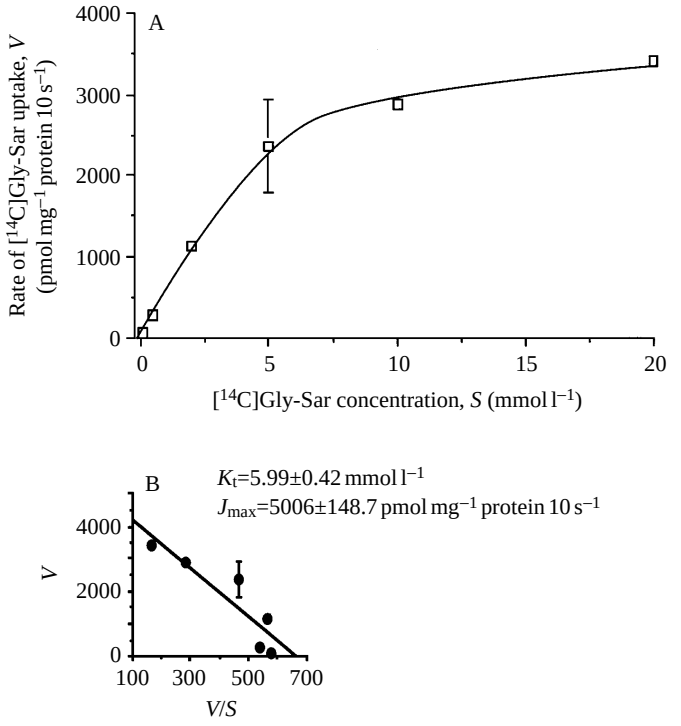

Fig. 4. (A) Influx (over 10 s) of [^{14}C]Gly-Sar into hepatopancreatic BBMVs as a function of substrate concentration. Vesicles were preloaded with 200 mmol l^{-1} mannitol, 100 mmol l^{-1} KCl, 20 mmol l^{-1} Hepes/Tris at pH 8.5 and 50 μmol l^{-1} valinomycin. External media contained 100 mmol l^{-1} KCl, 200 mmol l^{-1} mannitol and 20 mmol l^{-1} Mes/Tris at pH 5.5, with [^{14}C]Gly-Sar concentration ranging from 0.05 to 20 mmol l^{-1}. All influx values have been corrected for non-specific isotope binding using 'blank' uptake values obtained at each substrate concentration (see Materials and methods). (B) An Eadie–Hofstee plot of the same data; the line on this figure was drawn using linear regression analysis. *V*, rate of uptake of Gly-Sar; *S*, Gly-Sar concentration.

Effect of DEP and other dipeptides on [^{14}C]Gly-Sar influx

In order to investigate whether other dipeptides and drugs could influence the influx of [^{14}C]Gly-Sar, entry of the labelled substrate was measured in the presence of several unlabelled dipeptides and diethylpyrocarbonate (DEP), the latter being a known inhibitor of carrier-mediated dipeptide transport in intestinal BBMVs of the rabbit (Miyamoto *et al.* 1986) and teleosts (M. Thamotharan, J. Gomme, V. Zonno, M. Maffia, C. Storelli and G. A. Ahearn, in preparation). In these studies, membrane vesicles were preloaded with 200 mmol l^{-1} mannitol, 100 mmol l^{-1} KCl, 20 mmol l^{-1} Hepes/Tris at pH 8.5 and 50 μmol l^{-1} valinomycin. Influx of 0.1 mmol l^{-1} [^{14}C]Gly-Sar into hepatopancreatic BBMVs was measured as previously with an inwardly directed H^+ gradient for 10 s in the presence of 10 mmol l^{-1} test peptide or drug. Fig. 5 shows that a

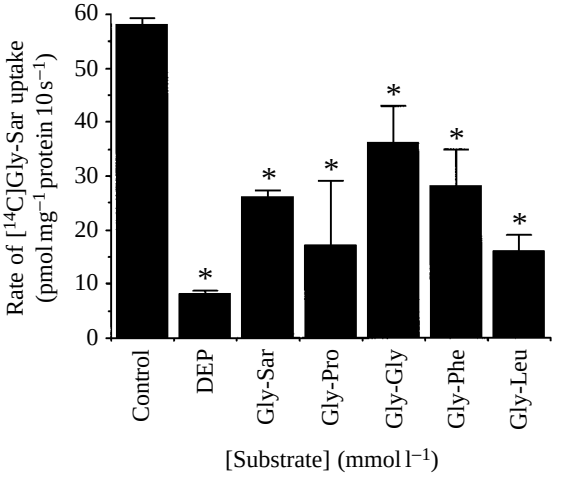

Fig. 5. Effect of 10 mmol l^{-1} external substrates on 10 s 0.1 mmol l^{-1} [^{14}C]Gly-Sar influx into hepatopancreatic BBMVs. Vesicles were preloaded with 200 mmol l^{-1} mannitol, 100 mmol l^{-1} KCl, 20 mmol l^{-1} Hepes/Tris at pH 8.5 and 50 μmol l^{-1} valinomycin. External media containing 0.1 mmol l^{-1} [^{14}C]Gly-Sar were 100 mmol l^{-1} KCl, 200 mmol l^{-1} mannitol, 20 mmol l^{-1} Mes/Tris at pH 5.5 and 10 mmol l^{-1} of the respective test substrate. For DEP treatment, vesicles were pretreated for 10 min with 10 mmol l^{-1} DEP before incubation in external medium. Values are means + S.E.M. (N=3) and asterisks indicate values that are significantly different (P<0.05) from the control value. No test substances were added to the control treatments.

significant (P<0.05) reduction in [^{14}C]Gly-Sar influx was caused by all unlabelled dipeptides used. In addition, DEP also strongly inhibited the entry of radiolabelled substrate under these conditions.

In order to determine whether unlabelled 10 mmol l^{-1} Gly-Sar reduced the influx of 0.1 mmol l^{-1} [^{14}C]Gly-Sar by competitive inhibition, the data were fitted to the competitive inhibition equation described in Segel (1975):

$$i = [I]/\{[I] + K_i(1 + [S]/K_m)\}, \quad (2)$$

where 100i is the percentage inhibition by a pure competitive inhibitor, [I] is the unlabelled Gly-Sar concentration (10 mmol l^{-1}), [S] is the labelled [^{14}C]Gly-Sar concentration (0.1 mmol l^{-1}), K_m (=K_t used in this paper) is the Michaelis–Menten apparent affinity constant for [^{14}C]Gly-Sar influx (5.90±0.13 mmol l^{-1}; see Fig. 4), and K_i is the apparent inhibitor constant for unlabelled Gly-Sar on [^{14}C]Gly-Sar influx (assumed to be identical to K_m in this case). Using these values and assumptions, 10 mmol l^{-1} Gly-Sar should inhibit the carrier influx of 0.1 mmol l^{-1} [^{14}C]Gly-Sar by 90 % by competitive inhibition. If 0.1 mmol l^{-1} [^{14}C]Gly-Sar influx in the presence of DEP approximates the diffusional entry of the dipeptide only and is therefore subtracted from control isotope influx in addition to the influx in the presence of 10 mmol l^{-1} Gly-Sar due to competition, a 64 % reduction of carrier-mediated [^{14}C]Gly-Sar can be calculated. This value is significantly less than the 90 % inhibition that would have occurred by pure competitive inhibition by unlabelled substrate on labelled substrate influx; therefore, the interaction between labelled and unlabelled Gly-Sar in this experiment is likely to represent a combination of both competitive *cis*-inhibition and *trans*-stimulation where the observed reduction in [^{14}C]Gly-Sar influx in the presence of unlabelled substrate largely reflects the predominance of *cis*-inhibition over *trans*-stimulation. The effects of the other dipeptides on 0.1 mmol l^{-1} [^{14}C]Gly-Sar influx in this experiment also probably involve a combination of *cis* and *trans* effects and imply that all these substrates are potentially able to employ this carrier for transapical transfer. The relative potency of each compound as a *cis* inhibitor will be determined in separate transport experiments.

Effect of external [H$^+$] on [^{14}C]Gly-Sar influx

In order to determine the effects of external [H$^+$] on [^{14}C]Gly-Sar influx, vesicles were preloaded with 200 mmol l^{-1} mannitol, 100 mmol l^{-1} KCl, 20 mmol l^{-1} Hepes/Tris at pH 8.5 and 50 μmol l^{-1} valinomycin. Influx of 0.1 mmol l^{-1} [^{14}C]Gly-Sar into BBMVs was measured with inwardly directed H$^+$ gradients for 10 s in the presence of different external H$^+$ concentrations. The resulting data are shown in Fig. 6A. The hyperbolic nature of proton-gradient-dependent [^{14}C]Gly-Sar influx suggests a single carrier system having an apparent H$^+$/dipeptide cotransport stoichiometry of 1:1 (Turner, 1983). Eadie–Hofstee linear transformation (Fig. 6B) of these hyperbolic data yielded the following kinetic constants for a high-affinity cotransporter: K_H=235±25 nmol l^{-1} (pK=6.6) and J_{max}=37.34±0.85 pmol mg^{-1} protein 10 s^{-1} (means ± S.E.M., N=3).

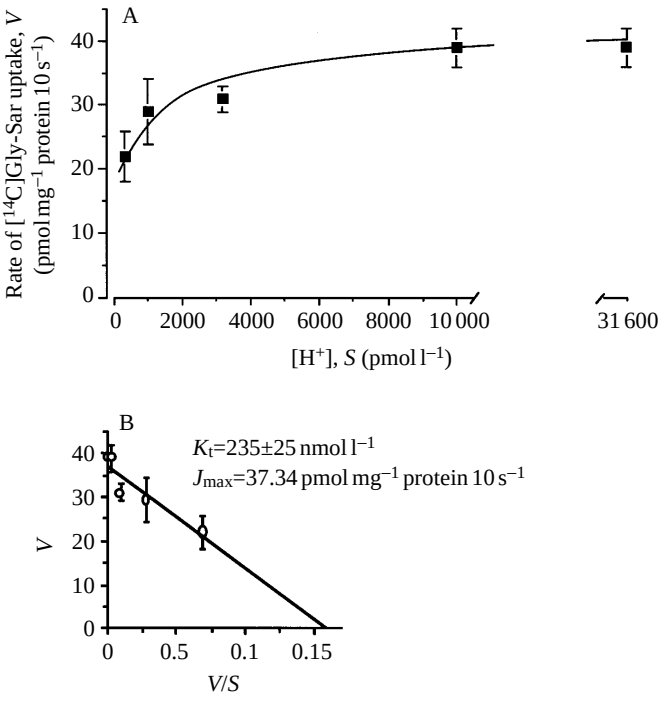

Fig. 6. (A) Effect of external proton concentration on 0.1 mmol l^{-1} [^{14}C]Gly-Sar influx into hepatopancreatic BBMVs. Vesicles were preloaded as described for Figs 4 and 5 and were incubated for 10 s in external media containing 100 mmol l^{-1} KCl, 200 mmol l^{-1} mannitol and either 20 mmol l^{-1} Hepes/Tris or 20 mmol l^{-1} Mes/Tris at pH 7.5, 6.5, 6.0, 5.5 or 4.5. (B) An Eadie–Hofstee plot of the same data; the line on the figure was drawn using linear regression analysis.

Discussion

This report demonstrates, for the first time, that the epithelium of the crustacean hepatopancreas has the ability to transport dipeptides. The results show that a transmembrane proton gradient provides the driving force for dipeptide transport in lobster hepatopancreatic BBMVs. Downhill movement of protons along their electrochemical gradient providing a driving force for electrogenic dipeptide uptake has been demonstrated in mammalian (Ganapathy and Leibach, 1983) and teleost (Verri *et al.* 1992; M. Thamotharan, J. Gomme, V. Zonno, M. Maffia, C. Storelli and G. A. Ahearn, in preparation) intestinal epithelia. Our data also showed that [^{14}C]Gly-Sar transport in lobster hepatopancreas BBMVs was electrogenic and that this electrogenicity was likely to be due to cotransport of a proton with an electroneutral peptide.

Lobster hepatopancreas BBMVs exhibited a high-capacity, low-affinity carrier with an apparent affinity constant (K_t) of 5.90±0.13 mmol l^{-1}. Previously, high- and low-affinity transport carriers for different peptides have been demonstrated in mammals (Daniel *et al.* 1991; Skopicki *et al.*

1991) and teleosts (M. Thamotharan, J. Gomme, V. Zonno, M. Maffia, C. Storelli and G. A. Ahearn, in preparation). Because the lobster is an omnivore, it is possible that its absorptive epithelium is exposed to high concentrations of dipeptides. For this reason, it would be advantageous for such an animal to have a low-affinity, high-capacity carrier that would enable it to absorb large quantities of dipeptides as they are broken down and released from proteins by proteases. This low-affinity K_t value quantitatively resembles reported values for analogous low-affinity carriers described in the intestinal tissues of carnivorous mammals (Mathews, 1991).

There are a number of studies that have focused on the effect of pH on the absorption of nutrients by the lobster hepatopancreas. A reduction in external vesicular pH has been shown to stimulate both glucose (Ahearn *et al.* 1985) and amino acid (Ahearn and Clay, 1988) transport in hepatopancreatic BBMVs. These studies found that the effects of pH on the uptake of these two classes of nutrients were different. Amino acids were protonated at low pH and became the preferred substrate for transport, whereas protonation of the glucose carrier resulted in an increased binding affinity of the carrier for glucose, leading to increased glucose uptake (Ahearn *et al.* 1985). In contrast, inositol transport by hepatopancreatic epithelium was shown to be maximal at pH values near neutrality (Siu and Ahearn, 1988), suggesting that there might be preferential nutrient absorption at different pH values by this animal as digestion of a meal proceeds.

Our study with dipeptides shows that lobster hepatopancreatic BBMVs possess a peptide transporter with a relatively high-affinity proton binding site (K_H= 235±25 nmol l^{-1}; pK=6.6) and an apparent 1:1 coupling stoichiometry between protons and Gly-Sar during transfer. In these animals, sea water (pH 8.2) is ingested during food intake. This can cause the gut luminal pH to rise from pH 4–5 prior to food intake towards a neutral pH (and hence lower proton concentration). As peptides would be early breakdown products during protein digestion, it would be beneficial to have peptide carriers with slightly acidic proton binding sites that would enable efficient absorption of peptides even at relatively low proton concentrations. At the slightly acidic conditions that would probably prevail in hepatopancreatic tubules during peptide digestion, it is likely that both a transmembrane proton gradient and a dipeptide gradient combine as driving forces for the net uptake of the nutrient into the cell.

The results reported here show that there was significant inhibition of 0.1 mmol l^{-1} [^{14}C]Gly-Sar influx into lobster hepatopancreatic BBMVs by a 10 mmol l^{-1} concentration of several other dipeptides and by the known dipeptide transport inhibitor DEP (Fig. 5). Investigations using ^{14}C-labelled Gly-Sar or Gly-Pro have indicated that both share the same transport system in rabbit intestine (Ganapathy *et al.* 1984), rabbit kidney (Ganapathy and Leibach, 1983) and teleost intestine (M. Thamotharan, J. Gomme, V. Zonno, M. Maffia, C. Storelli and G. A. Ahearn, in preparation). The findings reported here for the lobster hepatopancreatic epithelium, illustrating significant inhibition of Gly-Sar uptake by several other dipeptides, suggest that a broadly specific, shared peptide carrier is likely to be involved in the transport of these substrates in this tissue. The possible inclusion of *trans*-stimulatory effects by unlabelled Gly-Sar or other test dipeptides on [^{14}C]Gly-Sar influx also suggests that all these compounds may share a common transport system. The presence of an epithelial Gly-Sar transporter shared by a number of other neutral dipeptides has been demonstrated in mammals (Mathews, 1991).

In summary, this study is the first to describe the characteristics of a crustacean hepatopancreatic dipeptide transport mechanism. This class of nutrients in invertebrates deserves further research in order to assess the nature of the contribution that dipeptides make to invertebrate nutrition and development.

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References

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

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

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.* **32**, R472–R481.

AHEARN, G. A., GROVER, M. L. AND DUNN, R. E. (1985). Glucose transport by lobster hepatopancreatic brush border membrane vesicles. *Am. J. Physiol.* **248**, R133–R141.

DANIEL, H., MORSE, E. L. AND ADIBI, S. A. (1991). The high and low affinity transport system for dipeptides in kidney brush border membrane respond differently to alterations in pH gradient and membrane potential. *J. biol. Chem.* **263**, 19917–19924.

GANAPATHY, V., BURCKHARDT, G. AND LEIBACH, F. H. (1984). Characteristics of glycylsarcosine transport in rabbit intestinal brush border membrane vesicles. *J. biol. Chem.* **259**, 8954–8959.

GANAPATHY, V. AND LEIBACH, F. H. (1983). Role of pH gradient and membrane potential in dipeptide transport in intestinal and renal brush border membrane vesicles from the rabbit. Studies with L-carnosine and glycyl-L-proline. *J. biol. Chem.* **258**, 14189–14192.

GARDNER, M. L. G. (1984). Intestinal assimilation of intact peptides and proteins from the diet – A neglected field? *Biol. Rev.* **59**, 289–331.

GIBSON, R. AND BARKER, P. L. (1979). The decapod hepatopancreas. *Oceanogr. mar. Biol. A. Rev.* **17**, 285–346.

HOPFER, U., NELSON, K., PERROTTO, J. AND ISSELBACHER, K. J. (1973). Glucose transport in isolated brush border membrane from rat small intestine. *J. biol. Chem.* **243**, 25–32.

MATHEWS, D. M. (1991). *Protein Absorption – Development and Present State of the Subject*. New York: Wiley-Liss.

MIYAMOTO, Y., GANAPATHY, V. AND LEIBACH, F. H. (1986).

Identification of histidyl and thiol groups at the active site of rabbit renal dipeptide transporter. *J. biol. Chem.* **261**, 16133–16140.

SEGEL, I. H. (1975). *Enzyme Kinetics*. New York: John Wiley and Sons, Inc.

SIU, T. AND AHEARN, G. A. (1988). Inositol transport by hepatopancreas brush border membrane vesicles of the lobster *Homarus americanus*. *J. exp. Biol.* **140**, 107–121.

SKOPICKI, H. A., FISHER, K., ZIKOS, D., BLOCH, R., FLOURET, G. AND PETERSON, D. R. (1991). Multiple carriers for dipeptide transport: carrier-mediated transport of glycyl-L-proline in renal BBMV. *Am. J. Physiol.* **261**, F670–F678.

TURNER, R. J. (1983). Quantitative studies of cotransport systems: Models and vesicles. *J. Membr. Biol.* **76**, 1–15.

VERRI, T., MAFFIA, M. AND STORELLI, C. (1992). H^+/glycyl-glycine cotransport in eel intestinal brush border membrane vesicles: Studies with pH-sensitive dye acridine orange. *Biochim. biophys. Acta* **1110**, 123–126.