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*

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We examined the role of pH gradient and membrane potential in dipeptide transport in purified intestinal and renal brush-border membrane vesicles which were predominantly oriented right-side out. With an intravesicular pH of 7.5, changes in extravesicular pH significantly affected the transport of glycyl-L-proline and L-carnosine, and optimal dipeptide transport occurred at an extravesicular pH of 5.5-6.0 in both intestine and kidney. When the extravesicular pH was 5.5, glycyl-L-proline transport was accelerated 2-fold by the presence of an inward proton gradient. A valinomycin-induced K+ diffusion potential (interior-negative) stimulated glycyl-L-proline transport, and the stimulation was observed in the presence and absence of Na+. A carbonyl cyanide p-trifluoromethoxyphenylhydrazone-induced H+ diffusion potential (interior-positive) reduced dipeptide transport. It is suggested that glycyl-L-proline and proton(s) are cotransported in intestinal and renal brush-border membrane vesicles, and that the process results in a net transfer of positive charge.

Transport of di- and tripeptides has been demonstrated in the mammalian small intestine using a variety of intact tissue preparations. These studies have shown that intestinal peptide transport is Na+-dependent and occurs against a concentration gradient (1). Active transport of intact peptide in the mammalian kidney has also been demonstrated in vivo experiments, although the role of Na+ in the process was not examined (2). It was generally believed that the Na+ gradient across the brush-border membrane of the intestinal and renal epithelial cells is the driving force for the uphill transport of peptides, as is the case with sugars and amino acids. Recent studies with purified intestinal and renal brush-border membrane vesicles have demonstrated the Na+ gradient as the energy source in the active transport of sugars and amino acids (3), but similar studies have failed to demonstrate a role for a Na+ gradient in the transport of peptides (4). This apparent discrepancy can be explained if a hitherto unrecognized energy source other than the Na+ gradient is involved in driving the uphill transport of peptides in vivo in the mammalian intestine and kidney. In the present paper, we examined the role of pH gradient and membrane potential in the transport of Gly-Pro1 in purified intestinal and renal brush-border membrane vesicles.

EXPERIMENTAL PROCEDURES

Methods—The brush-border membrane vesicles were prepared from rabbit small intestine and renal cortex as described previously (5). In experiments in which the intravesicular medium was varied, the membrane vesicles were preloaded by resuspending the initial 43,000 × g pellet and carrying out the entire washing procedure in the desired medium. Transport measurements were performed by a rapid filtration technique using 0.45-µm Mericell membrane filters. The specific conditions for each experiment are given in the figure legends. Protein concentration in membrane suspensions was determined by Lowry assay with bovine serum albumin as the standard.

The purity of labeled dipeptides was checked by paper chromatography (Whatman No. 1; 14-h run) using n-butyl alcohol/acetic acid/water (4:1:1, by volume) as the solvent system. The radioactive spots on the paper were detected using a Packard radiochromatogram scanner.

Materials—Valinomycin and FCCP were purchased from Sigma. [1,14C]Glycyl-L-proline (specific radioactivity, 6.4 mCi/mmol) was obtained from the Radiochemical Center, Amersham, England. L-[2-3H]alanine-L-[1-14C]Carnosine (specific radioactivity, 11.4 mCi/mmol) was a gift from Dr. R. A. Roesel of this department. D-[U-14C]Glucose (specific radioactivity, 3.95 mCi/mmol) was from New England Nuclear. All labeled compounds were chromatographically pure. The membrane filters (pore size, 0.45 µm) were obtained from Gelman Sciences, Inc., Ann Arbor, MI.

RESULTS

Effect of Extravesicular pH on Gly-Pro and L-Carnosine Transport—The transport of Gly-Pro and L-carnosine in intestinal and renal brush-border membrane vesicles with internal pH 7.5 was measured as a function of external pH. These experiments were performed in the presence of intravesicular K+ (100 mM) and extravesicular Na+ (100 mM). The transport of both peptides in intestinal brush-border membrane vesicles was significantly affected by external pH (Fig. 1), with optimal peptide transport occurring at pH 5.5-6.0. As shown in Fig. 2, the transport of these peptides in renal brush-border membrane vesicles was also affected by external pH, although the effect was less pronounced compared to membrane vesicles from intestine. The optimal external pH for both peptides in kidney was, however, the same (pH 5.5-6.0) as in intestine.

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1 The abbreviations used are: Gly-Pro, glycyl-L-proline; FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazone, Heps, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; Mes, 2-(N-morpholino)ethanesulfonic acid.
In another experiment, the time course of Gly-Pro transport was studied at two different external pH values, 5.5 and 7.5, with the external pH fixed at 7.5. Both in intestine and kidney, the transport remained greater over a period of 30 min at external pH 5.5 compared to the transport at external pH 7.5 (Fig. 3).

There are two ionizable groups in Gly-Pro and three in L-carnosine. The concentration of the different ionic species of these dipeptides in solution would depend on pH. Since the rate of transport is a function of the concentration of the transportable ionic species, the external pH would be expected to influence the transport of Gly-Pro and L-carnosine. The data given in Figs. 1-3 clearly show that, with a fixed internal pH, the external pH has a marked effect on the transport of these dipeptides both in intestinal and renal brush-border membrane vesicles. However, the interpretation of the data from these experiments is made difficult because, in addition to external pH, the transmembrane pH gradients also vary in these experiments. In order to obtain specific information on the role of a transmembrane pH gradient in dipeptide transport, we compared the transport of Gly-Pro in the presence and absence of transmembrane proton gradients, but with a fixed external pH. The experiments were performed in the absence of both Na⁺ and K⁺. The results are given in Table I. With external pH 5.5, the presence of an inward proton gradient ([H⁺]o > [H⁺]i) stimulated Gly-Pro transport by 70% in intestinal brush-border membrane vesicles and by 108% in renal brush-border membrane vesicles. In the absence of a transmembrane proton gradient, the transport of Gly-Pro was almost the same at external pH 5.5 and 7.5 in both intestine and kidney. These results suggest that the increased dipeptide transport observed in previous experiments (Figs. 1-3) at external pH 5.5 compared to external pH 7.5 was due to the presence of an inward proton gradient rather than the change in external pH per se.

Role of Membrane Potential in Gly-Pro Transport—The effect of a K⁺ diffusion potential (interior-negative) generated by valinomycin on Gly-Pro transport was studied in renal brush-border membrane vesicles in the presence of an inward Na⁺ gradient. In this experiment, the membrane vesicles were preloaded with K₂SO₄, and the transport was initiated by adding 40 μl of the membrane suspension to 200 μl of the transport buffer containing Na₂SO₄. This resulted in an outward-directed K⁺ gradient. Addition of valinomycin under these conditions resulted in a 2-fold increase in Gly-Pro transport (Fig. 4). Since valinomycin generated an interior-negative membrane potential under the experimental conditions, the results indicate that Gly-Pro transport in renal brush-border membrane vesicles results in a net transfer of positive charge. Since Gly-Pro transport in renal brush-border membrane vesicles is Na⁺-independent (5), the experiment was also performed in the total absence of Na⁺. The K⁺ diffusion potential (interior-negative) produced by valinomycin stimulated Gly-Pro transport even in the absence of Na⁺.
Peptide Transport in Renal and Intestinal Membrane Vesicles

FIG. 4 (left). Effect of valinomycin-induced K+ diffusion potential (inside-negative) on Gly-Pro (0.3 mM) transport in renal brush-border membrane vesicles. The vesicles were preloaded with 5 mM Tris/Hepes buffer, pH 7.5, containing 100 mM K2SO4. Transport was assayed in 5 mM Tris/Hepes buffer, pH 7.5, containing either 300 mM mannitol (squares) or 106 mM Na2SO4 (circles). Valinomycin dissolved in absolute ethanol was added to the membrane suspension, with the addition of ethanol alone to the control. Final concentrations in the incubation medium of valinomycin and ethanol were 10 pg/mg of membrane protein and 0.17%, respectively (closed symbols), or 0.17% ethanol alone (open symbols).

FIG. 5 (right). Effect of valinomycin-induced K+ diffusion potential (inside-negative on Gly-Pro (0.3 mM) transport in intestinal brush-border membrane vesicles. The experimental details were as given in the legend for Fig. 4.

FIG. 6. Effect of FCCP-induced H+ diffusion potential (inside-positive) on D-glucose (30 mM) and Gly-Pro (0.3 mM) transport in intestinal brush-border membrane vesicles. The vesicles were preloaded with 40 mM Tris/Hepes buffer, pH 7.5, containing 75 mM K2SO4. Transport was assayed in 12 mM Tris/Mes buffer, pH 5.5, containing 75 mM Na2SO4. FCCP dissolved in absolute ethanol was added to the membrane suspension, with the addition of ethanol alone to the control. Final concentrations of FCCP and ethanol were 40 μM and 0.5%, respectively (closed symbols), or 0.5% ethanol alone (open symbols). The results obtained with intestinal brush-border membrane vesicles were essentially the same. Gly-Pro transport was significantly greater in mannitol medium than in NaCl medium.

The results obtained with intestinal brush-border membrane vesicles were essentially the same (Fig. 5). Gly-Pro transport in intestinal brush-border membrane vesicles is electrogenic, and an interior-negative membrane potential stimulated dipeptide transport both in the presence and absence of Na+. However, there was a significant difference between the two systems. The transport of Gly-Pro was the same in NaCl medium and in mannitol medium in renal brush-border membrane vesicles. But in intestinal brush-border membrane vesicles, Gly-Pro transport was significantly greater in mannitol medium than in NaCl medium.

Valinomycin did not have any effect on Gly-Pro transport in intestinal and renal brush-border membrane vesicles in the absence of K+ (data not shown).

The effect of a H+ diffusion potential (interior-positive) generated by the protonophore FCCP on Gly-Pro transport was studied in intestinal and renal brush-border membrane vesicles. The vesicles were preloaded with 40 mM Tris/Hepes buffer, pH 7.5, containing 75 mM K2SO4. Transport was measured in 12 mM Tris/Mes buffer, pH 5.5, containing 75 mM Na2SO4, in the presence and absence of 40 μM FCCP. The results obtained with intestinal brush-border membrane vesicles are given in Fig. 6. Essentially the same results were obtained with renal brush-border membrane vesicles (data not shown). That FCCP generates an interior-positive membrane potential in intestinal and renal brush-border membrane vesicles under these conditions is evident from the finding that the Na+ gradient-dependent D-glucose transport in these vesicles was reduced by FCCP. Under similar conditions, Gly-Pro transport was also significantly reduced by FCCP, further strengthening the idea that Gly-Pro transport is coupled to a net transfer of positive charge across the membrane.

DISCUSSION

There is considerable controversy over the exact role of Na+ in the transport of intact peptides. Many studies have demonstrated the dependence of peptide transport on Na+, and since the transport was active and Na+-dependent, these studies have led to the belief that, in an obvious analogy to sugars and amino acids, peptides are cotransported with Na+, and the Na+ gradient provides the energy for the uphill transport (1). However, recent investigations using intact tissue preparations as well as purified brush-border membrane
The Na\(^+-\)independent nature of peptide transport has been shown for a variety of dipeptides such as glycyl-L-leucine (6-8), L-leucylglycine (7), L-carnosine (9, 10), glycyl-L-phenylalanine (11), and glycyl-L-proline (5, 12).

The role of the membrane potential in renal and intestinal transport of sugars and amino acids has been studied in great detail (3). However, studies on the effects of small peptides on membrane potential are limited. Recently, Boyd and Ward (13) have examined the effects of some amino acids and dipeptides on the electrical properties of the brush-border membrane of the small intestine of the mud puppy, *Necturus maculosus*, using microelectrodes. They showed that L-carnosine, glycyl-L-proline, L-leucyl-L-leucine, and glycglycine caused an immediate and reversible depolarization of the brush-border membrane and that this depolarization was not due to amino acids resulting from surface hydrolysis of peptides. The peptide-induced depolarization, in contrast to the amino acid-induced depolarization, was observed even in a Na\(^+-\)free medium.

Gly-Pro exists predominantly as a dipolar ion with a net zero ionic charge at pH values 5.5 and 7.5 (14). Under these conditions, the transport of the dipeptide in intestinal and renal brush-border membrane vesicles is mediated by a process which results in a net transfer of positive charge across the membrane. Therefore, it appears that the flow of another ionic species is associated with the entry of Gly-Pro into membrane vesicles. In contrast to sugar and amino acid transport, Gly-Pro transport is totally Na\(^+-\)independent. In fact, the presence of an inward Na\(^+\) gradient strongly inhibited the dipeptide transport, at least in intestinal brush-border membrane vesicles. Since Gly-Pro transport was stimulated by an interior-negative membrane potential produced by valinomycin even in the absence of Na\(^+\), it follows that the dipeptide is transported with a cation other than Na\(^+\). The presence of an inward proton gradient accelerated the transport of Gly-Pro in the membrane vesicles. That the proton gradient is maintained in these vesicles under the experimental conditions is evident from the finding that FCCP reduced the Na\(^+-\)dependent D-glucose transport. These results strongly suggest that Gly-Pro and proton(s) are cotransported in renal and intestinal brush-border membrane vesicles.

The data from previous studies (for a review, see Ref. 4) show that carrier-mediated transport of dipeptides can be demonstrated in purified renal and intestinal brush-border membrane vesicles in the absence of transmembrane proton gradients. In the present study, we show that dipeptide transport in membrane vesicles can be stimulated by an inward proton gradient ([H\(^+\)]\(_i\) > [H\(^+\)]\(_o\)]. The existence of an inward proton gradient across brush-border membranes of intestinal and renal epithelial cells in *vivo* is indicated since the luminal pH is acidic compared to intracellular pH. There exists a Na\(^+-\)H\(^+\) antiport system in both intestinal and renal brush-border membrane vesicles (15) which mediates the uphill efflux of protons coupled to the downhill influx of sodium. This Na\(^+-\)H\(^+\) antiport is at least partially responsible for maintaining the proton gradient across the brush-border membrane of these epithelial cells. It is possible that the inward proton gradient is the *in vivo* driving force for active transport of intact peptides in the small intestine as well as kidney. The partial dependence of peptide transport on Na\(^+\) observed in intact tissue preparations may be related to this Na\(^+-\)H\(^+\) antiport. The presence of an inward Na\(^+\) gradient stimulates the Na\(^+-\)H\(^+\) antiport (16), thus maintaining the inward proton gradient which in turn drives dipeptide transport. In intact tissue preparations, (Na\(^+-\)K\(^+)\)-ATPase, present at the basal-lateral membrane of intestinal and renal epithelial cells, actively maintains the inward Na\(^+\) gradient. In purified brush-border membrane vesicles, the experimentally produced Na\(^+\) gradient dissipates rapidly because of the absence of (Na\(^+-\)K\(^+)\)-ATPase and, therefore, any dependence of dipeptide transport on Na\(^+\) could not be observed in these preparations. Since the cotransport of dipeptide and proton(s) is electrogenic, the presence of an inward Na\(^+\) gradient in membrane vesicles depolarizes the membrane which results in the inhibition rather than stimulation of dipeptide transport.

There is increasing evidence suggesting the presence of multiple dipeptide transport systems, at least, in small intestine (17). Therefore, it remains to be seen whether the observed stimulation of Gly-Pro transport by an inward proton gradient can be demonstrated for other dipeptides.

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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.

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