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Characteristics of tripeptide transport in human jejunal brush-border membrane vesicles

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These studies are aimed at characterizing the transport of the tripeptide, glycylglycyl-L-proline (GlyGlyPro) across human jejunal brush-border membrane vesicles. GlyGlyPro (0.65 mM) was hydrolyzed by brush-border membrane vesicles with the extent of hydrolysis per mg protein being 23% at 0.5 min, 57% at 1 min and complete hydrolysis at 60 min. Treatment of the membrane vesicles with gel-complexed papain (to remove membrane peptidases) resulted in minimal hydrolysis of GlyGlyPro up to 10 min of incubation. Measurement of GlyGlyPro influx with papain-treated vesicles in the presence of increasing medium osmolarity showed that uptake occurred into an osmotically reactive intravesicular space. Transport of GlyGlyPro with normal and papain-treated membrane vesicles was similar in the presence of an inward Na^+ or K^+ gradient. No overshoot phenomenon was observed in the presence of an inward proton gradient (extravesicular pH 5.5; intravesicular pH 7.5). An interior negative membrane potential induced by a K^+ diffusion potential in the presence of valinomycin stimulated the uptake of the peptide. The effect of increasing concentrations on initial rates of GlyGlyPro uptake revealed the presence of a saturable component as well as a diffusional component. Preloading the membrane vesicles with 20 mM glycylsarcosylsarcosine stimulated uptake by 4-fold. Uptake of GlyGlyPro was inhibited greater than 50% by dipeptides and tripeptides and less than 15% by free amino acids. These results indicate that GlyGlyPro uptake in jejunal brush-border membrane vesicles is not energized by a Na^+ or proton gradient and that transport occurs by carrier-mediated and diffusional processes.

Introduction

Intestinal peptide absorption plays a major role in human dietary protein assimilation. Studies on intestinal peptide absorption have been carried out employing in vivo and in vitro procedures [1–3]. Recently, purified brush-border membrane vesicles (BBMV) have been used to characterize intestinal peptide transport. Various dipeptides have been used to elucidate the transport mechanisms of peptides with BBMV [4–11]. Although the investigations have studied influx of dipeptides using rat, rabbit and human intestinal BBMV, there is considerable controversy concerning transport characteristics [4–11]. Studies in our laboratory using murine, rabbit and human BBMV indicate that the dipeptide, glycyl-L-proline, is transported by a Na^+-independent, nonconcentrative, carrier-mediated process [7]. Ganapathy et al., primarily using rabbit intestinal BBMV, suggested that peptide uptake is by an electrogenic H^+/peptide cotransport system [10,12].

Intestinal tripeptide influx has not been studied as extensively as dipeptide influx, primarily because of a lack of available radiolabeled tripeptides. Transport of glycylsarcosylsarcosine (GlySarSar) in hamster jejunal rings in vitro was shown to be active [2]. In humans, tripeptide absorption has been examined using in vivo perfusion methods [13–16], but tripeptide uptake using human intestinal BBMV has not been examined. Therefore, in an attempt to gain additional information on the transport mechanism of tripeptides, intestinal influx of glycylglycyl-L-proline (GlyGlyPro) into human BBMV was examined. Initial work indicated that GlyGlyPro was hydrolyzed, but papain treatment of the

Abbreviations: GlyGlyPro, glycylglycyl-L-proline; BBMV, brush-border membrane vesicles; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; GlySarSar, glycylsarcosylsarcosine; TMA, tetramethylammonium, Mes, 2-(*N*-morpholino)ethanesulfonic acid.

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BBMV reduced hydrolysis. Using the papain model, we demonstrated that GlyGlyPro was transported by a Na^+-independent, nonconcentrative, carrier-mediated process.

Materials and Methods

Preparation of BBMV. Approval of human studies was granted by the Human Research Review Committee of the Medical College of Wisconsin and the Zablocki Veteran Administration Medical Center. After obtaining the small intestine from organ donors maintained on life support systems, the tissue was rinsed with normal saline, cut longitudinally and the mucosa was scraped from the muscular layer. All processing of the tissue was done at temperatures less than 5°C. The mucosa was parafilmed airtight and frozen at −70°C until use. Studies in our laboratory have shown that the sucrase enrichment factor is 19–20 fold in purified human BBMV as compared with the crude mucosal homogenate [6].

Transport studies. Jejunal brush-border membranes were isolated by the $CaCl_2$ precipitation method of Schmitz et al. [17] and BBMV obtained by modification of the method of Hopfer et al. [18] as described by us earlier [19,20].

To load the vesicles with the appropriate intravesicular buffer, the pellet obtained from the centrifuged $CaCl_2$-treated supernatant was resuspended in the desired volume of the buffer with a tuberculin syringe and a 26-gauge needle. This solution was homogenized with a Potter-Elvejhem homogenizer and later subjected to differential centrifugation in the same intravesicular buffer according to previous methods [20]. The final pellet was resuspended in the same buffer to a protein concentration of 5–10 mg/ml. This suspension was then used for the papain digestion procedure as given below.

Papain digestion. Treatment of BBMV with gel-complexed papain was done using modification of the method of Berteloot et al. [4]. The gel-complexed papain was prepared as follows: 2 ml of Affi-Gel 10 (Bio-Rad) was washed on a Buchner funnel to remove the solvent, isopropanol, and then dried. The ligand solution was prepared by adding 5 ml of 0.1 M phosphate buffer (pH 7.0), 2 ml papain (two times crystallized in suspension in 0.05 M sodium acetate, from Sigma, spec. act. 25 U/mg protein) and 2 g of Affi-Gel 10. This mixture was stirred for 12 to 14 h at 4°C to allow coupling. Approx. 0.1 vol. of 1 M ethanolamine-HCl (pH 8.0) was added to block the unreacted sites, and this solution was stirred for an additional hour at 4°C. The mixture was washed on a Buchner funnel with 0.1 M phosphate buffer (pH 7.0) to remove reactants as verified by determination of absorbance at 260 nm on a Gilford Spectrophotometer (Model 260). The mixture was later washed with 100 ml 0.1 M phosphate buffer (pH 6.5) dried and weighed. The gel-complexed papain activity was assayed by a titrimetric determination of the acid produced during hydrolysis of benzoylarginyl ethyl ester. The papain was resuspended with 0.1 M phosphate buffer (pH 6.5) to give 25 U papain/ml buffer.

BBMV were incubated at 37°C in a shaking water bath for 10 min. The digestion medium consisted of 2 mM cysteine (pH 6.5)/1.5 mM EDTA (pH 6.5)/16.6 mM K_2HPO_4 (pH 6.5)/247 mM mannitol/10–12 U papain/mg protein. Digestion was stopped by filtration under vacuum through a Whatman paper (No. 4). The mixtures were centrifuged for 5 min at $2000 \times g$ to remove any remaining papain. The supernatant was centrifuged for 25 min at $31\,000 \times g$. The pellet was resuspended in the intravesicular buffer and homogenized with the Potter-Elvejhem homogenizer (six strokes) and centrifuged for 30 min at $31\,000 \times g$. The resulting pellet was resuspended to a final protein concentration of 1–2 mg/ml with the specific resuspension buffer. The conditions for papain digestion were established by determining solubilization of membrane oligopeptidases and glucose transport activity.

The vesicles were used for transport studies approx. 30 min after the final resuspension. This resuspension procedure used in our laboratory has been shown to result in membrane vesicles with the desired intravesicular medium [21]. An aliquot of the vesicles was added to the incubation media at room temperature (23°C) to initiate the transport studies. At various time intervals, 50-μl aliquots were removed from the incubation mixtures and transferred to 1 ml ice-cold stop solution (isoosmolar to incubation medium and containing L-[^{3}H]glucose to correct for nonspecific binding to the filters and insufficient washing of the filters). The mixture was filtered on prewetted 0.45 μm membrane filters. The filters were then washed with 5 ml of ice-cold stop solution and processed for counting as previously described. Protein was assayed by the method of Lowry et al. [22] using serum albumin as the standard. The composition of the various incubation media is given in the legend for the tables and figures.

Results are expressed as picomoles of GlyGlyPro uptake per milligram protein after correction of GlyGlyPro binding to the filters. This was done by filtering an aliquot of the incubation medium without the vesicles and processing the filters in the conventional manner for counting.

All experiments were performed in triplicates on three different BBMV preparations. The variations of the triplicates were always ±5% of the mean value. Although the studies yielded qualitatively similar results, only results of typical experiments are given due to differences in the equilibrium uptakes.

Materials. [1-^{14}C]Glycylglycyl-L-proline (GlyGlyPro) (spec. act. 9.8 mCi/mmol) was obtained from Amersham

(Arlington Heights, IL). D-[U-^{14}C]Glucose (spec. act. 265 mCi/mmol) and L-[^{3}H]glucose (20 Ci/mmol) were obtained from New England Nuclear (Boston, MA).

The membrane filters (pore size 0.45 μm) were obtained from Sartorious Filters (Hayward, CA). Papain, valinomycin, carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP), and all other chemicals were obtained from Sigma (St. Louis, MO) and were of the highest purity available. GlyGlyPro was obtained from Research Plus, Bayonne, NJ.

Membrane hydrolysis. Membrane hydrolysis was determined by using similar ionic media used for the various uptake studies (see legends for specific details). Aliquots (50 μl) were removed at 0.5, 1, 10 and 60 min and transferred immediately to 1.0 ml of boiling water. The solutions were concentrated using SEP-PAK C18 cartridges (Waters Associates, Milford, MA), lyophilized (Savant Speed Vac Concentrator, Hicksville, Long Island, NY) and analyzed on a Waters high-pressure liquid chromatography amino-acid system (Waters). Fractions corresponding to glycine and GlyGlyPro were collected using a fraction collector (ISCO Foxy, Lincoln, NE) and the radioactivity of the samples was determined. Radioactive [1-^{14}C]GlyGlyPro was used to standardize the HPLC method.

Intravesicular hydrolysis. The membrane vesicles were added to an incubation medium containing 100 mM choline chloride (ChCl)/100 mM mannitol/10 mM Hepes-Tris (pH 7.5)/0.65 mM [1-^{14}C]GlyGlyPro. The membrane filters obtained at 0.4 and 5 min incubation were added to 25 ml boiling water. A control was done by adding the filtered incubation medium and a calculated amount of GlyGlyPro equivalent to the transported amount to 25 ml boiling water. This control served to correct for any peptide hydrolysis during the procedure. The extracts were centrifuged at 35 000 $\times$ *g* for 60 min to remove membrane particles and debris. The supernatant solution was processed as mentioned above for membrane hydrolysis. Intravesicular hydrolysis was determined by analyzing collected fractions corresponding to glycine and GlyGlyPro.

Results

Hydrolysis of GlyGlyPro in control and papain-treated vesicles

Hydrolysis of GlyGlyPro under conditions used for transport was determined by measuring [^{14}C]glycine released from the peptide in the incubation medium at 0.5, 1, 10 and 60 min. There was 23% hydrolysis per mg protein at 0.5 min, 57% at 1 min and complete hydrolysis by 60 min. Treatment of the BBMV with gel-complexed papain showed only a 9% hydrolysis per mg protein at 10 min, but 100% hydrolysis at 60 min (Table I). The papain concentration used caused a 50–60% release of membrane tripeptidase activity (determined with phenylalanylglycylglycine as the substrate). Further, there was no change (in comparison to control vesicles) in glucose transport activity which is used primarily to monitor vesicular integrity and functionality.

TABLE I

Membrane hydrolysis of [1-^{14}C]GlyGlyPro in control and papain-treated BBMV

The membrane vesicles were preloaded with 100 mM ChCl/100 mM mannitol/10 mM Tris-Hepes (pH 7.5). The vesicles were added to a medium containing 100 mM ChCl/100 mM mannitol/10 mM Tris-Hepes (pH 7.5)/0.65 mM [1-^{14}C]GlyGlyPro. Hydrolysis was measured as described under Materials and Methods. Values are expressed as percent hydrolysis per mg BBMV protein. The amount of BBMV protein used was 0.8 mg for control vesicles and 0.6 mg for papain vesicles in 1 ml of incubation medium. Results represent the mean ± S.E. of five determinations.

Time (min)	Hydrolysis (%)	
	control	papain
0.5	22.8 ± 2.4	0
1.0	56.6 ± 8.1	0
10.0	100.0 ± 12.7	9.2 ± 1.8
60.0	100.0 ± 00.0	100.0 ± 4.4

Analysis of intravesicular contents for papain-treated vesicles

No [^{14}C]glycine was detected in the intravesicular medium after 0.4 and 5 min of incubation.

GlyGlyPro uptake with increasing medium osmolarity

Uptake of GlyGlyPro in papain-treated vesicles after 5 min of incubation with increasing osmolarity is shown in Fig. 1. Transport of GlyGlyPro decreased with increasing medium osmolarity, and when extrapolated to

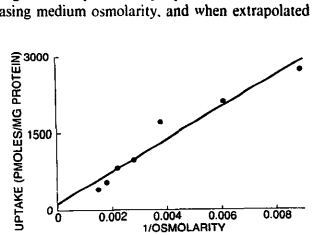

Fig. 1. Uptake of [1-^{14}C]GlyGlyPro as a function of the medium osmolarity. Membrane vesicles were preincubated with 25 mM ChCl/50 mM cellobiose/10 mM Tris-Hepes (pH 7.5). Uptake studies were done in media containing 25 mM ChCl/10 mM Tris-Hepes (pH 7.5), 1 mM GlyGlyPro and concentrations ranging from 50-600 mM of cellobiose to give the desired medium osmolarity. Values represent the mean of determinations at 5 min of incubation. A linear regression program was used to fit the data and the coefficient of determination was 0.9712.

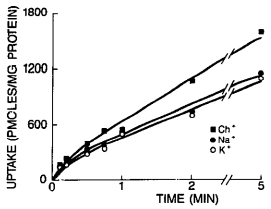

Fig. 2. Transport of [1-^{14}C]GlyGlyPro by jejunal papain-treated BBMV in the presence of a Na^+ or K^+ gradient. The BBMV were preincubated in 100 mM ChCl/100 mM mannitol/10 mM Tris-Hepes buffer (pH 7.5). The vesicles were assayed in a medium containing 100 mM mannitol/10 mM Tris-Hepes (pH 7.5)/0.65 mM GlyGlyPro and either 100 mM ChCl, ■, 100 mM NaCl ● or 100 mM KCl, ○.

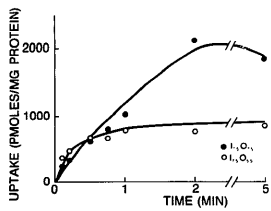

Fig. 3. Uptake of [1-^{14}C]GlyGlyPro by jejunal BBMV in the presence of a proton gradient (out > in). Papain-treated vesicles were equilibrated in 100 mM TMA gluconate/100 mM mannitol/50 mM Tris-Hepes (pH 7.5). The vesicles were added to a medium containing 100 mM TMA gluconate/100 mM mannitol/0.65 mM GlyGlyPro and 50 mM Tris-Hepes (pH 7.5) ● or 50 mM Tris-Mes buffer (5.5) ○. Osmolarity of the media was adjusted with mannitol when Tris-Mes buffer (pH 5.5) was used.

infinite osmolarity, uptake was negligible. This indicated that GlyGlyPro was transported into an osmotically reactive intravesicular space without significant binding to the surface of the vesicles.

Transport of GlyGlyPro in the presence of ionic gradients

The effect of ionic gradients on GlyGlyPro uptake was similar for control and papain vesicles. Therefore, only the results of the papain-treated vesicles will be shown. GlyGlyPro transport was similar in the presence of an inwardly directed Na^+ or K^+ gradient (Fig. 2). There was an increase in GlyGlyPro uptake with time, but transport was lower in the presence of the Na^+ and K^+ gradients than with the control ($ChCl_i = ChCl_o$) vesicles. To study the effect of an inwardly-directed proton gradient (out > in), BBMV were preincubated with 100 mM TMA gluconate, 100 mM mannitol, 50 mM Tris-Hepes buffer (pH 7.5) or 50 mM Tris-Mes buffer (pH 5.5). The vesicles were added to a medium containing 100 mM TMA gluconate, 100 mM mannitol, and 50 mM Tris-Hepes (pH 7.5) or Tris-Mes (pH 5.5) buffer and 0.65 mM GlyGlyPro. Fig. 3 shows that there was a slight stimulation of GlyGlyPro transport at early time points in the presence of the proton gradient (out > in), but there was no overshoot of uptake in the presence of the proton gradient. There was no stimulation of uptake with an outwardly directed proton gradient (5.5 in/7.5 out) (results not shown).

Role of membrane potential in GlyGlyPro transport

The effect of a K^+ diffusion potential (interior negative) produced by valinomycin was examined at initial time periods using papain-treated BBMV. Uptake was increased by 132% in the presence of the K^+ diffusion potential generated by valinomycin (interior-negative) at initial time points (Table II). Under voltage-clamped conditions, ($K_i = K_o$) no stimulation of GlyGlyPro was observed, indicating that the increase in uptake was due to the interior-negative membrane potential.

Concentration dependence of GlyGlyPro transport

The effect of increasing concentrations of GlyGlyPro on transport was investigated by varying concentrations of the peptide from 0.5 to 30 mM and measuring GlyGlyPro uptake. The Hofstee plot (Fig. 4a) illustrates that transport occurs via two systems. Correction for diffusion showed a saturable system (Fig. 4b) and further analysis of the data using the Lineweaver-Burk plot and weighted linear regression according to Wilkinson

TABLE II

The effect of a K^+ diffusion potential on GlyGlyPro transport in papain-treated BBMV

Papain-treated BBMV were loaded with 100 mM potassium gluconate/130 mM mannitol/10 mM Tris-Hepes (pH 7.5). The membrane vesicles were added to an incubation medium containing 100 mM TMA gluconate/100 mM mannitol/10 mM Tris-Hepes (pH 7.5)/21 μM valinomycin/0.65 mM GlyGlyPro. Voltage-clamped conditions were established by preincubating the vesicles with 100 mM potassium gluconate/100 mM mannitol/10 mM Tris-Hepes (pH 7.5). The membrane vesicles were diluted 10-fold in a medium containing 100 mM potassium gluconate/100 mM mannitol/10 mM Tris-Hepes (pH 7.5)/21 μM valinomycin/0.65 mM GlyGlyPro. Uptake of GlyGlyPro in the presence of valinomycin is expressed as a percentage of control conditions, i.e., K_i^+/TMA_o, and shown in parentheses in the table.

Time (min)	Uptake (pmol/mg protein)		
	K_i^+/TMA_o	K_i^+/TMA_{o+val}	K_i^+/K_{o+val}^+
0.05	106.6± 6.3	246.8±21.4 (132%)	86.1±3.0
0.1	221.0±15.0	383.9±23.9 (74%)	154.1±7.2

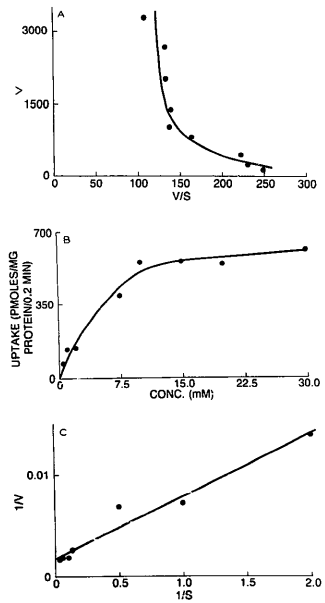

Fig. 4. Concentration dependence of [1-^{14}C]GlyGlyPro transport by human jejunal papain-treated BBMV. The BBMV were preincubated in media of 100 mM ChCl/100 mM mannitol/10 mM Tris-Hepes (pH 7.5). The vesicles were added to a medium containing 100 mM ChCl/10 mM Tris-Hepes buffer (pH 7.5), and various concentrations of GlyGlyPro and mannitol to maintain a medium osmolarity of 300 mM. Uptake was determined at 0.2 min to estimate initial rates. The uptake values obtained for 100 mM unlabeled GlyGlyPro were used as the diffusion correction for all the concentrations of the peptides similar to the studies of Stevens et al. [28]. The experiment was repeated three times using different vesicle preparation. All results for experiments gave similar kinetic constants. (a) Hofstee plot of the total uptake of GlyGlyPro in papain-treated BBMV. (b) GlyGlyPro uptake after correction for the diffusional component. (c) Lineweaver-Burk plot of uptake values.

(Fig. 4c) gave a K_m of 3.42 ± 0.15 mM and V_{max} of 576.3 ± 4.4 pm/mg protein per 0.2 min.

Effect of free amino acids and peptides on GlyGlyPro transport in papain-treated BBMV

To examine the specificity of GlyGlyPro transport, various free amino acids and peptides were studied to determine their inhibitory effect on GlyGlyPro uptake. No significant inhibition of GlyGlyPro uptake was noted in the presence of the free amino acids (60 mM) (Table III). Conversely, the range of inhibition for the dipeptides was 25–47% with glycyl-L-proline exhibiting the greatest inhibition on GlyGlyPro uptake (Table III). Inhibition by the various tripeptides (60 mM) was greater than 50%. The inhibition values were not corrected for the diffusional component of GlyGlyPro uptake.

TABLE III

[1-^{14}C]GlyGlyPro uptake in the presence of free amino acids and peptides

BBMV were preloaded with 100 mM ChCl/100 mM mannitol/10 mM Tris-Hepes (pH 7.5). The test medium contained 100 mM ChCl/40 mM mannitol/10 mM Tris-Hepes (pH 7.5)/60 mM free amino acids and peptides/2 mM [1-^{14}C]GlyGlyPro. Values represent the mean ± S.E. of triplicate assays for three determinations.

Test compounds	Inhibition (%)
Glycine	12.3 ± 3.7
L-Alanine	13.0 ± 0.1
L-Proline	13.5 ± 6.2
L-Leucine	5.9 ± 4.4
Glycyl-L-proline	47.7 ± 2.2
Glycylsarcosine	25.6 ± 1.1
Glycylglycine	42.0 ± 2.0
Glycylglycylglycine	39.3 ± 3.1
Glycylglycylsarcosine	35.6 ± 2.2
Glycyl-L-prolyl-L-alanine	44.0 ± 1.5
Glycyl-L-prolyl-L-hydroxyproline	38.5 ± 2.5
Glycyl-L-leucyl-L-tyrosine	51.6 ± 5.1
Glycyl-L-prolyl-L-proline	50.1 ± 3.3
Glycylsarcosylsarcosine	48.5 ± 5.0
Glycylglycyl-L-proline	57.3 ± 0.5

Transstimulation with GlySarSar

To confirm the presence of a carrier-mediated component for GlyGlyPro transport, transstimulation stud-

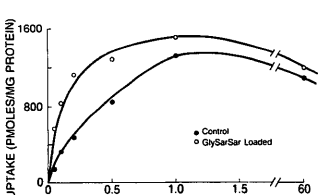

Fig. 5. Transstimulation of [1-^{14}C]GlyGlyPro uptake by GlySarSar in jejunal papain-treated BBMV. The membrane vesicles were equilibrated in 100 mM ChCl/120 mM mannitol/10 mM Tris-Hepes (pH 7.5) ●, or in 100 mM ChCl/100 mM mannitol/10 mM Tris-Hepes (pH 7.5)/20 mM GSS ○. Uptake of 1 mM GlyGlyPro was assayed in the presence of 100 mM ChCl/120 mM mannitol/10 mM Tris-Hepes (pH 7.5).

ies with GlySarSar were done. In this experiment, BBMV were preincubated with 100 mM ChCl/100 mM mannitol/10 mM Tris-Hepes (pH 7.5)/20 mM GlySarSar. Control vesicles were preincubated in a similar medium without the GlySarSar. In the presence of intravesicular GlySarSar, uptake was increased 300% at 3 s and 160% at 6 s in comparison to the control conditions (Fig. 5). This further confirms that GlyGlyPro transport is carrier-mediated.

Discussion

This is the first study which reports transport characteristics of a tripeptide using human jejunal BBMV. Most attempts to characterize intestinal peptide uptake using BBMV have been done using dipeptides. Influx of GlyGlyPro was examined, and initial investigations showed membrane hydrolysis of GlyGlyPro at early time periods. Therefore, papain-treated BBMV were used to prevent hydrolysis of GlyGlyPro. The papain-treated BBMV model has been effectively used in our laboratory to examine transport characteristics of dipeptides in murine intestinal BBMV [4,5]. Treatment of the BBMV with gel-complexed papain did not affect the functionality and integrity of the vesicles as monitored by glucose transport activity. In addition, osmolarity studies indicated that the vesicles were osmotically sensitive, and that GlyGlyPro was transported intravesicularly with minimal membrane binding. Influx of GlyGlyPro was studied in control and papain-treated vesicles in many of the studies.

For both control and papain-treated BBMV, GlyGlyPro transport was not dependent on a Na^+ gradient (extravesicular > intravesicular). Using a potential sensitive fluorescent dye to examine peptide transport in rabbit intestinal BBMV, Ganapathy et al., also showed that transport of β-alanylglycylglycine and L-ProGlyGly was Na^+-independent. [23] However, the uptake of GlySarSar and β-alanylglycylglycine was shown to be Na^+-dependent, using hamster jejunal rings in vitro [2]. There has been considerable debate concerning the role of Na^+ in peptide transport. Most studies using intact tissue indicate a Na^+ dependency for peptide transport, whereas uptake is shown to be Na^+-independent using BBMV [4–11]. Employing methods with intact intestinal tissue, it was difficult to differentiate between intact peptide transport and free amino acid uptake resulting from hydrolysis by either brush-border membrane or cytoplasmic peptidases, and, furthermore, contribution of intracellular peptide binding is not known.

The role of a proton gradient in intestinal transport of peptide remains controversial. The proposed peptide-proton cotransport hypothesis suggests that a proton gradient may possibly serve as the driving force for intestinal peptide influx [12]. In this study, there was a small stimulation of GlyGlyPro influx at early time points in the presence of the inwardly-directed proton gradient, but no overshoot phenomenon was observed for GlyGlyPro influx. Our recent studies have demonstrated that a 3–4-fold overshoot of Na^+ uptake energized by an outwardly directed proton gradient could be obtained in human jejunal BBMV [21]. The absence of overshoot of tripeptide uptake in the presence of proton-gradient is, therefore, not due to any problems of maintaining a proton gradient in human jejunal BBMV.

Uptake of the dipeptides, glycylglycine, glycylsarcosine and glycyl-L-proline with rabbit intestinal BBMV was demonstrated to be electrogenic but Na^+-independent [9–11]. Our results also indicate that GlyGlyPro influx is stimulated in the presence of an interior negative membrane potential (Table II). Parallel studies showed that GlyGlyPro transport was not energized by inwardly directed proton or Na^+ gradients. Some possible explanations for the stimulation of GlyGlyPro uptake in the presence of an interior negative membrane potential could be: (1) Ions other than Na^+ or proton(s) may be involved in the transport, or transport may be coupled to the antiport of an anion. (2) The peptide may be charged during experimental conditions, this possibility seems unlikely, since GlyGlyPro pI is 5.25 and at the working buffer pH, the peptide has no net charge. (3) The membrane carrier for GlyGlyPro may be charged. Berteloot showed that the protonated acidic amino-acid carrier enhanced glutamic acid uptake [24]. Further investigations with potential-sensitive dyes are warranted to fully evaluate the findings.

Both passive and facilitated diffusion processes were involved in GlyGlyPro uptake. It is not known whether the passive diffusion process represents a low-affinity–high-capacity carrier system. Such a low-affinity–high-capacity carrier system may not serve any physiological function in view of the reported peptide concentrations in the lumen after a protein meal [25]. A diffusional component in transport has been noted with glycylsarcosine and L-glutamyl-L-glutamic acid using hamster jejunal rings [26]. Previous studies showed diffusion as a component of total transport of glycyl-L-phenylalanine, glycyl-L-leucine and glycyl-L-proline with mouse and human BBMV, respectively [4–6]. The carrier-mediated component was confirmed by transstimulation of GlyGlyPro uptake by GlySarSar. Inhibitory studies with the various dipeptides and tripeptides gave further evidence for the presence of the carrier-mediated component of GlyGlyPro uptake. Among the dipeptides, glycyl-L-proline inhibited uptake by 47%, whereas the range of inhibition by the tripeptides was 30–58% (Table III). These results may show that dipeptides and tripeptides share a common transport system. A common uptake system for glycylsarcosine and GlySarSar has been demonstrated using hamster jejunal rings in vitro [27]. Additional kinetic studies on inhibi-

tion are needed to determine the specificity and multiplicity of peptide transport systems in human jejunum. Free amino acids showed less inhibition of GlyGlyPro uptake than the peptides, which agrees with other studies which indicate separate transport systems for amino acids and peptides [15,25].

Intestinal tripeptide uptake has not been investigated as extensively as dipeptide transport using either intact tissue or purified BBMV. Most information on the intestinal peptide uptake mechanism has been obtained from dipeptide studies. Whether a common peptide uptake system is present in the human jejunal BBMV is not presently known. In human perfusion studies, a common transport mechanism for di- and tripeptides has been suggested [15], and our results tend to agree with these studies. Also a common transport system for dipeptides and tripeptides has been demonstrated with hamster jejunal rings in vitro [27]. Using intestinal BBMV, we showed that GlyGlyPro uptake is Na^+-independent, nonconcentrative but carrier-mediated. Additional studies with hydrolysis-resistant tripeptides are needed to fully evaluate oligopeptide transport.

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