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TRANSPORT CHARACTERISTICS OF PAPAIN-TREATED BRUSH-BORDER MEMBRANE VESICLES

NON-INVOLVEMENT OF γ-GLUTAMYLTRANSFERASE IN LEUCINE TRANSPORT

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Summary

Papain treatment of isolated brush border membrane vesicles was carried out to correlate directly the solubilization of γ-glutamyltransferase with the uptake of leucine. Digestion of membrane vesicles with either soluble or gel-complexed papain resulted in nearly complete removal of γ-glutamyltransferase. However, the treated vesicles exhibited increased specific activity of leucine and glucose uptake, indicating the non-involvement of the transferase in leucine transport. The partial purification of amino acid and sugar transport function was better controlled with gel-complexed papain. In contrast to the digestion with soluble papain, the treatment with gel-complexed papain did not modify the diffusional components for solutes and ions and did not alter the intravesicular volume. It appears that controlled papain-digestion, resulting in nearly a 2-fold purification of the transport function with high reproducibility and quantitative recovery of uptake, should be useful in future attempts to purify the 'carrier' proteins.

Introduction

Over the years, studies have revealed the important role of the intestinal brush-border membrane in the digestion and absorption of nutrients [1]. An

Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; BAEE, benzoyl-arginyl ethyl ester.

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examination of the kinetics, ionic and structural requirements of sugar and amino acid transfer across the brush border membrane of intestinal epithelial cells [1—3] has led to the gradient hypothesis of carrier-mediated active transport [1,3—5]. These above-mentioned Na^+-coupled transport mechanisms have been localized to the brush-border membrane, as evident by in vitro experiments using brush-border membrane vesicles [6—8] or reconstituted vesicles [9]. Many enzymes have also been found localized to the brush-border membrane [4] but their physiological roles in relation to transport are not fully understood. In this context, an attractive hypothesis proposed the γ-glutamyltransferase (EC 2.3.2.2, formerly γ-glutamyl transpeptidase) as a membrane carrier for the transport of amino acids and possibly peptides in what has been called the γ-glutamyl-cycle hypothesis [10—13]. Though first proposed for the renal brush-border membrane [10], it has also been postulated that the transpeptidase and other enzymes of the γ-glutamyl cycle are involved in the transport of amino acids or peptides across epithelial surfaces [10—12]. In the gut, the hypothesis has been supported mainly by indirect evidence regarding the localizations of γ-glutamyl-cycle enzymes [14,15] and glutathione [14] along the villi and the characteristics of the intestinal γ-glutamyltransferase reaction [15]. However, no direct proof for the involvement of γ-glutamyltransferase in amino acid absorption has been reported so far.

In this paper, we report a direct evaluation of the role of γ-glutamyltransferase in intestinal transport of leucine. We used isolated brush-border membrane vesicles that display all the characteristics of Na^+ gradient-coupled monosaccharide and amino acid transport as shown by others [6—8] and took advantage of the efficiency of papain for the solubilization of brush border membrane hydrolases [16—21], so that a correlation between the specific activity for γ-glutamyl-*p*-nitroanilide hydrolysis and the specific activity of leucine uptake is made possible. Our data show that papain treatment of brush border membrane vesicles results in removal of γ-glutamyltransferase but also leads to a partial purification of leucine- and glucose-transport function, allowing the conclusion of non-requirement of γ-glutamyltransferase for leucine transport.

Materials and Methods

Preparation of brush-border membrane vesicles

Control mice (Strain C57-BL/Ks J-db/m) were killed by decapitation. The whole intestine was then removed, rinsed with cold NaCl 0.9%, and the mucosa was scraped with a spatula. Brush-border membranes were purified by the calcium chloride precipitation method of Schmitz et al. [22] and brush-border membrane vesicles were obtained by Hopfer's method [6]. The different steps are summarized in Fig. 1.

Transport studies

The purified brush-border membrane vesicles (P_6) were resuspended to a final protein concentration of 5—15 mg per ml with 1 mM Tris-Hepes buffer (pH 7.5), 100 mM mannitol and 0.1 mM $MgSO_4$. Incubation media contained, in a 250 μl final volume: the above buffer, 100 mM NaSCN or KSCN, 1 mM glucose or leucine, and either 3.3 μCi of D-[U-^{14}C]glucose (New England Nu-

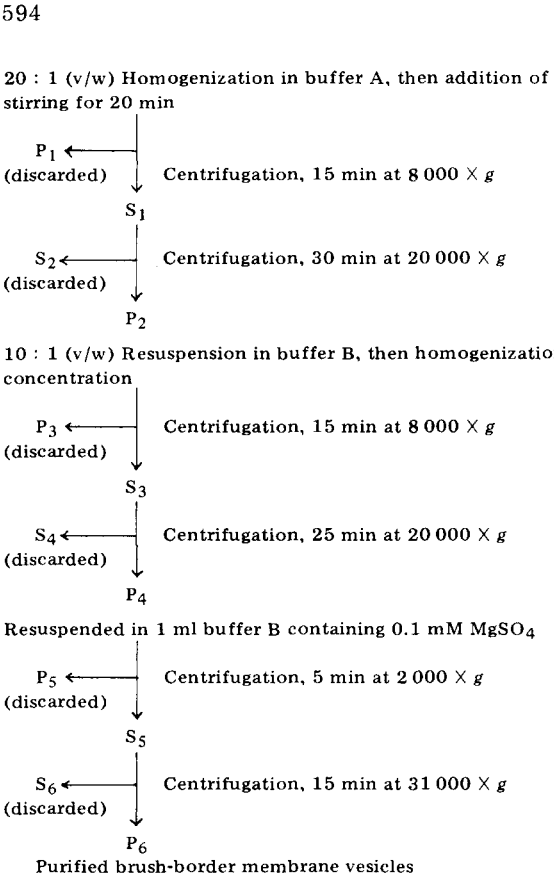

Fig. 1. Scheme of preparation of brush-border membrane vesicles from mouse intestine. Buffer A: 2 mM Tris-HCl (pH 7.0), 50 mM mannitol. Buffer B: 1 mM Tris-Hepes (pH 7.5), 100-mM mannitol.

clear, spec. act. 284.5 mCi/mmol) or 1.16 μCi of L-[U-^{14}C]leucine (New England Nuclear, spec. act. 344.0 mCi/mmol). Transport studies were initiated by the addition of 250—750 μg of brush-border membrane vesicles and were conducted at room temperature. At time intervals, 50 μl of the reaction mixture (50—150 μg of proteins) were mixed with 1 ml of cold stop solution containing 1 mM Tris-Hepes buffer, 115 mM mannitol, 0.1 mM $MgSO_4$, 100 mM NaCl and D-[1-^{3}H(n)]mannitol (New England Nuclear, spec. act. 22.4 Ci/mmol: 2.37 μCi for glucose studies and 0.9 μCi for leucine studies). This step allows the correction for nonspecific adsorption. The resulting mixtures were filtered through 0.45 μm Millipore or 0.60 μm Sartorius filters and were washed with 4 ml of non-radioactive stop solution. Filters were dissolved in scintillation vials by 1 ml ethyl acetate for 20 min and radioactivities were determined after addition of 16 ml aquasol in a Packard TriCarb scintillation counter (Model 3255). Results are expressed either as total (pmol) or specific (nmol/mg protein) activity of solute uptake.

Digestion of brush-border membrane vesicles with papain

P_4 fractions were divided into two equal aliquots, one being processed as out-

lined in the scheme of Fig. 1, the other being used for digestion, so that a direct comparison can be made between normal and papain-treated vesicles. Digestion with papain was conducted at room temperature (22°C) or 37°C in a shaking water bath for varying time periods of incubations. The methods of Eichholz [17] for soluble papain (two times crystallized and lyophilized powder type IV from Sigma, spec. act. 20.7 U/mg protein) and gel-complexed papain were used.

The gel-complexed papain was prepared as follows: 1 ml of Affi-Gel 10 (Bio-Rad Laboratories) was washed on a buchner funnel to remove completely the solvent, isopropanol, dried and weighed. The ligand solution was obtained by mixing 5 ml of 0.1 M phosphate buffer (pH 7.0) and 1 ml of papain (two times crystallized in suspension in 0.05 M sodium acetate from Sigma, spec. act. 28 U/mg protein) and was added to the Affi-Gel 10. The coupling reaction was performed by overnight stirring in the cold room. Unreacted sites were blocked by adding 0.1 volume of 1 M ethanolamine-HCl (pH 8.0) and reacting for 1 h at 4°C. The mixture was transferred to a buchner funnel and washed free of reactants with 0.1 M phosphate buffer (pH 7.0) as verified by determination of absorbance at 260 nm on a Beckman Spectrophotometer (Model 24), then washed with 100 ml of 0.1 M phosphate buffer (pH 6.5), dried and weighed. An aliquot was used for the determination of papain activity and the remaining resuspended in 0.1 M phosphate buffer (pH 6.5) to have 20 U papain/ml buffer. In these conditions, the efficiency of papain coupling was 20—25% and activity was preserved for at least 5 months at 4°C.

At time intervals, digestion with papain was stopped either by a 20 times dilution with cold 1 mM Tris-Hepes buffer (pH 7.5), 100 mM mannitol when soluble papain was used, or by filtration under vacuum through a Whatman paper (No. 4) when gel-complexed papain was used. $MgSO_4$ was then added to a final concentration of 0.1 mM and the resulting mixtures were centrifuged for 5 min at $2000 \times g$. The supernatants obtained were centrifuged for 15 min at $31\,000 \times g$ and the resulting pellets were processed as outline in the scheme of Fig. 1 to obtain P_6 which were used for transport studies. Incubations without papain were also done to check the effects of cysteine and EDTA present in the digestion medium.

Assays

Papain was assayed by a titrimetric determination of the acid produced during the hydrolysis of benzoyl-arginyl ethyl ester (BAEE). The enzyme solution was diluted to have 0.05 to 0.1 mg protein per ml of a medium containing 1.11 mM EDTA, 0.56 mM cysteine and 0.067 mM β-mercaptoethanol. The reaction was started by adding 1 ml of this solution to a mixture containing the substrate (5 ml, pH 6.2, 41 mM BAEE, 0.38 mM EDTA, and 0.19 mM cysteine), 5 ml of 3 M NaCl and 5 ml of water. The volume of 0.01 M NaOH required to maintain the pH at 6.2 was recorded every minute for a 5-min period. In these conditions, one unit of enzyme activity is equal to 1 μmol of BAEE hydrolysed per min at room temperature and corresponds to 1.4 Sigma units. The activities of papain reported here have been converted to Sigma units.

Sucrase was assyed by the method of Dahlqvist [23] as modified by Lloyd and Whelan [24] and γ-glutamyltransferase by the method of Naftalin et al.

[25]. After papain treatment, both enzymes were assayed in the 31 000 × *g* supernatant of digestion and in P_6, thus allowing a precise determination of the amount solubilized during papain digestion.

Protein was assayed by the method of Lowry et al. [26] using bovine serum albumin as standard.

Results

Transport characteristics of mouse brush-border membrane vesicles

As previously shown [27], the vesicles used in our studies exhibited Na^+-dependency of leucine and glucose transport and also the overshoot phenomenon in the presence of an Na^+ gradient (Table I, Figs. 2A and 3A) similar to membrane vesicles from other species [6—8,28,29].

The effect of soluble papain on leucine and glucose transport by brush-border membrane vesicles

Treatment of brush-border membrane vesicles with soluble papain led to an increase in specific activity of leucine and glucose uptake of 1.6- and 1.7-fold, respectively, as calculated from the maximum overshoot values (Table I). Under these conditions, sucrase and γ-glutamyltransferase activities were almost completely solubilized (90—100%). These results clearly show the non-involvement of γ-glutamyltransferase in leucine transport and also point to the partial purification of leucine and glucose carriers.

However, in the experiment shown in Table I, 54% of the total brush-border membrane proteins have been removed by papain treatment and so, if there was no loss of transport activity due to papain treatment, a more than 2-fold increase in specific activity of leucine and glucose uptake should have been obtained. It should also be noted that the peak to plateau ratios are reduced after papain treatment. The analysis of the total uptakes of solutes by the brush-border membrane vesicles before and after papain treatment (Table II) clearly shows a reduction in transport capacities of papain-treated vesicles (number of sites available for transport). This conclusion is supported by the similar pla-

TABLE I

EFFECT OF DIGESTION WITH SOLUBLE PAPAIN ON SPECIFIC ACTIVITY OF LEUCINE AND GLUCOSE UPTAKE BY BRUSH-BORDER MEMBRANE VESICLES

Digestion was done for 5 min at room temperature (22°C) with 0.8 U of papain/mg protein and brush-border membrane vesicles were prepared as described under Materials and Methods. Plateau values represent the mean of uptakes at 15 and 20 min of incubation with leucine or glucose.

Substrate	Treatment	Uptake (nmol/mg protein) as a function of time (min)					Peak to plateau ratio
		0.15	0.25	0.40	0.50	Plateau	
Leucine	None	1.40	1.53	1.63	1.62	0.60	2.72
	Soluble papain	2.27	2.30	2.59	2.24	1.46	1.77
Glucose	None	1.38	2.49	2.13	—	0.21	11.86
	Soluble papain	4.14	3.79	3.47	—	0.61	6.79

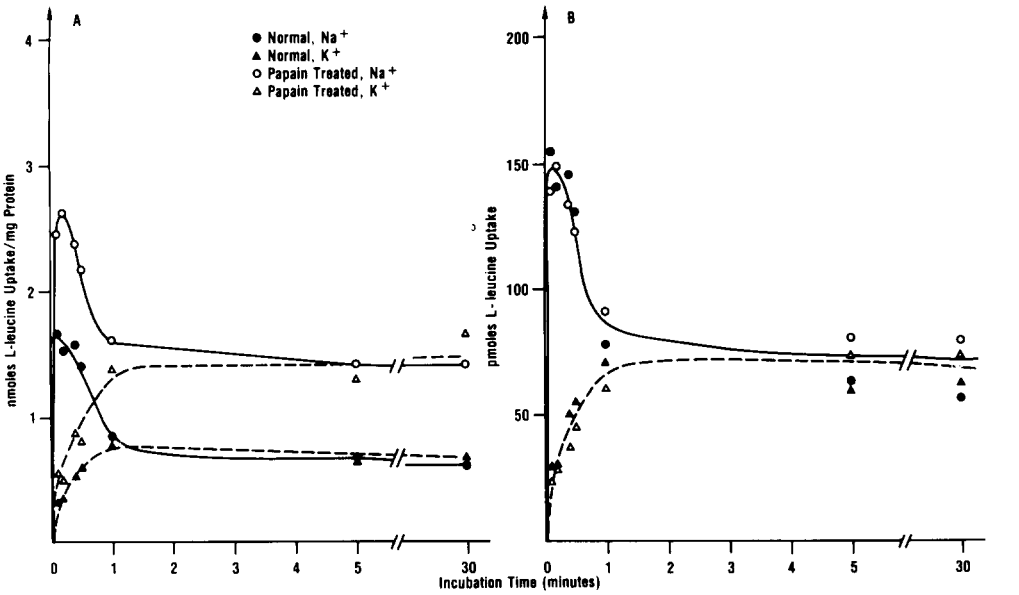

Fig. 2. The effect of digestion with gel-complexed papain on specific (A) and total (B) activities of leucine uptake by brush-border membrane vesicles. Conditions of digestion were as discussed in the text.

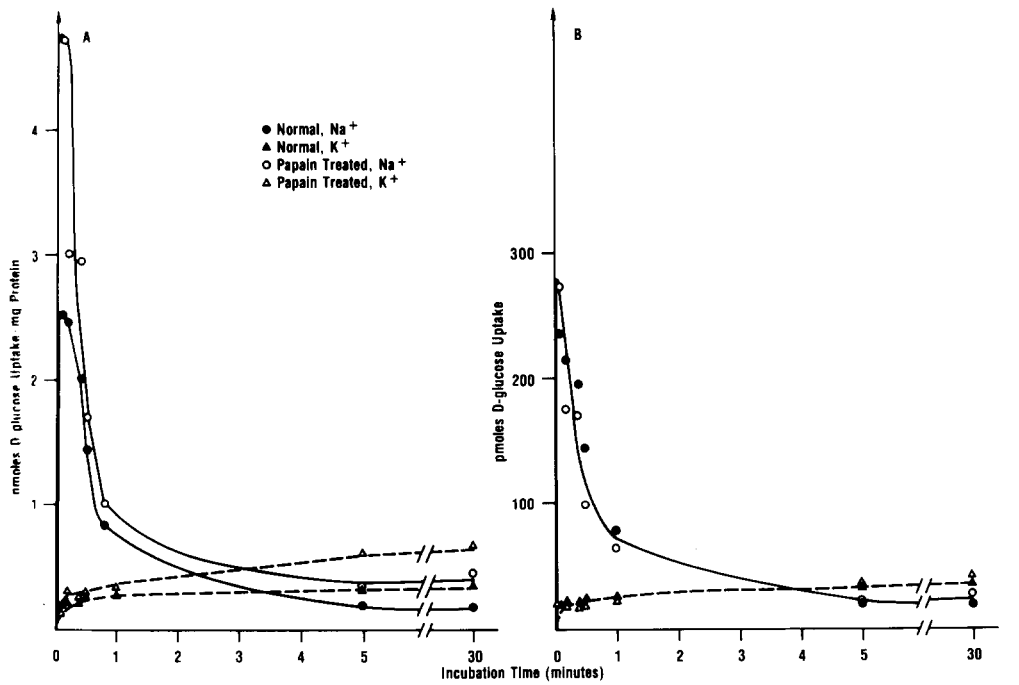

Fig. 3. The effect of digestion with gel-complexed papain on specific (A) and total (B) activities of glucose uptake by brush-border membrane vesicles. The same preparations of vesicles as for Fig. 2 were used.

TABLE II

EFFECT OF DIGESTION WITH SOLUBLE PAPAIN ON TOTAL UPTAKES OF LEUCINE AND GLUCOSE BY BRUSH-BORDER MEMBRANE VESICLES

Details were as under Table I.

Substrate	Treatment	Uptake (pmol) as a function of time (min)				
		0.15	0.25	0.40	0.50	Plateau
Leucine	None	186	204	217	216	82
	Soluble papain	139	140	158	137	89
Glucose	None	184	331	283	—	28
	Soluble papain	253	231	212	—	37

teau values prior to and after papain treatment, pointing to the integrity of papain-treated vesicles and to the availability of the same intravesicular volume for transport.

Further attempts with soluble papain treatment by varying the concentration of papain (0.2 to 1.1 U/mg protein), the time of digestion (0 to 60 min) and the membrane fraction digested (P_2 vs. P_4) gave almost similar results. It should be stressed, however, that incubations for a long time or with high concentrations of papain yielded much lower peak to plateau ratios compared to the results shown in Table I, probably due to the greater digestion of transport proteins. These results prompted us to use controlled papain-digestion of brush-border membrane vesicles with gel-complexed protease.

The effect of insoluble-papain on leucine and glucose transport by brush-border membrane vesicles

a. Effect of papain concentration on glucose transport. Table III shows that the highest specific activity of glucose uptake was obtained with 0.5 to 0.8 U papain/mg protein under the conditions described and that with further increase in papain concentration, the values returned to the level of non-treated vesicles.

TABLE III

EFFECT OF CONCENTRATION OF GEL-COMPLEXED PAPAIN ON SPECIFIC ACTIVITY OF GLUCOSE UPTAKE BY BRUSH-BORDER MEMBRANE VESICLES

Digestion was done at room temperature (22°C) for 5 min and brush-border membrane vesicles were prepared as described under Materials and Methods. Plateau values represent the mean of uptakes at 11.0 and 11.5 min of incubation with glucose.

Papain concentration (U/mg protein)	Uptake (nmol/mg protein) as a function of time (min)		
	0.15	0.40	Plateau
0	2.69	2.96	0.39
0.27	2.79	3.15	0.38
0.54	3.59	3.63	0.53
0.80	3.71	3.71	0.45
1.52	2.34	3.02	0.35

b. Effects of time and temperature of digestion by papain on glucose transport. Table IV shows that peak values of total uptake of glucose were nearly independent of the time of incubation with papain (0.6 U/mg protein) when the digestion was conducted at room temperature (22°C) but were extensively and increasingly depressed with the time of digestion at 37°C. Plateau values were also lowered to some extent after the digestion at 37°C. A complete recovery of transport sites and the integrity of vesicles were therefore achieved only at room temperature. The concomitant 20—25% removal of brush-border membrane proteins at this temperature (result not shown) led to an increased specific activity of glucose uptake which is maximum after 15 min of digestion with papain. That this is the result of the specific action of papain was clearly shown by the decreased total uptake of glucose recorded after an incubation of vesicles for 45 min in papain-free digestion medium. However, the reasons for the above decrease are not known but may be related to the presence of EDTA and/or cysteine in the incubation medium. It also has to be pointed out that despite the complete early solubilization of sucrase at both temperatures of digestion, γ-glutamyltransferase activity persisted to 25% of the level in the non-treated vesicles even after 45 min of digestion at room temperature while it was completely solubilized at 37°C (results not shown).

c. Leucine and glucose transport. From the experiments described above, the optimal conditions selected for these experiments were 10 min of digestion at room temperature with 0.95 U papain/mg protein. Figs. 2 and 3 show, respectively leucine and glucose transport activities of brush-border membrane vesicles before and after digestion with insoluble papain. Each curve represents the mean of four experiments. It can be seen from Figs. 2A and 3A that specific activity of leucine and glucose uptake were augmented to about the same extent by papain treatment, increases based on maximum overshoot values being 1.6- and 1.85-fold, respectively. Under these experimental conditions, Table V shows that 39 ± 4% of membrane proteins have been removed and that

TABLE IV

EFFECTS OF TEMPERATURE AND TIME OF DIGESTION WITH GEL-COMPLEXED PAPAIN ON TOTAL GLUCOSE UPTAKE BY BRUSH-BORDER MEMBRANE VESICLES

Digestions were done with 0.6 U of papain/mg protein for varying time periods after which brush-border membrane vesicles were prepared and assayed for transport as described under Materials and Methods. Plateau values represent the mean of uptakes at 11.0 and 11.5 min of incubation with glucose.

Incubation with papain	Uptake (pmol)			
	22°C		37°C	
	Peak	Plateau	Peak	Plateau
None	290	26	255	23
None, but 45 min in complete medium	152	15	7	5
0 min	211	17	40	13
5 min	243	24	34	15
10 min	271	27	—	—
15 min	284	28	30	16
30 min	233	26	20	10
45 min	220	30	15	10

TABLE V

EFFECT OF DIGESTION WITH GEL-COMPLEXED PAPAIN ON SOLUBILIZATION OF PROTEINS AND ENZYME ACTIVITIES OF BRUSH-BORDER MEMBRANE VESICLES

The values given represent the mean total amounts ± S.E. recovered in the respective fractions and were obtained with the four preparations of brush-border membrane vesicles used in Figs. 2 and 3. The activities referred to as soluble were determined in the supernatant recovered after 15 min centrifugation at 31 000 × *g* following papain digestion. See details under Materials and Methods.

Treatment	Proteins in P_6 (mg)	Sucrase (nmol glucose/min)		γ-Glutamyltransferase (nmol *p*-nitroanilide/min)	
		P_6	Soluble	P_6	Soluble
None	4.01 ± 0.1	4650 ± 116	—	786 ± 40	—
Gel-complexed papain	2.46 ± 0.13	5 ± 4	5341 ± 235	51 ± 8	630 ± 64

92 ± 2% of γ-glutamyltransferase and 99.9 ± 0.1% of sucrase activities have been solubilized with quantitative recoveries (87 ± 4% and 115 ± 3% for γ-glutamyltransferase and sucrase, respectively). The increased specific activity of leucine uptake with the concomitant 9.5 (±1.3)-fold reduction in specific activity of γ-glutamyltransferase (21 ± 2 compared to 196 ± 9) due to the solubilization (Table V) of this enzyme activity show clearly the non-requirement of the enzyme for leucine transport. That controlled papain-digestion gives vesicles with properties similar to normal vesicles is shown on Figs. 2B and 3B where results are expressed as total uptakes of solutes. The superposition of Na^+ curves shows the entirely quantitative recovery of carrier molecules while the superposition of K^+ curves shows the non-modification of the diffusional components in transport after papain treatment. Plateau values in each set of curves also coincide implying that papain digestion did not alter either the intravesicular space available for transport or the integrity of the vesicles. Lastly, initial velocities of transport (as estimated from values at 0.1 min) are not altered by papain treatment, indirectly pointing out that the permeabilities of the membrane to Na^+, K^+ and SCN^- are the same in both conditions. All these results strengthen the earlier conclusions that membrane γ-glutamyltransferase activity and leucine-transport function are independent, and that the increased specific activities of leucine and glucose uptake were the consequence of the partial purification of the leucine and glucose carriers by controlled papain-digestion of the brush-border membrane vesicles.

Discussion

Studies on the enzymology of glutathione have led to the recognition of the γ-glutamyl cycle and to the proposal that this cycle is one of the systems that functions in the transport of amino acids [10—13]. γ-Glutamyltransferase, which is a particulate enzyme localized in the renal brush border membrane and in other membranes involved in absorption or secretion, is postulated to interact with intracellular glutathione and extracellular amino acid to produce an intracellular γ-glutamyl amino acid [10—13]. Most of the data supporting the involvement of the γ-glutamyl cycle in amino acid transport have been ob-

tained using the kidney and include the high activities of γ-glutamyl-cycle enzymes and amino acid transport [10,30], the γ-glutamyltransferase localization to the proximal convoluted tubule (region believed to be involved in reabsorption of amino acids [31]), in vivo studies on experimental animals treated with inhibitors of γ-glutamyltransferase [32—36] and investigations on certain human disease [12]. The ubiquitous occurrence of glutathione and the wide distribution of γ-glutamyl-cycle enzymes led to the speculation that the γ-glutamyl cycle may play a role in the amino acid transport function of other epithelial cells, including enterocytes [10—13].

γ-Glutamyltransferase activity is relatively high in the small intestine [14, 15] and has been localized to the brush-border region of the villus tip cells [4, 14,15,22]. These cells were also shown to have low glutathione concentrations and high activities of amino acid transport [14]. All together, these results have been claimed compatible with a functional γ-glutamyl cycle in villus cells [14]. The comparison between certain characteristics of the gut-derived γ-glutamyltransferase activity like pH optimum, Na^+ stimulation and amino acid specificity with some of the known features of intestinal amino acid absorption, as well as the comparison of the localization of γ-glutamyltransferase activity in relation to sites of absorption, and the effect of an in vitro inhibitor of γ-glutamyltransferase on amino acid absorption in gut epithelial cells have been interpreted as a support for the hypothesis of γ-glutamyl-cycle involvement in the transport of amino acids by mammalian cells [15]. However, some characteristics of amino acid transport, like Na^+-dependency [2,3,5], cannot be accounted for by the γ-glutamyl-cycle hypothesis. Under certain conditions, γ-glutamyltransferase can be activated by monovalent cations including Na^+ and K^+ [15,37] but these effects have only been observed with model substrates such as γ-glutamyl-*p*-nitroanilide and not with glutathione [38]. The observation that none of the enzymes of the cycle is specifically activated by Na^+ suggested that the role of this cation in amino acid transport involves another step in transport or a different transport system [13]. Amino acid transport studies with isolated brush-border membrane vesicles also showed that transport may occur in the absence of the soluble enzymes of the cycle and of γ-glutamyl donor [6—8]. Recent findings showing the external localization of γ-glutamyltransferase [39—41] anchored to the membrane by a catalytically non-essential and protease-sensitive peptide also seemed to rule out the possibility of the transmembrane function of this enzyme essential for the operation of the γ-glutamyl cycle. It has also been shown that the high rates of transpeptidation observed in vitro resulted from the use of alkaline pH and high, non-physiological concentrations of amino acids, so that glutathione hydrolysis appeared to be the major reaction catalysed by γ-glutamyltransferase in vivo [44]. However, there is no direct evaluation of the role of γ-glutamyltransferase in amino acid transport.

The results of our studies demonstrate for the first time that amino acid transport may occur in isolated brush border membrane vesicles even in the nearly complete absence of γ-glutamyltransferase activity. This is clearly shown by the increased specific activity of leucine uptake concomitant with the reduction of specific activity of γ-glutamyl-*p*-nitroanilide hydrolysis after controlled papain digestion. The validity of this conclusion is strengthened by the follow-

ing considerations arising from a comparison of the results obtained before and after papain digestion. (1) The specific activity of glucose uptake was increased to a value similar to that of leucine, so that papain effect is not restricted to amino acid transport. (2) The increased specific activity of leucine and glucose uptake closely matched the extent of protein removal and the total uptakes in the presence of an Na^+ gradient were not altered, indicating a quantitative recovery in active-transport sites. (3) The total uptakes of leucine and glucose in the presence of a K^+ gradient were not changed, showing that diffusion was not modified. (4) The total amounts of leucine and glucose present at equilibrium uptake values were independent of papain treatment, showing the integrity of papain-treated vesicles and that the same intravesicular space was available for transport. (5) The initial velocities in solute uptake were not modified, pointing out the presence of the same driving force for active transport and indirectly supporting the non-modification in ionic permeabilities of the membranes. (6) The recovery of γ-glutamyltransferase activity was quantitative, indicating that the protein is efficiently solubilized and that transport after papain was not the result of an inactive form of γ-glutamyltransferase still present on the membranes. The last four points agree with the conclusions drawn by others that papain digestion does not appear to affect the lipid bilayer of the membranes [19,43] and that γ-glutamyltransferase is readily accessible from the outside of the membrane and entirely removed from the brush border membrane surface by papain digestion [19,41,43]. However, our results are at variance with those of Preston [45] who showed a reduced unidirectional influx of phenylalanine across the mucosal brush border of rabbit ileum by pretreatment with papain. The difference is easily explained by the use of a controlled papain-digestion in our study as different results may be obtained when changing the conditions for papain digestion. The conclusion of the non-involvement of γ-glutamyltransferase in leucine transport may probably be extended to neutral amino acids as it is known that neutral amino acids are the best amino acid acceptors in the γ-glutamyltransferase reaction [15,38]. However, our results do not rule out the possibility that the γ-glutamyl cycle might function with large amino acid loads [13] but the high K_m for amino acids (2—10 mM) at optimal alkaline pH (above 8.5) for transpeptidation makes this unlikely for a physiological role [46].

The susceptibility of sugar and amino acid 'carriers' to papain is shown by the loss of sites involved in the active transport process found in vesicles digested with soluble papain (Table II), with high concentrations of papain (Table III) and for long time periods (Table IV). However, none of these modifications of controlled papain-digestion (Figs. 2 and 3) reduced the intravesicular space available for transport. It may then be inferred that the 'carrier' molecules are proteins accessible to papain from the external side of the membrane but buried in the lipidic matrix. The peptide bond susceptible to the protease is therefore masked and inaccessible when controlled digestion is used. However, it cannot be assessed whether modified papain treatment results in the removal of the 'carrier' molecules from the membrane by the solubilization process observed with hydrolases [19] or their inactivation.

Finally, it has to be emphasized that the controlled papain digestion described herein led to the partial purification of the carriers for sugar and

amino acid and that this technique should be useful in any attempt to further purification of these entities. Its efficacy is assessed by the one step 40% removal of membrane proteins, the quantitative recovery of 'carrier' molecules and the high reproducibility. Such an attempt has been made previously by Tannenbaum et al. [29] which resulted in the partial negative purification of the glucose transport system. It can be inferred from our results that the conditions used for digestion in their studies were responsible for the non-reproducibility as we failed to show any increase in specific uptakes of solutes when digestion was conducted at 37°C.

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