Biochimica et Biophysica Acta, 394 (1975) 220–226

BBA 76985

DIPEPTIDE TRANSPORT IN ISOLATED INTESTINAL BRUSH BORDER MEMBRANE

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(Received January 22nd, 1975)

SUMMARY

Transport of glycyl-L-leucine into isolated brush border membrane vesicles was studied. On the basis of the following observations it was postulated that glycyl-L-leucine was transported intact by a specific dipeptide mechanism.

(1) The differing time course and Na^+ stimulation of glycine, L-leucine and glycyl-L-leucine.

(2) The failure of glycine and L-leucine to inhibit glycyl-L-leucine transport.

(3) Initial presence of dipeptide within the vesicle.

(4) Inhibition of glycyl-L-leucine uptake by other dipeptides.

(5) The occurrence of accelerated amino acid uptake in the presence of the dipeptide.

INTRODUCTION

Gastrointestinal digestion of a protein meal predominantly results in the production of low molecular weight peptides [1, 2]. Peptide-hydrolysing enzymes appear, for the greater part, to be associated with the mucosal cell [3–6]. The presence of certain peptidases in the luminal brush border membrane has, however, been substantiated [7–11]. An increasing amount of evidence has been appearing in the literature supporting the absorption of intact small peptides [12–17]. Because of the presence of intracellular peptidases it has been difficult to demonstrate the absorption of intact peptides in the mucosal cell. In vitro studies with glycylsarcosine [14] and carnosine [15], both being abnormally slowly hydrolysed, have overcome this obstacle and indicate that the dipeptides may be transported by an active process.

Intact separate transport systems for both glucose [18] and fructose [19] in highly purified brush border membranes from isolated epithelial cells of rat small intestine have recently been demonstrated. Hexose transport into the vesiculated membranes exhibited the same characteristics as reported for tissue preparations. The absence of intracellular contamination in the membrane preparation has enabled the study of dipeptide transport free from the disadvantages present with conventional tissue methods.

MATERIALS AND METHODS

The brush border membranes were isolated as reported elsewhere [18]. Sprague-Dawley rats (200–250 g) were utilized.

Uptake procedure

The isolated membranes were incubated at 25 °C in a buffer consisting of mannitol (100 mM), Tris-*N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (HEPES buffer adjusted with Tris hydroxide to pH 7.5, 1 mM) and $MgSO_4$ (0.1 mM). Further additions to the incubation medium are indicated in the legends with end concentrations of solutes given in parenthesis. The dipeptide glycyl L-leucine, obtained from Radiochemical Centre (Amersham), was present in the incubation medium as [1-^{14}C]glycyl-L-leucine or glycyl-L-[U-^{14}C]leucine. The amino acids [2-^{3}H]glycine and L-[4,5-$^{3}H_2$]leucine were purchased from New England Nuclear (Boston, Mass.). The uptake of the dipeptide or amino acid was terminated with the removal of an aliquot (20–80 μg protein) from the incubation medium and rapid 50-fold dilution with ice-cold buffer containing 200 mM NaCl, 100 mM mannitol, 10 mM Tris-HEPES, pH 7.5, 10 mM $MgSO_4$ and 0.4 μCi/ml D-[1-^{3}H]mannitol or D-[1-^{14}C]-mannitol when a single isotope was used in the incubation medium. The dilution medium was immediately filtered through a Satorius filter (No. 11305, 0.6 μM), the collected membranes were rinsed once with 4 ml of the dilution buffer without the isotope. The isotope in the dilution medium served as a correction for unspecific retention of either dipeptide or amino acid by the filter, due to insufficient washing. None of the labeled compounds alone were bound or retained by the filter. Uptake values from duplicate samples of the same membrane preparation were within 8 % of the mean.

Analytical procedures

The filters were dissolved in a fluor and counted in a liquid scintillation counter as described previously [19]. Protein determination was performed by the method of Resch et al. [20] as reported in an earlier publication [18].

Chemical identification of the dipeptide and amino acids was carried out by thin-layer chromatography. The solvent system employed was *n*-butanol/water/acetic acid (12 : 5 : 3, by vol.). Reference compounds were sprayed with a ninhydrin-cadmium reagent [21]. The chromatograph was then separated into 1-cm strips from the origin to the solvent front. The strips were eluted with 30 % ethanol and the eluate concentrated and counted in a liquid scintillation vial. In this system, the R_F value for glycine was 0.22, for L-leucine, 0.66 and for glycyl-L-leucine, 0.67. For determining the nature of the transported labeled compound, samples of membrane, taken after various times of incubation with the labeled substrate, were collected on the filter. The filters were extracted with 30 % ethanol and the extract concentrated and spotted. The labeled amino acids glycine and L-leucine, when present as substrate in the incubation medium, were recovered from the vesicles unchanged.

Sucrase (EC 3.2.1.48) activity was assayed as reported earlier [18]. Lactate dehydrogenase (EC 1.1.1.27) was measured by the UV Merkotest^{R} method. The aminopeptidase (EC 3.4.1.2) was determined according to Wacker et al. [22], leucine-*p*-nitroanilide being present as substrate.

RESULTS

Comparison of dipeptide and constituent amino acids uptake

Fig. 1 presents the time course for glycyl-L-leucine, labeled in the glycine and the leucine moiety, glycine and L-leucine uptake. At equilibrium, uptake values for all four compounds were the same. D-Glucose (not shown) reached the same level. That D-glucose enters into the brush border vesicle, an osmotically active space, and does not bind has been demonstrated [18]. Glycyl-L-leucine, glycine and L-leucine reaching the same equilibrium level as D-glucose indicates that they are entering into the same vesicular space. The uptake of both labeled species of dipeptide were similar, while the time courses for glycine and L-leucine were markedly different. Na^+ noticeably stimulated L-leucine transport and to a lesser degree the transport of the dipeptide. Glycine uptake was not affected by Na^+.

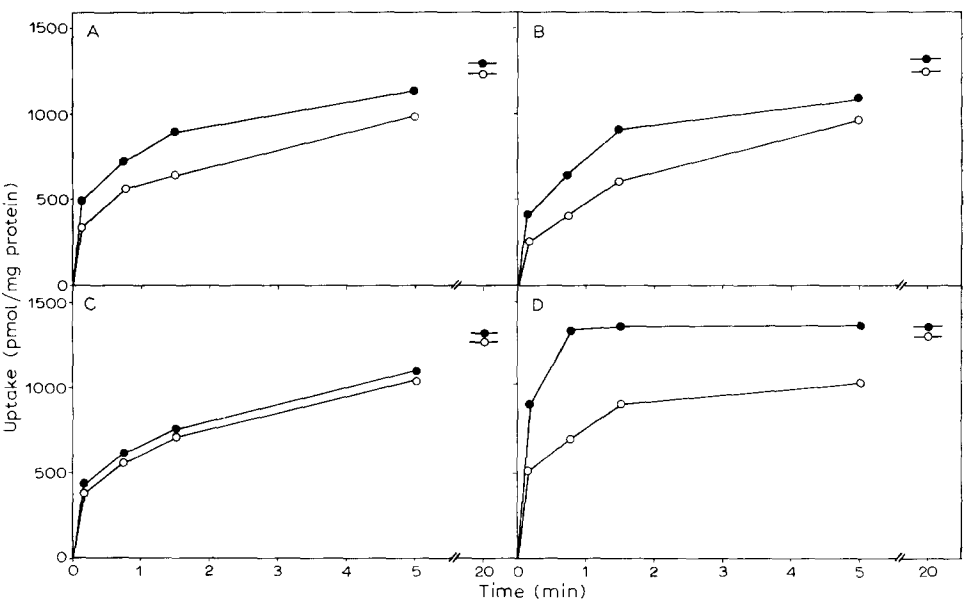

Fig. 1. Dipeptide and amino acid uptake by brush border membrane. Additions to incubation medium: NaSCN (100 mM, ●) or KSCN (100 mM, ○) and 1 mM (A) [1-^{14}C]glycyl-L-leucine, (B) glycyl-L-[U-^{14}C]leucine, (C) [2-^{3}H]glycine, (D) L-[4,5-$^{3}H_2$]leucine.

Vesicular contents after incubation with labeled dipeptide

When the membranes were incubated for various lengths of time with the radioactive dipeptide and the contents chromatographed, an interesting finding became apparent (Fig. 2). At the earliest time point taken, 10 s, 80 % of the radioactive vesicular contents was dipeptide and 20 % was glycine. After 5 min of incubation, 95 % of the radioactivity was in the form of glycine and only 5 % in the form of dipeptide.

Enzyme activity

The peptidase activity observed in Fig. 2 could be due to either membrane enzymes or contaminating cytoplasmic peptidases trapped within the vesicle. To

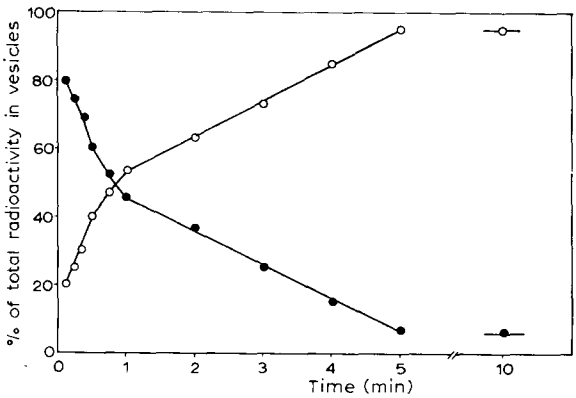

Fig. 2. Contents of vesicles incubated with [1-^{14}C]glycyl L-leucine. Radioactivity appearing as peptide, ●, or glycine, ○. Additions to incubation medium: NaSCN (100 mM), [1-^{14}C]glycyl-L-leucine (1 mM).

TABLE I
ENZYME ACTIVITY

Fraction	Sucrase		Aminopeptidase	
	Spec. act.*	Purification	Spec. act.*	Purification
Homogenate of intestinal scrapings	0.09	1.0	0.16	1.0
Purified brush border	2.38	26.4	3.90	24.4
Brush border membrane	3.33	37.0	5.70	35.6

* μmol sucrose or leucine *p*-nitroanilide split/min per mg protein.

check the latter possibility, a known cytoplasmic enzyme, lactate dehydrogenase, was measured. The membrane fraction was additionally assayed with 0.1 % Triton X-100 (final concentration) present. The detergent had no effect on the enzyme, but served to break down the vesicular compartmentation. Although high lactate dehydrogenase activity was present in the homogenate, none could be detected in the brush border or the membrane fraction.

However, the membrane fraction was rich in aminopeptidase. Table I gives the specific activities for sucrase, a marker for intestinal brush border membrane and aminopeptidase, both assayed in the same membrane preparation as lactate dehydrogenase. The purification of the two hydrolases is similar, suggesting that aminopeptidase is also a brush border membrane enzyme.

Effect of amino acids on dipeptide transport

The influence of amino acids on glycyl L-leucine is demonstrated in Table II. The constituent amino acids, at 40 times the concentration of the dipeptide, failed to inhibit the uptake of either labeled species of the dipeptide.

TABLE II

INFLUENCE OF AMINO ACIDS ON DIPEPTIDE TRANSPORT

Additions to medium: NaSCN (100 mM), test inhibitor (40 mM), [1-^{14}C]glycyl L-leucine (1 mM) or glycyl L-[U-^{14}C]leucine (1 mM). Time of incubation was 10 s/45 s. The figures are means ±S.E. of five experiments. Percentage of inhibition indicated in parenthesis.

Test inhibitors	Peptide uptake pmol/mg protein [1-^{14}C]Glycyl-L-leucine		Glycyl-L-[^{14}C]leucine	
Mannitol	299±23/557±54	(0/0)	309±28/540±59	(0/0)
Glycine	291±29/575±58	(3±1/3±2)	290±27/540±50	(6±1/2±1)
L-Leucine	310±32/570±56	(4±2/2±1)	295±27/553±52	(5±1/2±1)

TABLE III

INFLUENCE OF OTHER DIPEPTIDES ON GLYCYL-L-LEUCINE TRANSPORT

Additions to incubation medium: NaSCN (100 mM), [1-^{14}C]glycyl-L-leucine (1 mM) or glycyl-L-[U-^{14}C]leucine (1 mM) and test inhibitors (40 mM). Length of incubation was 10 s. The figures are means ± S.E. of six experiments. Percentage of inhibition is given in parenthesis.

Test inhibitor	Dipeptide uptake pmol/mg protein [1-^{14}C]Glycyl-L-leucine		Glycyl-L-[^{14}C]leucine	
Mannitol	302±28	(0)	294±25	(0)
L-Leucylglycine	149±12	(51±4)	145±13	(51±6)
L-Alanyl-L-leucine	139±14	(54±6)	147±10	(50±5)
Glycyl-L-methionine	187±12	(38±4)	171±16	(42±5)
Glycyl-L-alanine	208±18	(31±5)	212±12	(28±3)
D-Leucylglycine	245±16	(19±2)	250±18	(15±2)

TABLE IV

INFLUENCE OF DIPEPTIDES ON AMINO ACID TRANSPORT

Additions to incubation medium: NaSCN (100 mM), test inhibitor (40 mM) and [2-^{3}H]glycine (1 mM) or L-[4,5-$^{3}H_2$]leucine (1 mM). Length of incubation 10 s/45 s. The figures are means ±S.E. of six experiments. Percentage stimulation given in parenthesis.

Test inhibitor	Amino acid uptake pmol/mg protein Glycine		L-Leucine	
Mannitol	260±21/480±33	(0/0)	846±52/1056±110	(0/0)
Glycyl-L-leucine	327±33/755±81	(26±3/57±6)	1021±125/1717±157	(21±3/63±5)
L-Leucylglycine	319±37/748±74	(23±2/56±5)	998±95/1649±174	(18±3/56±4)

Influence of other dipeptides on glycyl L-*leucine transport*

Other dipeptides, when present in the incubation medium, inhibited the entry of glycyl-L-leucine (Table III). Inhibition was similar for both forms of the labeled peptide. Leucyl and alanyl peptides were more effective inhibitors than glycyl peptides. It is noteworthy that D-leucylglycine was not an effective inhibitor of glycyl L-leucine transport.

Influence of dipeptides on amino acid transport

When glycyl-L-leucine or L-leucylglycine were present in the incubation medium, no inhibition of either glycine or L-leucine transport occurred. To the contrary, a stimulation of amino acid uptake was observed, the stimulation being more pronounced at the 45 s time point than at the 10 s point.

DISCUSSION

The transport of glycyl-L-leucine and its constituent free amino acids in isolated brush border membrane appear to have differing characteristics. Dipeptide transport was identical, whether the labeled portion was the glycine or the L-leucine moiety. Both types of dipeptide were stimulated to the same extent by Na^+. Contrastingly, glycine and L-leucine transport demonstrated very different features. While L-leucine was strongly stimulated by Na^+, glycine transport was not noticeably affected. At 1 mM concentration and in the presence of an Na^+ gradient (medium > vesicle), L-leucine uptake had already attained equilibrium by 45 s. Under these same conditions, glycine was only approximately 46 % of the equilibrium level. These differences suggest that glycyl-L-leucine is transported by a system differing from that utilized by the free amino acids. Neither glycine nor L-leucine were successful in inhibiting either form of glycyl-L-leucine. This absence of competition between peptides and free amino acids has been reported by other investigators [12–15] and lends support to the concept of a separate transfer agency for dipeptides. Additionally, other dipeptides, with the exception of D-leucylglycine, were able to inhibit glycyl L-leucine transport. The failure of D-leucylglycine to inhibit glycyl L-leucine entry, while L-leucylglycine inhibited 51 % of its transport, points to a sterospecific uptake mechanism for glycyl-L-leucine.

That the dipeptide was transported intact could be affirmed by recovery of 80 % of the radioactivity in the form of dipeptide after 10 s of incubation. However, even at this early time point, 20 % of the radioactivity was in the form of amino acid. Contamination by cellular peptidases was ruled out due to the absence of lactate dehydrogenase activity. Aminopeptidase, present in intestinal and kidney brush border membrane [22, 25, 26], has been reported to hydrolyse glycyl peptides. Whether this enzyme is responsible for the observed hydrolysis of the peptide cannot at this time be certified.

Another interesting finding was that, when dipeptide was present in the incubation medium, a stimulation of the free amino acid transport was observed. This could be explained in terms of a counter-transport phenomenon; amino acids inside the vesicle, originating from the dipeptide, "loading" the vesicle and thus serving to stimulate the labeled amino acid transport on the trans side. The occurrence of counter-transport suggests that once the peptide is hydrolysed the freed amino acids are able to utilize the amino acid carrier.

In conclusion, several observations support the concept that glycyl-L-leucine is primarily transported intact across brush border membrane by a specific mechanism separate from that of amino acids. These observations are summarized as follows. (1) Initial presence of intact dipeptide within the vesicle; (2) failure of glycine and L-leucine to inhibit glycyl L-leucine transport; (3) inhibition of glycyl-L-leucine uptake by other dipeptides; (4) the occurrence of accelerated amino acid uptake in the presence of the dipeptide.

ACKNOWLEDGEMENTS

I wish to thank Mrs B. Parrish for excellent technical assistance, Dr Ulrich Hopfer for helpful comments on reviewing the manuscript and Drs H. Wacker and G. Semenza for their interest in this work. This study was supported by a grant from the Swiss National Science Foundation (grant no. 3.0090.73).

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