Biochimica et Biophysica Acta, 691 (1982) 362–366
Elsevier Biomedical Press

BBA Report

BBA 70062

PEPTIDE TRANSPORT IN RABBIT KIDNEY

STUDIES WITH L-CARNOSINE

VADIVEL GANAPATHY and FREDERICK H. LEIBACH *

Department of Cell and Molecular Biology, Medical College of Georgia, Augusta, GA 30912 (U.S.A.)

(Received May 26th, 1982)

Key words: Peptide transport; Structural requirement; Carnosine; Peptidase; Brush border; (Rabbit kidney)

L-Carnosine was shown to be transported into rabbit renal brush-border membrane vesicles by an Na^+-independent mechanism. The transport was competitively inhibited by glycyl-L-proline. Various di- and tripeptides inhibited L-carnosine transport, whereas free amino acids did not. Inhibition studies showed that blocking the free amino and carboxyl groups of the peptide reduced its affinity for the transport carrier. Under the conditions in which there was no detectable hydrolysis of L-carnosine in the medium, intravesicular contents showed a 30% hydrolysis of the peptide within the vesicles. Disruption of membrane vesicles with deoxycholate resulted in a 3-fold increase in L-carnosine hydrolyzing activity over untreated intact vesicles. Based on these observations, a model for peptide transport is proposed in which transport of the intact peptide across the membrane is followed by its partial or complete hydrolysis by a membrane peptidase whose active site is on the cytoplasmic side of the membrane.

Although the transport of peptides in the small intestine has been examined in great detail and with much interest, similar studies on peptide transport in kidney are very few. In fact, until recently there has been considerable controversy over the issue of whether or not mammalian kidney possesses a specific peptide transport system. Nutzenadel and Scriver [1] suggested that the kidney may transport small peptides, and the idea was further strengthened by the work of Adibi and his co-workers [2,3]. Direct evidence for the presence of a carrier-mediated dipeptide transport system in the brush-border membranes of renal tubular cells came from our laboratory [4–6]. We showed that purified renal brush-border membrane vesicles from rabbit can transport glycyl-L-proline by an Na^+-independent, non-concentrative mechanism. The present report deals with further characterization of this peptide transport system using L-carnosine (β-alanyl-L-histidine). The major advantage of this dipeptide is its relatively greater resistance to hydrolysis by rabbit renal brush-border membranes compared to glycyl-L-proline, and hence its potential use in the elucidation of the peptide transport mechanism.

Brush-border membrane vesicles were prepared from rabbit renal cortex by the method of Malathi et al. [7] and transport measurements were done as described in [5]. When the effect of other peptides and amino acids on L-carnosine transport was studied, the osmolarity of the transport buffer [5] was adjusted to 300 mosM by appropriately changing the concentration of mannitol, and the pH was adjusted to 7.5 with Tris base or HCl. All the peptides used in this study were chromatographically pure. The details for the recovery and identification of intravesicular contents were given in [4]. Hydrolysis of L-carnosine and glycyl-L-pro-

* To whom correspondence should be addressed

line by the purified brush-border membranes was studied as described in Ref. 4 using labeled peptides.

L-[β-alanyl-1-^{14}C]Carnosine (spec. act. 11.4 mCi/mmol) was a generous gift from R.A. Roesel of this department. The labeled peptide was chromatographically pure as checked by paper chromatography with *n*-butanol/acetic acid/water (4:1:1, v/v) as well as with 2-propanol/water (4:1, v/v) as solvent systems. [1-^{14}C]Glycyl-L-proline (spec. act. 7 mCi/mmol) was purchased from Amersham International, U.K. and β-[1-^{14}C]alanine (spec. act. 54.7 mCi/mmol) was from New England Nuclear Corporation, Boston, MA, U.S.A. All unlabeled peptides were obtained from Sigma.

The effect of ion gradients on the transport of L-carnosine (0.1 mM) and β-alanine (20 μM) is shown in Fig. 1. The presence of an Na^+-gradient resulted in a significant stimulation of β-alanine transport but it had only a minimal effect on L-carnosine transport. Thus, the characteristics of transport of radioactive label from L-carnosine and free β-alanine are quite different. The influence of various peptides and free amino acids on L-carnosine transport was also studied, and the results are given in Table I. Transport of radioactive label from labeled L-carnosine was not inhibited by either β-alanine or taurine. On the other hand, a number of dipeptides and tripeptides showed a significant inhibitory effect on L-carnosine transport. β-Alanine and taurine are known to share a common transport system in renal brush-border membrane vesicles [8]. If labeled L-carnosine were hydrolyzed to labeled free β-alanine before transport, unlabeled β-alanine and taurine would be expected to inhibit the transport of radioactive label. The lack of inhibition therefore shows that hydrolysis does not precede transport.

The inhibition by tripeptides is open to argument. Di- and tripeptides might share a common transport system in rabbit kidney as they do in intestine. In that case, tripeptides would be expected to cause significant inhibition of L-carnosine transport. However, it is also possible that the inhibition by tripeptides was due to the effect of dipeptides released by the hydrolysis of these tripeptides. However, since the incubation time was short (1 min), and the inhibition in certain cases was comparable to inhibition by dipeptides, the results strongly suggest that dipeptides and tripeptides may be transported by a common system in rabbit kidney.

TABLE I

EFFECTS OF AMINO ACIDS AND PEPTIDES ON L-CARNOSINE TRANSPORT

Purified brush-border membrane vesicles were incubated with 0.1 mM labeled L-carnosine for 1 min at 25°C in NaCl medium. The concentration of unlabeled amino acids or peptides was 5 mM. Data are expressed as mean ± S.D.

Addition	L-Carnosine transport	
	pmol/mg protein	%
None	78.5 ± 8.1	100
β-Alanine	67.1 ± 1.9	86
Taurine	84.7 ± 3.6	108
L-Carnosine	21.9 ± 0.6	28
Glycyl-L-proline	35.5 ± 6.6	45
Glycylsarcosine	19.5 ± 4.5	25
Glycyl-L-hydroxyproline	30.6 ± 0.2	39
Glycylglycine	84.0 ± 3.8	107
α-L-Glutamyl-L-alanine	46.0 ± 1.6	59
L-Prolyl-L-leucine	25.7 ± 5.4	33
Glycylglycylglycine	87.8 ± 1.5	112
L-Methionyl-L-alanyl-L-methionine	18.3 ± 1.0	23
L-Methionyl-L-methionyl-L-alanine	24.4 ± 0.4	31
α-L-Glutamyl-L-alanyl-L-alanine	52.8 ± 4.3	67
α-L-Glutamylglycyl-L-phenylalanine	30.0 ± 2.4	38
Glycyl-L-leucine	22.5 ± 1.1	29
Glycyl-D-leucine	58.2 ± 0.2	74
L-Leucinamide	69.2 ± 8.9	88
Glycyl-L-leucinamide	67.4 ± 2.3	86
Cbz-glycyl-L-leucine	65.2 ± 5.7	84
Cbz-glycyl-L-proline	68.0 ± 0.6	87

The inhibition studies with derivatives of glycyl-L-leucine show that free amino and carboxyl groups are necessary to make a dipeptide an effective substrate for the carrier system. When these groups were blocked, the dipeptide became a much less effective inhibitor. That glycyl-D-leucine was a poor inhibitor, compared to glycyl-L-leucine, shows that dipeptides with L-amino acids are preferred substrates for the renal peptide transport system.

Fig. 2 shows the effect of increasing concentration of L-carnosine on its transport. L-Carnosine

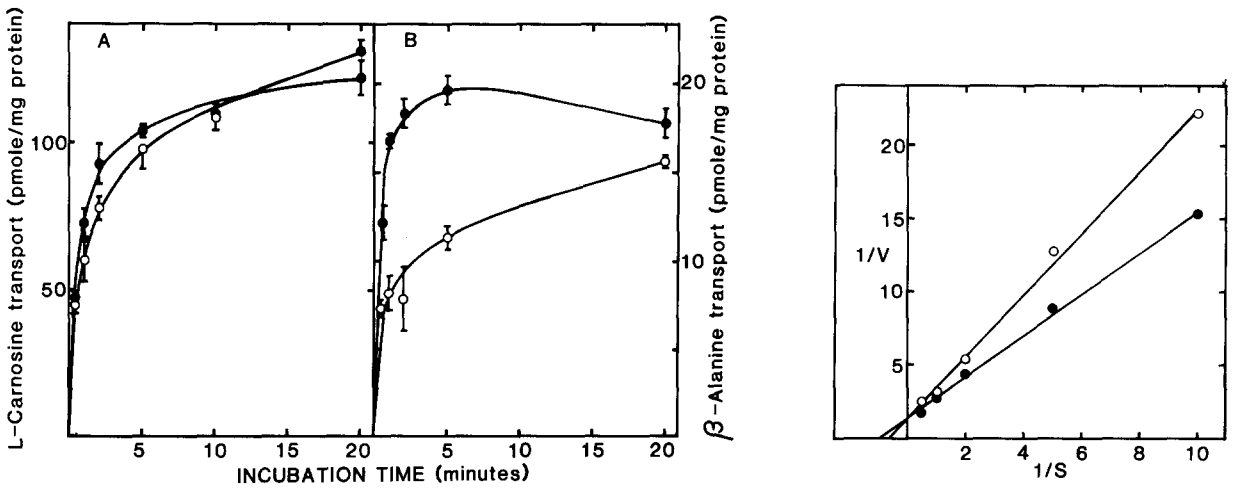

Fig. 1. Transport of 0.1 mM L-carnosine (A) and 20 μM β-alanine (B) into renal brush-border membrane vesicles in NaCl and KCl media over a period of 20 min. ●———●, NaCl medium, ○———○, KCl medium.

Fig. 2. Lineweaver-Burk plot of the inhibition of L-carnosine transport by glycyl-L-proline. Transport was studied with 1 min incubation in NaCl medium. Velocity is expressed in nmol/mg protein and substrate concentration in mM. The concentration of glycyl-L-proline was 1 mM. ●———●, glycyl-L-proline absent; ○———○, glycyl-L-proline present.

transport by rabbit renal brush-border membrane vesicles was saturable. The kinetic constants calculated by least-squares fit analysis of the data suggested that the apparent K_t for L-carnosine transport was 1.1 mM and V_{max} was 0.78 nmol/min per mg protein. Glycyl-L-proline competitively inhibited L-carnosine transport, causing a 2-fold increase in the K_t without altering the V_{max}.

Studies on the hydrolysis of L-carnosine by brush-border membrane vesicles show that the dipeptide was extremely resistant to hydrolysis. There was no detectable labeled β-alanine in the medium even after 20 min incubation under standard transport assay conditions. The extent of hydrolysis was minimal (11 ± 2%) after 1 h incubation. L-Carnosine was therefore a highly suitable substrate to study dipeptide transport in renal brush-border membrane vesicles from rabbit without the complication of hydrolysis.

The intravesicular contents were recovered and analyzed after 1 min incubation of the vesicles with labeled L-carnosine in NaCl medium. The results are given in Fig. 3. Even with such a short incubation time, about 30% of the radioactive label inside the vesicles was β-alanine, and the remaining 70% was the intact dipeptide. However, with 1 min incubation there was no detectable β-alanine in the medium. As shown in Fig. 1, the transport rates of L-carnosine and β-alanine in NaCl medium were comparable at 1 min incubation (73.2 ± 4.9 pmol/mg protein at 0.1 mM L-carnosine and 16.8 ± 0.1 pmol/mg protein at 20 μM β-alanine). Therefore, the free β-alanine inside the vesicles could not have been due to the transport of free amino acid after hydrolysis of L-carnosine in the medium. This result indicates that hydrolysis of L-carnosine should have occurred

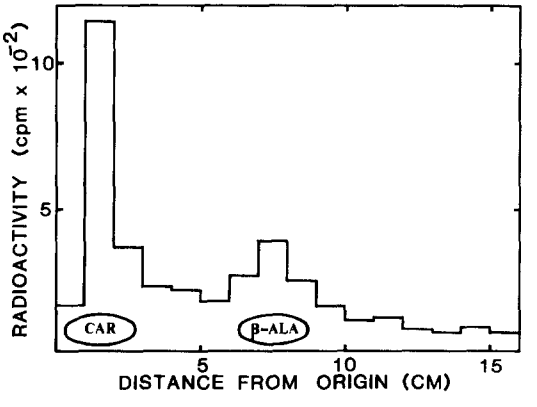

Fig. 3. Intravesicular contents of renal brush-border membrane vesicles incubated with 0.1 mM L-[*β-alanyl*-1-^{14}C]carnosine in NaCl medium.

after the transport of intact dipeptide into the vesicles. We have previously reported a similar observation with glycyl-L-proline in rabbit intestinal brush-border membrane vesicles [9].

The question then arises of which peptidase is responsible for this hydrolysis. It may be due to a membrane-bound peptidase, or traces of soluble carnosinase trapped inside the vesicles. To date, no one has reported the presence of L-carnosine hydrolyzing activity in the brush-border membrane of renal epithelial cells. The extent of cytosolic contamination in these membranes was estimated by measuring lactic acid dehydrogenase activity. Only $0.2 \pm 0.03\%$ of the total lactic acid dehydrogenase activity in the homogenate was present in the purified membrane preparations. The specific activity of lactic acid dehydrogenase was 0.49 ± 0.04 μmol/min per mg protein in the homogenate and 0.02 ± 0.01 μmol/min per mg protein in the purified membranes. To estimate the total lactic acid dehydrogenase and peptidase activities (intravesicular plus extravesicular), the vesicles were disrupted (0.1% deoxycholate, freezing and thawing) before the enzyme assays. Deoxycholate treatment resulted in a minimal increase (1.15-fold) of lactic acid dehydrogenase activity over the control value. On the other hand, there was a (3.2 ± 0.4)-fold increase in L-carnosine hydrolyzing activity and a (2.7 ± 0.2)-fold increase in glycyl-L-proline hydrolyzing activity. Thus, the cytosolic contamination could not fully account for the peptidase activities of the vesicles. Taken collectively, these data clearly show that there is a membrane-bound peptidase in the renal brush-border membrane, and the active site of the enzyme is on the cytoplasmic side of the membrane. In the control vesicles, the active site faces the intravesicular space and hence the transport of the peptide is the limiting factor in peptide hydrolysis. This adequately explains the absence of detectable hydrolysis of the peptide in the medium under short incubation conditions (up to 20 min), but a 30% hydrolysis of the peptide within 1 min inside the vesicles. Disruption of the vesicles with deoxycholate exposes the active site to the medium and thus results in an increase in the peptidase activity. A 3.2-fold increase in L-carnosine hydrolyzing activity due to deoxycholate treatment suggests that at least 70% of the vesicles are oriented right-side-out. However, the actual value will be more than 70%, because the presence of cytosolic peptidase contamination, though minimal, would result in significant underestimation of the actual value.

The specific acitivity of L-carnosine transport in the purified vesicles was 73.2 ± 4.9 pmol/min per mg protein at a peptide concentration of 0.1 mM. Under similar conditions, the specific acitivity of L-carnosine hydrolysis by the vesicles was 14.9 ± 2.3 pmol/min per mg protein. This hydrolytic activity was increased to 48.4 ± 6.8 pmol/min per mg protein after deoxycholate treatment. Thus, the specific activity of the transport system was much greater than the specific activity of hydrolysis of L-carnosine. These data clearly show that hydrolysis was not the rate-limiting step for the transport of L-carnosine.

Based on these observations, we propose a model for the transport of dipeptides across the brush-border membrane of renal epithelial cells (Fig. 4). According to this model, the peptide carrier system consists of a transport site on the external surface of the membrane which is responsible for binding and transport of the intact peptide. Before release from the membrane into the cell, the peptide comes in contact with the hydrolytic site of a membrane peptidase located on the cytoplasmic surface of the membrane. Depending upon the susceptibility of the peptide to hydrolysis by this site, the peptide may appear inside the cell as intact peptide or free amino acids or a mixture of both. The membrane peptidase may or may not be an integral part of the carrier system. We speculate, as shown in Fig. 4, that the

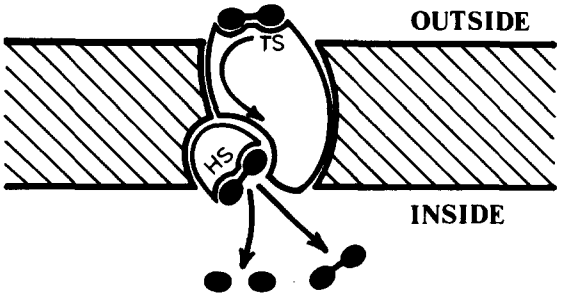

Fig. 4. A model for peptide transport. TS, transport site of the peptide carrier; HS, hydrolytic site of the membrane peptidase; ●——●, peptide; ●, amino acid.

membrane peptidase is closely associated with the peptide carrier because such a close integration of the carrier and peptidase would increase the efficiency of the peptide transport system. Even though direct proof is lacking, the model is nonetheless attractive in that it explains all the findings on peptide transport in purified brush-border membrane vesicles. This model differs significantly from other models proposed earlier [10].

The authors gratefully acknowledge the secretarial assistance of Ms. Lisa Padgett. This study was supported in part by National Institutes of Health Basic Research Support Grant RR 05365.

References

1 Nutzenadel, W. and Scriver, C.R. (1976) Am J. Physiol. 230, 643–651
2 Adibi, S.A., Krzysik, B.A. and Drash, A.L. (1977) Clin. Sci. Mol. Med., 52, 193–204
3 Adibi, S.A. and Krzysik, B.A. (1977) Clin. Sci. Mol. Med. 52, 205–213
4 Ganapathy, V., Mendicino, J., Pashley, D. and Leibach, F.H. (1980) Biochem. Biophys. Res. Commun. 97, 1133–1139
5 Ganapathy, V., Mendicino, J. and Leibach, F.H. (1981) J. Biol. Chem. 256, 118–124
6 Ganapathy, V., Mendicino, J. and Leibach, F.H. (1981) Biochim. Biophys. Acta 642, 381–391
7 Malathi, P., Preiser, H., Fairclough, P., Mallett, P. and Crane, R.K. (1979) Biochim. Biophys. Acta 554, 259–263
8 Hammerman, M.R. and Sacktor, B. (1978) Biochim. Biophys. Acta 509, 338–347
9 Ganapathy, V., Mendicino, J. and Leibach, F.H. (1981) Life Sci. 29, 2451–2457
10 Matthews, D.M. (1975) Physiol. Rev. 55, 537–608