Available online at www.sciencedirect.com

SCIENCE DIRECT

Biochimica et Biophysica Acta 1714 (2005) 134 – 140

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

http://www.elsevier.com/locate/bba

# Mechanism of inhibition of proton:dipeptide co-transport during chronic enteritis in the mammalian small intestine

Uma Sundaram[a,*], Sheik Wisel[b], Steven Coon[a]

[a]*Section of Digestive Diseases, Department of Medicine, West Virginia University School of Medicine, Medical Center Drive, Box 9161, Morgantown, WV 26506, USA*

[b]*Section of Digestive Diseases, Department of Medicine, The Ohio State University College of Medicine, Columbus, OH 43210, USA*

Received 9 April 2005; received in revised form 4 June 2005; accepted 20 June 2005
Available online 5 July 2005

**Abstract**

Amino acids, a critical energy source for the intestinal epithelial cells, are more efficiently assimilated in the normal intestine via peptide co-transporters such as proton:dipeptide co-transport (such as PepT1). Active uptake of a non-hydrolyzable dipeptide (glycosarcosine) was used as a substrate and PepT1 was found to be present in normal villus, but not crypt cells. The mRNA for this transporter was also found in villus, but not crypt cells from the normal rabbit intestine. PepT1 was significantly reduced in villus cells also diminished in villus cell brush border membrane vesicles both from the chronically inflamed intestine. Kinetic studies demonstrated that the mechanism of inhibition of PepT1 during chronic enteritis was secondary to a decrease in the affinity of the co-transporter for the dipeptide without an alteration in the maximal rate of uptake ($V_{max}$). Northern blot studies also demonstrated unaltered steady state mRNA levels of this transporter in the chronically inflamed intestine. Proton dipeptide transport is found in normal intestinal villus cells and is inhibited during chronic intestinal inflammation. The mechanism of inhibition is secondary to altered affinity of the co-transporter for the dipeptide.


*Keywords:* Proton; Dipeptide; Chronic enteritis; Amino acid absorption; Regulation of amino acid transport

## 1. Introduction

Amino acid assimilation is essential for the normal functioning of the small intestinal epithelium as it is a prime energy source for cell maintenance, turnover, and repair mechanisms [1–3]. Therefore, malabsorption of amino acids may seriously compromise the health of the small intestine [3]. In addition, altered amino acid absorption may further compromise the diseased intestinal epithelium and certainly diminish its ability to return to health. Dipeptide transporters represent a much faster and more efficient way of absorbing amino acids in the normal intestine and their regulation is therefore important to normal absorption of amino acids [1–3].

One of the most common, chronic and debilitating diseases of the small intestine is inflammatory bowel disease (IBD). IBD is characterized by malabsorption of nutrients, electrolytes and fluid in the small intestine [4,5]. This results in diarrhea, malnutrition, and weight loss in patients with this condition. Since amino acids are essential for the well being of the small intestine, better understanding of amino acid uptake alterations during chronic intestinal inflammation is important. In the normal small intestine, active amino acid absorption occurs primarily by Na-dependent amino acid co-transporters systems or peptide transporters such as proton dipeptide co-transport (i.e. PepT1; [1,2,6]). How the latter is affected during chronic enteritis is unknown. This due to the lack of suitable animal models of chronic small intestinal inflammation from which viable enterocytes suitable for the study of nutrient transport can be isolated.

In a model of chronic small intestinal inflammation, it has been demonstrated that Na-dependent amino acid co-

*Abbreviations:* BBM, Brush Border Membrane; BBMV, Brush Border Membrane Vesicles; EDTA, ethylene diamine tetraacetic acid

* Corresponding author. Fax: +1 304 293 2135.
*E-mail address:* usundaram@hsc.wvu.edu (U. Sundaram).

0005-2736/$ - see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.bbamem.2005.06.007

transport is inhibited [7]. The mechanism of inhibition at the level of the co-transporter on the BBM was due to a decrease in affinity for the amino acid without an alteration in the number of co-transporters [7]. The effect of chronic intestinal inflammation on proton:dipeptide assimilation pathway, specifically, PepT1, is unknown. Therefore, the aims of this study are first to determine the distribution of PepT1 along the villus-crypt axis of the rabbit small intestine. Next, the aim will be to determine the alterations in this co-transport process in the chronically inflamed intestine and to decipher the mechanisms of this alteration.

## 2. Materials and methods

### 2.1. Rabbit model of chronic small intestinal inflammation

Chronic intestinal inflammation was produced in rabbits as previously reported [8]. Pathogen-free New Zealand white male rabbits (Prince's Rabbitry, Oakvile, KY) were inoculated with *Eimeria magna* oocytes or sham inoculated with 0.9% NaCl (control animals). None of these sham inoculations and more than 80% of inoculation with coccidia resulted in chronic small intestinal inflammation during days 13–14. During the chronic phase of ileal inflammation histologically the intestine is free of the coccidia. Only enterocytes from those animals that had chronic small intestinal inflammation were utilized.

### 2.2. Cell isolation

Villus and crypt cells were isolated from the ileum by a calcium chelation technique as previously reported [8,9]. Previously established criteria were utilized to validate good separation of villus and crypt cells [8,9]. Previously established criteria were also utilized to study cells with good viability and to exclude those that showed evidence of poor viability [8,9].

### 2.3. Uptake studies in villus and crypt cells

Villus or crypt cells were washed and re-suspended in a Na–HEPES buffer containing (in mM) 8 μ1 $^{14}$C-Glycosarcosine (GlySar), 20 μM or 20 GlySar, 1 mM $MgSO_4$, 130 NaCl, 1.2 $K_2HPO_4$, 4.7 KCl, 1.25 $CaCl_2$, 20 HEPES–Tris, pH 6.9. The uptake was stopped by mixing with 3 ml of cold stop solution which consisted of the Na–HEPES buffer without GlySar. The mixture was filtered on 0.65 μm Millipore HAWP filters. After two washes the filter was dissolved in Liquiscient scintillation fluid and counted in a Beckman LS-5500 scintillation counter.

### 2.4. Brush border membrane vesicles preparation

Brush border membrane vesicles (BBMV) from rabbit intestinal villus cells were prepared by calcium precipitation and differential centrifugation as previously reported [10]. BBMV were re-suspended in the medium appropriate to each experiment. BBM purity was assured with appropriate marker enrichment (e.g. alkaline phosphatase).

### 2.5. Uptake studies in BBMV

BBMV uptake studies were performed by rapid filtration as previously described [10]. In brief, 5 μ1 of BBMV were re-suspended (mM) 50 HEPES–Tris, 0.1 $MgSO_4$, and 150 gluconate, pH 7.5, and incubated in 95 μ1 of reaction mix containing 8 μ1 $^{14}$C-GlySar, 20 μM GlySar, 0.1 mM $MgSO_4$ 150 mM gluconate, and either 50 mM HEPES–Tris, or 50 mM MES–Tris, pH 5.5. At desired times, the reaction was stopped with ice cold stop solution consisting (mM) 0.1 $MgSO_4$, 150

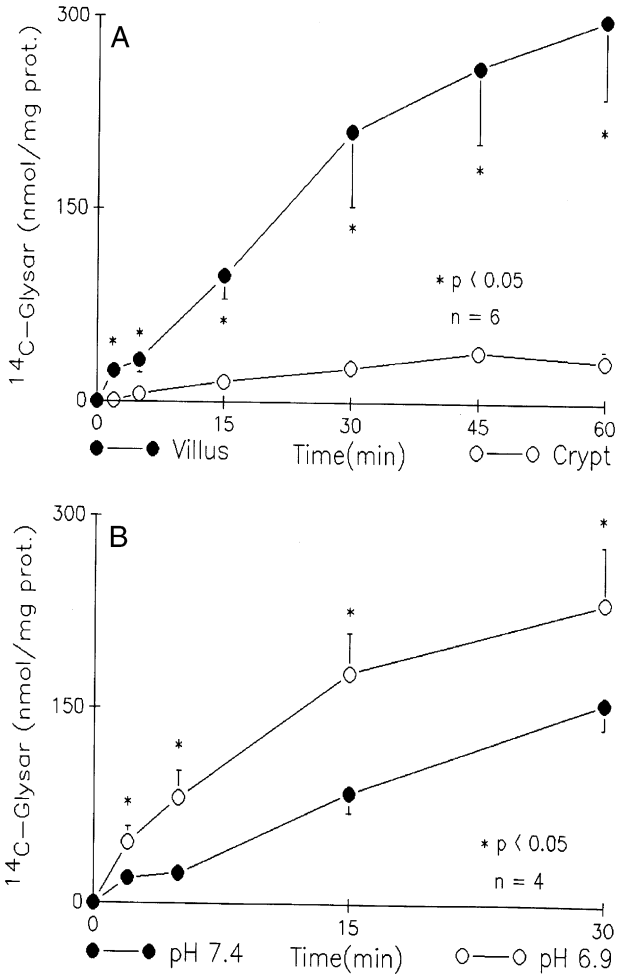


Fig. 1. Proton:dipeptide co-transport (PepT1) in the rabbit intestine. (A) The distribution of proton:dipeptide co-transport (PepT1) along the villus–crypt axis of the normal rabbit intestine. Active uptake of $^{14}$C-GlySar was defined as the uptake of $^{14}$C-GlySar in the presence of 20 μM unlabeled GlySar minus the uptake of $^{14}$C-GlySar in the presence of saturating levels of unlabeled GlySar (20 mM). Active $^{14}$C-GlySar is present in normal intestinal villus, but not crypt cells. (B) Effect of an inwardly directed proton gradient on the proton:dipeptide co-transporter in villus cells. An inwardly directed proton gradient, pH 6.9, stimulates the active uptake of $^{14}$C-GlySar in normal ileal villus cells.

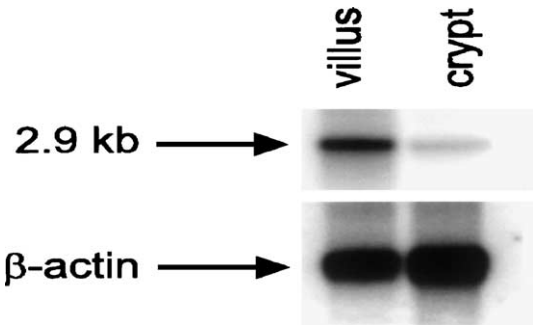


Fig. 2. Steady state messenger RNA levels of PepT1 along the villus–crypt axis of the normal rabbit intestine. The data are representative of 4 experiments. Northern blot analysis demonstrated that the mRNA for this co-transport process is found primarily in villus, but not crypt cells from the normal rabbit intestine. Densitometric quantitation also demonstrated the paucity of PepT1 mRNA in crypt cells (villus standardized to 1.0, crypt 0.16, $P<0.05$). β-actin was utilized to ensure equal loading of mRNA in gel.

NMG gluconate, and 50 HEPES–Tris, pH 7.5. The mixture was filtered on a 0.45-μm Millipore HAWP filters and washed twice with ice cold stop solution. Filters were then dissolved in Liquiscient and counted in a Beckman LS-5 liquid scintillation counter.

### 2.6. Northern blot studies

Total RNA was extracted from rabbit intestinal villus and crypt cells by the guanidinium isothiocyanate and cesium chloride methods as previously described [11]. Messenger RNA was prepared from total RNA using oligo-dT-cellulose chromatography columns [12]. mRNA was fractionated by electrophoresis on 1.8% agarose/formaldehyde gel, transferred to nylon plus membrane (Schleicher and Schuell, Keene, NH) and incubated with prehybridization solution. Membranes were hybridized with $^{32}$P labeled cDNA (pSPORT/PepT1, from Dr. V. Ganapathy, Augusta, GA), hybridized membranes were washed, dried and exposed to autoradiography film (NEN Research Products, Boston, MA). $^{32}$P labeled human β-actin (Clontech, CA) was used to ensure equal loading of RNA onto the electrophoresis gels.

### 2.7. Data presentation

When data are averaged, means±S.E.M. are shown except when error bars are inclusive within the symbol. All vesicle uptakes were done in triplicate. The *n* number for any set of experiments refers to vesicle or isolated cell preparation from different animals. Preparations in which cell viability was less than 85% were excluded from the analysis. Proton gradient stimulated uptake for cells and vesicles is defined as uptake in the presence of an inwardly directed proton-gradient minus uptake without such gradient. Student's *t*-test was used for statistical analysis.

## 3. Results

The distribution of proton dipeptide co-transport (PepT1) along the villus–crypt axis of the normal rabbit ileum was initially determined (Fig. 1A). Active uptake of $^{14}$C-GlySar defined as the uptake of $^{14}$C-GlySar in the presence of 20 μM GlySar minus the uptake of $^{14}$C-GlySar in the presence of saturating levels of unlabeled GlySar (20 mM) was determined in both cell types. As demonstrated in Fig. 1A, active uptake of $^{14}$C-GlySar was present in normal ileal villus cells. However, active uptake of $^{14}$C-GlySar was not present in crypt cells. Thus, PepT1 activity is present in villus, but not crypt cells from the normal ileum.

An inwardly directed proton-gradient would be expected to stimulate the active uptake of $^{14}$C-GlySar [13–

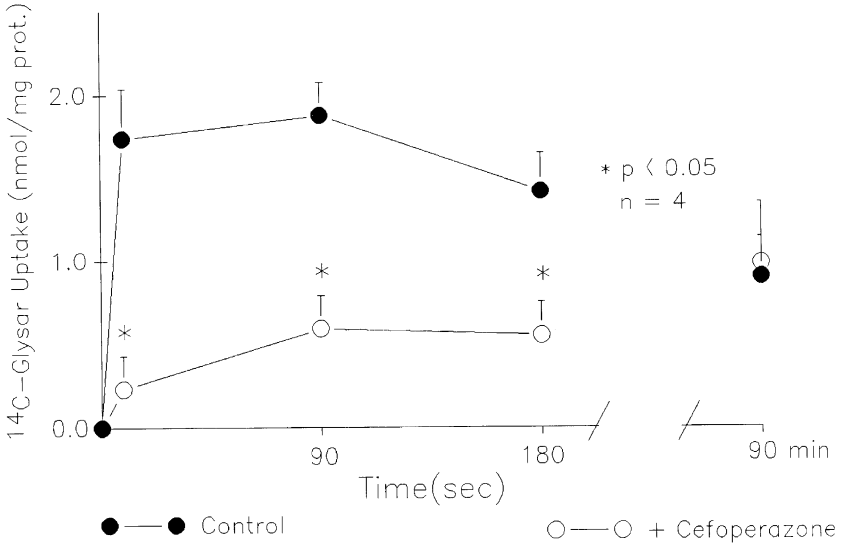


Fig. 3. PepT1 in villus cell BBMV. Proton gradient (pH 7.5 in/5.5 out) stimulated $^{14}$C-GlySar uptake in BBMV prepared from villus cells in the normal rabbit ileum. Villus cell BBMV proton gradient stimulated $^{14}$C-GlySar uptake is competitively inhibited by cefoperazone.

17]. Thus, the effect of acidic extra-cellular pH on PepT1 was determined in villus cells. As shown in Fig. 1B, an extra-cellular pH of 6.9 as compared to 7.4, did indeed stimulate active uptake of $^{14}$C-GlySar. Thus, the rabbit intestinal villus cell PepT1 is an active and proton dependent co-transporter.

Having demonstrated the presence of PepT1 activity in normal ileal villus, but not crypt cells, we next looked at the message for this co-transport process by Northern blot analysis. As shown in Fig. 2, the message for the PepT1 is present primarily in villus rather than in crypt cells from the normal rabbit ileum. Thus, the PepT1 message and the functional protein appears to be localized to the absorptive villus cells in the normal rabbit ileum.

Proton dipeptide co-transport was then further characterized at the level of the villus cell brush border membrane (BBM). In Fig. 3, the proton gradient-dependent $^{14}$C-GlySar uptake in villus cell BBMV from the normal rabbit ileum is shown. It has previously been demonstrated that certain antibiotics of the cephalosporin family may be transported by the proton dipeptide co-transport system [1,18]. If cephalosporins were to utilize this co-transporter in the normal rabbit intestine, then a cephalosporin would be expected to competitively inhibit this transport process. Thus, we next determined the effect of cefoperozone on $^{14}$C-GlySar uptake as demonstrated in Fig. 3. Cefoperozone significantly reduced the proton gradient-dependent uptake of $^{14}$C-GlySar. This indicates that the rabbit PepT1 may transport cephalosporin type of antibiotics.

Having determined the villus–crypt distribution and characteristics of the rabbit intestinal PepT1, we then studied the effect of chronic ileal inflammation on this system. Active uptake of $^{14}$C-GlySar was inhibited in intact villus cells from the chronically inflamed intestine as shown in Fig. 4A. In order to ensure that it is the proton dependent $^{14}$C-GlySar uptake that is affected rather than an increase in the nonspecific movement of $^{14}$C-GlySar during chronic intestinal inflammation, we then determined the effect of an acidic pH on PepT1 in intact villus cells from the chronically inflamed intestine. As demonstrated in Fig. 4B, the proton gradient-dependent $^{14}$C-GlySar uptake is indeed significantly reduced in villus cells from the chronically inflamed intestine. These data demonstrate that PepT1 is inhibited in villus cells during chronic enteritis.

To characterize the inhibition of PepT1 in villus cells during chronic enteritis is at the level of the transporter on the BBM, we studied the proton gradient-stimulated uptake of $^{14}$C-GlySar in BBMV prepared from villus cells from chronically inflamed intestine. As shown in Fig. 5, proton gradient-stimulated that $^{14}$C-GlySar uptake is significantly reduced in BBMV prepared from villus cells during chronic enteritis. To ensure that active uptake of $^{14}$C-GlySar was inhibited in BBM from the chronically inflamed intestine, peptide uptake in the absence of a proton gradient was measured. Indeed, peptide uptake in the absence of a proton gradient was also inhibited in BBM

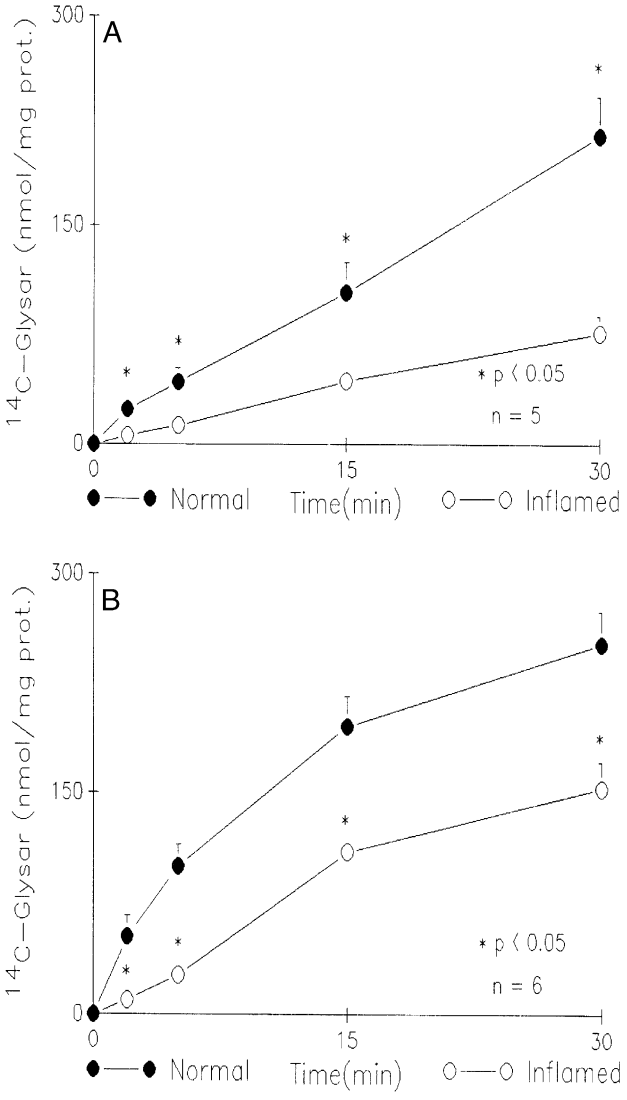


Fig. 4. The effect of chronic intestinal inflammation on intestinal PepT1. (A) The active uptake of $^{14}$C-GlySar is significantly reduced in villus cells from the chronically inflamed ileum. (B) The effect of chronic enteritis on proton gradient-dependent dipeptide co-transport. The proton gradient stimulated uptake of $^{14}$C-GlySar is diminished in villus cells from the chronically inflamed intestine.

from the chronically inflamed intestine (0.90±0.11 nmol/mg protein/9 s in normal and 0.28±0.06 in the chronically inflamed intestine, $n=3$, $P<0.05$). These data demonstrate that PepT1 is inhibited in villus cell BBM during chronic enteritis.

Kinetic studies were then performed to decipher the mechanism of inhibition of PepT1 inhibition in the chronic inflamed intestine. Uptake for various concentrations were carried out at 3 s since initial uptake studies for proton gradient-dependent $^{14}$C-GlySar uptake in BBMV was linear for at least 8 s (data not shown). Fig. 6A demonstrates the kinetics of $^{14}$C-GlySar uptake in villus cell BBMV from the normal and chronically inflamed ileum. As the concentrations of extra-vesicular GlySar was increased, proton gradient-dependent $^{14}$C-GlySar uptake was stimulated and

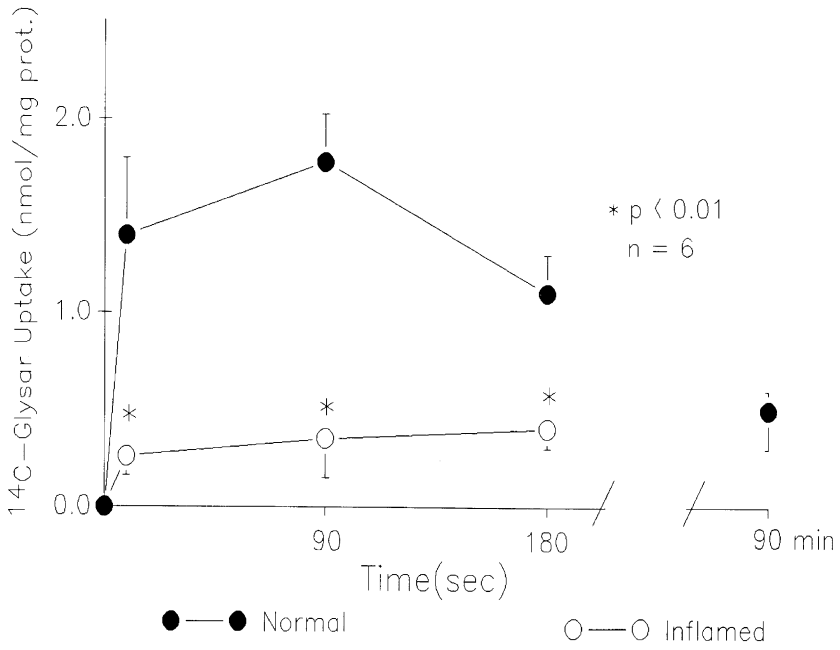


Fig. 5. The effect of chronic intestinal inflammation on PepT1 in villus cell BBMV. The proton gradient stimulated uptake of $^{14}$C-GlySar is markedly reduced in BBMV prepared from villus cells in the chronically inflamed intestine.

subsequently became saturated in all conditions. Using Enzfitter, kinetic parameters derived from these data demonstrated that the maximal of uptake of GlySar was not altered in the chronically inflamed ileum (Fig. 6B; $V_{max}$ for GlySar uptake in villus cell BBMV was 12.9±1.5 nmol/mg protein in normal and 13.1±1.9 in inflamed, $n$=4). However, the affinity ($1/K_m$) for GlySar was significantly reduced in the chronically inflamed ileum ($K_m$ for GlySar uptake in villus cell BBMV was 2.5±0.2 mM in normal and 5.7±0.5 in inflamed, $n$=4, $P$<0.01). These data indicated that PepT1 was inhibited in the chronically inflamed ileum secondary to a decrease in the affinity for GlySar rather than a change in the number of co-transporters.

To determine whether the message for this co-transporter may be altered during chronic enteritis we next measured the steady state mRNA levels of this co-transporter in the villus cells from the chronically inflamed intestine. As shown on Fig. 7, Northern blot studies showed that the steady state mRNA levels of PepT1 were unchanged in villus cells from the chronically inflamed intestine.

## 4. Discussion

These studies for the first time demonstrate the distribution of proton dipeptide co-transport (PepT1) along the villus–crypt axis of the normal rabbit intestine and the effect of chronic intestinal inflammation on this co-transport process. PepT1 is found in normal intestinal villus, but not crypt cells. This co-transport process is inhibited in villus cells from the chronically inflamed intestine. The mechanism of inhibition is secondary to a reduction in the affinity of the co-transporter for the dipeptide rather than an alteration in the number of co-transporters.

Peptide and Na–amino acid co-transport systems represent the primary means of assimilation of amino acids in the

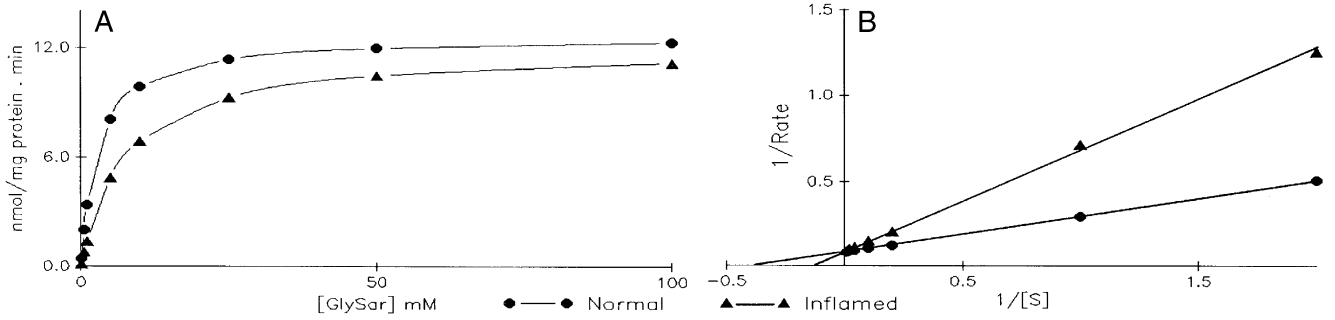


Fig. 6. Kinetic studies of proton dipeptide co-transport inhibition in the chronically inflamed intestine. (A) As the concentration of extra-vesicular GlySar was increased, the proton gradient-dependent $^{14}$C-GlySar uptake was stimulated and subsequently became saturated in all conditions. (B) Using Enzfititer, kinetic parameters derived from the data in panel A demonstrated the maximal rate of uptake ($V_{max}$) of GlySar was not altered in the chronically inflamed ileum. However, the affinity ($1/K_m$) for GlySar uptake was markedly reduced in villus cell BBMV from the chronically inflamed intestine.

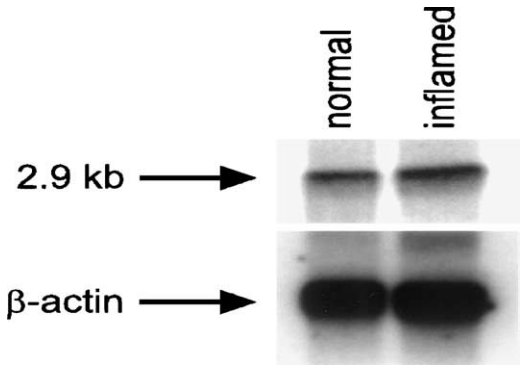


Fig. 7. Effect of chronic intestinal inflammation on steady state mRNA levels of PepT1. The data are representative of 3 experiments. Northern blot analysis demonstrated that steady state mRNA levels of the proton dipeptide co-transporter were unchanged in villus cells from the chronically inflamed intestine. Densitometric quantitation also demonstrated no significant change in PepT1 mRNA levels (villus from the normal intestine standardized to 1.0, chronically inflamed villus cells 1.04). β-actin was utilized to ensure equal loading of mRNA in gels.

small intestine [1,2,6]. It has been well established that amino acids are not only critical building blocks for the organism as a whole, but that they are also the essential source of energy for the small intestinal epithelium [1–3,6]. Thus, better understanding of the regulation of amino acid assimilation is important in the normal intestine and may be even more important in intestinal disorders such as inflammatory bowel disease (IBD). In pathological states such as (IBD) assimilation of key nutrients may be the most important step in recovery back to health. Given this background, our laboratory had previously studied Na–amino acid co-transport (specifically the ASCT1 system; 7). It was demonstrated that Na–amino acid co-transport was found in rabbit intestinal villus, but not crypt cells. This co-transport process was significantly diminished during chronic intestinal inflammation. At the cellular level, the inhibition was secondary to a decrease in Na-K-ATPase levels and secondary to an alteration at the co-transporter level. At the level of the co-transporter, the mechanism of inhibition during chronic enteritis was secondary to a reduction in the affinity of the co-transporter without a change in number of co-transporters [7].

In contrast to Na–amino acid co-transport, PepT1 has been less well studied. It has been demonstrated in mouse, rat, rabbit and human small intestine [14–17,19–24]. It is not only responsible for the absorption of amino acids, but it is also important in the assimilation of certain types of antibiotics such as beta lactams. Nevertheless, very little was previously known about the distribution of this transport process along the villus/crypt axis and how it may be altered in diarrheal diseases characterized by chronic intestinal inflammation.

A vast majority of currently available evidence would suggest that villus cells are absorptive, while crypt cells are secretory in the normal intestine [25]. Coupled NaCl absorption and a variety of Na-dependent nutrient co-transport processes have been demonstrated to be a feature of the absorptive villus, but not secretory crypt cells in the normal small intestine [25]. This study demonstrates that active uptake of dipeptide is present in villus, but not crypt cells in the normal rabbit intestine. As expected, a proton-gradient stimulated the uptake of the dipeptide in villus cells. The message for PepT1 was also found primarily in villus cells from the normal intestine. Taken together, these data demonstrate that proton dipeptide co-transport is also a feature of the absorptive villus, but not secretory crypt cells in the normal rabbit intestine.

Most nutrient assimilation transport processes are localized to the BBM of villus cells [7,10]. Indeed, this study demonstrates that PepT1 is also localized to the BBM of villus cells. It was previously demonstrated that certain types of antibiotics (e.g., beta lactams) may be assimilated via proton dipeptide co-transport [1,18]. This study demonstrates that cephalosporins may very well be transported by PepT1 on the BBM of villus cells in the normal intestine. This observation has clinical implications. Antibiotics are frequently utilized to treat IBD. However, if the transport process responsible for the uptake of an antibiotic is altered during IBD then treatment with that antibiotic is less likely to be efficacious. Information in this area of intestinal transport physiology was previously lacking. This study demonstrates that PepT1 is inhibited during chronic enteritis. This study would suggest that certain types of antibiotics may be less well absorbed during chronic intestinal inflammation and therefore, may be less efficacious than other types of antibiotics.

In addition to serving as a carrier for certain types of antibiotics, PepT1 serves as a primary means of assimilation of an important metabolic fuel for the normal small intestinal epithelial cells. Amino acid absorption may be even more important in the compromised epithelium as the inflamed intestine attempts to return to health. However, how the absorption of this important nutrient source may be altered in diarrheal diseases characterized by chronic intestinal inflammation was previously unknown. The results of these studies demonstrate that PepT1 is reduced in intact villus cells from the chronically inflamed intestine. The reduction was not simply secondary to an increase in the passive uptake of dipeptide. Indeed, active uptake dipeptide was inhibited in the intact villus cells during chronic enteritis. Further, it is the proton gradient-dependent uptake of peptide that is inhibited in the chronically inflamed intestine. At the level of the co-transporter on the BBM, proton dipeptide co-transport is also significantly diminished in the chronically inflamed intestine. Thus, PepT1 is inhibited in the chronically inflamed intestine.

Kinetic studies and molecular studies demonstrated the mechanism of inhibition of PepT1 during chronic enteritis. The affinity of PepT1 for the dipeptide was markedly reduced. There was no change in the maximal rate of uptake. Northern blot studies of villus cells from the normal and chronically inflamed intestine also demonstra-

ted no change in the message level of this co-transporter during chronic enteritis. Taken together, these data demonstrate that PepT1 is inhibited during chronic enteritis secondary to a decrease in the affinity of co-transporter for the dipeptide without a change in the number of co-transporters. Affinity changes in PepT1 are likely post-translational. Possible mechanisms which may alter the transporter's affinity could occur at the level of glycosylation of PepT1 during chronic enteritis. Alternatively, affinity changes occur at the level of phosphorylation of NAcT during chronic enteritis.

It is interesting to note that the two primary means of amino acid assimilation are markedly altered during chronic intestinal inflammation. This laboratory had previously demonstrated that the mechanism of inhibition of Na–amino acid co-transport in the chronically inflamed intestine was secondary to a reduction in the affinity of the co-transporter for the amino acid without a change in the number of co-transporters [7]. This study demonstrates a similar mechanism for the other major pathway of amino acid assimilation in the chronically inflamed intestine. Thus, two important means of assimilation of amino acids in the intestine are reduced during chronic intestinal inflammation and the mechanism of inhibition of these two dissimilar transport processes is similar during chronic intestinal inflammation.

## Acknowledgements

The authors thank Dr. V. Ganapathy, Augusta, GA for kindly providing the cDNA used in the Northern blots and J. Ward, Gastroenterology fellow for some of the preliminary uptake studies. Grant Support: This work was supported by National Institutes of Health Research Grants DK45062 and DK58034 to U. Sundaram.

## References

[1] V. Ganapathy, M. Brandsch, F.H. Leibach, Intestinal transport of amino acids and peptides, in: L.R. Johnson (Ed.), Physiology of the Gastrointestinal Tract, 3rd ed., Raven, New York, 1994, pp. 1773–1794.
[2] L.K. Munck, B.A. Munch, Amino acid transport in the small intestine, Physiol. Res. 43 (1994) 335–346.
[3] H.G. Windmueller, A.F. Spaeth, Respiratory fuels and nitrogen metabolism in vivo in small intestine of fed rats: quantitative importance of glutamine, glutamate, and aspartate, J. Biol. Chem. 255 (1980) 107–112.
[4] G.A. Castro, Immunological regulation of electrolyte transport, in: E. Lebenthan, M.E. Duffey (Eds.), Textbook of Secretory Diarrhea, Raven, New York, 1991, pp. 31–46.
[5] D.W. Powell, Immunophysiology of intestinal electrolyte transport, Handbook of Physiology, Am. Physiol. Soc., vol. IV, The Gastrointestinal System, Bethesda, MD, 1990, pp. 591–683.
[6] U. Hopfer, Membrane transport mechanisms for hexoses and amino acids in the small intestine, in: L.R. Johnson (Ed.), Physiology of the Gastrointestinal Tract, 2nd ed., Raven, New York, 1994, pp. 1499–1526.
[7] U. Sundaram, S. Wisel, J.J. Fromkes, Unique mechanism of inhibition of Na–amino acid co-transport during chronic ileal inflammation, Am. J. Physiol. 275 (1998) G483–G489.
[8] U. Sundaram, A.B. West, Effect of chronic ileal inflammation on electrolyte transport in rabbit ileal villus cells, Am. J. Physiol. 272 (35) (1997) G732–G741.
[9] U. Sundaram, R.G. Knickelbein, J.W. Dobbins, pH regulation in ileum: Na:H and Cl:$HCO_3$ exchange in isolated crypt and villus cells, Am. J. Physiol. 260 (23) (1991) G440–G449.
[10] U. Sundaram, S. Wisel, V.M. Rajendran, A.B. West, Mechanism of inhibition of Na-glucose co-transport in the chronically inflamed rabbit ileum, Am. J. Physiol. 272 (36) (1997) G913–G919.
[11] P. Chomcynski, N. Sacci, Single step method of RNA isolation by acid guanidinium thicyanate-phelol-chloroform extraction, Anal. Biochem. 162 (1987) 156–159.
[12] H. Aviv, P. Loder, Purification of biologically active globin mRNA by chromatography on oligothymidic acid-cellulose, Proc. Natl. Acad. Sci. U. S. A. 69 (1972) 1408–1412.
[13] Y.J. Fei, Y. Kanal, S. Nussberger, V. Ganapathy, F.H. Leibach, M.F. Romero, S.K. Singh, W.F. Boron, M.A. Hediger, Expression cloning of a mammalian proton coupled oligopeptide transporter, Nature 368 (1994) 563–566.
[14] V. Ganapathy, G. Burckhardt, F.H. Leibach, Peptide transport in rabbit intestinal brush border membrane vesicles studies with a potential-sensitive dye, Biochim. Biophys. Acta 816 (1985) 234–240.
[15] A. Berteloot, A.H. Khan, K. Ramaswamy, Characteristics of dipeptide transport in normal and papain-treated brush border membrane vesicles from mouse intestine: I. Uptake of glycyl-L-phenylalanine, Biochim. Biophys. Acta 649 (1981) 179–188.
[16] A. Berteloot, A.H. Khan, K. Ramaswamy, Characteristics of dipeptide transport in normal and papain-treated brush border membrane vesicles from mouse intestine: II. Uptake of glycyl-L-leucine, Biochim. Biopys. Acta 686 (1982) 47–54.
[17] V. Ganapathy, G. Burckhardt, F.H. Leibach, Characteristics of glycylsarcosine transport in rabbit intestinal brush border membrane vesicles, J. Biol. Chem. 259 (1984) 8954–8959.
[18] P. Sinko, G. Amidon, Characterization of the oral absorption of B-lactam antibiotics: II. Competitive absorption and peptide carrier specificity, J. Pharm. Sci. 78 (1989) 723–727.
[19] V. Ganapathy, F.H. Leibach, 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, J. Biol. Chem. 258 (1983) 14189–14192.
[20] V. Ganapathy, J.F. Mendicino, F.H. Leibach, Transport of glycyl-L-proline into intestinal and renal brush border vesicles from rabbit, J. Biol. Chem. 256 (1981) 118–124.
[21] V.M. Rajendran, S.A. Ansari, J.M. Harig, M.B. Adams, A.H. Khan, K. Ramaswamy, Transport of glycyl-L-proline by human intestinal brush border membrane vesicles, Gastroenterology 89 (1985) 1298–1304.
[22] V.M. Rajendran, A. Berteloot, V. Ishikawa, A.H. Khan, K. Ramaswamy, Transport of carnosine by mouse intestinal brush border vesicles, Biochim. Biophys. Acta 778 (1984) 443–448.
[23] V.M. Rajendran, A. Berteloot, K. Ramaswamy, Transport of glycyl-L-proline by mouse intestinal brush border membrane vesicles, Am. J. Physiol. 248 (1985) G682–G686.
[24] V.M. Rajendran, J.M. Harig, K. Ramaswamy, Characteristics of glycyl-L-proline transport in intestinal brush border membrane vesicles, Am. J. Physiol. 252 (1987) G281–G286.
[25] R. Kirckelbein, P.S. Aronson, J.W. Dobbins, Membrane distribution of sodium–hydrogen and chloride–bicarbonate exchangers in crypt and villus cell membranes from rabbit ileum, J. Clin. Invest. 82 (1988) 2158–2163.