γ -GLUTAMYLTRANSPEPTIDASE IN INTESTINAL BRUSH BORDER MEMBRANES

Norman P. CURTHOYS and Richard SHAPIRO
Department of Biochemistry, University of Pittsburgh, School of Medicine, Pittsburgh, Pa. 15261, USA

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1. Introduction

γ-Glutamyltranspeptidase is an enzyme of very broad specificity. It catalyzes the transfer of the γ-glutamyl group from glutathione as well as from a large number of other γ-glutamyl compounds including glutamine to a wide variety of amino acid acceptors. This enzyme has received considerable attention because of its clinical use as an indicator of hepatic dysfunction [1], its potential involvement in mercapturic acid biosynthesis [2], and its proposed role in amino acid transport [3]. The recent finding that the phosphate-independent glutaminase from rat kidney is a partial reaction of γ-glutamyltranspeptidase, and that maleate, which activates this glutaminase, acts by blocking transpeptidation, has lead to the suggestion that this enzyme may also be involved in renal ammonia formation [4,5]. This communication deals with the γ-glutamyltranspeptidase activity of small intestine. The activity in crude homogenates of villus cells isolated from rat small intestine exhibits a similar fold activation or inhibition by various combinations of amino acids and maleate as the γ-glutamyltranspeptidase from rat kidney. In addition, sequential elution of villus to crypt cells and preparation of intestinal brush border membranes indicate that the γ-glutamyltranspeptidase is localized primarily in the brush border membrane of the villus cells of the rat small intestine.

2. Materials and methods

White male rats (250–300 g) were obtained from Zivic-Miller and were maintained on Purina rat chow. γ-Glutamyl-*p*-nitroanilide and *p*-nitrophenylphosphate were obtained from Sigma Chemical Co.

Except where described in the text, the γ-glutamyltranspeptidase assay mixture contained: 3 mM γ-glutamyl-*p*-nitroanilide, 40 mM methionine, 50 mM Tris-Cl and 0.2 mM EDTA, pH 8.4. For kidney homogenates appearance of *p*-nitroanilide was followed continuously, but for homogenates of intestinal cells it was necessary to incubate samples at 37°C for 20–40 min, stop the reaction by heating to 90°C for 2 min and then centrifuging at 10 000 *g* to remove denatured protein and determining absorbance at 410 nm. Alkaline phosphatase was assayed by following the appearance of *p*-nitrophenol from a solution containing 1.5 mM *p*-nitrophenylphosphate, 4mM $MgCl_2$, 1mM $ZnCl_2$ and 40 mM glycine, pH 9.2. Sucrase [6] and galactosyl transferase [7] were assayed as described previously. Protein was determined by the method of Lowry et al. [8].

Intestinal epithelial cells were prepared according to the method of Weiser [9]. This procedure involves repeated filling of the small intestine with a phosphate buffered saline solution which contains EDTA and dithiothreitol. Fractions obtained from the initial incubations contain cells selectively released from the villus zone. Subsequent incubations result in release of cells in the form of a gradient from villus to crypt cells. Brush border preparations were obtained from villus cells (fractions 1–4) by the method of Forstner et al. [10]. Brush border membrane preparations were then obtained by the method of Hopfer et al. [11] which uses $MgSO_4$ precipitation to separate core from plasma membrane.

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Table 1
Comparison of the effect of amino acids and of maleate on γ-glutamyltranspeptidase activity from rat kidney and from intestinal villus cells

Acceptor	Kidney			Intestine		
	− Maleate (nmol · min^{-1} · mg^{-1})	+ Maleate	+/−[a]	− Maleate (nmol · min^{-1} · mg^{-1})	+ Maleate	+/−
None	156	271	1.74	2.01	3.76	1.87
Methionine	550 (3.5)[b]	336 (1.24)	0.61	6.55 (3.3)	4.14 (1.10)	0.63
Glutamine	531 (3.4)	165 (0.61)	0.31	6.45 (3.2)	2.72 (0.72)	0.42
Alanine	329 (2.1)	37 (0.14)	0.11	3.48 (1.7)	0.48 (0.13)	0.14

Crude homogenates of rat kidney and of isolated villus cells (fraction number 1) were homogenized in phosphate buffered saline. γ-Glutamyltranspeptidase activity was measured with 3 mM γ-glutamyl-*p*-nitroanilide, 50 mM imidazole, pH 7.2. When added, amino acid acceptors and maleate were present at 40 mM and 60 mM concentrations, respectively. [a] +/− Ratio of activity in the presence of maleate to that in the absence of maleate. [b] Numbers in parentheses represent fold effect of addition of amino acid acceptor on γ-glutamyltranspeptidase activity.

3. Results

The specific activity of γ-glutamyltranspeptidase in isolated villus cells of rat small intestine is about a 100-fold less than that observed in crude homogenates of rat kidney (table 1). But, in the absence of maleate, the addition of various amino acids cause a similar fold activation of γ-glutamyltranspeptidase in both preparations. In the absence of an amino acid acceptor, the addition of maleate causes a similar increase in *p*-nitroanilide production. The other product formed under these conditions is primarily glutamate [4]. Also, the presence of both maleate and amino acids result in similar effects on γ-glutamyltranspeptidase activity in both preparations, including the potent inhibition observed by the presence of alanine and maleate. In addition to the similar extents of activation and inhibition, the intestinal γ-glutamyltranspeptidase also exhibits maximal activity at slightly alkaline pHs, similar to the renal enzyme. The rat kidney γ-glutamyltranspeptidase can be solubilized by treatment with papain [4]. Similarly, incubation of rat small intestine with papain also results in release of γ-glutamyltranspeptidase with complete recovery of enzyme activity.

γ-Glutamyltranspeptidase is localized primarily in the villus cells of the small intestine (fig.1). The specific activity of γ-glutamyltranspeptidase in the initinal fraction of epithelial cells is 13-fold greater than that observed in the final fraction. The profile of γ-glutamyltranspeptidase activity is very similar to that observed for alkaline phosphatase activity which is a marker for villus cells [9] and is in contrast to the profile for galactosyl transferase activity which has been characterized as being contained primarily in

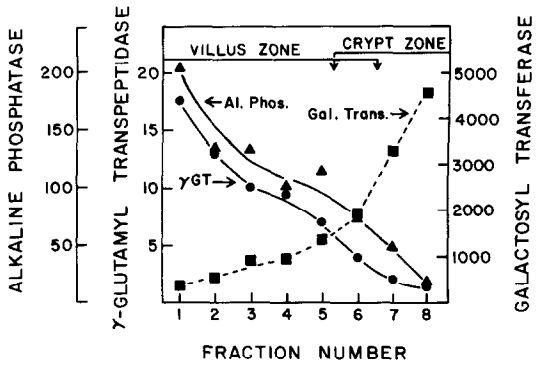

Fig.1. Localization of γ-glutamyltranspeptidase activity in the villus cells of the rat small intestine. A gradient of epithelial cells from villus zone to crypt zone was obtained by successive incubation of the small intestine filled with a phosphate buffered saline solution [9]. γ-Glutamyltranspeptidase (γGT) and alkaline phosphatase (Al. Phos.) are reported in units of nmol · min^{-1} · mg^{-1}, whereas galactosyl transferase (Gal. Trans.) is given in terms of cpm · mg^{-1}. The first five fractions of cells contained approximately the same amount of total protein, but the last three fractions, each contained about one-half that amount.

Table 2
Enrichment of γ-glutamyltranspeptidase and sucrase activities in preparations of intestinal brush border membranes

Fraction	Sucrase		γ-Glutamyl-transpeptidase	
	Total units	units/mg	Total units	units/mg
Crude cell homogenate	23 400	59	5520	14
'Purified' brush borders	16 400 (70)[a]	490	3320 (60)	100
Brush border membranes	11 500 (49)	1610	1930 (35)	270

Units are reported as nmol · min^{-1}. [a] Numbers in parentheses represent percent recovery.

crypt cells of the small intestine [7]. Assay of segments of small intestine before and after elution of epithelial cells indicate that this procedure results in release of greater than 85% of the total γ-glutamyltranspeptidase and alkaline phosphatase activities. As shown in table 2, isolation of brush border membranes from villus cells is accomplished with substantial retention of γ-glutamyltranspeptidase activity. The similar fold enrichment of γ-glutamyltranspeptidase (19-fold) and of sucrase activity (27-fold), a well characterized marker for intestinal brush border membrane [11], establishes that γ-glutamyltranspeptidase is also localized in the brush border membrane.

4. Discussion

The γ-glutamyltranspeptidase in rat small intestine appears to be identical to the transpeptidase found in rat kidney. Both preparations exhibit similar resistance to papain and nearly identical kinetics. These observations also indicate that the effect of maleate on γ-glutamyltranspeptidase activity is not unique to the kidney enzyme. In fact, γ-glutamyltranspeptidase in crude homogenates of rat brain, seminal vesicles, and pancreatic tissue are also similarly affected by maleate. They are also extensively inhibited by the combination of maleate and alanine. Using a purified preparation of γ-glutamyltranspeptidase from rat kidney [4], of 14 amino acids tested, only serine in combination with maleate produced a potent inhibition similar to that observed with alanine. This inhibition may be similar to the previously reported inhibition of γ-glutamyltranspeptidase that is produced by serine and borate [12]. But, understanding of the cause or mechanism of this inhibition will require further characterization.

Previous histochemical studies have indicated that γ-glutamyltranspeptidase activity is localized in the microvillus border of the columnar epithelial cells of the tips of the villi of the rat small intestine [13]. We have confirmed these observations by demonstrating that γ-glutamyltranspeptidase activity is concentrated in well characterized preparations of isolated villus cells and of brush border membranes. The proposal that γ-glutamyltranspeptidase may be involved in amino acid transport is very attractive and has been supported by considerable indirect experimentation [14,15]. But, to my knowledge no experiments have been reported which test directly the ability of γ-glutamyltranspeptidase to participate in amino acid transport. Both preparation of isolated intestinal cells [16] and of intestinal brush border membrane vesicles [11,17] have been used to study glucose and amino acid transport. The observation that these preparations are significantly enriched in γ-glutamyltranspeptidase activity, suggests that either or both of these preparations may prove useful in attempting to develop a system in which one could correlate the effect of various inhibitors or activators of γ-glutamyltranspeptidase with their effect on rates of amino acid transport.

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