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Glutathione and γ-glutamyl cycle enzymes in crypt and villus tip cells of rat jejunal mucosa

(amino-acid transport/γ-glutamyl transpeptidase/γ-glutamyl cyclotransferase/glutathione synthetase/γ-glutamyl cysteine synthetase)

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ABSTRACT Villus tip cells and crypt cells of rat jejunal mucosa were separated by the planing procedure of Imondi *et al.* and were studied with respect to their activities of the enzymes of the γ-glutamyl cycle and glutathione content. The villus tip cells exhibit much higher γ-glutamyl transpeptidase activities than do the crypt cells; thus, γ-glutamyl transpeptidase appears to be a villus-specific enzyme. γ-Glutamyl cyclotransferase and the enzymes required for glutathione synthesis are not specifically localized to either the crypt or villus tip cells but are present in both. The crypt cells have a high concentration of glutathione (4–5 mM) comparable to the levels found in liver and kidney; in contrast, the villus tip cells have much lower concentrations. On fasting, the glutathione concentration decreased markedly in both villus tip and crypt cells; feeding of protein, but not of sucrose, led to increased glutathione concentrations. The migration of cells from the undifferentiated crypt cell region to the villus tip is associated with structural and biochemical changes that equip the cell for its mature functional activities, which include transport. The present findings indicate that such cellular differentiation and migration is associated with a marked increase in γ-glutamyl transpeptidase activity and in the utilization of glutathione.

The jejunal epithelium is comprised of two functionally and morphologically distinct regions, the crypts and the villi. The undifferentiated crypt cells, which are mitotically active, migrate toward the intestinal lumen and differentiate into the absorptive villus cells. The normal life span of such cells from their origin in the crypt to their ultimate extrusion into the lumen is about 2 days (1, 2). Crypt and villus tip cells have been separated by several procedures (3–7) and examined for the presence of certain enzyme activities (8–16). Three classes of intestinal enzymes have been found: (*a*) Activities that are much higher in the crypt cells as compared to the villus cells, e.g., thymidine kinase, aspartate transcarbamylase, and uridine kinase. (*b*) Activities that are much higher in the villus tip cells than in the crypt cells, e.g., maltase, lactase, dipeptidases, adenosine deaminase, and alkaline phosphatase. (*c*) Activities that are about the same levels in the crypt and the villus regions, e.g., adenylate deaminase, cytochrome oxidase, and glucose-6-phosphate dehydrogenase.

In the present work, the micrometer planing technique devised by Imondi *et al.* (7) was used to separate the epithelial cells of rat jejunum. The intracellular concentration of glutathione and the activities of enzymes involved in glutathione metabolism were examined; two of these (γ-glutamyl cysteine synthetase, glutathione synthetase) catalyze the synthesis of glutathione from its constituent amino acids, and two (γ-glutamyl transpeptidase, γ-glutamyl cyclotransferase) are involved in the major degradative pathway of glutathione metabolism (17). The uptake of amino acids by the isolated crypt and villus cells was also studied.

EXPERIMENTAL PROCEDURE

Materials. Glutathione, 5,5′-dithiobis(2-nitrobenzoic acid), TPNH, ATP, L-γ-glutamyl-*p*-nitroanilide, glycylglycine, thymidine, thymidine 5′-phosphate, phosphocreatine, creatine kinase (Type I), glutathione reductase (Type III), and amino acids were obtained from Sigma Chemical Co. L-[*U*-^{14}C]Glutamate, L-[*U*-^{14}C]methionine, and [*methyl*-^{3}H]thymidine were purchased from New England Nuclear. L-[*U*-^{14}C]Valine and L-[*U*-^{14}C]glutamine were purchased from Schwarz/Mann. L-γ-Glutamyl-L-α-aminobutyrate was synthesized as described (18). L-γ-[*U*-^{14}C]Glutamyl-L-α-aminobutyrate was synthesized enzymatically with purified rat kidney γ-glutamyl-cysteine synthetase (kindly provided by Ronald Sekura of this laboratory); the product was isolated by paper electrophoresis (50 V/cm; 2 hr) in a buffer (pH 6.5) consisting of pyridine:acetic acid:water; 200:8:1792 (vol/vol). γ-Glutamyl cyclotransferase was purified from sheep brain (19).

Methods. Male Sprague-Dawley rats (250–300 g) were housed in stainless steel cages with wire mesh bottoms; the animals were fed ad lib. on standard laboratory rat chow. Groups of rats were fasted for 24 or 48 hr; some were fasted for 24 hr, and then fed ad lib. with cane sugar cubes. Other rats were fasted for 24 hours then fed ad lib. a protein mixture prepared by mixing 8.0 g of gelatin (Knox) and 10 g of casamino acids (Difco) with 400 ml of water.

The animals were sacrificed by decapitation and their small intestines were excised. The duodenum was removed and the first 18 cm of the jejunum was cut into three sections, each of which was mounted on the tissue planing device and planed at 125 μm depths according to the method of Imondi *et al.* (7). The fractions were quick-frozen in petri dishes placed on dry ice. Five fractions were obtained from the animals fed ad lib. For the enzyme studies the cells were thawed and homogenized in five volumes (weight/volume) of 10 mM $MgCl_2$, 10 mM Tris·HCl (pH 7.6). The homogenate was layered on 18% sucrose containing 10 mM $MgCl_2$ and 10 mM Tris·HCl (pH 7.6) and centrifuged at 100,000 × *g* to sediment γ-glutamyl transpeptidase. γ-Glutamyl transpeptidase was assayed after diluting the pellet to an appropriate volume with 0.1 M Tris·HCl (pH 8.0). The other enzymes were assayed in the supernatant collected at the top of the sucrose layer. Protein was determined by the method of Lowry (20) using bovine serum albumin as standard. Thymidine kinase was assayed by the method of Klemperer and Haynes (21) except that thymidine and thymidine 5′-phosphate were separated by ascending paper chromatography in tertiary butanol:methylethylketone:water:formic acid (44:44:11:0.26; vol/vol) (22). γ-Glutamyl transpeptidase was assayed with L-γ-glutamyl-*p*-nitroanilide and glycylglycine

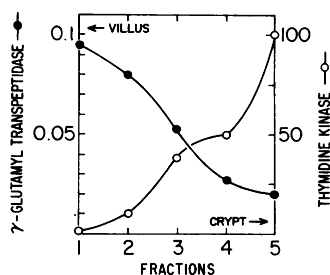

FIG. 1. γ-Glutamyl transpeptidase and thymidine kinase activities of villus tip and crypt cells. Thymidine kinase specific activity is expressed as percent of the activity of the lower crypt cells, and transpeptidase activity as μmol of product formed per min/mg of protein.

(23). γ-Glutamyl cyclotransferase, γ-glutamyl cysteine synthetase, and glutathione synthetase were assayed as described (24). "Total glutathione" (GSH + GSSG) was determined by measuring the reduction of 5,5′-dithiobis(2-nitrobenzoic acid) in the presence of TPNH and glutathione reductase (25). (Neither L-γ-glutamyl-L-cysteine nor the corresponding disulfide reacted in this system.) The frozen samples were thawed and homogenized in 50 volumes (weight/volume) of 5% trichloroacetic acid; the homogenate was extracted three times with 4 volumes of diethyl ether to remove trichloroacetic acid. Aliquots of the aqueous solution were assayed for glutathione.

Amino-acid uptake studies were carried out on suspensions of freshly obtained cells (1 to 2 × 10^7 cells per ml) in Earle's balanced salt solution (Gibco, Grand Island, N.Y.). The radioactive amino acids were brought to 30–50 mM in Earle's solution. The radioactive amino-acid solutions (10 μl) were added to 100 μl of cell suspension and the mixture was incubated at 37° for 5 min. The mixture was then layered on top of 0.10 ml of a 9:2 mixture of dibutylphthalate and mineral oil in a Microfuge tube (Bolab, Reading, Mass.), and centrifuged for 20 sec in a Beckman model 152 Microfuge. The cells sedimented rapidly to the bottom of the tube and the extracellular fluid remained at the top of the oil. The pellet was cut out with a razor blade and dissolved in a 0.75 ml of 0.1 M NaOH in a scintillation vial; solubilization took several hours at 27° with intermittent agitation. After solubilization, 10 ml of Bray's solution (26) was added and the radioactivity was determined in a scintillation counter.

RESULTS

Thymidine kinase was assayed as a marker enzyme to verify the separation of villus tip and crypt cells; this activity was found to increase sharply with increasing distance from the villus tip region as expected (6, 7, 9). On the other hand, γ-glutamyl transpeptidase was found to exhibit an opposite activity pattern in the crypt to villus gradient and thus appears to be a villus-specific enzyme (Fig. 1). γ-Glutamyl transpeptidase activity was also studied in several feeding states. After fasting for 24 hr, the activity per mg of protein increased 2- to 3-fold in both villus tip and crypt cells. In the villus tip cells no further change in transpeptidase activity was observed after fasting for 48 hr or after the 24 hr-fasted animals were fed either protein or carbohydrate for 24 hr; under these conditions, the activity in the crypt cells increased moderately. As indicated in Table 1, the activities of γ-glutamyl cyclotransferase, γ-glutamyl cysteine synthetase, and glutathione synthetase were somewhat lower in the villus tip cells than in the crypt cells, but substantial activities were found in both types of cells and in the cells of the mid-fraction. The differences observed between the two cell types were not as marked as found with γ-glutamyl transpeptidase and thymidine kinase.

Table 1. Activities of synthetases and cyclotransferase*

Activity	Villus tip cells	Mid fraction	Crypt cells
	(nmol/hr per mg of protein)		
Glutathione synthetase	400	490	730
γ-Glutamyl cysteine synthetase	160	180	380
γ-Glutamyl cyclotransferase	650	750	1500

* Averages of at least three separate determinations which agreed to within 10%; rats were fed normally.

Studies on the concentration of glutathione in the various cell fractions are summarized in Table 2. The concentration of glutathione in the crypt cells was about 16-fold greater than that of the villus tip cells. Fasting for 24 or 48 hr led to decreased levels of glutathione in all cell fractions. Feeding of carbohydrate after fasting was followed by some further decrease in the levels of glutathione, but feeding of protein increased the glutathione levels significantly.

Studies were also carried out on the capacities of the cells isolated from the crypt and villus tip regions to take up amino acids. It was found that when cells were suspended in a solution containing labeled amino acids, maximal uptake occurred within 5 min. Under the conditions given under *Methods*, the villus tip cells took up about twice as much amino acid as did the crypt cells. Thus, values for the villus tip cells of 20, 38, and 25 nmol per 10^7 cells were obtained, respectively, with methionine, valine, and glutamine; the respective values for the crypt cells were 11, 9, and 16. Similar values were obtained with both types of cells after fasting for 24 and 48 hr.

DISCUSSION

The present findings, which indicate that γ-glutamyl transpeptidase is a villus-specific enzyme, are in accord with earlier histochemical studies in which the products of this enzyme's activity were found to be localized in the brush border regions of the human (27), rabbit (28), and guinea pig (29) small intestine. In contrast to such localization of γ-glu-

Table 2. Effect of feeding state on glutathione concentration

Feeding state	Glutathione (μmol/g wet tissue) Villus tip cells	Mid-fractions*	Crypt cells
Fed ad lib.	0.26	0.43, 0.98, 1.7	4.3
Fasted 24 hr	0.02	0.36, 1.1	1.5
Fasted 48 hr	0.11	0.68, 0.86	1.2
Fasted 24 hr; then fed sucrose	0.02	0.27, 0.74	1.0
Fasted 24 hr; then fed protein	0.22	1.0, 1.3	2.3

* Four (rather than five) fractions were obtained after fasting because of the associated decrease in height of the villi.

tamyl transpeptidase, the present work suggests that γ-glutamyl cyclotransferase and the enzymes required for glutathione synthesis are not specifically localized to either the crypt or villus cells, but are present in both. The observed increase in the specific activity of γ-glutamyl transpeptidase on fasting may be related to an overall decrease in the protein content of the villus. During starvation intestinal cellular proteins may be catabolized for gluconeogenesis, and protein hydrolase activity increases (30). The membrane-bound glycoprotein γ-glutamyl transpeptidase is known to be relatively resistant to the action of proteases (31). Since intestinal cells exhibit relatively high protein hydrolase activities there may be some breakdown and inactivation of soluble enzymes such as γ-glutamyl cyclotransferase and the enzymes that catalyze glutathione synthesis during preparation of the cells by the planing procedure. Therefore, the actual values for these enzymes may be higher than those reported here, especially for the villus tip cells. Moderate decreases in the activities of the synthetases were observed after fasting; this probably contributes to the reduced levels of glutathione found after fasting. The decrease of glutathione concentration on fasting may also reflect utilization of glutathione for protein synthesis; the increase in glutathione after feeding protein (but not carbohydrate) is consistent with this interpretation.

The crypt cells have a high concentration of glutathione (4–5 mM) comparable in magnitude to that found in liver and kidney. However, the concentration of glutathione in the various cell fractions (from crypt to villus) decreases as the γ-glutamyl transpeptidase activity increases. The observed inverse relationship between the concentration of glutathione and the activity of γ-glutamyl transpeptidase is in accord with the view that the transpeptidase catalyzes the quantitatively major pathway of glutathione breakdown, and strongly suggests that such utilization of glutathione is connected with a significant physiological activity of the villus tip cells. On the other hand, the undifferentiated crypt cells, which are not very active in transport, have relatively high concentrations of glutathione and little γ-glutamyl transpeptidase activity. There is substantial evidence that the migration of cells from the crypt to the villus tip is associated with structural and biochemical changes which equip the cell for its mature functional activities, which include absorption of amino acids and peptides (32–34). It seems probable that the marked changes in γ-glutamyl transpeptidase activity and glutathione concentration observed here are representative of changes of this type. The findings are in accord with the hypothesis that the transpeptidase, via the γ-glutamyl cycle, functions in amino-acid transport (17). If this is the case, it would appear that the cycle is more important in relation to the function of the absorptive villus cells than to that of cells of the crypt.

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