Demonstration of a Difference in Expression of Maximal Lactase and Sucrase Activity Along the Villus in the Adult Rat Jejunum

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Lactase and sucrase are two disaccharidases that differ not only in their substrate specificity and developmental patterns, but also in their resistance to mucosal insult. In this experiment, we tested the hypothesis that there might be a dichotomy in expression of enzyme activity along the jejunal villus-crypt unit. Sectioning of the villus-crypt unit in a cryostat enabled direct comparison of the distribution of lactase and sucrase enzyme activities in the adult rat. There is a stepwise increase in mean lactase/sucrase ratio going from crypt to villus. The data indicate that unlike sucrase activity, which is expressed maximally in enterocytes along the entire villus, maximal lactase activity is not attained until midvillus. The delay in expression of maximal lactase activity might help to explain the vulnerability of this enzyme to acute mucosal insult such as occurs in viral gastroenteritis.

Since the work of Leblond and Stevens,[1] it has been recognized that the enterocytes mature both morphologically and biochemically while migrating from the crypts to become digestive-absorptive cells of the villus. Using various techniques to isolate crypt from villus cells, several different laboratories have shown in the rat that activity of enzymes asso-

ciated with DNA synthesis decrease,[2,3] activity of lysosomal enzymes remain unchanged,[2,4,5] and activity of microvillus enzymes such as alkaline phosphatase and disaccharidases increase[2-8] as cells migrate along the heights of the villus.

Early in this century, Finkelstein and Meyer recognized that infants with acute gastroenteritis could not tolerate "complex sugars."[9] Subsequent studies have shown that this is usually a manifestation of transient lactose intolerance,[10-12] whereas an ability to absorb sucrose is usually preserved.[13-15] Thus, these two disaccharidases differ not only in their substrate specificity and developmental patterns,[16,17] but also in their resistance to mucosal insult. Since the absolute activity of lactase is low compared to sucrase both in the human[18] and rat,[19] a comparable loss of both enzymes after mucosal injury may reduce lactase activity below a critical threshold to allow for physiologic hydrolysis of dietary lactose. However, the decrease in the ratio of lactase to sucrase activity in small bowel biopsies of patients who are lactose intolerant after acute mucosal injury indicates a higher vulnerability of lactase compared to sucrase.[13-15]

By studying incorporation and turnover of radiolabeled amino acids, James et al. have shown that microvillus proteins do not have a uniform rate of turnover.[20] Alpers showed that the largest molecular weight proteins (including the disaccharidases) have the fastest rate of turnover.[21] Therefore, both synthesis and degradation of disaccharidases are taking place along the entire length of the villus crypt. Differences in location where synthesis begins, or differences in the rate of turnover of lactase and sucrase might lead to a difference in enzyme distribution along the villus-crypt unit. Differences in localization of enzyme activity in turn might help explain the decrease in the ratio of lactase to sucrase
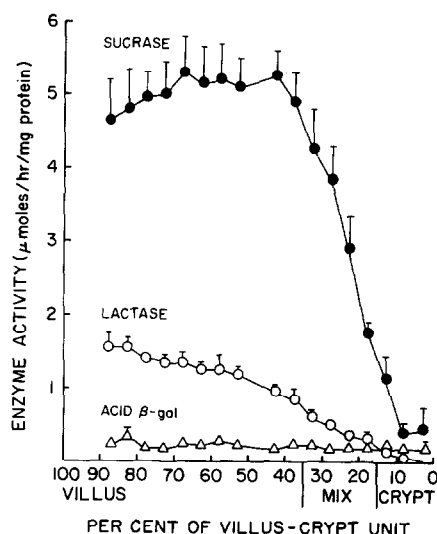
Figure 1. Specific activity (micromoles per hour per milligram protein) of sucrase (●), lactase (○), and acid β-galactosidase (△) along the villus-crypt unit. Abcissa depicts "idealized villus-crypt unit" as explained in the Methods section, with 100% representing the top-most part of the villus and 0% the bottom-most part of the crypt. Histologic examination of sections revealed villi to make up 65%; mixed villus crypt, 20%; and crypt, 15% of the total villus-crypt height. Because of very low protein in homogenates from apical 10% of villus crypt, the amount of absorbance measured by spectrophotometer was very low both for lactase and sucrase, and, therefore, enzyme activity was not considered accurate for presentation. *Short vertical lines* denote 1 SEM. Absence of vertical line indicates that value of SEM is smaller than the symbols used. All figures in the text represent data from eight rats; each *point* on graph represents mean data from 6 to 8 homogenates.

activity in small bowel biopsies after intestinal mucosal insult.

Although different investigators have described an apical localization of one or the other enzyme along the villus-crypt unit, no one to date has systematically compared the localization of lactase and sucrase activities on the same villus-crypt unit. In the present study, we compare localization of lactase and sucrase activity on the same villus-crypt unit after sequential sectioning in a cryostat by using the technique of Van Genderen and Engel[22] as modified by Nordstrom et al.[7] The data indicate that, unlike sucrase activity, which is maximally expressed in enterocytes along the entire villus, maximal lactase activity is not attained until midvillus.

Materials and Methods

Male rats of Charles River strain CD (Wilmington, Mass.) were shipped at 2 mo old and were kept 4–6 wk in our animal house before being used in experiments. Animals were fed a standard rat chow (Purina Co. No. 5001) ad libitum, and weighed between 300 and 350 g at time of

study. Fed rats were killed by decapitation at 9:00 AM, and a 5 × 5-mm segment of jejunum was sectioned within a cryostat at −18°C as previously described.[7] Horizontal sections were cut 10 μm thick (No. 5 setting on IEC microtome, Needham Hts., Mass.).

At various depths into the villus-crypt unit, a section was attached to the microscope slide for immediate inspection of histology under a phase-contrast microscope. The tissue blocks were sectioned through the submucosa to the proximal muscular layer. Commencing with counting of sections, every six consecutive sections were combined and homogenized in 0.5 cm³ distilled water by vortex shaking and sonification × 30 sec (Sonifier Cell Disrupter, model V185, Heat Systems-Ultrasonics, Inc., Plainview, N.J.). Assays of lactase and sucrase activities were performed on each homogenate according to the method of Dahlqvist.[23] The lactase assay mixture contained p-chloromercuriobenzoate (PCMB) (Aldrich Chemical Co., Milwaukee, Wis.) in order to inhibit any residual lysosomal acid β-galactosidase activity.[24] All enzyme determinations were made under conditions of linear activity with time and concentration of the enzyme. Protein was determined according to the method of Lowry et al.[25] All chemicals used were of reagent grade.

Three possible problems associated with the lactase assay were investigated (data not shown): (a) To test for the presence of an inhibitor of the glucose oxidase reaction, homogenates obtained from different heights of the villus-crypt unit were boiled and the same protein concentration as used in the enzyme assays were added to the glucose standards.[26] The resulting curves showed no significant inhibition. (b) The possibility of an inhibitor of enzyme activity in lower sections of the villus crypt was studied by combining homogenates of equal protein concentration from apical villus and lower villus-mixed crypt sections. The resulting activity for both lactase and sucrase approximated the arithmetic mean activity calculated to result from such a mixing. (c) The dependence of lactase activity on pH was determined in homogenates from apical, mid-, and lower villus-mixed villus-crypt areas of the villus-crypt unit. The pH optimum for lactase activity at all heights was between 5.0 and 6.0. In the presence of PCMB, all activity at pH 3.5 was abolished. Since the pH optimum of acid β-galactosidase is 3.5, this verifies that in homogenates of cryostat sections, PCMB totally inhibits the acid β-galactosidase activity as has also been shown in whole intestinal homogenates, and partially purified fractions of this lysosomal enzyme.[24,27,28]

Expression of Results

Since there was some variation in the number of sections obtained from different animals, the activities of various enzymes in serial homogenates were related in an "idealized villus-crypt unit."[17] This maneuver allowed data from a number of animals to be easily compared. The percent distance each homogenate represented of the total villus-crypt unit was determined in any given tissue block. Thus, enzyme activity in the sixth of 16 homogenates was considered to represent enzyme activity at a point between 35% and 40% of the distance between tip and base of an "idealized villus-crypt unit."
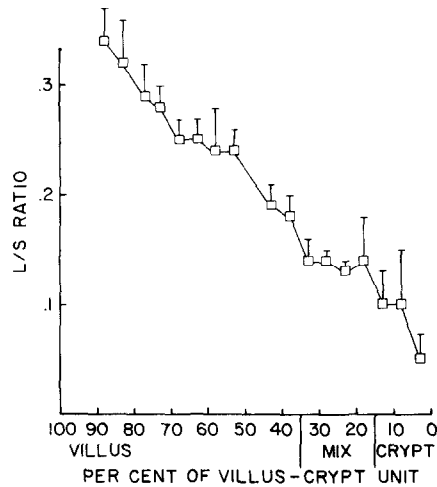
significant variation of mean lactose/sucrose (L/S) ratios at different heights of the villus crypt.

Results

Figure 1 shows the distribution of specific lactase, sucrase, and acid β-galactosidase activity along the villus-crypt unit. The general pattern of distribution of activity agrees with previous reports.[2-7] The mean L/S ratios at different heights of the villus crypt are plotted in Figure 2. Since both enzyme assays were performed on the same homogenate, the L/S ratio is independent of how enzyme activity is expressed. The results show that there is a definite gradient of increasing L/S ratio in going from crypt to apical villus. Using one-way analysis of variance, the ratio in lower villus is significantly less than in apical and midvillus (Table 1). The reason for this increase in L/S ratio can be seen if the specific activity of each enzyme is expressed as a percent of the maximal specific activity along a particular villus-crypt unit (Figure 3). Sucrase reaches maximal activity in enterocytes along the upper 70%–75% of the villus-crypt height, whereas lactase does not peak until the top 45%–50%. If the total activity of each enzyme in each homogenate is expressed as a percent of the total activity of the tissue block, the lag in expression of maximal lactase activity is also seen (data not shown). Total activity of both disaccharidases decreases sharply in the apical portions of the villus-crypt unit because of small amounts of protein in apical homogenates. The net result of the different patterns of distribution is that 63% ± 3% (mean ± SEM) of total lactase activity along the villus-crypt unit is present on the top 50% of the unit as compared with 51% ± 3% of sucrase activity (P < 0.02)

Discussion

This is the first report that directly compares distribution of lactase and sucrase activities along the crypt-villus unit of the adult rat. The technique

Figure 2. Mean L/S ratio along villus-crypt unit. Abcissa depicts villus-crypt unit as described in Figure 1. Short vertical lines denote 1 SEM. Statistical evaluation is given in Table 1.

The enzyme activity of each homogenate was calculated both as specific activity (micromoles of substrate hydrolyzed per hour per milligram protein) and as total activity (micromoles of substrate hydrolyzed per hour per homogenate). Since both lactase and sucrase activities were performed on the same homogenates, the lactase/sucrase ratio is independent of how the individual activities are expressed. However, to allow for statistical comparison between individual lactase and sucrase activities at any point along the villus-crypt unit, two alternative modes of expression of data were utilized: (a) The highest specific activity of each enzyme along the villus-crypt unit in any given rat was set at 100% and all other activities on the same unit expressed as a percent of that highest activity. (b) The total activity of each tissue block was determined by summation of total activities of consecutive homogenates. The total activity of each enzyme in each homogenate was then expressed as a percent of the total activity of the tissue block.

The data from each of eight rats were plotted along the idealized villus-crypt unit as explained above. Average values for each 5% of distance were calculated. When comparing lactase to sucrase activity at any given height of the villus-crypt, Student's t-test was used.[29] One-way analysis of variance[30] was used to determine if there was

Table 1. Significance of Mean L/S Ratios at Different Heights of Villus Crypt Compared with All other Heights

% Villus-crypt height	90%–81%	80%–71%	70%–61%	60%–51%	50%–41%	40%–31%	30%–21%	20%–11%	10%–0%
90%–81%				x	x, y	x, y	x, y	x, y	x, y
80%–71%					x	x, y	x, y	x, y	x, y
70%–61%						x	x, y	x, y	x, y
60%–51%	x						x	x, y	x, y
50%–41%	x	x, y							x, y

x = P < 0.05; y = P < 0.01. For statistical purposes, individual data for each 10% of height of the villus crypt were pooled. One-way analysis of variance confirmed that population means differ substantially, F = 18.9. Least significant difference (LSD) was used to compare individual means.
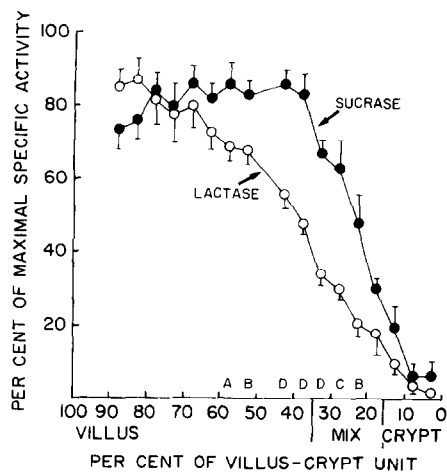
Figure 3. Relative activity expressed as mean percent maximal specific activity of sucrase (●) and lactase (○) along the villus-crypt unit. The highest specific activity in each tissue block was set at 100% and activities in all other homogenates expressed as a percent of that highest activity. Letters denote level of statistical significance between lactase and sucrase activities at any point along the villus-crypt unit. (A. $P < 0.05$; B. $P < 0.02$; C. $P < 0.01$; D. $P < 0.001$.) Absence of letter indicates no significant differences between maximal activities at a given height.

of cryostat sectioning of frozen intestine was chosen because it provides precise sequential separation of cells along the villus-crypt unit after minimal manipulation of bowel. The other accepted method of cell separation is the Weiser "washing" technique.[3] While this is an excellent method for obtaining isolated enterocytes from apical villus, lower villus, and crypt, we were concerned with the specificity of "washing" cells sequentially down the villus in order to determine a true gradient of enzyme activity. In addition, the method does not control for the possibility of differential binding of the two enzymes onto the microvillus. Selective solubilization of lactase in early washings from cells still adherent to the villus-crypt unit may result in erroneously low levels in lower portions of the villus.

In our present study, the general pattern of distribution of both lactase and sucrase along the villus crypt agrees with previous reports,[2-7] i.e., localization of activity to the villi and absence of activity in the crypts. However, by directly comparing enzyme activity in the same homogenate at each height of the villus crypt, we found that, unlike sucrase, lactase fails to achieve maximal activity until midvillus. This observation is based on the stepwise increase in mean L/S ratio going from crypt to midvillus. Since both lactase and sucrase enzyme assays were performed on the same homogenate, the L/S

ratio is independent of how enzyme activity is expressed.

The more apical localization of lactase activity compared to sucrase activity on the crypt-villus unit may help to explain the vulnerability of this enzyme to acute mucosal injury (e.g., acute gastroenteritis). Histologic studies in acute viral gastroenteritis have shown blunting of villus height and disorganization of surface epithelial cells.[31,32] In the event of apical destruction of villus cells carrying lactase activity, the remaining activity in cells in the mid to lower levels of the villus may be below critical threshold to allow for physiologic hydrolysis of lactose. It is important to remember that total jejunal lactase activity is between one-fifth (unpublished from our laboratory) and one-tenth[19] that of sucrase activity in the adult rat. The more even distribution of sucrase along the crypt-villus unit would then explain the usual normal ability to absorb sucrose in acute gastroenteritis.

Recent data suggest that invasion and damage of epithelial cells by the virus itself may not be the only factor in the pathogenesis of diarrhea in this disease. Study of transmissible gastroenteritis in piglets (corona virus) has suggested that intestinal epithelial cell migration rates increase in response to viral invasion.[33,34] While this leads to rapid desquamation of infected cells, the villi become populated by relatively immature cryptlike cells. Based on results of the present experiment, lactase activity, which is more dependent on cell maturity than sucrase, would be primarily affected by reparative events occurring after infection.

Nordstrom et al.[35] have described the distribution of disaccharidase activity along the crypt-villus unit in 1 human patient using the same technique as in our report. Difficulties in mounting and slicing human tissue restricted statistical comparison between a number of patients. Although it is not stated that the activities of all enzymes were assayed on the same villus-crypt unit, the authors report a more apical distribution of jejunal lactase activity when compared to sucrase and maltase. This observation lends further clinical credence to the significance of the present studies in the rat.

Further studies are needed to determine the mechanism responsible for the differences in distribution of lactase and sucrase activities. Differences in location where synthesis begins, or differences in the rate turnover of lactase and sucrase might lead to a difference in enzyme distribution along the villus-crypt unit. In view of the similarities of the lactase and sucrase distribution pattern between rat and human, the rat would be a suitable model for these studies.

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