Immunoelectrophoretic studies on human small-intestinal brush-border proteins

Relation between enzyme activity and immunoreactive enzyme along the villus–crypt axis

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The amounts of lactase (β -D-galactosidase, EC 3.2.1.23), sucrase (sucrose α -D-glucosidase, EC 3.2.1.48), maltase (α -D-glucosidase, EC 3.2.1.20), microvillus aminopeptidase (EC 3.4.11.2) and dipeptidyl peptidase IV (EC 3.4.14.-) in tangentially sectioned biopsies from jejunum were studied by quantitative immunoelectrophoresis and enzymic assays. All enzymes had their maximum activities near the mid-region of the villi and their lowest activities at the bases of the crypts. The ratio between enzyme activity and immunoreactive protein was constant along the villus–crypt axis. This result is consistent with a continuous brush-border-enzyme synthesis as the enterocytes migrate up the villi.

The human small-intestinal brush-border membrane contains several enzymes important for the final digestion of carbohydrates and polypeptides. The localization of some of the brush-border enzymes along the villus–crypt axis has been established by enzymic measurement either on successive horizontal sections of fresh frozen intestine (Nordström *et al.*, 1968) or on sequentially isolated cells (Webster & Harrison, 1969; Weiser, 1973). The brush-border-enzyme activity is low in crypt cells and seems to increase gradually as the cells migrate up the villi (Raul *et al.*, 1977; Gratecos *et al.*, 1978).

Immunofluorescent studies (Silverblatt *et al.*, 1974; Dubs *et al.*, 1975) have suggested the presence of inactive sucrase molecules in the crypts of human and rabbit small intestine, indicating that the enzyme is synthesized in the crypts as an inactive precursor that is converted to the active enzyme as the cells mature and migrate up the villi. On the other hand, labelling studies (James *et al.*, 1970) have shown a continuous synthesis and turnover of brush-border enzymes in the mature enterocyte.

Quantitative information of the relation between enzyme activity and amount of immunoreactive enzyme along the villus–crypt axis might contribute to the knowledge of the brush-border-enzyme synthesis. Such an estimation is possible by the use of crossed immunoelectrophoresis. Utilizing this method the present study reports on the relationship between enzyme activities and amount of immuno-

reactive protein on horizontal sectioned biopsies from human small intestine.

Experimental

Materials

Antibodies specific against brush-border proteins were raised in rabbits by using proteins solubilized from isolated human small-intestinal brush-border membranes as antigen (Skovbjerg *et al.*, 1978). All other chemicals were obtained as described in a previous paper (Skovbjerg *et al.*, 1979).

Methods

Biopsy preparation. 5 cm pieces of the proximal part of jejunum obtained during jejuno–ileal bypass operation for obesity from two patients were used. After removal the pieces were chilled in crushed ice, immediately opened and spread on a filter paper in a cold-room (+4°C). Biopsies 5 mm in diameter were punched out and frozen to a cryostat chuck on solid CO₂ by using distilled water as adhering material. The edges of the biopsy were trimmed with a razor blade (44 biopsies from one of the intestinal samples and 33 biopsies from the other were used in two experiments). Cryostat cutting into 10 μ m-thick slices was performed at –18°C (Nordström *et al.*, 1968). Each biopsy was cut into 80–90 slices. Every tenth section was stained with a 1% Methylene Blue solution for immediate microscopic inspection to ensure horizontal sectioning and estimate the distri-

bution of villi, crypts and connective tissue. The nine intermediate sections were pooled with the corresponding sections from the other biopsies. When all biopsies were cut, the pooled sections were homogenized for 3 min at 0°C in 175 μ l of a 1% Triton X-100 solution containing 1.4 mg of aprotinin/ml. The tube was incubated at 4°C for 60 min with frequent stirring. After incubation, 20 μ l of the homogenate was removed for protein analysis. A 130 μ l portion of the homogenate was centrifuged at 50000g for 1 h and the supernatant removed. The pellet was suspended in 50 μ l of the Triton X-100 solution, incubated at 4°C for 30 min with frequent stirring and subsequently centrifuged at 50000g for 1 h. The two supernatants were pooled.

Crossed immunoelectrophoresis. Crossed immunoelectrophoresis was performed in 0.02 M-sodium barbital buffer, pH 8.6, as described previously (Skovbjerg *et al.*, 1979). A 20 μ l portion of the pooled supernatants was mixed with 5 μ l of a papain solution (5 mg/ml) containing cysteine (1 mg/ml) and incubated at 37°C for 15 min. A 20 μ l sample was then analysed by crossed immunoelectrophoresis against the brush-border-specific antiserum. The lactase-phlorizin hydrolase [β -D-galactosidase (EC 3.2.1.23)—glycosylceramidase (EC 3.2.1.62)], sucrase-isomaltase [sucrose α -D-glucosylhydrolase (EC 3.2.1.48)—oligo-1,6-glucosidase (EC 3.2.1.10)], maltase (α -D-glucosidase, EC 3.2.1.20), microvillus aminopeptidase (EC 3.4.11.2) and dipeptidyl peptidase IV (EC 3.4.14.-) precipitates, which were previously identified by enzymic-staining methods (Skovbjerg *et al.*, 1978), were stained with Coomassie Brilliant Blue and their areas measured by an electronic integrator.

Assays. Lactase and sucrase were measured with the Tris/glucose reagent as described by Dahlqvist (1968), with potassium ferrocyanide as chromogen (Sjöström *et al.*, 1978). Maltase was not measured enzymically, since the assay of this enzyme also includes the maltose-splitting activity of at least the sucrase-isomaltase complex. The activities of microvillus aminopeptidase and dipeptidyl peptidase IV were determined by using L-alanine *p*-nitroanilide and glycyl-L-proline *p*-nitroanilide as substrates (Skovbjerg *et al.*, 1978). The protein concentration was measured as described by Wang & Smith (1975), with crystalline bovine serum albumin as a standard.

Results and discussion

The results of the experiments on the two biopsy groups (one of which is shown in Fig. 1) were similar.

Morphologic identification

As judged from microscopic examination of every

tenth section, all the biopsies were cut horizontally. The percentage distributions of villi, crypts and connective tissue in the microscopic examined sections are shown in Fig. 1(a).

Solubilization procedure

The solubilization procedure releases 90–100% of the studied enzymes in an amphiphilic form. The presence of aprotinin prevents a possible trypsin and chymotrypsin activation of hypothetical inactive enzymes during the preparation. For the same reason, papain was omitted in the solubilization procedure. To improve the quality of the immunoprecipitates (Skovbjerg *et al.*, 1979), papain was added to the pooled supernatants before the electrophoresis.

Immunoelectrophoretic patterns

The immunoelectrophoretic patterns obtained from the villus sections and the crypt sections are exemplified in Figs. 2(a) and 2(b). The lactase-phlorizin hydrolase, sucrase-isomaltase, maltase and microvillus aminopeptidase precipitates were visible in both plates, whereas the dipeptidyl peptidase IV precipitate cannot be identified in the crypt sections.

Distribution of the enzymes

The specific activities of all the enzymes studied were higher near the mid-region of the villi and had their lowest values at the bases of the crypts (Figs. 1b–1f). The distribution of brush-border maltase, which is specifically measured by the immunoelectrophoretic method, and dipeptidyl peptidase IV has not been reported before. Our results demonstrate that the distribution of these enzymes along the villus-crypt axis is similar to that of the other brush-border enzymes.

A decrease in activity was found in the tops of the villi, most pronounced in the case of sucrase and dipeptidyl peptidase IV. This might be due to an increased degradation rate caused by pancreatic enzymes (Alpers & Tedesco, 1975) near the villus top or to a decreased synthesis of the enzymes.

The distribution curves for the enzymes do not clearly parallel the percentage distribution of villi in the sections, suggesting that the crypt cells might contain some enzymic activity. This finding conflicts with that of Nordström *et al.* (1968), who found complete absence of brush-border enzymes in the crypts of rat intestine, but is in accordance with the results from several other studies (Raul *et al.*, 1977; Simon *et al.*, 1979), showing some sucrase activity in the crypt cells from rat intestine and suggesting that these cells are able to synthesize active sucrase. Also, another study on human small intestine (Nordström & Dahlqvist, 1973) suggested the presence of low sucrase, lactase and aminopeptidase activities in the crypts. However, in the

Fig. 1. *Distribution of enzymes along the villus-crypt axis*

(a) Percentage distribution of villi, crypts and connective tissue estimated by microscopic inspection of every tenth section. Each point represents the average result for slices from 44 biopsies from the same intestinal piece. ----, Connective tissue; Δ , crypts; $\blacktriangle$, villi. (b)–(f) Enzyme specific activities (●) and area under the precipitates (○) per mg of protein. The 44 biopsies were sliced and the corresponding sections pooled and analysed as described under 'Methods'.

present study, a sharp borderline between villi and crypts was not obtained, making a final conclusion of this question difficult.

Immunofluorescent studies (Silverblatt *et al.*, 1974; Dubs *et al.*, 1975) have previously indicated that crypt cells contained immunoreactive sucrase protein without enzymic activity. If this were the case, we would have expected our immunoreactive enzyme (area under the precipitate) (Figs. 1b–1f), to be relatively enlarged in the crypts compared with the enzyme activity. On the contrary, we found the same proportion between enzyme activity and immunoreactive enzyme in the sections containing

only villous cells and those containing a high percentage of crypt cells. Differences in methodological approach may account for the discrepancy in these studies. Cross-reaction with other brush-border proteins may affect the specificity of the immunofluorescent method. Alternatively in our study the amount of enzymically inactive enzyme protein might be very small and thereby not detected.

As the crypt cells compared with the villus cells do not contain a high proportion of inactive enzyme molecules, our results suggests that the brush-border enzymes are synthesized continuously as the entero-

Fig. 2. Immunelectrophoretic patterns

(a) Sections from the villus; (b) sections from the crypt. Nine sections (11–19 and 61–69 respectively) from each of 44 biopsies were pooled, the proteins solubilized and analysed by crossed immunelectrophoresis as described under 'Methods'. Abbreviations used: S, sucrase-isomaltase; L, lactase-phlorizin hydrolase; M, maltase; A, microvillus aminopeptidase; G, dipeptidyl peptidase IV.

cytes migrate up the villi. This suggestion is supported by labelling studies (James *et al.*, 1970), which showed a continuous synthesis and degradation of the brush-border enzymes as the cells mature.

Our conclusion also holds for intracellular proteins not yet inserted into the membrane. The existence of inactive enzymes lacking immunological reactivity is a possibility that could not be ruled out in the present study.

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